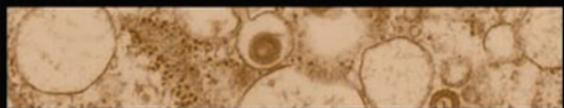


Alexander F. Voevodin
& Preston A. Marx



SIMIAN VIROLOGY



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Simian Virology

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Preface

Simian virology became a “centenarian” last year. It was conceived in 1908 by Carl Landsteiner and Erwin Popper who demonstrated that poliomyelitis was a viral disease transmissible to monkeys. Their work laid a foundation for a spectacular victory in the fight against poliomyelitis. During the next 40 years progress in simian virology was intermittent and generally slow. Nevertheless, some important achievements were made such as proving the viral etiology of yellow fever (Adrian Stokes, 1927; see Chapter 23) and identification of “herpes B” virus, a simian virus lethal for humans (Albert Sabin and Arthur Wright, 1934; see Chapter 12).

The first wave of explosive growth of simian virology was sparked in the early 1950s by the development of vaccines against poliomyelitis. Evaluation of the efficacy of the vaccine, large-scale propagation of the vaccine strains, and safety testing would have been impossible without the use of nonhuman primates (NHPs). As a by-product of polio vaccine development, a number of simian viruses were discovered and characterized in the 1950s and 1960s.

The advent of AIDS in 1981 ushered in the modern era of simian virology. Human immunodeficiency viruses type 1 (HIV-1) and type 2 (HIV-2) were discovered in 1982 and 1986, respectively (see Chapters 3 and 5). Both HIVs originated from simian ancestors, the simian immunodeficiency viruses (SIVs). These discoveries focused simian virology mainly on AIDS-related themes. Although other areas of simian virology moved at a slower pace compared to AIDS-related research, knowledge of simian viruses other than SIV advanced, particularly during last 10 years.

There are more than 20,000 simian virology-related publications. However, surprisingly, during past 40 years there has been no attempt to structure and inte-

grate this huge body of information into a single book. This book is an attempt to fill this gap by providing an up-to-date compendium for the readership ranging from experienced professionals interested in simian viruses and NHP models of viral diseases to advanced undergraduate students who may use the book as a stepping-stone to infectious disease research in NHPs.

To accommodate readers with different backgrounds the book is divided into two parts. Part I (Chapters 1 and 2) presents a minimalist approach to primate taxonomy and basic virology, a kind of primer for undergraduate and graduate students. Scientific and medical professionals who lack primatological and virological background or need a brief refresher may also benefit from reading Chapters 1 and 2.

Part II (Chapters 3–24) is mainly devoted to specific viral families that contain simian viruses. It is assumed that a reader of these chapters has a core knowledge of primatology and virology, at least at the level of Part I. Our aim in Part II is not simply the “cataloguing” of data relevant to the individual simian viruses, but rather we want to present a structured and critically assessed “state-of-the art” knowledge in these fields formatted to be useful for both professionals and students. We do not pretend to be comprehensive in each and every chapter of Part II. The specialists may find some omissions and we invite suggestions if the reader feels that the omissions were of central importance to the field. Several chapters in Part II stand apart. Chapter 5 contains a critical analysis of data and hypotheses relevant to the origin of HIV/AIDS pandemic. This chapter may be of interest not only to professionals, but also to students and even to lay readers. Chapter 23, in a very concise form, covers NHP models of human and animal diseases produced by the inoculation of NHPs with

nonsimian viruses. Chapter 24 contains examples of natural (nonexperimental) infections of NHPs with non-simian viruses, including human viruses.

Writing this book was a challenging and enriching experience. At the same time, one of the most striking findings we made was uncovering major gaps in the

knowledge of simian viruses, far more than we expected. We hope that our book will spur new investigations to close these gaps.

Alexander F. Voevodin
Preston A. Marx

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A special thanks to Robin Rodriguez for preparation of figures and images. The illustrations in this book add significantly to its weight and usefulness as a teaching tool and this would not have been possible without the help of Mrs. Rodriguez.

Part I:

Introduction to Primatology

and Virology

1

Classification of Nonhuman Primates

- 1.1 Introduction
- 1.2 Classification and nomenclature of primates
 - 1.2.1 Higher primate taxa (suborder, infraorder, parvorder, superfamily)
 - 1.2.2 Molecular taxonomy and molecular identification of nonhuman primates
- 1.3 Old World monkeys
 - 1.3.1 Guenons and allies
 - 1.3.1.1 African green monkeys
 - 1.3.1.2 Other guenons
 - 1.3.2 Baboons and allies
 - 1.3.2.1 Baboons and geladas
 - 1.3.2.2 Mandrills and drills
 - 1.3.2.3 Mangabeys
 - 1.3.3 Macaques
 - 1.3.4 Colobines
- 1.4 Apes
 - 1.4.1 Lesser apes (gibbons and siamangs)
 - 1.4.2 Great apes (chimpanzees, gorillas, and orangutans)
- 1.5 New World monkeys
 - 1.5.1 Marmosets and tamarins
 - 1.5.2 Capuchins, owl, and squirrel monkeys
 - 1.5.3 Howlers, muriquis, spider, and woolly monkeys
 - 1.5.4 Titis, sakis, and uakaris
- 1.6 Concluding remarks

1.1. INTRODUCTION

The order Primata includes humans and animals that are our closest living “relatives” in the animal kingdom. There are many definitions of a primate. None of them are completely satisfactory. As a result, there are disagreements regarding inclusion of some animal taxa in the order Primata. At the same time, it is indisputable

that the animals colloquially known as monkeys and apes are primates. From the zoological standpoint, humans are also apes, although the use of this term is usually restricted to chimpanzees, gorillas, orangutans, and gibbons.

1.2. CLASSIFICATION AND NOMENCLATURE OF PRIMATES

The classification of primates, as with any zoological classification, is a hierarchical system of taxa (singular form—taxon). The primate taxa are ranked in the following descending order:

Order	
Suborder	
Infraorder	
Parvorder	
Superfamily	
Family	
Subfamily	
Tribe	
Genus	
Species	
Subspecies	

Species is the “elementary unit” of biodiversity. Strictly speaking, the only natural grouping of animals is a population. The species is defined as a group of populations whose individual members interbreed and produce fertile offspring in their natural habitat. In practice, the use of this definition may be problematic, for example, for clearly distinct and fertile hybrids existing in the wild. As a result, there are multiple disagreements regarding species rank for the morphologically and/or behaviorally distinguishable nonhuman primate (NHP) populations.^{3,4,10,12}

The nomenclature of NHP species is binomial, that is, the scientific names of the species consist of two Latin words. The first capitalized word identifies the genus (plural—genera). The second, lowercase word identifies the species within the genus. The scientific names of species are *italicized*. For example, *Macaca mulatta* is the species *mulatta* within the genus *Macaca*.

The nomenclature of NHP subspecies is trinomial. The full scientific name of a subspecies name consists of three words: the binomial species name and the third word identifying the subspecies. For example, the common chimpanzee species (*Pan troglodytes*) is usually divided into four subspecies: *Pan troglodytes troglodytes*, *Pan troglodytes schweinfurthii*, *Pan troglodytes verus*, and *Pan troglodytes vellerosus*. The names of the subspecies are usually written in abbreviated form, for example, *P. t. troglodytes* and *P. t. schweinfurthii*. The notion of subspecies is very useful for understanding the natural history of simian viruses.

In addition to the scientific names, virtually all primate species and many subspecies have common or vernacular names. The common names are obviously different in various languages; however, the English names are used predominantly in the scientific literature.¹¹ For example, the common name for *Macaca mulatta* is rhesus monkey; its capitalized version, Rhesus Monkey, is also used. Some simian species have more than one common name; for example, *Papio hamadryas* is called the Sacred Baboon or Hamadryas Baboon. Despite the intrinsic ambiguity of common names, they are useful, for example, for describing NHPs whose taxonomic status is undetermined or controversial. Common names are also easier to pronounce and memorize than the Latin binomial designations.

Ideally, the hierarchy of primate taxa should reflect the evolutionary history. In such cases, classification would be invariant. However, the incompleteness of current knowledge allows multiple hierarchies of primate taxa—hence the existence of different classifications of primates. Most of the inconsistencies between various classifications are located at levels higher or lower than species. Taxa whose rank is higher than species are “artificial” in the sense that their definitions are based on subjectively chosen criteria.

In this book, we mainly follow Groves’ classification of primates which is the most widely used.¹⁰ This classification includes 375 simian species (Tables 1.1–1.4). Information on NHP subspecies is included only if it is relevant in a context of simian virology. At the sub-

species level, we follow the classification described in literature.^{4,12}

1.2.1. Higher Primate Taxa (Suborder, Infraorder, Parvorder, Superfamily)

The primates are divided into two suborders: Strepsirrhini and Haplorrhini (Figure 1.1). Strepsirrhini are divided into three infraorders: Lemuriformes (lemurs), Chiromyiformes (aye-ayes), and Lorisiformes (loris). Haplorrhini are divided into two infraorders: Tarsiiformes (tarsiers) and Simiiformes (simians, i.e., monkeys and apes). The problematic group is tarsiers, also called tree shrews. There is no agreement as to whether or not they belong to the primate order. Placing tarsiers together with simians is also disputed. Traditionally, lemurs, aye-ayes, lorises, and tarsiers (if the latter are included in the primates) are considered as prosimians.

The simian part of primate classification starts at the parvorder level. The simians are divided into Platyrhini (literally “broad or flat-nosed”) and Catarrhini (literally “downward-nosed”). All Platyrhini species live in South America—hence their common name the New World monkeys (NWMs). There are no Catarrhine species in the New World, except African green monkeys (AGMs), which were introduced to several Caribbean islands (St. Kitts, Nevis, and Barbados) in historically recent times (seventeenth century). All African and Asian simian species, except apes, are Old World monkeys (OWMs).

The division into monkeys and apes is formalized at the superfamily level: the members of Cercopithecoidea superfamily are monkeys while the members of Hominoidea superfamily are apes (Figure 1.2).

All 152 currently recognized OWM species are included in one family—Cercopithecidae. This family is divided into two subfamilies: Cercopithecinae and Colobinae (Figure 1.2). There are 11 Cercopithecinae genera and 10 Colobinae genera. Cercopithecinae genera with 42 chromosomes diploid karyotype (*Papio*, *Theropithecus*, *Mandrillus*, *Cercocebus*, *Lophocebus*, and *Macaca*) are combined in the tribe Papionini^{14,35} (Figure 1.3). The tribe level is not universally used in the classifications of primates. For instance, it is not included in Groves’ classification. However, the term Papionini is commonly used in the descriptions of simian immunodeficiency virus (SIV) and other simian retrovirus hosts.

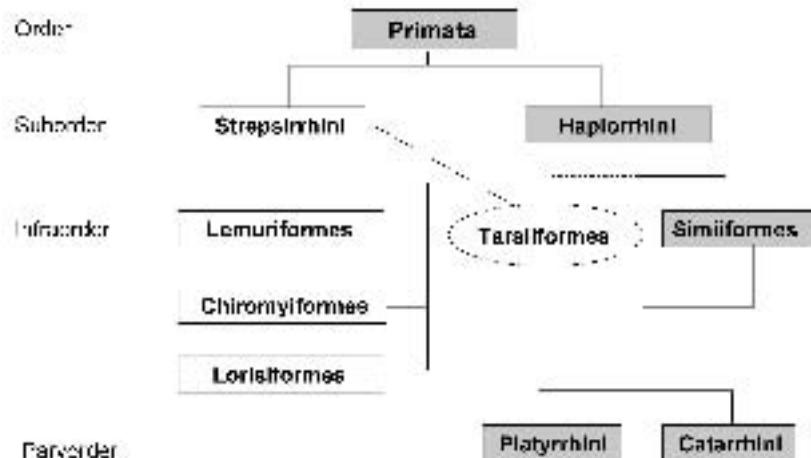


Figure 1.1. Higher primate taxa (order, suborder, infraorder, and parvorder). Shaded boxes—taxa which include monkeys and apes.

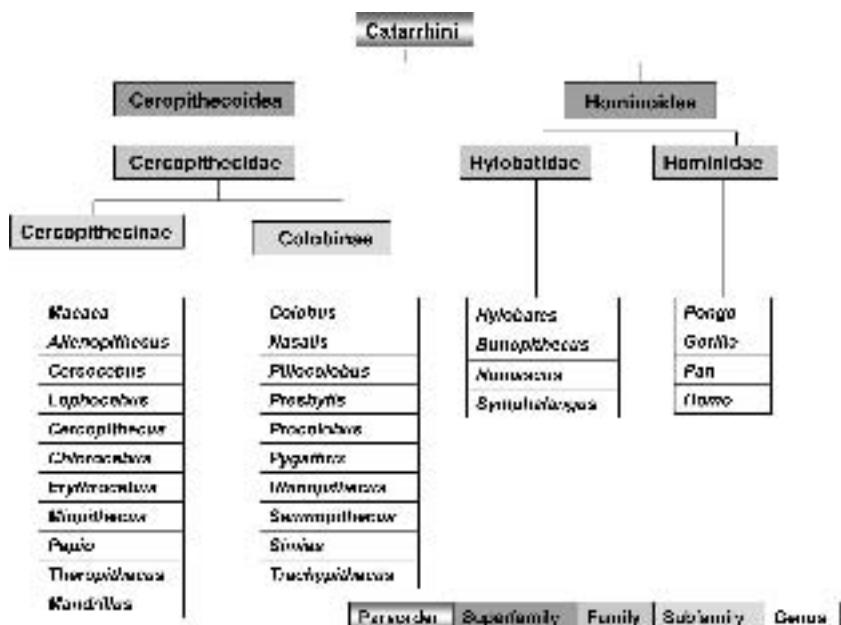


Figure 1.2. Catarrhini taxa (down to genus level).

Table 1.1. Old World Monkeys: Subfamily Cercopithecinae

Genus/Species	Common Name	Geographical Distribution
<i>Chlorocebus</i>	African green monkey	
<i>C. aethiops</i>	Grivet monkey or grivet	Sudan, Eritrea, Ethiopia
<i>C. cynosuros</i>	Malbrouck monkey	S DRC,* Angola, N Namibia, Zambia
<i>C. djamdamensis</i>	Bale Mountains vervet	Ethiopia
<i>C. pygerythrus</i>	Vervet monkey or vervet	Ethiopia, Somalia, Kenya, Tanzania, Zambia, Zimbabwe, RSA†
<i>C. sabaeus</i>	Green monkey	Senegal, Guinea-Bissau, Guinea, Sierra Leone, Liberia, Cote d'Ivoire, Ghana
<i>C. tantalus</i>	Tantalus monkey	Ghana, Togo, Benin, Nigeria, Cameroon, CAR,‡ Kenya
<i>Cercopithecus</i>		
<i>C. albogularis</i>	Sykes' monkey	Ethiopia to RSA, S&E DRC, NW Angola
<i>C. ascanius</i>	Red-tailed monkey	Uganda, DRC, Zambia, Angola, CAR, W Kenya
<i>C. campbelli</i>	Campbell's monkey	Senegal, Liberia, Cote d'Ivoire
<i>C. cephus</i>	Mustached guenon	Gabon, R of Congo,§ S Cameroon, Equatorial Guinea, SW CAR, NW Angola
<i>C. denti</i>	Dent's monkey	DRC, Rwanda, W Uganda, CAR
<i>C. diana</i>	Diana monkey	Sierra Leone, Liberia, Cote d'Ivoire
<i>C. doggetti</i>	Silver monkey	DRC, S Burundi, NW Tanzania, Rwanda, S Uganda
<i>C. dryas</i>	Dryas monkey	DRC
<i>C. erythrogaster</i>	White-throated guenon	S Nigeria, Benin
<i>C. erythrotis</i>	Red-eared guenon	S&E Nigeria, Cameroon, Bioko Isl (Equatorial Guinea)
<i>C. hamlyni</i>	Hamlyn's monkey	E DRC, Rwanda
<i>C. kandti</i>	Golden monkey	DRC, Uganda, Rwanda
<i>C. l'hoesti</i>	L'Hoest's monkey	E DRC, W Uganda, Rwanda, Burundi
<i>C. lowei</i>	Lowe's monkey	Cote d'Ivoire, Ghana
<i>C. mitis</i>	Blue monkey	DRC, Kenya, Rwanda, N Angola, NW Zambia
<i>C. mona</i>	Mona monkey	Ghana, Togo, Benin, Nigeria, Cameroon
<i>C. neglectus</i>	De Brazza's monkey	SE Cameroon, R of Congo, DRC, Equatorial Guinea, Gabon, Uganda, N Angola, W Kenya, SW Ethiopia, S Sudan
<i>C. nictitans</i>	Greater spot-nosed monkey	Liberia, Cote d'Ivoire, Nigeria, DRC, CAR, Equatorial Guinea, Cameroon
<i>C. petaurista</i>	Lesser spot-nosed monkey	Gambia, Guinea, Guinea-Bissau, Sierra Leone, Liberia, Cote d'Ivoire, Ghana, Togo
<i>C. pogonias</i>	Crowned monkey	SE Nigeria, Cameroon, Equatorial Guinea, N&W Gabon, W DRC
<i>C. preussi</i>	Preuss's monkey	Cameroon, Equatorial Guinea
<i>C. roloway</i>	Roloway monkey	Cote d'Ivoire, Ghana
<i>C. sclateri</i>	Sclater's guenon	SE Nigeria
<i>C. solatus</i>	Sun-tailed monkey	Gabon
<i>C. wolffii</i>	Wolf's monkey	DRC, NE Angola

Table 1.1. (Continued)

Genus/Species	Common Name	Geographical Distribution
<i>Erythrocebus</i>	Patas	
<i>E. patas</i>	Patas monkey	Savannahs, from W Africa to Ethiopia, Kenya, and Tanzania
<i>Miopithecus</i>	Talapoin	
<i>M. ogouensis</i>	Gabon talapoin	S Cameroon, Rio Muni (Equatorial Guinea), Gabon, Angola
<i>M. talapoin</i>	Angolan talapoin	Angola, SW DRC
<i>Allenopithecus</i>		
<i>A. nigroviridis</i>	Allen's swamp monkey	NW DRC, NE Angola
<i>Papio</i>	Baboon	
<i>P. hamadryas</i>	Hamadryas or sacred baboon	Red Sea coast of Ethiopia, Sudan, Eritrea, N Somalia, Yemen, Saudi Arabia
<i>P. anubis</i>	Olive or anubis baboon	Mali to Ethiopia, Kenya, NW Tanzania
<i>P. cynocephalus</i>	Yellow baboon	Somalia coast, Kenya, Tanzania to Zambezi River
<i>P. papio</i>	Guinea or red baboon	Senegal, Guinea and Guinea-Bissau to Mauritania, Mali
<i>P. ursinus</i>	Chacma baboon	S of Zambezi River to S Angola, SW Zambia
<i>Theropithecus</i>	Gelada	
<i>T. gelada</i>	Gelada	Highlands of N Ethiopia
<i>Mandrillus</i>	Mandrill and drill	
<i>M. sphinx</i>	Mandrill	Cameroon, S of Sanaga River, Rio Muni (Equatorial Guinea), Gabon, R of Congo
<i>M. leucophaeus</i>	Drill	SE Nigeria, Cameroon, N of Sanaga River, Bioko Isl (Equatorial Guinea)
<i>Cercopithecus</i>	Mangabey	
<i>C. agilis</i>	Agile mangabey	Rio Muni (Equatorial Guinea), Cameroon, NE Gabon, CAR, N R of Congo, DRC, N of Congo River
<i>C. atys</i>	Sooty mangabey	Senegal to Ghana
<i>C. chrysogaster</i>	Golden-bellied mangabey	DRC, S of Congo River
<i>C. galeritus</i>	Tana River mangabey	Lower Tana River (Kenya)
<i>C. sanjei</i>	Sanje mangabey	Tanzania
<i>C. torquatus</i>	Red-capped or collared mangabey	W Nigeria, Cameroon, Equatorial Guinea, Gabon
<i>Lophocebus</i>	Mangabey	
<i>L. albigena</i>	Gray-cheeked mangabey	Cross River (SE Nigeria), Cameroon, R of Congo, Gabon, Equatorial Guinea, NE Angola, CAR, DRC, N and E of Congo-Lualaba Rivers, W Uganda, Burundi
<i>L. aterrimus</i>	Black crested mangabey	DRC, S of Congo River
<i>L. opdenboschi</i>	Opdenbosch's mangabey	DRC, Angola

(Continued)

Table 1.1. (Continued)

Genus/Species	Common Name	Geographical Distribution
<i>Macaca</i>	Macaque	
<i>M. arctoides</i>	Stump-tailed macaque	Assam (India) to S China, N Malay Peninsula
<i>M. assamensis</i>	Assam macaque	Nepal to N Vietnam, S China
<i>M. cyclopis</i>	Formosan rock or Taiwan macaque	Taiwan
<i>M. fascicularis</i>	Crab-eating or cynomolgus macaque	S Indochina, Burma to Borneo and Timor, the Philippines
<i>M. fuscata</i>	Japanese macaque	Honshu, Shikoku, Kyushu and adjacent small islands, Yaku, Ryukyu (Japan)
<i>M. hecki</i>	Heck's macaque	N Sulawesi (Indonesia)
<i>M. leonina</i>	Northern pig-tailed macaque	Coast and Mergui Archipelago (Burma), N Thailand
<i>M. maura</i>	Moor macaque	S Sulawesi (Indonesia)
<i>M. mulatta</i>	Rhesus monkey	Afghanistan and India to N Thailand, China
<i>M. nemestrina</i>	Southern pig-tailed macaque or pig-tailed macaque	Malay Peninsula, Borneo, Sumatra and Bangka Isl (Indonesia), Thailand
<i>M. nigra</i>	Celebes crested macaque or Celebes black macaque or Celebes black ape [¶]	Sulawesi, east of Onggak Dumoga River, Lembeh Isl, Bacan Isl (Indonesia)
<i>M. nigrescens</i>	Gorontalo macaque	Sulawesi, E of Gorontalo to Onggak Dumoga River (Indonesia)
<i>M. ochreata</i>	Booted macaque	SE Sulawesi, Kabaena, Muna, Butung (Indonesia)
<i>M. pagensis</i>	Pagai Island macaque	Sipura, N&S Pagai Isl (Indonesia)
<i>M. radiata</i>	Bonnet macaque	S India
<i>M. siberu</i>	Siberut macaque	Siberut (Indonesia)
<i>M. silenus</i>	Lion-tailed macaque	SW India, W Ghats (India)
<i>M. sinica</i>	Toque macaque	Sri Lanka
<i>M. sylvanus</i>	Barbary macaque or Barbary ape [¶]	Morocco, Algeria, Gibraltar
<i>M. thibetana</i>	Milne-Edwards's macaque	E Tibet, Szechwan to Kwangtung (China)
<i>M. tonkeana</i>	Tonkean macaque	Sulawesi, Togian Isl (Indonesia)

Adapted from Groves.¹⁰

*Democratic Republic of Congo (DRC).

[†]Republic of South Africa (RSA).

[‡]Central African Republic (CAR).

[§]Republic of Congo (R of Congo).

[¶]Although the name ape has been used for these tailless monkeys, they are typical macaques, not apes.

Table 1.2. Old World Monkeys: Subfamily Colobinae

Genus/Species	Common Name	Geographical Distribution
Colobus	Colobus	
<i>C. angolensis</i>	Angola colobus	NE Angola, S&E DRC,* Burundi, NE Zambia, SE Kenya, E Tanzania
<i>C. guereza</i>	Mantled guereza	Nigeria to Ethiopia, Kenya, Uganda, Tanzania
<i>C. polykomos</i>	King colobus	Gambia to the Nzo-Sassandra system (Cote d'Ivoire)
<i>C. satanas</i>	Black colobus	SW Gabon, Rio Muni and Bioko (Equatorial Guinea), SW Cameroon, R of Congo [†]
<i>C. vellerosus</i>	Ursine colobus	Nzi-Bandama system (Cote d'Ivoire) to W Nigeria
Nasalis		
<i>N. larvatus</i>	Proboscis monkey	Borneo
Piliocolobus	Red colobus	
<i>P. badius</i>	Western red colobus	Senegal to Ghana
<i>P. foai</i>	Central African red colobus	Sangha, Oubangui (R of Congo), DRC (N of Congo River, E of Lualaba), Ngotto (CAR), [‡] S Sudan
<i>P. gordonorum</i>	Uzungwa red colobus	Uzungwa mountains and forests between Little Ruaha and Ulanga Rivers (Tanzania)
<i>P. kirkii</i>	Zanzibar red colobus	Zanzibar
<i>P. pennantii</i>	Pennant's red colobus	Bioko (Equatorial Guinea), Niger Delta (Nigeria), Sangha-Likouala (R of Congo)
<i>P. preussi</i>	Preuss's red colobus	Yabassi (Cameroon)
<i>P. rufomitratus</i>	Tana River red colobus	Lower Tana River (Kenya)
<i>P. tephrosceles</i>	Ugandan red colobus	Uganda, Rwanda, Burundi, W Tanzania to Lake Rukwa
<i>P. tholloni</i>	Thollon's red colobus	South of Congo River, W of Lomami River (DRC)
Presbytis	Surili/langur	
<i>P. chrysomelas</i>	Sarawak surili	Kalimantan, N of Kapuas River (Indonesia), Sarawak, Sabah (Malaysia)
<i>P. comata</i>	Javan surili	W and Central Java (Indonesia)
<i>P. femoralis</i>	Banded surili	S and NW of Malay Peninsula, peninsular part of Thailand and Burma, Singapore, NE Sumatra
<i>P. frontata</i>	White-fronted langur	Central and E Borneo, from Central Sarawak to S coast
<i>P. hosei</i>	Hose's langur	N and E Borneo, Brunei, E Sarawak, Sabah (Malaysia), S to Karangan River in Kalimantan (Indonesia)
<i>P. melalophos</i>	Sumatran surili	Sumatra (Indonesia)
<i>P. natunae</i>	Natuna Island surili	Bunguran Isl (Indonesia)
<i>P. potenziani</i>	Mentawai langur	Mentawai Isl (Indonesia)
<i>P. rubicunda</i>	Maroon leaf-monkey	Borneo, Karimat Isl (Indonesia)
<i>P. siamensis</i>	White-thighed surili	Malay Peninsula, except far S and NW, E Sumatra between Siak and Inderagiri Rivers, between Rokan and Barimun Rivers, Lake Toba region, Kundur, Bintang, Batam, and Galang Isl, Riau Archipelago (Indonesia)
<i>P. thomasi</i>	Thomas's langur	Sumatra/Aceh (Indonesia)
Procolobus		
<i>P. verus</i>	Olive colobus	Sierra Leone to Togo, Idah (E Nigeria)

(Continued)

Table 1.2. (Continued)

Genus/Species	Common Name	Geographical Distribution
<i>Pygathrix</i>	Shanked douc	
<i>P. cinerea</i>	Gray-shanked douc	Central Vietnam
<i>P. nemaeus</i>	Red-shanked douc	Central Vietnam, E Laos
<i>P. nigripes</i>	Black-shanked douc	S Vietnam, Cambodia, E of Mekong River
<i>Rhinopithecus</i>	Snub-nosed monkey	
<i>R. avunculus</i>	Tonkin snub-nosed monkey	NW Vietnam
<i>R. bieti</i>	Black snub-nosed monkey	Ridge of Mekong-Salween divide, Yunnan (China)
<i>R. brelichi</i>	Gray snub-nosed monkey	Guizhou (China)
<i>R. roxellana</i>	Golden snub-nosed monkey	Sichuan Mountains, S Ganssu, Hubei, Shaanxi (China)
<i>Semnopithecus</i>	Gray langur	
<i>S. ajax</i>	Kashmir gray langur	Dehradun (India) and W into Pakistani Kashmir
<i>S. dussumieri</i>	Southern Plains gray langur	SW and W and Central India
<i>S. entellus</i>	Northern Plains gray langur	Pakistan and India, lowlands N of Godavari and Krishna Rivers, S of Ganges
<i>S. hector</i>	Tarai gray langur	Kumaun (India) to Hazaria (Nepal)
<i>S. hypoleucos</i>	Black-footed gray langur	Kerala, South Coorg region (India)
<i>S. priam</i>	Tufted gray langur	SE India, Sri Lanka
<i>S. schistaceus</i>	Nepal gray langur	E of Gorkha to Sikkim (Nepal), and parts of southernmost Tibet (China)
<i>Simias</i>		
<i>S. concolor</i>	Simakobu or pig-tailed langurs	Mentawai Isl (Indonesia)
<i>Trachypithecus</i>	Lutung/langur	
<i>T. auratus</i>	Javan lutung	Java, Bali, Lombok (Indonesia)
<i>T. barbei</i>	Tenasserim lutung	N Burma, Thailand
<i>T. cristatus</i>	Silvery lutung	Borneo, Natuna Isl, Bangka, Belitung, Sumatra, Riau Archipelago (Indonesia), W coast of Malay Peninsula
<i>T. delacouri</i>	Delacour's langur	Vietnam, S of Red River
<i>T. ebenus</i>	Indochinese black langur	Lai Chau or Fan Si Pan chain, Hin Namno (Laos)
<i>T. francoisi</i>	Francois's langur	N Vietnam, C Laos, Kwangsi (China)
<i>T. geei</i>	Gee's Golden langur	Between Sankosh and Manas Rivers, Indo-Bhutan border (India, Bhutan)
<i>T. germaini</i>	Indochinese lutung	Thailand and Burma, Cambodia, Vietnam
<i>T. hatinhensis</i>	Hatinh langur	Quang Binh and neighboring regions (Vietnam)
<i>T. johnii</i>	Nilgiri langur	S India
<i>T. laotum</i>	Laotian langur	Central Laos
<i>T. obscurus</i>	Dusky leaf-monkey	S Thailand, Malay Peninsula, and small adjacent islands
<i>T. phayrei</i>	Phayre's leaf-monkey	Laos, Burma, Central Vietnam, Central and N Thailand, Yunnan (China)
<i>T. pileatus</i>	Capped langur	Assam (India)
<i>T. poliocephalus</i>	White-headed langur	Cat Ba Isl (Vietnam), Guangxi (China)
<i>T. shortridgei</i>	Shortridge's langur	Burma, E of Chindwin River, Gongshan, Yunnan (China)
<i>T. vetulus</i>	Purple-faced langur	Sri Lanka

Adapted from Groves.¹⁰

*Democratic Republic of Congo (DRC).

[†]Republic of Congo (R of Congo).

[‡]Central African Republic (CAR).

Table 1.3. Apes: Family Hominidae

Genus/Species	Common Name	Geographical Distribution
<i>Hylobates</i>	Gibbon	
<i>H. agilis</i>	Agile gibbon	Sumatra (Indonesia)
<i>H. albipilis</i>	Bornean white-bearded gibbon	Borneo (Indonesia)
<i>H. klossii</i>	Kloss's gibbon	Mentawai (Indonesia)
<i>H. lar</i>	Lar gibbon	Yunnan (China), Thailand, S Malaysia, Sumatra (Indonesia), SE Burma
<i>H. moloch</i>	Javan silvery gibbon	Java (Indonesia)
<i>H. muelleri</i>	Müller's Bornean gibbon	Borneo (Indonesia)
<i>H. pileatus</i>	Pileated gibbon	SE Thailand, Cambodia
<i>Bunopithecus</i>	Gibbon	
<i>B. hoolock</i>	Hoolock gibbon	Assam (India)
<i>Nomascus</i>	Gibbon	
<i>N. concolor</i>	Black crested gibbon	S Laos, S Vietnam
<i>N. gabriellae</i>	Red-cheeked gibbon	S Laos, S Vietnam, E Cambodia
<i>N. hainanus</i>	Hainan gibbon	Hainan (China), Vietnam
<i>N. leucogenys</i>	Northern white-cheeked gibbon	Yunnan (China), N Vietnam, N Laos
<i>N. siki</i>	Southern white-cheeked gibbon	Vietnam, Laos
<i>Sympthalangus</i>	Siamang	
<i>S. syndactylus</i>	Siamang	Sumatra (Indonesia), Malaysia
<i>Pongo</i>	Orangutan	
<i>P. abelii</i>	Sumatran orangutan	Sumatra (Indonesia)
<i>P. pygmaeus</i>	Bornean orangutan	Borneo (Indonesia)
<i>Gorilla</i>	Gorilla	
<i>G. gorilla</i>	Western gorilla	SE Nigeria, Cameroon, Equatorial Guinea, R of Congo,* SW CAR, [†] Gabon
<i>G. beringei</i>	Eastern gorilla	N&E DRC, [‡] SW Uganda, N Rwanda
<i>Pan</i>	Chimpanzee	
<i>P. troglodytes</i>	Common chimpanzee	S Cameroon, Gabon, S R of Congo, Uganda, W Tanzania, N DRC, W CAR, Guinea, Guinea-Bissau, Liberia
<i>P. paniscus</i>	Bonobo or pygmy chimpanzee	DRC, S of Congo River

Adapted from Groves.¹⁰

*Republic of Congo (R of Congo).

[†]Central African Republic (CAR).

[‡]Democratic Republic of Congo (DRC).

Table 1.4. New World Monkeys: Order Ptaicyrrhini

Genus/Species	Common Name	Geographical Distribution
<i>Catlithrix</i>	Marmoset	
<i>C. acariensis</i>	Rio Acari marmoset	Rios Acari and Sucunduri (Brazil)
<i>C. argentata</i>	Silvery marmoset	N and Central Brazil, E Bolivia
<i>C. airita</i>	Buffy-tufted marmoset	SE Brazilian coast
<i>C. chrysoleuca</i>	Gold-and-white marmoset	Between the Aripuana-Madeira and Canuma-Uraria, N to the Amazon (Brazil)
<i>C. emiliae</i>	Emilia's marmoset	Tapajos and Iriri, N to Maica, on the lower Tapajos (Brazil)
<i>C. flaviceps</i>	Buffy-headed marmoset	S Espirito Santo (Brazil)
<i>C. geoffroyi</i>	White-headed marmoset	Coastal Bahia (Brazil)
<i>C. humeralifera</i>	Santarem marmoset	S of the Amazon between the Maues-Acu and Tapajos Rivers (Brazil)
<i>C. humilis</i>	Roosmalens' dwarf marmoset	Between the Rios Aripuana and Madeira (Brazil)
<i>C. intermedia</i>	Hershkovitz's marmoset	Rios Aripuana and Roosevelt (Brazil)
<i>C. jacchus</i>	Common marmoset	Coast of Piaui, Ceara, and Pernambuco (Brazil)
<i>C. kuhlii</i>	Wied's marmoset	Between Rio de Contas and Rio Jequitinhonha, SW Brazil
<i>C. leucippe</i>	White marmoset	Rios Tapajos and Cupari (Brazil)
<i>C. manicorensis</i>	Manicore marmoset	Rios Aripuana and Manicore, from the Rio Madeira S to the Rio Roosevelt (Brazil)
<i>C. marcai</i>	Marca's marmoset	Amazonas, Fozdo Rio Castanho (Brazil)
<i>C. mauesi</i>	Maues marmoset	Rios Uraria-Abacaxis and Maues-Ac'u (Brazil)
<i>C. melanura</i>	Black-tailed marmoset	S Brazil, between the Rios Aripuana and Juruena, SW to the Rio Beni in Bolivia
<i>C. nigriceps</i>	Black-headed marmoset	Rios Marmelos and Madeira, N of the Ji-Parana River (Brazil)
<i>C. penicillata</i>	Black-tufted marmoset	Brazilian coast, Bahia to Sao Paulo, inland to Goias
<i>C. pygmaea</i>	Pygmy marmoset	N and W Brazil, N Peru, Ecuador
<i>C. saterei</i>	Satere marmoset	Rios Abacaxis and Canuma-Sucunduri (Brazil)
<i>Callimico</i>	Marmoset	
<i>C. goeldii</i>	Goeldi's marmoset	W Brazil, N Bolivia, E Peru, Colombia, Upper Amazon rainforests
<i>Saguinus</i>	Tamarin	
<i>S. bicolor</i>	Pied tamarin	N Brazil, possibly NE Peru
<i>S. fuscicollis</i>	Brown-mantled tamarin	N and W Brazil, N Bolivia, E Peru, E Ecuador, SW Colombia
<i>S. geoffroyi</i>	Geoffroy's tamarin	SE Costa Rica to NW Colombia
<i>S. graellsi</i>	Graells's tamarin	Peru, Ecuador, Colombia, W of Rio Napo, from Rio Putumayo S to Rio Maranon, W to Rio Santiago
<i>S. imperator</i>	Emperor tamarin	W Brazil, E Peru, Bolivia
<i>S. inustus</i>	Mottle-faced tamarin	NW Brazil, SW Colombia
<i>S. labiatus</i>	White-lipped tamarin	W Brazil, E Peru, Bolivia

Table 1.4. (Continued)

Genus/Species	Common Name	Geographical Distribution
<i>S. leucopus</i>	White-footed tamarin	N Colombia
<i>S. martinsi</i>	Martins's tamarin	Very small area N of the Amazon, on both sides of the Rio Nhamunda (Brazil)
<i>S. melanoleucus</i>	White-mantled tamarin	Between Rios Jurua and Tarauca (Brazil)
<i>S. midas</i>	Red-handed tamarin	Brazil, Guyana, Cayenne, Surinam, N of the Amazon, E of the Rio Negro
<i>S. mystax</i>	Moustached tamarin	W Brazil, Peru, S of Amazon–Solimoes–Maranon, between lower Rio Huallaga and Rio Madeira
<i>S. niger</i>	Black tamarin	Brazil, S of the Amazon, E of the Rio Xingu, including Marajo Isl
<i>S. nigricollis</i>	Black-mantled tamarin	W Brazil, E Peru, E Ecuador
<i>S. oedipus</i>	Cottontop tamarin	N Colombia, Panama
<i>S. pileatus</i>	Red-capped tamarin	W Brazil, E of Rio Tefe, W of Rio Purus
<i>S. tripartitus</i>	Golden-mantled tamarin	E of Rio Curaray, Brazil–Colombia border
<i>Leontopithecus</i>	Lion tamarin	
<i>L. caissara</i>	Superagui lion tamarin	Superagui Isl and a small region on the opposite mainland (Brazil)
<i>L. chrysomelas</i>	Golden-headed lion tamarin	Coastal Bahia (Brazil)
<i>L. chrysopygus</i>	Black lion tamarin	Sao Paulo region (Brazil)
<i>L. rosalia</i>	Golden lion tamarin	SE Brazil, Rio Doce (Espirito Santo), S of Rio de Janeiro and Guanabara
<i>Cebus</i>	Capuchin	
<i>C. albifrons</i>	White-fronted capuchin	Venezuela, Colombia, Ecuador, N Peru, NW Brazil, Trinidad, Bolivia
<i>C. apella</i>	Tufted capuchin	N and W South America, from Guyana, Venezuela (S from the Rio Orinoco delta) and Colombia south across the Amazon in Brazil
<i>C. capucinus</i>	White-headed capuchin	W Ecuador to Honduras
<i>C. kaapori</i>	Kaapori capuchin	Between Rios Gurupi and Pindare (Brazil)
<i>C. libidinosus</i>	Black-striped capuchin	Highland region of S Brazil to Bolivia and Paraguay
<i>C. nigratus</i>	Black capuchin	Brazilian coast 16–30°S
<i>C. olivaceus</i>	Weeper capuchin	Guyana, French Guiana, Surinam, N Brazil, Venezuela, possibly N Colombia
<i>C. xanthosternos</i>	Golden-bellied capuchin	Brazil, formerly between Rio Sao Francisco and Rio Jequitinhonha, now much reduced
<i>Saimiri</i>	Squirrel monkey	
<i>S. boliviensis</i>	Black-capped squirrel monkey	Upper Amazon in Peru, SW Brazil, Bolivia
<i>S. oerstedii</i>	Central American squirrel monkey	Panama, Costa Rica
<i>S. sciureus</i>	Common squirrel monkey	N Brazil, N of the Amazon–Jurua system, S of the Amazon E, east Rio Xingu or the Rio Iriri, Marajo Isl (Brazil), Guyana, French Guiana, Surinam, Venezuela, Colombia, E Ecuador, NE Peru

(Continued)

Table 1.4. (Continued)

Genus/Species	Common Name	Geographical Distribution
<i>S. ustus</i>	Bare-eared squirrel monkey	S Brazil, S of Rio Amazon, probably from Rio Xingu to Lage Tefe
<i>S. vanzolinii</i>	Black squirrel monkey	Between Rios Japura, Solimoes and (probably) Paranadojaraua (Brazil), Tarara and Capucho IIs (Brazil)
<i>Aotus</i>	Night or owl monkey	
<i>A. azarae</i>	Azara's night monkey	Bolivia S of Amazon, between Rios Tocantins and Tapajos-Juruena, S to Paraguay and N Argentina
<i>A. hershkovitzi</i>	Hershkovitz's night monkey	Colombia
<i>A. lemurinus</i>	Gray-bellied night monkey	Panama, Ecuador, and Colombia W of Cordillera Oriental
<i>A. miconax</i>	Peruvian night monkey	Between Rio Ucayali and the Andes, S of Rio Maranon (Peru)
<i>A. nancymaae</i>	Nancy Ma's night monkey	Loreto (Peru) to Rio Jandiatuba, S of Rio Solimoes (Brazil), enclave between Rios Tigre and Pastaza (Peru)
<i>A. nigriceps</i>	Black-headed night monkey	Brazil, S of Rio Solimoes, W of Rio Tapajos Juruena, W into Peru, Bolivia
<i>A. trivirgatus</i>	Three-striped night monkey	Venezuela, S of Rio Orinoco, S to Brazil N of Rios Negro and Amazon
<i>A. vociferans</i>	Spix's night monkey	Colombia, E of Cordillera Oriental, W of Rio Negro, S to Brazil (N of Amazon–Solimoes Rivers)
<i>Alouatta</i>	Howler	
<i>A. belzebul</i>	Red-handed howler	N Brazil (mainly S of Lower Amazon, E of Rio Madeira), Mexiana Isl, Para Province (Brazil), N of Amazon
<i>A. caraya</i>	Black howler	N Argentina to Mato Grosso (Brazil), Bolivia
<i>A. coibensis</i>	Coiba Island howler	Coiba Isl and Azuero Peninsula (Panama)
<i>A. guariba</i>	Brown howler	N Bolivia, SE and EC Brazil, N to the Rio Sao Francisco
<i>A. macconnelli</i>	Guyanan red howler	Guyana, coast region
<i>A. nigerrima</i>	Amazon black howler	N Brazil, E of the Rio Trombetas to the Rio Tapajos, possibly to the Rio Tocantins
<i>A. palliata</i>	Mantled howler	W Ecuador to Veracruz and Oaxaca (Mexico)
<i>A. pigra</i>	Guatemalan black howler	Yucatan and Chiapas (Mexico) to Belize and Guatemala
<i>A. sara</i>	Bolivian red howler	Bolivia (Sara Province), Peru, and Brazil to the Rio Negro and Rondonia
<i>A. seniculus</i>	Venezuelan red howler	Colombia to Venezuela and NW Brazil
<i>Ateles</i>	Spider monkey	
<i>A. belzebuth</i>	White-fronted spider monkey	Cordillera Oriental, Colombia to Venezuela and N Peru
<i>A. chamek</i>	Peruvian spider monkey	NE Peru, E Bolivia to Brazil west of Rio Jurua and S of Rio Solimoes
<i>A. fusciceps</i>	Black-headed spider monkey	SE Panama to Ecuador, Colombia to W Cordillera (Paraguay)

Table 1.4. (Continued)

Genus/Species	Common Name	Geographical Distribution
<i>A. geoffroyi</i>	Geoffrey's spider monkey	S Mexico to Panama
<i>A. hybrida</i>	Brown spider monkey	N Colombia and NW Venezuela
<i>A. marginatus</i>	White-cheeked spider monkey	S of Lower Amazon, Rio Tapajos to Rio Tocantins (Brazil)
<i>A. paniscus</i>	Red-faced spider monkey	Guianas and Brazil, N of the Amazon, E of Rio Negro
<i>Lagothrix</i>	Woolly monkey	
<i>L. cana</i>	Gray woolly monkey	Brazil, S of Amazon, S highlands of Peru, an isolated population in northern Bolivia
<i>L. lagotricha</i>	Brown woolly monkey	Brazil N of Rio Napo-Amazon system, SE Colombia, extreme N Peru and NE Ecuador
<i>L. lugens</i>	Colombian woolly monkey	Colombia, headwaters of Orinoco tributaries, Venezuela, Sarare River drainage
<i>L. poeppigii</i>	Silvery woolly monkey	Highlands of E Ecuador and N Peru, to about 70°W, 5°S in Brazil
<i>Oreonax</i>	Woolly monkey	
<i>O. flavicauda</i>	Yellow-tailed woolly monkey	E Andes in San Martin (Peru) and Amazonas (Brazil)
<i>Brachyteles</i>	Muriqui	
<i>B. arachnoides</i>	Southern muriqui	SE Brazil, states of Rio de Janeiro and Sao Paulo
<i>B. hypoxanthus</i>	Northern muriqui	E Brazil, Bahia, Minas Gerais, Espírito Santo
<i>Callicebus</i>	Titi	
<i>C. baptista</i>	Baptista Lake titi	Central Brazil, N of the Parana do Uraria and Parana do Ramos and S of the Amazon and lowermost Rio Madeira, a small wedge between the Rio Uira-Curupa and Rio Andira
<i>C. barbarabrownae</i>	Barbara Brown's titi	E Brazil, between Rio Paraguacu and Rio Itapicuru, except where <i>C. coimbrai</i> is found
<i>C. bernhardi</i>	Prince Bernhard's titi	Brazil, Amazonas, and Rodonia states, between Rios Madeira-Ji-Parana and Rios Aripuanã-Roosevelt
<i>C. brunneus</i>	Brown titi	Middle to upper Madeira basin in Peru and Brazil, to upper Rio Purus (Brazil) and Ucayali (Peru), Bolivia
<i>C. caligatus</i>	Chestnut-bellied titi	S of the Rio Solimões from Rio Purus to Rio Madeira (Brazil)
<i>C. cinerascens</i>	Ashy black titi	Rio Madeira basin (Brazil)
<i>C. coimbrai</i>	Coimbra Filho's titi	NE Brazil, between Rio São Francisco and Rio Real
<i>C. cupreus</i>	Coppery titi	S of the Amazon from Rio Purus to Rio Ucayali, Brazil, and Peru, probably Bolivia

(Continued)

Table 1.4. (Continued)

Genus/Species	Common Name	Geographical Distribution
<i>C. discolor</i>	White-tailed titi	Upper Amazonian region in Peru, Ecuador, and Colombia, and possibly into Brazil, between the Rios Ucayali and Huallaga and N of Rio Maranon across the Rio Napo to the Rio Putumayo and Rio Guames
<i>C. donacophilus</i>	White-eared titi	WC Bolivia, El Beni, and Santa Cruz, Upper Rios Marmore-Grande and San Miguel basins
<i>C. dubius</i>	Hershkovitz's titi	Brazil, Ituxi River or the Mucuim River, E to the Madeira River S of Humaita, and W to the Purus River
<i>C. hoffmannsi</i>	Hoffmanns's titi	Central Brazil, S of Amazon, between Rios Canuma and Tapajos-Jurena, S to the Rio Sucunduri
<i>C. lucifer</i>	Lucifer titi	Peru, Ecuador, and Brazil, between Rios Caqueta-Japua and Rios Napo-Solimoes
<i>C. lugens</i>	Black titi	Brazil, Colombia, and Venezuela, W of Rio Branco and N of Rios Negro/Uaupes/Vaupes, then W of Rio Apaporis and N of Rio Caqueta, E of Andes N to Rio Tomo, possibly to Rio Orinoco (only between Rio Caura and Rio Caroni)
<i>C. medemi</i>	Colombian black-handed titi	Amazonian region of Colombia
<i>C. melanochir</i>	Coastal black-handed titi	Between Rio Mucuri and Rio Itapicuru (Brazil)
<i>C. modestus</i>	Rio Beni titi	Upper Rio Beni basin (Bolivia)
<i>C. moloch</i>	Red-bellied titi	Central Brazil, S of Amazon, between Rios Tapajos and Tocantins-Araguaia
<i>C. nigrifrons</i>	Black-fronted titi	SE Brazil, states of Rio de Janeiro, Sao Paulo (north of Rio Tiete), and S Minas Gerais
<i>C. oenanthe</i>	Rio Mayo titi	Rio Mayo valley (N Peru)
<i>C. olallae</i>	Ollala Brothers' titi	Bolivia, El Beni Province, La Laguna
<i>C. ornatus</i>	Ornate titi	Colombia, headwaters of Rio Meta and Rio Guiviare
<i>C. pallescens</i>	White-coated titi	Paraguay, W of Rio Paraguay in Gran Chaco, Mato Grosso do Sul, in the Pantanal (Brazil), probably Bolivia
<i>C. personatus</i>	Atlantic titi	SE Brazil, Espirito Santo, possibly into NW Minas Gerais
<i>C. purinus</i>	Rio Purus titi	Brazil S of the Rio Solimoes between the Rio Tapaua and Rio Jurua
<i>C. regulus</i>	Red-headed titi	Brazil, between Rios Javari/Solimoes and Rio Jurua
<i>C. stephennashi</i>	Stephen Nash's titi	Brazil, probably along the right bank of the Rio Purus, in between the distributions of <i>C. caligatus</i> and <i>C. dubius</i>
<i>C. torquatus</i>	Collared titi	Brazil, between Rios Negro/Uaupes and Rios Solimoes/Japura/Apaporis

Table 1.4. (*Continued*)

Genus/Species	Common Name	Geographical Distribution
Pithecia	Saki	
<i>P. aequatorialis</i>	Equatorial saki	Napo (Ecuador) to Loreto (Peru)
<i>P. albicans</i>	White-footed saki	S bank of Amazon, between lower Jurua and lower Purus Rivers
<i>P. irrorata</i>	Rio Tapajos saki	S of the Amazon in SW Brazil, SW Peru, E Bolivia
<i>P. monachus</i>	Monk saki	W of Rio Jurua and Rio Japura-Caquetá (Brazil), Colombia, Ecuador and Peru
<i>P. pithecia</i>	White-faced saki	Guyana, French Guiana, Surinam, N Amazon, E of Rio Negro and Rio Orinoco (Brazil), S Venezuela
Chiropotes	Saki	
<i>C. albinasus</i>	White-nosed saki	N and Central Brazil
<i>C. chiropotes</i>	Red-backed bearded saki	Guyana, French Guiana, Surinam, Brazil E of the Rio Branco
<i>C. israelita</i>	Brown-backed bearded saki	Brazil N of the Amazon and E of the Rio Branco, S Venezuela E of the Rio Orinoco
<i>C. satanas</i>	Black bearded saki	Brazil S of Amazon estuary, between Rios Tocantins and Gurupi
<i>C. utahickae</i>	Uta Hick's bearded saki	N Brazil, S of Amazon, between Rios Xingu and Tocantins, S to Serra dos Carajás and Rio Itacaiuna
Cacajao	Uacari	
<i>C. calvus</i>	Bald uakari	NW Brazil, E Peru
<i>C. melanocephalus</i>	Black-headed uakari	SW Venezuela, NW Brazil

Adapted from Groves.¹⁰

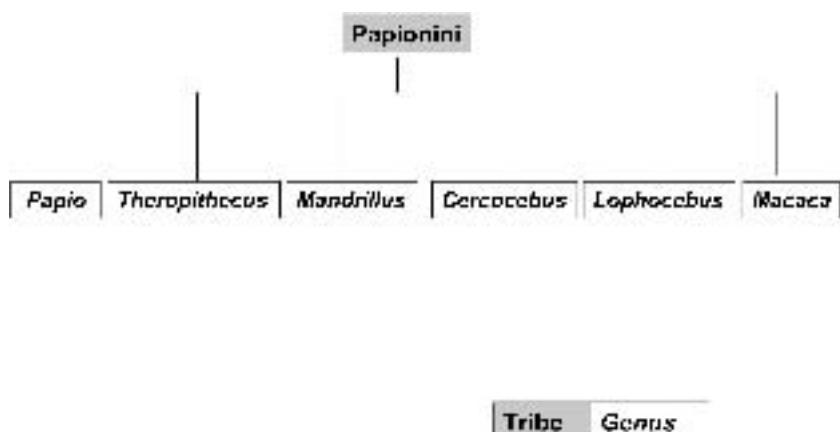


Figure 1.3. Genera included in Papionini tribe.

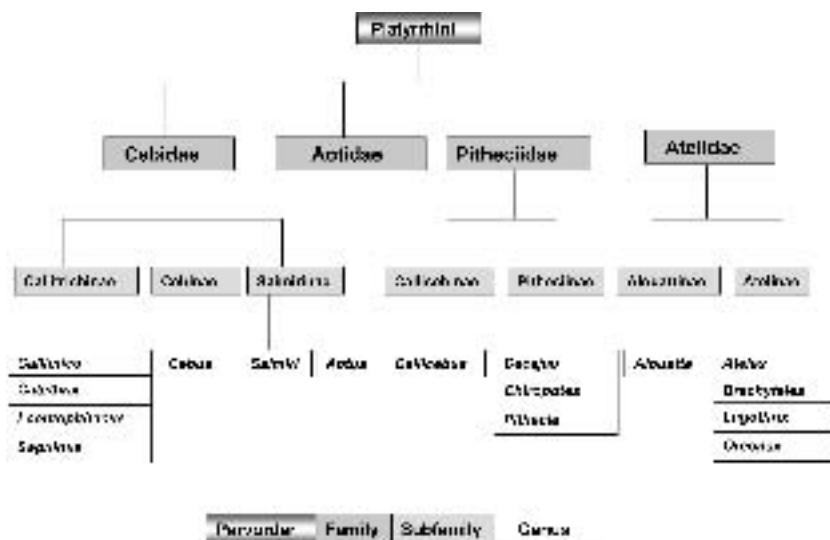


Figure 1.4. Platyrrhini taxa (down to genus level).

Ape is a generic common name for species included in the superfamily Hominoidea. There are two types of simian apes: the lesser apes (species belonging to the family Hylobatidae) and the great apes (species belonging to the family Hominidae). The subfamily level in the classification of hominoids is omitted. The list of simian ape genera and species is presented in Table 1.3.

The NWMs (Platyrrhini) are divided into four families: Cebidae, Pitheciidae, Atelidae, and Aotidae (Figure 1.4). The first three of those families are divided into subfamilies. The subfamilies within Cebidae are Cebinae, Callitrichinae, and Saimiriinae; within Pitheciidae—Pitheciinae and Callicebinae; and within Atelidae—Atelinae and Alouattinae. There are no subfamilies in the Aotidae family. The list of NWM genera and species is presented in Table 1.4.

1.2.2. Molecular Taxonomy and Molecular Identification of NHPs

Traditionally, taxonomic identification of NHPs was based on the description and analysis of external morphological and anatomical traits. Many early descriptions of simian species were made entirely from museum specimens. Gradually, purely morphological identifications were supplemented with more detailed and reliable information on the geographical distribution and

behavioral characteristics of simian species, subspecies, and local populations obtained in field studies.

Although morphology, behavioral characteristics, and habitat data continue to be important, they are supplemented with molecular taxonomic data. As a concept, molecular taxonomy, an approach that is based on protein and DNA sequence data analyses, is not new. It has been explored since the early 1960s. However, with the advent of polymerase chain reaction (PCR) and automatic DNA sequencing, the use of molecular taxonomy has grown explosively. The wide availability of sophisticated phylogenetic analysis programs and powerful computers has also contributed to the “popularity” of this approach. Molecular taxonomy has proven to be a powerful tool for resolving uncertainties and controversies in the classification of NHPs that were based on classical approaches.^{14,17,22,30,35,37,39,43–45} Importantly, molecular taxonomic studies provide estimates of timing for divergence of various primate taxa; although these estimates should be treated with caution because they are based on many assumptions that may or may not be correct.

The practical application of molecular taxonomy allows accurate species identification of specimens containing extractable DNA.^{15,20} Such genotyping is extremely important for samples collected in the field. Moreover, genotyping allows unequivocal individual

identification of “anonymous” samples, such as fecal specimens⁵ or hairs from wild monkeys and apes. A few hairs pulled from a specimen or live animal usually contain sufficient DNA for these analyses. Another important practical application of genotyping is tracing the origin of imported wild-caught or captive-born monkeys or apes when relevant information is insufficient or unreliable.^{7,18,28}

In this chapter, where possible, we provide brief descriptions of the latest molecular taxonomy data relevant to specific NHP taxa.

1.3. OLD WORLD MONKEYS

1.3.1. Guenons and Allies

The common name “guenons” was used originally for “tree-dwelling” monkeys grouped into *Cercopithecus* genus. Its current meaning is broader. The guenons are monkeys grouped into the tribe Cercopithecini that includes *Cercopithecus*, *Chlorocebus*, *Erythrocebus*, *Miopithecus*, and *Allenopithecus* genera. Some of these guenons are “arboreal” (tree-dwelling), others are “terrestrial” (ground-dwelling). Molecular taxonomic evidence indicates that both arboreal and terrestrial groups are monophyletic; that is, they evolved from a single common ancestor. The terrestrial lineage is more ancient. Evolutionarily, the oldest guenon species is *Allenopithecus nigroviridis*, a terrestrial monkey.⁴⁴ Among arboreal guenons, the oldest evolutionarily species are the talapoin monkeys (*Miopithecus* spp.).

The division into arboreal and terrestrial species does not coincide completely with taxonomic boundaries. Most arboreal guenons belong to one genus *Cercopithecus*; only one *Cercopithecus* species *C. l'hoesti* is terrestrial. The terrestrial group also includes the *Chlorocebus* and *Patas* species.

1.3.1.1. AFRICAN GREEN MONKEYS

AGM is the common name for a group of species in which the prototype is the grivet monkey (*Cercopithecus aethiops* or *Chlorocebus aethiops*). The *Chlorocebus* genus was introduced recently and some authors still continue to place AGMs into the *Cercopithecus* genus.¹² Both scientific names, that is *Chlorocebus* spp. and *Cercopithecus* spp., are used for AGMs, but the former is gradually replacing the latter.

There are four clearly distinguishable major forms of AGMs: grivet (*Chlorocebus aethiops*), vervet (*C. pygerythrus*) (Figure 1.5), green or sabaeus monkey



Figure 1.5. Vervet monkeys (*Chlorocebus pygerythrus*, possibly *C. p. callidus*), the Lake Nakuru region, Kenya. (Image is kindly provided by Dr. Jean P. Boubli.) See color version page 1.

(*C. sabaeus*), tantalus monkey (*C. tantalus*) (Figure 1.6); and two minor forms: Bale Mountains grivet (*C. djamda-jamensis*) and Malbrouck monkey (*C. cynosuros*). These forms are ranked as separate species.^{10,12} Within three of these species (*aethiops*, *pygerythrus*, and *tantalus*), there are distinguishable forms, particularly numerous in *C. pygerythrus*. Taxonomic ranking of these forms is disputable; however, usually they are classified as subspecies (Table 1.5).¹²

There are large feral populations of *sabaeus* AGMs on the Caribbean islands of Barbados, St. Kitts, Nevis, and St. Martin. The founders of these populations were brought by slave traders in the seventeenth and eighteenth centuries. Genetically, Caribbean AGMs are much more homogenous than their counterparts in Africa. Caribbean AGMs are also free of several viruses found in AGMs on the African continent; most notably, they are SIV-free (see Chapter 3).



Figure 1.6. Tantalus monkey (*Chlorocebus tantalus*), Nigeria. (Image is kindly provided by Dr. Janette Wallis.) See color version page 1.

The oldest AGM lineage leading to modern green monkeys is estimated to have diverged 2.78 ± 0.29 Mya. The common ancestor of other AGMs is dated at 1.53 ± 0.15 Mya. Apparently, the closest relatives among the AGMs are the grivets and tantalus monkeys. The timing of the divergence of velvet and grivet/tantalus lineages is not well resolved in currently available estimates.³⁹

Table 1.5. African Green Monkeys Subspecies Groups*

Aethiops	Pygerythrus	Tantalus
<i>C. a. aethiops</i>	<i>C. p. pygerythrus</i>	<i>C. t. tantalus</i>
<i>C. a. hilgerii</i>	<i>C. p. cloetei</i>	<i>C. t. budgetti</i>
<i>C. a. ellenbecki</i>	<i>C. p. helvescens</i>	<i>C. t. marrensis</i>
	<i>C. p. ngamiensis</i>	
	<i>C. p. marjoriae</i>	
	<i>C. p. whytei</i>	
	<i>C. p. ruboviridis</i>	
	<i>C. p. johnstoni</i>	
	<i>C. p. rubellus</i>	
	<i>C. p. centralis</i>	
	<i>C. p. callidus</i>	
	<i>C. p. nesiotes</i>	
	<i>C. p. excubitor</i>	
	<i>C. p. aranarius</i>	
	<i>C. p. zavattarii</i>	

Adapted from Grubb *et al.*¹²

*There is no subspecies of sabaeus monkey (*C. sabaeus*).

The AGMs, particularly grivets, vervets, and Caribbean *sabaeus*, are widely used in biomedical experiments.

1.3.1.2. OTHER GUENONS

This remarkably diverse group of guenons includes multiple *Cercopithecus* species, patas monkeys (*Erythrocebus patas*) (Figure 1.7), two species of talapoin monkeys (*Miopithecus talapoin* and *M. ogouensis*), and Allen's swamp monkey (*Allenopithecus nigroviridis*).¹⁹

Two schemes are used by different authors for classifying cercopithecini guenons: “species–subspecies” and “superspecies–species–subspecies.” There are many classifications of non-AGM guenons, and it is unlikely that a consensus will be reached in the foreseeable future. At the same time, it is generally accepted that the arboreal *Cercopithecus* can be divided into seven species/subspecies groups: Cephus, Mitis, Mona, Neglectus, Diana, Dryas, and Hamlini (Table 1.6, Figures 1.8 and 1.9). Phylogenetic analysis strongly supports the monophyletic origin for the Cephus–Mitis and Mona–Neglectus–Diana aggregated groups, Arboreal Clades I and II, respectively.³⁴ Two arboreal species, *C. hamlyni* and *C. dryas*, stand apart from all other arboreal guenons.

The terrestrial *Cercopithecus* guenons are represented by l’Hoesti species group, also named Preussi group (Table 1.6). One species in this group, the Preuss’s monkey is divided into two subspecies: *C. preussi preussi* and *C. p. insularis*.¹² The l’Hoesti guenons are related



Figure 1.7. Male patas monkey (*Erythrocebus patas*), Nigeria. (Image is kindly provided by Dr. Janette Wallis.) See color version page 1.

Table 1.6. *Cercopithecus* Species Groups

Group	Species	Phylogenetic Clade ³⁴
Cephus	<i>C. cephush</i>	Arboreal Clade I (Cephus-Mitis)
	<i>C. ascanius</i>	
	<i>C. petaurista</i>	
	<i>C. erythrotis</i>	
	<i>C. erythrogaster</i>	
	<i>C. sclateri</i>	
Mitis	<i>C. mitis</i>	Arboreal Clade I (Cephus-Mitis)
	<i>C. albogularis</i>	
	<i>C. nictitans</i>	
	<i>C. kandti</i>	
	<i>C. doggetti</i>	
Mona	<i>C. mona</i>	Arboreal Clade II (Mona-Neglectus-Diana)
	<i>C. campbelli</i>	
	<i>C. pogonias</i>	
	<i>C. wolfi</i>	
	<i>C. denti</i>	
	<i>C. lowei</i>	
Neglectus	<i>C. neglectus</i>	Arboreal Clade II (Mona-Neglectus-Diana)
Diana	<i>C. diana</i>	Arboreal Clade II (Mona-Neglectus-Diana)
	<i>C. roloway</i>	
Hamlini	<i>C. hamlyni</i>	Uncertain
Dryas	<i>C. dryas</i>	Uncertain
I'Hoesti	<i>C. l'hoesti</i>	Terrestrial Clade I (I'Hoesti-Aethiops-Patas)
	<i>C. preussi</i>	
	<i>C. solatus</i>	

to AGMs, patas, and talapoin monkeys. However, phylogenetic analysis does not resolve relationships between these groups. According to phylogeny based on Y and X chromosome sequences, *C. l'hoesti* has a common origin with *E. patas* and *C. aethiops*, whereas mtDNA phylogeny suggests a monophyletic origin for *C. l'hoesti* and *M. talapoin*.^{34,36}

Although there is a clear variation in the phenotype of patas monkeys from different locales, only one species (*Erythrocebus patas*) is currently recognized. However, the division of this species into subspecies—*E. p. patas*, *E. p. pyrrhonotus*, *E. p. baumstarki*, and *E. p. villiersi*—has been suggested.¹²

There are two forms of talapoin monkeys ranked either as species or subspecies: *M. talapoin/M. t. talapoin* (northern talapoin monkey) and *M. ogouensis/M. t. ogouensis* (southern talapoin monkey). They are separated by the Ogooué River.

Allen's swamp monkey (*Allenopithecus nigroviridis*) is the only species in the genus *Allenopithecus*.

1.3.2. Baboons and Allies

This group includes large, “strongly-built” monkeys commonly called baboons, geladas, mandrills, drills, and the much smaller, but related, mangabeys. All these monkeys live in Africa.

1.3.2.1. BABOONS AND GELADAS

The baboon is a common name for the species included in genus *Papio* (Figure 1.10). They are distributed throughout most of sub-Saharan Africa. There are five major morphologically distinguishable forms of baboons: Hamadryas, Olive, Yellow, Guinea, and Chacma. In Groves' classification,¹⁰ they are classified as species (Table 1.1). In some other classifications,



Figure 1.8. Juvenile red-eared guenon (*Cercopithecus erythrotis*), Limbe Zoo, Limbe, Cameroon. (Photo by Preston Marx.) See color version page 1.



Figure 1.10. Olive baboons (*Papio anubis*), Yankari Game Reserve, Nigeria. (Image is kindly provided by Dr. Janette Wallis.) See color version page 2.

these forms are ranked as subspecies. In this case, a single baboon species based on the priority rule is designated *P. hamadryas* (this name was introduced first). As a result, both binomial and trinomial nomenclatures are used. For example, *P. cynocephalus* and *P. hamadryas cynocephalus* (*P. h. cynocephalus*) are scientific names



Figure 1.9. Juvenile greater spot-nosed monkey (*Cercopithecus nictitans*), Medical Research Station, Kumba, Cameroon. (Photo by Preston Marx.) See color version page 2.

for Yellow baboon; *P. anubis* and *P. hamadryas anubis* for Olive baboon and so on. In this book, binomial nomenclature is used.

The variability of baboons is not limited to the major five species. Indeed, there are distinctive morphological variants of *P. cynocephalus* (Typical, Ibean, and Kinda Yellow baboons) and *P. ursinus* (Typical, Grey-footed, Transvaal, and Kalahari Chacma baboons). In addition, morphologically distinguishable natural hybrids of different baboon species and intraspecific forms are observed at boundary zones.

Most of the territory populated by baboons comprises a continuum in which neighboring baboon populations are not strictly isolated from each other. The transition from one species to another can be described as the geographical series.¹⁶ The north to south series is: Anubis → Ibean Yellow → Typical Yellow → Gray-footed Chacma → Transvaal Chacma → Typical Chacma. The Anubis segment of the series overlaps with Typical Guinea, Kinda, and Kalahari Chacma in the west and Hamadryas in the east. In addition, there are three baboon enclaves. Two of these, Anubis Air and Anubis Tibesti, are located in the Sahara desert, the third enclave, Arabian Hamadryas, is located in the southwest corner of the Arabian Peninsula.⁴²

The oldest extant *Papio* lineage is Chacma (estimated divergence time: 1.69–2.09 Mya). The Guinea lineage diverged next (1.23–1.51 Mya) followed by the

Hamadryas lineage (577–660 Tya). The youngest extant baboon lineages are the Olive and Yellow (150–172 Tya).²¹

Geladas (*Theropithecus gelada*) are the closest relatives of the baboons. They are quite large monkeys, approximately the same size as baboons. The eye-catching morphological feature of geladas is an hourglass-shaped area of naked pink skin on the neck and chest. In the females, it is framed by “fringed” vesicles, which swell during estrus. The natural habitat of geladas is restricted to the highlands of Ethiopia (2,000–4,000 m altitude). There are two morphologically distinguishable forms, recognized as subspecies: western gelada (*T. gelada gelada*) and eastern gelada (*T. g. obscurus*).¹² However, there is no clear-cut boundary between these subspecies. In addition, there is a distinct form, so-called Wabe Shabelle gelada found in a “gelada enclave” located in Wabe Shebelle gorge. Wabe Shabelle geladas are not formally recognized as a subspecies.

It is estimated that the gelada lineage diverged from a common *Papio*–*Theropithecus* ancestor 3.5–4.0 Mya.²⁰

The geladas do not breed well in captivity. In the 1960s–1980s, wild-caught geladas were widely used in biomedical experiments but this was discontinued. In contrast, baboons breed very well in captivity and are among the monkeys most commonly used in biomedical research.³⁸

1.3.2.2. MANDRILLS AND DRILLS

Mandrills (*Mandrillus sphinx*) and drills (*M. leucophaeus*) are the closest relatives of baboons. Male mandrills are the biggest, heaviest, and the most spectacular in appearance among all OWMs. Their face is fascinatingly colored in red, white, and blue; the bare skin in the perianal area and the penis are also brightly colored (Figure 1.11).

Mandrills are hunted for bush-meat, which is considered a delicacy, which poses a major danger to these animals.

The mandrill habitat is located in the West African coastal rainforest between the Sanaga and Zaire Rivers and expands up to 300 km inland. Mandrill populations separated by the Ogooué River are genetically distinct.³³

The natural habitat of drills is located north of the mandrill range. It is noncontinuous, consisting of two parts: the continental (between the Cross and Sanaga Rivers) and Bioko Island (Equatorial Guinea). They are populated by different subspecies, the mainland drills



Figure 1.11. Adult male mandrill (*Mandrillus sphinx*) (center), International Center for Medical Research, Franceville, Gabon. (Photo by Preston Marx.) See color version page 2.

(*M. leucophaeus leucophaeus*) (Figure 1.12) and Bioko drills (*M. l. poensis*).

Mandrills and drills are rarely used in biomedical research; although viruses harbored by wild mandrills, particularly retroviruses and herpesviruses are quite extensively studied.



Figure 1.12. Adult male drill (*Mandrillus leucophaeus*), Limbe Zoo, Limbe, Cameroon. (Photo by Preston Marx.) See color version page 2.

1.3.2.3. MANGABEYS

There are two types of mangabeys, drill-mangabeys (*Cercocebus* spp.) and baboon-mangabeys (*Lophocebus* spp.). Drill-mangabeys and baboon-mangabeys are less related to each other than to mandrills/drills and baboons, respectively. However, the general “look” of all mangabeys is similar. They are much smaller than their “grand” relatives and do not have the eye-catching sexual dimorphic characteristics of baboons and mandrills. Mangabeys live in the rainforest in and around equatorial West and Central Africa.

A distinctive trait of drill-mangabeys is white upper eyelids—hence their nickname “eyelid monkeys” (Figures 1.13 and 1.14).

Groves’ classification includes six *Cercocebus* species (Table 1.1). However, more than six morphologically distinguishable forms of drill-mangabeys are known and there is no consensus regarding ranking some of them as species or subspecies.¹² The most controversial is taxonomic identification of the white-naped mangabey. This mangabey is classified as a subspecies of sooty mangabey (*C. atys lunulatus*); at the same time, there are data supporting its closer relatedness to red-capped mangabey (*C. torquatus*).¹²

Taxonomy of baboon-mangabeys is less complicated than that of drill-mangabeys; however, a consensus in



Figure 1.13. Adult sooty mangabey (*Cercocebus atys*), Tulane National Primate Research Center, USA. (Image is kindly provided by Mrs. Robin Rodrigues.) See color version page 3.



Figure 1.14. Adult red-capped mangabey (*Cercocebus torquatus*), Yaoundé, Cameroon. (Photo by Preston Marx.) See color version page 3.

this field has also not been reached. Three species of baboon-mangabeys are distinguished in Groves’ classification: gray-cheeked (*L. albigena*), black-crested (*L. aterrimus*), and Opdenbosch’s (*L. opdenboschi*) mangabeys. The latter is considered as a subspecies of *L. aterrimus* by others.¹²

A new baboon-mangabey species, the highland mangabey (*L. kipunji* or *Rungwecebus kipunji*), has recently been described.⁶ This is the first description of a new OWM species in 85 years. Very little is known about these extremely rare mangabeys which have been identified in two mountain sites in Tanzania. However, available data indicate that the difference between classical baboon-mangabeys and this species may be sufficient for placing highland mangabeys in a new genus. The name *Rungwecebus* has been suggested for this genus.⁶

1.3.3. Macaques

Macaques (*Macaca* spp.) live almost exclusively in Asia. The only exception among 21 macaque species is a barbary macaque (*M. sylvanus*) whose natural habitat is located in the western corner of North Africa. However, the macaque lineage originated in Africa—hence the inclusion of the macaques in the Papionini tribe. A common ancestor of macaques, baboons, and geladas lived in Africa approximately 8.6–10.9 Mya.²⁴ The migration of common ancestors of extant Asian macaque species to Eurasia is dated at 6–5.5 Mya. All ancient European macaque species became extinct. The same also happened to all African ancient macaques, except the lineage leading to *M. sylvanus*.

Asian macaque species can be divided into four groups: Mulatta, Nemestrina, Fascicularis, and Sinica (Table 1.7, Figures 1.15–1.17). The prototype species for three of these groups, *M. mulatta*, *M. fascicularis*, and *M. nemestrina*, are the monkeys most commonly used in biomedical research. Importantly, susceptibility of different macaque species to different medically important viruses varies significantly.

Rhesus monkey (*M. mulatta*), undoubtedly, is the best-studied primate species, next to *Homo sapiens* (Figure 1.15). Suffice it to mention that *M. mulatta* is the only monkey species for which virtually the complete genome sequence is known.²⁵ Rhesus monkeys originating from different regions of Asia are quite distinct. Seven *M. mulatta* subspecies have been formally recognized (Table 1.8).⁴ Most of the rhesus monkeys used in biomedical experiments before the 1980s were



Figure 1.15. Adult female and infant rhesus monkeys (*Macaca mulatta*), Swoyambhu Temple, Kathmandu, Nepal. (Image is kindly provided by Prof. Randall C. Kyes.) See color version page 3.

either imported from India or originated from breeding colonies, whose founders were Indian-origin rhesus macaques. The situation changed when India introduced a ban on the export of monkeys. Currently, most imported rhesus macaques available for biomedical experiments are of Chinese origin. The Chinese or Indian origin of rhesus monkeys can be validated by genetic analysis.^{18,28,31}

Table 1.7. Macaque Species Groups

Mulatta	Fascicularis	Sinica	Nemestrina	Sylvanus
<i>M. mulatta</i>	<i>M. fascicularis</i>	<i>M. sinica</i>	<i>M. nemestrina</i>	<i>M. sylvanus</i>
<i>M. cyclopis</i>	<i>M. arctoides</i>	<i>M. radiata</i>	<i>M. silenus</i>	
<i>M. fuscata</i>		<i>M. assamensis</i>	<i>M. nigra</i>	
		<i>M. thibetana</i>	<i>M. nigrescens</i>	
			<i>M. hecki</i>	
			<i>M. tonkeana</i>	
			<i>M. maura</i>	
			<i>M. ochreata</i>	
			<i>M. leonina</i>	
			<i>M. pagensis</i>	
			<i>M. siberu</i>	

Adapted from Groves.¹⁰



Figure 1.16. Adult male cynomolgus macaque (*Macaca fascicularis*), Tinjil Island, Indonesia. (Image is kindly provided by Prof. Randall C. Kyes.) See color version page 3.

Cynomolgus macaques (*M. fascicularis*), also named crab-eating or long-tailed macaques, are second to rhesus monkeys in their significance to biomedical research (Figure 1.16).



Figure 1.17. Juvenile male Celebes black macaque (*Macacanigra*), Tangkoko Nature Reserve, North Sulawesi, Indonesia. (Image is kindly provided by Prof. Randall C. Kyes.) See color version page 4.

The largest captive monkey colony in the world (Nafovanny, Vietnam) houses about 30,000 cynomolgus macaques. Cynomolgus macaques are smaller than rhesus monkeys and they breed in captivity year around (rhesus monkeys are seasonal breeders). Genetically, rhesus and cynomolgus macaques are closely related; for instance, they share many single nucleotide polymorphisms (SNPs).³¹ At the same time, cynomolgus macaques originating from different geographical regions are sufficiently distinct to be classified as subspecies (Table 1.8).^{4,29}

1.3.4. Colobines

Colobines (*Colobinae* spp.) live both in Africa and Asia. These monkeys are strict “vegetarians”—hence their colloquial name “leaf-eaters.” In addition, different colobines are known under different common names: colobuse, langur, surili, lutung. There are 10 genera of colobines: *Colobus* (5 species), *Presbitis* (11 species), *Trachypithecus* (17 species), *Semnopithecus* (7 species), *Nasalis* (1 species), *Pygathrix* (3 species), *Pilicolobus* (9 species), *Procolobus* (1 species), *Simias* (1 species), and *Rhinopithecus* (4 species).

Colobus are medium-sized monkeys with small heads, disproportionately large bodies, and long limbs. They live in forested areas of Africa, from the west to east coast. Their body composition and long “acrobatic balancer” tails are well suited for the arboreal “lifestyle.” Colobus monkeys are also well adapted for processing “hard-to-digest” plant food. They have powerful jaw muscles, large salivary glands, and very large multi-chambered stomachs, containing microorganisms that ferment cellulose. A distinctive feature of colobus monkeys is the lack of a thumb—hence their name—a derivative of the Greek *colobe* for cripple. Colobus species are grouped into three genera: *Colobus*, *Pilicolobus*, and *Procolobus*. It is estimated that colobuses diverged from other African monkeys 14.7 ± 1.5 Mya.³⁰

It is very difficult to breed colobus monkeys in captivity and they are not used as experimental animals.

Asian colobines are a more diverse group than the colobus.² It is estimated that they diverged from their African “relatives” 10.8 ± 1 Mya.³⁰

Gray langurs (*Semnopithecus* spp.) are the largest among Asian colobines. They inhabit many regions of the Indian subcontinent including urban areas (Figure 1.18).

Table 1.8. Subspecies of *M. mulatta* and *M. fascicularis*

Species/Subspecies	Common Name	Geographical Distribution
<i>Macaca mulatta</i>	Rhesus monkey	
<i>M. mulatta mulatta</i>	Indian rhesus monkey	E Afghanistan, Bangladesh, Bhutan, N peninsular India, Nepal, N Pakistan
<i>M. mulatta lasiota</i>	West Chinese rhesus monkey	China (SE Qinghai, W Sichuan, NE Yunnan)
<i>M. mulatta littoralis</i>	South Chinese rhesus monkey	China (Fujian, Guangdong, Far E Guangxi)
<i>M. mulatta sanctijohannis</i>	Insular Chinese rhesus monkey	China (Hainan, Islands around Hong Kong, Wanshan Isl)
<i>M. mulatta siamica</i>	Indochinese rhesus monkey	Burma, China (Anhui, NW Guangxi, Guizhou, Hubei, Hunan, C&E Sichuan, W&S and Central Yunnan), Laos, N Thailand, N Vietnam
<i>M. mulatta tcheliensis</i>	North Chinese rhesus monkey	China (Hebei, N Henan, and S Shanxi)
<i>M. mulatta vestita</i>	Tibetan rhesus monkey	China (SE Tibet and NW Yunnan)
<i>Macaca fascicularis</i>	Cynomolgus macaque*	
<i>M. fascicularis atriceps</i>	Dark-crowned long-tailed macaque	SE Thailand (Khram Yai Island)
<i>M. fascicularis aurea</i>	Burmese long-tailed macaque	S Bangladesh, S Burma, W and Central Thailand
<i>M. fascicularis condorensis</i>	Con Son long-tailed macaque	Vietnam (Con Son)
<i>M. fascicularis fusca</i>	Simeulue long-tailed macaque	Indonesia (Simeulue Isl)
<i>M. fascicularis karimondjawaee</i>	Karimunjawa long-tailed macaque	Indonesia (Karimunjawa Isl)
<i>M. fascicularis lasiae</i>	Lasia long-tailed macaque	Indonesia (Lasia Isl)
<i>M. fascicularis philippinensis</i>	Philippine long-tailed macaque	Philippines (Balabac, Culion, Leyte, Luzon, NE Mindanao, Mindoro, Palawan, Samar)
<i>M. fascicularis tua</i>	Maratua long-tailed macaque	Indonesia (Maratua Isl)
<i>M. fascicularis umbrosa</i>	Nicobar long-tailed macaque	India (Katchall Isl, Little Nicobar Isl)

Adapted from Brandon-Jones *et al.*⁴

*Synonymous names: long-tailed or crab-eating macaque.

In contrast to most colobines, gray langurs are mainly terrestrial animals. The gray langur is a diverse group. However, there is no consensus regarding formal classification of various forms. Groves' classification distinguishes 76 species of gray langurs (Table 1.2). Others recognize 14 *Semnopithecus* subspecies combined in 3 species (*S. entellus*, *S. jorpii*, and *S. vetulus*); particularly, numerous are the *S. entellus* subspecies.⁴ The classification of the purple-faced langur (*S. vetulus*) and

Nilgiri langur (*S. jorpii*) as *Semnopithecus* species is disputable. In Groves' classification, they are included in the *Trachypithecus* genus. However, this placement is not supported by molecular taxonomic data.¹⁷

Surilis (*Presbytis* spp.) are arboreal colobine monkeys living in the southern part of the Malay Peninsula, on Sumatra, Java, Borneo, and adjacent small islands. Some *Presbytis* species are called surilis, others are called langurs (Table 1.2). Surilis and surili-langurs



Figure 1.18. Adult female Northern Plains gray langur (*Semnopithecus entellus*), Jodhpur, India. (Image is kindly provided by Prof. Randall C. Kyes.) See color version page 4.

have a characteristic hair tuft on their heads—hence their nickname “capped langur.” Groves’ classification includes 11 *Presbytis* species. However, the number of distinguishable forms in this genus is much greater; 29 surili taxa ranked as species or subspecies have been suggested.⁴

Lutungs are colobine monkeys belonging to the *Trachypithecus* genus. This genus also includes some langurs and so-called leaf monkeys (Table 1.2). Seventeen *Trachypithecus* species are included in Groves’s classification. The natural habitat of these species is “disjunctive”; that is there are two widely separated areas populated by the trachypithecines: southwest of India and Sri Lanka and northeast of India and Southeast Asia. The inclusion of some species into the *Trachypithecus* genus has been challenged by molecular taxonomic data, which indicate that southwest Indian and Sri Lankan langurs (Nilgiri and purple faced) are more related to the gray langurs (*Semnopithecus*) than to other *Trachypithecus* species.¹⁷

The “odd-nosed” group of species includes *Nasalis*, *Simias*, *Pygathrix*, and *Rhinipithecus* species. The group is diverse, but as its name implies, all these monkeys have characteristic morphological traits in the facial area. The most distinctive in this respect is the long-nosed or proboscis monkey (*Nasalis larvatus*). Males of this species have a very large protruding nose. Long-nosed monkeys live mostly in trees in swamps of the

costal areas of Borneo. They swim well and can walk upright. The latter characteristic is exceptional for OWMs.

The closest relatives of the long-nosed monkeys are pig-tailed langurs or simakobu monkeys (*Simias concolor*),^{30,41} a species endemic on the Mentawai Islands, Indonesia. These monkeys, with a characteristic upwards pointed nose, are the most endangered species among Asian NHPs.

1.4. APES

1.4.1. Lesser Apes (Gibbons and Siamangs)

Lesser apes inhabit rainforests throughout Southeast Asia, from eastern India to Vietnam, southern China, and Indonesia. They are divided into four groups ranked as genera in most classifications: *Hylobates*, *Bunopithecus*, *Nomascus*, and *Sympalangus*. A diploid number of chromosomes is characteristic of each genus: 44, 38, 52, and 50 for *Hylobates*, *Bunopithecus*, *Nomascus*, and *Sympalangus*, respectively. Molecular phylogeny based on the analysis of mtDNA sequences also supports this classification.³²

Most of the gibbon species belong to the *Hylobates* genus (Table 1.3). There are many gibbon subspecies (Table 1.9). *Hylobates* gibbons are commonly named the Lar group, after the type species *Hylobates lar*.

The Lar gibbons inhabit central and southern parts of Indochina, including the Malay Peninsula, Sumatra, Borneo, and western Java. Within this habitat, *H. lar* and *H. moloch* (Figure 1.19) are the northernmost and southernmost species. Accurate species identification of Lar gibbons is possible based on the D-loop mitochondrial DNA sequence.⁴⁰

Crested gibbons (*Nomascus* spp.) inhabit eastern Indochina and southern China. Crested gibbons are also named the Concolor group, after a type species *Nomascus concolor*. The natural boundary separating the Lar and Concolor gibbons is the Mekong River. There are many subspecies of crested gibbons.

The *Bunopithecus* genus includes only one species, hoolock (*B. hoolock*). Hoolock gibbons inhabit an area northeast of the continental gibbon habitat, the territory between the Brahmaputra and Salween rivers. There are two subspecies of the hoolocks, western (*B. hoolock hoolock*) and eastern (*B. hoolock leucopenedys*).

The *Sympalangus* genus also includes only one species, siamang (*Sympalangus syndactylus*). Siamangs inhabit the southern part of the Malay Peninsula and the mountains of the Indian Ocean coast of Sumatra.

Table 1.9. Ape Subspecies

Species/Subspecies	Common Name
<i>Hylobates agilis</i>	Agile gibbon
<i>H. agilis agilis</i>	Mountain agile gibbon
<i>H. agilis albibarbis</i>	Bornean agile gibbon
<i>H. agilis unko</i>	Lowland agile gibbon
<i>Hylobates lar</i>	Lar gibbon or white-handed gibbon
<i>H. lar lar</i>	Malayan white-handed gibbon
<i>H. lar carpenteri</i>	Carpenter's white-handed gibbon
<i>H. lar entelloides</i>	Central white-handed gibbon
<i>H. lar vestitus</i>	Sumatran white-handed gibbon
<i>H. lar yunnanensis</i>	Yunnan white-handed gibbon
<i>Hylobates moloch</i>	Javan silvery gibbon
<i>H. moloch moloch</i>	West Javan silvery gibbon
<i>H. moloch pongoalsoni</i>	Central Javan silvery gibbon
<i>Hylobates muelleri</i>	Müller's Bornean gibbon
<i>H. muelleri muelleri</i>	Müller's gray gibbon
<i>H. muelleri abbotti</i>	Abbott's gray gibbon
<i>H. muelleri funerus</i>	Nothern gray gibbon
<i>Nomascus concolor</i>	Black crested gibbon
<i>N. concolor concolor</i>	Tonkin black crested gibbon
<i>N. concolor furvogaster</i>	West Yunnan black crested gibbon
<i>N. concolor jingdongensis</i>	Central Yunnan black crested gibbon
<i>N. concolor lu</i>	Laotian black crested gibbon
<i>Nomascus leucogenys</i>	Northern white-cheeked gibbon
<i>N. leucogenys leucogenys</i>	Northern white-cheeked gibbon*
<i>N. leucogenys siki</i>	Southern white-cheeked gibbon*
<i>Bunopithecus hoolock</i>	Hoolock gibbon
<i>B. hoolock hoolock</i>	Western hoolock gibbon
<i>B. hoolock leuconedys</i>	Eastern hoolock gibbon
<i>Sympalangus syndactylus</i>	Siamangs
<i>S. syndactylus syndactylus</i>	Sumatran siamang
<i>S. syndactylus continentis</i>	Malayan siamang
<i>Pongo pygmaeus</i>	Bornean orangutan
<i>P. pygmaeus pygmaeus</i>	Western Bornean orangutan
<i>P. pygmaeus wurmbii</i>	Southern Bornean orangutan
<i>Gorilla gorilla</i>	Western gorilla
<i>G. gorilla gorilla</i>	Western lowland gorilla
<i>G. gorilla diehli</i>	Cross River gorilla
<i>Gorilla beringei</i>	Eastern gorilla
<i>G. beringei beringei</i>	Mountain gorilla
<i>G. beringei graueri</i>	Grauer's gorilla
<i>P. troglodytes</i>	Common chimpanzee
<i>P. troglodytes troglodytes</i>	Common or robust chimpanzee
<i>P. troglodytes vellerosus</i>	Nigeria chimpanzee
<i>P. troglodytes schweinfurthii</i>	Eastern chimpanzee
<i>P. troglodytes verus</i>	Western chimpanzee

Adapted from Brandon-Jones *et al.*⁴ and Grubb *et al.*¹²*Classified as species by Groves.¹⁰



Figure 1.19. Adult female and infant Javan silvery gibbon (*Hylobates moloch*), Primate Research Center at Bogor Agricultural University, Bogor, Indonesia. (Image is kindly provided by Prof. Randall C. Kyes.) See color version page 4.



Figure 1.20. Adult common chimpanzee (*Pan troglodytes*), Bakumba, Gabon. (Photo by Preston Marx.) See color version page 4.

It is estimated that the common ancestor of all four extant gibbon lineages diverged from the common ancestor of the other apes 16–23 Mya. Apparently, the *Hylobates* lineage is the youngest, whereas the *Bunopithecus* lineage is the oldest.³²

Gibbons are susceptible to human viruses and may carry oncogenic retroviruses.

1.4.2. Great Apes (Chimpanzees, Gorillas, and Orangutans)

Chimpanzees (genus *Pan*) undoubtedly are our closest relatives. The natural habitat of these remarkably intelligent animals extends from West to Equatorial Africa. Most chimpanzees, both wild and captive, belong to the common chimpanzee species (*P. troglodytes*) (Figure 1.20). Common chimpanzees are also called robust chimpanzees, but this name is rarely used.

The second chimpanzee species, the pygmy chimpanzee, also named gracile chimpanzee (*P. paniscus*), has a much more restricted range.

Within the *P. troglodytes* species, there are several morphologically and geographically distinguishable forms classified as subspecies: central chimpanzee (*P. t. troglodytes*), Nigeria chimpanzee (*P. t. vellerosus*), eastern chimpanzee (*P. t. schweinfurthii*), and western chimpanzee (*P. t. verus*; Table 1.9).^{12,23} It is worth mentioning that the classification of common chimpanzees into subspecies has been challenged by the proponents of molecular taxonomy. The extent of genetic diver-

sity in chimpanzees belonging to different subspecies is comparable to that in various human populations.⁸ Phylogenetic analysis of mitochondrial DNA sequences supports the division of common chimpanzees into two groups: western (*P. t. verus*) and central-eastern (*P. t. troglodytes/P. t. schweinfurthii*).¹³ Analysis of another set of chimpanzee mitochondrial DNA sequences indicates that the major phylogenetic break between common chimpanzee lineages separates chimps along the Sanaga River in Cameroon.⁹

The captive chimpanzee population in the United States includes approximately 1,000 animals, most of which were born in captivity. These chimpanzees originated predominantly from *P. t. verus*, with 95% of the population founders belonging to this subspecies.⁷ Many thousands of common chimpanzees were used in biomedical experiments in the United States from the 1950s to the 1990s. In 2007, the NIH announced a permanent ban on the breeding of chimpanzees in US government-funded facilities. Although the scale of biomedical experiments using common chimpanzees is steadily decreasing, it is unlikely that use of chimpanzee models of human diseases will be completely terminated. Unfortunately, there is no alternative to the chimpanzee model for preclinical testing of the efficacy of candidate vaccines extremely important for public health, for instance, the development of a vaccine against hepatitis C.



Figure 1.21. Adult male lowland gorilla, International Center for Medical Research, Franceville, Gabon. (Photo by Preston Marx.) See color version page 5.



Figure 1.22. Adult male Bornean orangutan (*Pongo pygmaeus*), Woodland Park Zoo, Seattle, Washington, USA. (Image is kindly provided by Prof. Randall C. Kyes.) See color version page 5.

Gorillas (genus *Gorilla*) are the largest and the heaviest NHPs (height up to 180 cm, weight 90–180 kg) (Figure 1.21).

Genetically, gorillas are the closest relatives to humans, after chimpanzees. Unfortunately, gorillas are endangered species. The size of the natural gorilla population has decreased dramatically over the last 20 years, partly due to human activities and partly as a result of devastating outbreaks of Ebola hemorrhagic fever in wild gorilla populations.¹

Taxonomically, gorillas are divided into two species: western gorilla (*G. gorilla*) and eastern gorilla (*G. beringei*). Within each of these species, there are distinguishable forms classified as subspecies in the latest classification (Table 1.9).¹² The western gorilla species is divided into western lowland gorilla (*G. g. gorilla*) and Cross River gorilla (*G. g. diehli*). Two subspecies are also distinguished within eastern gorilla species: mountain gorilla (*G. b. beringei*) also known as *G. b. bwindi* and Grauer's gorilla (*G. b. graueri*).

Asian great apes, orangutans (*Pongo spp.*), are large (115–160 cm, 40–100 kg), mostly arboreal, animals living in the tropical rainforests on two islands, Borneo (Kalimantan) and Sumatra (Figure 1.22). Orangutan males are much larger than females. Their lifestyle is solitary. Orangutans reproduce slowly and their life span is quite long (the longest recorded in captive orangutans is 57 years). Orangutans' diet is mainly veg-

etarian; however, occasionally they eat bird eggs and may prey on small vertebrate animals. Bornean and Sumatran orangutans are recognized as separate species, *P. pygmaeus* and *P. abelii*, respectively (Table 1.3). Bornean orangutans are significantly larger than Sumatran orangutans. There are at least two distinguishable forms of Bornean orangutans. In some classifications, they are ranked as subspecies: *P. p. pygmaeus* (western Bornean orangutan) and *P. p. wurmblii* (eastern Bornean orangutan; Table 1.9).⁴ Orangutans are also endangered species and they are not bred in captivity for research purposes. However, they adapt well to zoo conditions and a number of orangutans have been born in captivity. Orangutans in zoos invariably attract attention for their eye-catching reddish color, size (captive orangutans are overweight, sometimes exceeding 200 kg in weight), and “intellectual” facial expressions.

1.5. NEW WORLD MONKEYS

According to Groves' classification, there are 121 extant species of the NWMs divided into four families and seven subfamilies¹⁰ (Figure 1.3, Table 1.4). These monkeys live in continental Central and South America as well as Trinidad and Tobago. The characteristic morphological feature of NWMs is a flat nose with circular side-facing nostrils spaced far apart—hence their names “flat-nosed” or “broad-nosed.” The other characteristic

traits are a distinctive dental formula, absence of buttock pads, and cheek pouches. All NWMs have long tails; in some species the tail serves as an additional “arm,” called prehensile tails. In general, NWMs are smaller than OWMs; although howlers and muriquis are quite large. Virtually all NWMs are arboreal species.

The common ancestor of NWMs, presumably, lived in Africa. The divergence of the NWM lineage from the other primates occurred approximately 35 Mya.²² How common ancestors of NWMs (“flat-nosed Eve and Adam”) arrived in South America is a matter of speculation. The “floating vegetation raft” hypothesis appears to be a likely scenario. According to molecular phylogenetic reconstructions, the divergence of NWM species started approximately 25 Mya. The most ancient is the lineage leading to the Pitheciidae family. A common ancestor of three other NWM families (Cebidae, Atelidae, and Aotidae) is estimated to have lived 23 Mya. The diversification of NWM taxa was particularly extensive during the Miocene 15–10 Mya.^{22,27} The “youngest” NWM genera are *Cacajao* and *Chiropotes*; their common ancestor is dated at approximately 7 Mya. Molecular phylogenetic analysis separates extant NWMs into four groups that are not equidistant and not fully concordant with the families and subfamilies in the zoological classification.²²

1.5.1. Marmosets and Tamarins

Marmosets and tamarins are generally smaller than other NWMs (body length without tail 17–40 cm). Their distinguishing morphological traits are the presence of claws instead of nails on all digits except the big toe, nonopposable thumbs, and characteristic dental make-up. Both marmosets and tamarins are arboreal, omnivorous monkeys.

All but one marmoset species belong to the *Callithrix* genus. The exception is Goeldi’s marmoset (*Callimico goeldii*) which is the only marmoset species in the *Callimico* genus. The marmosets are usually born as twins. Interestingly, all tissues, including ovaries and testicles, in the marmoset twins (*Callithrix kuhlii*) are chimeric. As a consequence, marmosets can transmit sibling gametes to the offspring, a unique situation among primates in which the biological parent cannot be identified by genetic analysis.²⁶

Common marmosets (*Callithrix jacchus*) adapt and breed well in captivity, assuming proper husbandry (Figure 1.23). That is why these monkeys are widely used



Figure 1.23. Common marmosets (*Callithrix jacchus*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.) See color version page 5.

in biomedical research. A number of marmoset viruses are known, most notably various herpesviruses.

Most tamarins belong to the *Saguinus* genus (17 species). Less numerous are lion tamarins species belong to the *Leontopithecus* genus (4 species). Characteristic morphological traits of tamarins are mustache-like facial hairs and long lower canine teeth. On average, tamarins are larger than marmosets. Although tamarins can be adapted to captivity, their maintenance is more demanding than that of marmosets. For this reason, tamarins are much less used in biomedical research; although sometimes virological experiments are performed in captive tamarins (Figure 1.24).

1.5.2. Capuchins, Owl, and Squirrel Monkeys

Capuchins (*Cebus* spp.) are so named because their coloration resembles cowls of Franciscan Capuchins, an order of monks. Capuchins are relatively small (30–55 cm without tail, weight 1–4 kg), but very intelligent monkeys. They are frequently kept as pets and perform as “organ grinder” monkeys. More importantly, they can be trained to assist quadriplegics. Capuchins are rarely used in virological research (Figure 1.25).

Squirrel monkeys (*Saimiri* spp.), as their name suggests, are small, squirrel-size animals (25–35 cm, 0.75–1 kg) (Figure 1.26).



Figure 1.24. Golden-headed lion tamarin (*Leontopithecus chrysomelas*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.) See color version page 5.



Figure 1.26. Common squirrel monkey (*Saimiri sciureus*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.) See color version page 6.

Interestingly, squirrel monkey brain mass relative to body weight is the largest among all primates. However, this is not translated into remarkable intelligence. Common squirrel monkeys (*S. sciureus*) are widely used in virological research.



Figure 1.25. White-fronted capuchin (*Cebus albifrons*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.) See color version page 6.

Owl monkeys, also named night monkeys, have small external ears—hence the name of the genus *Aotus*—which literally means “earless” (Figure 1.27).

As their common name suggests, these animals are nocturnal; that is, they are active at night. An interesting behavioral characteristic of owl monkeys is their remarkably wide repertoire of vocal sounds. Their vision is well adapted to low light conditions so they can move and feed efficiently at night. Quite unusually for NHPs, owl monkeys in natural conditions are monogamous. The three-striped night monkey (*Aotus trivirgatus*) has been used in biomedical research, usually under the name “owl monkey.”

1.5.3. Howlers, Muriquis, Spider, and Woolly Monkeys

All these monkeys belong to the family Pitheciidae. They are relatively large as compared to other NWMs. The largest in terms of body size are howlers (55–90 cm without tail) (Figures 1.28 and 1.29). Howlers can produce a very loud barking sound figuratively named “the howl”—hence their name. Unlike most of the NWM species, howlers are “phlegmatic,” they rest most of the time in trees. Although fights between howlers may happen, in general, they are very tranquil animals. Exceptionally for NWMs howlers have trichromatic color vision.

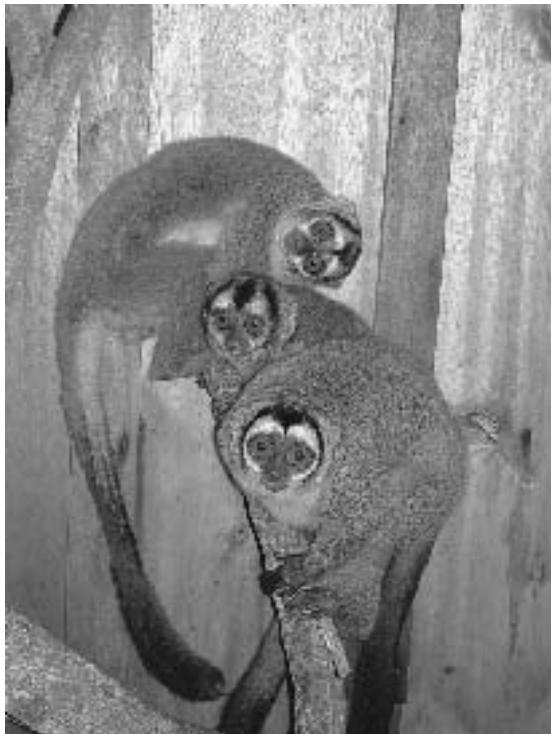


Figure 1.27. Black-headed owl monkey, also called night monkey (*Aotus nigriceps*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.) See color version page 6.



Figure 1.29. Black howler (*Alouatta pigra*), Balancan, Tabasco, Mexico. (Image is kindly provided by Dr. Juan Carlos Serio Silva.) See color version page 7.



Figure 1.28. Brown howler monkey (*Alouatta guariba*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.) See color version page 6.



Figure 1.30. Spider monkeys (*Ateles geoffroyi vellerosus*), Balancan, Tabasco, Mexico. (Image is kindly provided by Dr. Juan Carlos Serio Silva.) See color version page 7.



Figure 1.31. Gray woolly monkey (*Lagothrix cana*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.) See color version page 7.



Figure 1.32. Adult male Northern muriqui (*Brachyteles hypoxanthus*), Caratinga Biological Station, Minas Gerais, Brazil. (Image is kindly provided by Dr. Jean P. Boubli.) See color version page 7.

resembling a penis. Spider monkeys are very intelligent animals, perhaps the most intelligent among NWMs.

Spider monkeys are rarely used in biomedical research. However, several herpesviruses harbored by these monkeys have been isolated and studied.

Woolly monkeys are, on average, larger than spider monkeys and more uniform in size (51–69 cm without tail) (Figure 1.31). Woolly monkeys are divided into two genera, the “classical” genus *Lagothrix* with four species and the recently introduced genus *Oreonax*. The latter includes only one species, the yellow-tailed woolly monkey (*O. flavicauda*). Woolly monkeys are rarely used in biomedical research. However, it happens that this name is better known to virologists than the names of most NWMs. This is because the only known primate acutely transforming retrovirus, the simian sarcoma virus 1 (SSV-1), has been isolated from woolly monkeys.

Two rare species of NWM, the muriquis (*Brachyteles* spp.), share characteristics of both spider and woolly monkeys—hence their second name woolly spider monkeys. The word “muriqui” in Tupi, an Amerindian language, means “very large monkey.” Indeed, these monkeys, although shorter than the howlers (46–63 cm) are much heavier (12–15 kg versus 4–10 kg). Muriquis are an endangered species and are not used in biomedical research (see Figure 1.32).

1.5.4. Titis, Sakis, and Uakaris

Titis (*Callicebus* spp.) are NWM-related species (28 species) with long soft fur and long furry tails that are not prehensile (Figure 1.33). The size and coloring of titis significantly varies (24–61 cm, weight 0.5–2 kg). Titis are very good jumpers, being referred to as “jumping



Figure 1.33. Collared titi (*Callicebus torquatus*), Sustainable Development Reserve Amanã, Lake Amanã, Amazonas, Brazil. (Image is kindly provided by Marcela Alvares Oliveira.) See color version page 8.



Figure 1.34. Rio Tapajos saki (*Pithecia irrorata*), Belo Horizonte Zoo, Minas Gerais, Brazil. (Image is kindly provided by Eduardo Franco.) See color version page 8.

monkeys” in German. Titis are known for their life-long monogamous mating. Titis are not used in biomedical research.

Sakis (*Pithecia* spp.) (Figure 1.34) and bearded sakis (*Chiropotes* spp.) are related species which have characteristic head hairs resembling a hood or a cap. These monkeys are extremely well adapted to life on trees. Similar to titis, sakis are monogamous. Sakis are not used in biomedical research.

Uakaris (*Cacajao* spp.) are medium-sized monkeys (30–50 cm, weight 2.5–3.5 kg) living in the Amazon Basin. Their common name, as well as the name of the genus, is believed to originate from indigenous Amerindian languages, although the exact meaning of these words is not known. The distinctive morphological features of these NWMs are a hairless, “skull-like” face, very little subcutaneous fat and an unusually short tail (Figure 1.35). Uakaris adapt poorly to captivity and are not used in biomedical research.

1.6. CONCLUDING REMARKS

It should be emphasized that taxonomic and biogeographical information presented in this chapter are adapted for the novice in Primatology. Those interested in deeper knowledge will find relevant information in the experts’ reviews.^{4,10,12} A large collection of NHP images is available at Primate Info Net (<http://pin.primate.wisc.edu/av/images/index.html>).



Figure 1.35. Neblina black-headed uakari (*Cacajao melanocephalus*), the Pico da Neblina National Park, Brazil; classified also as a separate species (*C. hosomi*). (Image is kindly provided by Dr. Jean P. Boubli.). See color version page 8.

REFERENCES

1. Bermejo, M., J. D. Rodriguez-Teijeiro, G. Illera, A. Barroso, C. Vila, and P. D. Walsh. 2006. Ebola outbreak killed 5000 gorillas. *Science* 314(5805):1564.
2. Brandon-Jones, D. 2004. A taxonomic revision of the langurs and leaf monkeys (Primates: Colobinae) of South Asia. *Zoos' Print J.* 19(8):1552–1594.
3. Brandon-Jones, D. 2006. The pros and cons of a consensus list of Asian primate subspecies. *Primate Conserv.* 20:89–93.
4. Brandon-Jones, D., A. A. Eudey, T. Geissmann, C. P. Groves, D. J. Melnick, J. C. Morales, M. Shekelle, and C. B. Stewart. 2004. Asian primate classification. *Int. J. Primatol.* 25(1):97–164.
5. Constable, J. J., C. Packer, D. A. Collins, and A. E. Pusey. 1995. Nuclear DNA from primate dung. *Nature* 373(6513):393.
6. Davenport, T. R., W. T. Stanley, E. J. Sargis, D. W. De Luca, N. E. Mpunga, S. J. Machaga, and L. E. Olson. 2006. A new genus of African monkey, Rungwecebus: morphology, ecology, and molecular phylogenetics. *Science* 312(5778):1378–1381.
7. Ely, J. J., B. Dye, W. I. Frels, J. Fritz, P. Gagneux, H. H. Khun, W. M. Switzer, and D. R. Lee. 2005. Subspecies

- composition and founder contribution of the captive U.S. chimpanzee (*Pan troglodytes*) population. *Am. J. Primatol.* 67(2):223–241.
8. Fischer, A., J. Pollack, O. Thalmann, B. Nickel, and S. Paabo. 2006. Demographic history and genetic differentiation in apes. *Curr. Biol.* 16(11):1133–1138.
 9. Gonder, M. K., T. R. Disotell, and J. F. Oates. 2006. New genetic evidence on the evolution of chimpanzee populations and implications for taxonomy. *Int. J. Primatol.* 27(4):1103–1127.
 10. Groves, C. P. 2005. Order primates. In: Wilson, Don E. and DeeAnn M. Reeder (eds), *Mammal Species of the World: A Taxonomic and Geographical Reference*, 3rd edn. Baltimore: Johns Hopkins University Press, pp. 111–184.
 11. Grubb, P. 2006. English common names for subspecies and species of African primates. *Primate Conserv.* 20:65–73.
 12. Grubb, P., T. M. Butynski, J. F. Oates, S. K. Bearder, T. R. Disotell, C. P. Groves, and T. T. Struhsaker. 2003. Assessment of the diversity of African primates. *Int. J. Primatol.* 24(6):1301–1357.
 13. Guillen, A. K. Z., G. M. Barrett, and O. Takenaka. 2005. Genetic diversity among African great apes based on mitochondrial DNA sequences. *Biodivers. Conserv.* 14(9):2221–2233.
 14. Harris, E. E. 2000. Molecular systematics of the Old World monkey tribe Papionini: analysis of the total available genetic sequences. *J. Hum. Evol.* 38(2):235–256.
 15. Herke, S. W., J. Xing, D. A. Ray, J. W. Zimmerman, R. Cordaux, and M. A. Batzer. 2007. A SINE-based dichotomous key for primate identification. *Gene* 390(1–2):39–51.
 16. Jolly, C. J. 1993. Species, subspecies and baboon systematics. In: Kimbel, W. and L. Martin (eds), *Species, Species Concepts and Primate Evolution*. New York: Wiley, pp. 67–107.
 17. Karanth, K. P., L. Singh, R. V. Collura, and C. B. Stewart. 2008. Molecular phylogeny and biogeography of langurs and leaf monkeys of South Asia (Primates: Colobinae). *Mol. Phylogenet. Evol.* 46(2):683–694.
 18. Kyes, R. C., L. Jones-Engel, M. K. Chalise, G. Engel, J. Heidrich, R. Grant, S. S. Bajimaya, J. McDonough, D. G. Smith, and B. Ferguson. 2006. Genetic characterization of rhesus macaques (*Macaca mulatta*) in Nepal. *Am. J. Primatol.* 68(5):445–455.
 19. Lernould, J.-M. 1988. Classification and geographical distribution of guenons: a review. In: Gautier-Hion, A., F. Bourlier, and J. P. Gautier (eds), *A Primate Radiation: Evolutionary Biology of African Guenons*. Cambridge: Cambridge University Press, pp. 54–78.
 20. Lorenz, J. G., W. E. Jackson, J. C. Beck, and R. Hanmer. 2005. The problems and promise of DNA barcodes for species diagnosis of primate biomaterials. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 360(1462):1869–1877.
 21. Newman, T. K., C. J. Jolly, and J. Rogers. 2004. Mitochondrial phylogeny and systematics of baboons (*Papio*). *Am. J. Phys. Anthropol.* 124(1):17–27.
 22. Opazo, J. C., D. E. Wildman, T. Prychitko, R. M. Johnson, and M. Goodman. 2006. Phylogenetic relationships and divergence times among New World monkeys (Platyrrhini, Primates). *Mol. Phylogenet. Evol.* 40(1):274–280.
 23. Pilbrow, V. 2006. Population systematics of chimpanzees using molar morphometrics. *J. Hum. Evol.* 51(6):646–662.
 24. Raaum, R. L., K. N. Sterner, C. M. Noviello, C. B. Stewart, and T. R. Disotell. 2005. Catarrhine primate divergence dates estimated from complete mitochondrial genomes: concordance with fossil and nuclear DNA evidence. *J. Hum. Evol.* 48(3):237–257.
 25. Rhesus Macaque Genome Sequencing and Analysis Consortium. 2007. Evolutionary and biomedical insights from the rhesus macaque genome. *Science* 316(5822):222–234.
 26. Ross, C. N., J. A. French, and G. Ortí. 2007. Germ-line chimerism and paternal care in marmosets (*Callithrix kuhlii*). *Proc. Natl. Acad. Sci. U. S. A* 104(15):6278–6282.
 27. Schrago, C. G. 2007. On the time scale of New World primate diversification. *Am. J. Phys. Anthropol.* 132(3):344–354.
 28. Smith, D. G., D. George, S. Kanthaswamy, and J. McDonough. 2006. Identification of country of origin and admixture between Indian and Chinese rhesus macaques. *Int. J. Primatol.* 27(3):881–898.
 29. Smith, D. G., J. W. McDonough, and D. A. George. 2007. Mitochondrial DNA variation within and among regional populations of longtail macaques (*Macaca fascicularis*) in relation to other species of the fascicularis group of macaques. *Am. J. Primatol.* 69(2):182–198.
 30. Sterner, K. N., R. L. Raaum, Y. P. Zhang, C. B. Stewart, and T. R. Disotell. 2006. Mitochondrial data support an odd-nosed colobine clade. *Mol. Phylogenet. Evol.* 40(1):1–7.
 31. Street, S. L., R. C. Kyes, R. Grant, and B. Ferguson. 2007. Single nucleotide polymorphisms (SNPs) are highly conserved in rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques. *BMC Genomics* 8(1):480.
 32. Takacs, Z., J. C. Morales, T. Geissmann, and D. J. Melnick. 2005. A complete species-level phylogeny of the

- Hylobatidae based on mitochondrial ND3-ND4 gene sequences. *Mol. Phylogenet. Evol.* 36(3):456–467.
- 33. Telfer, P. T., S. Souquiere, S. L. Clifford, K. A. Abernethy, M. W. Bruford, T. R. Disotell, K. N. Sterner, P. Roques, P. A. Marx, and E. J. Wickings. 2003. Molecular evidence for deep phylogenetic divergence in *Manodrillus sphinx*. *Mol. Ecol.* 12(7):2019–2024.
 - 34. Tosi, A. J., K. M. Detwiler, and T. R. Disotell. 2005. X-chromosomal window into the evolutionary history of the guenons (Primates: Cercopithecini). *Mol. Phylogenet. Evol.* 36(1):58–66.
 - 35. Tosi, A. J., T. R. Disotell, J. C. Morales, and D. J. Melnick. 2003. Cercopithecine Y-chromosome data provide a test of competing morphological evolutionary hypotheses. *Mol. Phylogenet. Evol.* 27(3):510–521.
 - 36. Tosi, A. J., D. J. Melnick, and T. R. Disotell. 2004. Sex chromosome phylogenetics indicate a single transition to terrestriality in the guenons (tribe Cercopithecini). *J. Hum. Evol.* 46(2):223–237.
 - 37. Tosi, A. J., J. C. Morales, and D. J. Melnick. 2000. Comparison of Y chromosome and mtDNA phylogenies leads to unique inferences of macaque evolutionary history. *Mol. Phylogenet. Evol.* 17(2):133–144.
 - 38. VandeBerg, J. L., S. Williams-Blangero, and S. D. Tardif (eds). 2009. *The Baboon in Biomedical Research*. New York: Springer.
 - 39. Wertheim, J. O. and M. Worobey. 2007. A challenge to the ancient origin of SIVagm based on African green monkey mitochondrial genomes. *PLoS Pathog.* 3(7):e95.
 - 40. Whittaker, D. J., J. C. Morales, and D. J. Melnick. 2007. Resolution of the *Hylobates* phylogeny: congruence of mitochondrial D-loop sequences with molecular, behavioral, and morphological data sets. *Mol. Phylogenet. Evol.* 45(2):620–628.
 - 41. Whittaker, D. J., N. Ting, and D. J. Melnick. 2006. Molecular phylogenetic affinities of the simakobu monkey (*Simias concolor*). *Mol. Phylogenet. Evol.* 39(3):887–892.
 - 42. Wildman, D. E., T. J. Bergman, A. al-Aghbari, K. N. Sterner, T. K. Newman, J. E. Phillips-Conroy, C. J. Jolly, and T. R. Disotell. 2004. Mitochondrial evidence for the origin of hamadryas baboons. *Mol. Phylogenet. Evol.* 32(1):287–296.
 - 43. Xing, J., H. Wang, K. Han, D. A. Ray, C. H. Huang, L. G. Chemnick, C. B. Stewart, T. R. Disotell, O. A. Ryder, and M. A. Batzer. 2005. A mobile element based phylogeny of Old World monkeys. *Mol. Phylogenet. Evol.* 37(3):872–880.
 - 44. Xing, J., H. Wang, Y. Zhang, D. A. Ray, A. J. Tosi, T. R. Disotell, and M. A. Batzer. 2007. A mobile element-based evolutionary history of guenons (tribe Cercopithecini). *BMC Biol.* 5:5.
 - 45. Xing, J., D. J. Witherspoon, D. A. Ray, M. A. Batzer, and L. B. Jorde. 2007. Mobile DNA elements in primate and human evolution. *Am. J. Phys. Anthropol.* 45(Suppl):2–19.

2

Principles of Virology

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2.1. WHAT IS A VIRUS?

Viruses are obligate intracellular parasites that “hijack” cellular machinery for the purpose of their replication. They are transmitted from cell to cell as infectious particles containing a nucleic acid genome enclosed in a protective protein “shell” called a capsid. In some viruses, the shell is surrounded by an envelope (Figure 2.1) which consists of viral and cellular proteins and cellular lipids.

The major feature that distinguishes viruses from other microorganisms is a lack of protein synthesis machinery and metabolism of their own. Viral genomes in the infectious virions are represented by either DNA or

RNA, hence the common designation, RNA and DNA viruses.

2.2. VIRAL GENOMES

The viral genome encodes all the information required for virus replication and transmission from cell to cell. Different types of nucleic acids comprise viral genomes: “ordinary” double-stranded DNA (dsDNA) and single-stranded RNA (ssRNA) as well as “non-ordinary” single-stranded DNA (ssDNA) and double-stranded RNA (dsRNA). The length of viral genomes varies from a few thousand nucleotides (nt) or base pairs (bp) to more than 300 kb (1 kb = 1,000 nt or bp); the number of genes coded by a genome vary from a few to several hundred.

There are two categories of viral genes: structural and nonstructural. The structural genes encode proteins that make up the virion, the structural viral proteins. The nonstructural genes encode viral proteins which are required for replication. These proteins operate only intracellularly and are not incorporated in the virion. In some viral genomes, the genes are arranged sequentially in a “head-to-tail” fashion. However, more commonly, genes overlap to allow for “economical” use of the genome capacity when the same string of nucleotides encodes different proteins, depending on the reading frame. Viral genes can also be “split,” consisting of several exons. Apart from the protein-coding genes, viral genomes have untranslated regions which contain regulatory sequences, commonly called signals or motifs. Viral genome organization is presented graphically in the form of a genome map (Figure 2.2).

An important feature of a viral genome is its polarity. By convention, positive (+) polarity is assigned to the strand of nucleic acid whose sequence is identical to that of the mRNA. Thus, mRNA is actually transcribed from

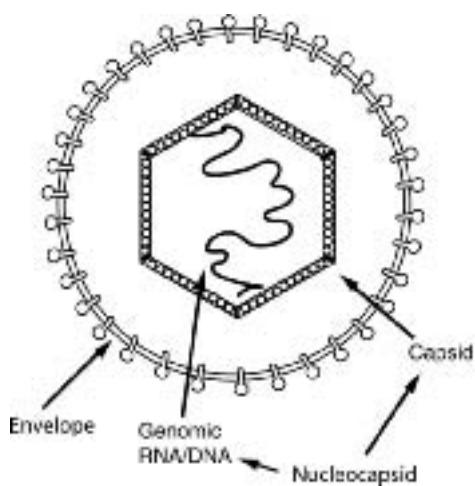


Figure 2.1. Major components of a virion. The **capsid** encloses the viral genome; the capsid, together with the RNA or DNA genome, is called the **nucleocapsid**. Some viruses have an **envelope** that surrounds the nucleocapsid.

the strand which is complementary to the (+) polarity strand, that is negative (−) polarity strand.

The single-stranded viral genomes (mostly RNA) are of positive (+) or negative (−) polarity. The (+) polarity RNA genomes can be translated directly because cellular translation machinery treats them as mRNA. Copying genomic RNA requires RNA-dependent RNA

polymerase. Host cells do not have this enzyme; it is encoded in the genomes of all RNA viruses, except retroviruses. Virions are assembled from newly synthesized viral proteins (translated directly from genomic RNA) and genomic RNAs.

The replication strategy is more complicated in the case of (−) polarity RNA genomes. In order to be translated, (−) polarity RNA genomes have to be converted into (+) polarity; that is a complementary copy of the genomic RNA must be synthesized. RNA-dependent RNA polymerase required for that is absent in the host cells. The viruses with (−) polarity RNA genomes overcome this problem by incorporating the RNA-dependent RNA polymerase in the virions. Thus, the enzyme is delivered into the cell together with the genomic RNA. After uncoating, the RNA-dependent RNA polymerase copies the genomic RNA. The complementary copies [(+) polarity] are produced preferentially. The viral proteins, including the RNA-dependent RNA polymerase, are translated from these (+) polarity RNAs. Then, newly synthesized RNA-dependent RNA polymerase, which is now present in much larger quantity, starts copying; that is newly synthesized (−) polarity genomic RNA is produced. The assembly of virions starts when a sufficient quantity of both viral proteins and genomic RNA has accumulated. The question of how “The First (−) RNA Virion” acquired RNA-dependent RNA polymerase remains unanswered.

Another important feature of viral genomes is their “geometry”. The genomes can be linear or circular. In terms of molecular integrity, the viral genomes can be composed of a single molecule or consist of several non-covalently linked molecules (segments), each containing different genes. Importantly, the segmented genomes are much more prone to recombination than nonsegmented genomes.

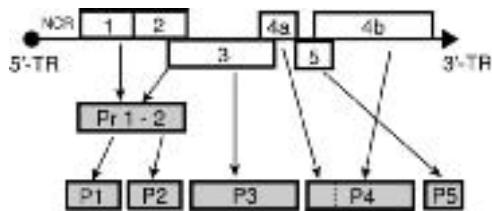


Figure 2.2. Schematic map of a viral genome. Boxes 1, 2, 3, 5 represent viral genes encoding viral proteins P1, P2, P3, and P5; protein P4 is encoded by a “split” gene consisting of two exons (4a and 4b). Only gene 1 is nonoverlapping. Genes 1 and 2 are translated as a precursor polyprotein Pr1-2 that is cleaved into P1 and P2. Precursor proteins in various viruses are cleaved by either viral or cellular proteases.

2.3. VIRAL “ARCHITECTURE”

The size of virions varies from about 10 to 500 nanometers (nm). Among simian viruses, the smallest are parvoviruses (ca. 20 nm) and the largest are poxviruses (ca. 400 nm). Virions can be visualized only under an electron microscope.

The shape of virions is usually ball-like (circular in thin sections) and uniform. However, the size and shape of some viruses can vary significantly (pleomorphism). Unusual shapes, rod-like and elongated spheres, are characteristic of filo- and pox- viruses, respectively.

The main structural element of the virion is the capsid. The “filled” capsid, containing genomic DNA or RNA, is called the nucleocapsid. The building blocks of the capsid are the capsomeres, structural units distinguishable by electron microscopy. The number of capsomeres is characteristic of viruses belonging to the same family. For example, all herpesviruses and adenoviruses have 162 and 252 capsomeres, respectively.

Capsids of many viruses are assembled from the capsomeres in such a way that their shape is icosahedral; that is they have 20 identical triangular faces (Figure 2.3). Such viruses are said to have an icosahedral symmetry.

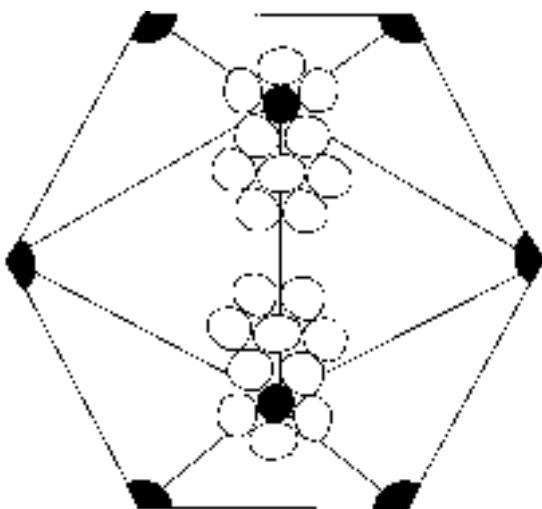


Figure 2.3. Schematic structure of an icosahedral capsid or nucleocapsid. Capsids and nucleocapsids of many viruses have icosahedral symmetry: their structural units (capsomeres) are assembled to form icosahedron (polyhedron with 20 triangular faces and 12 vertices). Note that 12 capsomeres located at the vertices are different from other capsomeres. Usually, but not always, vertex capsomeres are surrounded by 5 capsomeres—hence the name pentamer (black circles). Each nonvertex capsomeres is surrounded by six capsomeres—hence the name hexamer (white circles). Examples of two types of capsomeres arrangement are shown in the figure. In real capsids and nucleocapsids the entire icosahedral shell is composed of the capsomeres.

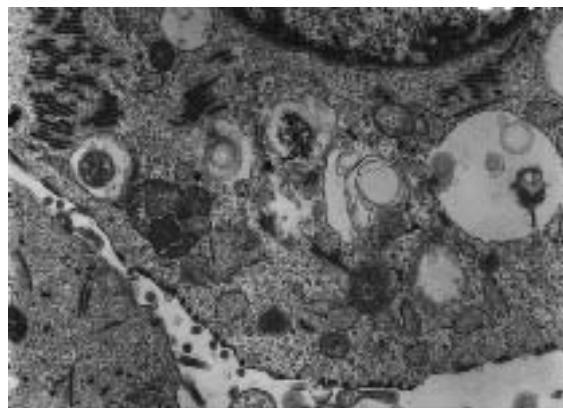


Figure 2.4. Ebola virus detected by EM in thin sections. Etiologic agent of Ebola hemorrhagic fever (filovirus). Filoviruses have unusual filamentous morphology (black arrows) that helps their identification by EM. Round particles with ring-like core in the intracellular space (gray arrows) are cross sections of the filamentous virions. (Image was kindly provided by Prof. Hans R. Gelderblom.)

The alternative type of capsid “architecture” is helical. In this case, the capsomeres are connected in such a way that they comprise a cylindrical structure resembling a condensed spiral. Such viruses are said to have a helical symmetry (Figure 2.5).

The largest and most complex viruses are in the poxvirus family and do not fit into either of the two symmetry groups. They have complex symmetry (Figure 2.5).

The nucleocapsids of some viruses are “naked,” whereas other viruses have an outer cover surrounding the nucleocapsid called the envelope. Accordingly, viruses can be enveloped or nonenveloped. The enveloped viruses are less resistant to the environment, because the envelope is more fragile than the nucleocapsid, mainly due to the presence of lipids.

2.4. FUNDAMENTALS OF VIRUS REPLICATION

The general scheme of replication is common to all viruses. The virus enters the host cell, uncoats its genome, then multiple copies of genomic nucleic acid

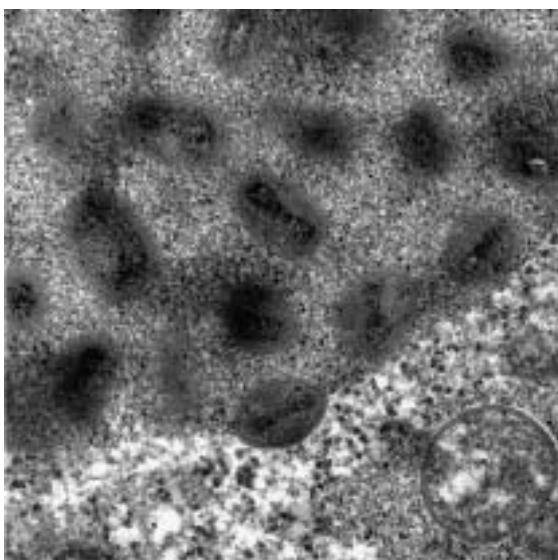


Figure 2.5. Poxvirus detected by EM in thin sections. Characteristically shaped (oval with dumbbell-like core) enveloped virions in a cell form in a skin lesion of a marmoset (*Callithrix jacchus*) naturally infected with cowpox virus (detail in Section 24.3.2). (Image is kindly provided by Dr. Kerstin Mätz-Rensing.)

and viral proteins are produced, and the virions are assembled from these main components (genomic RNA or DNA and structural viral proteins). Finally, newly made virions are released and the conditions for a new cycle of infection are set (Figure 2.6).

Cellular entry starts by physical contact between the virion and a susceptible cell. The major requirement for the binding of a virion to the cell membrane is the presence of a cell surface receptor for the virus. The interaction between the receptor-binding site (RBS) on the virion and the cell is specific for a particular virus, akin to the interaction between antigen and antibody. The RBS is bound by the viral surface protein(s), oriented outwards. Envelope proteins are the attachment sites in the enveloped viruses. The viral receptors are normal components of the cellular membrane. They belong to different classes of macromolecules and have normal physiological functions. The use of these molecules is a typical example of the “hijacking” lifestyle characteristic of viruses. The most striking example is the use of the

CD4 molecule, a key component of the immune system, by human and simian immunodeficiency viruses (HIVs and SIVs) as a receptor.

In some cases, the binding to the receptor is necessary, but insufficient for entry. The second essential step is the binding of a co-receptor, as is the case for HIV and SIV.

The binding to the receptor and in some cases a co-receptor, triggers the process of viral entry. There are several general mechanisms for this process. The most common among them is the fusion of the viral envelope with the cellular membrane resulting in the internalization of the nucleocapsids.

In order to expose viral genomic RNA or DNA to the cellular components necessary for replication, the internalized nucleocapsids must be “stripped.” The uncoating process in many viruses is poorly understood, but the end-result is clear—the appearance of a “naked” genome in the cytoplasm or nucleus. From the beginning of uncoating the parental virion ceases to exist as an infectious entity (except under special artificial conditions: e.g., genomic RNA of (+) polarity RNA viruses is infectious for susceptible cells *in vitro*). The virions also disappear as recognizable particles, hence the term eclipse phase, which describes the part of the viral replication cycle when there are no infectious virions. During the eclipse phase, the building blocks for the viral progeny are produced by the joint efforts of viral enzymes and the cellular protein synthesis machinery including the transcription mechanism consisting of ribosomes, tRNA, energy supply, and posttranslational modification mechanisms. A virus may also depend on cellular components for its genome replication.

The expression (transcription/translation) of the viral genome is divided into two stages: early and late. The early stage precedes the replication of the viral genome. The viral proteins produced during the early stage (early proteins) are those that are required for copying the genomic RNA or DNA. The early proteins, as a rule, are nonstructural proteins.

There are different strategies for the replication of viral genomes. Broadly, they can be divided into two groups. First, the replication is mediated by cellular polymerase. This group includes small DNA viruses, such as parvo-, polyoma-, and papilloma- viruses. Second, the replication is mediated by viral polymerase. This group includes all RNA viruses and large DNA viruses, such as adeno-, herpes-, and pox- viruses. The capacity of a polymerase to copy error-free is termed

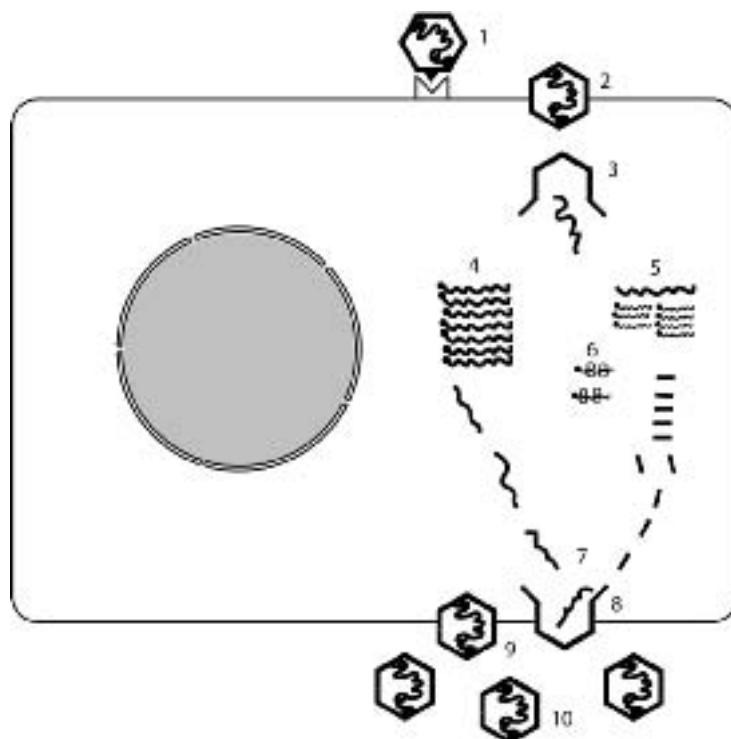


Figure 2.6. Schematic viral replication cycle. Viral replication takes place entirely in the cytoplasm for some viruses. Genomic replication takes place in the nucleus for some viruses with viral assembly in the cytoplasm or the nucleus. The general steps of replication are: 1—attachment to a cell surface receptor; 2—entry in the cell (internalization); 3—uncoating and releasing genomic nucleic acid; 4—replication of viral genome; 5—transcription of mRNAs encoding viral proteins; 6—translation of viral proteins; 7—transport of newly synthesized viral genomes and proteins to the site of assembly; 8—virion assembly; 9—release (egress) of virions; 10—maturation of virions.

fidelity. The fidelity of RNA polymerases is lower than that of DNA polymerases. This accounts for the greater genomic variability in RNA viruses compared to DNA viruses. In extreme cases (RNA viruses such as HIV/SIV or hepatitis C virus), a carrier of the virus is infected with millions of minor variants, called quasispecies, and the composition of the quasispecies changes continuously.

After completion of genome replication, the late genes are transcribed and late proteins translated. As a rule, the late proteins are structural proteins. The nomenclature of viral proteins is dual. On the one hand, they are named according to their function (mostly viral enzymes, e.g.: reverse transcriptase, integrase, protease), or location in the virion (mostly structural proteins, e.g.: capsid

protein, envelope protein). On the other hand, the proteins have a second name, containing information about their biochemical properties including posttranslational modifications and the molecular mass/weight. For example, p24 stands for viral protein with a molecular mass of 24 kDa; gp70—glycoprotein, 70 kDa; pp65—phosphoprotein, 65 kDa.

During the late stage, all required structural proteins are accumulated in the proper proportion and “delivered” to the site of virion assembly. The assembly process is enigmatic; nevertheless, multiple building blocks composing the virion (nucleic acid and various proteins) are assembled in an orderly manner. Most often assembly takes place in the vicinity of cellular membranes.

However, some viruses are assembled in the cytoplasm or in the nucleus.

The release of progeny virus, also called egress, completes the replication cycle. There are two major pathways by which virions are released. Some viruses produce such a huge quantity of progeny in each cell they infect that the cell becomes literally “stuffed” with the “new-born” virions and ruptures. This type of replication is named *cytocidal*. Other viruses can replicate without causing visible damage to the host cell. Such noncytocidal viruses use the cellular secretory machinery or are released through poorly understood “budding” from the plasma membrane.

2.5. CULTIVATION OF VIRUSES IN VITRO

Until the early 1950s, viruses were propagated in animals or in the embryos of chicken eggs. In 1949, J. Enders, T. Weller, and F. Robbins developed the first tissue culture system for propagation of viruses in vitro. This discovery, for which they received the Nobel Prize in 1954, revolutionized virology. There are two types of culture media for growing cells in vitro: those that require the addition of “crude” growth factors (e.g., fetal calf serum) and those whose components are chemically defined. Culture media containing serum, in general, are better for the growth of cells. However, in certain situations, chemically defined media are preferable, for example, when the proteins secreted by the cultured cells are to be studied.

There are three main types of tissue culture used for the isolation and propagation of viruses: primary, diploid, and continuous or permanent. Primary cultures are prepared from organs or tissues usually by “disrupting” tissues into single-cell suspensions or clumps of a few cells with the help of proteolytic enzymes like trypsin. Primary cultures from monkey kidneys were used, and are still used, for preparation of polio vaccine. The characteristics of cells in primary cultures are similar, but not identical, to the characteristics of the same cells before explantation; however, the life span of primary cultures is limited to 5–20 divisions in vitro. Many types of cells will not grow in primary culture. The standardization of primary cell culture is difficult. These cultures can be contaminated with unrecognized viruses, as happened in the early years of polio vaccine production when rhesus monkey kidney cultures were contaminated by the simian virus 40 (SV40).

Diploid cell strains are derivatives of primary cell cultures. As the name implies these cells are diploid; however, contrary to the primary cultured cells, they can divide in vitro approximately 50 times. In order to maintain diploid cell strains, multiple stocks of early-passage cells (earlier than approximately 10 divisions) are cryopreserved. With some simplification, the cells of diploid cultures can be considered “normal.”

Continuous or permanent cell lines can be serially passaged indefinitely. The cells in such cell lines are substantially different from the cells of origin. Usually, they do not possess specialized morphological and biochemical features that are characteristic for these cells *in vivo*. For example, they are less differentiated and aneuploid, that is having multiple chromosomal abnormalities. Thus, the cells of continuous cell lines can be considered as transformed or neoplastic. Permanent cell lines are widely used for isolation and propagation of viruses, but not for vaccine production.

There are two types of cell cultures, monolayer and suspension, depending on the growth characteristics. Cells in a monolayer culture adhere to the solid surface and normally grow until they reach contact with neighboring cells when the cell monolayer covers the surface of the culture flask and becomes confluent. The cessation of cell division after formation of the monolayer is referred to as contact inhibition of growth. There are two major morphological types of monolayer cell cultures—fibroblastoid (i.e., resembling fibroblasts, elongated, “spindle-shaped”) and epithelioid (i.e., resembling epithelial cells, rounded).

Suspension cell cultures can be prepared from various tissues, but mostly originate from hematopoietic cells. A common type of such culture is lymphoblastoid.

When viruses replicate in cell cultures, they may cause visible changes in the host cells called *cytopathic effects* (CPEs), culminating in cell death. The CPE can be observed under an optical microscope in live, unstained cultures (Figure 2.7).

There are many morphological manifestations of the CPE: rounding and detachment of cells, vacuolization of the cytoplasm, formation of multinucleated cells (*syncytia*), nuclear shrinkage (*pyknosis*) and others. The experienced observer can provisionally identify the virus (at the family/genus levels) based on the characteristics of the CPE in a particular cell line. However, the type of CPE is always preliminary to an unambiguous identification using molecular, immunological, or electron microscopy methods.

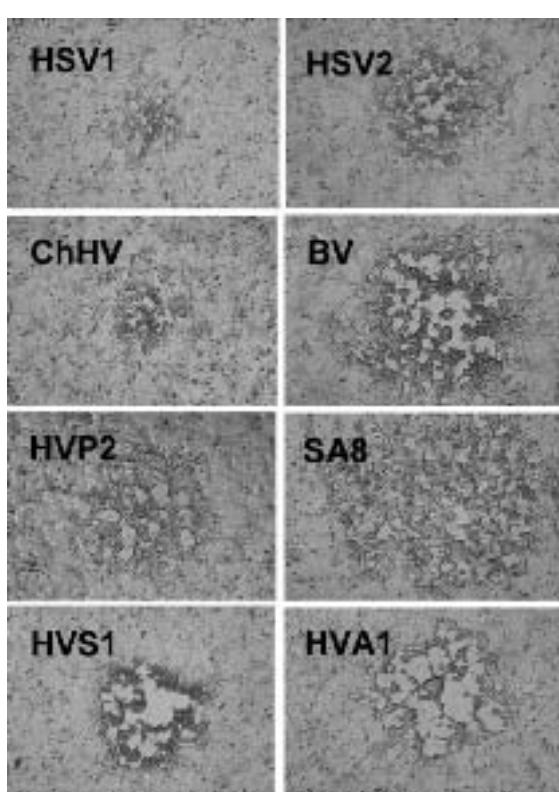


Figure 2.7. Virus-induced cytopathic effects (CPE). CPE induced by different primate simplex viruses in African green monkey Vero cells (24-h postinoculation). HSV1, herpes simplex virus type 1; HSV2, herpes simplex virus type 2; ChHV, chimpanzee herpes virus; BV, B virus; HVP2, herpesvirus papio 2; SA8, simian agent 8, HVS1, herpesvirus saimiri 1; HVA1, herpesvirus atelis 1. (Images are kindly provided by Dr. Richard Eberle.)

2.6. CLASSIFICATIONS OF VIRUSES

Until the early 1960s, classification and naming of viruses was unsystematic. Many viruses carried the name of the diseases they caused, but others were named after discoverers. Numerous competing ad hoc classifications of viruses coexisted and multiple names for the same agent were common.

In 1962, A. Lwoff, R. Horne, and P. Tournier suggested using a classical Linnaean hierarchical scheme for the classification of viruses, based on the properties

of virions. The International Committee on Taxonomy of Viruses (ICTV) was formed and charged with the task of creating and maintaining the classification of viruses. The first edition of the ICTV classification, named the Report, was issued in 1971. Updates are issued approximately every 3 years. The current version of the ICTV classification is the 8th Report (2005).

In the ICTV classification, all viruses are attributed to viral taxa, regardless of host species. The taxa are ranked hierarchically based on characteristics such as the nature of the nucleic acid in the virion, symmetry of the capsid, presence/absence of envelope, and the replication strategy.

The “elementary unit” of classification is the viral species. Species names are italicized. For example, *simian immunodeficiency virus* is a species name. The members of viral species are referred to as “isolates” or “strains.” The isolate/strain names are not italicized. There are no universal criteria for distinguishing the “species” from the “isolate/strain.” The taxonomic rank of a virus is determined by a panel of the ICTV and may be reconsidered with the accumulation of new data.

In addition to the ICTV names, viral species have “vernacular” or trivial names. Usually, these names have a historical basis. Often, the vernacular names are so deeply rooted that their official counterparts are seldom used. For example, the official name of Epstein–Barr virus (EBV) is human herpesvirus 4 (HHV-4), but in practice, the latter name is rarely used.

Viral taxa higher than species in the ascending inclusive order are genus (ending with *-virus*), subfamily (ending with *-virinae*), family (ending with *-viridae*), and order (ending with *-virales*). The definitions of higher viral taxa are also based on the current opinion of the ICTV panel. Taxonomic changes are not uncommon, particularly at the genus and subfamily levels. The list of viral families, subfamilies, and genera, which include the ICTV-recognized simian viral species, is presented in Table 2.1.

There are useful classifications of viruses that are “unofficial”; that is they are not a part of the ICTV classification. Taxonomically different viruses are sometimes grouped according to the system affected (respiratory, enteric, etc.), mode of transmission (blood-borne, water-borne, sexually transmitted), the disease (hepatitis, hemorrhagic fever), the vector (arboviruses), and tissue tropism (lymphotropic, neurotropic). In some situations these groupings are informative and helpful, which is why they remain in use.

Table 2.1. Viral Families, Subfamilies, and Genera which Include ICTV-recognized Simian Viruses

DNA Viruses	RNA Viruses
Family	Family
Subfamily	Subfamily
Genus	Genus
<i>Papillomaviridae</i> (dsDNA)	<i>Arteriviridae</i> (ssRNA+)
<i>Alphapapillomavirus</i>	<i>Arterivirus</i>
<i>Polyomaviridae</i> (dsDNA)	<i>Picornaviridae</i> (ssRNA+)
<i>Polyomavirus</i>	<i>Enterovirus</i> <i>Hepadnavirus</i>
<i>Adenoviridae</i> (dsDNA)	<i>Paramixoviridae</i> (ssRNA-)
<i>Mastadenovirus</i>	<i>Paramyxovirinae</i> <i>Respirovirus</i> <i>Rubulavirus</i>
<i>Herpesviridae</i> (dsDNA)	<i>Retroviridae</i> (ssRNA/RT)
<i>Alphaherpesvirinae</i>	<i>Orthoretrovirinae</i>
<i>Simplexvirus</i>	<i>Betaretrovirus</i>
<i>Varicellovirus</i>	<i>Gammaretrovirus</i>
<i>Betaherpesvirinae</i>	<i>Deltaretrovirus</i>
<i>Cytomegalovirus</i>	<i>Lentivirus</i>
<i>Gammaherpesvirinae</i>	<i>Spumaretrovirinae</i>
<i>Lymphocryptovirus</i>	<i>Spumavirus</i>
<i>Rhinadinovirus</i>	
<i>Poxviridae</i> (dsDNA)	<i>Reoviridae</i> (dsRNA)
<i>Chordopoxvirinae</i>	<i>Rotavirus</i>
<i>Yatapoxvirus</i>	<i>Orthoreovirus</i>
<i>Parvoviridae</i> (ssDNA)	
<i>Parvovirinae</i>	
<i>Erythroivirus</i>	
<i>Hepadnaviridae</i>	
(dsDNA/RT)	
<i>Orthohepadnavirus</i>	

Special cases are classifications based on the phylogenetic analysis of viral genomic sequences. The groupings that are defined by phylogenetic analysis are referred to as subtypes, genotypes, clades. The ICTV is increasingly adopting this approach.

2.7. VIRAL PATHOGENESIS

Many viruses are pathogenic, that is capable of causing disease. The term *viral pathogenesis*, in a narrow, “conventional” sense, describes mechanisms which are directly involved in the development of viral diseases. However, for many viral infections a pathogenic out-

come is not predetermined. Viral infections may remain subclinical for many years, even decades, and the demarcation between the “harmless” and disease stages may be blurred. Reflecting these complex infection–disease relationships, the term *viral pathogenesis* in a broad sense describes the entire spectrum of virus–host interactions and interplay of viral, host, and environmental factors which may potentially result in disease development.

Viral infection can be initiated if three requirements are met: (1) a sufficient quantity of infectious virus is available at a potential site of entry into the host; (2) the cells at the site of entry are accessible and permissive for the virus; and (3) host defenses are not capable of preventing the “seeding” of virus in the host.

The potential sites of virus entry are located in the mucosal lining of respiratory, gastrointestinal, and urogenital tracts; eye conjunctiva and cornea; and the skin. The cells potentially permissive to virus are directly accessible at mucosal and eye surfaces, whereas intact skin, thanks to the outer keratinized layer (the epidermis) cannot be “penetrated” by viruses. The virus-permissive cells within the skin are located in the basal germinal layer and in the underlying dermis. Viruses can gain access to them only if the integrity of the skin is damaged by scratches, wounds, punctures, and so on. The latter can be “natural” (e.g., arthropod bites, and consequences of animal fights) or iatrogenic (e.g., injections or transfusions).

The port of entry for most simian viruses is usually at the respiratory or gastrointestinal tracts. Entry through damaged skin is also common, particularly in NHP species whose “life style” includes aggressive social interactions resulting in skin traumas mainly from bites. The cells permissive for virus at the site of entry are called primary targets. Common primary targets are epithelial cells, mucosal macrophages, and, in the case of viruses transmitted by insect vectors, various cells that make up the peripheral blood mononuclear cells (PBMCs).

A virus that successfully replicates at the site of entry can be contained as a localized infection or it can spread within the host resulting in generalized infection, also called systemic or disseminated. The most common route of dissemination is hematogenic, that is, spread through the blood stream. The presence of virus in blood is called viremia. Viruses may also spread through the lymphatic and nervous systems, lymphatogenic and neural spread, respectively. The neural spreading is typical for some herpesviruses

(simplex- and varicella-viruses). The cells harboring viruses during its dissemination are sometimes called secondary targets, although the distinction between the primary and secondary targets is not absolute.

The spread of virus within the host culminates in its “settling” in target organs or tissues. Such preferences are described by the term *viral tropism*. The tropism to certain types of cells, tissues, and organs is rarely exclusive. However, the preferences of many viruses are quite characteristic and this is reflected in commonly used terms hepatotropic, neurotropic, B-lymphotropic, T-lymphotropic viruses, and so on. Multiple viral and host factors underlie the tropism. The cellular distribution of receptors (and co-receptors) used by a virus to enter the cell is one of the most important determinants. Experimental expression of a viral receptor in receptor-negative cells frequently confers susceptibility to this virus. Alternatively, blocking the receptor inhibits replication of the virus. However, the tissue and organ distribution of viral receptors per se are not sufficient to explain viral tropism. It is not uncommon for viruses to exhibit tropism for a certain cell type whereas the receptor to this virus is expressed on a much wider range of cells. Thus, viral-intracellular interactions located “downstream” to the receptor/co-receptor binding are also important for determining viral tropism and outcome of infection. Blocking of virus replication in receptor-positive resistant cells may be mediated by intrinsic cellular resistance mechanisms, such as interferons, TRIM and APOBEC proteins, and others.

Replication of some viruses requires factors which are present only in highly differentiated cells. For example, papillomaviruses can complete their replication cycle only in squamous epithelial cells, the uppermost layer of skin epithelium. Such differentiation-dependent viruses are usually “fastidious”; that is, they cannot be propagated *in vitro* in permanent cell lines.

If the invading virus is not “repelled” at the initial site of infection, several outcomes are possible (Figure 2.8). The simplest pattern is acute infection. In this case, the duration of infection is relatively short (days to weeks); the virus is either cleared by an immune response or replicates uncontrollably until it kills the host.

The alternative to acute infection is chronic infection that can be persistent and latent. Persistent chronic infection is characterized by the continuous production of infectious virus and a steady quantity of virus (viral load) in a host compartment that preferentially harbors the virus. During latent infections no infectious virus is produced. The virus stays “dormant” in the latently infected cells which harbor the complete viral genome, most of which is not expressed. A latent virus may be reactivated at any time; that is, the production of infectious virus is resumed.

As a rule, latent and persistent viral infections remain subclinical for years, even decades. Clinically healthy virus-positive hosts are called virus carriers, inapparent carriers, or simply carriers. Importantly, carriers are the main source of new host-to-host transmissions.

Pathogenicity is the capacity to cause a disease. The related term *virulence* is usually used when the pathogenicity is quantifiable, that is, when there is a clear relationship between quantity of the virus and severity of the disease. The term *pathogenicity* may also be used in a quantitative context, for example, high pathogenicity versus low pathogenicity. However, the quantitative component is greater in the term virulence.

There are many ways of measuring virulence. They are based on the assumption of a direct relationship between the infecting dose and different parameters reflecting the severity of the disease, such as death, length of incubation period, appearance of certain clinical manifestations, and extent of tissue/organ damage. The most commonly used virulence index is the 50% lethal dose or LD_{50} , the dose of virus causing death in 50% of

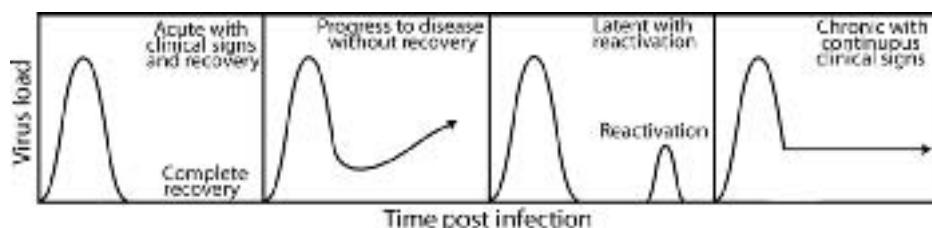


Figure 2.8. Outcomes of viral infection.

inoculated animals. Importantly, the LD₅₀ for the virus may vary significantly depending on the route of infection, age, sex, and genetic make-up of the inoculated animals. Therefore, a “side-by-side” comparison of virulence must be conducted in controlled experiments and is meaningful only if closely related viruses are compared, for example, different strains of the same virus.

Viruses which do not show any pathogenicity are called apathogenic, nonpathogenic, or avirulent. The term *attenuated* virus is reserved for strains that have lost virulence through experimental manipulations. All live viral vaccine strains are attenuated.

Viral infection develops into disease if and when the equilibrium between virus and host cannot be maintained. This happens when cell death as a result of viral infection exceeds the limit of “adaptability” of corresponding organs or systems and their functions become compromised.

Two major processes contribute to virus-dependent cell death *in vivo*: directly induced cell death and cell death due to the host’s antiviral responses. The replication of many viruses by itself is destructive for host cells. On the other hand, cells infected by viruses express viral and virus-induced antigens which are recognized as foreign by the host immune system. The immune system uses all its “weaponry” for killing and elimination of these cells and sometimes causes “collateral damage” to the infected organs and tissues.

An important aspect of viral pathogenesis is the struggle between the virus and host immune system. Viruses have developed multiple mechanisms of “immune evasion,” that is, “diverting” or suppressing antiviral immune responses. Virus-induced immunosuppression is, perhaps, the most powerful of weaponries in this regard. An extreme example of the destructive power of viruses on the immune system is simian and humans AIDS. In less dramatic forms, immunosuppressive activity is a characteristic of other viruses, for example, the measles virus. Immunosuppression caused by nonviral factors (malnutrition, stress, aging, bacterial, and parasitic infections) also plays important roles in the pathogenesis of viral diseases, particularly those caused by latent and persistent viruses.

2.8. VIRAL DIAGNOSTICS

Detection of a virus in tissues or body fluids is diagnostic for viral infection. Diagnosing viral disease requires integration of virological and clinical data.

Viral diagnostic methods can be divided into two groups: those which target a virus or its components and those which target virus-specific host responses. The first group includes virus isolation, detection of viral antigens or nucleic acids, and electron microscopy. The second group includes methods detecting humoral and cellular antiviral responses, although the latter is used mostly in research settings.

Viral diagnostic tests are based on different methodologies, for example, immunofluorescence, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR); they may be screening or confirmatory; their results can be qualitative or quantitative.

Screening methods are the most suitable for testing large numbers of samples. However, these tests usually have lower specificity, as compared with the confirmatory tests. In other words, screening tests tend to produce false-positives. Confirmatory tests are least prone to false-positive results; however, they are more expensive than the screening tests and cannot be used for rapid testing of large numbers of samples. A common viral diagnostic algorithm includes consecutive use of screening and confirmatory tests. The screening test is used first, then only positive samples are tested by the confirmatory test. The distinction between screening and confirmatory tests is not absolute. The diagnostic specificity of some screening tests has improved markedly and high-throughput confirmatory tests are being developed. However, the distinction between screening and confirmatory tests remains informative and useful. A typical example of a screening test is ELISA for antiviral antibodies, whereas commonly used confirmatory tests are western blot (WB) assay for antiviral antibodies, or PCR for viral nucleic acids.

Viral diagnostic tests can be qualitative or quantitative. The results of a qualitative test are expressed as positive or negative. The results of a quantitative test are numerical values. By setting cut-off values, any quantitative test may be used in the qualitative mode. Quantitative tests in general are more informative than qualitative tests. However, the latter are usually cheaper, simpler, and tend to have higher sensitivity. The major drawback of the qualitative methods is subjectivity in discriminating positives from negatives. For those tests in which results are scored by an observer, the subjectivity is obvious. Discrimination between positives and negatives in quantitative tests is also subjective because it is based on arbitrarily chosen cut-off values. Ideal discrimination of results into positive and negative is

rarely observed in real life. Usually some results cannot be scored unequivocally. Such “gray-zone” results are called indeterminate.

An important characteristic of viral infection and disease is *viral load*, an estimate of the quantity of virus in body fluids or tissues. Viral load is usually approximated by measuring the quantity of viral genomic RNA or DNA in peripheral blood.

Nonhuman primates harbor many viruses which have counterparts in humans. The degree of relatedness between some simian and human viruses is sufficiently high to permit the use of reagents for the detection of human viruses in testing for their simian counterparts. However, even in such cases the reagents based on simian viruses are preferable. Unfortunately, as a rule such reagents are not available commercially. Some simian viruses, however, cannot be detected by the reagents designed for human diagnostics. In such cases, there is no alternative to the development of in-house tests for simian viruses.

Proper controls are essential for any viral diagnostic test. In addition to obvious positive and negative controls, each viral diagnostic test requires “customized” controls or procedures aimed at reducing bias that is, to some extent, “built in” to every method. For example, the results of subjective tests, such as immunofluorescence, are more reliable if they are scored blindly by several observers. External quality control is of paramount importance in ensuring reliability of test results and standardization of tests used by different laboratories for detection of the same agents. The principle of external quality control is simple: a panel of well-characterized reference samples is tested blindly (samples are coded) in each participating laboratory; the concordance of each laboratory’s results with the reference data is determined; each participating laboratory receives a feedback report showing the results of its performance compared to the reference (“gold standard”) and other participating laboratories. Laboratory identifiers are usually kept anonymous. Despite conceptual simplicity, external quality control programs are quite complicated logistically and they require substantial funding. Although such programs are used in simian viral diagnostics they clearly require more attention.

An important part of viral diagnostics is specimen collection. The most common specimen for detection of simian viruses is blood and its derivatives such as serum, plasma PBMCs, peripheral blood lymphocytes

(PBLs). Saliva and urine are also used, although much less frequently. Tissue samples are used more commonly than in human diagnostics. Usually, tissue samples are obtained from euthanized experimental animals. However, biopsies are performed frequently. Specimen collection from wild animals is particularly challenging. In such cases, so-called *noninvasive sampling* may be the only alternative. Methods for extraction of nucleic acids and immunoglobulins from simian feces and urine are now available.

2.8.1. Electron Microscopy

Electron microscopy (EM), more precisely transmission EM (TEM), is used for visualization of viral particles. Two methodologies, ultra-thin sections and negative-staining, are most commonly used.

The ultra-thin sections are made from fixed cells or tissues embedded in a special polymer. The sections are “stained” with the salts of heavy metals that bind to the proteins and make them impenetrable to electrons, that is, forming “electron-dense” material. Virions and precursor viral particles (empty capsids, nucleocapsids) can be visualized inside cells. Viruses can be seen in the cytoplasm or nucleus, at the cell membranes (budding virions), and in the intercellular spaces (immature and mature virions) depending on the virus type. The morphology of virus particles on thin section depends on the orientation of a particle and the section plane. EM of ultra-thin sections is a time-consuming procedure. It also has low sensitivity in detecting viruses and therefore is rarely used for diagnostic purposes. However, EM may provide a lead when a material presumably containing an unknown viral agent is studied (Figures 2.4 and 2.5).

The negative-staining technique can be used when virions are suspended in a liquid. The “stain,” consisting of the salts of heavy metals, fills the “empty” spaces outside and inside viral particle. Obviously, the stain can penetrate inside the virion only if there are “holes” in the viral envelope and capsid shell. The appearance of negatively stained virus particles is opposite to what is observed in thin sections; that is, structural elements are “electron-lucent” whereas empty spaces are “electron-dense.” Negative-staining allows generic identification of many viruses. However, individual viral species as a rule cannot be distinguished. Negative-staining is technically simpler than thin section EM and the sample preparation is rapid. This technique is sometimes called a “catch-all” method. Indeed, if several different viruses

are present, they can be recognized by an experienced observer. However, the detection threshold for negative-staining EM is relatively low. The major field for the diagnostic use of negative-staining EM is viral gastroenteritis (Figure 2.9).

Negative-staining can be used in combination with specific antibodies, termed immune electron microscopy. In this case, a virus to be negatively stained is preincubated with antibodies specific for the envelope antigens. If the antigen is present, the antibodies cross-link virus particles and characteristic aggregates are detected in the negatively stained preparations. This technique is used mainly for the identification of gastroenteritis viruses. The other version of immune electron microscopy allows visualization of viral antigens in the thin sections using antibodies tagged with the electron dense, characteristically shaped labels, such as ferritin and colloidal gold. This method is techni-

cally very demanding and it is used only in research settings.

2.8.2. Virus Isolation

Virus isolation is the cornerstone of virology. Before the 1950s, this term implied that the disease could be transmitted to a susceptible host by cell-free filtrates and then transferred by serial passage. Inoculation of experimental animals with a presumptive virus-containing material remains important for the isolation of unknown viruses. However, after introduction of tissue culture the term *virus isolation* is used almost exclusively for virus growth in vitro in tissue culture, either established directly from an infected host, or by inoculation with material presumed to contain virus.

The most common method of virus isolation in vitro is the inoculation of permanent cell lines with

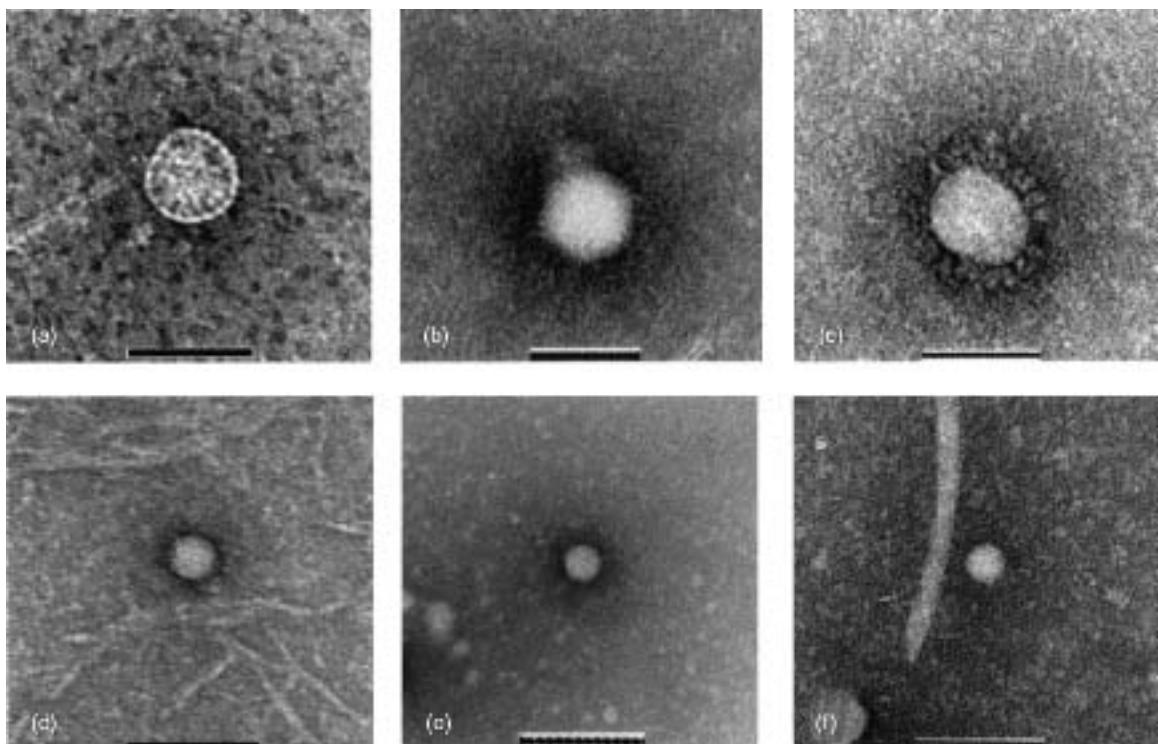


Figure 2.9. Negatively stained virions of different enteric viruses. Various viruses detected by negative staining EM in fecal specimens of monkeys with diarrhea: (a) rotavirus, (b) adenovirus, (c) coronavirus, (d) norovirus, (e) enterovirus, (f) picobirnavirus. Bar = 100 nm. (From Wang *et al.*, *J. Med. Primatol.* 36, 2, 102–107, 2007; with permission.)

virus-containing materials. The primary cell cultures are also used for virus isolation. Many viruses cause CPE in the infected cells (Figure 2.7). The kinetics and appearance of CPE are quite characteristic for some viruses, and “old school” virologists, almost an extinct breed, can identify many viruses by just observing CPE. The lack of such skill among “modern school” virologists is compensated for by the availability of multiple tools for the detection and identification of viruses in tissue culture, using viral antigens or nucleic acids as the targets. These tools are particularly important for detecting viruses which replicate *in vitro* without causing any visible cellular damage.

In vitro host range, that is, susceptibility of different cell lines to a virus, is highly variable. Tissue cultures that support replication of a particular virus are called permissive. Availability of permissive cell lines greatly facilitates characterization and quantification of viruses.

Virus isolation is not used for diagnosis of infection with “fastidious” viruses, for example, papillomaviruses or hepaciviruses. These viruses do not replicate in conventional cell cultures.

Use of virus isolation as a diagnostic tool is declining because modern methods allow direct detection of viruses in the uncultured specimens. These methods are also more rapid and less laborious than classical virus isolation. However, virus isolation is indispensable when biological properties of a virus are investigated, for instance, virus neutralization, and resistance to antiviral drugs.

2.8.3. Antigen Detection

The key reagent in any antigen detection test is antibodies specific to the antigen of interest. Binding of the antibodies to the antigen by itself is not “visible.” It is revealed through a label. When the label is attached to the specific antibody the test is called direct. If diagnostic antibodies are not labeled, a secondary labeled reagent is required. This reagent should bind specifically to the diagnostic antibodies without interfering with their antigen-binding function and be conjugated to the label—hence the name *conjugate*. The most commonly used conjugates are antibodies against immunoglobulins of the species from which the specific antibodies are obtained or protein A, the bacterial protein which binds to mammalian immunoglobulin G (IgG) with high affinity. There are many types of labels; the most common are fluorescent or enzymatic tags. All

labels are recognizable either by the naked eye or by using proper instrumentation.

Antigen detection methods can be divided into two categories: *in situ* methods when the antigens are “visualized” directly in the cells/tissues and the methods when the antigens are determined “in solution,” that is; in the body fluids, cell/tissue extracts, and so on.

Depending on the type of signal, either color staining or fluorescent staining, the *in situ* methods are termed immunocytochemistry and immunofluorescence, respectively.

In situ antigen detection methods provide opportunities to determine intracellular localization of viral antigens (nuclear, cytoplasmic, diffuse, granular, etc.), to localize virus positive cells in tissues, and to identify the type of cells which harbor the virus (Figure 2.10). The main disadvantage of *in situ* methods is their subjectivity, although this can be corrected by the use of computer-assisted image analysis.

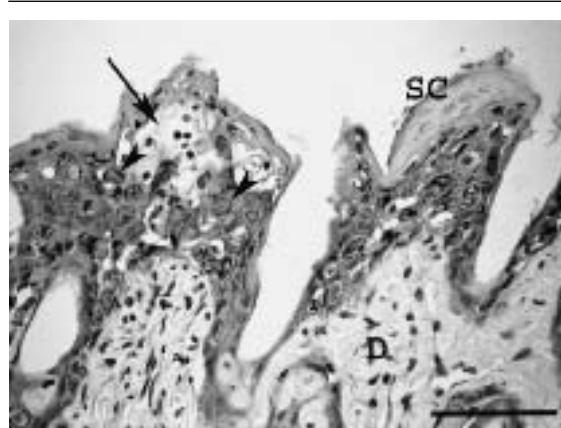


Figure 2.10. *In situ* detection of viral antigens by immunohistochemistry (IHC). Skin of a mouse inoculated with baboon simplexvirus (*Herpesvirus papio* 2). Epithelial cells beneath the stratum corneum (SC) are positive for viral antigens (red-brown staining). Positive cells exhibit degeneration, necrosis, and margination of chromatin (arrowheads). Virus-induced damage results in vesicle formation (arrow) and mild infiltrates of inflammatory cells in the dermis (D). Bar = 75 µm. (Image is kindly provided by Dr. Jerry W. Ritchey. See color version page 9.)

When viral antigens are detected in solution, the information about the cells which harbor them is lost. This is the major disadvantage, as compared to the *in situ* methods. On the other hand, antigen detection in solution has a number of advantages: soluble antigens can be quantified easily, they can be detected in very low concentration (pg/mL), and the detection of soluble antigens is more amenable to automation.

There are many formats for detecting soluble viral antigens. The most common is a version of the ELISA, termed “antigen-capture” ELISA. Two types of specific antibodies are used in such an ELISA, each against the nonoverlapping epitopes of the antigen to be detected. The first-capture antibodies are linked to a solid phase. The second detector antibodies are labeled. Antigen-capture methods are highly specific and quantitative. However, even the most sensitive antigen detection methods cannot match the sensitivity of methods that target viral nucleic acids.

2.8.4. Viral Nucleic Acid Detection

Diagnostic tests for detection of viral genomic DNA or RNA are based on the specific binding of two complementary strands, which is called molecular hybridization or annealing. These tests are commonly referred to as “molecular,” although this name is a misnomer. Antigen/antibody detection tests strictly speaking are also molecular because they, too, are based on the specific interaction of protein molecules. However, traditionally the attribute “molecular” is used for nucleic acid-based tests.

There are two major groups of molecular viral diagnostic tests: either with or without target nucleic acid amplification. The tests employing target amplification are most commonly used. Among a number of inventive nucleic acid amplification methods, the most commonly used, undoubtedly, is the PCR. The detailed description of the PCR principle is beyond the scope of this book. Simplistically, a fragment of viral genome, usually a few hundred bp, is copied by a special thermostable DNA polymerase. The fragment is defined by two primers, the oligonucleotides complementary to the sense and anti-sense strands of target DNA. PCR amplification produces more than a million identical copies of the target, named *amplimers*. There are many ways to detect amplimers. The simplest, historically the first, and still commonly used, is agarose gel electrophoresis coupled with the staining of DNA with double-strand specific

dyes (such as ethidium bromide). A positive result is the presence of amplimers seen as bands in the gel of the expected molecular size (Figure 2.11).

However, the specificity of PCR, based only on the size of the amplimer may not be reliable. Preferably, the

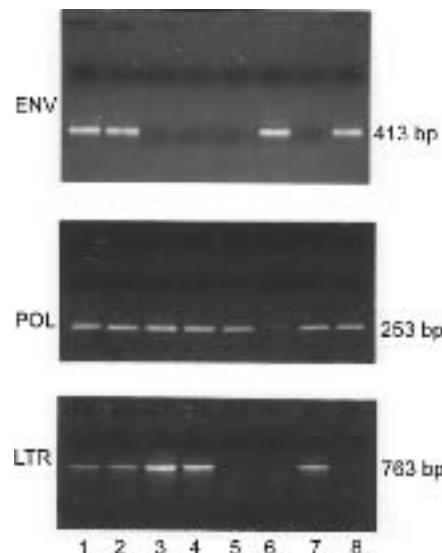


Figure 2.11. PCR amplified fragments detected by agarose gel electrophoresis. PCR amplification of three different regions of simian T-lymphotropic virus type 1 (STLV-1) genome in baboon peripheral blood lymphocytes DNA. Amplimers are detected by agarose gel electrophoresis and staining with the ethidium bromide. 1 to 8: DNA samples extracted from peripheral blood lymphocytes of 8 wild yellow baboons from Mikumi National Park, Tanzania; 413 bp, 253 bp, and 763 bp are the sizes of amplimers for ENV, POL, and LTR PCR tests, respectively. Of note, the sensitivity of PCR amplification of different fragments in the genome of the same virus may differ. POL-PCR clearly detects 7 of 8, possibly even all 8 samples (faint band in sample 6). ENV-PCR was the least sensitive detecting STLV-1 in 4 of 8 samples. However, such sensitivity ranking of PCR tests may be misleading at the individual sample level—sample 6 is the most clearly detected by the “least sensitive” PCR.

specificity of amplimer should be confirmed either by molecular hybridization with a probe complementary to the target sequence, or by direct DNA sequencing of the amplimer. Sequencing is most commonly used now due to low cost and widespread availability of commercial sequencing services.

Among numerous versions of the PCR method, the most commonly used in viral diagnostics are nested and real-time PCR. Nested PCR employs two sets of primers, external and internal, which are used in two consecutive rounds of amplification. The advantage of nested PCR is its extremely high specificity approaching a single copy of the target per sample. However, such sensitivity is a double-edged sword. False-positive results in nested PCR due to “carry-over” contamination with amplimers from previous amplifications are not uncommon. In real-time PCR, the processes of amplification and detection are coupled in such a way that the accumulation of amplimers can be followed and quantified in real time. There are many formats of real-time PCR. The simplest is detection of double-stranded amplimers with dsDNA-specific dyes, like SYBR Green. More complex formats require use of labeled probes; some of them, like energy transfer probes, are quite sophisticated. Real-time PCR which includes detection of amplimers with probes is more specific than tests relying only on dsDNA-specific dyes. Without going into methodological details, it is worth mentioning that real-time PCR is more rapid than classical PCR and that instrumentation for high-throughput real-time PCR testing is available. Real-time PCR is commonly used for determination of viral load.

PCR allows amplification of DNA targets. In order to amplify RNA it first must be converted into DNA and then amplified by PCR. This RNA→DNA conversion is achieved by using reverse transcriptase (RT); hence, the name RT-PCR for PCR tests adapted for the amplification of RNA targets.

Conceptually, molecular tests without target amplification are simpler than tests employing target amplification. In this case, the specific probe is single-stranded nucleic acids complementary to the target. Importantly, the most commonly used oligonucleotide probes can be easily designed (assuming that the target sequence is known) and synthesized. Visualization of probe binding to the target is achieved through the labels attached to the probe. The specific binding of the probe to the target is “converted” into a signal which is detected visually or by an instrument. Some methods for the

detection of viral nucleic acids using probes are conceptually straightforward—one-labeled probe binds to the target RNA or DNA and emits the signal. Examples of such methods are different types of “blot” assays (dot-blot, line-blot, Southern blot) and *in situ* molecular hybridization. More sophisticated methods, such as the branched DNA assay, employ signal amplification instead of target amplification (Figure 2.12). The key reagent for the branched DNA assay is a complex probe that includes a segment which is complementary to the target, and a “branched” segment consisting of multiple copies of the same artificial sequence. When this probe hybridizes to the target through its specific moiety, each target molecule becomes covered by a “bush” consisting of identical “branches.” The secondary-labeled probe is complementary to these branches. Thus, the number of secondary probes attached is much larger than the number of targets. Each secondary probe will emit a signal. Thus, figuratively speaking, the signal is amplified. The sensitivity of the branched DNA assay is lower than that of quantitative PCR. However, due to the constant numerical relationship between the number of targets and the number of labeled probes linked through the branches to each target, the precision of quantification in the branched DNA assay is very good, better than in other methods of viral load determination.

2.8.5. Nucleic Acid Sequencing and Sequence Analysis

In the 1970s–1980s, DNA sequencing was available only in a few “elite” laboratories. The situation changed dramatically in the mid-1990s when automatic DNA sequencing became routine. Since then the amount of viral genome sequence information has been growing exponentially. A number of complete genome sequences of simian viruses are now available. Unfortunately, for many simian viruses even fragmentary genomic sequence data are absent.

Genomic sequences per se are just strings of A, T, C, and G nucleotides, of greater or lesser length. In order to “extract” meaningful information from the sequences they have to be subjected to an analysis typically aiming at:

1. Identification of sequences encoding proteins, termed open reading frames (ORFs), and prediction of amino acid sequences of viral proteins.
2. Identification of various regulatory sequences, commonly called “motifs” or “signals.”

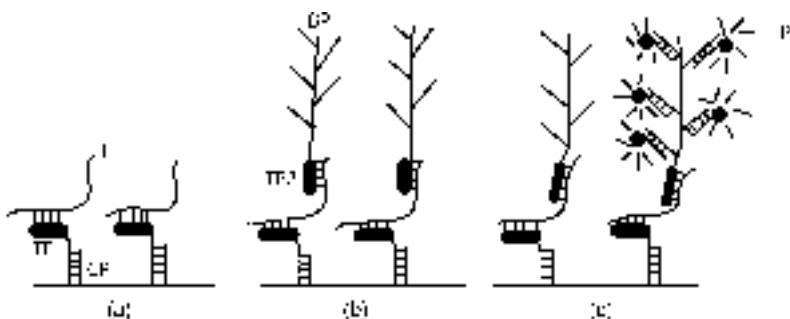


Figure 2.12. Scheme of branched DNA assay. (a) CP, capture probe; TP, target probe; T, target viral nucleic acid. TP consists of two parts that are complementary to CP and T, respectively. CP is linked to a solid phase. The first part of TP anneals to CP, whereas the second part anneals to T. As a result, viral nucleic acid (T) becomes bound to a solid phase and at the same time remains accessible to another target-specific probe. (b) Complex probe consisting of the target-specific part (TP2) and multiple branches (BP) of the same artificial sequence binds to the viral nucleic acid (T). As a result, each viral nucleic acid molecule bound to the solid phase becomes “covered” by extending BP branches. (c) Labeled probes complementary to branches of BP anneal to the branches. Signal emitted by the label is proportional to the number of target molecules.

3. Comparison of different sequences for their similarity, homology, and presence of insertions and deletions (indels).
4. Identification of mutational “hot spots” and highly conserved regions.
5. Identification of viruses, strains, isolates, and mutants (e.g., drug-resistant mutants).

Many computer algorithms have been invented for sequence analysis. Even greater is the diversity of software tools implementing these algorithms in different combinations. Most sequence analysis programs are now freely available on the internet. Perhaps the most commonly used among these programs is BLAST (www.ncbi.nlm.nih.gov/blast). BLAST allows comparison of the sequence of interest with all sequences deposited in the major sequence databases, such as GenBank, EMBL, and others, and delivers the results within seconds.

One of the most common tasks performed on viral genomic sequences is phylogeny inference or, simplistically, “building” viral phylogenetic trees. Ideally, a phylogenetic tree inferred from the genomic sequences of different viruses should accurately reflect their evolution. However, phylogenetic inferences are highly dependent on the values of numerous parameters which rarely, if ever, can be reliably estimated. As a result,

some conclusions derived from the phylogenetic analysis of sequences, for instance, divergence time estimates, are inherently inaccurate. At the same time, phylogenetic analysis of sequences is the best way to establish the degree of relatedness between viruses, and the results of phylogenetic analysis are increasingly used for the classification of viruses.

The number of potentially possible trees, even for a relatively small set of sequences, is astronomically high; it rapidly becomes unmanageable as the number of sequences included in the analysis increases. That is why phylogenetic analysis programs use heuristic searches for the best trees in the “tree space,” a kind of “virtual forest” consisting of phylogenetic trees. The algorithms used in these programs can generally be divided into three categories: neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML). NJ-phylogenetic analysis is the least robust. At the same time, it is computationally less demanding and as a result NJ-analysis is relatively fast and can be performed on the larger data sets. ML-phylogenetic analysis is, theoretically, the most accurate. However, it is also the most demanding computationally and limits the size of data sets that can be analyzed within a reasonable time frame. Availability of faster desktop and laptop computers has greatly increased the capacity to perform these analyses.

A very important component of phylogenetic analysis, regardless of the tree-building algorithm, is statistical evaluation of the reliability of tree nodes, that is, the points where the trees form branches. The statistical method most commonly used for this purpose is called “bootstrapping” (BS). Simplistically, the end-result of bootstrapping is a BS-value for each node of the “consensus tree,” which integrates in one tree the results of many repetitive computations. The higher the BS-value, the more reliable is the node. Importantly, the significance threshold for the BS-value is arbitrary. At the same time, it is generally accepted that nodes with BS-values 90% and higher are reliable. Nodes with bootstrap values in the 80–90% range may be considered as significant, whereas nodes with BS-values less than 80% are usually qualified as unreliable. Phylogenetic trees can be rooted or unrooted (Figure 2.13). Rooted trees are built on the assumption that they “grew” from the most ancient node, the root. A time parameter is openly or silently built into the rooted tree. In order to construct a rooted tree, a related sequence outside the group being analyzed (and therefore known to have diverged first), termed the outgroup, must be known or appointed based

on common sense or other considerations. An unrooted tree reveals a mutual relatedness of sequences under analysis without stepping on the shaky ground of making assumptions regarding the chronology of tree nodes. Most of the phylogenetic trees which can be found in the virological literature are unrooted trees.

2.8.6. Detection of Antibodies Against Viruses (Serology)

The presence of antibodies against viral antigens in serum is the marker that is most commonly used in viral diagnostics—hence, the terms *serology*, *serological tests*, *serodiagnosis*, *seroprevalence*, and so forth. Antibody positivity (seropositivity) may indicate either ongoing virus infection or prior resolved infection with this virus. In addition to serum and plasma, antibodies can also be determined in saliva, urine, cerebrospinal fluid (CSF), vaginal and rectal swabs. The immunoglobulin class (IgG, IgM, and IgA) to which the antibodies belong may be diagnostically informative. Antiviral IgM antibodies are typically present during primary infection and decline thereafter. Antibodies in the mucosal secretions are IgA and IgG, with IgG predominating. The

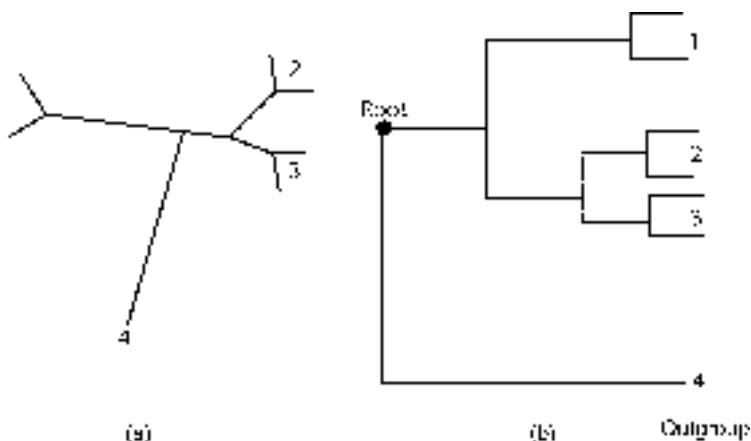


Figure 2.13. Rooted and unrooted phylogenetic trees. (a) Unrooted tree. No assumption is made regarding which of the species (sequences) subjected to phylogenetic analysis diverged first. The length of the branches between any two species is proportional to the genetic distance. (b) Rooted tree (based on the analysis of the same species). It is assumed that the tree “grew” from the most ancient node, the root. The species which diverged first (in this example 4), named outgroup, must be known or appointed based on common sense or other considerations. The horizontal distance between any two viruses is proportional to the genetic distance. Vertical distance is for clarity only. Clades 1, 2, and 3 are identified in both phylogenetic analyses. Clades 2 and 3 are the most closely related; species (sequence) 4 is the most distant.

most commonly measured are IgG antibodies which are maintained at detectable levels in serum for years, sometimes decades.

The key factor determining the specificity of an antibody test is the antigen, which must be well defined. Ideally the antigen should react only with the antibodies being determined. This is hard to achieve even with highly purified viral antigens because some antigenic determinants (also named epitopes) may be cross-reactive. An obvious disadvantage of serological diagnosis is its reliance on the strength and kinetics of the immune response. Detectable antibodies appear in weeks, sometimes months, after virus entry or may not appear at all. Thus, negative results of serological assays, particularly in immunocompromised hosts, should be interpreted with caution and verified using confirmatory tests based on the direct detection of the virus. There are a number of methodologies used for the detection of antiviral antibodies. The most widely used are covered below.

2.8.6.1. NEUTRALIZATION TEST

The neutralization test is a classical serological assay which not only detects antiviral antibodies, but also reveals their potential significance for controlling viral infection. For this reason, the neutralization assay remains an important test, despite being quite cumbersome. A live virus serves as the antigen in the neutralization test. The virus is incubated with serum or another source of antibodies and then tested for residual infectivity. If infectivity is diminished, the neutralization test is positive.

Various virus-induced effects can be used as indicators of neutralization. The classical version of the neutralization test is based on CPE as a marker of virus replication. Virus neutralization can also be measured by a number of other indicators, such as the presence of viral antigens or nucleic acids in the infected cells or culture medium and presence of enzymatic activity induced by virus. The neutralizing antibodies are directed against surface viral proteins. The neutralization test typically detects type-specific antibodies because, as a rule, the virus envelope proteins are more variable than the inner viral proteins. However, in some cases, neutralizing antibodies can cross-react with related, but different, viruses. Neutralizing antibodies are an important component of protective immunity and in some viral infections (for instance, poliovirus) their presence is a reliable indicator that the host is protected. However, the presence of neutralizing antibodies per se cannot always be equated with protective immunity.

2.8.6.2. ENZYME-LINKED IMMUNOSORBENT ASSAY

ELISA is the most commonly used method for detection of antibodies against viruses. There are many ELISA formats, but two features are common to all of them: (1) a solid phase “coated” with the antigen; (2) an enzymatic label emitting signal proportional to the amount of antibody being measured. The simplest, most versatile, and most commonly used ELISA format for the detection of antibodies is called the indirect antibody ELISA (Figure 2.14). This test includes three steps (washing

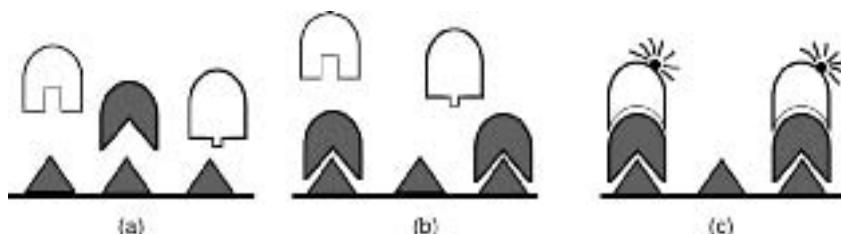


Figure 2.14. Scheme of indirect ELISA for detection of antibodies. (a) Antigen (gray triangles) is bound to a solid phase; test serum containing antibodies of different specificities (white and gray) is added. (b) Specific antibodies (gray) bind to the antigen; all unbound antibodies (white) and other serum proteins are washed away. (c) Second-labeled antibodies (conjugate) are added that are specific for the immunoglobulin in the test serum used (e.g., labeled anti-macaque IgG antibody); the conjugate binds to the antigen–antibody complexes. After washing away unbound-labeled antibodies, the signal emitted by the conjugate label (radiating black dot) is detected. The signal is proportional to the amount of specific antibodies bound.

steps in between are not counted): (1) a fluid sample to be tested for antibody (serum, saliva, etc.) is brought into contact with the antigen-coated solid-phase surface (plastic microplate wells, beads, etc.); if the antibodies in question are present in the sample they bind to the antigen; all unbound serum proteins are washed away; (2) anti-immunoglobulin conjugate (usually, anti-IgG) labeled with enzyme is added; if specific antibodies are bound to the antigen they react with the conjugate; unbound conjugate is washed away; (3) substrate is added, the enzyme present in the conjugate decomposes the substrate resulting in the emission of signal. Most commonly used ELISA substrates are colorogenic; that is, the signal is color development.

2.8.6.3. IMMUNOBLOT AND RELATED METHODS

Immunoblot is the most commonly used confirmatory method for detection of antibodies against viruses. The hallmark of the immunoblot is simultaneous detection of antibodies against several viral proteins. The classical version of this assay is the WB. This name has a decidedly unscientific origin. The first blotting technique, the detection of DNA fragments bound ("blotted") to a nitrocellulose membrane by molecular hybridization with complimentary probes, was invented by Sir E. M. Southern in 1975. The method was nicknamed "Southern blot" and became very popular. In 1977, the Southern blot was adapted for the detection of RNA. With tongue in cheek, the RNA blot was named northern blot. This name was accepted in the research community. In 1981, when an analogous technique was described for the immunodetection of proteins, predictably, it was named the WB. There were attempts to introduce the term Eastern and even Far Eastern blot for variations of the WB, but the key "vacancies" (DNA, RNA, and proteins) had already been filled and these terms did not survive. Given the history of blot nomenclature only Southern should be capitalized.

WB includes separation of viral proteins by polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE) and subsequent electrophoretic transfer of separated proteins from the gel slab onto the surface of a membrane, which is the replica of the gel, is then cut into strips. Each strip contains "bands" of attached viral proteins "arranged" according to their molecular weights. Each strip is incubated in diluted serum; if there is a spectrum of antiviral antibodies in the serum, each antibody binds to the corresponding

antigen band on the membrane. The following steps are exactly the same as in the indirect ELISA, except for the type of substrate. The substrates used in the WB produce insoluble deposits when decomposed by the enzyme. Typical results of WB are presented in Figure 2.15.

Interpretation of WB is straightforward when the result is clearly negative (no bands at all) or clearly positive (bands corresponding to all major viral proteins are present). Unfortunately, results which do not fit into these clear-cut categories are not uncommon. Such results are called indeterminate. Individual criteria for

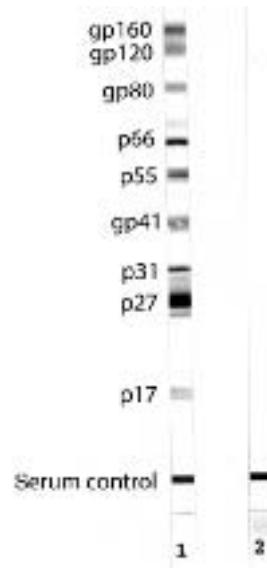


Figure 2.15. Western blot (WB) assay for antibodies against simian immunodeficiency virus (SIVmac). 1—positive result; 2—negative result. SIV-infected rhesus monkey serum tested on strip 1 contains antibodies against all structural SIV proteins. Viral proteins are separated according to their molecular weights. Note that bands corresponding to various viral proteins have different appearances; glycoprotein bands tend to be diffuse. gp160, gp120, gp80, gp41—*env* proteins; p27, p17, p55—*gag* proteins; p31, p66—*pol* proteins; serum control—reactivity with this band confirms that test serum was added and that conjugate was working.

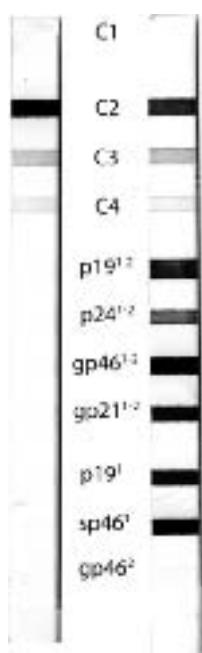


Figure 2.16. Line assay for antibodies reacting with the antigens of human T-lymphotropic virus type 1 and type 2 (HTLV-1 and HTLV-2). 1—negative result; 2—positive result. Serum of STLV-1-infected monkey tested on Inno-LIA HTLV1/II strip (Innogenetics) containing “spotted” recombinant proteins and peptides derived from HTLV-1 and HTLV-2 proteins ($p19^{1-2}$, $p24^{1-2}$, $gp46^{1-2}$, $gp21^{1-2}$, $p19^1$, $gp46^1$, $gp46^2$). Antigens $p19^{1-2}$, $p24^{1-2}$, $gp46^{1-2}$, $gp21^{1-2}$ are cross-reactive; that is, they react with antibodies against different human and simian T-lymphotropic retroviruses. Antigens $p19^1$, $gp46^1$ are specific for HTLV-1 and related simian viruses (STLV-1). Antigen $gp46^2$ is specific for HTLV-2; however, antibodies against STLV-3s may react with this antigen. C2, C3, C4—positive controls [strong (3+), medium (1+), and weak (+/−)]; C1—conjugate control.

interpretation of WB results have to be established for each viral infection.

Line-assays, similar to WBs, determine antibodies against several viral antigens simultaneously. The antigens used in line assays are recombinant proteins and/or

peptides. They contain immunodominant epitopes and are designed to minimize nonspecific reactions. In contrast to WB, the position of bands on the line assay strips is not a result of electrophoretic separation and is not related to the molecular weight of the antigens; the bands are of identical shape and evenly spaced, unlike in WBs (Figure 2.16). Technically, line assay methodology is the same as for WBs.

Commercially available WB and line assays are commonly used for the diagnosis of retroviral infections in NHPs.

2.8.6.4. IMMUNOFLUORESCENCE

Immunofluorescence is another classical method for the detection of antibodies against viruses. Technologically the immunofluorescence assay (IFA) for antibodies is very similar to *in situ* antigen detection. The principal difference is that in antigen detection tests, the known component is antibody, whereas in the antibody detection method the known component is antigen. In other words, the cells used for IFA must be known to contain

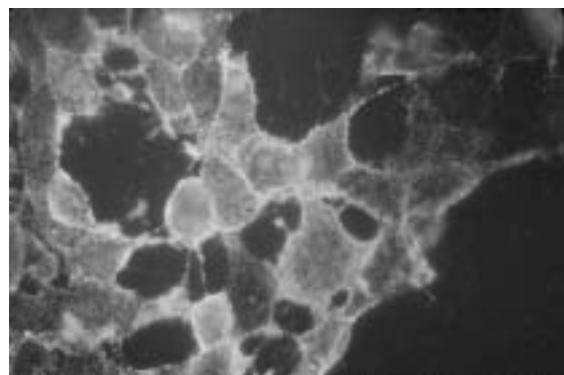


Figure 2.17. Indirect immunofluorescence assay. Acetone-fixed herpes simplex virus type 1 (HSV-1)-infected Vero cells were used as targets for detection of antibodies against the virus. Anti-HSV antibodies bound to the viral antigens are visualized by the secondary anti-IgG antibodies labeled with fluorescent dye (FITC—fluorescein isothiocyanate). Bright green fluorescence indicates positive reaction. (Image is kindly provided by Dr. Richard Eberle. See color version page 9.)

the viral antigens of interest. Usually the virus-infected culture cells are used as antigen. It is highly desirable, although not always possible, that the same uninfected cells are simultaneously used as a control. The cells are fixed on a glass surface, usually multi-well slides are used. Sera to be tested are added to the wells and after incubation the unbound proteins are washed away. Then anti-immunoglobulin conjugate labeled with fluorochrome is added; after incubation the unbound conjugate is washed away. Ideally, each serum has to be tested in two neighboring wells, containing virus-positive and virus-negative cells. The IFA results are scored under a fluorescent microscope (Figure 2.17). Preferably, this should be done blindly by two or more observers.

Although the use of indirect IFA has largely been substituted by ELISA, in some fields, for instance for the detection of antibodies against lymphocryptoviruses, this methodology remains a viable option.

2.9. CONCLUDING REMARKS

Material presented in this chapter is introductory; only the major concepts and terminology are covered. The aim of this chapter is to facilitate learning of the more detailed and advanced information presented in Part II. Those who are interested in deeper knowledge of different aspects of basic virology should turn to more comprehensive texts, such as the corresponding chapters of the latest edition of *Fields Virology*.

Part II:

Simian Viruses and Nonhuman Primate Models of Viral Infections

Introduction to Part II

ORGANIZATION OF MATERIAL

Part II of *Simian Virology* is devoted to the viruses whose natural hosts are simian species; the material is organized primarily on viral taxonomy. The major subdivisions of Part II describe RNA and DNA viruses, respectively. The “RNA viruses” subdivision (IIA) is divided into two sections: “Simian Retroviruses” and “Other Simian RNA Viruses.” Similarly, “DNA Viruses” subdivision (IIB) contains “Simian Herpesviruses” and “Other Simian DNA Viruses” sections. Retro- and herpesviruses are singled-out because these groups are the most extensively studied. For the same reason, general properties of retroviruses and herpesviruses are described in separate introductions preceding Chapters 3 through 9 and 12 through 16, respectively.

Each chapter, with a few exceptions, contains material relevant to simian viruses belonging to a certain viral family. The amount of information on simian immunodeficiency viruses (SIVs) far exceeds the knowledge of other simian viruses, and condensing this voluminous information in a single chapter would complicate its perception. For this reason, SIVs are covered in three chapters; two of these (Chapters 3 and 4) are devoted to SIVs in the natural hosts and nonhuman primate (NHP) models of AIDS, respectively. The main focus of Chapter 5 is on factors that may have been responsible for turning “dead-end” human SIV infections into pandemic and epidemic forms of human immunodeficiency viruses (HIV-1 and HIV-2). Chapter 22 “Miscellaneous Viruses” (subdivision IIC) covers those simian (or presumed simian) viruses which are insufficiently well characterized.

The organization of all taxonomy-based chapters follows the same general plan. Each chapter starts with a brief “free-style” introduction highlighting the most interesting facts or features of a particular viral group or its “celebrity” members. This is followed by sections describing the general features of the viral family. The purpose of these general sections is to provide the reader

with a basic knowledge of viral classification, nomenclature, genomic organization, gene products, and replication cycles. Where possible the descriptions are based on the data directly relevant to simian viruses. However, not uncommonly, the generic features of simian viruses are “deduced” from their better studied human counterparts. These general descriptions are followed by sections that contain information on the specific simian viruses. A short summary concludes each chapter.

Chapters 23 and 24 (subdivision IID) stand apart. They concisely cover experimental and natural infections of NHPs with human and nonsimian animal viruses.

CLASSIFICATION AND NOMENCLATURE OF SIMIAN VIRUSES

Numerous simian viruses were isolated in the 1950s–1960s and were given the designations “simian virus” (SV) or “simian agents” (SA), followed by a number (SV1, SV2, etc.; SA1, SA2, etc.). The SV and SA names generally were used for the isolates from Asian and African monkeys, respectively. The relics of the SV/SA nomenclature can still be found in the literature. In the first classification of simian viruses, they were grouped according to the type of cytopathic effect in vitro.² In the 1960s, the general principles of virus classification were developed and the International Committee on Taxonomy of Viruses (ICTV) began the classification of viruses. The first attempt to bring the classification of simian viruses into compliance with the ICTV-approved taxonomy of viruses was made in 1968; however, the SV/SA nomenclature largely remained.¹

The next major overhaul of the simian virus classification and nomenclature was suggested by the Simian Virus Working Team in 1980.³ Starting from the early 1980s, simian viruses were integrated into the ICTV classification of viruses. However, there was and is no general rule for the naming of simian viruses under

the ICTV classification schemes; naming practices differ for various viral families. Usually, a simian virus name contains two attributes: the descriptor of the host (simian genus or species) and the virus (viral family). Regrettably, the host descriptors are not standardized. As a result, multiple names and abbreviations are used for the same host. In addition, viral family descriptors may be too broad. For instance, the descriptor “herpesvirus” is used for simplex-, varicella-, cytomegalo-, lymphocryptic-, and rhadinoviruses.

The adage, “rules are made to be broken” certainly applies to the naming of viruses. The published literature is replete with virus names that ignore the conventions of the ICTV; the “vernacular” names of simian viruses are widely used and this practice is not likely to be abandoned in the foreseeable future. In some cases, the vernacular names are more informative and easier to remember than the ICTV names. However, the ICTV nomenclature must be used in parallel for unequivocal identification, even when vernacular names appear to be preferable.

An unofficial “classification novelty” widely used in this book is the notion of “frag-virus” and the corresponding term. This term has been suggested for cate-

gorizing presumptive viruses known primarily from sequence data with no biological characterization to confirm that an infectious virus actually exists.⁴ Some areas of simian virology have been inundated with the descriptions of such “frag-viruses” and clear distinction between preliminary genome-based evidence and conclusive proof by biological isolation and characterization of a replication-competent virus rectifies the meaning of new virus that is blurred otherwise.

REFERENCES

1. Hull, R. N. 1968. The simian viruses. *Virol. Monogr.* 2:1–16.
2. Hull, R. N., J. R. Minner, and J. W. Smith. 1956. New viral agents recovered from tissue cultures of monkey kidney cells. I. Origin and properties of cytopathogenic agents S.V.1, S.V.2, S.V.4, S.V.5, S.V.6, S.V.11, S.V.12 and S.V.15. *Am. J. Hyg.* 63(2):204–215.
3. Kalter, S. S., D. Ablashi, C. Espana, R. L. Heberling, R. N. Hull, E. H. Lennette, H. H. Malherbe, S. McConnell, and D. S. Yohn. 1980. Simian virus nomenclature, 1980. *Intervirology* 13(6):317–330.
4. Voevodin, A. and P. A. Marx. 2008. Frag-virus: a new term to distinguish presumptive viruses known primarily from sequence data. *Virol. J.* 5(1):34.

Section 1:

RNA Viruses

Section 1.1:

Simian Retroviruses

Introduction to Retroviruses

Retroviruses are the most extensively studied group among all simian viruses and simian immunodeficiency viruses (SIVs); the simian counterpart of human immunodeficiency viruses (HIV-1 and HIV-2), undoubtedly, attracts the most attention.

The defining features of retroviruses are two essential steps in their replication: the conversion of the RNA genome into a DNA copy and the integration of this DNA copy into cellular chromosomal DNA. The integrated chromosomal DNA copy of the RNA genome is called the provirus. Copying the genomic RNA into a DNA copy is called reverse transcription, and is mediated by a specialized multifunctional enzyme named reverse transcriptase (RT). The integration of the DNA copy is mediated by the integrase (IN) region of RT. The combination of reverse transcription and integration into the host genome is unique to the retroviruses.

CLASSIFICATIONS

All retroviruses belong to the family *Retroviridae* which contains two subfamilies: *Orthoretrovirinae* and *Spumavirinae*. Within the *Orthoretrovirinae* subfamily, there are genera named *Alpharetrovirus*, *Betaretrovirus*, and so on. Simian orthoretroviruses belong to four of these genera: *Betaretrovirus*, *Gammaretrovirus*, *Deltaretrovirus*, and *Lentivirus*. Within the *Spumavirinae* subfamily, there is a single genus *Spumaretrovirus* which includes simian spumaviruses (Table RI.1).

In addition to the International Committee on Taxonomy of Viruses (ICTV) classification, there are numerous supplementary classifications of retroviruses which are based on different characteristics of these agents. Historically, the first was a morphological classification which distinguished four types of retroviral particles: A, B, C, and D (Figure RI.1). They differ from each other by the position and shape of the core. Both type-C and type-B particles have the spherical core. However, the core is located differently: centrally in type-C and eccentrically in type-B particles. Simian gamma- and deltaretro-

viruses belong to type-C, whereas no simian retrovirus belongs to type-B. The core of type-D particles is cylindrical. Simian betaretroviruses belong to type-D. The type-A particle is defined as a ring of electron-dense material with an electron-lucent center. None of the infectious retroviral particles have type-A morphology. However, there are historical reasons as to why type-A particles have the same “status” as type-B, -C, and -D infectious particles. Originally, it was thought that particles with type-A morphology were infectious virions. It was shown later that this morphological type was limited to immature retroviral particles. Among simian retroviruses, immature “precursor-virions” of betaretroviruses are classified as type-A particles. Morphological types of spumaviruses and lentiviruses do not fit into C or D types. They are sufficiently unique to be distinguishable morphologically from other retroviruses.

Retroviruses are also classified as exogenous or endogenous. Endogenous retroviruses are integrated into the genome of germ cells and are transmitted from generation to generation via gametes. In a host infected by an endogenous retrovirus, every single cell carries the integrated genome of the virus. There are thousands of endogenous retroviral sequences integrated in primate genomes. Most of these “viruses” are defective. However, some endogenous simian viruses can be rescued in the infectious form.

Simian exogenous retroviruses are transmitted from individual to individual horizontally through host contact via sexual, bite, or other exposure to body fluids. They can be distinguished from the endogenous viruses by the presence of both provirus-positive and provirus-negative cells in the infected host. All simian counterparts of medically important viruses are exogenous. Endogenous retroviruses in one host may become exogenous in another host. This transition occurred with the gibbon ape lymphoma virus (see Chapter 7).

Retroviruses that dramatically change the growth characteristics of host cells in vitro are classified as

Table RI.1. Simian Retroviruses

Subfamily Genus	Simian Retroviruses	Human Analogues
<i>Orthoretrovirinae</i>		
<i>Lentivirus</i>	Simian immunodeficiency virus (SIV)	Human immunodeficiency viruses 1 and 2 (HIV-1 and HIV-2)
<i>Deltaretrovirus</i>	Simian T-lymphotropic virus 1 (STLV-1) Simian T-lymphotropic virus 2 (STLV-2) Simian T-lymphotropic virus 3 (STLV-3) None	Human T-lymphoma/Leukemia virus 1 (HTLV-1) Human T-lymphotropic virus 2 (HTLV-2) Human T-lymphotropic virus 3 (HTLV-3) Human T-lymphotropic virus 4 (HTLV-4)
<i>Betaretrovirus</i>	Mason-Pfizer monkey virus (MP-MV) and simian retroviruses (SRVs)	None
<i>Gammaretrovirus</i>	Gibbon ape lymphoma virus (GALV) Simian sarcoma virus 1 (SSV-1)	None None
<i>Spumavirinae</i>		
<i>Spumavirus</i>	Simian foamy viruses (SFVs)	None

acutely transforming. Cell transformation induced by such viruses appears as foci of multilayer growth in monolayer cell cultures. Simian sarcoma virus type 1 (see Chapter 7) is the only acutely transforming retrovirus found in primates. Nontransforming retroviruses replicate without causing visible changes in host cells *in vitro*.

It has to be mentioned that the term “transformation” is also used for describing indefinite growth of T cells *in vitro*, which is caused by some simian deltaretroviruses (see Chapter 8). The mechanism and phenomenology of such transformations are completely different from those of acutely transforming retroviruses.

GENOME COMPOSITION AND GENE PRODUCTS

The retroviral genome is diploid in the sense that it consists of two identical RNA molecules. These molecules are linked by dimer linkage structures (DLS), a self-complementarity region located near the 5'-end of each molecule. A single retroviral genomic RNA molecule spans 6,000–13,000 nt. It is depicted schematically in Figure RI.2. Retroviral genomic RNA has the characteristic features of mRNA: the 5'-end of this molecule is “capped” by an m7G5'-ppp5'-G_mp chain, whereas the 3'-end is “tailed” by a poly-A sequence. At both ends of the genomic RNA, next to the cap and upstream to the poly-A tract, there are identical repeated sequences (R). Downstream to the 5'-R lays a unique, that is, non-

repeated sequence U5. Similarly, upstream to the 3'-R resides another unique sequence U3. The initiation of reverse transcription occurs when the primer binds to the primer-binding site (PBS) located immediately downstream to the U5. Reverse transcription is primed by a cellular tRNA. Various retroviruses use different tRNAs as their primers. Packaging of genomic RNA into the nucleocapsid requires a specific sequence, the encapsulation signal Ψ (psi) which is located downstream to the PBS. At the other end of the genomic RNA, upstream to U3, resides the polypurine tract (*ppt*). The space between the Ψ and *ppt* is occupied by the genes encoding viral proteins.

In a simple retrovirus genome the genes are positioned in the following order:

5'-R/U5-gag-pro-pol-env-U3/R-3'

Complex retroviruses have additional genes, called auxiliary or accessory genes, which are located immediately before and after the *env* gene.

Reverse transcription of the viral RNA results in the formation of a double-stranded DNA copy, the provirus. Proviral DNA is not an identical copy of genomic RNA (Figure RI.3).

The difference is in the structure of nonprotein-coding terminal sequences. In proviral DNA, protein-coding genes are flanked by long terminal repeats (LTRs). Each LTR consist of U3, R, and U5 positioned in the following order: 5'-U3-R-U5-3'. Thus, the general structures of

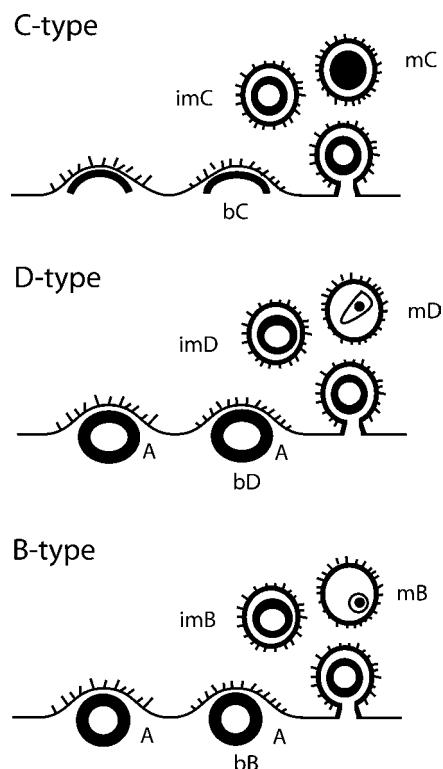


Figure RI.1. Morphological types of retroviruses. C-type: Characteristic features of C-type morphology are crescent shape of the core at the early stages of budding (bC), electron-lucent center of the core of immature virions (imC), electron-dense central core in mature virions. D-type: Characteristic features of D-type morphology are ring-shaped core (A-type particles) at all stages of budding (bD) and immediate after release (imD), condensed cylindrical core in the mature virions (mD). B-type: Characteristic feature of B-type morphology is small eccentric core in the mature virions (mB). There is no simian retrovirus with this type of morphology. bB, B-type budding; imB, immature B-type particles.

retroviral RNA and DNA genomes are as follows:

5'-R/U5-gag-pro-pol-env-U3/R-3'
 5'-LTR-gag-pro-pol-env-LTR-3'
 LTR = U3/R/U5

RNA genome
 RNA genome

The complete sequence of a retrovirus genome is usually deposited in GenBank, and in other nucleic acid sequence databases, as a proviral DNA sequence. Similarly, the genomic maps of retroviruses usually reflect the structure of the DNA genome. A typical map of a retroviral genome is presented in Figure RI.4.

The LTRs contain a number of functionally important elements required for genome expression, most of which are located in U3, the longest and the most diverse part of the LTR. The functionally important sequences are well conserved.

The *gag* gene in all retroviruses is located in the 5'-proximal part, downstream to the 5'-LTR, from which it is separated by a short sequence. The *gag* gene encodes three Gag proteins: matrix or membrane-associated (MA), capsid (CA), and nucleocapsid (NC). These proteins are produced through the cleavage of precursor polyproteins Gag, Gag-Pol, and Gag-Pro-Pol by viral protease (Figure RI.4).

The *pro* gene, located between *gag* and *pol*, encodes the protease enzyme (PR). This viral enzyme belongs to the family of aspartyl proteases.

The *pol* gene encodes the enzymes: RT, RNAase H (RH), and IN (Figure RI.4). Sometimes the IN-encoding component of the *pol* gene is considered as a separate gene and is designated as *int*. The RT/RH protein consists of two domains: amino-terminal polymerase (RT per se) and carboxyl-terminal RH. The RNA/DNA copying mediated by RT is error-prone, on average 10^{-4} errors per incorporated base. The IN protein consists of three domains. Three highly conserved amino acid residues, which are crucial for the function of IN, are located in the central domain.

The *env* gene, located downstream to the *pol* gene encodes two envelope proteins, the surface (SU) and the transmembrane (TM) glycoproteins (Figure RI.4).

The auxiliary genes typically contain two or more exons. They are located before or after the *env* gene, or they overlap with *env*. The products of these genes are important regulators of the expression of complex retrovirus genomes.

REPLICATION CYCLE

The major steps of the retrovirus replication cycle are receptor binding and entry, uncoating, reverse transcript-

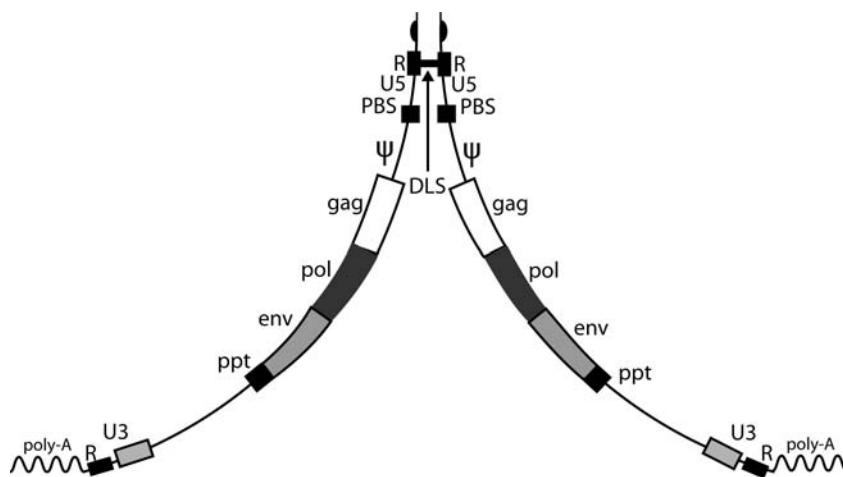


Figure RI.2. Scheme of RNA genome of retroviruses. The RNA genome in retrovirus virions is diploid: two identical genomic RNA molecules are linked by the dimer linkage structure (DLS). Each RNA molecule has a cap structure and a poly-A tail at the 5'- and 3'-ends, respectively. R, terminal repeats; U5, unique sequence at the 5'-end of each genomic RNA; PBS, primer-binding site; Ψ , encapsulation signal; gag, pol, env, minimal set of retroviral genes; ppt, polyuridine tract; U3, unique sequence at the 3'-end of each genomic RNA.

tion, transport of viral DNA into the nucleus, integration of DNA provirus into chromosomal DNA, transcription of proviral DNA, splicing of primary transcripts, export of mRNAs to the cytoplasm, translation of mRNAs, assembly, release, and maturation (Figure RI.5).

The receptors for retroviruses belong to various classes of cellular macromolecules. The receptor-binding site (RBS) is located in the SU glycoprotein. Binding evokes conformational changes in both the re-

ceptor and viral envelope. This leads to the fusion of the viral and cellular membranes and the internalization of the viral core. The main viral player in the fusion process is the TM protein which contains a fusogenic domain. A coreceptor is required for the entry of some retroviruses, for example SIV.

The uncoating process, simplistically perceived as release of the “naked” genomic RNA, includes complex changes of internalized nucleocapsids preceding

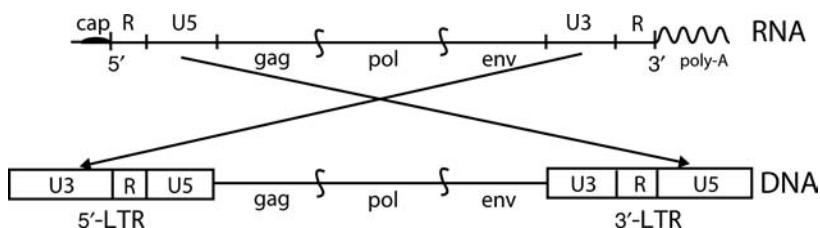


Figure RI.3. Scheme of proviral DNA genome of retroviruses and comparison with the RNA genome of the virion. Major distinguishing feature of retroviral proviral DNA genome is the presence of two identical long terminal repeats (LTRs) that flank the coding part of the genome. Each LTR is formed during the reverse transcription and includes U3, R, and U5 regions.

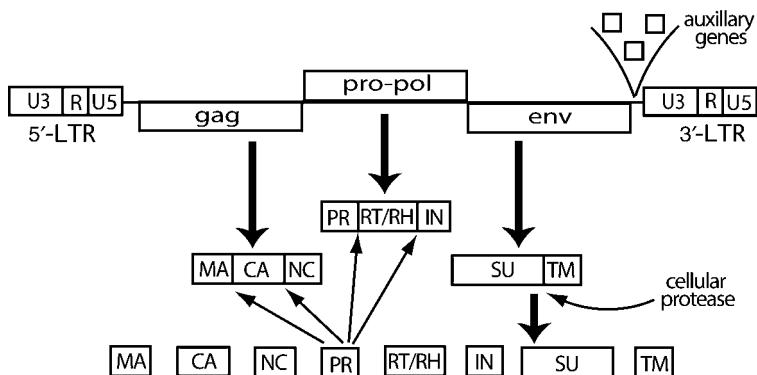


Figure RI.4. Scheme of retroviral genome and major proteins. *Gag*, *pro*, *pol*, and *env* genes encode Gag, Pro-Pol, and Env polyproteins, respectively. The Gag polyprotein is cleaved by the viral protease (PR) into matrix or membrane-associated (MA), capsid (CA), and nucleocapsid (NC) proteins. Pro-Pol polyprotein is autocleaved into the protease (PR), reverse transcriptase/RNAase H (RT/RH), and integrase proteins (IN). Env precursor protein is glycosylated and then cleaved by a cellular protease into surface glycoprotein (SU) and transmembrane glycoprotein (TM). Auxiliary genes are present in the genomes of the complex retroviruses (lentiviruses, deltaretroviruses, and spumaviruses).

the start of reverse transcription. The presence of the processed CA proteins, that is, the final products of CA protein cleavage, is required for uncoating.

Reverse transcription takes place in the cytoplasm in complexes which include the genomic RNA dimer, the enzymes (RT/RH and IN), and the NC protein. The signal triggering the start of reverse transcription is unknown. Reverse transcription is a multistep process resulting in the formation of a linear dsDNA copy of the genomic RNA.

The dsDNA provirus synthesized in the cytoplasm is transported to the nucleus. The simple and complex retroviruses use different pathways for nuclear entry. The simple retroviruses typically infect only actively dividing cells. At least partly, this is due to their inability to engage mechanisms of active transport via the nuclear membrane. As a result, they are dependent on the breakdown of the nuclear membrane during mitosis. The complex retroviruses are capable of delivering their DNA to the nucleus through the intact nuclear membrane and can infect nondividing cells.

Linear DNA is the dominant form of viral DNA in the nucleus. However, circular forms of viral DNA are also present. The synthesis of these circles from unintegrated

linear viral DNA is mediated by cellular enzymes. The circular forms of viral DNA are believed to be dead-end products of retroviral DNA “trafficking.” However, detection of circular viral DNA can be used as a marker of DNA genome nuclear entry.

Only full-length linear DNA is integrated into chromosomal DNA. The sequence termed *att* in the LTR is required for integration. The *att* sites contain a CA dinucleotide, which is conserved in all retroviruses.

The integration of provirus is essential for the efficient expression of the retroviral genome and the formation of infectious viral progeny. Once it is integrated, the provirus becomes an integral part of the chromosomal DNA. It replicates synchronously with cellular DNA and is “passed” to the progeny cells. Theoretically, the only way to eradicate retroviral infection is to eliminate all provirus-positive cells.

The integrated provirus is a few bases shorter than the unintegrated full-length viral dsDNA. Usually, the last two base pairs are deleted at each terminus of the provirus during the integration. The host sequences flanking the integrated provirus also undergo modification—the duplication of a few bases. The exact number of duplicated bases varies for different retroviruses from 4 to 6.

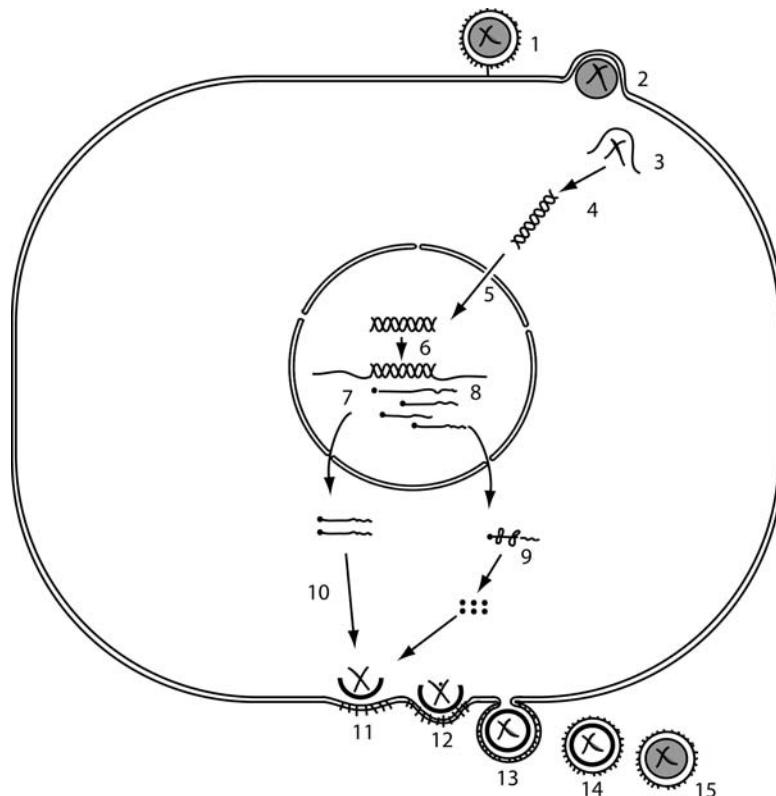


Figure RI.5. Scheme of retroviral replication cycle. 1, attachment to the cell surface receptor; 2, internalization by fusion of viral envelope and plasma membrane; 3, core disassembly and release of genomic RNA; 4, reverse transcription of genomic RNA into genomic DNA; 5, transport of genomic DNA into the nucleus; 6, formation of the provirus by integration of linear genomic DNA in the cellular chromosomal DNA; 7, synthesis of full length RNA transcripts and transport of newly synthesized genomic RNAs to the cytoplasm; 8, transcription of mRNA encoding viral proteins and transport of these transcripts in the cytoplasm; 9, translation of viral proteins; 10, transport of newly synthesized structural viral proteins and genomic RNAs to the sites of assembly in the vicinity of the plasma membrane; 11–13, assembly and budding of virions; 14, immature extracellular virions; 15, mature infectious virions.

The integration of provirus is a complex and orderly process in which the IN enzyme is the major player. Other viral and cellular factors are also involved.

The distribution of integration sites along the host genome is quasi-random: there are no specific integration sites, but some preferences are observed. For example, the tendency to integrate into the transcribed regions of the host genome is characteristic of HIV. On the other hand, spumaviruses integrate mostly in transcriptionally inactive sites.

The transcription of the provirus is mediated by cellular transcription machinery. Major types of retroviral transcripts are depicted in Figure RI.6.

The primary transcript is full-length RNA. Transcription is initiated by the promoter for cellular RNA polymerase II, located in the U3 region, and starts near the U3/R border in the 5'-LTR and continues to a point located in the downstream flanking cellular sequence. The extra sequence at the 3'-end of the full-length transcript is cleaved and a poly-A tail is added. Polyadenylation

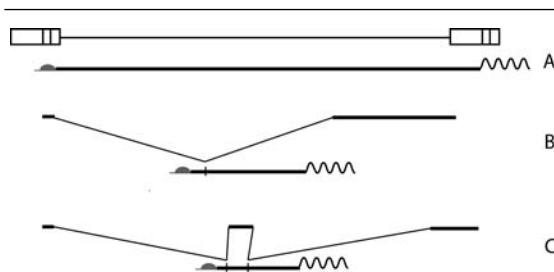


Figure RI.6. Major types of retroviral transcripts. A, full genome-length transcript; B, single-spliced transcript; C, double-spliced transcript.

at the 3'-end of transcribed RNA is “triggered” by the polyadenylation signal, usually the AAUAAA sequence within R region. The exact site where the poly-A tail is added is not critical for virus replication.

The full-length nuclear RNA transcripts are divided into two sets: spliced and unspliced. Both sets of viral RNAs are transported from the nucleus to the cytoplasm. The unspliced transcripts are packaged into virions as genomic RNAs, or translated into Gag, Gag-Pro, and Gag-Pro-Pol precursor polyproteins by the cellular protein synthesis machinery. Various combinations of ribosomal frameshift and translational read-through mechanisms are used by different retroviruses for translation of these precursor polyproteins. All spliced transcripts are translated. Env protein synthesis is directed by singly-spliced mRNA. Doubly-spliced mRNAs direct synthesis of the auxiliary proteins.

The Gag precursor is translated as an individual polyprotein and as a part of the “compound” Gag-Pro, and Gag-Pro-Pol, precursors. The MA protein occupies an N-terminal position in Gag; the NC protein occupies a C-terminal position in the Gag polyprotein, or position bordering protease in Gag-Pro and Gag-Pro-Pol; the CA protein is located between the MA and NC. During maturation, the final Gag proteins are cleaved from the precursors by viral protease.

The Env precursor protein contains hydrophobic residues at the N-terminus serving as a signal peptide for transport to the rough endoplasmic reticulum (ER). At the ER, the signal peptide is cleaved by the cellular proteases and the Env precursor is heavily glycosylated. The Env precursor is oligomerized, presumably into a trimer, which is transported from the ER to the Golgi apparatus. The trimer precursor is cleaved by furin pro-

teases into another trimer consisting of separate SU and TM glycoproteins connected by disulfide bonds. The SU-TM trimers are then transported from the Golgi apparatus to the cell membrane.

There are two sites in the host cell where retroviruses are assembled: at the plasma membrane and at the cytoplasm. Gammaretroviruses and deltaretroviruses are assembled at the plasma membrane. Betaretroviruses and spumaviruses are assembled in the cytoplasm.

The assembly of retrovirus virions is driven primarily by the Gag precursor protein. The Gag-Pro-Pol precursor is coassembled with the Gag precursor, but the number of these molecules is lower (100–150 versus 1,200–1,800 Gag molecules per virion).

Three domains within the Gag polyprotein are crucial for assembly: a membrane binding (M), an interaction (I), and a late assembly (L). These domains exist as functional entities only within Gag polyprotein and are destroyed by proteolytic cleavage.

The M domain is mainly located within the MA part of the Gag precursor and is responsible for Gag binding to the cell membrane. In many, but not all retroviruses, this domain contains myristylated residues which are important for the plasma membrane targeting of the Gag precursor.

The I domain is located predominately in the NC part of the Gag precursor and is responsible for Gag-to-Gag interaction during the assembly. Mutations in the I domain either block the assembly, or result in the production of defective viral particles. The I domain contains the RNA-binding sites. The binding of genomic RNA to these sites is believed to be important for efficient assembly.

The L domain is critical for the late stages of the assembly. Mutations in this domain lead to the production of virus-like structures which accumulate under the plasma membrane or remain “tethered” to the cell membrane (incomplete budding). Different regions of the Gag precursor participate in the formation of the L domain in various retroviruses. The L domain is the target for binding to multiple components of the cellular machinery, responsible for protein sorting and delivery into endosomal compartments.

The Env proteins SU and TM are accumulated preferentially in cellular membranes in the vicinity of the nucleocapsid assembly sites. The orientation of Env in the membrane is “polarized.” The SU faces outwards, whereas the TM has three parts: the extracellular moiety interacting with the SU which also includes the

trimerization and fusion domains; the transmembrane domain which is embedded in the membrane; and a portion protruding into cytoplasm, named the cytoplasmic tail. Proteolytic cleavage of the Env precursor, in contrast to other retroviral protein precursors, is mediated by cellular protease. This cleavage is essential for virus infectivity.

Genomic RNA is incorporated into the nucleocapsids by binding to the NC part of the Gag precursor. The assembled nucleocapsids contain a genomic RNA dimer. Whether the dimerization of RNA occurs before or after its incorporation is not clear.

Packaging of host tRNAs is essential for the infectivity of retroviruses. The assembled virions contain 50–100 tRNA molecules. For some retroviruses, the composition of the tRNA pool is not substantially different from that in the host cell. Other retroviruses preferentially package those tRNA molecules that serve as primers for reverse transcription for this particular virus species.

Virions are not infectious immediately after release. They acquire infectivity through the “maturation” process. The key event in maturation is the proteolytic cleavage of the Gag and Gag-Pro-Pol precursors mediated by viral protease. Presumably, the early stage of cleavage is mediated by the PR included in the Gag-

Pro-Pol precursor. Accumulation of “free” PR, that is, the PR which is cleaved from the precursor, accelerates cleavage. Cleavage begins only in the assembled virions and continues until all polyproteins in the virion are replaced by individual viral proteins. The dimerization of PR, induced by the close contact between Gag-Pro-Pol precursors during the assembly, is believed to be a contributing factor to cleavage/maturation. Another important molecular event occurring during maturation is the stabilization of the genomic RNA dimer. Dimer stabilization is believed to be mediated by free NC protein released as a result of Gag cleavage. Maturation is accompanied by a characteristic change in the morphology of virions. The doughnut-like core of “immature” virions (containing electron-lucent region in the center) transforms into an electron-dense core.

The introduction to the retroviral chapters (3 through 9) covers the most general features of retrovirus genome composition and replication cycle. There are many specifics in the properties of retroviruses belonging to different genera and species which are not discussed here. This material, as well as the information on the pathogenicity and other characteristics of simian retrovirus infections *in vivo*, is presented in the corresponding chapters.

3

Lentiviruses in Their Natural Hosts*

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- 3.2 ICTV classification and nomenclature
- 3.3 SIV and primate species radiation
- 3.4 SIV phylogenetic classification
 - 3.4.1 SIVs from arboreal guenons
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 - 3.4.3 SIVs from the four species of AGMs (SIVagm)
 - 3.4.4 SIVs from L'Hoest supergroup (*C. l'hoesti*, *C. solatus*, *C. preusi*) and mandrill (*Mandrillus sphinx*): SIVlhoest, SIVsun, SIVpre, SIVmnd-1
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 - 3.10.2 Serological testing

* Co-authored with Cristian Apetrei, Tulane National Primate Research Center, Covington, Louisiana, USA.

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3.1. INTRODUCTION

Simian immunodeficiency viruses (SIVs) belong to the genus *Lentivirus* of the family *Retroviridae*. Lentiviruses infect the equine, ovine, bovine, and feline families in addition to simian species and humans. All known lentiviruses are exogenous and are distinguished by lentiviral-specific virion morphology and a relatively complex retroviral genome. The study of lentiviral diseases was first among retroviruses and predates the modern era of virology. Equine infectious anemia (EIA) was described in 1904 by Henri Vallee and Henri Carre as having an infectious nature. Vallee and Carre also showed that EIA was caused by a “filterable virus,” therefore bestowing on the equine infectious anemia virus (EIAV) the honor of being the first retrovirus discovered. The name lentivirus, which means slow virus from the Latin word *lenti*, was introduced by Bjorn Sigurdsson who studied scrapie or rida, a slow chronic disease of sheep in Iceland.^{252,253} The diseases described in sheep were named maedi and visna. Both EIAV and maedi–visna viruses were cultured in the 1960s and classified as lentiviruses.^{252,253}

The SIVs are a relatively new group of lentiviruses, having first been reported in macaques with AIDS in 1985.⁶² The original SIV nomenclature may be confusing to some, even to an accomplished virologist. In early reports, the human immunodeficiency virus (HIV) was named human T-cell leukemia virus III in the belief that the human T-cell lymphoma/leukemia viruses (HTLVs) (see Chapter 8) and HIV were closely related. The first reports on SIV followed the HTLV-III nomenclature with the name STLV-III for SIV.^{62,160} The simian T-lymphotropic viruses (STLVs) are separate retroviral species and the name of simian lentiviruses was changed to SIV in keeping with the HIV nomenclature.⁵²

The first discovery of SIV was in the Asian macaque colony at the New England Primate Research Center.⁶² At first, SIV was thought to be a natural infection of the macaques, rhesus, and cynomolgus—hence the name SIVmac—for SIV from macaques.⁶³ However, in 1987 a group from the Tulane National Primate Research Center reported that the natural host of SIVmac-like viruses was more likely to be the sooty mangabey (SM), *Cercocebus atys*.¹⁹⁴ This finding in SMs was confirmed at the Yerkes National Primate Research Center.⁸⁸ This salient observation was that SIV likely had an African origin and that SIV-infected SMs were not observed to develop clinical signs of an AIDS-like disease.^{160,194} Since the discovery of SIVmac in 1985, there has been an explosion of information in this field consisting of thousands of publications listed in the US National Library of Medicine database (www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed). This chapter will focus on the research on the natural hosts of SIV.

Besides primate species, lentiviruses have been identified in other mammals. They are feline immunodeficiency virus (FIV), equine infectious anemia virus, caprine arthritis–encephalitis virus (also known as maedi–visna virus) of sheep and goats, and bovine immunodeficiency virus (BIV). FIV induces an AIDS-like disease in domestic cats. Neurological disorders, arthritis, and pneumonia are seen in sheep and goats. Recurrent fever and blood dyscrasias characterize equine lentiviral infections. BIV infection does not induce disease in cattle, despite its name.¹⁸⁷

Lentiviruses have a cone-shaped core or nucleocapsid that morphologically distinguishes them from other retroviruses (Figure 3.1). Lentiviruses have a complex genome compared to other retroviruses. In addition to the *gag*, *pol*, and *env* structural genes present in all

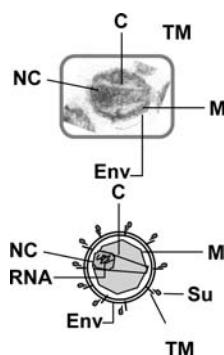


Figure 3.1. (Top) Electron micrograph of simian immunodeficiency virus (SIV) isolated from a sooty mangabey in West Africa (SIVsm).¹⁷⁹ (Bottom) Diagram showing structures of the SIV virion. C, capsid–internal protein shell structure in lentiviruses that encloses the genome; RNA, the genome; NC, nucleocapsid–capsid plus the RNA genome; M, matrix–encloses the nucleocapsid; env, outer lipid-containing bilayer that surrounds the virion and anchors the glycoprotein spikes; Su, surface external spikes that bind susceptible cells; TM, transmembrane protein that anchors the spikes.

retroviruses (see Introduction to Retroviruses), lentiviruses have an additional five or six accessory genes. The number and composition of the accessory genes vary according to the SIV group (Figure 3.2). All viruses in the lentiviral family have the *vif* accessory gene (virus infectivity factor) and *rev* (regulatory virus factor or regulator of expression of virus),^{170,187,301} both of which have been implicated as facilitators of viral transcription and activation. FIVs also encode for one or two small open reading frames (ORF1 and ORF2). ORF2 has been associated with vpr-like function in domestic cat FIV isolates.⁹³ Three accessory genes are specific for primate lentiviruses: *vpr*, *vpx*, and *vpu*; SIVs also include *nef* and *tat* genes.

All known lentiviruses are exogenous. At least 40 species of NHPs, all of African origin, carry species-specific lentiviral strains. Natural SIV infections of NHPs rarely result in immunodeficiency. The majority of these infections are clinically silent.

3.2. ICTV CLASSIFICATION AND NOMENCLATURE

The simian lentiviruses consist of three species, *human immunodeficiency virus 1* (HIV-1), *human immunodeficiency virus 2* (HIV-2), and *simian immunodeficiency virus* (SIV). Although only 10 SIVs naturally found in African nonhuman primates (NHPs) are recognized by the International Committee on Taxonomy of Viruses (ICTV), SIV infection has been described in 42 simian species and subspecies (Table 3.1). Partial or

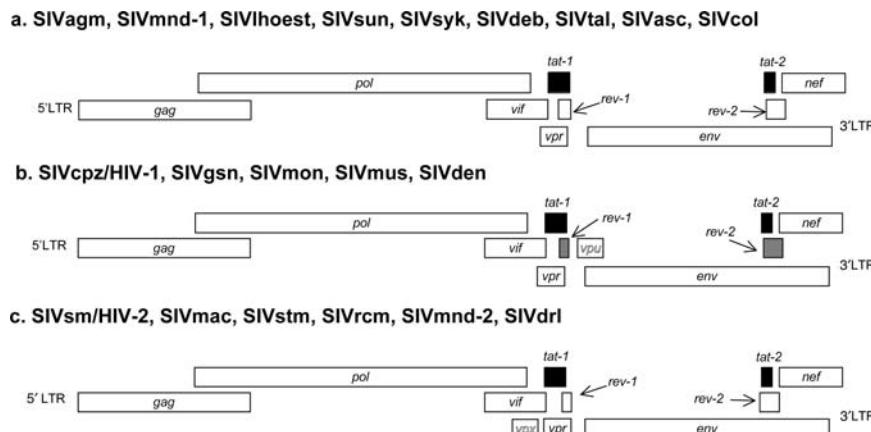


Figure 3.2. Three different genomic organizations for simian immunodeficiency viruses. Boxes show the order and approximate relative size of the genes. Gene functions are described in Section 3.5.

Table 3.1 African Nonhuman Primates Infected with SIV

Species/Subspecies	Virus Name*	Where Found	Seroprevalence	Sequence	Pathogenicity	Cross-Species Transmission	References
Common chimpanzee, <i>Pan troglodytes troglodytes</i>	SIVcpz.Pt ^t	Cameroon, Gabon, Democratic Republic of Congo (DRC)	<10%	Complete genomes and partial sequences	Not reported	Human (HIV-1)	55, 91, 135, 147, 199, 224
Eastern chimpanzee, <i>Pan troglodytes schweinfurthii</i>	SIVcpz.Pts	Tanzania, DRC	<10%	Complete genomes and partial sequences	Thrombocytopenia	Not reported	223, 237, 241
<i>Pan troglodytes verlorus</i>	SIVcpz.Pt ^t	Zoo in Cameroon	Not applicable	Complete sequence	Not reported	Not reported	55
Gorilla, <i>Gorilla gorilla</i>	SIVgor	Cameroon	Not known	Complete sequence	Not reported	HIV-1 group O-like	282
Sooty mangabey, <i>Cercopithecus atys</i>	SIVsmm	Sierra Leone, Liberia, Ivory Coast	20–58%	Complete genomes and partial sequences	Rare cases of AIDS in captivity	Human (HIV-2) macaques, SIVmac, SIVptm, SIVstm	9, 12, 46, 47, 48, 88, 124, 128, 165, 179, 194, 240
Red-capped mangabey, <i>Cercopithecus torquatus</i>	SIVrcm	Gabon, Cameroon, Nigeria	10–20%	Complete genomes and partial sequences	Not reported	Experimental transmission to <i>Macaca mulatta</i> and <i>M. fascicularis</i> (no AIDS); potential source for SIVcpz	14, 21, 94, 273
White-crowned mangabey, <i>Cercopithecus lunctatus</i>	SIVagnm.ver ^t	Primate Center in Kenya	Not applicable*	Partial sequence	Not reported	Not reported	205
Gray-cheeked mangabey, <i>Lophocebus albigena</i>	None given	Central Africa	Not reported	Serological evidence	Not reported	Not reported	272
Black mangabey, <i>Lophocebus aterrimus</i>	SIVbkm	DRC	Not reported	Partial sequence	Not reported	Not reported	

Mandrill, <i>Mandrillus sphinx</i>	SiVmnd-1	Gabon	50%	Complete genomes and partial sequences	AIDS in captivity	Not reported	264, 278
	SiVmnd-2	Cameroon, Gabon	50%	Complete genomes and partial sequences	AIDS in captivity	Transient infection in Rh upon experimental inoculation	134, 264
Drill, <i>Mandrillus leucophaeus</i>	SiVdrl	Nigeria, Cameroon	Not reported	Complete genomes and partial sequences	Not reported	Not reported	51, 134
Yellow baboon, <i>Papio cynocephalus</i>	SiVagnm.ver [†]	Tanzania	Not reported	Partial sequence	Not reported	Not reported	139
Chacma baboon, <i>Papio ursinus</i>	SiVagnm.ver [‡]	South Africa	Not reported	Partial sequence	Not reported	Not reported	283
Allen's monkey, <i>Allenopithecus nigroviridis</i>	None given	Central Africa	Not reported	Serological evidence	Not reported	Not reported	205
Talapoin, <i>Miopithecus talapoin, M. ougonensis</i>	SiVtal	Gabon, Angola, Cameroon	11%	Complete genomes and partial sequences	Not reported	Transient infection in Rh upon experimental transmission	162, 209
Patas, <i>Erythrocebus patas</i>	SiVagnm.ver [†]	West Africa	Not reported	Partial sequence	Not reported	Not reported	28
Grievet, <i>Chlorocebus aethiops</i>	SiVagnm.gri	East Africa	>50%	Complete genomes and partial sequences	Not reported	Not reported	85
Vervet, <i>Chlorocebus pygerythrus</i>	SiVagnm.ver	East and South Africa	>50%	Complete genomes and partial sequences	AIDS in a monkey coinfecte with STLV	Naturally transmitted to white-crowned mangabey in captivity; experimental transmission to pig-tailed macaques (AIDS) and Rh (no AIDS)	64, 87

(Continued)

Table 3.1. African Nonhuman Primates Infected with SIV

Species/Subspecies	Virus Name*	Where Found	Seroprevalence	Sequence	Pathogenicity	Cross-Species Transmission	References
Tantalus, <i>Chlorocebus tantalus</i>	SIVagn.tan	Central Africa	>50%	Complete genomes and partial sequences	Not reported	Not reported	127, 191
Sabaeus, <i>Chlorocebus sabaeus</i>	SIVagn.sab	West Africa	>60%	Complete genomes and partial sequences	Not reported	Naturally transmitted to Patas (no AIDS); experimentally transmitted to Rh (no AIDS)	3, 138
Diana, <i>Cercopithecus diana</i>	None given	West-central Africa	Not reported	Serological evidence	Not reported	Not reported	205
Greater spot-nosed monkey, <i>Cercopithecus nictitans</i>	SIVgsn	Central Africa	4–20%	Complete genomes and partial sequences	Not reported	Potential source virus for SIVcpz	56, 59
Blue monkey, <i>Cercopithecus mitis</i>	SIVblu	Central-east Africa	>60%	Partial sequences	Not reported	Not reported	27
Syke's monkey, <i>Cercopithecus albogularis</i>	SIVsyk	East Africa	30–60%	Complete genomes and partial sequences	Not reported	Transient infection in Rh upon experimental transmission	78
Mona, <i>Cercopithecus mona</i>	SIVmon	West Cameroon, Nigeria	Not reported	Complete genome	Not reported	Not reported	15, 56
Dent's mona, <i>Cercopithecus denti</i>	SIVden	Central Africa	10%	Not reported	Not reported	Not reported	65
Crested mona, <i>Cercopithecus pogonias</i>	None given	West Africa	Not reported	Serological evidence	Not reported	Not reported	205
Campbell's mona, <i>Cercopithecus campbelli</i>	None given	West Africa	Not reported	Serological evidence	Not reported	Not reported	172
Lowe's mona, <i>Cercopithecus lowei</i>	None given	Zoo in the United States	Not reported	Serological evidence	Not reported	Not reported	172
Mustached monkey, <i>Cercopithecus cephus</i>	SIVmus	Central Africa	3%	Complete genomes and partial sequences	Not reported	Potential source virus for SIVcpz	2, 56

Red-tailed monkey, <i>Cercopithecus ascanius</i>	SIVasc/SIVschm	Central Africa	Not reported	Complete genome and partial sequence	Not reported	Not reported	288
De Brazza's monkey, <i>Cercopithecus neglectus</i>	SIVdeb	West-central and Central Africa	40%	Complete genomes and partial sequences	Not reported	Not reported	27
Owl-faced monkey, <i>Cercopithecus hamlyni</i>	None given	Zoo in the United States	Not reported	Serological evidence	Not reported	Not reported	172
L'Hoest's monkey, <i>Cercopithecus lhoesti</i>	SIVlhoest/ SIVlho	East Africa	50%	Complete genomes and partial sequences	Not reported	Experimental infection of pig-tailed macaques (AIDS)	18, 123, 238
Sun-tailed monkey, <i>Cercopithecus solatus</i>	SIVsun	Central Africa	Not known	Complete genomes and partial sequences	Not reported	Source virus for SIVmnd-1; experimental infection of pig-tailed macaques (AIDS)	19
Mantled colobus, <i>Colobus guereza</i>	SIVcol	Central Africa	28%	Complete genomes and partial sequences	Not reported	Not reported	58
Western red colobus, <i>Piliocolobus hadius</i>	SIVwrc	West Africa	40%	Partial sequence	Not reported	Not reported	57, 168, 169
Olive colobus, <i>Procolobus verus</i>	SIVolc	West Africa	40%	Partial sequence	Not reported	Not reported	57

None given—names for new SIVs are not given when evidence is serological only
 * SIV names are derived from common or scientific species and/or subspecies name, for example, SIVcpz is simian immunodeficiency virus chimpanzee and SIVagm sab is simian immunodeficiency virus sabaeus African green monkey

† Cross-species transmitted in captivity
 ‡ Cross-species transmitted in the wild

complete viral sequences are available for 36 species, and 6 additional species have been reported to harbor SIV-specific antibodies. In almost all cases, infected NHP species of African origin represent the natural reservoir of that particular SIV. The SIVs from naturally infected NHPs of African origin hosts are designated by a three-letter abbreviation of the host primate species. There are many experimentally derived strains of SIV and they have a different nomenclature (see Chapter 4). The first known SIV from an African NHP is SIVsmm. The name designates the SIV that naturally infects the SM monkey, albeit somewhat redundantly since all SMs are monkeys. In the case of SIVs from African green monkey (AGM) species in the genus *Chlorocebus* (SIVagm, the common name of the genus and the species are included in the virus designation. The common names of the four species of AGMs are vervet, grivet, tantalus, and sabaeus, and are infected by SIVagm.Ver, SIVagm.Gri, SIVagm.Tan, and SIVagm.Sab, respectively. For chimpanzee subspecies infected with SIVs, there is an exception to this rule; each SIVcpz isolate is named from the known or last known country of origin of the chimpanzee. Thus, the *Pan troglodytes troglodytes* subspecies is infected by SIVcpzGAB (Gabon), SIVcpzCAM (Cameroon), and SIVcpzUS (a captive chimpanzee in the United States), whereas *P. t. schweinfurthii* is infected by SIVcpzANT [this SIV-infected chimpanzee originated from the Democratic Republic of Congo (DRC) but SIVcpzANT was found in a captive chimpanzee in Antwerp], SIVcpzTAN (Tanzania), and SIVcpzDRC1.^{55,91,199,223,224,238,295} Some authors have adopted the abbreviations SIVcpzPtt and SIVcpzPts to differentiate between the SIV strains infecting these two subspecies of chimpanzees.^{122,251}

Names of the individual isolates of different SIVs may include the country of origin; thus, SIVmnd-1GB1 and SIVrcmGAB1 are viruses isolated from a mandrill and a red-capped mangabey in Gabon, respectively,^{94,279} whereas SIVrcmNG409 originates from Nigeria.²¹ The sampling year may also be included as for SIVsmmSL92a which is an SM virus isolated from samples collected in Sierra Leone in 1992.⁴⁸ Subsequent isolates in the same year were designated b, c, d, e, and f.⁴⁸ This feature is useful in tracing the origin of viruses allowing for a better understanding of their evolution. In a recent paper, an attempt has been made to rename SIVs using a three-letter code. This method would introduce modifications to the current nomenclature, that

is, SIVsm becoming SIVsmm, while SIVlhoest would become SIVlho.²⁷

The list of SIV sequences in the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide&itool=toolbar>) is ever changing, so any listing is immediately out of date almost as soon as it is written. In mid-2008, there were 48 fully sequenced SIV genomes representing 25 different African NHP species. Partial genomic sequences are available for 11 additional SIVs, and serological evidence of SIV infection has been obtained for 6 primate species for which no sequence information has been obtained. Ten infectious molecular SIV clones have been derived from different NHP species, namely, the rhesus macaque (Rh), an experimental host (see Chapter 4), and the natural hosts, SM and AGM. The rhesus macaque, *Macaca mulatta*, is of Asian origin and is an experimental host. SIV does not naturally infect macaque species.

3.3. SIV AND PRIMATE SPECIES RADIATION

SIV is a virus with an African origin. Asian species of Old World monkeys, the Asian colobines, and macaques are not naturally infected with SIV, which suggests that the last common ancestors of the catarrhines (all Old World monkeys and apes) were not infected by SIV 25 million years ago.^{20,105} Therefore, SIV emerged after radiation by these species, possibly from a nonprimate source.²⁴⁸

3.4. SIV PHYLOGENETIC CLASSIFICATION

The phylogeny of SIVs partially follows the phylogeny of NHPs, but there are significant exceptions. When the classification of SIVs and their natural host are collinear, the SIVs diverged in parallel with their natural hosts. A good example is the arboreal guenons (Figure 3.4). They are infected with SIVs sharing biological properties, structural features, and form a single phylogenetic cluster.²⁷ The arboreal guenons form a cluster²⁷⁴ as do the SIVs that infect them (Figure 3.4). Conversely, each of the terrestrial genera is infected with different viral lineages, with the exception of *Erythrocebus*, which carries a cross-species-transmitted SIVagm.²⁸

The Papionini group, mangabeys, mandrills, and drills, are infected with related viruses, though a higher proportion of recombinant viruses can be observed in these monkeys (Figure 3.2c).^{134,264}

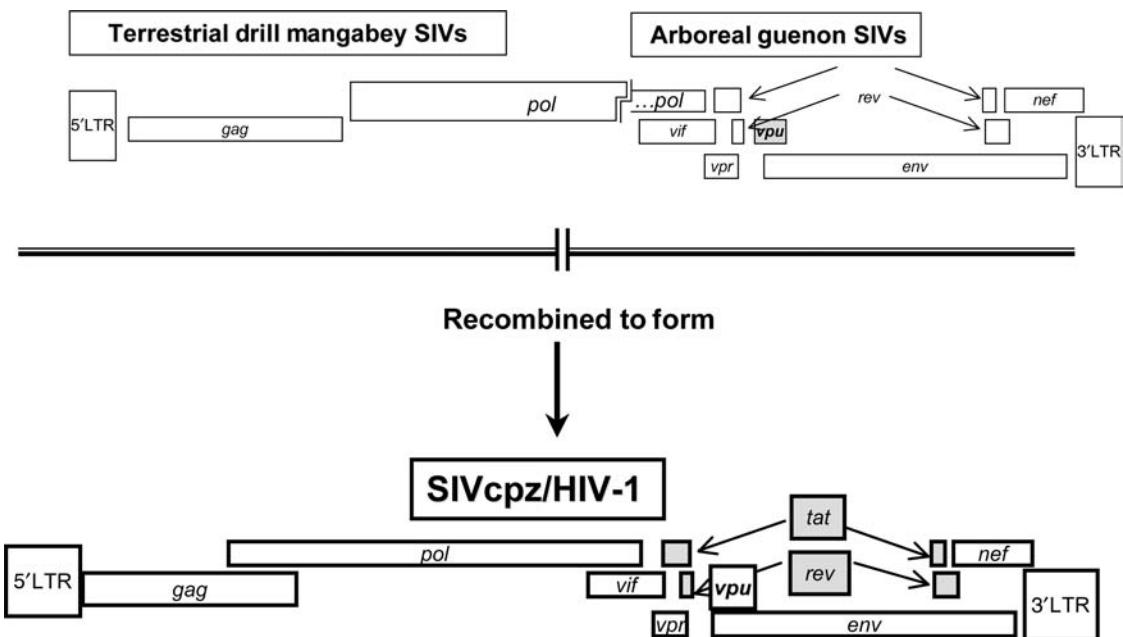


Figure 3.3. Left- and right-half of the SIVcpz genome. SIVcpz is a recombinant virus formed by SIVs from two different SIV lineages.¹⁴ The left-half of an SIV from the drill mangabey group (e.g., SIVrcm) recombined with the right-half of an SIV from the arboreal guenon group (e.g., SIVmsn) to form SIVcpz, which in turn crossed to humans to form HIV-1. Recombination is a key mechanism for generating new SIVs that can infect new hosts.

Phylogenetic analyses of SIV have identified a starburst phylogenetic pattern, which suggests SIV's evolution from a single ancestor.^{10,285} The phylogenetic distances among the major SIV lineages do not always match the phylogenetic relationships among their hosts. SIV in a particular species may be recombinants between highly divergent viruses,²⁵¹ or cross-species transmissions.²⁸ Therefore, such recombinant SIVs complicate phylogenetic trees because parts of their genome have originated from different SIVs. SIVcpz is the best-known example of recombination between highly divergent. Early classifications identified six phylogenetic lineages: SIVcpz/HIV-1, SIVsmm/HIV-2, SIVagnm, SIVlhoest, SIVsyk, and SIVcol.^{20,58,250} The concept of “pure” viruses was introduced and means an SIV that diverged over time as one virus without recombination. However, it has been suggested that the definition of “pure” versus “recombinant” lineages is primarily a matter of chronology and that each of the “classical” lineages might in fact be recombinants.²³⁶ With the discovery of new SIVs, some of the classical

lineages were indeed shown to be formed by recombinant strains, most notably the SIVcpz/HIV-1 lineage (Figure 3.3). The remaining “nonrecombinant” strains cluster into six lineages approximately equidistant, with genetic distances of up to 40% in Pol proteins. These lineages are described below.

3.4.1. SIVs from Arboreal Guenons

Guenons are in the *Cercopithecus* genus and are naturally infected with nine distinct SIVs: (1) SIVsyk (Sykes’ monkey),^{27,125} (2) SIVblu (blue monkey),²⁷ (3) SIVgsn (greater spot-nosed guenon),^{56,59} (4) SIVdeb (deBrazza monkey),²⁷ (5) SIVmon (mona monkey),⁵⁶ (6) SIVden (Dent’s mona),⁶⁵ (7) SIVmus (mustached monkey),^{1,56} (8) SIVasc (ascalus monkey),²⁸⁸ and (9) SIVtal (talapoin monkey).^{162,209} Partial sequences available for SIVbkm from the black mangabey cluster in this lineage.²⁷²

This SIV lineage is of particular importance because it contains one of the two ancestral viruses of SIVcpz.²⁵¹

SIVcpz is the immediate ancestor of HIV-1, emerging from cross-species transmission to humans in the recent past. A key to understanding the ancestry of SIVcpz and HIV-1 is the origin of the *vpu* gene. Four SIVs from the arboreal guenon group have the *vpu* gene, SIVgsn, SIVmon, SIVmus, and SIVden. One of these SIVs likely contributed the 3'-half of the SIVcpz genome (Figure 3.3). An SIV from the baboon–mangabey group provided the 5'-half of the genome (Figures 3.2c and 3.3).

3.4.2. SIV from Sooty Mangabeys

SIV naturally infects SMs (*Cercocebus atys*) (SIVsmm). The closely related viruses SIVmac, SIVb670, and HIV-2 are all derived from the sooty mangabey SIV lineage.^{9,124,194,240} SMs from the Ivory Coast harbor SIVsmm strains related to the epidemic HIV-2 groups A and B; SIVsmm from SMs in Sierra Leone and Liberia are the sources of HIV-2 groups C–H. SIVmac was discovered in Asian macaque monkeys that had been inadvertently infected by inoculation of tissues from SMs in the 1970s and 1980s.^{10,176,194}

3.4.3. SIVs from the Four Species of AGMs (SIVagsm)

Four different but closely related SIVs have been described for each of four species in the *Chlorocebus* genus: (a) SIVagsm.Ver for *C. pygerythrus*, the vervet; (b) SIVagsm.Tan for *C. tantalus*, the tantalus monkey; (c) SIVagsm.Gri for *C. aethiops*, the grivet; and (d) SIVagsm.Sab for *C. sabaeus*, the sable monkey.^{3,85,87,127,138,191} The geographical range of SIVagsm in their natural hosts is from the West African coast across to East Africa and south to Southern Africa encompassing virtually the entire African continent south of the Sahara desert. The distribution and phylogenetic relationships suggest that SIVagsm diverged through host-dependent evolution. There is one recombinant in the group, SIVagsmSab, which resulted from a recombination event between an SIVagsm ancestor and an SIVrcm-like virus.¹⁴

3.4.4. SIVs from L'Hoest Supergroup (*C. l'hoesti*, *C. solatus*, *C. preussi*) and Mandrill (*Mandrillus sphinx*): SIVlhoest, SIVsun, SIVpre, SIVmnd-1

This lineage emerged through host-dependent evolution of monkeys in the *C. l'hoesti* supergroup.^{18,19,123,278,279}

Cross-species transmission from the solatus guenon to mandrills was at the origin of SIVmnd-1.¹⁹

3.4.5. SIV from Red-Capped Mangabeys (*Cercocebus torquatus*): SIVrcm

Originally, this virus was considered to be a recombinant,^{21,94} however, further phylogenetic analysis places SIVrcm as a “pure” virus.¹⁴ The origin of SIVmnd-2 is from a recombination between an SIVrcm-like virus and SIVmnd-1;²⁶⁴ SIVcpz resulted from a recombination between an SIVrcm-like virus and one of the SIVgsn/SIVmon/SIVmus viruses.¹⁴

3.4.6. SIV from the Mantled Colobus (*Colobus guereza*): SIVcol

This virus was the first SIV isolated from the *Colobinae* family; other viruses from the Western colobus species do not cluster with SIVcol.^{57,58,168}

All SIV lineages have two or more strains (Figure 3.4). The l'hoesti lineage is formed by SIVs circulating in distantly related species. These phylogenetic clusters can be partially superimposed on the NHP phylogenetic trees.

SIVs that infect apes contain the *vpu* gene, whereas papionini-infected SIVs contain a *vpx* gene.^{11,13} Three of eight guenon species have been shown to harbor *vpu*-containing viruses (*C. mona*, *C. mitis*, and *C. cephus* groups). *Chlorocebus*, *C. l'hoesti* supergroup, and *Miopithecus* monkeys have an eight-gene organization, whereas *Allenopithecus* and *Erythrocebus* have no specific SIV. These findings point to the Cercopithecini as the origin of SIVs or at least as the major reservoir of SIVs. Since *vpu* first appeared in cercopithecines, Cercopithecini also appear to be an ancestral to viruses in the SIVcpz/HIV-1 lineage.¹⁴

SIV phylogenetic lineages have become difficult to superimpose on primate phylogeny as new strains are identified. It may ultimately be more effective to classify primate lentiviruses based on the genomic organization (i.e., accessory genes present).

3.5. SIV GENOMIC ORGANIZATION

3.5.1. Simian Lentivirus Genes and their Gene Products

The majority of the gene products follow the description in the “Introduction to Retroviruses.” This chapter focuses on the additional features of the genome not held

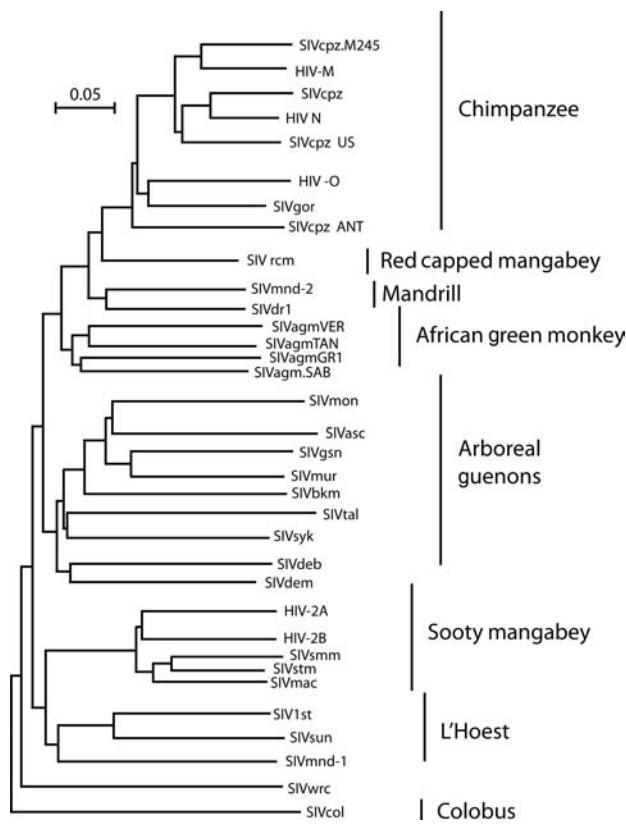


Figure 3.4. Phylogenetic tree of the simian immunodeficiency viruses (SIVs). SIVs segregate into discrete groups or lineages. This tree is constructed from *gag* gene relationships, but other genes may be used to construct trees of SIV. SIV clusters are marked by vertical lines. Viruses in the clusters are more closely related than to viruses in other branch clusters. For example, SIVagm isolates are closely related to each other and are distant from SIVcpz. (See Chapter 1 for general description of phylogenetic trees.)

in common with other retroviruses, in particular the accessory genes, *tat*, *rev*, *vpu*, *vpx*, *vpr*, *vif*, and *nef*. These accessory genes are present in complex retroviruses.

3.5.1.1. GAG

This gene encodes the capsid proteins and is an abbreviation of group-specific antigen. The p55 myristylated precursor protein is cleaved into the virion proteins matrix (MA), capsid (CA), and nucleocapsid (NC) as described in Introduction to Retroviruses. An additional protein, the p6 protein, is also cleaved from the SIV p55 precursor.

3.5.1.2. POL

The viral enzymes: protease, reverse transcriptase, and integrase are encoded by the *pol* gene. They are produced as a Gag-Pol precursor polyprotein, which is generated by ribosomal frameshifting near the 3'-end of *gag* and it is processed by the viral protease.

3.5.1.3. ENV

This gene codes for the two viral glycoproteins (SU and TM) that are produced from a gp160 precursor which is processed to generate surface glycoprotein (SU) (gp120) and a transmembrane glycoprotein (TM) (gp41) as described in Introduction to Retroviruses. The mature gp120-gp41 proteins are bound by noncovalent interactions and form a trimer on the virion surface.²³⁵ The gp120 mediates entry into a susceptible cell in a two-step process (Figure 3.5). First gp120 binds to the CD4 molecule, the cell surface attachment site on T cells and macrophages.¹⁵⁰ The second step involves binding to a coreceptor that mediates fusion of the virion to the cell membrane. The main coreceptor is CCR5, a member of the seven transmembrane domain chemokine receptor family. SIVrcm is the exception in that uses CCR2 as its coreceptor.⁴⁵

3.5.1.4. TAT

Tat is the transactivator of SIV gene expression and together with *rev* is one of the two regulatory genes of

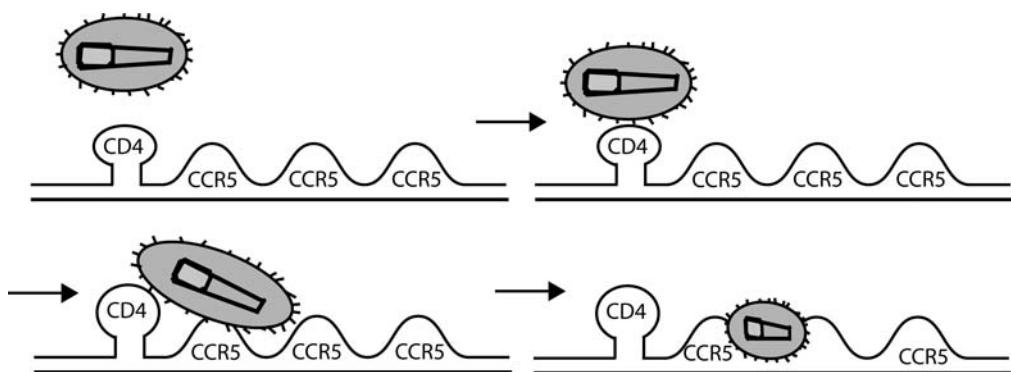


Figure 3.5. Binding of simian immunodeficiency virus (SIV) to cell surface receptor and coreceptor. (Top left) SIV in proximity to surface CD4 molecule on the cytoplasmic membrane of a susceptible cell, a T cell or a macrophage. (Top right) Virion binds to CD4 molecule. (Bottom left) Interaction of the virion with the CD4 molecule leads to binding of the CCR5 surface membrane molecule. (Bottom right) Virion envelope fuses with cell membrane leading to entry into the cell and the initiation of the infection. Some simian lentiviruses use coreceptor other than CCR5, such as CXCR4 and CCR2 (45,246).

SIV gene expression. There are two *tat* exons: exon 1 (minor form) which is composed of 72 amino acids and exon 2 (major form) of 86 amino acids.¹⁵⁵ Persistently SIV-infected cells contain low levels of both proteins in the nucleolus/nucleus. Tat binds the target sequence for viral transactivation (TAR) RNA element and activates transcription initiation and elongation from the long terminal repeat (LTR) promoter. Thus, Tat prevents premature termination of transcription and polyadenylation by the 5'-LTR AATAAA polyadenylation signal.¹⁵⁵ Tat protein can be released in the extracellular media and be taken up by other cells in culture.

3.5.1.5. REV

Rev is the second regulatory protein of SIV expression. *Rev* codes for a 19-kDa phosphoprotein that is localized in the nucleolus/nucleus.¹⁷⁴ Rev is the most functionally conserved regulatory protein among the SIVs. It binds to the rev responsive element (RRE) and promotes the nuclear export of unspliced genomic RNAs. Rev functions in the stabilization and utilization of the viral mRNAs containing RRE.²³³ Rev cycles rapidly between the nucleus and the cytoplasm.

3.5.1.6. VIF

The *vif* gene codes for a viral infectivity factor, a protein of 23 kDa, which is present in the majority of

lentiviruses. This protein promotes the infectivity but not the production of viral particles. In the absence of Vif, viral particles are defective, although cell-to-cell transmission of virus is not affected significantly. Vif is a cytoplasmic protein, which can be found both soluble in cytosol and tightly associated to the inner side of the plasma membrane.³⁰⁰ Vif prevents the action of the cellular resistance factor, called the APOBEC protein system. APOBEC proteins deaminate DNA:RNA heteroduplexes in the cytoplasm.⁹⁰ Therefore, Vif is required for bypassing host restrictions by cross-species-transmitted SIVs.

3.5.1.7. VPR

This gene codes for a Vpr (viral protein R), which is a 96-amino acid (14 kDa) protein incorporated into the virion. In the cell, Vpr is localized in the nucleus. It interacts with the Pr55 Gag precursor (p6 Gag component). Vpr is involved in targeting nuclear import of preintegration complexes, cell growth arrest, transactivation of cellular genes, and induction of cellular differentiation.^{6,96} The *vpx* gene resulted from a *vpr* gene duplication event by recombination.²⁴⁹

3.5.1.8. VPU

This gene encodes Vpu (viral protein U), an 81-amino acid (16 kDa) type I integral membrane protein,⁵³ which

is only present in HIV-1, SIVcpz (the simian ancestor of HIV-1), SIVgsn, SIVmus, SIVmon, and SIVden. There is no similar gene in other SIVs. Vpu biological functions consist of degradation of CD4 in the endoplasmic reticulum, enhancement of virion release from the plasma membrane of HIV-1-infected cells, and Env maturation.¹³² A bicistronic mRNA codes for Env and Vpu. Vpu possesses an N-terminal hydrophobic membrane anchor and a hydrophilic moiety and it is phosphorylated by casein kinase II.¹³² Vpu is not present in the virion. Recently, Vpu was found to increase susceptibility of HIV-1-infected cells to Fas killing. It has been suggested that Vpu is involved in virus released from infected cells.¹⁹⁷

3.5.1.9. NEF

The product of *nef* is 27-kDa-myristylated protein coded by a gene located at the 3'-end of SIVs. Nonmyristylated variants are also described. Nef is a cytoplasmic protein that binds to the plasma membrane by the myristyl residue.²²⁸ Additional locations described for Nef are in the nucleus and associated with the cytoskeleton. Nef is the most immunogenic of the accessory proteins and is one of the first viral proteins produced in infected cells.¹⁴⁸ SIV Nef is dispensable in vitro.¹⁹⁰ Nef has not been well studied in vivo in its natural host. SIVmac *nef* in the Rh experimental host is essential for efficient viral spread and disease progression in vivo,¹⁴⁹ being necessary for the maintenance of high virus loads (VLs) and disease progression in macaques.^{61,133} Whether or not *nef* has similar functions in its natural host seems doubtful since natural SIV infection rarely progresses to disease. Nef is reported to be responsible for the lack of immune activation observed in natural SIV infections.²⁴²

Nef interacts with components of host cell signal transduction and clathrin-dependent, protein-sorting pathways.¹⁶⁷ It increases viral infectivity. Nef downregulates CD4, the primary viral receptor, and MHC class I molecules. The PxxP motifs that bind to SH3 domains of Src kinases are present in Nef and are required for the enhanced growth of HIV but not for CD4 downregulation.

3.5.1.10. VPX

Vpx is only present in HIV-2, SIVsmm, SIVrcm, SIVmnd-2, and SIVdrl and is not in HIV-1 or other SIVs. *vpx* is a homolog of *vpr*, and viruses with Vpx carry both *vpr* and *vpx*. This gene codes for a virion protein of 12 kDa. Vpx function is not fully elucidated; both

Vpx and Vpr are incorporated into virions at levels comparable to Gag proteins. Vpx is necessary for efficient replication of SIVsmm in peripheral blood mononuclear cells (PBMCs).¹²⁹

3.5.2. CLASSES OF SIV PROTEINS

3.5.2.1. STRUCTURAL PROTEINS/VIRAL ENZYMES

These gene products are coded by the structural *gag*, *pol*, and *env* genes. All these products are essential components of the retroviral particle and follow the general scheme of expression and function described in Sections 3.5.1.5 and 3.5.1.6, and Introduction to Retroviruses.

3.5.2.2. REGULATORY PROTEINS

Tat and Rev proteins modulate transcriptional and post-transcriptional steps of virus gene expression. They are essential for virus propagation and are described in Section 3.5.1.5.⁶⁰

3.5.2.3. ACCESSORY/AUXILIARY PROTEINS

Vif, Vpr, Vpu, Vpx, and Nef are represented by additional virion- and nonvirion-associated proteins. In general, the accessory proteins are not necessary for viral propagation in tissue cultures; however, they are conserved in the different isolates, which suggests that their role in vivo is essential.

3.5.3. SIV GENOMIC STRUCTURAL ELEMENTS

3.5.3.1. LONG TERMINAL REPEATS

LTRs are the DNA sequences that flank the genome of integrated proviruses. LTRs contain important regulatory regions, especially those involved in transcription initiation and polyadenylation²³⁴ (see Introduction to Retroviruses).

3.5.3.2. TARGET SEQUENCE FOR VIRAL TRANSACTIVATION

TAR is the binding site for Tat protein and for cellular proteins. TAR consists of the first 100 nucleotides in the SIV genome (it is the first 45 nucleotides in HIV-1).²⁴ TAR RNA has a hairpin stem-loop structure with a side bulge, which is necessary for Tat binding and function.²⁴

3.5.3.3. REV RESPONSIVE ELEMENT

RRE is an RNA element that consists of approximately 200 nucleotides spanning the border of gp120 and gp41.

RRE is necessary for Rev function. RRE contains seven binding sites for Rev existing within the RRE RNA.²⁰⁶

3.5.3.4. Cis-Acting Repressive Sequences

Cis-acting repressive sequences (CRS) are postulated to inhibit structural protein expression in the absence of Rev. One such site was mapped within the *pol* gene of HIV-1.³⁹ The exact function has not been defined; splice sites may act as CRS sequences.

3.5.3.5. Inhibitory/Instability RNA Sequences

Inhibitory/instability RNA sequences (INS) are found within the structural genes of SIVs. There are multiple INS elements in the genome and they can act independently. They have been defined by functional assays as elements that inhibit posttranscriptional expression.²⁴⁵ Mutation of the RNA elements leads to INS inactivation and upregulation of gene expression.

3.5.4. SIV Diversity Relative to Viral Genomic Structure

Using gene and ORF structure as a second method for determining relatedness, three groupings of SIVs are identified. All primate lentiviruses harbor five regulatory genes (*vif*, *rev*, *tat*, *vpr*, and *nef*) that generally fall in the same regions of the SIV genome. *Tat* and *rev* each consists of two exons. The presence of two other regulatory genes (*vpx* and *vpu*) is variable and thus defines three patterns of genomic organization (Figure 3.2).

- SIVsyk, SIVasc, SIVdeb, SIVblu, SIVtal, SIVagn, SIVmnd-1, SIVhoest, SIVsun, and SIVcol contain five accessory genes, the fewest among the SIVs (Figure 3.2a). They are *tat*, *rev*, *nef*, *vif*, and *vpr*.^{18,19,27,58,64,123,125,127}
- SIVcpz, SIVgsn, SIVmus, SIVmon, SIVden, and HIV-1 genomes have an additional supplementary gene, *vpu*.^{56,65,135,290} (Figure 3.2b). This group lacks *vpx*.
- SIVsmm, SIVrcm, SIVmnd-2, SIVdrl, SIVmac, and HIV-2 form the third genomic group, which is characterized by the presence of the *vpx* gene and the absence of *vpu*.^{21,42,112,128,134,264} (Figure 3.2c). Both HIV-2 and SIVmac were derived directly from SIVsmm. Thus far, *vpx* appears to be specific for SIVs infecting the Papionini group of monkeys (mangabeys, drills, and mandrills) and was acquired by a nonhomologous recombination which resulted in a duplication of the *vpr*.²⁴⁹

- SIVblu, SIVvolc,⁵⁷ SIVwrc,⁵⁷ SIVasc,²⁸⁸ and SIVbkm²⁷² are frag-viruses²⁸⁹ and have not been completely sequenced or cultured in vitro. It is not yet possible to characterize these viruses by genomic organization.

3.6. PREVALENCE OF SIV IN THE NATURAL HOSTS

3.6.1. SIV Infection in Apes

Studies of SIV seroprevalence in great apes revealed that SIV naturally occurs in only two subspecies of chimpanzees: *Pan troglodytes troglodytes* and *P. t. schweinfurthii*.^{55,91,135,223,224,237–239,241} In addition, gorillas (*Gorilla gorilla*) are infected with specific SIV strain, SIVgor.²⁸² Based on phylogenetic relationships (Figure 3.4), the prevailing view is that HIV-1 originated from SIVcpzPtt, which naturally infects the common chimpanzee (*P. t. troglodytes*). SIVgor, which naturally infects gorillas in Cameroon, is closely related to HIV-1 group O.²⁸² It is not known if gorillas were infected by cross-species transmission of SIVcpz, but this possibility seems likely. SIVcpz-infected chimpanzees are known in Gabon, Cameroon, and Democratic Republic of Congo.^{55,91,135,223,224,237–239,241} SIVcpz.US was identified in a captive chimpanzee in the United States (SIVcpzUS).^{91,98}

The prevalence of SIVcpz was believed to be very low.^{91,98}

Foci of endemic SIVcpz infections were documented in eastern chimpanzees, *P. t. schweinfurthii*.^{237,239,241} However, an extensive seroepidemiological study conducted in Cameroon using a noninvasive sampling approach¹⁴⁷ reported a higher seroprevalence (10%) in the common chimpanzee.^{147,281} Moreover, in this study, SIVcpz strains closely related to HIV-1 group M and group N were described.²⁸¹ These findings are consistent with the emergence of the three HIV-1 groups (M, N, and O) in this region of Africa.^{66,111,258,284}

Despite extensive testing, naturally occurring lentiviruses have not been detected in West African chimpanzees (*P. t. verus*)²²⁹ or *P. t. vellerosus* subspecies, though one *P. t. vellerosus* contracted SIV from *P. t. troglodytes* in captivity.^{55,229,271} The lack of SIV in West African *P. t. verus* suggests that the origin of SIVcpz was after the split of *P. t. verus*.

Seroepidemiological studies failed to produce evidence of SIV infection in bonobos (*Pan paniscus*).²⁸⁰ Given that bonobos are frugivorous, they do not

experience interspecies aggression and predation, suggesting this behavior might mitigate against lentiviral infection.^{91,98}

3.6.2. SIV in Guenons

Due to their number, genetic diversity, and large distribution in sub-Saharan Africa, guenons (tribe Cercopithecini) are the largest reservoir for SIV. Studies of hundreds of wild-born AGMs belonging to different subspecies reveal a prevalence of SIVagm infection of 40–50%.^{121,172,205} These prevalence levels are similar in all four AGM subspecies, vervet (*Chlorocebus pygerythrus*), grivet (*C. aethiops*), tantalus (*C. tantalus*), and sabaeus (*C. sabaeus*), and are independent of geographic origin.¹⁹¹ Interestingly, seroepidemiological studies showed that AGMs from Caribbean Islands extensively imported from Africa in the seventeenth and eighteenth centuries⁶⁷ are not infected with SIV.^{63,121} This lack of exposure has been attributed to the capture and movement of these animals as unexposed juveniles; an alternative and far less probable explanation is that SIVagm was not yet present in the AGM population two centuries ago.

High prevalence levels (30–60%) were also reported for SIVs infecting other species of African guenons: SIVlho from l’Hoest monkeys (*Cercopithecus l’hoesti*),¹⁸ SIVsyk from Sykes’ monkeys (*Cercopithecus albogularis*),^{78,125,273} SIVblu from blue monkeys (*Cercopithecus mitis*),¹¹⁵ and SIVdeb from de Brazza’s monkeys (*Cercopithecus neglectus*).² Prevalence might be lower (5–20%) for SIVs infecting other species: SIVmus from mustached monkeys (*C. cephus*)⁵⁶ and SIVgsn from greater spot-nosed monkeys (*C. nictitans*).^{2,59} No prevalence study exists thus far for the SIVs infecting different species of mona monkeys (*C. mona*); however, virus has been isolated from naturally infected mona monkeys (*C. mona mona*) from Nigeria and Cameroon,^{15,56} and from Dent’s monkey (*C. mona denti*) from the Democratic Republic of Congo.⁶⁵

3.6.3. SIV Infection in the Papionini Tribe

Several studies have produced compelling evidence that SMs are infected at high prevalence in the wild (25–55%) and also have suggested that the major route of transmission in this species is sexual.^{12,48,240} Pet SMs in Sierra Leone and Liberia have a 4–8% seroprevalence, probably as a consequence of being separated from feral populations as juveniles.^{12,48,179}

Prevalence of SIVrcm from red-capped mangabeys (RCMs, *Cercocebus torquatus*) is relatively low, 20%.^{94,259} SIV has not been found in a number of mangabey species. Sera from three-dozen gray-cheeked mangabeys (*Lophocebus albigena*) did not reveal any seropositive animals. No data are available concerning SIV prevalence in Eastern mangabeys, Tana river mangabey (*Cercocebus galeritus*), Sanje mangabey (*C. sanjei*), or the newly described highland mangabey (*Lophocebus kipunji*).¹⁴²

A high prevalence (>60%) was described for both SIVmnd-1 and SIVmnd-2 infection in wild mandrills (MNDs, *Mandrillus sphinx*) from Gabon and Cameroon, respectively,^{2,264} with a lower prevalence in juvenile MNDs.^{95,172,277}

To date, there is no proof that baboons (genus *Papio*) are naturally infected with SIVs. Several studies reported relatively frequent serological reactivities on both ELISA and Western blot when testing baboon sera.^{23,151,259} However, no species-specific virus was recovered from different baboon species. Two baboon species (*Papio anubis* and *P. hamadryas*) have been shown to carry SIVagm from sympatric green monkeys.¹³⁹

3.6.4. Colobinae

A relatively high prevalence of SIVcol (30%) was reported for wild-born mantled colobus (*Colobus guereza*) monkeys from Cameroon.⁵⁸ Three species of West African colobines have been evaluated for the presence of SIV seroreactivity and >40% of animals tested seropositive. Two isolates were characterized from this population; SIVolc from olive colobus (*Procolobus verus*) and SIVwrc from western red colobus (*Piliocolobus badius*).^{57,168} To date, there is no study reporting SIV’s presence in Asian species of colobus.

Serosurveys of other species groups including *Cercopithecus hamlyni*, *Allenopithecus nigroviridis*, *Lophocebus albigena*, *C. pogonias*, and *C. lowei* have detected serum antibodies reactive against SIV, but no genetic evaluations have been performed.^{116,172,222} These viruses, therefore, cannot be placed in any of the SIV groups.

3.6.5. SIV Distribution in Simian Species and Subspecies

Comparative analysis of simian lentiviral distribution revealed that, similar to the recent emergence of HIV in

humans, chimpanzee lentiviral infections appear to be relatively recent, with more limited distribution and a lower seroprevalence compared to infections of AGMs and SMs. Interestingly, SIV infection has been confined to species of African origin. Because the dynamics of lentiviral evolution occurs on a shorter timeframe than species evolution, these infections may be useful in tracking species dispersion, radiations, and population declines or expansions.²⁹ In populations with high seroprevalence, these viruses may drive species evolution, as has been suggested may occur in regions with high HIV seroprevalence.²⁸⁵

3.7. SIV ROUTES OF TRANSMISSION

The routes of transmission in the wild are difficult to establish. However, some information can be derived from studies of wild-caught animals showing that transmission primarily occurs mostly after sexual maturity.

3.7.1. Sexual Transmission

Serological surveys in AGMs, SMs, and MNDs revealed higher prevalence in adult monkeys than in juveniles, indicating a horizontal route of transmission. SIVagm prevalence in adult AGMs may reach 80%, fourfold higher than in juveniles aged 1–3 years, suggesting SIV transmission during mating.^{70,225}

Studies of a semi-free colony of MNDs found no evidence of sexual transmission after 16 years of follow-up.^{54,80,95,264} Two of the founders (one female and one male) were infected with different viral types, SIVmnd-1 and SIVmnd-2, respectively.²⁶⁴ SIVmnd-1 had been transmitted to four offsprings (males and females) of the SIVmnd-1-infected female founder. SIVmnd-2 had been transmitted from the infected male founder to four other males, following aggressive contacts for dominance.^{198,264} Interestingly, two of the dominant males became SIVmnd-2-infected, without evidence of sexual transmission. However, in wild MNDs in Gabon, SIVmnd-1 infection has been diagnosed in both sexes.²⁶⁴

Several cases of horizontal transmission occurring by biting have been described in captive monkeys: AGMs,^{70,225} SMs,²³⁰ and chimpanzees belonging to two different subspecies.⁵⁵ SIVsmm has reportedly been transmitted among macaques by biting.¹⁷¹ The lower prevalence in pet monkeys¹⁷⁹ also supports SIV transmission after sexual maturity.

3.7.2. Vertical Transmission

SIV vertical transmission seems less frequent than horizontal transmission, and if it does occur, the point of transmission (in utero, perinatally, or via breast milk) has not been identified. Some recent studies, however, suggest vertical transmission as a potential mechanism of SIVsm transmission, based on phylogenetic clustering of strains.^{12,240} In a recent prospective study, experimental mother-to-offspring transmission by breastfeeding was not observed in MNDs,²¹⁸ while another study did not demonstrate vertical transmission in AGMs.²¹⁰

3.7.3. Transmission within Species

Epidemiologic patterns of seroconversion in wild NHP populations endemically infected with SIVs suggest that these agents are most efficiently transmitted by contact among adults, including aggressive contact. Considering that it is well established that HIV is spread by sexual contact through mucosal exposure, it is likely that naturally occurring SIVs are spread through this route as well. While mother-to-offspring transmissions have been reported in NHPs, this is relatively rare compared to horizontal transmissions.

3.8. CROSS-SPECIES TRANSMISSION OF SIVs

Cases of SIV infections that have crossed species barriers have been documented; however, these events are relatively rare, despite ample opportunity for cross-species transmission to occur.

Clear evidence of the potential of SIVagm cross-species transmission has been proven in the wild, by isolation of SIVagm from a yellow baboon (*Papio cynocephalus*),¹³⁹ a chacma baboon (*P. ursinus*),²⁸³ and a patas monkey (*Erythrocebus patas*).²⁸ In captivity, in Kenya, SIVagmVer was transmitted to a white-crowned mangabey (*Cercocebus lunulatus*) housed at the same primate center, although the route or means of transmission was not established.²⁷³ Systematic prevalence studies have not been carried out to determine if SIVagm is established as an endemic virus in these species, or if the isolation of these strains is the result of unique, artefactual transmissions. There has been no long-term follow-up to determine if SIVagm is pathogenic in African NHP species following cross-species transmission. It is noteworthy that none of these species were reported to date to carry their own species-specific SIV, which may explain a higher susceptibility to cross-species-transmitted infections.

In some instances, described in the next section, cross-species transmission of SIVs may be followed by recombination in the new host. Recombination events have been documented to occur in SIV infections, both during cross-species transmission and between different viral subtypes within the same species.

3.9. CROSS-SPECIES TRANSMISSION FOLLOWED BY RECOMBINATION

The most interesting recombination of SIVs appears to be that involving SIVgsn/mon/mus and SIVrcm, resulting in the emergence of a new SIV, the chimpanzee virus SIVcpz.^{14,251} This recombination between two monkey SIVs was likely responsible for the emergence of SIV into apes and subsequently to humans. Cross-species transmission from chimpanzees to humans was the origin of the HIV/AIDS pandemic.^{55,91,135,147,223,224,237–239,241,282}

Although chimpanzees have been shown to be a reservoir for SIV,²⁴¹ available data fail to demonstrate that SIVcpz coevolved with its host.^{158,229,241,271} Therefore, it has been postulated that chimpanzees acquired SIVcpz after their divergence into western, central African, and east African subspecies about 1.5 million years ago.²⁵¹

Genomic analysis offers strong support that SIVcpz is a recombinant virus. In the 5'-region of the genome, SIVcpz's closest relative is SIVrcm,^{21,94} whereas in the 3'-half of its genome, it most closely aligns with SIVgsn.⁵⁹ SIVgsn was the first monkey SIV identified with the *vpu* gene, an accessory gene also found in SIVcpz.^{59,135} Initially, SIVrcm and SIVgsn were believed to be recombinant viruses resulting from recombination of SIVcpz and unidentified SIV lineages;^{21,59} however, SIVs from other NHPs (mustached monkeys and mona monkeys) provide evidence of other SIV types similar to SIVgsn.^{15,56,65} Therefore, it appears that the SIVgsn lineage predates SIVcpz, which arose by interstrain recombination events.

Four scenarios may explain SIVcpz occurrence and emergence:²⁵¹ (i) chimpanzees acquired ancestral SIVs through monkey hunting, resulting in exposure to more than one SIV type,^{188,292} which then recombined in the new host; (ii) the two SIVs that generated SIVcpz were transmitted independently to different chimpanzees and then each spread separately in the new host population until coinfection occurred, resulting in SIVcpz; (iii) an ancestral SIV established itself as a chimpanzee virus, and following superinfection with a new SIV evolved

into SIVcpz present today; or (iv) the recombinant virus was generated in another monkey host species yet to be identified, and was subsequently transmitted to chimpanzees.

3.10. DIAGNOSING SIV INFECTIONS

3.10.1. Sample Collection

In general, simian species are very difficult to approach in the wild. There are well-known habituated groups of chimpanzees and gorillas, but taking blood samples from them is not an acceptable practice. Capture in the forest or savannah is possible, but difficult, disruptive, and perhaps even a dangerous practice for the well-being of free-ranging monkeys and apes. Several approaches, some quite innovative, have been used to estimate SIV's prevalence in African NHP hosts: (i) testing of monkeys in zoos and research colonies;^{7,9,88,172,194,273} (ii) testing of pet monkeys within their natural range;^{94,179,222} (iii) testing bushmeat in African markets;^{12,222} and (iv) use of noninvasive samples, such as urine and feces, for serological and molecular diagnostic purposes in wild or captive primates.^{136,166,237–241,281,282} All of these methods have limitations mainly in sensitivity and therefore generate a bias toward underestimation in prevalence estimates.

3.10.1.1. CAPTIVE MONKEYS AS A MODEL FOR THE STUDY OF SIV PREVALENCE

The study of monkeys in zoos or colonies may not reflect prevalence in the wild because most were captured as juveniles or bred in captivity. In wild monkeys, a significant increase in SIV prevalence has been found in adult wild monkeys as compared to juveniles.^{225,264} Also, close contact between captive monkeys in zoos and colonies provides opportunities for cross-species transmission.^{9,55,273} To overcome these problems it was proposed that the study of pet monkeys sampled within their natural range could provide significant information concerning the diversity of SIVs.¹⁷⁹ Although most of these pet monkeys are captured when young, and the prevalence levels might be lower than those in wild animals, pet monkey testing is a relevant approach for the study of SIV prevalence. They are wild-captured, thus they reflect the seroprevalence of feral animals. A main advantage is that the virus can be readily isolated from blood as opposed to fecal specimens that have not provided infectious SIV thus far. New SIVs have been discovered by studying household pets: the

closest simian counterpart of HIV-2, in an SM from Sierra Leone^{46,48} and SIVrcm, a virus which naturally infects the red-capped mangabey.⁹⁴ Testing of pet samples has confirmed previously obtained results with monkeys in zoos and colonies. Although mortality rates are high for pet monkeys in Africa, they may sometimes be resampled if subsequent analysis or confirmation of the infecting virus is required.

3.10.1.2. STUDY OF THE BUSHMEAT: ADVANTAGES AND LIMITATIONS

Reports have suggested that zoonotic transfers of primate lentiviruses to humans are due to bushmeat consumption and exposure to simian blood during hunting and food preparation.¹¹⁶ If this hypothesis is proven, humans in Central Africa, especially in rural regions, are at high risk for cross-species transmission of simian lentiviruses. Extensive studies conducted in Sierra Leone, Cameroon, and Gabon to evaluate the magnitude of this exposure^{12,222} showed high levels of prevalence, the overall seroprevalence of SIV infection in 11 tested species being 18%, ranging between 5 and 40% for different species.^{2,222} These prevalence levels are within the same range as previous estimates of SIV prevalence in the wild.⁴⁸ Therefore, results from bushmeat samples provide strong evidence of human exposure to SIV; however, the major limitation is that postmortem samples are in poor condition, thus not allowing virus isolation and further biological characterization. Its main advantage is that it offers large numbers of samples in a short period of time and provides estimations of prevalence in the wild.

3.10.1.3. SAMPLING URINE AND FECES

Most of the natural hosts of SIV are highly endangered species and therefore sampling blood from these animals is not usually feasible. Alternative noninvasive diagnostic strategies have been developed for testing SIV prevalence in wild NHPs. These strategies involve using urine and feces for serological diagnosis and viral RNA amplification. Initial evaluation showed that urine is highly sensitive (100%) and specific for the detection of anti-SIVcpz antibodies, whereas feces, which had a lower sensitivity for antibody detection (65%), were useful for polymerase chain reaction (PCR) amplification of viral RNA (positive result in 66% of cases).^{136,166,241} Despite the obvious advantages of a method that allows access to large numbers of samples, there are limitations: (i) the

nature of sample precludes standardization; thus, in spite of previous reports, feces from SIVmnd-1-infected mandrills in Central Gabon have been negative thus far, even though there is a very high seroprevalence of SIVmnd infection in that area²⁸⁵ and (ii) a positive animal cannot be readily tracked, so the virus cannot be isolated nor can its in vivo pathogenesis be investigated.

3.10.2. SEROLOGICAL TESTING

Serology is the gold standard for studying the prevalence of SIVs in NHPs. In the past, most laboratories screened NHPs for anti-SIV antibodies using commercial HIV and SIV ELISA and Western blot kits.^{21,47,78,88,94,191} ELISA screening tests are based on antigens consisting of viral lysates, recombinant proteins, or synthetic peptides corresponding to immunodominant epitopes of the two subtype HIV-1 B variants (strains LAI and MN) and HIV-2 group A (ROD strain). These "mixed" tests are therefore able to detect SIV antibodies that cross-react with HIV-1 and HIV-2.¹³ Cross-reactivity with other lentiviral lineages enables the use of these tests for screening NHP samples. For a more sensitive detection of SIVs in NHPs, two strategies have been developed. The first uses a highly sensitive line assay (INNO-LIA HIV, Innogenetics, Ghent, Belgium) as a screening test. Using this strategy, more than 10 different new SIV types have been identified in NHPs.²²² A second strategy uses synthetic peptides from gp41/36 and V3 peptides, allowing for increased sensitivity (Gp41/36) and specificity (V3 peptides).^{222,259} This technique has also led to the discovery of several SIVs.

3.10.3. CHARACTERIZATION OF NEW SIVS

Full-length sequences are required to fully describe a new SIV. The database is increasing each year and presently 45 full-length SIV genomes are available (<http://hiv-web.lanl.gov>) and their number is expected to increase, as significant advances have been recently reported in characterizing SIV diversity.^{13,285} The requirement of full-length sequencing of newly identified viruses is necessary to characterize their phylogenetic relationships and to identify recombinant structures. Also, full genome analysis will place new SIVs into one of the three established genomic groups (Figure 3.2). By this analysis, SIVs containing *vpx* or *vpu* have been recently identified.²⁸⁵ Altogether, these analyses will clarify the molecular evolutionary history of primate lentiviruses, as well as the potential risk for

infection to exposed humans. In order to “diagnose” a new virus, most investigators use PCR (or RT-PCR) for the amplification of the integrase region, which is the most conserved region in the SIV species. Several sets of primers have been evaluated for this purpose: Unipol,¹³ Hpol,¹³ DR,⁵¹ or Or/Is4.⁵⁸ Once the diagnostic fragment is characterized, specific primers can be designed to target the circular genomic forms in a “genome walking” approach.^{1,27,56,58,59,162,169}

3.10.4. Virus Isolation

The efficiency of the isolation of SIVs varies widely. Their ability to replicate in human PBMC or T-cell lines has been evoked as a major argument in favor of these viruses infecting the exposed human population. This ability has been documented for SIVcpz,²²³ SIVsm/SIVmac,⁹² SIVagm,¹⁰⁸ SIVlhoest,^{108,123} SIVmnd-1,²⁷⁷ SIVrcm,^{21,94} SIVmnd-2, and SIVdrl.¹³⁴ Altogether, these data confirm that SIV may have an intrinsic capacity to enter the human population through activated CD4⁺ T lymphocytes. The ability of SIV to infect human macrophages has been also reported for SIVagm and SIVmnd.¹⁰⁸ For HIV-1, it was shown that macrophage tropism was linked to the use of chemokine receptor CCR5, which is the major coreceptor in vivo for viral entry for most SIVs.¹⁸⁹ Human macrophages were infected by 75% of the tested SIV isolates.¹⁰⁸ The exceptions were SIVsun and SIVsyk, which did not replicate in human PBMC or macrophages. SIVagm also presents discordant patterns with low or absent replication in human PBMCs.¹⁰⁸ In addition, another study provided evidence that SIVmac239 replicated in human macrophages, which is remarkable, since this virus is considered T tropic and unable to replicate in human or rhesus macaque macrophages.³¹ Also, SIVmnd-1 GB1 was reported to replicate in human PBMCs but not in macrophages.¹³ These data are consistent with previous studies reporting exclusive use of CXCR4 as a coreceptor by this virus.²⁴⁶ However, SIVmnd-1 GB1 in the NIAID repository (<https://www.aidsreagent.org/Index.cfm>) had been previously adapted on SupT1 cells, which may have affected coreceptor usage. Some SIVs might require special culture conditions. The in vitro tropism of SIVsyk is highly restricted. This virus grows in Syke’s monkey PBMC, but only after CD8 cell depletion.¹²⁵ Data on in vivo viral replication showed that only one (SIVlhoest) out of four cercopithecini SIVs has the ability to

grow on human PBMCs and macrophages, suggesting that extensive in vitro studies on SIV’s properties are needed in order to correctly evaluate the risk for human population following exposure to SIV.

3.11. PATHOGENESIS OF NATURAL SIV INFECTION

SIV infection in the natural host has been studied in only three species: the SM, the AGM, and mandrill. SIV is not pathogenic in the vast majority of infected individuals. However, exceptions have been observed in a few naturally infected individuals after many years of SIV infections.

3.11.1. Pathogenicity of SIV Infection in African NHPs

For over 20 years, it has been the belief that SIV infections are nonpathogenic in their natural hosts.^{71,72,88,101–104,153,194,212,214–216,218,219,255–257} This observation depicted a major paradox in the face of active viral replication and high prevalence levels. More long-term studies have demonstrated that SIV infection in natural hosts can eventually lead to the development of immunodeficiency. However, clinical disease occurs in a minority of infected individuals and only after many years of infection. A few examples of immunodeficiency disease have been reported in mandrills and SMs.^{8,165,182,204,217,276}

Cases of naturally occurring SIV infections that progress to AIDS are rare, possibly because host and virus adaptation has occurred in favor of persistent infection with an incubation period that exceeds the normal life span of the naturally infected animal.²¹⁷ This hypothesis is supported by the observation that all cases of AIDS have occurred in monkeys significantly older than the mean life span of the species in nature.

3.11.2. SIV Receptor Use and Tropism

Most of the SIVs naturally infecting NHPs in Africa are reported to use the same system of receptors as HIV-1; that is, CD4 on the T-cell surface that binds *env* and a seven transmembrane chemokine as the cell surface coreceptor^{49,299} (Figure 3.5). For HIV-1 replication in vivo, the most relevant coreceptors are CCR5 and CXCR4;¹⁸⁹ in 50% of cases, progression to AIDS is characterized by a switch in viral tropism from CCR5 (so-called “macrophage” tropic) to CXCR4 (so-called “lymphocyte” tropic) viruses.¹⁸⁹ Most of the SIVs

naturally infecting African NHPs use CCR5 as the main coreceptor.^{49,299} However, in NHPs, no correlation can be established between the coreceptor usage and pathogenesis *in vivo*. Thus SIVmac, the SIV derived from SMs that is pathogenic for macaques, is CCR5-tropic in spite of being more virulent than HIV-1.²⁹⁹ SIVmnd-1, SIVagm.Sab, and some strains of SIVsmm were reported to use CXCR4, but no pathologic correlation has been described in these monkey species.^{211,215,246} Moreover, experimental infection of sabeaus AGMs with CXCR4-tropic SIVagmSab does not show a different pattern of viral replication or disease progression compared to other SIV infections in natural NHP hosts.^{153,212,215} SIVrcm uses CCR2b instead of CCR5 or CXCR4 as the coreceptor for viral entry.^{21,45} This finding is host-related in that the CCR5 gene of most RCMs contains a 24-bp deletion in the *env*-binding region of the CCR5.⁴⁵ As such, this may constitute an example of convergent evolution, similar to humans who possess the delta-32 mutation in the CCR5 gene.

The natural hosts of SIV infection, such as SMs, AGMs, MNDs, and chimpanzees, express lower levels of CCR5 on memory CD4⁺ T cells in PBMC and mucosal tissues, compared to disease-susceptible hosts, such as macaques, baboons, and humans.²¹³ Moreover, chimpanzees, which are considered a more recent host of SIV, show an intermediate level of CD4⁺CCR5⁺ T cells.²¹³ A hallmark of pathogenic primate lentiviral infections is early and persistent depletion of mucosal memory/activated CD4⁺CCR5⁺ T cells,^{38,161,181,184,226,287} and virus coreceptor usage is a key factor in determining which CD4⁺ T-cell subsets are depleted. As CCR5 has been shown to be the main coreceptor used by SIV in the natural hosts,²⁹⁹ African species with endemic naturally occurring SIVs may be less susceptible to pathogenic disease because they have fewer receptor targets for infection.²¹³ This hypothesis would provide an elegant evolutionary mechanism of “peaceful coexistence” between SIV replication and natural host immune system function.²⁵⁶

While this possibility presents an attractive explanation for species-specific differences in SIV disease expression, the fact that SIV VLs in nonpathogenic infections are equivalent to pathogenic levels does not support a simple association between the number of susceptible target cells and disease. Moreover, a dramatic depletion of CD4⁺ T cell in the intestine has been reported in SMs and AGMs during acute SIV infection.^{104,214} More studies of the *in vivo* dynamics of SIVagm and SIVsmm

replication are needed to investigate if the primary cell target of these viruses differs from those of SIVmac and HIV.

3.11.3. Viral Determinants of Pathogenicity in the Natural Host

Several viral factors were reported to be related to the lack of virulence in naturally occurring SIV infections. SIVmac replicates poorly and has low pathogenicity in macaques when the *nef* gene is deleted.¹⁴⁹ This observation was corroborated by the description of *nef* gene mutations in HIV-1-infected long-term nonprogressors.¹⁷⁸ Studies have shown that some Nef functions include the ability to downregulate CD4, CD28, and MHC class I,²⁹³ which will result in virus immune evasion.¹⁴¹ Nef may also enhance the responsiveness of T cells to activation,^{84,86} but this effect is not uniformly observed among primate lentiviruses.^{22,133,193} However, all SIVs from African monkeys have ORFs corresponding to a functional Nef; therefore, *nef* structure may not entirely account for differences in pathogenicity. It was recently suggested that Nef from the great majority of primate lentiviruses, including HIV-2, down-modulates TCR-CD3 in infected T cells in natural hosts, which subsequently blocks T-cell activation. In contrast, Nef derived from HIV-1 and a subset of closely related SIVs does not induce CD3 downregulation, which may have predisposed the simian precursor of HIV-1 to greater pathogenicity in humans.²⁴²

3.11.4. Viral Load as an Indicator of Pathogenicity in the Natural Host

In HIV-infected patients and SIVmac/smm-infected macaques, levels of plasma VLs are the best predictor of disease progression.^{69,106,126,163,173,185,186,203,221,262} Asymptomatic patients show low levels of VL, while progression to AIDS in patients resulting from failure of the immune system to control HIV replication, or failure of antiretroviral treatments, is always associated with significant increases in VLs.¹⁷³ The corollary is that immune or therapeutic control of virus should result in low VLs,^{130,185} and therefore the study of viral replication is of interest in species that have a largely nonpathogenic lentiviral infections.

However, in captive African NHPs naturally infected with SIVs, such as SIVsmm, SIVagm, SIVmnd-1, and SIVmnd-2, VLs quantified during the chronic phase of infection were higher than those in HIV-1 chronically

infected asymptomatic patients.²²⁰ The only investigation of SIV VLs in wild animals showed similarly high levels of viral replication.²¹⁶ Longitudinal analyses of the dynamics of plasma viremia in natural SIV infections suggest that the level of viral replication is relatively constant over time.^{7,165,216,217} Interestingly, the SIVmnd-1-infected mandrill and the SIVsmm-infected SM progressed to AIDS when the set point level of viral replication was higher than average.^{7,165,217}

Some species-specific differences could be observed in comparative studies.²²⁰ AGMs naturally infected with SIVagm display a considerably wider range of VLs than those observed in SMs.^{102,255,257} Proviral load in lymph node mononuclear cells from AGMs are 100-fold lower than the viral DNA loads observed in naturally infected SMs or MNDs lymph nodes.^{17,207,208} Altogether these data suggest that VL in SIVagm-infected AGM is generally lower than that observed in other African NHP species, and that viral replication kinetics may differ

among African NHP natural hosts of SIV without significant pathogenic consequences.

A series of studies has investigated the dynamics of early SIV replication by performing experimental SIV infections in natural hosts, such as SMs, AGMs, and MNDs.^{71,101,104,153,207,208,212,214,215,218–220,255} These studies showed a consistent pattern of SIV VL dynamics consisting of a peak of viremia (10^6 – 10^9 copies/mL of plasma) occurring around days 9–11 postinfection²²⁰ (Figure 3.6). Peak viremia is followed by a sharp decline of 10- to 100-fold and attainment of a stable level of viral replication (set point), which is maintained during the chronic phase of infection.²²⁰ Lower levels of peak viremia (10^4 – 10^6 copies/mL), but similar chronic set-point viremia, were found after experimental infection of vervets with SIVagm.ver644.^{212,215,220}

Thus, it does not appear that the lack of disease in naturally infected monkeys is associated with effective host containment of viral replication in these species.

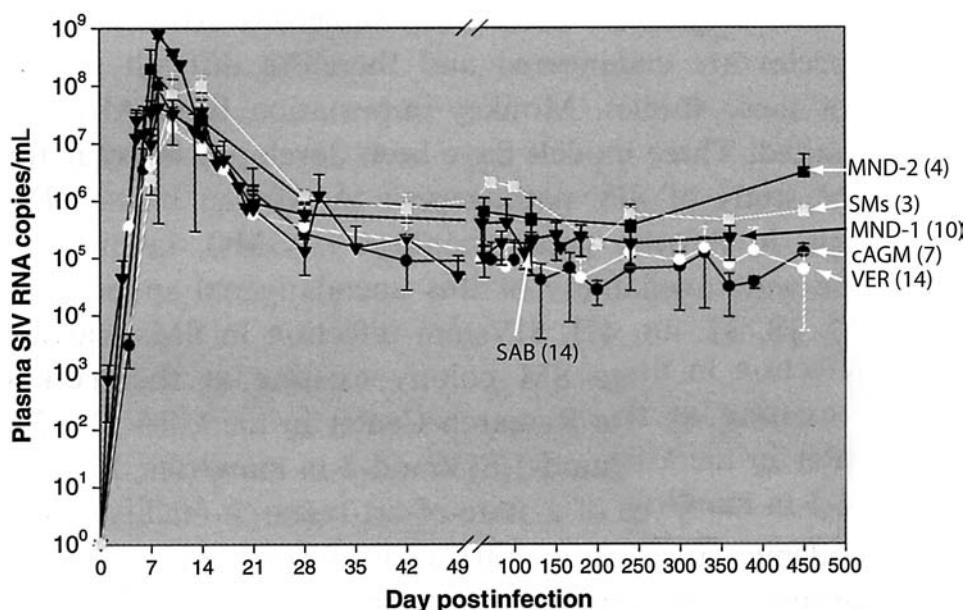


Figure 3.6. Plasma virus loads in acute and chronic infection of the natural host. African green monkey (AGM) infection: cAGM, SIVagmSAB infection of Caribbean origin AGMs (*Chlorocebus sabaeus*); VER, SIVagmVER infection of vervet AGMs (*C. pygerythrus*). MND-1, SIVmnd-1 infection of mandrills (*Mandrillus sphinx*); MND-2, SIVmnd-2 infection of mandrills (*M. sphinx*); SMs,³ SIVsmm infection of sooty mangabeys (*Cercocebus atys*). (Numbers in parenthesis are the number of animals in the group.) (Reprinted with permission from Pandrea *et al.*²¹²)

3.11.5. CD4⁺ T-Cell Dynamics in Relation to SIV Pathogenicity

SIV infection of natural hosts may be associated with normal or even increased CD4⁺ T-cell regeneration that eventually plays a key role in determining the lack of disease progression in naturally infected monkeys. This possibility is intriguing as, in HIV infection, a failure of the lymphoid regenerative capacity has been proposed as an important factor in the pathogenesis of the immunodeficiency.^{36,109,114,183,226,227} In particular, bone marrow suppression, reduced thymic output, and loss of naive T cells have all been observed in HIV-infected individuals.^{74,75,120,177,232} In marked contrast with this picture, studies in SIV-infected SMs showed that the regenerative capacity of the CD4⁺ T-cell compartment is fully preserved.²⁵⁷ Acute SIV infection in the natural host induces a massive mucosal CD4⁺ T-cell depletion that is of the same magnitude as in pathogenic HIV/SIV infections.^{104,214} However, in spite of persistently high viral replication, there is a partial immune restoration of CD4⁺ T cells in natural hosts during chronic infection as a consequence of the preservation of mucosal immunologic barrier and baseline levels of immune activation.^{104,214} In marked contrast with HIV infection, in which a failure of the lymphoid regenerative capacity is an important factor in the pathogenesis of the immunodeficiency, the regenerative capacity of the CD4⁺ T-cell compartment is fully preserved in natural hosts and may play a key role in determining the lack of disease progression. Interleukin-7 (IL-7)-dependent preserved T-cell regeneration plays a critical role in the avoidance of CD4 T-cell depletion and disease progression in SIV-infected SMs.^{195,196}

3.12. ADAPTIVE IMMUNE RESPONSE AND RELATIONSHIP TO DISEASE

The lack of disease progression in naturally infected NHPs is not related to immune control. This lack of immune control is not surprising considering the high levels of SIV replication found in the natural host.

3.12.1. Immune Response During SIV Infection

Some authors have reported that high viral replication during the steady-state SIV infection in natural hosts is associated with low immunologic pressures,^{192,230} although not all agree with this interpretation.¹⁴⁴ In addi-

tion, levels of T-cell activation, proliferation, and apoptosis which are indistinguishable from uninfected animals have been observed during chronic SIV infection in natural hosts,^{44,79,214,257} resulting in a limited bystander pathology.^{216,257,286} It is believed that in natural hosts of SIVs, a high VL results mainly from active viral replication, since immunological destruction is ineffective. Thus, African NHPs are not confronted with the consequences of chronic immune activation—namely, destruction of infected tissues. Upon cross-species transmission of SIVs, this equilibrium may be disrupted, inducing different immune characteristics.

Both HIV and SIVmac infection induce immune responses that are characterized by robust antibody and cellular immune responses.^{34,68,231} However, continuous immune escape is the hallmark of HIV/SIV infection in pathogenic models.⁴⁰ In natural infections of African NHPs, *de novo* immune responses are not superior to those observed during pathogenic infections of macaques.^{77,144} Some reports show that natural host species develop tolerance to their virus or to specific antigens or epitopes,^{83,200,202} or immune responses that differ qualitatively and quantitatively from those observed in pathogenic infections. Thus, AGMs, SMs, and l'hoesti monkeys generate antibody responses to their respective SIVs; however, the predominant responses are directed to Env rather than Gag, which differs from what is typically noted in HIV-1/SIVmac pathogenic infections.^{122,123,202} This observation was confirmed experimentally in AGMs and macaques infected with an SIVagm molecular clone.²⁰¹ While it is unlikely that Gag-specific antibodies per se are harmful, the lack of such antibody responses in naturally infected species may suggest differences in the host immune responses that may explain the lack of disease progression in these animals. However, induction of anti-Gag responses in SIVagm-infected AGMs did not result in any detectable change in the pathogenesis of SIV infection.

Another difference lies in the intensity of the response, which appears to be weaker in the natural host; for equivalent VL, SMs have antibody titers that are about 10 times lower than Rh.⁴³

Relatively few studies have investigated the neutralizing antibody activity in African natural hosts of SIVs, and results are generally conflicting. SIVagm was reported to not be neutralized by sera from infected AGMs.²⁰² Another study reported that specific SIVagm isolates are susceptible to neutralization

depending on the cell line used in the assay.⁹⁷ SIVagm infectivity is enhanced by the addition of soluble CD4, in contrast to HIV-1.^{4,294} This enhanced infectivity can be abrogated by SIVagm-specific antibodies.⁴ Replacement of the portion of the Env glycoprotein responsible for the coreceptor tropism with the corresponding region of a CXCR4-tropic HIV isolate conferred CXCR4 tropism, and rendered the variant SIVagm susceptible to neutralization.¹⁵² Neutralizing antibodies are rarely detected in SIV-infected SMs.⁸⁹ The failure of very high amounts of passively transferred specific immunoglobulin to prevent SIVagm infection suggests that the humoral immune response in AGMs is largely ineffective.²⁰¹ Experimental ablation of humoral immune responses in AGMs had little impact on the dynamics of SIVagm infection.

Several lines of evidence emphasize that generation of HIV-/SIV-specific cellular immune responses, and in particular those mediated by CD8⁺ T cells, protect from disease progression in pathogenic HIV/SIV infections:^{107,243,244} (i) experimental CD8 depletion in SIV-infected macaques results in an increased viral replication and rapid disease progression;^{140,164,180,243} (ii) the postpeak decline of viremia in acute HIV/SIV infection is coincidental with the expansion of HIV-/SIV-specific cytotoxic T lymphocytes (CTLs);^{32,154} and (iii) immunologic pressure exerted by CTLs results in viral escape mutations.^{5,33,81,82,107,159} Therefore, it was initially postulated that natural hosts for SIV do not progress to AIDS because they are able to exert better immune control of the virus, most probably through CTLs. The main focus on cytotoxic T-cell response characterization was in SMs. Clearly, SIVsmm-infected SMs develop CTL responses^{77,146,291} and CTL escape can also be documented,¹⁴⁴ which shows that the CTL responses are functional to some degree in controlling viral replication. It is difficult to compare the strength of CTL responses in macaques and SMs; however, while SIV-specific T-cell responses can be detected in the majority of naturally SIVsmm-infected SMs, their magnitude is generally lower than that determined, using the same technique, in HIV-infected patients.²⁶ In addition, no correlation was found between breadth or magnitude of SIV-specific T-cell responses and either VLs or CD4⁺ T-cell counts.⁷⁷ Moreover, the magnitude of the SIV-specific cellular response is not related to the level of T-cell activation and proliferation in SIVsmm-infected SMs.⁷⁷ Thus, the presence of a strong and broadly reactive T-cell response to SIV antigens is not a require-

ment for the lack of disease progression in SIVsmm-infected SMs; conversely, the complete suppression of SIV-specific T-cell responses (i.e., immunologic tolerance and/or naivety) is not required for the low levels of T-cell activation that are likely instrumental in avoiding AIDS in these animals.⁷⁷

Studies to dissect the role of CD8⁺ T cells in controlling viral replication in SMs and AGMs show only minor changes in the pattern of viral replication.¹⁶ Note that the anti-CD8 monoclonal antibody treatment used also had the potential to deplete other cell populations, most notably CD8⁺ NK cells, which may also be involved in the control of viral replication. It is also conceivable that the increase in SIV VLs after anti-CD8 treatment may be partially related to an increase in the availability of activated CD4⁺ T cells residing in lymphoid or mucosal tissues, as CD4⁺ T cells activated by exposure to non-self-immunoglobulin (i.e., anti-CD8) would serve as highly fertile targets for SIV infection.¹⁶ The proinflammatory signal resulting from the loss of large numbers of CD8⁺ T cells (in lymphoid tissues), the reactivation of latent viruses, cytomegalovirus for instance, resulting from the loss of CD8⁺ T cells, or increased CD4⁺ T-cell proliferation resulting from an attempt of the T-lymphocyte compartment to restore the overall T-cell homeostasis could also contribute to the rise in VLs observed in these experiments.²⁶⁶

Efforts to measure SIVagm-specific CTLs using traditional assays have been unsuccessful, due either to reduced activity or lack of reagent specificity to detect responses in AGMs. However, there is evidence of massive expansion of CD8⁺ T cells in infected AGMs^{131,153,212,215} and SIVmnd-infected mandrills.^{207,208} Although flow-cytometric analysis is not specific for the functional analysis of these cells, this observation provides a strong indication that the immune system of African NHPs is influenced by SIV infection. It is not yet clear if this CD8⁺ T-cell expansion results from active stimulation of the specific immune response or from a nonspecific stimulation of the immune system in general.²⁰¹

The conflicting findings of the levels and character of neutralizing antibody responses, and T-cell subset depletion or expansion in pathogenic versus nonpathogenic SIV have not elucidated an obvious adaptive immune response correlating with disease. A more targeted dissection of the role of antibodies and CTLs in controlling SIV infection in natural hosts is warranted to clarify this issue.²⁵⁴

3.12.2. Cytokine Induction and Immune Activation During Natural SIV Infection

Studies performed in SMs, AGMs, and mandrills indicate that both acute and chronic SIV infections of natural hosts are associated with lower levels of T-cell activation, proinflammatory responses, immunopathology, and bystander apoptosis than pathogenic HIV/SIV infection.^{103,104,213,214,216,218,219} When SIV infection in natural hosts is compared to SIVmac infection in Rh, natural infections differ in the following aspects: (i) only mild increases in the fraction of proliferating CD4⁺ T cells in the blood, with normal levels of proliferating CD8⁺ T cells; (ii) normal levels of proliferating CD4⁺ and CD8⁺ T cells in the lymph node (LN); (iii) normal production of proinflammatory cytokines by T cells; (iv) a low frequency of apoptotic T cells in the LN; and (v) normal in vitro susceptibility to apoptosis.^{103,104,214,216,218,219} In addition, naturally infected African NHPs maintain a capacity to regenerate T cells, with normal bone marrow morphology and function, normal levels of T-cell receptor excision circle (TREC)-expressing T cells, and preserved LN architecture.²⁵⁷ A statistically significant inverse correlation between CD4⁺ T-cell count and the level of T-cell activation was described in SIVsmm-infected SMs.^{257,269}

The kinetics of immune cell activation was investigated in LNs and blood of SIVsmm-infected SMs, SIVagm-infected AGMs, and SIVmnd-infected mandrills.^{103,104,153,207,208,212,214,219,255,257} In all three species, increased numbers of activated CD4⁺ and CD8⁺ T cells were detected in blood and LNs at the time of peak viral replication.^{145,153,208,214,255} The level of activated T cells then returned to preinfection values despite continuously high viremia in the chronic phase of SIV infection. In chronic SIVmnd-1 and SIVsmm infections there is only a minor increase of CD8⁺ T-cell activation and no increase in CD4⁺ T-cell activation.^{145,153,208,214,255}

Comparative studies on SIVsmm pathogenesis in SMs and Rh, as well as of SIVagm pathogenesis in AGMs and pig-tailed macaques²⁸⁵ showed that when SMs/Rh are infected with the same viral strain (SIVsmm/SIVmac239) or AGMs/PTMs are infected with the same SIVagm strain, high VLs are observed in both species. However, macaques develop chronic immune activation and increased T-cell apoptosis^{145,214,255} whereas minimal immune activation and T-cell apoptosis are observed in SMs and AGMs. The animals re-

main clinically normal,^{153,212,215,257} consistent with the hypothesis that chronic immune activation is a main determinant of disease progression during HIV and SIV infection.^{99,119,265} SIVagm-infected macaques develop lymphadenopathy with paracortical and follicular hyperplasia and progression to AIDS and CD4⁺ T-cell depletion and an increase in the number of T cells expressing activation and proliferation markers.¹²² In contrast, SIVagm-infected AGMs display normal lymph node morphology without evidence of either hyperplasia or depletion. Activation and proliferation markers are generally not upregulated in natural infections.^{44,214,216,257} Altogether, these data offer strong proof that the major difference in pathology between the natural hosts of SIVs and the experimental Asian macaque hosts derives indirectly from hyperimmune activation in the latter, which drives excessive activation-induced apoptosis. These observations lead to the hypothesis that impaired regeneration of T cells and the steady loss of CD4⁺ T cells by direct virus-induced cytopathic effects, along with chronic generalized immune activation seen in HIV infection, contribute to AIDS-associated T-cell depletion,^{109,110,113,114,118,183,257} disease progression,^{99,119,265} and ultimately to the collapse of the immune system and AIDS.^{36,73,76}

Conversely, in nonpathogenic infection in naturally SIV-infected monkeys, downregulation of the immune response favors preservation of CD4⁺ T-cell homeostasis.

These studies suggest that the level of immune activation is higher in the few SIV-infected African monkeys that ultimately progress to AIDS, compared to animals that retain their CD4⁺ T cells. Further studies that test this hypothesis in natural host models of SIV infection may uncover the mechanisms of SIV pathogenesis. More direct approaches that stimulate immune activation may result in disease progression in natural hosts for SIV infection.

The low immune activation levels in AGMs infected with SIVagm are due to a strong induction of TGF- β 1 and FOXP3, followed by a significant increase in IL-10 expression which occurs early in the SIVagm infection.¹⁵³ In HIV-infected humans and SIV-infected Rh, significant increases of β -chemokine expression have been reported^{41,50} to contribute to nonspecific inflammation and immune activation, as well as to high VLs in lymphoid tissues.¹⁵⁶ In sharp contrast to pathogenic lentiviral infections, only a transient increase of IFN- γ expression and no changes in the levels

of TNF- α and MIP-1 α/β expression were observed in SIVagm-infected AGMs.¹⁵³ These results, combined with the finding of an early increase in the levels of CD4 $^{+}$ CD25 $^{+}$ T cells, suggest that SIVagm infection of AGMs is associated with the rapid establishment of an anti-inflammatory environment which may prevent the host from developing the aberrant chronic T-cell hyperactivation that is correlated with progression to AIDS during HIV-1 infection.¹⁵³ CD4 $^{+}$ CD25 $^{+}$ T cells are maintained in chronically infected AGMs and SMs.²⁷⁰

Taken together, these data further support the hypothesis of a protective role for the downregulation of T-cell activation in natural hosts infected with species-specific SIVs. In particular, it is becoming clear that the establishment of anti-inflammatory profiles of gene expression early in the immune response to SIV antigens is associated with protection against AIDS. These early studies suggest that cytokine and chemokine signaling mechanisms may be key elements differentiating nonpathogenic versus pathogenic SIV disease, and point to another area where study of the natural course of these infections may be informative for pathogenesis and interventional strategy development.

With regard to the mechanism of immune activation observed in pathogenic SIV/HIV infection, the current view is that viral replication during acute infection results in rapid, massive mucosal CD4 $^{+}$ T-cell depletion in both progressive and nonprogressive SIV infections.^{104,109,161,181,214} During pathogenic HIV/SIV infection, immunologic and structural damage to the mucosal barrier results in microbial translocation from the gut lumen into the systemic circulation, which contributes to chronic immune activation and progression to AIDS.^{35–37} In stark contrast, African NHPs (such as AGMs, mandrills, and SMs) are able to maintain their intestinal barrier integrity,^{104,214} perhaps through the suppression of inflammation¹⁵³ and lack of enteropathy as the chronic phase begins, despite significant mucosal CD4 $^{+}$ T-cell depletion.^{104,214} Consequently, natural hosts maintain normal levels of T-cell activation, proliferation, and apoptosis and show significant recovery of mucosal CD4 $^{+}$ T cells during chronic infection despite high levels of viral replication.^{104,214}

3.12.3. Immune Responses and Lentiviral Disease—Overview

The lack of disease associated with naturally occurring lentiviral infections has not been clearly ascribed to an effective adaptive immune response to infection;

in contrast, the evidence to date suggests that these nonpathogenic infections in fact elicit a more measured and less robust immune response, potentially allowing the host to avoid chronic immune stimulation and eventual exhaustion seen in HIV infections. One may speculate that this host–virus adaptation that results in low levels of lentivirus-specific cellular immune responses will lead to a chronic, yet nonpathogenic infection. This observation emphasizes the tremendous challenge of artificially inducing protective immunity with an AIDS vaccine that has not been selected after thousands of years of evolutionary pressure on the human immune system. Further, studies of cytokine and chemokine innate protective mechanisms, and mechanisms for preserving CD4 $^{+}$ T-cell populations suggest innate immune parameters appear to be critical elements of the host–lentiviral adaptation.

3.13. INTRACELLULAR RESTRICTION FACTORS LIMITING CROSS-SPECIES LENTIVIRAL TRANSMISSION

Lentiviral species-specificity has typically been ascribed to factors such as virus–host receptor compatibility and cellular machinery needed to direct viral replication. However, host factors have been identified that prevent SIV cross-species infections *in vitro*. Although this field is only in its initial phase of development, data are rapidly accumulating, demonstrating new mechanisms by which cross-species transmission of the viruses can be effectively blocked in a potentially new host. The viral adaptation required for replication in a new host species is highly relevant to pathogenesis and therapeutic intervention of lentiviral disease.

3.13.1. Cellular Cytidine Deaminase and the SIV *vif* Gene

Initial studies postulated the role of *vif* in species-specificity of SIVs. This accessory gene was also determined to be essential for efficient FIV replication^{260,261} in cross-species transmission. The identification of an SIV-*vif* cellular target, a member of the cytidine deaminase APOBEC family, has permitted a better understanding of species restrictions on emergence of new lentiviral infections. A cellular deaminase is incorporated into the lentiviral virion during the reverse transcription to direct the deamination of cytidine to uridine on the minus-strand of viral DNA.¹⁴³ This deamination results in catastrophic G-to-A mutations in the viral genome, resulting in its inactivation^{117,157,175,298} and/or

degradation²⁶¹ (so-called catastrophic mutation). Two primate APOBEC family members (APOBEC3G and APOBEC3F) are believed to play a central role in antagonizing viral replication because they are expressed in the natural target cells of HIV-1 infection, including lymphocytes and macrophages. However, lentiviruses are able to successfully infect and replicate in host target cells containing APOBEC when host-adapted viral Vif interferes with this mechanism. This Vif activity is species-specific; that is, human APOBEC3G is inhibited by HIV-1 Vif but not by SIVagm Vif, whereas AGM APOBEC3G is inhibited by SIVagm Vif, but not by HIV-1 Vif. Several studies have identified the mechanism of this specificity and showed that a single amino acid change can alter the ability of Vif to interfere with APOBEC activity.^{30,247,296} The mechanism of this reaction is complex and still being intensively investigated.

3.13.2. TRIM5- α

A second cellular restriction factor is represented by the cytoplasmic body component TRIM5- α which restricts HIV-1 infection of monkey cells.²⁶⁷ TRIM5- α is now considered to be the cellular factor that mediates the antiretroviral restriction to infection previously referred to as REF-1 and LV-1.^{25,275,276} Although the mechanism of antiviral action is not fully understood, it is believed that TRIM5- α interferes with the viral uncoating step that is required to liberate viral nucleic acids into the cytoplasm upon viral binding and fusion with the target cell.¹⁰⁰ Sensitivity to TRIM5- α restriction is dictated by a small region in the viral capsid gene which has previously been shown to be involved in cyclophilin A binding.²⁷⁴ Therefore, subtle amino acid differences in this region of capsid influence the strength of binding to TRIM5- α and, hence, relative sensitivity to its restriction.^{137,268} HIV-2, but not closely related SIVmac, is highly susceptible to Rh TRIM5- α .²⁹⁷ Furthermore, HIV-2 was weakly restricted by human TRIM5- α , which may contribute to the lower pathogenic potential of the HIV-2 versus HIV-1 in human hosts.²⁹⁷ These experiments will build strategies to exploit TRIM5- α restriction for intervention of HIV-1 replication.²⁶³

3.14. SUMMARY

SIV is a species within the lentivirus genus. The virus naturally infects more than 40 species of NHPs in sub-Saharan Africa. Simian species outside of Africa are not infected. Two SIVs, one from the common chimpanzee

and the other from the SM, are ancestral to HIV-1 and HIV-2, respectively. The SIV group has a relatively complex genome consisting of the major retroviral structural genes plus accessory genes. SIVs are divided into three groups based on the number of accessory genes. The virus infects CD4 $^{+}$ T cells and macrophages in systemic and mucosal immune system. During acute infection, CD4 $^{+}$ cells in the gut mucosa are depleted, similar to pathogenic SIV and HIV infections. However, CD4 $^{+}$ cells are restored in the nonpathogenic SIV-infected natural host. SIV is relatively common in the wild with prevalence estimates in nature deduced from the study of wild-caught household pets and the analysis of fecal specimens collected in the field. The most striking feature of this group is the very low rate of AIDS in natural SIV infections despite high virus loads in blood and tissues. This finding is in marked contrast to HIV infection in human beings which is highly pathogenic. Theories proposed for resistance to AIDS in naturally infected hosts include lower immune activation, lower densities of cell receptors, and preservation of CD4 cell regenerative capacity after depletion during acute infection.

REFERENCES

1. Aghokeng, A. F., E. Bailes, S. Loul, V. Courgaud, E. Mpoudi-Ngolle, P. M. Sharp, E. Delaporte, and M. Peeters. 2007. Full-length sequence analysis of SIVmus in wild populations of mustached monkeys (*Cercopithecus cephus*) from Cameroon provides evidence for two co-circulating SIVmus lineages. *Virology* 360(2):407–418.
2. Aghokeng, A. F., W. Liu, F. Bibollet-Ruche, S. Loul, E. Mpoudi-Ngole, C. Laurent, J. M. Mwenda, D. K. Langat, G. K. Chege, H. M. McClure, E. Delaporte, G. M. Shaw, B. H. Hahn, and M. Peeters. 2006. Widely varying SIV prevalence rates in naturally infected primate species from Cameroon. *Virology* 345(1):174–189.
3. Allan, J. S., M. Short, M. E. Taylor, S. Su, V. M. Hirsch, P. R. Johnson, G. M. Shaw, and B. H. Hahn. 1991. Species-specific diversity among simian immunodeficiency viruses from African green monkeys. *J. Virol.* 65(6):2816–2828.
4. Allan, J. S., J. Strauss, and D. W. Buck. 1990. Enhancement of SIV infection with soluble receptor molecules. *Science* 247(4946):1084–1088.
5. Allen, T. M., D. H. O'Connor, P. Jing, J. L. Dzuris, B. R. Mothe, T. U. Vogel, E. Dunphy, M. E. Liebl, C. Emerson, N. Wilson, K. J. Kunstman, X. Wang, D. B.

- Allison, A. L. Hughes, R. C. Desrosiers, J. D. Altman, S. M. Wolinsky, A. Sette, and D. I. Watkins. 2000. Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. *Nature* 407(6802):386–390.
6. Andersen, J. L. and V. Planelles. 2005. The role of Vpr in HIV-1 pathogenesis. *Curr. HIV Res.* 3(1):43–51.
7. Apetrei, C., R. Gautam, B. Sumpter, A. C. Carter, T. Gaufin, S. I. Staprans, J. Else, M. Barnes, R. Cao Jr., S. Garg, J. M. Milush, D. L. Sodora, I. Pandrea, and G. Silvestri. 2007. Virus-subtype specific features of natural SIVsmm infection in sooty mangabeys. *J. Virol.* 81(15):7913–7923.
8. Apetrei, C., B. Gormus, I. Pandrea, M. Metzger, P. ten Haaf, L. N. Martin, R. Bohm, X. Alvarez, G. Koopman, M. Murphey-Corb, R. S. Veazey, A. A. Lackner, G. Baskin, J. Heeney, and P. A. Marx. 2004. Direct inoculation of simian immunodeficiency virus from sooty mangabeys in black mangabeys (*Lophocebus aterimus*): first evidence of AIDS in a heterologous African species and different pathologic outcomes of experimental infection. *J. Virol.* 78(21):11506–11518.
9. Apetrei, C., A. Kaur, N. W. Lerche, M. Metzger, I. Pandrea, J. Hardcastle, S. Fakelstein, R. Bohm, J. Kohler, V. Traina-Dorge, T. Williams, S. Staprans, G. Plauche, R. S. Veazey, H. McClure, A. A. Lackner, B. Gormus, D. L. Robertson, and P. A. Marx. 2005. Molecular epidemiology of SIVsm in US Primate Centers unravels the origin of SIVmac and SIVstm. *J. Virol.* 79(14):8991–9005.
10. Apetrei, C., N. W. Lerche, I. Pandrea, B. Gormus, M. Metzger, G. Silvestri, A. Kaur, R. Bohm, D. L. Robertson, J. Hardcastle, A. A. Lackner, and P. A. Marx. 2006. Kuru experiments triggered the emergence of pathogenic SIVmac. *AIDS* 20(3):317–321.
11. Apetrei, C. and P. A. Marx. 2005. African lentiviruses related to HIV. *J. Neurovirol.* 11(Suppl 1):33–49.
12. Apetrei, C., M. J. Metzger, D. Robinson, B. Ling, P. T. Telfer, P. Reed, D. L. Robertson, and P. A. Marx. 2005. Detection and partial characterization of new simian immunodeficiency virus (SIVsm) strains from bush meat samples from rural Sierra Leone. *J. Virol.* 79(4): 2631–2636.
13. Apetrei, C., D. L. Robertson, and P. A. Marx. 2004. The history of SIVs and AIDS: epidemiology, phylogeny and biology of isolates from naturally SIV infected non-human primates (NHP) in Africa. *Front Biosci.* 9:225–254.
14. Bailes, E., F. Gao, F. Bibollet-Ruche, V. Courgaud, M. Peeters, P. A. Marx, B. H. Hahn, and P. M. Sharp. 2003. Hybrid origin of SIV in chimpanzees. *Science* 300(5626):1713.
15. Barlow, K. L., A. O. Ajao, and J. P. Clewley. 2003. Characterization of a novel simian immunodeficiency virus (SIVmonNG1) genome sequence from a mona monkey (*Cercopithecus mona*). *J. Virol.* 77(12):6879–6888.
16. Barry, A. P., G. Silvestri, J. T. Safrit, B. Sumpter, N. Kozyr, H. M. McClure, S. I. Staprans, and M. B. Feinberg. 2007. Depletion of CD8⁺ cells in sooty mangabey monkeys naturally infected with simian immunodeficiency virus reveals limited role for immune control of virus replication in a natural host species. *J. Immunol.* 178(12):8002–8012.
17. Beer, B., J. Scherer, J. zur Megede, S. Norley, M. Baier, and R. Kurth. 1996. Lack of dichotomy between virus load of peripheral blood and lymph nodes during long-term simian immunodeficiency virus infection of African green monkeys. *Virology* 219(2):367–375.
18. Beer, B. E., E. Bailes, G. Dapolito, B. J. Campbell, R. M. Goeken, M. K. Axthelm, P. D. Markham, J. Bernard, D. Zagury, G. Franchini, P. M. Sharp, and V. M. Hirsch. 2000. Patterns of genomic sequence diversity among their simian immunodeficiency viruses suggest that L'Hoest monkeys (*Cercopithecus lhoesti*) are a natural lentivirus reservoir. *J. Virol.* 74(8):3892–3898.
19. Beer, B. E., E. Bailes, R. Goeken, G. Dapolito, C. Coulibaly, S. G. Norley, R. Kurth, J. P. Gautier, A. Gautier-Hion, D. Vallet, P. M. Sharp, and V. M. Hirsch. 1999. Simian immunodeficiency virus (SIV) from sun-tailed monkeys (*Cercopithecus solatus*): evidence for host-dependent evolution of SIV within the *C. lhoesti* superspecies. *J. Virol.* 73(9):7734–7744.
20. Beer, B. E., E. Bailes, P. M. Sharp, and V. M. Hirsch. 1999. Diversity and evolution of primate lentiviruses. In: Kuiken, C. L., B. Foley, B. Hahn, P. A. Marx, F. McCutchan, J. W. Mellors, J. I. Mullins, S. Wolinsky, and B. Korber (eds), *Human Retroviruses and AIDS 1999*. Los Alamos: Los Alamos National Laboratory, pp. 460–474.
21. Beer, B. E., B. T. Foley, C. L. Kuiken, Z. Tooze, R. M. Goeken, C. R. Brown, J. Hu, M. St Claire, B. T. Korber, and V. M. Hirsch. 2001. Characterization of novel simian immunodeficiency viruses from red-capped mangabeys from Nigeria (SIVrcm-NG409 and -NG411). *J. Virol.* 75(24):12014–12027.
22. Bell, I., C. Ashman, J. Maughan, E. Hooker, F. Cook, and T. A. Reinhart. 1998. Association of simian immunodeficiency virus Nef with the T-cell receptor (TCR) zeta chain leads to TCR down-modulation. *J. Gen. Virol.* 79(Pt 11):2717–2727.
23. Benveniste, R. E., R. W. Hill, W. B. Knott, C. C. Tsai, L. Kuller, and W. R. Morton. 1993. Detection

- of serum antibodies in Ethiopian baboons that cross-react with SIV, HTLV-I, and type D retroviral antigens. *J. Med. Primatol.* 22(2–3):124–128.
24. Berkhout, B. 1992. Structural features in TAR RNA of human and simian immunodeficiency viruses: a phylogenetic analysis. *Nucleic Acids Res.* 20(1):27–31.
 25. Berthoux, L., G. J. Towers, C. Gurer, P. Salomoni, P. P. Pandolfi, and J. Luban. 2003. As₂O₃ enhances retroviral reverse transcription and counteracts Ref1 antiviral activity. *J. Virol.* 77(5):3167–3180.
 26. Betts, M. R., D. R. Ambrozak, D. C. Douek, S. Bonhoeffer, J. M. Brenchley, J. P. Casazza, R. A. Koup, and L. J. Picker. 2001. Analysis of total human immunodeficiency virus (HIV)-specific CD4⁺ and CD8⁺ T-cell responses: relationship to viral load in untreated HIV infection. *J. Virol.* 75(24):11983–11991.
 27. Bibollet-Ruche, F., E. Bailes, F. Gao, X. Pourrut, K. L. Barlow, J. Clewley, J. M. Mwenda, D. K. Langat, G. K. Chege, H. M. McClure, E. Mpoudi-Ngole, E. Delaporte, M. Peeters, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 2004. A new simian immunodeficiency virus lineage (SIVdeb) infecting de Brazza's monkeys (*Cercopithecus neglectus*): evidence for a *Cercopithecus* monkey virus clade. *J. Virol.* 78(14):7748–7762.
 28. Bibollet-Ruche, F., A. Galat-Luong, G. Cuny, P. Sarni-Manchado, G. Galat, J. P. Durand, X. Pourrut, and F. Veas. 1996. Simian immunodeficiency virus infection in a patas monkey (*Erythrocebus patas*): evidence for cross-species transmission from African green monkeys (*Cercopithecus aethiops sabaeus*) in the wild. *J. Gen. Virol.* 77:773–781.
 29. Biek, R., A. J. Drummond, and M. Poss. 2006. A virus reveals population structure and recent demographic history of its carnivore host. *Science* 311(5760):538–541.
 30. Bogerd, H. P., B. P. Doehle, H. L. Wiegand, and B. R. Cullen. 2004. A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. *Proc. Natl. Acad. Sci. U. S. A.* 101(11):3770–3774.
 31. Borda, J. T., X. Alvarez, I. Kondova, P. Aye, M. A. Simon, R. C. Desrosiers, and A. A. Lackner. 2004. Cell tropism of simian immunodeficiency virus in culture is not predictive of in vivo tropism or pathogenesis. *Am. J. Pathol.* 165(6):2111–2122.
 32. Borrow, P., H. Lewicki, B. H. Hahn, G. M. Shaw, and M. B. Oldstone. 1994. Virus-specific CD8⁺ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J. Virol.* 68(9):6103–6110.
 33. Borrow, P., H. Lewicki, X. Wei, M. S. Horwitz, N. Pefter, H. Meyers, J. A. Nelson, J. E. Gairin, B. H. Hahn, M. B. Oldstone, and G. M. Shaw. 1997. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat. Med.* 3(2):205–211.
 34. Brander, C. and B. D. Walker. 2003. Gradual adaptation of HIV to human host populations: good or bad news? *Nat. Med.* 9(11):1359–1362.
 35. Brenchley, J. M. and D. C. Douek. 2008. HIV infection and the gastrointestinal immune system. *Mucosal Immunol.* 1(1):23–30.
 36. Brenchley, J. M., D. A. Price, and D. C. Douek. 2006. HIV disease: fallout from a mucosal catastrophe? *Nat. Immunol.* 7(3):235–239.
 37. Brenchley, J. M., D. A. Price, T. W. Schacker, T. E. Asher, G. Silvestri, S. Rao, Z. Kazzaz, O. Lambotte, D. Altmann, B. R. Blazar, B. Rodriguez, L. Teixeira-Johnson, A. Landay, J. N. Martin, F. M. Hecht, L. J. Picker, M. Lederman, S. G. Deeks, and D. C. Douek. 2006. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* 12(12):1365–1371.
 38. Brenchley, J. M., T. W. Schacker, L. E. Ruff, D. A. Price, J. H. Taylor, G. J. Beilman, P. L. Nguyen, A. Khoruts, M. Larson, A. T. Haase, and D. C. Douek. 2004. CD4⁺ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J. Exp. Med.* 200(6):749–759.
 39. Brighty, D. W. and M. Rosenberg. 1994. A cis-acting repressive sequence that overlaps the Rev-responsive element of human immunodeficiency virus type 1 regulates nuclear retention of env mRNAs independently of known splice signals. *Proc. Natl. Acad. Sci. U. S. A.* 91(18):8314–8318.
 40. Burns, D. P. and R. C. Desrosiers. 1994. Envelope sequence variation, neutralizing antibodies, and primate lentivirus persistence. *Curr. Top. Microbiol. Immunol.* 188:185–219.
 41. Caufour, P., R. Le Grand, A. Cheret, O. Neildez, F. Theodoro, B. Boson, B. Vaslin, and D. Dormont. 1999. Secretion of beta-chemokines by bronchoalveolar lavage cells during primary infection of macaques inoculated with attenuated nef-deleted or pathogenic simian immunodeficiency virus strain mac251. *J. Gen. Virol.* 80:767–776.
 42. Chakrabarti, L., M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiollais, and P. Sonigo. 1987. Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. *Nature* 328(6130):543–547.

43. Chakrabarti, L. A. 2004. The paradox of simian immunodeficiency virus infection in sooty mangabeys: active viral replication without disease progression. *Front Biosci.* 9:521–539.
44. Chakrabarti, L. A., S. R. Lewin, L. Zhang, A. Gettie, A. Luckay, L. N. Martin, E. Skulsky, D. D. Ho, C. Cheng-Mayer, and P. A. Marx. 2000. Normal T-cell turnover in sooty mangabeys harboring active simian immunodeficiency virus infection. *J. Virol.* 74(3):1209–1223.
45. Chen, Z., D. Kwon, Z. Jin, S. Monard, P. Telfer, M. S. Jones, C. Y. Lu, R. F. Aguilar, D. D. Ho, and P. A. Marx. 1998. Natural infection of a homozygous delta24 CCR5 red-capped mangabey with an R2b-tropic simian immunodeficiency virus. *J. Exp. Med.* 188(11):2057–2065.
46. Chen, Z., A. Luckay, D. L. Sodora, P. Telfer, P. Reed, A. Gettie, J. M. Kanu, R. F. Sadek, J. Yee, D. D. Ho, L. Zhang, and P. A. Marx. 1997. Human immunodeficiency virus type 2 (HIV-2) seroprevalence and characterization of a distinct HIV-2 genetic subtype from the natural range of simian immunodeficiency virus-infected sooty mangabeys. *J. Virol.* 71(5):3953–3960.
47. Chen, Z., P. Telfer, P. Reed, L. Zhang, A. Gettie, D. D. Ho, and P. A. Marx. 1995. Isolation and characterization of the first simian immunodeficiency virus from a feral sooty mangabey (*Cercopithecus atys*) in West Africa. *J. Med. Primatol.* 24(3):108–115.
48. Chen, Z., P. Telfer, A. Gettie, P. Reed, L. Zhang, D. D. Ho, and P. A. Marx. 1996. Genetic characterization of new West African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop. *J. Virol.* 70(6):3617–3627.
49. Chen, Z., P. Zhou, D. D. Ho, N. R. Landau, and P. A. Marx. 1997. Genetically divergent strains of simian immunodeficiency virus use CCR5 as a coreceptor for entry. *J. Virol.* 71(4):2705–2714.
50. Cheret, A., R. Le Grand, P. Caufour, O. Neildez, F. Matheux, F. Theodoro, B. Vaslin, and D. Dormont. 1999. RANTES, IFN-gamma, CCR1, and CCR5 mRNA expression in peripheral blood, lymph node, and bronchoalveolar lavage mononuclear cells during primary simian immunodeficiency virus infection of macaques. *Virology* 255(2):285–293.
51. Clewley, J. P., J. C. Lewis, D. W. Brown, and E. L. Gadsby. 1998. A novel simian immunodeficiency virus (SIVdrl) pol sequence from the drill monkey, *Mandrillus leucophaeus*. *J. Virol.* 72(12):10305–10309.
52. Coffin, J., A. Haase, J. A. Levy, L. Montagnier, S. Oroszlan, N. Teich, H. Temin, K. Toyoshima, H. Varmus, P. Vogt, and R. Weiss. 1986. Human immunodeficiency viruses. *Science* 232(4751):697.
53. Cohen, E. A., E. F. Terwilliger, J. G. Sodroski, and W. A. Haseltine. 1988. Identification of a protein encoded by the vpu gene of HIV-1. *Nature* 334(6182):532–534.
54. Cooper, R., A. Feistner, S. Evans, H. Tsujimoto, and M. Hayami. 1989. A lack of evidence of sexual transmission of a simian immunodeficiency agent in a semifree-ranging group of mandrills. *AIDS* 3(11):764.
55. Corbet, S., M. C. Muller-Trutwin, P. Versmisse, S. Delarue, A. Ayouba, J. Lewis, S. Brunak, P. Martin, F. Brun-Vezinet, F. Simon, F. Barre-Sinoussi, and P. Maucclere. 2000. env sequences of simian immunodeficiency viruses from chimpanzees in Cameroon are strongly related to those of human immunodeficiency virus group N from the same geographic area. *J. Virol.* 74(1):529–534.
56. Courgaud, V., B. Abela, X. Pourrut, E. Mpoudi-Ngole, S. Loul, E. Delaporte, and M. Peeters. 2003. Identification of a new simian immunodeficiency virus lineage with a vpu gene present among different cercopithecus monkeys (*C. mona*, *C. cephus*, and *C. nictitans*) from Cameroon. *J. Virol.* 77(23):12523–12534.
57. Courgaud, V., P. Formenty, C. Akoua-Koffi, R. Noe, C. Boesch, E. Delaporte, and M. Peeters. 2003. Partial molecular characterization of two simian immunodeficiency viruses (SIV) from African colobids: SIVwrc from Western red colobus (*Piliocolobus badius*) and SIVVolc from olive colobus (*Procolobus verus*). *J. Virol.* 77(1):744–748.
58. Courgaud, V., X. Pourrut, F. Bibollet-Ruche, E. Mpoudi-Ngole, A. Bourgeois, E. Delaporte, and M. Peeters. 2001. Characterization of a novel simian immunodeficiency virus from guereza colobus monkeys (*Colobus guereza*) in Cameroon: a new lineage in the nonhuman primate lentivirus family. *J. Virol.* 75(2):857–866.
59. Courgaud, V., M. Salemi, X. Pourrut, E. Mpoudi-Ngole, B. Abela, P. Auzel, F. Bibollet-Ruche, B. Hahn, A. M. Vandamme, E. Delaporte, and M. Peeters. 2002. Characterization of a novel simian immunodeficiency virus with a vpu gene from greater spot-nosed monkeys (*Cercopithecus nictitans*) provides new insights into simian/human immunodeficiency virus phylogeny. *J. Virol.* 76(16):8298–8309.
60. Cullen, B. R. and E. D. Garrett. 1992. A comparison of regulatory features in primate lentiviruses. *AIDS Res. Hum. Retroviruses* 8(3):387–393.

61. Daniel, M. D., F. Kirchhoff, S. C. Czajak, P. K. Sehgal, and R. C. Desrosiers. 1992. Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. *Science* 258(5090):1938–1941.
62. Daniel, M. D., N. L. Letvin, N. W. King, M. Kan-nagi, P. K. Sehgal, R. D. Hunt, P. J. Kanki, M. Essex, and R. C. Desrosiers. 1985. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* 228(4704):1201–1204.
63. Daniel, M. D., N. L. Letvin, P. K. Sehgal, D. K. Schmidt, D. P. Silva, K. R. Solomon, F. S. Hodi Jr., D. J. Ringler, R. D. Hunt, N. W. King, and R. C. Desrosiers. 1988. Prevalence of antibodies to 3 retroviruses in a captive colony of macaque monkeys. *Int. J. Cancer* 41(4):601–608.
64. Daniel, M. D., Y. Li, Y. M. Naidu, P. J. Durda, D. K. Schmidt, C. D. Troup, D. P. Silva, J. J. MacKey, H. W. Kestler 3rd, P. K. Sehgal, N. W. King, Y. Ohta, M. Hayami, and R. C. Desrosier. 1988. Simian immunodeficiency virus from African green monkeys. *J. Virol.* 62(11):4123–4128.
65. Dazza, M. C., M. Ekwalanga, M. Nende, K. Bin Shamamba, P. Bitshi, and S. Saragosti. 2005. Characterization of novel simian immunodeficiency virus from *Cercopithecus mona denti* (SIVden) from the Democratic Republic of Congo. *J. Virol.* 79:8560–8571.
66. De Leys, R., B. Vanderborgh, M. Vanden Haesevelde, L. Heyndrickx, A. van Geel, C. Wauters, R. Bernaerts, E. Saman, P. Nijs, B. Willem, H. Taelman, G. Van der Groen, P. Piroy, T. Tersmette, J. G. Huisman, and H. Van Heuverswyn. 1990. Isolation and partial characterization of an unusual human immunodeficiency retrovirus from two persons of west-central African origin. *J. Virol.* 64(3):1207–1216.
67. Denham, W. W. 1981. History of green monkeys in West Indies: migration from Africa. *J. Barbados Museum Historical Soc.* 36:210–229.
68. Derdeyn, C. A., J. M. Decker, F. Bibollet-Ruche, J. L. Mokili, M. Muldoon, S. A. Denham, M. L. Heil, F. Kasolo, R. Musonda, B. H. Hahn, G. M. Shaw, B. T. Korber, S. Allen, and E. Hunter. 2004. Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. *Science* 303(5666):2019–2022.
69. Diehl, L. J., C. K. Mathiason-Dubard, L. L. O’Neil, L. A. Obert, and E. A. Hoover. 1995. Induction of accelerated feline immunodeficiency virus disease by acute-phase virus passage. *J. Virol.* 69(10):6149–6157.
70. Diop, O. M., A. Gueye, A. Ayouba, E. Nerrienet, S. Corbet, P. Mauclere, F. Simon, F. Barre-Sinoussi, and M. C. Muller-Trutwin. 2002. Simian immunode-ficiency viruses and the origin of HIVs. In: Essex, M., S. M’Boup, P. Kanki, R. Marlink, and S. D. Tlou (eds), *AIDS in Africa*, 2nd edn. Kluwer Academic/Plenum Publishers.
71. Diop, O. M., A. Gueye, M. Dias-Tavares, C. Kornfeld, A. Faye, P. Ave, M. Huerre, S. Corbet, F. Barre-Sinoussi, and M. C. Müller-Trutwin. 2000. High levels of viral replication during primary simian immunodeficiency virus SIVagm infection are rapidly and strongly controlled in African green monkeys. *J. Virol.* 74(16):7538–7547.
72. Diop, O. M., M. J.-Y. Ploquin, L. Mortara, A. Faye, B. Jacquelin, D. Kunkel, P. Lebon, C. Butor, A. Hosmalin, F. Barré-Sinoussi, and M. C. Müller-Trutwin. 2008. Plasmacytoid dendritic cell dynamics and alpha interferon production during simian immunodeficiency virus infection with a nonpathogenic outcome. *J. Virol.* 82(11):5145–5152.
73. Douek, D. C. 2003. Disrupting T-cell homeostasis: how HIV-1 infection causes disease. *AIDS Rev.* 5(3):172–177.
74. Douek, D. C., M. R. Betts, B. J. Hill, S. J. Little, R. Lempicki, J. A. Metcalf, J. Casazza, C. Yoder, J. W. Adelsberger, R. A. Stevens, M. W. Baseler, P. Keiser, D. D. Richman, R. T. Davey, and R. A. Koup. 2001. Evidence for increased T cell turnover and decreased thymic output in HIV infection. *J. Immunol.* 167(11):6663–6668.
75. Douek, D. C., R. D. McFarland, P. H. Keiser, E. A. Gage, J. M. Massey, B. F. Haynes, M. A. Polis, A. T. Haase, M. B. Feinberg, J. L. Sullivan, B. D. Jamieson, J. A. Zack, L. J. Picker, and R. A. Koup. 1998. Changes in thymic function with age and during the treatment of HIV infection. *Nature* 396(6712):690–695.
76. Douek, D. C., L. J. Picker, and R. A. Koup. 2003. T cell dynamics in HIV-1 infection. *Annu. Rev. Immunol.* 21:265–304.
77. Dunham, R., P. Pagliardini, S. Gordon, B. Sumpter, J. Engram, A. Moanna, M. Paiardini, J. N. Mandl, B. Lawson, S. Garg, H. M. McClure, H. Xian-Xu, C. Ibegbu, K. Easley, N. Katz, I. Pandrea, C. Apetrei, D. L. Sodora, S. Staprans, M. B. Feinberg, and G. Silvestri. 2006. The AIDS resistance of naturally SIV-infected sooty mangabeys is independent of cellular immunity to the virus. *Blood* 108(1):209–217.
78. Emau, P., H. M. McClure, M. Isahakia, J. G. Else, and P. N. Fultz. 1991. Isolation from African Sykes’ monkeys (*Cercopithecus mitis*) of a lentivirus related to human and simian immunodeficiency viruses. *J. Virol.* 65(4):2135–2140.
79. Estaquier, J., T. Idziorek, F. de Bels, F. Barre-Sinoussi, B. Hurtrel, A. M. Aubertin, A. Venet, M. Mehtali, E.

- Muchmore, P. Michel, Y. Mouton, M. Girard, and J. C. Ameisen. 1994. Programmed cell death and AIDS: significance of T-cell apoptosis in pathogenic and nonpathogenic primate lentiviral infections. *Proc. Natl. Acad. Sci. U. S. A.* 91(20):9431–9435.
80. Estaquier, J., M. Peeters, L. Bedjabaga, C. Honore, P. Bussi, A. Dixson, and E. Delaporte. 1991. Prevalence and transmission of simian immunodeficiency virus and simian T-cell leukemia virus in a semi-free-range breeding colony of mandrills in Gabon. *AIDS* 5(11):1385–1386.
81. Evans, D. T. and R. C. Desrosiers. 2001. Immune evasion strategies of the primate lentiviruses. *Immunol. Rev.* 183:141–158.
82. Evans, D. T., D. H. O'Connor, P. Jing, J. L. Dzuris, J. Sidney, J. da Silva, T. M. Allen, H. Horton, J. E. Venham, R. A. Rudersdorf, T. Vogel, C. D. Pauza, R. E. Bontrop, R. DeMars, A. Sette, A. L. Hughes, and D. I. Watkins. 1999. Virus-specific cytotoxic T-lymphocyte responses select for amino-acid variation in simian immunodeficiency virus Env and Nef. *Nat. Med.* 5(11):1270–1276.
83. Feinberg, M. 2002. Ignorance is bliss: how natural hosts for SIV remain healthy despite long-term high level virus replication. *J. Human Virol.* 5:564.
84. Fenard, D., W. Yonemoto, C. de Noronha, M. Cavrois, S. A. Williams, and W. C. Greene. 2005. Nef is physically recruited into the immunological synapse and potentiates T cell activation early after TCR engagement. *J. Immunol.* 175(9):6050–6057.
85. Fomsgaard, A., J. Allan, M. Gravell, W. T. London, V. M. Hirsch, and P. R. Johnson. 1990. Molecular characterization of simian lentiviruses from east African green monkeys. *J. Med. Primatol.* 19(3–4):295–303.
86. Fortin, J. F., C. Barat, Y. Beausejour, B. Barbeau, and M. J. Tremblay. 2004. Hyper-responsiveness to stimulation of human immunodeficiency virus-infected CD4⁺ T cells requires Nef and Tat virus gene products and results from higher NFAT, NF-kappaB, and AP-1 induction. *J. Biol. Chem.* 279(38):39520–39531.
87. Fukasawa, M., T. Miura, A. Hasegawa, S. Morikawa, H. Tsujimoto, K. Miki, T. Kitamura, and M. Hayami. 1988. Sequence of simian immunodeficiency virus from African green monkey, a new member of the HIV/SIV group. *Nature* 333(6172):457–461.
88. Fultz, P. N., H. M. McClure, D. C. Anderson, R. B. Swenson, R. Anand, and A. Srinivasan. 1986. Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (*Cercocebus atys*). *Proc. Natl. Acad. Sci. U. S. A.* 83(14):5286–5290.
89. Fultz, P. N., R. B. Stricker, H. M. McClure, D. C. Anderson, W. M. Switzer, and C. Horaist. 1990. Humoral response to SIV/SMM infection in macaque and mangabey monkeys. *J. Acquir. Immune Defic. Syndr.* 3(4):319–329.
90. Gaddis, N. C., A. M. Sheehy, K. M. Ahmad, C. M. Swanson, K. N. Bishop, B. E. Beer, P. A. Marx, F. Gao, F. Bibollet-Ruche, B. H. Hahn, and M. H. Malim. 2004. Further investigation of simian immunodeficiency virus Vif function in human cells. *J. Virol.* 78(21):12041–12046.
91. Gao, F., E. Bailes, D. L. Robertson, Y. Chen, C. M. Rodenburg, S. F. Michael, L. B. Cummins, L. O. Arthur, M. Peeters, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 1999. Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. *Nature* 397(6718):436–441.
92. Gautam, R., A. C. Carter, N. Katz, I. F. Butler, M. Barnes, A. Hasegawa, M. Ratterree, G. Silvestri, P. A. Marx, V. M. Hirsch, I. Pandrea, and C. Apetrei. 2007. In vitro characterization of primary SIVsmm isolates belonging to different lineages. In vitro growth on rhesus macaque cells is not predictive for in vivo replication in rhesus macaques. *Virology* 362(2):257–270.
93. Gemeniano, M. C., E. T. Sawai, and E. E. Sparger. 2004. Feline immunodeficiency virus Orf-A localizes to the nucleus and induces cell cycle arrest. *Virology* 325(2):167–174.
94. Georges-Courbot, M. C., C. Y. Lu, M. Makuwa, P. Telfer, R. Onanga, G. Dubreuil, Z. Chen, S. M. Smith, A. Georges, F. Gao, B. H. Hahn, and P. A. Marx. 1998. Natural infection of a household pet red-capped mangabey (*Cercocebus torquatus torquatus*) with a new simian immunodeficiency virus. *J. Virol.* 72(1):600–608.
95. Georges-Courbot, M. C., P. Moisson, E. Leroy, A. M. Pingard, E. Nerrienet, G. Dubreuil, E. J. Wickings, F. Debels, I. Bedjabaga, V. Poaty-Mavoungou, N. T. Hahn, and A. J. Georges. 1996. Occurrence and frequency of transmission of naturally occurring simian retroviral infections (SIV, STLV, and SRV) at the CIRMF Primate Center, Gabon. *J. Med. Primatol.* 25(5):313–326.
96. Gibbs, J. S., A. A. Lackner, S. M. Lang, M. A. Simon, P. K. Sehgal, M. D. Daniel, and R. C. Desrosiers. 1995. Progression to AIDS in the absence of a gene for vpr or vpx. *J. Virol.* 69(4):2378–2383.
97. Gicheru, M. M., M. Otsyula, P. Spearman, B. S. Graham, C. J. Miller, H. L. Robinson, N. L. Haigwood, and D. C. Montefiori. 1999. Neutralizing antibody responses in Africa green monkeys naturally infected with simian immunodeficiency virus (SIVagm). *J. Med. Primatol.* 28(3):97–104.
98. Gilden, R. V., L. O. Arthur, W. G. Robey, J. C. Kelliher, C. E. Graham, and P. J. Fischinger. 1986.

- HTLV-III antibody in a breeding chimpanzee not experimentally exposed to the virus. *Lancet* 1(8482):678–679.
99. Giorgi, J. V., L. E. Hultin, J. A. McKeating, T. D. Johnson, B. Owens, L. P. Jacobson, R. Shih, J. Lewis, D. J. Wiley, J. P. Phair, S. M. Wolinsky, and R. Detels. 1999. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J. Infect. Dis.* 179(4):859–870.
 100. Goff, S. P. 2004. HIV: replication trimmed back. *Nature* 427(6977):791–793.
 101. Goldstein, S., C. R. Brown, I. Ourmanov, I. Pandrea, A. Buckler-White, C. Erb, J. S. Nandi, G. J. Foster, P. Autissier, J. E. Schmitz, and V. M. Hirsch. 2006. SIVagmVer replicates more efficiently in vervet than sabaeus African green monkeys but both maintain peripheral CD4⁺ T cells. *J. Virol.* 80:4868–4877.
 102. Goldstein, S., I. Ourmanov, C. R. Brown, B. E. Beer, W. R. Elkins, R. Plishka, A. Buckler-White, and V. M. Hirsch. 2000. Wide range of viral load in healthy African green monkeys naturally infected with simian immunodeficiency virus. *J. Virol.* 74(24):11744–11753.
 103. Gordon, S., R. M. Dunham, J. C. Engram, J. Estes, N. R. Klatt, I. Pandrea, C. Apetrei, D. L. Sodora, H. Y. Lee, A. T. Haase, M. Miller, A. Kaur, S. I. Staprans, A. S. Perelson, M. B. Feinberg, and G. Silvestri. 2008. Short-lived infected cells support the bulk of virus replication in naturally SIV-infected sooty mangabeys: implications for AIDS pathogenesis. *J. Virol.* 82:3725–3735.
 104. Gordon, S., N. R. Klatt, J. M. Milush, J. Engram, R. M. Dunham, M. Paiardini, E. A. Strobert, C. Apetrei, I. Pandrea, S. Staprans, D. L. Sodora, and G. Silvestri. 2007. Severe depletion of mucosal CD4⁺ T cells in AIDS-free SIV-infected sooty mangabeys. *J. Immunol.* 179:3026–3034.
 105. Gordon, S., I. Pandrea, R. Dunham, C. Apetrei, and G. Silvestri. 2005. The call of the wild: what can be learned from studies of SIV infection of natural hosts? In: Leitner, T., B. Foley, B. Hahn, P. Marx, F. McCutchan, J. Mellors, S. Wolinsky, and B. Korber (eds), *HIV Sequence Compendium 2004*. Los Alamos, NM: Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, pp. 2–29.
 106. Goto, Y., Y. Nishimura, K. Baba, T. Mizuno, Y. Endo, K. Masuda, K. Ohno, and H. Tsujimoto. 2002. Association of plasma viral RNA load with prognosis in cats naturally infected with feline immunodeficiency virus. *J. Virol.* 76(19):10079–10083.
 107. Goulder, P. J. and D. I. Watkins. 2004. HIV and SIV CTL escape: implications for vaccine design. *Nat. Rev. Immunol.* 4(8):630–640.
 108. Grimm, T. A., B. E. Beer, V. M. Hirsch, and K. A. Clouse. 2003. Simian immunodeficiency viruses from multiple lineages infect human macrophages: implications for cross-species transmission. *J. Acquir. Immune Defic. Syndr.* 32(4):362–369.
 109. Grossman, Z., M. Meier-Schellersheim, W. E. Paul, and L. J. Picker. 2006. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. *Nat. Med.* 12(3):289–295.
 110. Grossman, Z., M. Meier-Schellersheim, A. E. Sousa, R. M. Victorino, and W. E. Paul. 2002. CD4⁺ T-cell depletion in HIV infection: are we closer to understanding the cause? *Nat. Med.* 8(4):319–323.
 111. Gurtler, L. G., P. H. Hauser, J. Eberle, A. von Brunn, S. Knapp, L. Zekeng, J. M. Tsague, and L. Kapte. 1994. A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon. *J. Virol.* 68(3):1581–1585.
 112. Guyader, M., M. Emerman, P. Sonigo, F. Clavel, L. Montagnier, and M. Alizon. 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. *Nature* 326(6114):662–669.
 113. Haase, A. T. 1999. Population biology of HIV-1 infection: viral and CD4⁺ T cell demographics and dynamics in lymphatic tissues. *Annu. Rev. Immunol.* 17:625–656.
 114. Haase, A. T. 2005. Perils at mucosal front lines for HIV and SIV and their hosts. *Nat. Rev. Immunol.* 5(10):783–792.
 115. Hahn, B. 2002. Bernard Fields Memorial Lecture: SIV reservoirs and human zoonotic risk. In: *9th Conference on Retroviruses and Opportunistic Infections*. Seattle WA, Abstr. # L1.
 116. Hahn, B. H., G. M. Shaw, K. M. De Cock, and P. M. Sharp. 2000. AIDS as a zoonosis: scientific and public health implications. *Science* 287(5453):607–614.
 117. Harris, R. S., K. N. Bishop, A. M. Sheehy, H. M. Craig, S. K. Petersen-Mahrt, I. N. Watt, M. S. Neuberger, and M. H. Malim. 2003. DNA deamination mediates innate immunity to retroviral infection. *Cell* 113(6):803–809.
 118. Hazenberg, M. D., D. Hamann, H. Schuitemaker, and F. Miedema. 2000. T cell depletion in HIV-1 infection: how CD4⁺ T cells go out of stock. *Nat. Immunol.* 1(4):285–289.
 119. Hazenberg, M. D., S. A. Otto, B. H. van Benthem, M. T. Roos, R. A. Coutinho, J. M. Lange, D. Hamann, M. Prins, and F. Miedema. 2003. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS* 17(13):1881–1888.

120. Hellerstein, M., M. B. Hanley, D. Cesar, S. Siler, C. Papageorgopoulos, E. Wieder, D. Schmidt, R. Hoh, R. Neese, D. Macallan, S. Deeks, and J. M. McCune. 1999. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. *Nat. Med.* 5(1):83–89.
121. Hendry, R. M., M. A. Wells, M. A. Phelan, A. L. Schneider, J. S. Epstein, and G. V. Quinnan. 1986. Antibodies to simian immunodeficiency virus in African green monkeys in Africa in 1957–62. *Lancet* 2(8504):455.
122. Hirsch, V. M. 2004. What can natural infection of African monkeys with simian immunodeficiency virus tell us about the pathogenesis of AIDS? *AIDS Rev.* 6(1):40–53.
123. Hirsch, V. M., B. J. Campbell, E. Bailes, R. Goeken, C. Brown, W. R. Elkins, M. Axthelm, M. Murphey-Corb, and P. M. Sharp. 1999. Characterization of a novel simian immunodeficiency virus (SIV) from L'Hoest monkeys (*Cercopithecus l'hoesti*): implications for the origins of SIVmnd and other primate lentiviruses. *J. Virol.* 73(2):1036–1045.
124. Hirsch, V. M., G. Dapolito, C. McGann, R. A. Olmsted, R. H. Purcell, and P. R. Johnson. 1989. Molecular cloning of SIV from sooty mangabey monkeys. *J. Med. Primatol.* 18(3–4):279–285.
125. Hirsch, V. M., G. A. Dapolito, S. Goldstein, H. McClure, P. Emau, P. N. Fultz, M. Isahakia, R. Lenroot, G. Myers, and P. R. Johnson. 1993. A distinct African lentivirus from Sykes' monkeys. *J. Virol.* 67(3):1517–1528.
126. Hirsch, V. M., T. R. Fuerst, G. Sutter, M. W. Carroll, L. C. Yang, S. Goldstein, M. Piatak Jr., W. R. Elkins, W. G. Alvord, D. C. Montefiori, B. Moss, and J. D. Lifson. 1996. Patterns of viral replication correlate with outcome in simian immunodeficiency virus (SIV)-infected macaques: effect of prior immunization with a trivalent SIV vaccine in modified vaccinia virus Ankara. *J. Virol.* 70(6):3741–3752.
127. Hirsch, V. M., C. McGann, G. Dapolito, S. Goldstein, A. Ogen-Odoi, B. Biryawaho, T. Lakwo, and P. R. Johnson. 1993. Identification of a new subgroup of SIVagm in tantalus monkeys. *Virology* 197(1):426–430.
128. Hirsch, V. M., R. A. Olmsted, M. Murphey-Corb, R. H. Purcell, and P. R. Johnson. 1989. An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature* 339(6223):389–392.
129. Hirsch, V. M., M. E. Sharkey, C. R. Brown, B. Brichacek, S. Goldstein, J. Wakefield, R. Byrum, W. R. Elkins, B. H. Hahn, J. D. Lifson, and M. Stevenson. 1998. Vpx is required for dissemination and pathogenesis of SIV(SM) PBj: evidence of macrophage-dependent viral amplification. *Nat. Med.* 4(12):1401–1408.
130. Ho, D. D. 1995. Time to hit HIV, early and hard. *N. Engl. J. Med.* 333(7):450–451.
131. Holznagel, E., S. Norley, S. Holzammer, C. Coulibaly, and R. Kurth. 2002. Immunological changes in simian immunodeficiency virus SIVagm-infected African green monkeys (AGM): expanded cytotoxic T lymphocyte, natural killer and B cell subsets in the natural host of SIVagm. *J. Gen. Virol.* 83:631–640.
132. Hout, D. R., E. R. Mulcahy, E. Pacyniak, L. M. Gomez, M. L. Gomez, and E. B. Stephens. 2004. Vpu: a multifunctional protein that enhances the pathogenesis of human immunodeficiency virus type 1. *Curr. HIV Res.* 2(3):255–270.
133. Howe, A. Y., J. U. Jung, and R. C. Desrosiers. 1998. Zeta chain of the T-cell receptor interacts with nef of simian immunodeficiency virus and human immunodeficiency virus type 2. *J. Virol.* 72(12):9827–9834.
134. Hu, J., W. M. Switzer, B. T. Foley, D. L. Robertson, R. M. Goeken, B. T. Korber, V. M. Hirsch, and B. E. Beer. 2003. Characterization and comparison of recombinant simian immunodeficiency virus from drill (*Mandrillus leucophaeus*) and mandrill (*Mandrillus sphinx*) isolates. *J. Virol.* 77(8):4867–4880.
135. Huet, T., R. Cheynier, A. Meyerhans, G. Roelants, and S. Wain-Hobson. 1990. Genetic organization of a chimpanzee lentivirus related to HIV-1. *Nature* 345(6273):356–359.
136. Israel, Z. R. and P. A. Marx. 1995. Nonclassical mucosal antibodies predominate in genital secretions of HIV-1 infected chimpanzees. *J. Med. Primatol.* 24(2):53–60.
137. Javanbakht, H., F. Diaz-Griffero, M. Strelzlau, Z. Si, and J. Sodroski. 2005. The contribution of RING and B-box 2 domains to retroviral restriction mediated by monkey TRIM5alpha. *J. Biol. Chem.* 280(29):26933–26940.
138. Jin, M. J., H. Hui, D. L. Robertson, M. C. Muller, F. Barre-Sinoussi, V. M. Hirsch, J. S. Allan, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 1994. Mosaic genome structure of simian immunodeficiency virus from West African green monkeys. *EMBO J.* 13(12):2935–2947.
139. Jin, M. J., J. Rogers, J. E. Phillips-Conroy, J. S. Allan, R. C. Desrosiers, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 1994. Infection of a yellow baboon with simian immunodeficiency virus from African green monkeys: evidence for cross-species transmission in the wild. *J. Virol.* 68(12):8454–8460.
140. Jin, X., D. E. Bauer, S. E. Tuttleton, S. Lewin, A. Gettie, J. Blanchard, C. E. Irwin, J. T. Safrit, J. Mittler, L. Weinberger, L. G. Kostrikis, L. Zhang,

- A. S. Perelson, and D. D. Ho. 1999. Dramatic rise in plasma viremia after CD8⁺ T cell depletion in simian immunodeficiency virus-infected macaques. *J. Exp. Med.* 189(6):991–998.
141. Johnson, W. E. and R. C. Desrosiers. 2002. Viral persistence: HIV's strategies of immune system evasion. *Annu. Rev. Med.* 53:499–518.
142. Jones, T., C. L. Ehardt, T. M. Butynski, T. R. Davenport, N. E. Mpunga, S. J. Machaga, and D. W. De Luca. 2005. The highland mangabey *Lophocebus kipunji*: a new species of African monkey. *Science* 308(5725):1161–1164.
143. Kaiser, S. M. and M. Emerman. 2004. Controlling lentiviruses: single amino acid changes can determine specificity. *Proc. Natl. Acad. Sci. U. S. A.* 101(11):3725–3736.
144. Kaur, A., L. Alexander, S. I. Staprans, L. Denekamp, C. L. Hale, H. M. McClure, M. B. Feinberg, R. C. Desrosiers, and R. P. Johnson. 2001. Emergence of cytotoxic T lymphocyte escape mutations in non-pathogenic simian immunodeficiency virus infection. *Eur. J. Immunol.* 31(11):3207–3217.
145. Kaur, A., R. M. Grant, R. E. Means, H. McClure, M. Feinberg, and R. P. Johnson. 1998. Diverse host responses and outcomes following simian immunodeficiency virus SIVmac239 infection in sooty mangabeys and rhesus macaques. *J. Virol.* 72(12):9597–9611.
146. Kaur, A., J. Yang, D. Hempel, L. Gritz, G. P. Mazzara, H. McClure, and R. P. Johnson. 2000. Identification of multiple simian immunodeficiency virus (SIV)-specific CTL epitopes in sooty mangabeys with natural and experimentally acquired SIV infection. *J. Immunol.* 164(2):934–943.
147. Keele, B. F., F. Van Heuverswyn, Y. Li, E. Bailes, J. Takehisa, M. L. Santiago, F. Bibollet-Ruche, Y. Chen, L. V. Wain, F. Liegeois, S. Loul, E. M. Ngole, Y. Bienvenue, E. Delaporte, J. F. Brookfield, P. M. Sharp, G. M. Shaw, M. Peeters, and B. H. Hahn. 2006. Chimpanzee reservoirs of pandemic and non-pandemic HIV-1. *Science* 313(5786):523–526.
148. Kestler, H. W., K. Mori 3rd, D. P. Silva, T. Kodama, N. W. King, M. D. Daniel, and R. C. Desrosiers. 1990. Nef genes of SIV. *J. Med. Primatol.* 19(3–4):421–429.
149. Kestler, H. W., D. J. Ringler 3rd, K. Mori, D. L. Pinali, P. K. Sehgal, M. D. Daniel, and R. C. Desrosiers. 1991. Importance of the nef gene for maintenance of high virus loads and for development of AIDS. *Cell* 65(4):651–662.
150. Klatzmann, D., E. Champagne, S. Chamaret, J. Gruet, D. Guetard, T. Hercend, J. C. Gluckman, and L. Montagnier. 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature* 312(5996):767–768.
151. Kodama, T., D. P. Silva, M. D. Daniel, J. E. Phillips-Conroy, C. J. Jolly, J. Rogers, and R. C. Desrosiers. 1989. Prevalence of antibodies to SIV in baboons in their native habitat. *AIDS Res. Hum. Retroviruses* 5(3):337–343.
152. Konig, R. R., E. Flory, S. Steidl, J. Neumann, C. Coulibaly, E. Holznagel, S. Holzammer, S. Norley, and K. Cichutek. 2002. Engineered CD4- and CXCR4-using simian immunodeficiency virus from African green monkeys is neutralization sensitive and replicates in nonstimulated lymphocytes. *J. Virol.* 76(21):10627–10636.
153. Kornfeld, C., M. J. Ploquin, I. Pandrea, A. Faye, R. Onanga, C. Apetrei, V. Poaty-Mavoungou, P. Rouquet, J. Estaquier, L. Mortara, J. F. Desoutter, C. Butor, R. Le Grand, P. Roques, F. Simon, F. Barre-Sinoussi, O. M. Diop, and M. C. Muller-Trutwin. 2005. Antiinflammatory profiles during primary SIV infection in African green monkeys are associated with protection against AIDS. *J. Clin. Invest.* 115(4):1082–1091.
154. Koup, R. A., J. T. Safrit, Y. Cao, C. A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, and D. D. Ho. 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J. Virol.* 68(7): 4650–4655.
155. Kuppuswamy, M., T. Subramanian, A. Srinivasan, and G. Chinnadurai. 1989. Multiple functional domains of Tat, the trans-activator of HIV-1, defined by mutational analysis. *Nucleic Acids Res.* 17(9):3551–3561.
156. LaFranco-Scheuch, L., K. Abel, N. Makori, K. Rothaeusler, and C. J. Miller. 2004. High beta-chemokine expression levels in lymphoid tissues of simian/human immunodeficiency virus 89.6-vaccinated rhesus macaques are associated with uncontrolled replication of simian immunodeficiency virus challenge inoculum. *J. Virol.* 78(12):6399–6408.
157. Lecossier, D., F. Bouchonnet, F. Clavel, and A. J. Hance. 2003. Hypermutation of HIV-1 DNA in the absence of the Vif protein. *Science* 300(5622):1112.
158. Leitner, T., M. C. Dazza, M. Ekwalanga, C. Apetrei, and S. Saragosti. 2007. Sequence diversity among chimpanzee simian immunodeficiency viruses (SIVcpz) suggests that SIVcpzPts was derived from SIVcpzPtt through additional recombination events. *AIDS Res. Hum. Retroviruses* 23(9):1114–1118.
159. Leslie, A. J., K. J. Pfafferott, P. Chetty, R. Draenert, M. M. Addo, M. Feeney, Y. Tang, E. C. Holmes,

- T. Allen, J. G. Prado, M. Altfeld, C. Brander, C. Dixon, D. Ramduth, P. Jeena, S. A. Thomas, A. St John, T. A. Roach, B. Kupfer, G. Luzzi, A. Edwards, G. Taylor, H. Lyall, G. Tudor-Williams, V. Novelli, J. Martinez-Picado, P. Kiepiela, B. D. Walker, and P. J. Goulder. 2004. HIV evolution: CTL escape mutation and reversion after transmission. *Nat. Med.* 10(3):282–289.
160. Letvin, N. L., M. D. Daniel, P. K. Sehgal, R. C. Desrosiers, R. D. Hunt, L. M. Waldron, J. J. MacKey, D. K. Schmidt, L. V. Chalifoux, and N. W. King. 1985. Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. *Science* 230(4721):71–73.
161. Li, Q., L. Duan, J. D. Estes, Z. M. Ma, T. Rourke, Y. Wang, C. Reilly, J. Carlis, C. J. Miller, and A. T. Haase. 2005. Peak SIV replication in resting memory CD4⁺ T cells depletes gut lamina propria CD4⁺ T cells. *Nature* 434(7037):1148–1152.
162. Liegeois, F., V. Courgaud, W. M. Switzer, H. W. Murphy, S. Loul, A. Aghokeng, X. Pourrut, E. Mpoudi-Ngole, E. Delaporte, and M. Peeters. 2006. Molecular characterization of a novel simian immunodeficiency virus lineage (SIVtal) from northern talapoins (*Miopithecus ogouensis*). *Virology* 349(1):55–65.
163. Lifson, J. D., M. A. Nowak, S. Goldstein, J. L. Rossio, A. Kinter, G. Vasquez, T. A. Wiltzout, C. Brown, D. Schneider, L. Wahl, A. L. Lloyd, J. Williams, W. R. Elkins, A. S. Fauci, and V. M. Hirsch. 1997. The extent of early viral replication is a critical determinant of the natural history of simian immunodeficiency virus infection. *J. Virol.* 71(12):9508–9514.
164. Lifson, J. D., J. L. Rossio, M. Piatak Jr., T. Parks, L. Li, R. Kiser, V. Coalter, B. Fisher, B. M. Flynn, S. Czajak, V. M. Hirsch, K. A. Reimann, J. E. Schmitz, J. Ghayeb, N. Bischofberger, M. A. Nowak, R. C. Desrosiers, and D. Wodarz. 2001. Role of CD8⁺ lymphocytes in control of simian immunodeficiency virus infection and resistance to rechallenge after transient early antiretroviral treatment. *J. Virol.* 75(21):10187–10199.
165. Ling, B., C. Apetrei, I. Pandrea, R. S. Veazey, A. A. Lackner, B. Gormus, and P. A. Marx. 2004. Classic AIDS in a sooty mangabey after an 18-year natural infection. *J. Virol.* 78(16):8902–8908.
166. Ling, B., M. L. Santiago, S. Meleth, B. Gormus, H. M. McClure, C. Apetrei, B. H. Hahn, and P. A. Marx. 2003. Noninvasive detection of new simian immunodeficiency virus lineages in captive sooty mangabeys: ability to amplify virion RNA from fecal samples correlates with viral load in plasma. *J. Virol.* 77(3):2214–2226.
167. Littman, D. R. 1994. Immunodeficiency viruses. Not enough sans Nef. *Curr. Biol.* 4(7):618–620.
168. Locatelli, S., B. Lafay, F. Liegeois, N. Ting, E. Delaporte, and M. Peeters. 2008. Full molecular characterization of a simian immunodeficiency virus, SIVwr-cpbt from Temminck's red colobus (*Piliocolobus badius temminckii*) from Abuko Nature Reserve, The Gambia. *Virology* 376(1):90–100.
169. Locatelli, S., F. Liegeois, B. Lafay, A. D. Roeder, M. W. Bruford, P. Formenty, R. Noe, E. Delaporte, and M. Peeters. 2008. Prevalence and genetic diversity of simian immunodeficiency virus infection in wild-living red colobus monkeys (*Piliocolobus badius badius*) from the Tai forest, Côte d'Ivoire SIVwrc in wild-living western red colobus monkeys. *Infect. Genet. Evol.* 8(1):1–14.
170. Lockridge, K. M., S. Himathongkham, E. T. Sawai, M. Chienand, and E. E. Sparger. 1999. The feline immunodeficiency virus vif gene is required for productive infection of feline peripheral blood mononuclear cells and monocyte-derived macrophages. *Virology* 261(1):25–30.
171. Lowenstein, L. J., N. W. Lerche, J. L. Yee, A. Uyeda, M. B. Jennings, R. J. Munn, H. M. McClure, D. C. Anderson, P. N. Fultz, and M. B. Gardner. 1992. Evidence for a lentiviral etiology in an epizootic of immune deficiency and lymphoma in stump-tailed macaques (*Macaca arctoides*). *J. Med. Primatol.* 21(1):1–14.
172. Lowenstein, L. J., N. C. Pedersen, J. Higgins, K. C. Pallis, A. Uyeda, P. Marx, N. W. Lerche, R. J. Munn, and M. B. Gardner. 1986. Seroepidemiologic survey of captive Old-World primates for antibodies to human and simian retroviruses, and isolation of a lentivirus from sooty mangabeys (*Cercocebus atys*). *Int. J. Cancer* 38(4):563–574.
173. Lyles, R. H., A. Munoz, T. E. Yamashita, H. Bazmi, R. Detels, C. R. Rinaldo, J. B. Margolick, J. P. Phair, and J. W. Mellors. 2000. Natural history of human immunodeficiency virus type 1 viremia after seroconversion and proximal to AIDS in a large cohort of homosexual men. Multicenter AIDS Cohort Study. *J. Infect. Dis.* 181(3):872–880.
174. Malim, M. H., S. Bohnlein, R. Fenrick, S. Y. Le, J. V. Maizel, and B. R. Cullen. 1989. Functional comparison of the Rev trans-activators encoded by different primate immunodeficiency virus species. *Proc. Natl. Acad. Sci. U. S. A.* 86(21):8222–8226.
175. Mangeat, B., P. Turelli, G. Caron, M. Friedli, L. Perrin, and D. Trono. 2003. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature* 424(6944):99–103.

176. Mansfield, K. G., N. W. Lerche, M. B. Gardner, and A. A. Lackner. 1995. Origins of simian immunodeficiency virus infection in macaques at the New England Regional Primate Research Center. *J. Med. Primatol.* 24(3):116–122.
177. Marandin, A., A. Katz, E. Oksenhendler, M. Tulliez, F. Picard, W. Vainchenker, and F. Louache. 1996. Loss of primitive hematopoietic progenitors in patients with human immunodeficiency virus infection. *Blood* 88(12):4568–4578.
178. Mariani, R., F. Kirchhoff, T. C. Greenough, J. L. Sullivan, R. C. Desrosiers, and J. Skowronski. 1996. High frequency of defective nef alleles in a long-term survivor with nonprogressive human immunodeficiency virus type 1 infection. *J. Virol.* 70(11):7752–7764.
179. Marx, P. A., Y. Li, N. W. Lerche, S. Sutjipto, A. Gettie, J. A. Yee, B. H. Brotman, A. M. Prince, A. Hanson, R. G. Webster, and R. C. Desrosiers. 1991. Isolation of a simian immunodeficiency virus related to human immunodeficiency virus type 2 from a West African pet sooty mangabey. *J. Virol.* 65(8):4480–4485.
180. Matano, T., R. Shibata, C. Siemon, M. Connors, H. C. Lane, and M. A. Martin. 1998. Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. *J. Virol.* 72(1):164–169.
181. Mattapallil, J. J., D. C. Douek, B. Hill, Y. Nishimura, M. Martin, and M. Roederer. 2005. Massive infection and loss of memory CD4⁺ T cells in multiple tissues during acute SIV infection. *Nature* 434(7037):1093–1097.
182. McClure, H. M., D. C. Anderson, T. P. Gordon, A. A. Ansari, P. N. Fultz, S. A. Klumpp, P. Emau, and M. Isahakia. 1992. Natural simian immunodeficiency virus infection in nonhuman primates. *Top. Primatol.* 3:425–438.
183. McCune, J. M. 2001. The dynamics of CD4⁺ T-cell depletion in HIV disease. *Nature* 410(6831):974–979.
184. Mehandru, S., M. A. Poles, K. Tenner-Racz, A. Horowitz, A. Hurley, C. Hogan, D. Boden, P. Racz, and M. Markowitz. 2004. Primary HIV-1 infection is associated with preferential depletion of CD4⁺ T lymphocytes from effector sites in the gastrointestinal tract. *J. Exp. Med.* 200(6):761–770.
185. Mellors, J. W. 1998. Viral-load tests provide valuable answers. *Sci. Am.* 279(1):90–93.
186. Mellors, J. W., C. R. Rinaldo Jr., P. Gupta, R. M. White, J. A. Todd, and L. A. Kingsley. 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 272(5265):1167–1170.
187. Miller, R. J., J. S. Cairns, S. Bridges, and N. Sarver. 2000. Human immunodeficiency virus and AIDS: insights from animal lentiviruses. *J. Virol.* 74(16):7187–7195.
188. Mitani, J. C. and D. P. Watts. 1999. Demographic influences on the hunting behavior of chimpanzees. *Am. J. Phys. Anthropol.* 109(4):439–454.
189. Moore, J. P., S. G. Kitchen, P. Pugach, and J. A. Zack. 2004. The CCR5 and CXCR4 coreceptors-central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. *AIDS Res. Hum. Retroviruses* 20(1):111–126.
190. Muller, M. C. and F. Barre-Sinoussi. 2003. SIVagm: genetic and biological features associated with replication. *Front Biosci.* 8:D1170–D1185.
191. Muller, M. C., N. K. Saksena, E. Nerrienet, C. Chappey, V. M. Herve, J. P. Durand, P. Legal-Campodonico, M. C. Lang, J. P. Digoutte, A. J. Georges, M.-C. Georges-Courbot, P. Sonigo, and F. Barré-Sinoussi. 1993. Simian immunodeficiency viruses from central and western Africa: evidence for a new species-specific lentivirus in tantalus monkeys. *J. Virol.* 67(3):1227–1235.
192. Muller-Trutwin, M. C., S. Corbet, M. D. Tavares, V. M. Herve, E. Nerrienet, M. C. Georges-Courbot, W. Saurin, P. Sonigo, and F. Barre-Sinoussi. 1996. The evolutionary rate of nonpathogenic simian immunodeficiency virus (SIVagm) is in agreement with a rapid and continuous replication in vivo. *Virology* 223(1):89–102.
193. Munch, J., M. Schindler, S. Wildum, E. Rucker, N. Bailer, V. Knoop, F. J. Novembre, and F. Kirchhoff. 2005. Primary sooty mangabey simian immunodeficiency virus and human immunodeficiency virus type 2 nef alleles modulate cell surface expression of various human receptors and enhance viral infectivity and replication. *J. Virol.* 79(16):10547–10560.
194. Murphey-Corb, M., L. N. Martin, S. R. Rangan, G. B. Baskin, B. J. Gormus, R. H. Wolf, W. A. Andes, M. West, and R. C. Montelaro. 1986. Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. *Nature* 321(6068):435–437.
195. Muthukumar, A., A. Wozniakowski, M. C. Gauduin, M. Paiardini, H. M. McClure, R. P. Johnson, G. Silvestri, and D. L. Sodora. 2004. Elevated interleukin-7 levels not sufficient to maintain T-cell homeostasis during simian immunodeficiency virus-induced disease progression. *Blood* 103(3):973–979.
196. Muthukumar, A., D. Zhou, M. Paiardini, A. P. Barry, K. S. Cole, H. M. McClure, S. I. Staprans, G. Silvestri, and D. L. Sodora. 2005. Timely triggering of homeostatic mechanisms involved in the regulation

- of T-cell levels in SIVsm-infected sooty mangabeys. *Blood* 106(12):3839–3845.
197. Neil, S. J., T. Zang, and P. D. Bieniasz. 2008. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 451(7177):425–430.
 198. Nerrienet, E., X. Amouretti, M. C. Muller-Trutwin, V. Poaty-Mavoungou, I. Bedjebaga, H. T. Nguyen, G. Dubreuil, S. Corbet, E. J. Wickings, F. Barre-Sinoussi, A. J. Georges, and M. C. Georges-Courbot. 1998. Phylogenetic analysis of SIV and STLV type I in mandrills (*Mandrillus sphinx*): indications that intracolony transmissions are predominantly the result of male-to-male aggressive contacts. *AIDS Res. Hum. Retroviruses* 14(9):785–796.
 199. Nerrienet, E., M. L. Santiago, Y. Fouopouapouognigni, E. Bailes, N. I. Mundy, B. Njinku, A. Kfutwah, M. C. Muller-Trutwin, F. Barre-Sinoussi, G. M. Shaw, P. M. Sharp, B. H. Hahn, and A. Ayoubia. 2005. Simian immunodeficiency virus infection in wild-caught chimpanzees from Cameroon. *J. Virol.* 79(2):1312–1319.
 200. Norley, S., B. Beer, S. Holzammer, J. zur Megede, and R. Kurth. 1999. Why are the natural hosts of SIV resistant to AIDS? *Immunol. Lett.* 66(1–3):47–52.
 201. Norley, S. and R. Kurth. 2004. The role of the immune response during SIVAgm infection of the African green monkey natural host. *Front Biosci.* 9:550–564.
 202. Norley, S. G., G. Kraus, J. Ennen, J. Bonilla, H. Konig, and R. Kurth. 1990. Immunological studies of the basis for the apathogenicity of simian immunodeficiency virus from African green monkeys. *Proc. Natl. Acad. Sci. U. S. A.* 87(22):9067–9071.
 203. Nowak, M. A., A. L. Lloyd, G. M. Vasquez, T. A. Wiltztrout, L. M. Wahl, N. Bischofberger, J. Williams, A. Kinter, A. S. Fauci, V. M. Hirsch, and J. D. Lifson. 1997. Viral dynamics of primary viremia and anti-retroviral therapy in simian immunodeficiency virus infection. *J. Virol.* 71(10):7518–7525.
 204. O’Neil, S. P., F. J. Novembre, A. B. Hill, C. Suwyn, C. E. Hart, T. Evans-Strickfaden, D. C. Anderson, J. deRosario, J. G. Herndon, M. Saucier, and H. M. McClure. 2000. Progressive infection in a subset of HIV-1-positive chimpanzees. *J. Infect. Dis.* 182(4):1051–1062.
 205. Ohta, Y., T. Masuda, H. Tsujimoto, K. Ishikawa, T. Kodama, S. Morikawa, M. Nakai, S. Honjo, and M. Hayami. 1988. Isolation of simian immunodeficiency virus from African green monkeys and seroepidemiologic survey of the virus in various non-human primates. *Int. J. Cancer* 41(1):115–122.
 206. Olsen, H. S., S. Beidas, P. Dillon, C. A. Rosen, and A. W. Cochrane. 1991. Mutational analysis of the HIV-1 Rev protein and its target sequence, the Rev responsive element. *J. Acquir. Immune Defic. Syndr.* 4(6):558–567.
 207. Onanga, R., C. Kornfeld, I. Pandrea, J. Estaquier, S. Souquiere, P. Rouquet, V. P. Mavoungou, O. Bourry, M’Boup, S., F. Barre-Sinoussi, F. Simon, C. Apetrei, P. Roques, and M. C. Muller-Trutwin. 2002. High levels of viral replication contrast with only transient changes in CD4⁺ and CD8⁺ cell numbers during the early phase of experimental infection with simian immunodeficiency virus SIVmnd-1 in Mandrillus sphinx. *J. Virol.* 76(20):10256–10263.
 208. Onanga, R., S. Souquiere, M. Makwua, A. Mouinga-Ondeme, F. Simon, C. Apetrei, and P. Roques. 2006. Primary simian immunodeficiency virus SIVmnd-2 infection in mandrills (*Mandrillus sphinx*). *J. Virol.* 80:3303–3309.
 209. Osterhaus, A. D., N. Pedersen, G. van Amerongen, M. T. Frankenhuus, M. Marthas, E. Reay, T. M. Rose, J. Pamungkas, and M. L. Bosch. 1999. Isolation and partial characterization of a lentivirus from talapoin monkeys (*Myopithecus talapoin*). *Virology* 260(1):116–124.
 210. Otsyula, M., J. Yee, M. Jennings, M. Suleman, A. Gettie, R. Tarara, M. Isahakia, P. Marx, and N. Lerche. 1996. Prevalence of antibodies against simian immunodeficiency virus (SIV) and simian T-lymphotropic virus (STLV) in a colony of non-human primates in Kenya, East Africa. *Ann. Trop. Med. Parasitol.* 90(1):65–70.
 211. Owen, S. M., S. Masciotra, F. Novembre, J. Yee, W. M. Switzer, M. Ostyula, and R. B. Lal. 2000. Simian immunodeficiency viruses of diverse origin can use CXCR4 as a coreceptor for entry into human cells. *J. Virol.* 74(12):5702–5708.
 212. Pandrea, I., C. Apetrei, J. Dufour, N. Dillon, J. Barbercheck, M. Metzger, B. Jacquelin, R. Bohm, P. A. Marx, F. Barre-Sinoussi, V. M. Hirsch, M. C. Muller-Trutwin, A. A. Lackner, and R. Veazey. 2006. Simian immunodeficiency virus (SIV) SIVAgm.sab infection of Caribbean African green monkeys: new model of the study of SIV pathogenesis in natural hosts. *J. Virol.* 80(10):4858–4867.
 213. Pandrea, I., C. Apetrei, S. Gordon, J. Barbercheck, J. Dufour, R. Bohm, B. Sumpter, P. Roques, P. A. Marx, V. M. Hirsch, A. Kaur, A. A. Lackner, R. S. Veazey, and G. Silvestri. 2007. Paucity of CD4⁺CCR5⁺ T cells is a typical feature of natural SIV hosts. *Blood* 109:1069–1076.
 214. Pandrea, I., R. Gautam, R. Ribeiro, J. M. Brenchley, I. F. Butler, M. Pattison, T. Rasmussen, P. A. Marx, G. Silvestri, A. A. Lackner, A. S. Perelson, D. C. Douek, R. S. Veazey, and C. Apetrei. 2007. Acute

- loss of intestinal CD4⁺ T cells is not predictive of SIV virulence. *J. Immunol.* 179:3035–3046.
215. Pandrea, I., C. Kornfeld, M. J.-I. Ploquin, C. Apetrei, A. Faye, P. Rouquet, P. Roques, F. Simon, F. Barré-Sinoussi, M. C. Müller-Trutwin, and O. M. Diop. 2005. Impact of viral factors on very early in vivo replication profiles in SIVagm-infected African green monkeys. *J. Virol.* 79(10):6249–6259.
 216. Pandrea, I., R. Onanga, C. Kornfeld, P. Rouquet, O. Bourry, S. Clifford, P. T. Telfer, K. Abernethy, L. T. White, P. Ngari, M. Muller-Trutwin, P. Roques, P. A. Marx, F. Simon, and C. Apetrei. 2003. High levels of SIVmnd-1 replication in chronically infected Mandrillus sphinx. *Virology* 317(1):119–127.
 217. Pandrea, I., R. Onanga, P. Rouquet, O. Bourry, P. Ngari, E. J. Wickings, P. Roques, and C. Apetrei. 2001. Chronic SIV infection ultimately causes immunodeficiency in African non-human primates. *AIDS* 15(18):2461–2462.
 218. Pandrea, I., R. Onanga, S. Souquiere, A. Mouinga-Ondéme, O. Bourry, M. Makwua, P. Rouquet, G. Silvestri, F. Simon, P. Roques, and C. Apetrei. 2008. Paucity of CD4⁺CCR5⁺ T-cells may prevent breast-feeding transmission of SIV in natural non-human primate hosts. *J. Virol.* 82(11):5501–5509.
 219. Pandrea, I., R. M. Ribeiro, R. Gautam, T. Gaufin, M. Pattison, M. Barnes, C. Monjure, C. Stoulig, G. Silvestri, M. Miller, A. S. Perelson, and C. Apetrei. 2008. Simian immunodeficiency virus SIVagm dynamics in African green monkeys. *J. Virol.* 82(7):3713–3724.
 220. Pandrea, I., G. Silvestri, R. Onanga, R. S. Veazey, P. A. Marx, V. M. Hirsch, and C. Apetrei. 2006. Simian immunodeficiency viruses replication dynamics in African non-human primate hosts: common patterns and species-specific differences. *J. Med. Primatol.* 35(4):194–201.
 221. Pedersen, N. C., C. M. Leutenegger, J. Woo, and J. Higgins. 2001. Virulence differences between two field isolates of feline immunodeficiency virus (FIV-APetaluma and FIV-CPGammair) in young adult species-specific pathogen free cats. *Vet. Immunol. Immunopathol.* 79(1–2):53–67.
 222. Peeters, M., V. Courgnaud, B. Abela, P. Auzel, X. Pourrut, F. Bibollet-Ruche, S. Loul, F. Liegeois, C. Butel, D. Koulagna, E. Mpoudi-Ngole, G. M. Shaw, B. H. Hahn, and E. Delaporte. 2002. Risk to human health from a plethora of simian immunodeficiency viruses in primate bushmeat. *Emerg. Infect. Dis.* 8(5):451–457.
 223. Peeters, M., K. Fransen, E. Delaporte, M. Van den Haesevelde, G. M. Gershy-Damet, L. Kestens, G. van der Groen, and P. Piot. 1992. Isolation and characterization of a new chimpanzee lentivirus (simian immunodeficiency virus isolate cpz-ant) from a wild-captured chimpanzee. *AIDS* 6(5):447–451.
 224. Peeters, M., C. Honore, T. Huet, L. Bedjabaga, S. Ossari, P. Bussi, R. W. Cooper, and E. Delaporte. 1989. Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon. *AIDS* 3(10):625–630.
 225. Phillips-Conroy, J. E., C. J. Jolly, B. Petros, J. S. Allan, and R. C. Desrosiers. 1994. Sexual transmission of SIVagm in wild grivet monkeys. *J. Med. Primatol.* 23(1):1–7.
 226. Picker, L. J., S. I. Hagen, R. Lum, E. F. Reed-Inderbitzin, L. M. Daly, A. W. Sylvester, J. M. Walker, D. C. Siess, M. Piatak Jr., C. Wang, D. B. Allison, V. C. Maino, J. D. Lifson, T. Kodama, and M. K. Axthelm. 2004. Insufficient production and tissue delivery of CD4⁺ memory T cells in rapidly progressive simian immunodeficiency virus infection. *J. Exp. Med.* 200(10):1299–1314.
 227. Picker, L. J. and D. I. Watkins. 2005. HIV pathogenesis: the first cut is the deepest. *Nat. Immunol.* 6(5):430–432.
 228. Piguet, V. and D. Trono. 1999. The Nef protein of primate lentiviruses. *Rev. Med. Virol.* 9(2):111–120.
 229. Prince, A. M., B. Brotman, D. H. Lee, L. Andrus, J. Valinsky, and P. Marx. 2002. Lack of evidence for HIV type 1-related SIVcpz infection in captive and wild chimpanzees (*Pan troglodytes verus*) in West Africa. *AIDS Res. Hum. Retroviruses* 18(9):657–660.
 230. Rey-Cuille, M. A., J. L. Berthier, M. C. Bomsel-Demontoy, Y. Chaduc, L. Montagnier, A. G. Hovanessian, and L. A. Chakrabarti. 1998. Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. *J. Virol.* 72(5):3872–3886.
 231. Richman, D. D., T. Wrin, S. J. Little, and C. J. Petropoulos. 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc. Natl. Acad. Sci. U. S. A.* 100(7):4144–4149.
 232. Roederer, M., J. G. Dubs, M. T. Anderson, P. A. Raju, L. A. Herzenberg, and L. A. Herzenberg. 1995. CD8 naive T cell counts decrease progressively in HIV-infected adults. *J. Clin. Invest.* 95(5):2061–2066.
 233. Sakai, H., R. Shibata, T. Miura, M. Hayami, K. Ogawa, T. Kiyomasu, A. Ishimoto, and A. Adachi. 1990. Complementation of the rev gene mutation among human and simian lentiviruses. *J. Virol.* 64(5):2202–2207.
 234. Sakuragi, J., M. Fukasawa, R. Shibata, H. Sakai, M. Kawamura, H. Akari, T. Kiyomasu, A. Ishimoto, M. Hayami, and A. Adachi. 1991. Functional analysis of long terminal repeats derived from four strains of

- simian immunodeficiency virus SIVAGM in relation to other primate lentiviruses. *Virology* 185(1):455–459.
235. Sakuragi, S., J. Sakuragi, and A. Adachi. 1995. Both SU and TM env proteins are responsible for monkey cell tropism of simian immunodeficiency virus SIV mac. *Arch. Virol.* 140(12):2255–2260.
236. Salemi, M., T. De Oliveira, V. Courgaud, V. Moulton, B. Holland, S. Cassol, W. M. Switzer, and A. M. Vandamme. 2003. Mosaic genomes of the six major primate lentivirus lineages revealed by phylogenetic analyses. *J. Virol.* 77(13):7202–7213.
237. Santiago, M. L., F. Bibollet-Ruche, E. Bailes, S. Kamenya, M. N. Muller, M. Lukasik, A. E. Pusey, D. A. Collins, R. W. Wrangham, J. Goodall, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 2003. Amplification of a complete simian immunodeficiency virus genome from fecal RNA of a wild chimpanzee. *J. Virol.* 77(3):2233–2242.
238. Santiago, M. L., F. Bibollet-Ruche, N. Gross-Camp, A. C. Majewski, M. Masozera, I. Munanura, B. A. Kaplin, P. M. Sharp, G. M. Shaw, and B. H. Hahn. 2003. Noninvasive detection of simian immunodeficiency virus infection in a wild-living L'Hoest's monkey (*Cercopithecus lhoesti*). *AIDS Res. Hum. Retroviruses*. 19(12):1163–1166.
239. Santiago, M. L., M. Lukasik, S. Kamenya, Y. Li, F. Bibollet-Ruche, E. Bailes, M. N. Muller, M. Emery, D. A. Goldenberg, J. S. Lwanga, A. Ayouba, E. Nerrienet, H. M. McClure, J. L. Heeney, D. P. Watts, A. E. Pusey, D. A. Collins, R. W. Wrangham, J. Goodall, J. F. Brookfield, P. M. Sharp, G. M. Shaw, and B. H. Hahn. 2003. Foci of endemic simian immunodeficiency virus infection in wild-living eastern chimpanzees (*Pan troglodytes schweinfurthii*). *J. Virol.* 77(13):7545–7562.
240. Santiago, M. L., F. Range, B. F. Keele, Y. Li, E. Bailes, F. Bibollet-Ruche, C. Fruteau, R. Noe, M. Peeters, J. F. Brookfield, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 2005. Simian immunodeficiency virus infection in free-ranging sooty mangabees (*Cercocebus atys atys*) from the Tai Forest, Côte d'Ivoire: implications for the origin of epidemic human immunodeficiency virus type 2. *J. Virol.* 79(19):12515–12527.
241. Santiago, M. L., C. M. Rodenburg, S. Kamenya, F. Bibollet-Ruche, F. Gao, E. Bailes, S. Meleth, S. J. Soong, J. M. Kilby, Z. Moldoveanu, B. Fahey, M. N. Muller, A. Ayouba, E. Nerrienet, H. M. McClure, J. L. Heeney, A. E. Pusey, D. A. Collins, C. Boesch, R. W. Wrangham, J. Goodall, P. M. Sharp, G. M. Shaw, and B. H. Hahn. 2002. SIVcpz in wild chimpanzees. *Science* 295(5554):465.
242. Schindler, M., J. Munch, O. Kutsch, H. Li, M. L. Santiago, F. Bibollet-Ruche, M. C. Muller-Trutwin, F. J. Novembre, M. Peeters, V. Courgaud, E. Bailes, P. Roques, D. L. Sodora, G. Silvestri, P. M. Sharp, B. H. Hahn, and F. Kirchhoff. 2006. Nef-mediated suppression of T cell activation is a fundamental property of primate lentiviruses that was lost in the lineage that gave rise to HIV-1. *Cell* 125:1055–1067.
243. Schmitz, J. E., M. J. Kuroda, S. Santra, V. G. Sasseville, M. A. Simon, M. A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B. J. Scallan, J. Ghrayeb, M. A. Forman, D. C. Montefiori, E. P. Rieber, N. L. Letvin, and K. A. Reimann. 1999. Control of viremia in simian immunodeficiency virus infection by CD8⁺ lymphocytes. *Science* 283(5403):857–860.
244. Schmitz, J. E., M. A. Simon, M. J. Kuroda, M. A. Lifton, M. W. Ollert, C. W. Vogel, P. Racz, K. Tenner-Racz, B. J. Scallan, M. Dalesandro, J. Ghrayeb, E. P. Rieber, V. G. Sasseville, and K. A. Reimann. 1999. A nonhuman primate model for the selective elimination of CD8⁺ lymphocytes using a mouse-human chimeric monoclonal antibody. *Am. J. Pathol.* 154(6):1923–1932.
245. Schneider, R., M. Campbell, G. Nasioulas, B. K. Felber, and G. N. Pavlakis. 1997. Inactivation of the human immunodeficiency virus type 1 inhibitory elements allows Rev-independent expression of Gag and Gag/protease and particle formation. *J. Virol.* 71(7):4892–4903.
246. Schols, D. and E. De Clercq. 1998. The simian immunodeficiency virus mnd(GB-1) strain uses CXCR4, not CCR5, as coreceptor for entry in human cells. *J. Gen. Virol.* 79(Pt 9):2203–2205.
247. Schrofelbauer, B., D. Chen, and N. R. Landau. 2004. A single amino acid of APOBEC3G controls its species-specific interaction with virion infectivity factor (Vif). *Proc. Natl. Acad. Sci. U. S. A.* 101(11):3927–3932.
248. Sharp, P. M., E. Bailes, F. Gao, B. E. Beer, V. M. Hirsch, and B. H. Hahn. 2000. Origins and evolution of AIDS viruses: estimating the time-scale. *Biochem. Soc. Trans.* 28(2):275–282.
249. Sharp, P. M., E. Bailes, M. Stevenson, M. Emerman, and B. H. Hahn. 1996. Gene acquisition in HIV and SIV. *Nature* 383(6601):586–587.
250. Sharp, P. M., D. L. Robertson, F. Gao, and B. H. Hahn. 1994. Origins and diversity of human immunodeficiency viruses. *AIDS* 8(Suppl 1):S27–S42.
251. Sharp, P. M., G. M. Shaw, and B. H. Hahn. 2005. Simian immunodeficiency virus infection of chimpanzees. *J. Virol.* 79(7):3891–3902.

252. Sigurdsson, B., P. Palsson, and H. Grimsson. 1957. Visna, a demyelinating transmissible disease of sheep. *J. Neuropathol. Exp. Neurol.* 16(3):389–403.
253. Sigurdsson, B. and P. A. Palsson. 1958. Visna of sheep: a slow, demyelinating infection. *Br. J. Exp. Pathol.* 39(5):519–528.
254. Silvestri, G. 2005. Naturally SIV-infected sooty mangabeys: are we closer to understanding why they do not develop AIDS? *J. Med. Primatol.* 34(5–6):243–252.
255. Silvestri, G., A. Fedanov, S. Germon, N. Kozyr, W. J. Kaiser, D. A. Garber, H. McClure, M. B. Feinberg, and S. I. Staprans. 2005. Divergent host responses during primary simian immunodeficiency virus SIVsm infection of natural sooty mangabey and nonnatural rhesus macaque hosts. *J. Virol.* 79(7):4043–4054.
256. Silvestri, G., M. Paiardini, I. Pandrea, M. M. Lederman, and D. L. Sodora. 2007. Understanding the benign nature of SIV infection in natural hosts. *J. Clin. Invest.* 117(11):3148–3154.
257. Silvestri, G., D. L. Sodora, R. A. Koup, M. Paiardini, S. P. O’Neil, H. M. McClure, S. I. Staprans, and M. B. Feinberg. 2003. Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. *Immunity* 18(3):441–452.
258. Simon, F., P. Maclere, P. Roques, I. Loussert-Ajaka, M. C. Muller-Trutwin, S. Saragosti, M. C. Georges-Courbot, F. Barre-Sinoussi, and F. Brun-Vezinet. 1998. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. *Nat. Med.* 4(9):1032–1037.
259. Simon, F., S. Souquiere, F. Damond, A. Kfutwah, M. Makuwa, E. Leroy, P. Rouquet, J. L. Berthier, J. Rigoulet, A. Lecu, P. T. Telfer, I. Pandrea, J. C. Plantier, F. Barre-Sinoussi, P. Roques, M. C. Muller-Trutwin, and C. Apetrei. 2001. Synthetic peptide strategy for the detection of and discrimination among highly divergent primate lentiviruses. *AIDS Res. Hum. Retroviruses* 17(10):937–952.
260. Simon, J. H. and M. H. Malim. 1996. The human immunodeficiency virus type 1 Vif protein modulates the postpenetration stability of viral nucleoprotein complexes. *J. Virol.* 70(8):5297–5305.
261. Simon, J. H., D. L. Miller, R. A. Fouchier, M. A. Soares, K. W. Peden, and M. H. Malim. 1998. The regulation of primate immunodeficiency virus infectivity by Vif is cell species restricted: a role for Vif in determining virus host range and cross-species transmission. *EMBO J.* 17(5):1259–1267.
262. Smith, S. M., B. Holland, C. Russo, P. J. Dailey, P. A. Marx, and R. I. Connor. 1999. Retrospective analysis of viral load and SIV antibody responses in rhesus macaques infected with pathogenic SIV: predictive value for disease progression. *AIDS Res. Hum. Retroviruses* 15(18):1691–1701.
263. Song, B., H. Javanbakht, M. Perron, D. H. Park, M. Stremlau, and J. Sodroski. 2005. Retrovirus restriction by TRIM5alpha variants from Old World and New World primates. *J. Virol.* 79(7):3930–3937.
264. Souquiere, S., F. Bibollet-Ruche, D. L. Robertson, M. Makuwa, C. Apetrei, R. Onanga, C. Kornfeld, J. C. Plantier, F. Gao, K. Abernethy, L. J. White, W. Karesh, P. Telfer, E. J. Wickings, P. Maclere, P. A. Marx, F. Barre-Sinoussi, B. H. Hahn, M. C. Muller-Trutwin, and F. Simon. 2001. Wild Mandrillus sphinx are carriers of two types of lentivirus. *J. Virol.* 75(15):7086–7096.
265. Sousa, A. E., J. Carneiro, M. Meier-Schellersheim, Z. Grossman, and R. M. Victorino. 2002. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J. Immunol.* 169(6):3400–3406.
266. Staprans, S. I. and M. B. Feinberg. 2004. The roles of nonhuman primates in the preclinical evaluation of candidate AIDS vaccines. *Expert Rev. Vaccines* 3(Suppl 4):S5–S32.
267. Stremlau, M., C. M. Owens, M. J. Perron, M. Kiessling, P. Autissier, and J. Sodroski. 2004. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* 427(6977):848–853.
268. Stremlau, M., M. Perron, S. Welikala, and J. Sodroski. 2005. Species-specific variation in the B30.2(SPRY) domain of TRIM5alpha determines the potency of human immunodeficiency virus restriction. *J. Virol.* 79(5):3139–3145.
269. Sumpter, B., R. Dunham, S. Gordon, J. Engram, M. Hennessy, A. Kinter, M. Paiardini, B. Cervasi, N. Klatt, H. McClure, J. M. Milush, S. Staprans, D. L. Sodora, and G. Silvestri. 2007. Correlates of preserved CD4⁺ T cell homeostasis during natural, nonpathogenic simian immunodeficiency virus infection of sooty mangabeys: implications for AIDS pathogenesis. *J. Immunol.* 178(3):1680–1691.
270. Sumpter, B., R. Dunham, S. Gordon, J. Engram, M. Hennessy, A. Kinter, M. Paiardini, B. Cervasi, N. Klatt, H. McClure, J. M. Milush, S. Staprans, D. L. Sodora, and G. Silvestri. 2007. Correlates of preserved CD4⁺ T cell homeostasis during natural, nonpathogenic simian immunodeficiency virus infection of sooty mangabeys: implications for AIDS pathogenesis. *J. Immunol.* 178:1680–1691.
271. Switzer, W. M., B. Parekh, V. Shanmugam, V. Bhullar, S. Phillips, J. J. Ely, and W. Heneine. 2005. The

- epidemiology of simian immunodeficiency virus infection in a large number of wild- and captive-born chimpanzees: evidence for a recent introduction following chimpanzee divergence. *AIDS Res. Hum. Retroviruses* 21(5):335–342.
272. Takemura, T., M. Ekwalanga, B. Bikandou, E. Ido, Y. Yamaguchi-Kabata, S. Ohkura, H. Harada, J. Takehisa, H. Ichimura, H. J. Parra, M. Nende, E. Mubwo, M. Sepole, M. Hayami, and T. Miura. 2005. A novel simian immunodeficiency virus from black mangabey (*Lophocebus aterrimus*) in the Democratic Republic of Congo. *J. Gen. Virol.* 86(Pt 7):1967–1971.
273. Tomonaga, K., J. Katahira, M. Fukasawa, M. A. Hassan, M. Kawamura, H. Akari, T. Miura, T. Goto, M. Nakai, M. Suleman, M. Isahakia, and M. Hayami. 1993. Isolation and characterization of simian immunodeficiency virus from African white-crowned mangabey monkeys (*Cercopithecus torquatus lunulatus*). *Arch. Virol.* 129(1–4):77–92.
274. Tosi A. J., D. J. Melnick, and T. R. Disotell. 2004. Sex chromosome phylogenetics indicate a single transition to terrestriality in the guenons (tribe Cercopithecini). *J. Hum. Evol.* 46:223–237.
275. Towers, G. J. and S. P. Goff. 2003. Post-entry restriction of retroviral infections. *AIDS Rev.* 5(3):156–164.
276. Towers, G. J., T. Hatziloannou, S. Cowan, S. P. Goff, J. Luban, and P. D. Bieniasz. 2003. Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. *Nat. Med.* 9(9):1138–1143.
277. Traina-Dorge, V., J. Blanchard, L. Martin, and M. Murphey-Corb. 1992. Immunodeficiency and lymphoproliferative disease in an African green monkey dually infected with SIV and STLV-I. *AIDS Res. Hum. Retroviruses* 8(1):97–100.
278. Tsujimoto, H., R. W. Cooper, T. Kodama, M. Fukasawa, T. Miura, Y. Ohta, K. Ishikawa, M. Nakai, E. Frost, G. E. Roelants, J. Roffi, and M. Hayami. 1988. Isolation and characterization of simian immunodeficiency virus from mandrills in Africa and its relationship to other human and simian immunodeficiency viruses. *J. Virol.* 62(11):4044–4050.
279. Tsujimoto, H., A. Hasegawa, N. Maki, M. Fukasawa, T. Miura, S. Speidel, R. W. Cooper, E. N. Moriyama, T. Gojobori, and M. Hayami. 1989. Sequence of a novel simian immunodeficiency virus from a wild-caught African mandrill. *Nature* 341(6242):539–541.
280. Van Dooren, S., W. M. Switzer, W. Heneine, P. Goubaud, E. Verschoor, B. Parekh, W. De Meirchy, C. Furley, M. Van Ranst, and A. M. Vandamme. 2002. Lack of evidence for infection with simian immunodeficiency virus in bonobos. *AIDS Res. Hum. Retroviruses* 18(3):213–216.
281. Van Heuverswyn, F., Y. Li, E. Bailes, C. Neel, B. Lafay, B. F. Keele, K. S. Shaw, J. Takehisa, M. H. Kraus, S. Loul, C. Butel, F. Liegeois, B. Yangda, P. M. Sharp, E. Mpoudi-Ngole, E. Delaporte, B. H. Hahn, and M. Peeters. 2007. Genetic diversity and phylogeographic clustering of SIVcpzPtt in wild chimpanzees in Cameroon. *Virology* 368(1):155–171.
282. Van Heuverswyn, F., Y. Li, C. Neel, E. Bailes, B. F. Keele, W. Liu, S. Loul, C. Butel, F. Liegeois, Y. Bienvenue, E. M. Ngolle, P. M. Sharp, G. M. Shaw, E. Delaporte, B. H. Hahn, and M. Peeters. 2006. Human immunodeficiency viruses: SIV infection in wild gorillas. *Nature* 444(7116):164.
283. van Rensburg, E. J., S. Engelbrecht, J. Mwenda, J. D. Laten, B. A. Robson, T. Stander, and G. K. Chege. 1998. Simian immunodeficiency viruses (SIVs) from eastern and southern Africa: detection of a SIVagn variant from a chacma baboon. *J. Gen. Virol.* 79:1809–1814.
284. Vanden Haesevelde, M., J. L. Decourt, R. J. De Leys, B. Vanderborght, G. van der Groen, H. van Heuverswijn, and E. Saman. 1994. Genomic cloning and complete sequence analysis of a highly divergent African human immunodeficiency virus isolate. *J. Virol.* 68(3):1586–1596.
285. VandeWoude, S. and C. Apetrei. 2006. Going wild: lessons from T-lymphotropic naturally occurring lentiviruses. *Clin. Microbiol. Rev.* 19:728–762.
286. Veazey, R., B. Ling, I. Pandrea, H. McClure, A. Lackner, and P. Marx. 2003. Decreased CCR5 expression on CD4⁺ T cells of SIV-infected sooty mangabeys. *AIDS Res. Hum. Retroviruses* 19(3):227–233.
287. Veazey, R. S., M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner. 1998. Gastrointestinal tract as a major site of CD4⁺ T cell depletion and viral replication in SIV infection. *Science* 280(5362):427–431.
288. Verschoor, E. J., Z. Fagrouch, I. Bontjer, H. Niphuis, and J. L. Heeney. 2004. A novel simian immunodeficiency virus isolated from a Schmidt's guenon (*Cercopithecus ascanius schmidtii*). *J. Gen. Virol.* 85(Pt 1):21–24.
289. Voevodin, A. and P. Marx. 2008. Frag-virus: a new term to distinguish presumptive viruses known primarily from sequence data. *Virol. J.* 5:34.
290. Wain-Hobson, S., P. Sonigo, O. Danos, S. Cole, and M. Alizon. 1985. Nucleotide sequence of the AIDS virus, LAV. *Cell* 40(1):9–17.
291. Wang, Z., B. Metcalf, R. M. Ribeiro, H. McClure, and A. Kaur. 2006. Th-1-type cytotoxic CD8⁺ T-lymphocyte responses to simian immunodeficiency virus (SIV) are a consistent feature of natural SIV

- infection in sooty mangabeys. *J. Virol.* 80(6):2771–2783.
292. Watts, D. P. and J. C. Mitani. 2002. Hunting behaviour of chimpanzees at Ngogo, Kibale National Park, Uganda. *Int. J. Primatol.* 23(1):1–28.
293. Wei, B. L., V. K. Arora, J. L. Foster, D. L. Sodora, and J. V. Garcia. 2003. In vivo analysis of Nef function. *Curr. HIV Res.* 1(1):41–50.
294. Werner, A., G. Winskowsky, and R. Kurth. 1990. Soluble CD4 enhances simian immunodeficiency virus SIVagm infection. *J. Virol.* 64(12):6252–6256.
295. Worobey, M., M. L. Santiago, B. F. Keele, J. B. Ndjango, J. B. Joy, B. L. Labama, A. B. Dhed, A. Rambaut, P. M. Sharp, G. M. Shaw, and B. H. Hahn. 2004. Origin of AIDS: contaminated polio vaccine theory refuted. *Nature* 428(6985):820.
296. Xu, H., E. S. Svarovskaia, R. Barr, Y. Zhang, M. A. Khan, K. Strelbel, and V. K. Pathak. 2004. A single amino acid substitution in human APOBEC3G antiretroviral enzyme confers resistance to HIV-1 virion infectivity factor-induced depletion. *Proc. Natl. Acad. Sci. U. S. A.* 101(15):5652–5657.
297. Ylinen, L. M., Z. Keckesova, S. J. Wilson, S. Ranasinghe, and G. J. Towers. 2005. Differential restriction of human immunodeficiency virus type 2 and simian immunodeficiency virus SIVmac by TRIM5alpha alleles. *J. Virol.* 79(18):11580–11587.
298. Zhang, H., B. Yang, R. J. Pomerantz, C. Zhang, S. C. Arunachalam, and L. Gao. 2003. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* 424(6944):94–98.
299. Zhang, Y., B. Lou, R. B. Lal, A. Gettie, P. A. Marx, and J. P. Moore. 2000. Use of inhibitors to evaluate coreceptor usage by simian and simian/human immunodeficiency viruses and human immunodeficiency virus type 2 in primary cells. *J. Virol.* 74(15):6893–6910.
300. Zou, J. X. and P. A. Luciw. 1996. The requirement for Vif of SIVmac is cell-type dependent. *J. Gen. Virol.* 77(Pt 3):427–434.
301. Zou, L., M. C. Barr, W. A. Hoose, and R. J. Avery. 1997. Characterization of the transcription map and Rev activity of a highly cytopathic feline immunodeficiency virus. *Virology* 236(2):266–278.

4

Lentiviruses AIDS Models

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4.1. INTRODUCTION

The discovery of simian immunodeficiency virus (SIV) for AIDS animal model research was serendipitous. The two major SIVs used in AIDS experiments both resulted from the unintended consequences of animal model research unrelated to AIDS, and in fact took place before AIDS was reported in humans in 1981. The law of unintended consequences states that “the purposeful actions of people and especially of governments always have effects that are unintended.”²¹¹

SIVs have their natural origins in healthy African monkeys and apes (see Chapter 3). SIV was first discovered in two Asian macaque species, rhesus (RhM) and cynomolgus macaques.⁵³ Because the infection is mostly silent in African species,^{112,178,206,223} the infection first manifested itself in Asian species after they developed a typical AIDS clinical syndrome.⁵³ The combination of the close genetic relationship between SIV and HIV-2¹¹² and the induction of AIDS in commonly available laboratory monkey species, led to the development

of SIV infection in macaques (SIVmac) as the model most widely used for AIDS research.

The number of publications on AIDS nonhuman primate (NHP) models exceeds 5,000 and increases every day. This chapter will describe the major findings that made SIV in RhM the model of choice for AIDS vaccines, drug testing, and pathogenesis. Notably, several important discoveries were made in the SIVmac model before they were found in human AIDS. For example, early immunopathogenesis of SIV was characterized in SIV-infected RhM^{43,283} before the same studies were done in humans.²¹⁸ Proof that AIDS was a disease of mucosal immune system was also done first in SIV-infected macaques,^{272,274} showing the decline in mucosal CD4⁺ cells in acute infection.

There has much discussion in the literature about the value of AIDS animal models.^{69,278} The viewpoint as to what constitutes a “perfect” AIDS model is a matter of opinion. The ideal model for AIDS and for any other infectious disease is the induction of the human disease in a common laboratory animal with a virus isolated from human tissue. Although idea model conditions exist for some human diseases, they are not rigorously met by any of the AIDS animal models. The most common AIDS virus is HIV-1 and it does not reproducibly infect or cause AIDS in any animal species, although many species were tested.^{4,152,203} Although HIV-1 readily infects chimpanzees, AIDS is a relatively rare outcome and chimpanzees are hardly a common laboratory animal.^{80,228,242} There are reports of HIV-1 infection in pig-tailed macaques and HIV-2 infection in baboons and cynomolgus macaques. HIV-1 infection in pig-tailed macaques has been of limited value due to the transient nature of the HIV infection.¹³¹ Infection for baboons and cynomolgus macaques with HIV-2 has not been widely used because of transient infections.^{25,163,277} SIVmac in macaques represents an animal model compromise because SIVmac is related to HIV-2, the less prevalent human AIDS virus. Nevertheless, SIV readily induces AIDS in macaques and macaques are a common laboratory species. Therefore, the SIV-macaque model is widely acknowledged as the most appropriate model of human AIDS.

4.2. NOMENCLATURES AND SIV PHYLOGENETIC CLASSIFICATION

The nomenclature for SIV has always followed that of HIV. However, the human AIDS virus was named and renamed in the early days of AIDS research and SIV

followed suit. A French team at the Pasteur Institute in Paris discovered HIV. Francoise Barre-Sinoussi and Luc Montagnier won the Noble prize in Medicine in 2008 for their discovery. The French group named the virus lymphadenopathy-associated virus (LAV) because the isolate was from a patient with lymphadenopathy.²⁶ The first American publication on HIV renamed LAV the human T-cell lymphotropic virus-III (HTLV-III) in the erroneous belief that HTLV-III was different from LAV and that HTLV-III was closely related to the human T-cell lymphoma/leukemia virus I (HTLV-I) family, genus *Deltaretrovirus*.²²⁷ Following suit, the first publication of the simian version of HIV was named simian T-cell lymphotropic virus III (STLV-III) because by analogy with HTLV-III, STLV-III should also be related to simian T-cell leukemia virus I group.⁵³ Both the nomenclature and the science were further confused by the isolation of a virus from African green monkey cells that was named STLV-IIIagm.¹²⁷ In the following year, the same group reported finding the STLV-IIIagm in persons in West Africa.¹²⁸ This “human virus” was named HTLV-IV. In 1989, the Desrosiers group came to the rescue and showed that both HTLV-IV and STLV-IIIagm were laboratory contaminations by SIVmac251.¹³⁵ The STLV-IIIagm reports were responsible for the widely held view by the press and their readers that African green monkeys were the source of HIV. The genuine SIV from African green monkeys is covered in Chapter 3 and is not related to HIV-1 or HIV-2. When HIV and SIV were sequenced, neither were related to the genus *Deltaretrovirus* (HTLV/STLV), but belonged to the entirely different *Lentivirus* genus.^{41,112,166,235} LAV/HTLV-III was renamed HIV and the STLV-III was renamed SIV.⁴⁸

4.3. SIVs AND SIV/HIV HYBRID VIRUSES (SHIV) USED FOR INDUCTION OF AIDS, VACCINE, AND DRUG STUDIES

Three different SIV and SIV-derived viruses are commonly used in AIDS animal model research. They are the SIVmac group, SIVb670, and simian–human immunodeficiency viruses (SHIVs). SHIVs are hybrid viruses derived from SIV and HIV-1 genes. Figure 4.1 shows the genomic maps of these viruses. Table 4.1 provides a list of the virus and their characteristics with regard to pathogenesis and sensitivity to neutralizing antibody.

4.3.1. SIVmac Group—SIVmac251, SIVmac239

The commonly used SIV macaque AIDS models employ SIVs derived from sooty mangabeys (SMs). Although

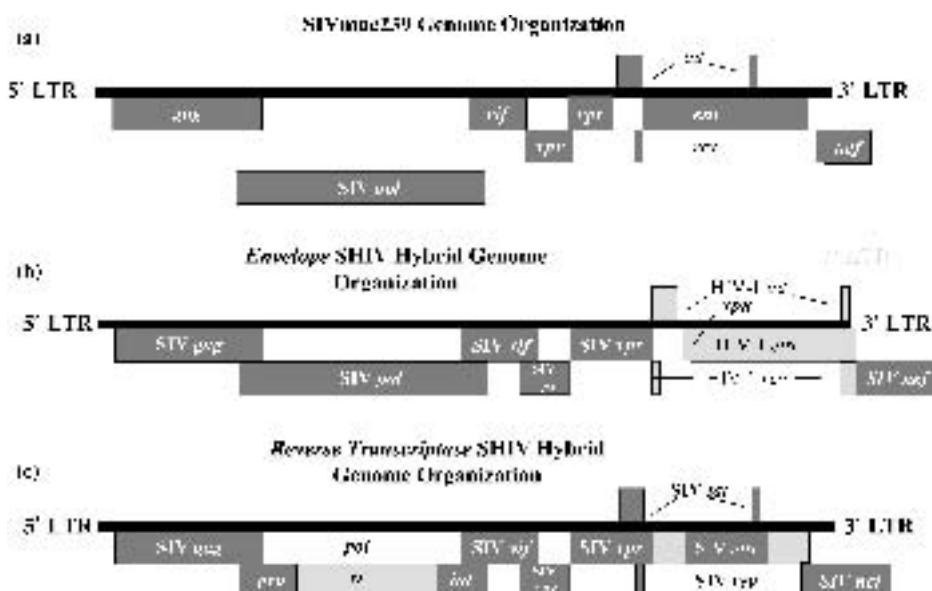


Figure 4.1. Genomic organization of simian immunodeficiency virus (SIV) and simian–human immunodeficiency virus (SHIV). The genome organization for SIVmac239 (a), SHIV-containing HIV-1 *env* (b), and HIV-1 reverse transcriptase (c). The SIV genes are dark gray and the HIV-1 genes are light gray.¹⁷

nine separate SIVsmm phylogenetic lineages are known, two lineages, the SIVmac lineage 8 and the SIVb670/H5 lineage 1, account for the vast majority of AIDS animal model research.¹⁷

The SIVmac group is the most used of the AIDS animal model viruses. The SIVmac group originated from an SM colony that was at the California National Primate Research Center (California NRPC)^{17,172} in the 1970s (Figure 4.2). The history of SIVmac is an excellent example of the law of unintended consequences. In the 1970s, this SM colony was used by the Carlton Gajdusek group to develop a kuru animal model.^{18,85,89} In the course of their experiments, tissues from kuru-inoculated SMs were passaged in rhesus macaques (RhM) to simply the development of the kuru model in a more commonly available monkey species, i.e., the rhesus macaque. This passage was responsible for an outbreak of B-cell lymphomas in outdoor rhesus colonies at the CNPRC.^{34,85,181,260}

The apparently healthy animals from the outbreak at the California center were sent to the New England Primate Research Center where infected tissues were

subjected to additional serial passages, monkey to monkey resulting in the highly pathogenic viruses SIVmac251 (passage 4) and SIVmac239 (passage 7).¹⁷² Figure 4.3 outlines the serial passage at the New England Primate Center.¹⁷² When HIV AIDS was described in 1981,⁹⁴ the disease in these rhesus macaques was recognized as AIDS.¹²⁰ SIVmne, a less pathogenic member of the SIVmac group, surfaced in pig-tailed macaques (*M. nemestrina*).²⁰⁴ SIVmne was also derived from the same rhesus lymphoma outbreak at the California NRPC. The phylogenetic relationships between SMs at the California NRPC, SIVmac at the New England Primate Research Center, and SIVmne at the Washington National Primate Research Center are shown in Figure 4.2. SIVmne is less pathogenic compared to SIVmac239 because SIVmne was not serially passaged to the same extent as SIVmac239.¹⁷² Figure 4.1 shows the genome map of SIVmac. The map is the same as the SIVmac/HIV-2 genome group described in Chapter 3. An additional virus derived from SIVmac is SIV32H²⁴⁰ which was a reisolated strain of SIVmac251 (Figure 4.2).

Table 4.1. SIVs and SHIVs Commonly Used in AIDS Animal Model Research

SIVs Commonly Used	Major Coreceptors in Vitro*/In Vivo	In Vivo Pathogenesis [†] in Rhesus	Remarks
SIVmac group lineage 8 (see Figure 4.4)	CCR5, BONZO/CCR5		
SIVmac251 ⁵³	CCR5, BONZO/CCR5	Medium pathogenic swarm	4 serial passages
SIVmac251 TCLA ¹⁹⁹	CCR5, BONZO/CCR5	Not tested in vivo	T-cell-line-adapted neutralization sensitive
SIVmac251 CX-1 ¹⁸⁹	CCR5, BONZO/CCR5	Pathogenic swarm	Moderately neutralization sensitive
SIVmac239 ¹⁷²	ND	Highly pathogenic swarm	7 serial passages [‡]
SIVmac239 (clone) ¹³⁴	CCR5, BONZO/CCR5	Highly pathogenic molecular clone [§]	7 serial passages [‡] neutralization resistant
SIVmac142 ¹⁷²	ND	Less commonly used	
SIVmne ²⁰⁴	CCR5, BONZO/CCR5	Least pathogenic of SIVmac group	
SIVmac32H ²⁴⁰	CCR5, BONZO/CCR5	Pathogenic molecular clone derived	
SIVb670 group lineage 1 (see Figure 4.4)	CCR5, BONZO/CCR5		
SIVb670 ²⁰⁶	CCR5, BONZO/CCR5	Pathogenic swarm	Neutralization resistant
SIVe660 ³⁸	CCR5, BONZO/CCR5	Pathogenic swarm	Moderately sensitive to neutralization
SHIV group			Clade/HIV-1 donor strain
SHIV vpu ⁻¹⁵⁴	CXCR4/CXCR4		B/HXBc2
SHIV89.6P ²³⁶	CCR5+, CXCR4/CxCR4	Pathogenic	B/89.6
SHIVKu-1, KU-2 ¹²⁴	CXCR4/CXCR4	Pathogenic	B/HXBc2
SHIV162p3/4 ¹⁰⁵	CCR5/CCR5	Transient infection	B/162p
SHIV-SF33A ¹⁰⁵	CXCR4/CXCR4	Moderate	B/SF33
SHIV DH12 ⁶³	CCR5, CxCR4/CxCR4	Pathogenic	B/HIV-1 DH12
RT-SHIV ²¹⁰	CCR5/CCR5 (same as SIVmac239)	Moderately pathogenic	B/HIV-1 HXBc2
SHIV1157 ²⁵⁴	CCR5/CCR5	Transient or moderately pathogenic	C
RT-SHIVrti/3mP ⁷	CXCR4	Transient infection	B

References for the viruses are in superscript.

*SIV and SHIV are promiscuous in vitro. Coreceptors used in vitro but are not major coreceptors in vivo BOB (gpr15) and BONZO (STRL33).

[†]Relative pathogenesis defined as time to AIDS and plasma virus load—highly pathogenic strains induce AIDS in shortest with higher virus loads than moderately pathogenic strains²⁵⁰.

[‡]Passaged in rhesus.

[§]This clone is also used for SIV vaccine vectors.

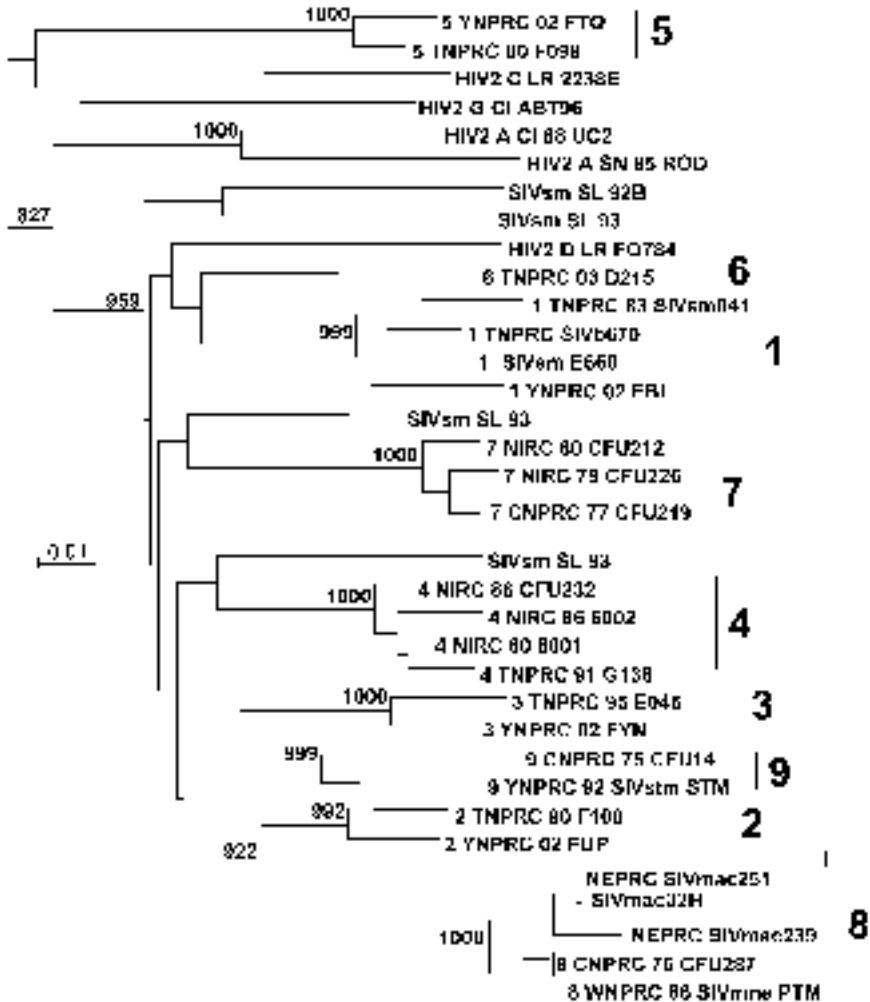


Figure 4.2. The *env* phylogenetic tree of simian immunodeficiency viruses derived from sooty mangabeys. SIV falls into nine phylogenetic lineages. Lineage 8, the SIVmac group, and lineage 1—the SIVb670 group, are most commonly used. Numbers at the branching points or nodes (1000, 999, etc.) are bootstrap support values (see Chapter 2); only significant numbers are shown. Viruses are labeled by the primate center where they were found. Note that SIV 8 CNPRC 76 CFU287 (boxed) is from an SM in the California Primate Center colony. Sample was taken in 1976. This virus is the ancestor of the SIVmac group and SIVmne. 9 CNPRC 75 CFU14 is the source of SIVstm¹⁶⁴ that caused a lymphoma outbreak in stump-tailed macaques at the Yerkes Primate Center. SIVsm 041 was isolated from one of the few infected sooty mangabeys to develop AIDS. SL93/SL92 designate unassigned SIVs from Sierra Leone. HIV-2 groups A, D, C, and D are shown. Abbreviations: SM, sooty mangabey; mac, macaque; stm, stump-tailed macaque; mne, *M. nemestrina* pig-tailed macaque. YNPRC, Yerkes National Primate Research Center; NEPRC, New England Primate Research Center; CNPRC, California National Primate Research Center; HIV, human immunodeficiency virus; NIRC, New Iberia Research Center; WNPRC, Washington National Primate Research Center. Nomenclature for 8 CNPRC 76 CFU287: 8 is the phylogenetic lineage; CNPRC location of animal at time of sampling; 76—year of sample is 1976; CFU287—animal identification number. Other SIV names are from the published literature. The scale bar indicates nucleotide substitutions per site (Adapted from Apetrei *et al.*¹⁷ and Apetrei *et al.*¹⁸).

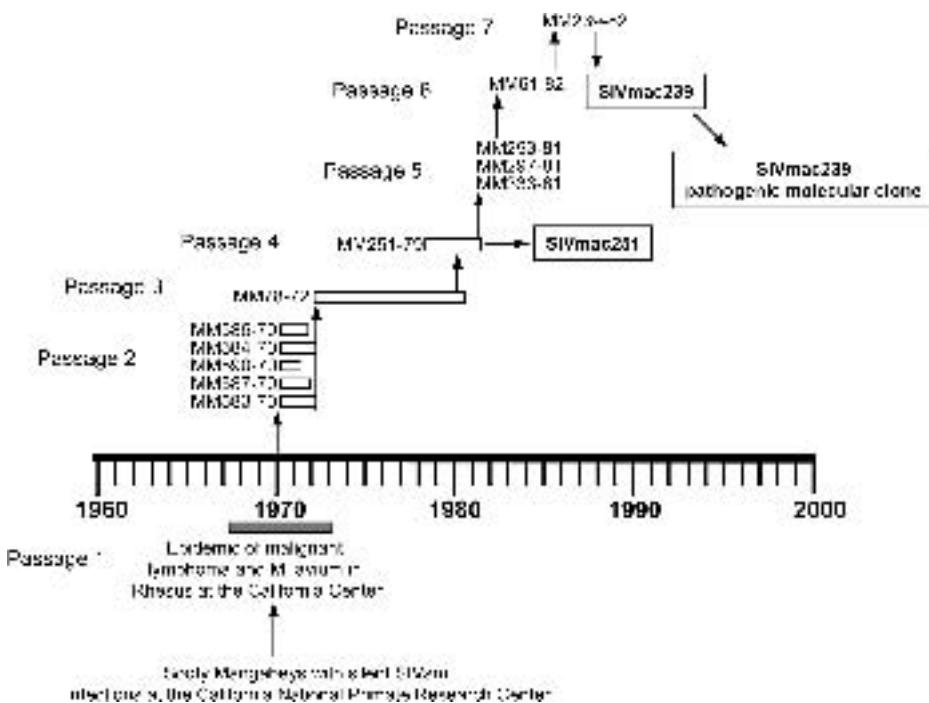


Figure 4.3. Serial passage history of simian immunodeficiency viruses macaque group (SIVmac) commonly used in rhesus models of AIDS. The SIVmac group originated from a sooty mangabey (SM) at the California National Primate Research Center. The SMs received clinical material from kuru patients.¹⁸ Rhesus macaques (RhM) were used to serially passage tissue from the kuru-material exposed SMs. These RhM developed AIDS B-cell lymphomas and *Micobacterium avium* opportunistic infections.²⁶⁰ The clinically healthy RhM remaining from the outbreak were transferred to the New England Primate Research Center where lymphoma developed and the unknown agent was passaged six additional times to generate SIVmac251 (4th passage) and SIVmac239 (7th passage). The causative agent of AIDS was passaged in RhM resulting in highly pathogenic viruses that were identified later in 1985 as SIV.⁵³ (Adapted from Mansfield *et al.*¹⁷² with permission.)

Sequence data of SIVmac142 indicates that it was derived from the same series of animal passages at the New England Primate Center that generated SIVmac142.¹⁷ SIVmac142 is not widely used because the molecular clone was less pathogenic and did not protect monkeys from superinfection with pathogenic SIVmac251.^{58,208}

4.3.2. SIVb670/H4/H9 Group

The SIVb670/H4/H9 group was derived from three SMs in the colony at the Tulane National Primate Research Center (Tulane NRPC) (Figure 4.2, lineage group 1).

Again, the discovery was the unintended consequence of a completely different set of experiments. The research group at the Tulane NRPC developed a leprosy model using the SM colony in which a naturally occurring case of leprosy appeared in SM A022 in the colony.^{76,176} As in the kuru experiments by the Gajdusek group,^{18,65} the Tulane group passaged affected tissues from SMs to rhesus macaques with the goal of further developing the model in a more common laboratory NHP.²⁸¹ After a period of time, immunosuppression was seen in some of the rhesus macaques and the disease was later characterized as SIV-induced AIDS.^{28,206}

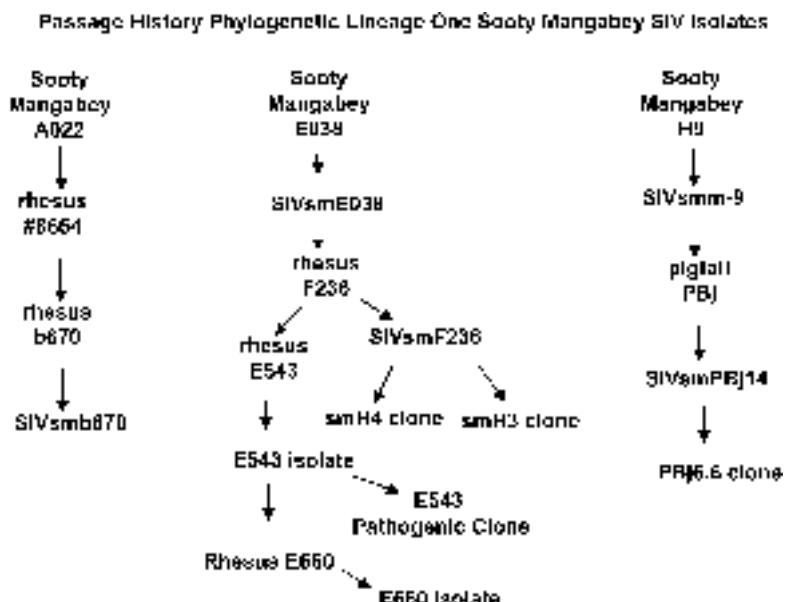


Figure 4.4. Passage history of commonly used phylogenetic lineage 1 simian immunodeficiency viruses (SIVs), SIVb670, SIVe660, and SIVe543. SIVb670 was originally obtained in leprosy experiments at the Tulane National Primate Research Center (Tulane NRPC) that were designed to adapt the leprosy bacillus to RhM. SM A022 was the original donor of SIVb670. SIVe660 group was derived from SME038, also at the Tulane NRPC. The SIVpbj group was derived at the Yerkes National Primate Research Center. All are phylogenetic lineage 1 viruses (Figure was kindly provided by Dr. V. Hirsch.).

Commonly used members of the lineage 1 group are SIVb670,²⁰⁶ SIV-E660^{38,109} an uncloned isolate that is pathogenic and moderately susceptible to neutralizing antibody,^{109–111} and SIVpbj.^{78,245} A pathogenic molecular clone of lineage 1, E543-3, is also available.¹⁰⁹ SIVpbj induces a particularly virulent and frequently fatal infection in pig-tailed macaques that is characterized by extensive lymphoid hyperplasia of T-cell zones in the gut-associated lymphoid tissue.⁸⁴ The names b670, E660, pbj, and E543-3 are derived from the monkey numbers. B670 was from one of the RhM in the original Leprosy serial passage group (Figure 4.3). Figure 4.4 summarizes the history of lineage 1 SIVs.

The SIVb670 strain was derived from SM-022 (Figure 4.4). SM-022 was followed for clinical signs and was shown to be free of AIDS. The same observation was also made at the Yerkes National Primate Research Center (Yerkes NRPC) in their SM colony.⁷⁷ These studies were the first to associate SIV with SMs that were

free of disease and led to the theory that SIV was not pathogenic in its natural host. This theory holds largely true with only a few exceptions^{158,184} in which AIDS developed in a few SMs after many years of infection.

SIV H4 virus was derived from SM-038, an SM in the Tulane NRPC colony. SIV H4 was passaged in rhesus (Figure 4.4) and was the first SIV sequenced that could be directly traced to an SM.¹¹² This seminal paper showed that SIVsm was the most likely source of HIV-2. This theory was proved by further studies on SMs in West Africa.¹⁷⁸

4.3.3. SHIV Group

SHIVs are hybrids of HIV-1 and SIV. The concept of simian–human hybrid viruses was developed in the Sodroski laboratory in the early 1990s.¹⁵⁴ The first SHIV consisted of clade B HIV-1 *env*, *tat*, and *rev* genes. Because this SHIV lacked a functional HIV-1 *vpu* gene,

it was named vpu⁻SHIV and was nonpathogenic.¹⁵⁵ Since vpu⁻SHIV, many more SHIVs have been made and the major ones are summarized in Table 4.1. Most env SHIVs produced after vpu⁻SHIV had a functional vpu and were designated vpu⁺ SHIV. Since env SHIVs that followed had functional vpu genes, the early distinctions of vpu⁻ and vpu⁺ are no longer used. The HIV-1 genes of vpu⁻SHIV were from the CXCR4 HIV-1 strain HXBc2 and the rest of the genome was from the pathogenic molecular clone of SIVmac239 (Figure 4.1b).^{154,155}

The next generation of SHIVs was dual tropic CCR5/CXCR4 in vitro, but behaved like CXCR4 tropic in vivo (SHIV89.6p, SHIVDH12, KU-SHIVs, SHIV-SF33A).^{63,105,106,124,130,236} The need for a pure CCR5 tropic env-SHIV was met by SHIV162, which is CCR5 tropic both in vitro and in vivo.^{105,116} SHIV162 was passaged up to four times to increase pathogenicity for rhesus macaques.²⁶² SHIV162 (passage 3) and SHIV162p3/p4 (passage 4) are commonly used; however, the SHIV162 viruses are not as pathogenic as other SHIVs and some infected rhesus monkeys may suppress the infection.²¹⁹

HIV-1 strains are divided into phylogenetic clades, A, B, C, and so forth. Although clade B is common in the United States and Europe, other clades are more prevalent in other countries.²⁷⁶ Therefore, clade C and clade E HIV-1 env-SHIVs were developed to provide a model to assess vaccines against an HIV-1 clade that is common in Africa and Asia.^{21,44,98,108,254}

Attempts continued to expand the HIV-1 genes that could be used in SHIV macaque models. SHIVs containing the RT coding region of HIV-1 were made. These viruses are used for in vivo testing of RT inhibitors that are restricted to blocking HIV-1 RT activity, such as nonnucleoside RT inhibitors (NNRTI).^{11,12,24,114,201,210,252,270}

SHIVrti/3rnP combines an env-SHIV with an RT-SHIV and also includes the *int* region of *pol*.⁷ This SHIV contains the most HIV-1 genes attempted thus far in an NHP model. Only the *gag*, *prt* (*pro*), *vif* are of SIV origin. After serial passaged virus in RhM SHIVrti/3rnP is capable of transient replication in rhesus macaques of Chinese origin. Since SIVmac239 replicates to higher virus loads in RhM of Indian origin compared to RhM of Chinese origin,^{160,264} use of SHIVrti/3rnP in RhM of Indian origin may confer better in vivo replication.

4.4. NHP MODELS OF HUMAN HIV TRANSMISSION

The most acceptable model for AIDS would consist of the inoculation of HIV into small animals that become persistently infected and progress to AIDS. However, extensive studies to develop such models were unsuccessful.²⁰³ From very early in the AIDS epidemic, NHPs were actively pursued as viable HIV animal models because of their close relationship to humans.

4.4.1. HIV Infection of Chimpanzees

The only animal species routinely susceptible to HIV-1 infection is the common chimpanzee (*Pan troglodytes*). The two theories for this unique susceptibility are (1) chimpanzees are the nearest human relatives in the animal kingdom and (2) chimpanzees are the natural host of HIV-1 ancestral viruses and therefore, HIV is a natural or near natural infection. Both theories are probably correct. Chimpanzees are persistently infected by HIV-1, either by intravenous or mucosal routes.^{9,79,82} However, infected chimpanzees develop only transient lymphadenopathy and immunosuppression.⁸⁰ Infected chimpanzees maintain immunocompetence and mount cellular immune responses to HIV-1 antigens.⁶² AIDS develops only rarely in this model.²¹³ HIV-1 subtype B-infected chimpanzees can be superinfected with HIV-1 subtype E.⁹¹ Moreover, recombination between the two viruses was observed⁸³ providing a demonstration of divergence by recombination, a major mechanism for evolution and divergence of HIV. Chimpanzees are used much less frequently for AIDS research compared to the earlier days of the epidemic. High cost is frequently cited, but the fact that the vast majority of chimpanzees do not develop persistent immunosuppression and AIDS is also a major factor.

4.4.2. HIV-1 Infection of Pig-Tailed Macaques

A series of studies reported the infection of HIV-1 in pig-tailed macaques.^{5,75} HIV-1 infection induces cell-mediated immune (CMI) responses. Such a model would have undoubtedly been accepted as the best model; however, infection was not persistent.¹³¹

4.5. PATHOGENESIS OF SIV AND SHIV

Infection of macaques with pathogenic strains of SIV mimics HIV pathogenesis. HIV and SIV infect the same target cells in vivo, establish persistent infections, are

resistant to neutralizing antibody, and are fatal in the vast majority of animals. With the exception of Kaposi sarcoma, they induce the same spectrum of tumors and opportunistic infections. The SHIVs vary in their target cell infection, some being infectious for CD4⁺/CCR5⁺ cells and others infecting CD4⁺/CXCR4⁺ cells. SHIVs are generally more susceptible to neutralizing antibody compared to primary isolates of HIV-1.^{8,46,199}

4.5.1. Routes of Infection

There are four routes of infection that serve as entry points for HIV transmission: (1) mucosal tissue, (2) blood, (3) placenta, and (4) solid tissue injection. Mucosal transmission is further divided into genital, oral, and rectal transmission routes. NHP models have been developed for each of the four routes of transmission.

The simplest and most commonly used transmission mode is intravenous (i.v.) injection. This route of infection is the most efficient and requires the least amount of infectious virus¹⁸⁹ because blood presents no barrier to infection, unlike mucous membranes that provide an epithelial barrier to virus penetration.

Vaginal transmission is frequently used, especially in vaccine experiments in which the vaccine is being tested for efficacy in a sexual transmission model. Typically, 1–4 mL of titrated SIV or SHIV is placed atraumatically into the vaginal vault.¹⁸⁹ The volume of the macaque vagina is approximately 4 cc. Atraumatic exposure is used to model the natural penetration of the virus through the intact mucosa. Chimpanzees can be infected with HIV by this route.⁷⁹ Vaginal infection requires a higher dose of virus compared to i.v., as much as a 100- to 1,000-fold increase in the dose compared to the i.v. route.¹⁸⁹ Susceptibility to vaginal transmission can be increased by pretreating macaques with progesterone or progestins such as Depo-Provera.^{179,180} The correlate of increased transmission is the thinning of the vaginal epithelium.^{179,249,251} Estrogen thickens the vaginal epithelium and renders macaques more resistant to infection. Progesterone, an estrogen antagonist, causes the epithelium to thin and therefore increases susceptibility. Other factors in the vagina may also play a role, such as vaginal pH and thickening of mucous. Progestins are reported to be immunosuppressive in macaque AIDS models and may contribute diseases progression or breaking vaccine protection.^{3,86,265}

The intact rectal mucosa is also susceptible to infection and this model is widely used for vaccine efficacy studies.^{165,222} The outcome of infection is the same by any of these infection routes. The foreskin and urethra of the macaque penis is also susceptible to infection.^{189,195}

Oral transmission of HIV to infants by breast milk is an important route of transmission in humans.^{268,289} This route has been effectively modeled in SIVb670-infected lactating females in which 10 of 14 transmitted SIV to their sucking infants in 1 year. Four infants remained SIV-negative despite their mothers progressing to AIDS.^{13,14,241}

SIVb670 transplacental transmission has been demonstrated in dams inoculated mid-gestation.¹⁷⁵ However, in another study, infants delivered by C-section were SIV-negative.⁵⁶

4.5.1.1. MECHANISMS OF MUCOSAL TRANSMISSION

Mechanisms of mucosal transmission have been investigated. The site of entry of SIV is the vaginal epithelium. The cervix is not required for infection. This was proven by surgical removal of the cervix to create a blind vaginal pouch. After healing, SIVmac was shown to be capable of infecting the blind vaginal pouch, following nontraumatic inoculation.¹⁹⁰

In the vaginal mucosa, SIV infection is likely to be facilitated by dendritic cells. Dendritic cells are a family of antigen-processing cells that are present in various tissues.²⁶⁷ Langerhan cells are dendritic cells found in the epithelium (Figure 4.5). These cells were hypothesized by Miller *et al.*¹⁹⁰ to transport SIV through mucosal tissues to be “handed off” to activated T cells in tissues subjacent to the epithelium. T cells become productively infected as a result of the interaction.^{117,191,287} Additional support for the role of dendritic cells in vaginal tissue was provided by showing SIVmac251 penetration of the vagina epithelium in 60 min. SIVmac-positive Langerhan/intraepithelial dendritic cells were found in vaginal tissues within 24 h.¹¹⁷

4.5.2. SIV-Induced AIDS in Macaques

4.5.2.1. PLASMA VIRUS LOADS—ACUTE AND CHRONIC INFECTION

Plasma virus loads (PVLs) for SIVmac239 and SIVmac251 in RhM macaques are shown in comparison with typical PVL in HIV-1-infected humans (Figure 4.6). Generally, the higher the PVL the shorter the time

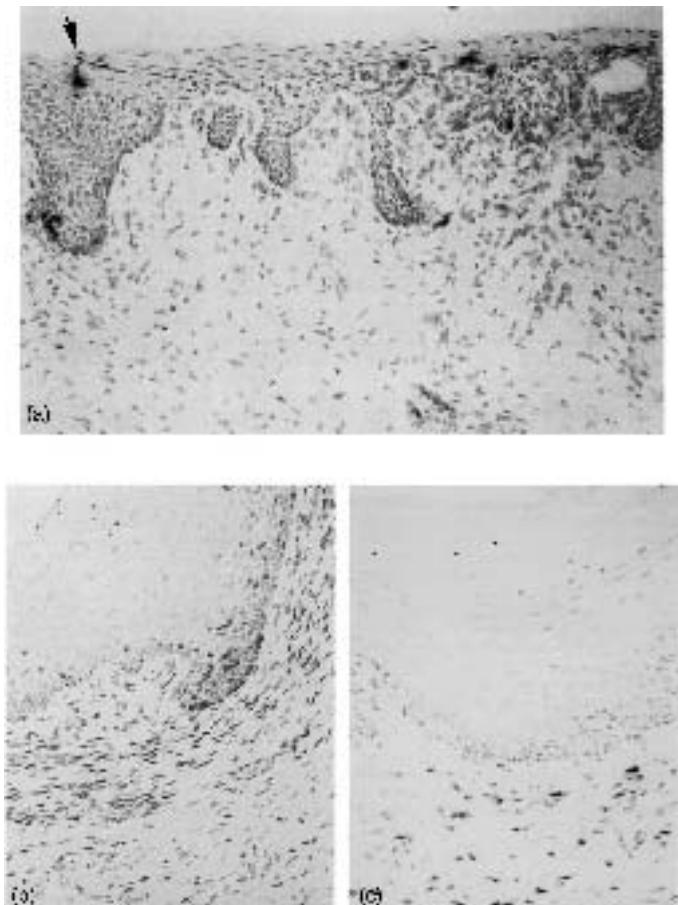


Figure 4.5. Immunohistochemistry staining of Langerhan cells, T cell, and macrophages in the vaginal mucosa. Simian immunodeficiency virus susceptible cells in the normal vaginal mucosa: (a) Langerhan cells (LCs); (b) T cells and (c) macrophages. Note in panel (a) (arrow), an LC at the vaginal surface with dendritic process sampling the lumen of the vagina. (Adapted from Miller *et al.*¹⁹⁶.) See color version page 10.

course to AIDS.²⁵⁰ SIVmac239 and SIVb670 infections of rhesus of Indian (Ind Rh) origin are highly pathogenic models. Full-blown AIDS can occur very early postinfection in rapid progressors (Figure 4.5). Monkeys infected with SIVmac239 may also progress relatively slowly to AIDS, 1 year or more postinfection.²⁵⁰ Plasma viral loads of progressors are lower than rapid progressors (Figure 4.6). SIVmac251 is generally slower in disease progression compared to SIVmac239 in Rh of Ind origin. The PVL in HIV-1-infected humans is shown for comparison (Figure 4.6).

SIVmac239 infection of Chinese origin rhesus (Ch Rh) displays lower PVLs with AIDS developing in 1–2 years.¹⁶⁰ A significant subset of SIVmac-infected Ch Rh will become elite long-term nonprogressors (LTNPs), suppressing infection for 6 years or longer¹⁵⁹ (Figure 4.6). The presence of LTNPs in vaccine studies may be

a confounding variable since it may appear that protection from diseases and low virus loads was achieved by vaccination. Thus far, there is no method to identify LTNPs prospectively. Ind Rh will also become LTNPs, but the percentage is low compared to Ch origin Rh.^{95,169}

4.5.3. Clinical Signs of AIDS in Macaques

The clinical signs of AIDS seen in SIVmac or SIVb670-infected rhesus macaques are mostly the same as that seen in HIV-1-infected humans (Table 4.2).^{28,43,146,149} The time course of the appearance clinical signs and AIDS varies as it does in humans. The principal early signs are lymphadenopathy and depletion of CD4⁺ cells in the intestinal mucosa.^{272,274} Giant cell pneumonia or encephalitis may also appear early, particularly in rapid progressors.^{29,32,149,280} In studies of RhM

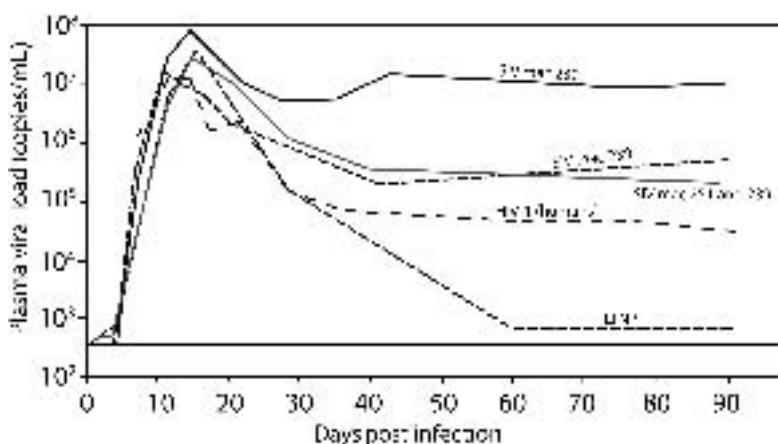


Figure 4.6. Typical plasma virus loads in Rhesus macaques (RhM) of Chinese (Ch) and Indian (Ind) origin. SIVmac239 (solid line labeled SIVmac239) rapid progressor in Ind origin Rh. SIVmac239 (dashed line labeled SIVmac239) Ch origin Rh. SIVmac251 and SIVmac239 (gray line) progressor in Rh of Ind origin, HIV-1 in humans (dashed line HIV-1 labeled), long-term nonprogressor (LTNP) (dotted line labeled LTNP) Ch or Ind origin Rh.

inoculated with a low dose SIVmac by the vaginal route, some animals had transient viremia but developed AIDS in the long term.^{183,193}

Other areas examined include ocular pathology¹²⁵ and even diet.¹⁷¹ The effects of a high fat diet on AIDS progression may be of interest to dieters. Disease progression in SIVmac-infected macaques fed an atherogenic diet, high in saturated fatty acids and cholesterol, was

compared to macaques fed the standard diet. Macaques fed the atherogenic diet had significantly more rapid disease progression.¹⁷¹

There is an acutely lethal variant of SIV known as SIVpbj. This virus induces a fatal disease within a few weeks of infection causing extensive T-lymphocyte activation and characterized by severe diarrhea, rash, and extensive lymphoid proliferation in the gastrointestinal tract.²⁴⁵ Du *et al.* reported that a mutation in the *nef* gene that substituted an arginine for a tyrosine at position 17 in *nef* was responsible for this dramatic change in SIVpbj pathogenesis.⁶¹

Table 4.2. Common Clinical Signs of Simian AIDS*

- Generalized lymphadenopathy
- Lymphoid hyperplasia followed by lymphoid depletion
- CD4⁺ cell depletion especially in the intestinal mucosa
- Multinucleated giant cell pneumonia
- Wasting >10% weight loss
- Diarrhea unresponsive to treatment
- Pneumocytis carnii pneumonia
- Atypical tuberculosis
- B-cell lymphoma
- Generalized cytomegalovirus infection
- Cryptosporidiosis
- Retroviral encephalitis

*Kaposi sarcoma-like disease and NOMA are associated with type D retrovirus infections (see Chapter 6).

4.5.4. Malignancies

The most common malignancy seen in SIV-infected macaques is lymphoma of B cell origin,^{27,42,99,100} which is analogous to non-Hodgkin's lymphoma in humans, the second most common malignancy in HIV/AIDS. The most common HIV-associated malignancy is Kaposi sarcoma, which is not found in SIV-infected macaques even when coinfecting with macaque rhadinoviruses.¹⁷³ However, a Kaposi-like disease associated with rhadinovirus is found in betaretrovirus (SRV-2)-infected macaques³⁶ (see Chapter 6).

Non-Hodgkins lymphoma is 60–100 times more common in HIV-infected persons compared to the general

population. SIV-infected macaques demonstrate a similar increase.²⁷ These lymphomas tend to appear in macaques that are progressing to AIDS relatively slowly. The majority of the lymphomas are classified as Burkitt's-like lymphomas, immunoblastic lymphomas, or diffuse large cell lymphomas.²⁷ Testing the lymphomas by immunohistochemistry and polymerase chain reaction (PCR) failed to detect SIV in the tumor cells, but did show that SIV was present in infiltrating T cells and macrophages, suggesting that SIV is a cofactor.^{99,100} Testing tumors for rhesus lymphocryptovirus (LCV) revealed a strong association with the B-cell tumors. Rhesus LCV is covered in Chapter 15.

4.5.5. Latent Infections

HIV-infected persons treated with highly active anti-retroviral therapy (HAART) may have undetectable levels of HIV in peripheral blood. However, these HIV antibody-positive persons are not cured and replication competent HIV remains latent in resting CD4⁺ cells.^{47,73} To test for SIV latency, infected RhM treated with a combination of anti-retroviral drugs suppressed SIV to undetectable levels.²⁴⁷ CD4-positive and HLA-DR-negative resting T cells were isolated from various tissues of these macaques and shown to harbor replication competent SIV. SIV-infected macaques, therefore, are a good model for studying HIV latency.

4.5.6. SIV/SHIV Immupathogenesis

Progression to AIDS is caused by the destruction of the immune system by HIV in infected humans or by SIV in macaques. The value of the macaque AIDS model lies in its similarity to human AIDS. Both SIV and the NHP host have advantages that have been developed to study how infections progress to AIDS. Each section below covers a particular phase of AIDS progression in macaques.

4.5.6.1. SIV TARGET CELLS IN THE IMMUNE SYSTEM IN BLOOD AND MUCOSAL TISSUES

The infection of the immune system and its subsequent destruction depends on the infection of cells of the immune system. The cell surface receptor and coreceptor for SIV are CD4 and CCR5 cell surface proteins, respectively. These coreceptors must appear together on cells for infection to occur (see Chapter 3; Figure 3.5). Susceptible cells are present throughout the immune system and infection is widespread in end stage diseases,¹¹³ but are particularly prevalent in the gut mu-

cosa. Gut CD4⁺/CCR5⁺ T cells are largely destroyed in the first few weeks of infection.^{272,274} However, depletion of these cells does not set the final course to AIDS, since susceptible cells are also destroyed in non-pathogenic SIV infections of the natural host (see Chapter 3). The key to disease progression is whether or not the CD4⁺/CCR5⁺ T cells are restored in the immune tissue. In nonpathogenic SIV infections of the natural host²¹⁷ (see Chapter 3) and in LTNPs,¹⁵⁹ CD4⁺/CCR5⁺ cells are replenished. In pathogenic HIV human and SIV macaque infections CD4⁺/CCR5⁺ T cells are not restored.²⁷²

In macaques, the predominant CD4⁺ cell expresses CXCR4 in the peripheral blood. Therefore, there is a rapid depletion of peripheral CD4⁺ cells by viruses with CXCR4 tropism, such as certain SHIV viruses, SHIV33 (Table 4.1).^{105,106} In contrast to the CXCR4 tropic SHIVs, SHIV162p3/p4 is CCR5 tropic and depletes CD4⁺/CCR5⁺ cells in the gut and does not show peripheral blood CD4⁺ lymphocyte destruction to any great extent.¹⁰³

SIV infection of macrophages is well established.^{168,196} However, in vitro macrophage tropism does not correlate with in vivo replication. SIVmac239 does not replicate in vitro in rhesus macrophages, but does infect these cells in vivo.^{35,192,195}

4.5.6.2. ROLE OF APOPTOSIS IN SIV INFECTIONS

Apoptosis or programmed cell death is a normal cellular process in which a multicellular organism's own cells are removed in a controlled fashion.¹⁵ Apoptosis occurs in response to cell stress and is a way of eliminating unhealthy cells. Apoptotic cells are detected in NHPs by the Tunel method, an immunohistochemistry technique.⁷² Apoptosis has been widely studied in SIV AIDS models^{19,51,66,72,121,198} and is a key feature in lymph nodes of the pathogenic models. Apoptosis is hypothesized to play a key role in the pathogenic models leading to a loss of immune cells that leads to AIDS. Pathogenic SIVmac infections of Chinese and Indian origin RhM display different levels of apoptosis. The more pathogenic Indian origin RhM model displays the higher levels of apoptosis.

4.5.6.3. SURROGATE MARKERS OF IMMUNOSUPPRESSION AND ENCEPHALITIS

The surrogate markers of disease progression frequently used in SIV-induced AIDS are decreases in CD4⁺ cell numbers, weight loss, and PVL. Other less frequently

used surrogate markers of SIV infection may help in identifying certain clinical syndromes or disease progression associated with SIV infection. Neopterin, one such biomarker, is a small molecule derived from guanosine triphosphate and used as a marker of immune activation. It is released by macrophages and is an early marker in serum and urine of SIV-infected macaques.^{70,226} The role of immune activation is a key difference between nonpathogenic and pathogenic SIV infections and is covered in Chapter 3.

Markers for SIV-related central nervous system (CNS) disease are of particular interest in predicting clinical outcomes.¹⁴² YKL-40, named for its N-terminal motif of tyrosine lysine leucine (YKL) and its 40-kDa molecular weight, is specifically elevated in the cerebrospinal fluid of SIV-infected macaques with CNS disease. Increases in YKL-40 correlated with increase virus load in cerebrospinal fluid.³⁴ The osteopontin receptor, CD44v6, is another marker of CNS diseases. It is present on monocytes and the level of expression is reported to be a correlate of encephalitis in SIV-infected macaques.¹⁷⁴

4.6. IMMUNE CONTROL OF SIV AND SHIV

4.6.1. Cell-Mediated Immunity

The principal means of controlling SIV infection resides with the CMI system^{96,145,157,243,244} and not with neutralizing antibody.^{109,111,148} CD8⁺ T cells carry out this major function. CMI acts by inducing lysis of SIV- or SHIV-infected cells. Infected cell killing between CD8⁺ cells and infected cell is specific and requires a series of recognition steps involving genes of the major histocompatibility complex (MHC) in that both CD8⁺ effector cells and infected target cell must match. A role for CD8⁺ cells has been demonstrated by the *in vivo* depletion of CD8⁺ T cells, which results in a loss of control of virus.^{123,145,157,182,243,244} Other mechanisms besides CD8 effector cell functions may be involved in virus control.¹⁵⁰

Certain genes in the MHC are efficient controllers of SIV PVL. The best characterized MHC genes are Mamu-A01, B8, and Mamu-B*17.^{144,162,169} Indian origin RhM with these genes typically control SIV PVL and progress more slowly to AIDS than RhM lacking these genes.

4.6.2. Humoral Immunity

Viruses that are well controlled by acquired immunity are susceptible to antibody neutralization and there are

effective vaccines against these viruses. Unfortunately, SIV and HIV are largely resistant to neutralizing antibody. One could argue that if HIV and SIV were neutralization sensitive, they would not exist. HIV and SIV are inefficiently transmitted. Since neutralizing antibody appears in 6–8 weeks postinfection, simian lentiviruses must be antibody resistant in order to persist in the host long enough to transmit. Humoral antibody appears in SIV-infected macaques,¹²⁹ but does not control infection to any significant extent.^{109,199} However, it is possible to render SIV sensitive to neutralizing antibody through serial passage in tissue culture lines.¹⁸⁶

Antibody is induced and is the basis for enzyme-linked immunoassays (ELISA)²⁴⁸ and Western blots for detection of active or past infections.¹⁷⁸

4.6.3. Innate Immunity

Innate immunity is rapidly activated after SIV/SIV infection. It is preexisting and does not require immunization or prior exposure to the infecting agent. Innate immunity involves cells, soluble substances, and genetic factors that can control infections.^{6,31} Innate factors such as chemokines increase resistance in tissues. Interferon-alpha is a well known and broadly active antiviral factor and that is active in SIVmac infections. Increased alpha- and beta-interferon gene expression correlates with lower virus loads.¹⁹⁷ However, treatment with pegylated interferon was not highly active against SIVmac in RhM.²⁰ Other innate factors, CXCL9 and CXCL10, were also associated with slower progression to AIDS. IL-4, IL-10, MIP-1 a, MIP-1 b MCP-1, and RANTES are induced early in SIV-infected rhesus macaques.²⁹⁰

Cells of the immune system function in diverse roles in innate immunity.⁶ Dendritic cells, macrophages, neutrophils, natural killer (NK) cells, and gamma-delta T cells play significant roles. CD8⁺ T cells are active in a noncytolytic role. Innate immune cells carry out lysis of SIV-infected cells directly without preexisting immunity. NK cells are activated early in SIV infection.⁸⁸

Genetic factors like CCR5 gene deletions certainly play a role, but it has not been extensively studied in monkeys. The mangabey group, but not macaques, contains a 24-bp deletion in the CCR5 gene which confers resistance to CCR5 tropic SIV in homozygous CCR5-deleted T cells.^{45,216} CCR5-deleted heterozygotes are known in SMs but it is not known how heterozygosity (one wild-type and one delta-24 CCR5 gene) affects SIV infection. Polymorphisms in cytokine promoters in Rh and SM may contribute to controlling disease

progression; however, it has not been shown.³⁷ Polymorphisms in eight macaque genes (CCR5, CXCR6, GPR15, RANTES, IL-10, APOBEC3G, TNF-alpha, TSG101) known to control HIV infections, did not correlate with control in the SIVmac system.²⁷⁹

4.7. AIDS VACCINE MODELS

The greatest challenge for NHP models is the successful testing of a safe and effective AIDS vaccine against a relevant immunosuppressive primate lentivirus. The key word is “relevant” since protection in NHPs can be achieved against tissue culture-adapted HIVs and SHIVs that are neutralization sensitive. The search for this vaccine began with simple killed virus vaccines and now encompasses numerous sophisticated replicating/single-cycle vectors and DNA vaccines. The number of publications reporting on AIDS vaccines in NHP models is over 1,000. There is no single source covering the entire field of vaccine design and testing. The major issues of vaccine design, correlates of protection, escape from vaccine-induced immunity, and vaccine safety are dealt with in a series of reviews.^{96,118,141,185} Representative vaccine approaches in the NHP models are covered. The chapter only serves as a starting point for understanding how to use this model and what has been done.

The gold standard for vaccines is prophylaxis of infection, often called sterilizing immunity. Protection from diseases is also a criterion for judging vaccine candidates, if sterilizing immunity is not achieved. Classically, protective immunity is achieved through priming with the vaccine, followed by one or more boosts with the same vaccine or a different vaccine that carries the same antigens of interest. Table 4.3 is a list of the major types of vaccines that have been tested in the SIV and SHIV macaque models.

4.7.1. Killed Virus Vaccines

Studies began with simple killed virus vaccines published in high-profile journals and reporting sterilizing immunity.^{54,60,205} Protection was achieved using a low dose challenge of SIV grown in human cells and inactivated with formalin or nonionic detergent. Another study was unable to show protection using a relatively high challenge dose.²⁶¹ A major setback in the progress toward a safe and effective AIDS vaccine occurred when the mechanism of protection by killed virus vaccines was shown to be anti-human antibody.^{258,259} The anti-human antibodies were induced by human cell antigens

that coated SIV when virions budded from the human cells used to make the vaccine. These human cell antigens on the killed SIV virions induced antibody that was able to neutralize the challenge virus. The necessary control consisting of cellular antigens from the cells used to grow the vaccine was omitted in earlier studies. In a controlled experiment, it was shown that the anti-human cell antibody alone was capable of protecting against SIV challenge. When these vaccine studies were repeated using SIV grown in macaque cells, no protection was found.²⁵⁹

Killed virus vaccines induced SIV antibody that was largely ineffective against SIV and HIV.¹⁹⁹ After these initial studies with killed vaccines, the criteria for efficacy were ratcheted down from complete protection provided by sterilizing immunity to delay of disease and lower virus loads in immunized groups compared to control groups.

4.7.2. Subunit Protein Vaccines

Soluble HIV and SIV proteins are effective in inducing antibody. Using the HIV-1 chimpanzee model, protection was shown with a soluble gp120 *env*-based vaccine. However, the challenge HIV-1 was a tissue culture-adapted strain of HIV-1 that was highly susceptible to neutralizing antibody.³³ A similar vaccine was tested in humans and failed to elicit protection because HIV-1 strains representative of the HIV-1 transmitted between persons are highly resistant to neutralizing antibody.^{209,225}

After these failures, emphasis shifted to vaccines that were capable of inducing CMI. These include DNA plasmids encoding SIV and SHIV proteins and live virus vectors. DNA plasmids that replicate *in vivo* and express SIV proteins *in vivo* are best at inducing CMI. DNA plasmids are taken up by cells and carry out intracellular expression of the encoded antigens. These antigens are processed by the CMI system and result in the induction of CD8⁺ cytotoxic T cells. DNA plasmids have also been used to express cytokine adjuvants, such as IL-2, IL-12, and others.^{138,139,237} These plasmids have been used in the SIVmac model to show enhanced CMI responses and amelioration of PVL.^{138,139,207,237}

4.7.3. Replication-Competent Vaccine Vectors

Replicating vaccine vectors based on replication-competent viruses are hybrid viruses containing SIV or HIV genes. To be viable as a candidate AIDS vaccine,

Table 4.3. Major Types of Vaccines Tested in Nonhuman Primate Models

Vaccine Type/NHP Model	Prime/Boost	Adjuvant	Challenge	Protection	Ab/CMI*
Killed whole virus/Rh ^{60.156.205}	Killed whole virus/same	MDP, 60.205 Alum, 205	SIVmac, SIVmneE11S SIVb670	Y ^{†,+}	+/ND [‡]
Soluble antigen/Cpz ³² HIV protein cocktail/Cpz ³¹	HIV env/same HIV virion proteins, peptide/same	Aluminum hydroxide SAF-1	HIV-1 HIV-II.Lai virus followed by infected Cpz cells	Y [§] Y [¶]	+/ND +/ND
Attenuated live SIVmac ^{***/Rh^{52.246}}	None	None	Various SIVmac strains	Y	+/+
Nonpathogenic HIV-2 SBL-6669 ^{233.277/cyno}	Virus alone or with canarypox HIV-2 env, gag, pol	None	SIVsm	Y	+/+
DNA/Rh ^{57.138.207.214} Attenuated SHIV/Rh ¹ VLP/Rh ²¹²	DNA/DNA or MVA SHIV/hone VLP/Semliki forest virus vector	Cytokine ^{††} /Bivucaine None None or alum	SIVmac251 SIVmac239 SHIV-4	Y [¶] Y [¶] Y [¶]	+/ +/ +/ +
Fowlpox virus (FPV) ¹³²	DNA/FPV	ipofectamine or DOTAP None	SHIV162p3 SHIV89.6p	Y [¶] Y [¶]	+/ +/ +
Modified vaccinia Ankara (MVA)/Rh ¹⁰ Vesicular stomatitis virus (VSV)/Rh ²³⁸	MVA/DNA plasmid VSV/VSV ^{‡‡} (different serotype)	None	SHIV89.6p	Y [¶]	+/ +/ +

Table 4.3. (Continued)

Vaccine Type/NHP Model	Prime/Boost	Adjuvant	Challenge	Protection	Ab/CMI*
Canarypox vector ⁹⁰	Canarypox/canarypox	None	HIV-1 IIIB-infected cells/HIV IIIB virus	N	+/-
Adenovirus vector/Rh ^{57,220}	Adeno/soluble viral protein or DNA prime/adeno/SIV prime/adeno/SIV proteins	IL-12-IL-15 ^{††}	SIVmac/SIV89.6P	Y [¶]	++/+
Semliki forest virus/cynomolgus ³⁰	Same	None	SHIV-4	Y [¶]	++/+
Replicon Venezuelan equine encephalitis virus/Rh ⁵⁵	VEE replicon/same	None	SIVsme660	Y [¶]	++/+
Bacterial vectors					
Salmonella ²⁵⁵ /Rh	Salmonella/same or p27	None/polyphosphazene for p27	SHIV89.6P	N	+/-
(BCG) ²⁸⁵ /Rh	BCG/p11c peptide	None/lipid A liposome and alum	SIVmne	N	weak/+

Representative references are in superscript.

* Antibody-/cell-mediated immunity ++ antibody induced/CMI induced.

† Protection due to anti-cellular antibody induced by vaccine prepared in human cell lines.^{60,156}

‡ No data.

§ Protection was from a neutralization sensitive laboratory-adapted challenge virus. Primary HIV-1 strains (not adapted to tissue culture) are neutralization resistant.

¶ Virus load reduced, no protection from transmission of virus.

** Attenuation of SIVs by deletions in accessory genes, deletion in nef or multiply deleted viruses in vpr, vpx, and other regions. Viruses tested in newborn rhesus were pathogenic.²²

†† Cytokine expression by vector, for example, IL-2, IL-15.

Boosting with different VSV serotypes, so boosting vector is not neutralized by antibody induced by priming vector.

+ Partial protection was not due to anti-cellular antibody. CMI induced, no protection against heterologous SIV660 challenge.¹⁵⁶

the vectors must have low pathogenic potential, the capability to express HIV and SIV genes in vivo, and induce both antibody- and cell-mediated immunity. Replicating vectors are superior for inducing CMI. Commonly used DNA virus vectors are vaccinia^{119,167} and its derivative, modified vaccinia Ankara (MVA),²¹⁵ fowlpox,³⁹ and adenovirus.^{40,253} RNA virus vectors include vesicular stomatitis virus (VSV),^{234,238} Semliki forest virus, and simian foamy viruses among others.^{30,64,140,177} These vectors have shown at least limited promise in either SIVmac or SHIV models. Typically, these vectors are used in various combinations of prime and boost. For example, VSV vectors containing an HIV-1 env gene and the SIVmac gag gene were used to prime RhM against an SHIV challenge (Table 4.3). The boost consisted of the same vectors, but with the G surface protein replaced with the G protein from a different serotype.²³⁸ Therefore, the boost is resistant to antibody induced by the priming vaccine allowing for replication of the boosting vector. A variation on this approach is to prime with VSV and boost with MVA. This approach has the advantage of boosting with a completely unrelated virus.²³⁴

Replicating adenovirus-SIV and adenovirus-HIV vaccine vectors have also shown promise in both the SIV and SHIV systems.^{40,57,92,93,220,221,224,288} Studies in chimpanzees showed that replicating adenovirus vectors were superior to nonreplicating vectors in inducing HIV immune responses. Immunization with replicating adenovirus type-5 vector containing SIVmac env/rev/gag and or nef genes and boosted with soluble gp120 was capable of suppressing SIVmac251 PVLs compared to empty vector controls. Further studies showed that antibody-dependent cell cytotoxicity correlated with lower PVLs in RhM immunized with adenovirus-SIV hybrid vectors. This study is one of the few to show an immune correlate of vaccine efficacy.⁹³

4.7.4. Single-Cycle Vaccine Vectors

Single-cycle vectors are vaccines that carry out only a single round of replication, as the name implies.^{67,143,263} The vectors contain the SIV or HIV antigens of interest that are encoded within their genomes. The vector viruses contain partial gene deletions that do not allow for complete rounds of replication. Essential missing functions may be provided by proteins from other viruses. For example, one such vector was created by mutating 27 codons in multiple genes of SIVsm. Envelope functions were provided with the G protein from

VSV.²⁶³ The end result is a vaccine candidate that provides all of the viral proteins for priming and boosting the immune systems without the risk of disease. It remains to be determined if this approach is potent enough for an effective HIV vaccine.

4.7.5. Replicons

Replicons are DNA or RNA self-replicating vectors encoding a viral replicase that confers the capacity to replicate in vitro and in vivo. RNA replicons derived from RNA viruses have been engineered to express SIV/HIV proteins for testing as candidate vaccines in NHP models. The capacity to produce particles released by replicon “infected” cells is accomplished through the use of a helper gene⁵⁵ or encoded within the replicon itself.²³⁹ Replication of the vector amplifies antigen expression, activates the innate immune system, and induces strong immune responses. Replicons developed for AIDS vaccine testing in NHPs were derived from Venezuelan equine encephalitis,^{55,126} Semliki forest virus,²³⁹ poliovirus,²⁰² Kunjin virus,¹³³ and Sindbis virus.²⁸⁴ Thus far these systems have not been widely used for efficacy testing. One vector tested, the Kunjin vector, did not induce SIV immunity in pig-tailed macaques and no protection was observed from SIVmac251 challenge.¹³³

4.7.6. Bacteria-Based AIDS Vaccines

Bacterial expression systems have been used as live vectors for AIDS vaccine models in macaques. Expression is adequate for induction of both CMI and antibody; however, antigens will not be glycosylated, which may impair humoral immune responses. The attenuated tuberculosis bacillus, Bacille Calmette-Guérin (BCG) has been developed as a candidate vaccine vector in macaques.^{16,71,151,187,285} In one study, intradermally inoculated BCG expressing SIV gag and boosting with the mamu Ao1-specific gag peptide epitope p11c, induced cell-mediated immunity in RhM but offered no protection against SIVmne.^{80,286}

Salmonella expression systems are another example of a bacterial vector in use in AIDS.^{68,74,255–257} SIV-specific immunity is induced in the Salmonella system. Test for protection with Salmonella vector with and without p27 antigen boosting did not show protection against SHIV89.6P challenge.²⁵⁷ Other bacteria have been used as vaccine vectors, including *Listeria monocytogenes*.¹²²

4.7.7. Attenuated Live Virus Vaccines

The attenuated live SIVmac approach is one vaccine strategy that has shown remarkable success in protection of macaques against highly pathogenic SIVmac strains. Unfortunately, after early successes it was shown that attenuated SIVs were capable of inducing AIDS. Attenuated SIV vaccines have not met, as yet, the minimum criteria of being both safe and effective.

Attenuated SIVs are obtained by deleting genes that are nonessential for replication. These gene-deleted SIVs replicate *in vitro* and *in vivo*, but their capacity to establish persistent infections and cause disease is greatly reduced compared to wild-type virus. Using a pathogenic molecular clone of SIVmac239, the *nef* gene was deleted and shown to be nonessential for transient *in vivo* replication.^{136,137} This virus was named SIVmac239Δ*nef* to indicate the gene deleted. This nomenclature has been adopted by the field. SIVmac239Δ*nef* infection of adult RhM induced lower virus loads compared to the wild-type SIVmac239 clone.¹³⁷ The RhM inoculated with SIVmac239Δ*nef* survived 3 years without evidence of infection or disease, providing preliminary safety data. These results spurred the development of a *nef*-deleted SIV as a candidate for an attenuated AIDS vaccine. For the immunization study, adult RhM were inoculated with SIVmac239Δ*nef*. The infection was suppressed and was undetectable for more than 2 years. When challenged with pathogenic SIVmac239 or SIVmac251, vaccinated animals did not have detectable virus in blood, achieving the “sterilizing immunity,” the “Holy Grail” of vaccinology.⁵² Unfortunately, this most promising of vaccine approaches was shown to be unsafe in neonatal rhesus monkeys²² and eventually, AIDS was shown in SIVmacΔ*nef*-infected adults as well.^{23,115} Multiply deleted SIVmac239 mutants, lacking *nef*, *vpr*, and upstream sequences in U3, protected against pathogenic SIVmac251²⁸² but were also pathogenic.²³ Other combinations of gene deletions were either too weak to induce protection or were pathogenic.⁵⁹ Deletion of the V1-V2 region or deglycosylation of SIVmac239 molecular clone also attenuated SIVmac239. Protection against pathogenic wild-type SIVmac239 challenge was observed.^{170,200} Importantly, protection was achieved in face of low antibody and cell-mediated immunity, suggesting that protection was due to other mechanisms.²⁴⁶ Nevertheless, proof of the correlates of protection from attenuated SIVs remained elusive.

The negative impact of these attenuated SIV vaccine studies on AIDS vaccine development was great. Protection was remarkably strong, and all vaccines that followed have been judged in comparison to live attenuated SIV vaccines. The gold standard of protection was established but not equaled by subsequent vaccines. Finally, the correlates of protection by attenuated SIVs have not been found. Should they be discovered, it may be possible to engineer a safe and effective vaccine based on the promising results obtained by gene deleted SIVs.

Another approach to attenuated primate lentiviruses as vaccines is the use of low-pathogenic SHIVs as an attenuated vaccine. The nonpathogenic form of SHIV89.6 has been used in this regard and provided protection against pathogenic SIVmac. This model has also been used to explore the correlates of protection provided by attenuated primate lentiviruses.^{1,2,87,147,194}

4.8. PRE- AND POSTEXPOSURE PROPHYLAXIS

Prevention of infection has been achieved in the NHPs AIDS models using anti-retroviral drugs, microbicides applied to the vagina of monkeys and treatment with specific antibody. These studies have provided important data for developing methods of prevention and treatment. There are numerous publications on the subject, so only an introduction to the field is provided here.

4.8.1. Anti-Retroviral Drugs

Early studies effectively showed the value of the SIVmac model for evaluation of potential drug therapies. 3'-azido-3'-deoxythymidine (AZT) was used to prevent oral SIVmac transmission to infants, paving the way for effective prevention of transmission of HIV from mothers to infants.²⁷¹ New drugs, not yet tested in humans, were also used successfully in the SIV system. One of the major successes was with R-9-(2-phosphonylmethoxypropyl) adenine (PMPA). PMPA was used to treat cynomolgus macaques subcutaneously, beginning at 48 h before and either 4 or 24 h after intravenous inoculation with SIVmne.²⁶⁶ Treatment was continued for 4 weeks after inoculation. Doses ranged between 20 and 30 mg/kg. All treated animals were protected and all controls were infected. PMPA (Tenofovir) was approved for treatment of HIV-infected persons. Numerous other studies have followed the same

approach of prevention of infection by treatment before infection or shortly thereafter.²⁶⁹ The advantage of the SIV model is that the precise time of infection is known and drug administration timing can be chosen to test the selected hypothesis.

4.8.2. Microbicides

The development of a safe and effective vaginal and rectal microbicides has received an enormous amount of attention.⁴⁹ The primary reason for this interest is that AIDS vaccine development is progressing very slowly and microbicides are seen as a stop-gap measure until a vaccine is available. The concept is to prevent HIV transmission with a relatively inexpensive drug that can be used in resource poor countries and administered in private to genital or rectal mucosa. The SIV/SIV macaque model has been widely used to test microbicide formulations and strategies.

The first to be tested in macaques was nonoxynol-9 (N-9), a contraceptive spermicide in gel and foam formulations. A single treatment of N-9 was applied to the vaginas of RhM about 30 min before vaginal exposure to SIVmac251. Five of 12 animals were protected.¹⁸⁸ However, repeated exposure to N-9 causes irritation to the vaginal epithelium and may actually enhance SIV or HIV transmission.¹⁰⁷ Results of a clinical trial with N-9 actually enhanced HIV transmission.⁹⁷

Numerous candidate microbicides have been studied, including polyamions,¹⁵³ anti-retroviral drugs,^{50,273} antibodies,²⁷⁵ and vaginal flora expressing HIV/SIV inhibitors.¹⁶¹ The overall strategy remains the same in that the candidate microbicide is placed into the vagina or rectum, followed by atraumatic SIV or SHIV inoculation to the mucosa. This microbicide field showed much early promise that has gone largely unrealized. Scientists in the field recommend that more emphasis be given to NHP models.⁹⁷

4.8.3. Passive Immunization

Passive immunotherapy is the administration of HIV-/SIV-/SHIV-specific antibody in the form of serum, purified gamma-globulin, or monoclonal antibody followed by challenge with virus. Cynomolgus macaques have been treated with hyperimmune anti-HIV-2 serum obtained from a cynomolgus macaque donor. This monkey had been immunized with killed HIV-2 vaccine and had resisted with HIV-2 challenge. Another group

of cynomolgus monkeys in the same study received serum from an SIVsm-infected cynomolgus macaque that had controlled infection. In both experiments, significant protection was obtained.^{231,232} Because viruses were grown in human cells, anti-human antibody may have played a role in protection. Nevertheless, this study served as a proof of concept for passive antibody studies. Immune globulin from an LTNP was effective in SIVe660-infected rhesus, resulting in slower development of AIDS and lowered PVLs.^{101–103}

Protection was also shown in the HIV-1 chimpanzee model using HIVIG, HIV immune globulin collected from patients with high titered antibody. One chimpanzee was protected from 10 TCID50 of HIV-1 IIIB, but two were infected after receiving 400 TCID50. Protection was likely due to using a tissue culture-adapted HIV-1 strain that is sensitive to neutralizing antibody.^{229,230}

4.9. IMMUNOTHERAPY WITH VACCINES

Vaccine immunotherapy is the treatment of infected individuals with vaccines to promote immunity and reduce progression to disease. However, effective immunotherapy will require highly effective vaccines that are not yet available. Therefore, most studies have focused on immune stimulation. SIV-DNA vaccines have been tested with and without cytokines. DNA vaccines expressing IL-12 stimulated SIV-specific CD8 effector memory T cells.¹⁰⁴ Using anti-retroviral therapy (ART) consisting of PMPA and d4T, SIVmac239 PVLs were suppressed to <200 copies/mL. Following ART, SIV DNA vaccines, with and without IL-2, stimulated CMI with increases in gag-specific responses that persisted after cessation of therapy. However, virus loads rebounded to the same levels preimmunotherapy.²⁹¹ Taking the optimistic view, there may be long-term benefits to vaccine immunotherapy, but these are not known.

4.10. SUMMARY

The emergence of pandemic HIV spawned spectacular growth in NHP models of AIDS. Since the discovery of HIV in 1983 and SIV in 1985, there have been over 5,000 publications in SIV monkey models and HIV models in chimpanzees. The most commonly used SIVs are SIVmac and SIVb670. These SIVs are derived from SMs, a West African species from which HIV-2 arose. Although disease is rare in the natural SM host, AIDS is readily induced in macaque monkeys, genus *Macaca*, causing

AIDS in 1–2 years in most instances. SIV hybrid viruses, SHIV-containing HIV-1 and SIV genes were developed to test HIV-1 antigens, particularly in vaccine studies in monkeys. Lastly, chimpanzees have been used since they can be infected with HIV-1. However, the limited availability and the lack of diseases in the vast majority of infected chimpanzees diminish the value of this model. The models have led the way in understanding many of the primary features of AIDS pathogenesis. Descriptions of the early dissemination of the virus and immunopathogenesis in the intestinal mucosa were elucidated before being found in HIV infections.

SIV and SHIV replication are the same as HIV and they infect the same CD4⁺ T cells and macrophages that are infected by HIV-1. With a few exceptions, SIVmac uses the same coreceptor, CCR5, as do primary HIV isolates. Some SHIVs infect CD4⁺/CCR5⁺ cells while others infect CD4+/CXCR4⁺-positive cells like some HIV-1 strains.

AIDS in macaques is virtually indistinguishable from AIDS in humans. Key features are persistent lymphadenopathy, CD4⁺ T-cell depletion, weight loss, and opportunistic infections such as *Pneumocystis carinii*, atypical tuberculosis, generalized cytomegalovirus infection, unresponsive diarrhea, and lymphoma. AIDS NHP models are highly accepted for studies in pathogenesis, immunity, vaccines, and therapy.

REFERENCES

- Abel, K., L. Compton, T. Rourke, D. Montefiori, D. Lu, K. Rothaeusler, L. Fritts, K. Bost, and C. J. Miller. 2003. Simian-human immunodeficiency virus SHIV89.6-induced protection against intravaginal challenge with pathogenic SIVmac239 is independent of the route of immunization and is associated with a combination of cytotoxic T-lymphocyte and alpha interferon responses. *J. Virol.* 77(5):3099–3118.
- Abel, K., L. La Franco-Scheuch, T. Rourke, Z. M. Ma, V. De Silva, B. Fallert, L. Beckett, T. A. Reinhart, and C. J. Miller. 2004. Gamma interferon-mediated inflammation is associated with lack of protection from intravaginal simian immunodeficiency virus SIVmac239 challenge in simian–human immunodeficiency virus 89.6-immunized rhesus macaques. *J. Virol.* 78(2):841–854.
- Abel, K., T. Rourke, D. Lu, K. Bost, M. B. McChesney, and C. J. Miller. 2004. Abrogation of attenuated lentivirus-induced protection in rhesus macaques by administration of depo-provera before intravaginal challenge with simian immunodeficiency virus mac239. *J. Infect. Dis.* 190(9):1697–1705.
- Adachi, A., H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. A. Martin. 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and non-human cells transfected with an infectious molecular clone. *J. Virol.* 59:284–291.
- Agy, M. B., L. R. Frumkin, L. Corey, R. W. Coombs, S. M. Wolinsky, J. Koehler, W. R. Morton, and M. G. Katze. 1992. Infection of *Macaca nemestrina* by human immunodeficiency virus type-1. *Science* 257(5066):103–106.
- Ahmed, R. K., G. Biberfeld, and R. Thorstensson. 2005. Innate immunity in experimental SIV infection and vaccination. *Mol. Immunol.* 42(2):251–258.
- Akiyama, H., M. Ishimatsu, T. Miura, M. Hayami, and E. Ido. 2008. Construction and infection of a new simian/human immunodeficiency chimeric virus (SHIV) containing the integrase gene of the human immunodeficiency virus type 1 genome and analysis of its adaptation to monkey cells. *Microbes Infect.* 10(5):531–539.
- Albert, J., B. Abrahamsson, K. Nagy, E. Aurelius, H. Gaines, G. Nyström, and E. M. Fenyö. 1990. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. *AIDS* 4(2):107–112.
- Alter, H. J., J. W. Eichberg, H. Masur, W. C. Saxinger, R. Gallo, A. M. Macher, H. C. Lane, and A. S. Fauci. 1984. Transmission of HTLV-III infection from human plasma to chimpanzees: an animal model for AIDS. *Science* 226(4674):549–552.
- Amara, R. R., F. Villinger, J. D. Altman, S. L. Lydy, S. P. O’Neil, S. I. Staprans, D. C. Montefiori, Y. Xu, J. G. Herndon, L. S. Wyatt, M. A. Candido, N. L. Kozyr, P. L. Earl, J. M. Smith, H. L. Ma, B. D. Grimm, M. L. Hulsey, J. Miller, H. M. McClure, J. M. McNicholl, B. Moss, and H. L. Robinson. 2001. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. *Science* 292(5514):69–74.
- Ambrose, Z., V. Boltz, S. Palmer, J. M. Coffin, S. H. Hughes, and V. N. Kewalramani. 2004. In vitro characterization of a simian immunodeficiency virus–human immunodeficiency virus (HIV) chimera expressing HIV type 1 reverse transcriptase to study antiviral resistance in pigtail macaques. *J. Virol.* 78(24):13553–13561.
- Ambrose, Z., S. Palmer, V. F. Boltz, M. Kearney, K. Larsen, P. Polacino, L. Flanary, K. Oswald, M. Piatak Jr., J. Smedley, W. Shao, N. Bischofberger, F. Maldarelli, J. T. Kimata, J. W. Mellors, S. L. Hu,

- J. M. Coffin, J. D. Lifson, and V. N. KewalRamani. 2007. Suppression of viremia and evolution of human immunodeficiency virus type 1 drug resistance in a macaque model for antiretroviral therapy. *J. Virol.* 81(22):12145–12155.
13. Amedee, A. M., N. Lacour, and M. Ratterree. 2003. Mother-to-infant transmission of SIV via breastfeeding in rhesus macaques. *J. Med. Primatol.* 32(4–5):187–193.
 14. Amedee, A. M., J. Rychert, N. Lacour, L. Fresh, and M. Ratterree. 2004. Viral and immunological factors associated with breast milk transmission of SIV in rhesus macaques. *Retrovirology* 14(1):17.
 15. Ameisen, J. C., O. Pleskoff, J. D. Lelièvre, and F. De Bels. 2003. Subversion of cell survival and cell death: viruses as enemies, tools, teachers and allies. *Cell Death Differ.* 10(1):S3–S6.
 16. Ami, Y., Y. Izumi, K. Matsuo, K. Someya, M. Kanekiyo, S. Horibata, N. Yoshino, K. Sakai, K. Shinozaki, S. Matsumoto, T. Yamada, S. Yamazaki, N. Yamamoto, and M. Honda. 2005. Priming-boosting vaccination with recombinant *Mycobacterium bovis* bacillus Calmette-Guérin and a nonreplicating vaccinia virus recombinant leads to long-lasting and effective immunity. *J. Virol.* 79(20):12871–12879.
 17. Apetrei, C., A. Kaur, N. W. Lerche, M. Metzger, I. Pandrea, J. Hardcastle, S. Falkenstein, R. Bohm, K. Koehler, V. Traina-Dorge, T. Williams, S. Staprans, G. Plauche, R. S. Veazey, H. McClure, A. A. Lackner, B. Gormus, D. L. Robertson, and P. A. Marx. 2005. Molecular epidemiology of simian immunodeficiency virus SIVsm in U.S. primate centers. *J. Virol.* 79(14):8991–9005.
 18. Apetrei, C., N. W. Lerche, I. Pandrea, B. Gormus, G. Silvestri, A. Kaurf, D. L. Robertson, J. Hardcastle, A. A. Lackner, and P. A. Marx. 2006. Kuru experiments triggered the emergence of pathogenic SIVmac. *AIDS* 20:317–321.
 19. Arnoult, D., F. Petit, J. D. Lelièvre, D. Lecossier, A. Hance, V. Monceaux, B. Hurtrel, R. Ho Tsong Fang, J. C. Ameisen, and J. Estaquier. 2003. Caspase-dependent and -independent T-cell death pathways in pathogenic simian immunodeficiency virus infection: relationship to disease progression. *Cell Death Differ.* 10(11):1240–1252.
 20. Asmuth, D. M., K. Abel, M. D. George, S. Dandekar, R. B. Pollard, and C. J. Miller. 2008. Pegylated interferon-alpha 2a treatment of chronic SIV-infected macaques. *J. Med. Primatol.* 37:26–30.
 21. Ayash-Rashkovsky, M., A. L. Chenine, L. N. Steele, S. J. Lee, R. Song, H. Ong, R. A. Rasmussen, R. Hofmann-Lehmann, J. G. Else, P. Augostini, H. M. McClure, W. E. Secor, and R. M. Ruprecht. 2007. Coinfection with *Schistosoma mansoni* reactivates viremia in rhesus macaques with chronic simian-human immunodeficiency virus clade C infection. *Infect. Immun.* 75(4):1751–1756.
 22. Baba, T. W., Y. S. Jeong, D. Pennick, R. Bronson, M. F. Greene, and R. M. Ruprecht. 1995. Pathogenicity of live, attenuated SIV after mucosal infection of neonatal macaques. *Science* 267(5205):1820–1825.
 23. Baba, T. W., V. Liska, A. H. Khimani, N. B. Ray, P. J. Dailey, D. Penninck, R. Bronson, M. F. Greene, H. M. McClure, L. N. Martin, and R. M. Ruprecht. 1999. Live attenuated, multiply deleted simian immunodeficiency virus causes AIDS in infant and adult macaques. *Nat. Med.* 5(2):194–203.
 24. Balzarini, J., M. Weeger, M. J. Camarasa, E. De Clercq, and K. Uberla. 1995. Sensitivity/resistance profile of a simian immunodeficiency virus containing the reverse transcriptase gene of human immunodeficiency virus type 1 (HIV-1) toward the HIV-1-specific non-nucleoside reverse transcriptase inhibitors. *Biochem. Biophys. Res. Commun.* 211:850–856.
 25. Barnett, S. W., K. K. Murthy, B. G. Herndier, and J. A. Levy. 1994. An AIDS-like condition induced in baboons by HIV-2. *Science* 266(5185):642–646.
 26. Barré-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vézinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 220(4599):868–871.
 27. Baskin, G. B., K. J. Cremer, and L. S. Levy. 2001. Comparative pathobiology of HIV- and SIV-associated lymphoma. *AIDS Res. Hum. Retroviruses* 17(8):745–751.
 28. Baskin, G. B., L. N. Martin, S. R. Rangan, B. J. Gormus, M. Murphey-Corb, R. H. Wolf, and K. F. Soike. 1986. Transmissible lymphoma and simian acquired immunodeficiency syndrome in rhesus monkeys. *J. Natl. Cancer Inst.* 77(1):127–139.
 29. Baskin, G. B., M. Murphey-Corb, L. N. Martin, K. F. Soike, F. S. Hu, and D. Kuebler. 1991. Lentivirus-induced pulmonary lesions in rhesus monkeys (*Macaca mulatta*) infected with simian immunodeficiency virus. *Vet. Pathol.* 28(6):506–513.
 30. Berglund, P., M. Quesada-Rolander, P. Putkonen, G. Biberfeld, R. Thorstensson, and P. Liljeström. 1997. Outcome of immunization of cynomolgus monkeys with recombinant Semliki forest virus encoding human immunodeficiency virus type 1 envelope protein and challenge with a high dose of SHIV-4 virus. *AIDS Res. Hum. Retroviruses* 13(17):1487–1495.

31. Bergmeier, L. A. and T. Lehner. 2006. Innate and adaptive mucosal immunity in protection against HIV Infection. *Adv. Dent. Res.* 19:21–28.
32. Berman, N. E., L. G. Sheffield, J. Purcell, S. V. Joag, O. Narayan, and P. Cheney. 1998. Gradient of microglial activation in the brain of SIV infected macaques. *J. NeuroAIDS* 2(1):43–54.
33. Berman, P. W., T. J. Gregory, L. Riddle, G. R. Nakamura, M. A. Champe, J. P. Porter, F. M. Wurm, R. D. Hershberg, E. K. Cobb, and J. W. Eichberg. 1990. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. *Nature* 345(6276):622–625.
34. Bonneh-Barkay, D., S. J. Bissel, G. Wang, K. N. Fish, G. C. Nicholl, S. W. Darko, R. Medina-Flores, M. Murphey-Corb, P. A. Rajakumar, J. Nyaundi, J. W. Mellors, R. Bowser, and C. A. Wiley. 2008. YKL-40, a marker of simian immunodeficiency virus encephalitis, modulates the biological activity of basic fibroblast growth factor. *Am. J. Pathol.* 173(1):130–143.
35. Borda, J. T., X. Alvarez, I. Kondova, P. Aye, M. A. Simon, R. C. Desrosiers, and A. A. Lackner. 2004. Cell tropism of simian immunodeficiency virus in culture is not predictive of in vivo tropism or pathogenesis. *Am. J. Pathol.* 165(6):2111–2122.
36. Bosch, M. L., E. Harper, A. Schmidt, K. B. Strand, S. Thormahlen, M. E. Thouless, and Y. Wang. 1999. Activation in vivo of retroperitoneal fibromatosis-associated herpesvirus, a simian homologue of human herpesvirus-8. *J. Gen. Virol.* 80(2):467–475.
37. Bostik, P., M. Watkins, F. Villinger, and A. A. Ansari. 2004. Genetic analysis of cytokine promoters in non-human primates: implications for Th1/Th2 profile characteristics and SIV disease pathogenesis. *Clin. Dev. Immunol.* 11(1):35–44.
38. Brown, C. R., M. Czapiga, J. Kabat, Q. Dang, I. Ourmanov, Y. Nishimura, M. A. Martin, and V. M. Hirsch. 2007. Unique pathology in simian immunodeficiency virus-infected rapid progressor macaques is consistent with a pathogenesis distinct from that of classical AIDS. *J. Virol.* 81(11):5594–5606.
39. Bublot, M., N. Pritchard, D. E. Swayne, P. Selleck, K. Karaca, D. L. Suarez, J. C. Audonnet, and T. R. Mickle. 2006. Development and use of fowlpoxes, vectored vaccines for avian influenza. *Ann. N. Y. Acad. Sci.* 1081:193–201.
40. Buge, S. L., L. Murty, K. Arora, V. S. Kalyanaraman, P. D. Markham, E. S. Richardson, K. Aldrich, L. J. Patterson, C. J. Miller, S. M. Cheng, and M. Robert-Guroff. 1999. Factors associated with slow disease progression in macaques immunized with an adenovirus–simian immunodeficiency virus (SIV) envelope priming-gp120 boosting regimen and challenged vaginally with SIVmac251. *J. Virol.* 73(9):7430–7440.
41. Chakrabarti, L., M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiollais, and P. Sonigo. 1987. Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. *Nature* 328(6130):543–547.
42. Chalifoux, L. V., N. W. King, M. D. Daniel, M. Kanagai, R. C. Desrosiers, P. K. Sehgal, L. M. Waldron, R. D. Hunt, and N. L. Letvin. 1986. Lymphoproliferative syndrome in an immunodeficient rhesus monkey naturally infected with an HTLV-III-like virus (STLV-III). *Lab. Invest.* 55(1):43–50.
43. Chalifoux, L. V., D. J. Ringler, N. W. King, P. K. Sehgal, R. C. Desrosiers, M. D. Daniel, and N. L. Letvin. 1987. Lymphadenopathy in macaques experimentally infected with the simian immunodeficiency virus (SIV). *Am. J. Pathol.* 128(1):104–110.
44. Chen, Z., Y. Huang, X. Zhao, E. Skulsky, D. Lin, J. Ip, A. Gettie, and D. D. Ho. 2000. Enhanced infectivity of an R5-tropic simian/human immunodeficiency virus carrying human immunodeficiency virus type 1 subtype C envelope after serial passages in pig-tailed macaques (*Macaca nemestrina*). *J. Virol.* 74(14):6501–6510.
45. Chen, Z., D. Kwon, Z. Jin, S. Monard, P. Telfer, M. S. Jones, C. Y. Lu, R. F. Aguilar, D. D. Ho, and P. A. Marx. 1998. Natural infection of a homozygous delta24 CCR5 red-capped mangabey with an R2b-tropic simian immunodeficiency virus. *J. Exp. Med.* 188(11):2057–2065.
46. Cheng-Mayer, C., A. Brown, J. Harouse, P. A. Luciw, and A. J. Mayer. 1999. Selection for neutralization resistance of the simian/human immunodeficiency virus SHIVSF33A variant in vivo by virtue of sequence changes in the extracellular envelope glycoprotein that modify N-linked glycosylation. *J. Virol.* 73(7):5294–5300.
47. Chun, T. W., L. Stuyver, S. B. Mizell, L. A. Ehler, J. M. Mican, M. Baseler, A. L. Lloyd, M. A. Nowak, and A. S. Fauci. 1997. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc. Natl. Acad. Sci. U. S. A.* 94:13193–13197.
48. Coffin, J., A. Haase, J. A. Levy, L. Montagnier, S. Oroszlan, N. Teich, H. Temin, K. Toyoshima, H. Varma, P. Vogt, and R. Weiss. 1986. Human immunodeficiency viruses. *Science* 232(4751):697.
49. Cohen, J. 2008. AIDS research. Microbicide fails to protect against HIV. *Science* 319(5866):1026–1027.
50. Cranage, M., S. Sharpe, C. Herrera, A. Cope, M. Dennis, N. Berry, C. Ham, J. Heeney, N. Rezk, A.

- Kashuba, P. Anton, I. McGowan, and R. Shattock. 2008. Prevention of SIV rectal transmission and priming of T cell responses in macaques after local pre-exposure application of tenofovir gel. *PLoS Med.* 5(8):e157; discussion e157.
51. Cumont, M. C., O. Diop, B. Vaslin, C. Elbim, L. Vioillet, V. Monceaux, S. Lay, G. Silvestri, R. Le Grand, M. Müller-Trutwin, B. Hurtrel, and J. Estaquier. 2008. Early divergence in lymphoid tissue apoptosis between pathogenic and nonpathogenic simian immunodeficiency virus infections of nonhuman primates. *J. Virol.* 82(3):1175–1184.
52. Daniel, M. D., F. Kirchhoff, S. C. Czajak, P. K. Sehgal, and R. C. Desrosiers. 1992. Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. *Science* 258(5090):1880–1881.
53. Daniel, M. D., N. L. Letvin, N. W. King, M. Kanagai, P. K. Sehgal, R. D. Hunt, P. J. Kanki, M. Essex, and R. C. Desrosiers. 1985. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* 228(4704):1201–1204.
54. Daniel, M. D., P. K. Sehgal, T. Kodama, M. S. Wyand, D. J. Ringler, N. W. King, D. K. Schmidt, C. D. Troup, and R. C. Desrosiers. 1990. Use of simian immunodeficiency virus for vaccine research. *J. Med. Primatol.* 19(3–4):395–399.
55. Davis, N. L., A. West, E. Reap, G. MacDonald, M. Collier, S. Dryga, M. Maughan, M. Connell, C. Walker, K. McGrath, C. Cecil, L. H. Ping, J. Frelinger, R. Olmsted, P. Keith, R. Swanstrom, C. Williamson, P. Johnson, D. Montefiori, and R. E. Johnston. 2002. Alphavirus replicon particles as candidate HIV vaccines. *IUBMB Life* 53(4–5):209–211.
56. Davison-Fairburn, B., J. Blanchard, F. S. Hu, L. Martin, R. Harrison, M. Ratterree, and M. Murphey-Corb. 1990. Experimental infection of timed-pregnant rhesus monkeys with simian immunodeficiency virus (SIV) during early, middle, and late gestation. *J. Med. Primatol.* 19(3–4):381–393.
57. Demberg, T., J. D. Boyer, N. Malkevich, L. J. Patterson, D. Venzon, E. L. Summers, I. Kalisz, V. S. Kalyanaraman, E. M. Lee, D. B. Weiner, and M. Robert-Guroff. 2008. Sequential priming with SIV DNA vaccines, with or without encoded cytokines, and a replicating Ad-SIV recombinant followed by protein boosting does not control a pathogenic SIVmac251 mucosal challenge. *J. Virol.* 82(21):10911–10921.
58. Denesvre, C., R. Le Grand, F. Boissin-Cans, L. Chakrabarti, B. Hurtrel, B. Vaslin, D. Dormont, and P. Sonigo. 1995. Highly attenuated SIVmac142 is immunogenic but does not protect against SIVmac251 challenge. *AIDS Res. Hum. Retroviruses* 11(11):1397–1406.
59. Desrosiers, R. C., J. D. Lifson, J. S. Gibbs, S. C. Czajak, A. Y. Howe, L. O. Arthur, and R. P. Johnson. 1998. Identification of highly attenuated mutants of simian immunodeficiency virus. *J. Virol.* 72(2):1431–1437.
60. Desrosiers, R. C., M. S. Wyand, T. Kodama, D. J. Ringler, L. O. Arthur, P. K. Sehgal, N. L. Letvin, N. W. King, and M. D. Daniel. 1989. Vaccine protection against simian immunodeficiency virus infection. *Proc. Natl. Acad. Sci. U. S. A.* 86(16):6353–6357.
61. Du, Z., S. M. Lang, V. G. Saserville, A. A. Lackner, P. O. Ilyinskii, M. D. Daniel, J. U. Jung, and R. C. Desrosiers. 1995. Identification of a nef allele that causes lymphocyte activation and acute disease in macaque monkeys. *Cell* 82(4):665–674.
62. Eichberg, J. W., J. M. Zarling, H. J. Alter, J. A. Levy, P. W. Berman, T. Gregory, L. A. Lasky, J. McClure, K. E. Cobb, P. A. Moran, S. Hu, R. C. Kennedy, T. C. Chanh, and G. R. Dreesman. 1987. T-cell responses to human immunodeficiency virus (HIV) and its recombinant antigens in HIV-infected chimpanzees. *J. Virol.* 61:3804–3808.
63. Endo, Y., T. Igarashi, Y. Nishimura, C. Buckler, A. Buckler-White, R. Plishka, D. S. Dimitrov, and M. A. Martin. 2000. Short- and long-term clinical outcomes in rhesus monkeys inoculated with a highly pathogenic chimeric simian/human immunodeficiency virus. *J. Virol.* 74(15):6935–6945.
64. Eriksson, K. K., N. J. King, M. Bublot, and A. Bukreyev. 2006. Towards a coronavirus-based HIV multigene vaccine. *Clin. Dev. Immunol.* 13(2–4):353–360.
65. Espana, C., D. C. Gajdusek, C. J. Gibbs Jr., B. I. Osburn, D. H. Gribble, G. H. Cardinet, and R. M. Chanock. 1975. Transmission of Creutzfeldt–Jakob disease to the stump-tail macaque (*Macaca arctoides*). *Proc. Soc. Exp. Biol. Med.* 149:723–724.
66. Estaquier, J. T., T. Idziorek, F. de Bels, F. Barré-Sinoussi, B. Hurtrel, A. M. Aubertin, A. Venet, M. Mehtali, E. Muchmore, and P. Michel. 1994. Programmed cell death and AIDS: significance of T-cell apoptosis in pathogenic and nonpathogenic primate lentiviral infections. *PNAS* 91(20):9431–9435.
67. Evans, D. T., J. E. Bricker, and R. C. Desrosiers. 2004. A novel approach for producing lentiviruses that are limited to a single cycle of infection. *J. Virol.* 78(21):11715–11725.
68. Evans, D. T., L. M. Chen, J. Gillis, K. C. Lin, B. Harty, G. P. Mazzara, R. O. Donis, K. G. Mansfield, J. D. Lifson, R. C. Desrosiers, J. E. Galán, and R. P. Johnson. 2003. Mucosal priming of simian immunodeficiency virus-specific cytotoxic T-lymphocyte responses in

- rhesus macaques by the *Salmonella* type III secretion antigen delivery system. *J. Virol.* 77(4):2400–2409.
69. Fauci, A. S., M. I. Johnston, C. W. Dieffenbach, D. R. Burton, S. M. Hammer, J. A. Hoxie, M. Martin, J. Overbaugh, D. I. Watkins, A. Mahmoud, and W. C. Greene. 2008. HIV vaccine research: the way forward. *Science* 321(5888):530–532.
 70. Fendrich, C., W. Lüke, C. Stahl-Hennig, O. Herchenröder, D. Fuchs, H. Wachter, and G. Hunsmann. 1989. Urinary neopterin concentrations in rhesus monkeys after infection with simian immunodeficiency virus (SIVmac 251). *AIDS* 3(5):305–307.
 71. Feurst, T. R., C. K. Stover, and V. F. de la Cruz. 1991. Development of BCG as a live recombinant vector system: potential use as an HIV vaccine. *Biotechnol. Ther.* 2(1–2):159–178.
 72. Finkel, T. H., G. Tudor-Williams, N. K. Banda, M. F. Cotton, T. Curiel, C. Monks, T. W. Baba, R. M. Ruprecht, and A. Kupfer. 1995. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. *Nat. Med.* 1:129–134.
 73. Finzi, D., M. Hermankova, T. Pierson, L. M. Carruth, C. Buck, R. E. Chaisson, T. C. Quinn, K. Chadwick, J. Margolick, R. Brookmeyer, J. Gallant, M. Markowitz, D. D. Ho, D. Richman, and R. F. Siliciano. 1997. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278:1295–1300.
 74. Franchini, G., M. Robert-Guroff, J. Tartaglia, A. Aggarwal, A. Abimiku, J. Benson, P. Markham, K. Limbach, G. Hurteau, J. Fullen, K. Aldrich, N. Miller, J. Sadoff, E. Paoletti, and R. C. Gallo. 1995. Highly attenuated HIV type 2 recombinant poxviruses, but not HIV-2 recombinant *Salmonella* vaccines, induce long-lasting protection in rhesus macaques. *AIDS Res. Hum. Retroviruses* 11(8):909–920.
 75. Frumkin, L. R., M. B. Agy, R. W. Coombs, L. Panther, W. R. Morton, J. Koehler, M. J. Florey, J. Dragavon, A. Schmidt, M. G. Katze, and L. Corey. 1993. Acute infection of *Macaca nemestrina* by human immunodeficiency virus type 1. *Virology* 195(2):422–431.
 76. Fukunishi, Y., W. M. Meyers, C. H. Binford, G. P. Walsh, F. B. Johnson, P. J. Gerone, R. H. Wolf, B. J. Gormus, and L. N. Martin. 1984. Electron microscopic study of leprosy in a mangabey monkey (natural infection). *Int. J. Lepr. Other Mycobact. Dis.* 52(2):203–207.
 77. Fultz, P. N. 1986. Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (*Cercocebus atys*). *Proc. Natl. Acad. Sci. U. S. A.* 83:5286–5290.
 78. Fultz, P. N., H. M. McClure, D. C. Anderson, and W. M. Switzer. 1994. Unique lentivirus-host interactions: SIVsmmPBj14 infection of macaques. *Virus Res.* 32(2):205–225.
 79. Fultz, P. N., H. M. McClure, H. Daugherty, A. Brodie, C. R. McGrath, B. Swenson, and D. P. Francis. 1986. Vaginal transmission of human immunodeficiency virus (HIV) to a chimpanzee. *J. Infect. Dis.* 154(5):896–900.
 80. Fultz, P. N., H. M. McClure, R. B. Swenson, C. R. McGrath, A. Brodie, J. P. Getchell, F. C. Jensen, D. C. Anderson, J. R. Broder, and D. P. Francis. 1986. Persistent infection of chimpanzees with human T-Lymphotropic virus type III/Lymphadenopathy-associated virus: a potential model for acquired immunodeficiency syndrome. *J. Virol.* 58:116–124.
 81. Fultz, P. N., P. Nara, F. Barre-Sinoussi, A. Chaput, M. L. Greenberg, E. Muchmore, M. P. Kiely, and M. Girard. 1992. Vaccine protection of chimpanzees against challenge with HIV-1-infected peripheral blood mononuclear cells. *Science* 256(5064):1687–1690.
 82. Fultz, P. N., Q. Wei, and L. Yue. 1999. Rectal transmission of human immunodeficiency virus type 1 to chimpanzees. *J. Infect. Dis.* 179(Suppl 3):S418–S421.
 83. Fultz, P. N., L. Yue, Q. Wei, and M. Girard. 1997. Human immunodeficiency virus type 1 intersubtype (B/E) recombination in a superinfected chimpanzee. *J. Virol.* 71(10):7990–7995.
 84. Fultz, P. N. and P. M. Zack. 1994. Unique lentivirus-host interactions: SIVsmmPBj14 infection of macaques. *Virus Res.* 32(2):205–225.
 85. Gajdusek, D. C. and C. J. Gibbs Jr. 1972. Transmission of kuru from man to rhesus monkey (*Macaca mulatta*) 8 and one-half years after inoculation. *Nature* 240:351.
 86. Genescà, M., J. Li, L. Fritts, P. Chohan, K. Bost, T. Rourke, S. A. Blozis, M. B. McChesney, and C. J. Miller. 2007. Depo-Provera abrogates attenuated lentivirus-induced protection in male rhesus macaques challenged intravenously with pathogenic SIVmac239. *J. Med. Primatol.* 36(4–5):266–275.
 87. Genescà, M., T. Rourke, J. Li, K. Bost, B. Chohan, M. B. McChesney, and C. J. Miller. 2007. Live attenuated lentivirus infection elicits polyfunctional simian immunodeficiency virus Gag-specific CD8⁺ T cells with reduced apoptotic susceptibility in rhesus macaques that control virus replication after challenge with pathogenic SIVmac239. *J. Immunol.* 179(7):4732–4740.
 88. Giavedoni, L. D., M. C. Velasquillo, L. M. Parodi, G. B. Hubbard, and V. L. Hodara. 2000. Cytokine expression, natural killer cell activation, and phenotypic changes in lymphoid cells from rhesus macaques

- during acute infection with pathogenic simian immunodeficiency virus. *J. Virol.* 74:1648–1657.
89. Gibbs, C. J., Jr. and D. C. Gajdusek. 1972. Transmission of scrapie to the cynomolgus monkey (*Macaca fascicularis*). *Nature* 236:73–74.
90. Girard, M., E. van der Ryst, F. Barré-Sinoussi, P. Nara, J. Tartaglia, E. Paoletti, C. Blondeau, M. Jennings, F. Verrier, B. Meignier, and P. N. Fultz. 1997. Challenge of chimpanzees immunized with a recombinant canarypox-HIV-1 virus. *Virology* 232(1):98–104.
91. Girard, M., L. Yue, F. Barré-Sinoussi, E. van der Ryst, B. Meignier, E. Muchmore, and P. N. Fultz. 1996. Failure of a human immunodeficiency virus type 1 (HIV-1) subtype B-derived vaccine to prevent infection of chimpanzees by an HIV-1 subtype E strain. *J. Virol.* 70(11):8229–8233.
92. Gómez-Román, V. R., G. J. Grimes Jr., G. K. Potti, B. Peng, T. Demberg, L. Gravlin, J. Treece, R. Pal, E. M. Lee, W. G. Alvord, P. D. Markham, and M. Robert-Guroff. 2006. Oral delivery of replication-competent adenovirus vectors is well tolerated by SIV- and SHIV-infected rhesus macaques. *Vaccine* 24(23):5064–5072.
93. Gómez-Román, V. R., L. J. Patterson, D. Venzon, D. Liewehr, K. Aldrich, R. Florese, and M. Robert-Guroff. 2005. Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. *J. Immunol.* 174(4):2185–2189.
94. Gottlieb, M. S., R. Schroff, H. M. Schanker, J. D. Weisman, P. T. Fan, R. A. Wolf, and A. Saxon. 1981. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N. Engl. J. Med.* 305(24):1425–1431.
95. Goulder, P. J. and D. I. Watkins. Impact of MHC class I diversity on immune control of immunodeficiency virus replication. *Nat. Rev. Immunol.* 8(8):619–630.
96. Goulder, P. J. and D. I. Watkins. 2004. HIV and SIV CTL escape: implications for vaccine design. *Nat. Rev. Immunol.* 4(8):630–640.
97. Grant, R. M., D. Hamer, T. Hope, R. Johnston, J. Lange, M. M. Lederman, J. Lieberman, C. J. Miller, J. P. Moore, D. E. Mosier, D. D. Richman, R. T. Schooley, M. S. Springer, R. S. Veazey, and M. A. Wainberg. 2008. Whither or wither microbicides? *Science* 321(5888):532–534.
98. Gruber, A., A. S. Chalmers, R. A. Rasmussen, H. Ong, S. Popov, J. Andersen, S. L. Hu, and R. M. Ruprecht. 2007. Dendritic cell-based vaccine strategy against human immunodeficiency virus clade C. skewing the immune response toward a helper T cell type 2 profile. *Viral Immunol.* 20(1):160–169.
99. Habis, A., G. B. Baskin, M. Murphrey-Corb, and L. S. Levy. 1999. Simian AIDS-associated lymphoma in rhesus and cynomolgus monkeys recapitulates the primary pathobiological features of AIDS-associated non-Hodgkin's lymphoma. *AIDS Res. Hum. Retroviruses* 15(15):1389–1398.
100. Habis, A., G. Baskin, L. Simpson, I. Fortgang, M. Murphrey-Corb, and L. S. Levy. 2000. Rhesus lymphocryptovirus infection during the progression of SAIDS and SAIDS-associated lymphoma in the rhesus macaque. *AIDS Res. Hum. Retroviruses* 16(2):163–171.
101. Haigwood, N. L., L. Misher, S. M. Chin, M. Blair, V. Planelles, C. J. Scandella, K. S. Steimer, M. B. Gardner, T. Yilma, V. M. Hirsch, and P. R. Johnson. 1992. Characterization of group specific antibodies in primates: studies with SIV envelope in macaques. *J. Med. Primatol.* 21(2–3):82–90.
102. Haigwood, N. L., D. C. Montefiori, W. F. Sutton, J. McClure, A. J. Watson, G. Voss, V. M. Hirsch, B. A. Richardson, N. L. Letvin, S. L. Hu, and P. R. Johnson. 2004. Passive immunotherapy in simian immunodeficiency virus-infected macaques accelerates the development of neutralizing antibodies. *J. Virol.* 7811:5983–5995.
103. Haigwood, N. L., A. Watson, W. F. Sutton, J. McClure, A. Lewis, J. Ranchalis, B. Travis, G. Voss, N. L. Letvin, S. L. Hu, V. M. Hirsch, and P. R. Johnson. 1996. Passive immune globulin therapy in the SIV/macaque model: early intervention can alter disease profile. *Immunol. Lett.* 51(1–2):107–114.
104. Halwani, R., J. D. Boyer, B. Yassine-Diab, E. K. Haddad, T. M. Robinson, S. Kumar, R. Parkinson, L. Wu, M. K. Sidhu, R. Phillipson-Weiner, G. N. Pavlakis, B. K. Felber, M. G. Lewis, A. Shen, R. F. Siliciano, D. B. Weiner, and R. P. Sekaly. 2008. Therapeutic vaccination with simian immunodeficiency virus (SIV)-DNA + IL12 or IL-15 induces distinct CD8 memory subsets in SIV-infected macaques. *J. Immunol.* 180(12):7969–7979.
105. Harouse, J. M., A. Gettie, R. C. Tan, J. Blanchard, and C. Cheng-Mayer. 1999. Distinct pathogenic sequela in rhesus macaques infected with CCR5 or CXCR4 utilizing SHIVs. *Science* 284(5415):816–819.
106. Harouse, J. M., R. C. Tan, A. Gettie, P. Dailey, P. A. Marx, P. A. Luciw, and C. Cheng-Mayer. 1998. Mucosal transmission of pathogenic CXCR4-utilizing SHIVSF33A variants in rhesus macaques. *Virology* 248(1):95–107.
107. Hillier, S. L., T. Moench, R. Shattock, R. Black, P. Reichelderfer, and F. Veronese. 2005. In vitro and in

- vivo: the story of nonoxynol 9 [Abstract]. *J. Acquir. Immune Defic. Syndr.* 39(1):1–8.
108. Himathongkham, S., N. S. Halpin, J. Li, M. W. Stout, C. J. Miller, and P. A. Luciw. 2000. Simian-human immunodeficiency virus containing a human immunodeficiency virus type 1 subtype-E envelope gene: persistent infection, CD4(+) T-cell depletion, and mucosal membrane transmission in macaques. *J. Virol.* 74(17):7851–7860.
 109. Hirsch, V., D. Adger-Johnson, B. Campbell, S. Goldstein, C. Brown, W. R. Elkins, and D. C. Montefiori. 1997. A molecularly cloned, pathogenic, neutralization-resistant simian immunodeficiency virus, SIVsmE543-3. *Virology* 71(2):1608–1620.
 110. Hirsch, V. M., T. R. Fuerst, G. Sutter, M. W. Carroll, L. C. Yang, S. Goldstein, M. J. Piatak, W. R. Elkins, W. G. Alvord, D. C. Montefiori, B. Moss, and J. D. Lifson. 1996. Patterns of viral replication correlate with outcome in simian immunodeficiency virus (SIV)-infected macaques: effect of prior immunization with a trivalent SIV vaccine in modified vaccinia virus Ankara. *J. Virol.* 70:3741–3752.
 111. Hirsch, V. M. and P. R. Johnson. 1994. Pathogenic diversity of simian immunodeficiency viruses. *Virus Res.* 32:183–203.
 112. Hirsch, V. M., R. A. Olmsted, M. Murphey-Corb, R. H. Purcell, and P. R. Johnson. 1989. An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature* 339(6223):389–392.
 113. Hirsch, V. M., P. M. Zack, A. P. Vogel, and P. R. Johnson. 1991. Simian immunodeficiency virus infection of macaques: end-stage disease is characterized by widespread distribution of proviral DNA in tissues. *J. Infect. Dis.* 163:976–988.
 114. Hofman, M. J., J. Higgins, T. B. Matthews, N. C. Pedersen, C. Tan, R. F. Schinazi, and T. W. North. 2004. Efavirenz therapy in rhesus macaques infected with a chimera of simian immunodeficiency virus containing reverse transcriptase from human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* 48(9):3483–3490.
 115. Hofmann-Lehmann, R., J. Vlasak, A. L. Williams, A. L. Chenine, H. M. McClure, D. C. Anderson, S. O’Neil, and R. M. Ruprecht. 2003. Live attenuated, nef-deleted SIV is pathogenic in most adult macaques after prolonged observation. *AIDS* 17(2):157–166.
 116. Hsu, M., J. M. Harouse, A. Gettie, C. Buckner, J. Blanchard, and C. Cheng-Mayer. 2003. Increased mucosal transmission but not enhanced pathogenicity of the CCR5-tropic, simian AIDS-inducing simian/human immunodeficiency virus SHIV_{SF162P3} maps to envelope gp120. *J. Virol.* 277(2):989–998.
 117. Hu, J., M. B. Gardner, and C. J. Miller. 2000. Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells. *J. Virol.* 74(13):6087–6095.
 118. Hu, S. L. 2005. Non-human primate models for AIDS vaccine research. *Curr. Drug Targets Infect. Disord.* 5(2):193–201.
 119. Hu, S. L., P. N. Fultz, H. M. McClure, J. W. Eichberg, E. K. Thomas, J. Zarling, M. C. Singhal, S. G. Kosowski, R. B. Swenson, D. C. Anderson, and G. Todaro. 1987. Effect of immunization with a vaccinia-HIV env recombinant on HIV infection of chimpanzees. *Nature* 328(6132):721–723.
 120. Hunt, R. D., B. J. Blake, L. V. Chalifoux, P. K. Sehgal, N. W. King, and N. L. Letvin. 1983. Transmission of naturally occurring lymphoma in macaque monkeys. *Proc. Natl. Acad. Sci. U. S. A.* 80(16):5085–5089.
 121. Hurtrel, B., F. Petit, D. Arnoult, M. Muller-Trutwin, G. Silvestri, and J. Estaquier. 2005. Apoptosis in SIV infection. *Cell Death Differ.* 12(Suppl 1):979–990.
 122. Jiang, S., R. A. Rasmussen, K. M. Nolan, F. R. Frankel, J. Lieberman, H. M. McClure, K. M. Williams, U. S. Babu, R. B. Raybourne, E. Strobert, and R. M. Ruprecht. 2007. Live attenuated Listeria monocytogenes expressing HIV Gag: immunogenicity in rhesus monkeys. *Vaccine* 25(42):7470–7479.
 123. Jin, X., D. E. Bauer, S. E. Tuttleton, S. Lewin, A. Gettie, J. Blanchard, C. E. Irwin, J. T. Safrit, J. Mittler, L. Weinberger, L. G. Kostrikis, L. Zhang, A. S. Perelson, and D. D. Ho. 1999. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J. Exp. Med.* 189(6):991–998.
 124. Joag, S. V., Z. Li, L. Foresman, E. B. Stephens, L. J. Zhao, I. Adany, D. M. Pinson, H. M. McClure, and O. Narayan. 1996. Chimeric simian/human immunodeficiency virus that causes progressive loss of CD4⁺ T cells and AIDS in pig-tailed macaques. *J. Virol.* 70(5):3189–3197.
 125. Johnson, J. K., K. A. Warren, N. E. Berman, O. Narayan, E. B. Stephens, S. V. Joag, R. Raghavan, J. K. Marcario, and P. D. Cheney. 2004. Manifestations of SIV-induced ocular pathology in macaque monkeys. *J. NeuroAIDS* 2(4):1–13.
 126. Johnston, R. E., P. R. Johnson, M. J. Connell, D. C. Montefiori, A. West, M. L. Collier, C. Cecil, R. Swanstrom, J. A. Frelinger, and N. L. Davis. 2005. Vaccination of macaques with SIV immunogens delivered by Venezuelan equine encephalitis virus replicon particle vectors followed by a mucosal challenge with SIVsmE660. *Vaccine* 23(42):4969–4979.

127. Kanki, P. J., J. Alroy, and M. Essex. 1985. Isolation of T-lymphotropic retrovirus related to HTLV-III/LAV from wild-caught African green monkeys. *Science* 230(4728):951–954.
128. Kanki, P. J., F. Barin, S. M'Boup, J. S. Allan, J. L. Romet-Lemonne, R. Marlink, M. F. McLane, T. H. Lee, B. Arbeille, F. Denis, and M. Essex. 1986. New human T-lymphotropic retrovirus related to simian T-lymphotropic virus type III (STLV-IIIAGM). *Science* 232(4747):238–243.
129. Kannagi, M., M. Kiyotaki, R. C. Desrosiers, K. A. Reimann, N. W. King, L. M. Waldron, and N. L. Letvin. 1986. Humoral immune responses to T cell tropic retrovirus simian T lymphotropic virus type III in monkeys with experimentally induced acquired immune deficiency-like syndrome. *J. Clin. Invest.* 78(5):1229–1236.
130. Karlsson, G. B., M. Halloran, J. Li, I. W. Park, R. Gomila, K. A. Reimann, M. K. Axthelm, S. A. Iliff, N. L. Letvin, and J. Sodroski. 1997. Characterization of molecularly cloned simian-human immunodeficiency viruses causing rapid CD4⁺ lymphocyte depletion in rhesus monkeys. *J. Virol.* 71(6):4218–4225.
131. Kent, S. J., L. Corey, M. B. Agy, W. R. Morton, M. J. McElrath, and P. D. Greenberg. 1995. Cytotoxic and proliferative T cell responses in HIV-1-infected *Macaca nemestrina*. *J. Clin. Invest.* 95(1):248–256.
132. Kent, S. J., C. J. Dale, C. Ranasinghe, I. Stratov, R. De Rose, S. Chea, D. C. Montefiori, S. Thomson, I. A. Ramshaw, B. E. Coupar, D. B. Boyle, M. Law, K. M. Wilson, and A. J. Ramsay. 2005. Mucosally-administered human-simian immunodeficiency virus DNA and fowlpoxvirus-based recombinant vaccines reduce acute phase viral replication in macaques following vaginal challenge with CCR5-tropic SHIVSF162P3. *Vaccine* 23(42):5009–5021.
133. Kent, S. J., R. De Rose, V. V. Mokhonov, E. I. Mokhonova, C. S. Fernandez, S. Alcantara, E. Rollman, R. D. Mason, L. Loh, V. Peut, J. C. Reece, X. J. Wang, K. M. Wilson, A. Suhrbier, and A. Khromykh. 2008. Evaluation of recombinant Kunjin replicon SIV vaccines for protective efficacy in macaques. *Virology* 374(2):528–534.
134. Kestler, H., T. Kodama, D. Ringler, M. Marthas, N. Pedersen, A. Lackner, D. Regier, P. Sehgal, M. Daniel, N. King, and R. C. Desrosiers. 1990. Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. *Science* 248(4959):1109–1112.
135. Kestler, H. W., 3rd, Y. Li, Y. M. Naidu, C. V. Butler, M. F. Ochs, G. Jaenel, N. W. King, M. D. Daniel, and R. C. Desrosiers. 1988. Comparison of simian immunodeficiency virus isolates. *Nature* 331(6157):619–622.
136. Kestler, H. W., 3rd, K. Mori, D. P. Silva, T. Kodama, N. W. King, M. D. Daniel, and R. C. Desrosiers. 1990. Nef genes of SIV. *J. Med. Primatol.* 19(3–4):421–429.
137. Kestler, H. W., 3rd, D. J. Ringler, K. Mori, D. L. Panicali, P. K. Sehgal, M. D. Daniel, and R. C. Desrosiers. 1991. Importance of the nef gene for maintenance of high virus loads and for development of AIDS. *Cell* 65(4):651–662.
138. Kim, J. J., L. K. Nottingham, A. Tsai, D. J. Lee, H. C. Maguire, J. Oh, T. Dentchev, K. H. Manson, M. S. Wyand, M. G. Agadjanyan, K. E. Ugen, and D. B. Weiner. 1999. Antigen-specific humoral and cellular immune responses can be modulated in rhesus macaques through the use of IFN-gamma, IL-12, or IL-18 gene adjuvants. *J. Med. Primatol.* 28(4–5):214–223.
139. Kim, J. J., J. S. Yang, T. C. VanCott, D. J. Lee, K. H. Manson, M. S. Wyand, J. D. Boyer, K. E. Ugen, and D. B. Weiner. 2000. Modulation of antigen-specific humoral responses in rhesus macaques by using cytokine cDNAs as DNA vaccine adjuvants. *J. Virol.* 74(7):3427–3429.
140. King, N. J., D. R. Getts, M. T. Getts, S. Rana, B. Shrestha, and A. M. Kesson. 2007. Immunopathology of flavivirus infections. *Immunol. Cell Biol.* 85(1):33–42.
141. Koff, W. C., P. R. Johnson, D. I. Watkins, D. R. Burton, J. D. Lifson, K. J. Hasenkrug, A. B. McDermott, A. Schultz, T. J. Zamb, R. Boyle, and R. C. Desrosiers. 2006. HIV vaccine design: insights from live attenuated SIV vaccines. *Nat. Immunol.* 7(1):19–23.
142. Kolson, D. L. 2008. YKL-40: a candidate biomarker for simian immunodeficiency virus and human immunodeficiency virus encephalitis? *Am. J. Pathol.* 173(1):25–29.
143. Kuate, S., C. Stahl-Hennig, P. ten Haaft, J. Heeney, and K. Uberla. 2003. Single-cycle immunodeficiency viruses provide strategies for uncoupling in vivo expression levels from viral replicative capacity and for mimicking live-attenuated SIV vaccines. *Virology* 313(2):653–662.
144. Kuroda, M. J., J. E. Schmitz, D. H. Barouch, A. Craiu, T. M. Allen, A. Sette, D. I. Watkins, M. A. Forman, and N. L. Letvin. 1998. Analysis of Gag-specific cytotoxic T lymphocytes in simian immunodeficiency virus-infected rhesus monkeys by cell staining with a tetrameric major histocompatibility complex class I-peptide complex. *J. Exp. Med.* 9:1373–1381.
145. Kuroda, M. J., J. E. Schmitz, W. A. Charini, C. E. Nickerson, M. A. Lifton, C. I. Lord, M. A.

- Forman, and N. L. Letvin. 1999. Emergence of CTL coincides with clearance of virus during primary simian immunodeficiency virus infection in rhesus monkeys. *J. Immunol.* 162(9):5127–5133.
146. Lackner, A. A., M. O. Smith, R. J. Munn, D. J. Martfeld, M. B. Gardner, P. A. Marx, and S. Dandekar. 1991. Localization of simian immunodeficiency virus in the central nervous system of rhesus monkeys. *Am. J. Pathol.* 139(3):609–621.
147. LaFranco-Scheuch, L., K. Abel, N. Makori, K. Rothaeusler, and C. J. Miller. 2004. High beta-chemokine expression levels in lymphoid tissues of simian/human immunodeficiency virus 89.6-vaccinated rhesus macaques are associated with uncontrolled replication of simian immunodeficiency virus challenge inoculum. *J. Virol.* 78(12):6399–6408.
148. Langlois, A. J., M. G. Lewis, V. N. KewalRamani, D. R. Littman, J. Y. Zhou, K. Manson, D. P. Bolognesi, M. S. Wyand, R. C. Desrosiers, and D. C. Montefiori. 1998. Neutralizing antibodies in sera from macaques immunized with attenuated simian immunodeficiency virus. *J. Virol.* 72:6950–6955.
149. Letvin, N. L., M. D. Daniel, P. K. Sehgal, R. C. Desrosiers, R. D. Hunt, L. M. Waldron, J. J. MacKey, D. K. Schmidt, L. V. Chalifoux, and N. W. King. 1985. Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. *Science* 230(4721):71–73.
150. Letvin, N. L. and B. D. Walker. 2003. Immunopathogenesis and immunotherapy in AIDS virus infections. *Nat. Med.* 9(7):861–866.
151. Leung, N. J., A. Aldovini, R. Young, M. A. Jarvis, J. M. Smith, D. Meyer, D. E. Anderson, M. P. Carlos, M. B. Gardner, and J. V. Torres. 2000. The kinetics of specific immune responses in rhesus monkeys inoculated with live recombinant BCG expressing SIV Gag, Pol, Env, and Nef proteins. *Virology* 268(1):94–103.
152. Levy, J. A., C. Cheng-Mayer, D. Dina, and P. A. Luciw. 1986. AIDS retrovirus (ARV-2) clone replicates in transfected human and animal fibroblasts. *Science* 232(4753):998–1001.
153. Leydet, A., C. Moullet, J. P. Roque, M. Witvrouw, C. Pannecouque, G. Andrei, R. Snoeck, J. Neyts, D. Schols, and E. De Clercq. 1998. Polyanion inhibitors of HIV and other viruses. 7. Polyanionic compounds and polyzwitterionic compounds derived from cyclodextrins as inhibitors of HIV transmission. *J. Med. Chem.* 41(25):4927–4932.
154. Li, J., C. I. Lord, W. Haseltine, N. L. Letvin, and J. Sodroski. 1992. Infection of cynomolgus monkeys with a chimeric HIV-1/SIVmac virus that expresses the HIV-1 envelope glycoproteins. *J. Acquir. Immune Defic. Syndr.* 5(7):639–646.
155. Li, J. T., M. Halloran, C. I. Lord, A. Watson, J. Ranchalis, M. Fung, N. L. Letvin, and J. G. Sodroski. 1995. Persistent infection of macaques with simian–human immunodeficiency viruses. *J. Virol.* 69(11):7061–7077.
156. Lifson, J. D., J. L. Rossio, M. Piatak Jr., J. Bess Jr., E. Chertova, D. K. Schneider, V. J. Coalter, B. Poore, R. F. Kiser, R. J. Imming, A. J. Scarzello, L. E. Henderson, W. G. Alvord, V. M. Hirsch, R. E. Benveniste, and L. O. Arthur. 2004. Evaluation of the safety, immunogenicity, and protective efficacy of whole inactivated simian immunodeficiency virus (SIV) vaccine with conformationally and functionally intact envelope glycoproteins. *AIDS Res. Hum. Retroviruses* 20(7):772–787.
157. Lifson, J. D., J. L. Rossio, M. Piatak Jr., T. Parks, L. Li, R. Kiser, V. Coalter, B. Fisher, B. M. Flynn, S. Czajak, V. M. Hirsch, K. A. Reimann, J. E. Schmitz, J. Ghrayeb, N. Bischofberger, M. A. Nowak, R. C. Desrosiers, and D. Wodarz. 2001. Role of CD8(+) lymphocytes in control of simian immunodeficiency virus infection and resistance to rechallenge after transient early antiretroviral treatment. *J. Virol.* 75:10187–10199.
158. Ling, B., C. Apetrei, I. Pandrea, R. S. Veazey, A. A. Lackner, B. Gormus, and P. A. Marx. 2004. Classic AIDS in a sooty mangabey after an 18-year natural infection. *J. Virol.* 78(16):8902–8908.
159. Ling, B., R. S. Veazey, M. Hart, A. A. Lackner, M. Kuroda, B. Pahar, and P. Marx. 2007. Early restoration of mucosal CD4⁺ T cells in the gut of SIV-infected rhesus predicts long term nonprogression. *AIDS* 21(18):2377–2385.
160. Ling, B., R. S. Veazey, A. Luckay, C. Penedo, K. Xu, J. D. Lifson, and P. A. Marx. 2002. SIV(mac) pathogenesis in rhesus macaques of Chinese and Indian origin compared with primary HIV infections in humans. *AIDS* 16(11):1489–1496.
161. Liu, X., L. A. Lagenaar, D. A. Simpson, K. P. Essenmacher, C. L. Frazier-Parker, Y. Liu, D. Tsai, S. S. Rao, D. H. Hamer, T. P. Parks, P. P. Lee, and Q. Xu. 2006. Engineered vaginal lactobacillus strain for mucosal delivery of the human immunodeficiency virus inhibitor cyanovirin-N. *Antimicrob. Agent Chemother.* 50(10):3250–3259.
162. Loffredo, J. T., A. T. Bean, D. R. Beal, E. J. León, G. E. May, S. M. Piaskowski, J. R. Furlott, J. Reed, S. K. Musani, E. G. Rakasz, T. C. Friedrich, N. A. Wilson, D. B. Allison, and D. I. Watkins. 2008. Patterns of CD8⁺ immunodominance may influence the ability of Mamu-B*08-positive macaques to

- naturally control simian immunodeficiency virus SIVmac239 replication. *J. Virol.* 82(4):1723–1738.
163. Looney, D. J., J. McClure, S. J. Kent, A. Radaelli, G. Kraus, A. Schmidt, K. Steffy, P. Greenberg, S. L. Hu, W. R. Morton, and F. Wong-Staal. 1998. A minimally replicative HIV-2 live-virus vaccine protects M. nemestrina from disease after HIV-2(287) challenge. *Virology* 242(1):150–160.
 164. Lowenstein, L. J., N. W. Lerche, J. L. Yee, A. Uyeda, M. B. Jennings, R. J. Munn, H. M. McClure, D. C. Anderson, P. N. Fultz, and M. B. Gardner. 1992. Evidence for a lentiviral etiology in an epizootic of immune deficiency and lymphoma in stump-tailed macaques (*Macaca arctoides*). *J. Med. Primatol.* 21:1–14.
 165. Lu, Y., C. D. Pauza, X. Lu, D. C. Montefiori, and C. J. Miller. 1998. Rhesus macaques that become systemically infected with pathogenic SHIV 89.6-PD after intravenous, rectal, or vaginal inoculation and fail to make an antiviral antibody response rapidly develop AIDS. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 19:6–18.
 166. Luciw, P. A., S. J. Potter, K. Steimer, D. Dina, and J. A. Levy. 1984. Molecular cloning of AIDS-associated retrovirus. *Nature* 312(5996):760–763.
 167. Mackett, M., G. L. Smith, and B. Moss. 1982. Vaccinia virus: a selectable eukaryotic cloning and expression vector. *Proc. Natl. Acad. Sci. U. S. A.* 79(23):7415–7419.
 168. Mandell, C. P., N. C. Jain, C. J. Miller, and S. Dandekar. 1995. Bone marrow monocyte/macrophages are an early cellular target of pathogenic and nonpathogenic isolates of simian immunodeficiency virus (SIVmac) in rhesus macaques. *Lab. Invest.* 72(3):323–333.
 169. Maness, N. J., L. J. Yant, C. Chung, J. T. Loffredo, T. C. Friedrich, S. M. Piaskowski, J. Furlott, G. E. May, T. Soma, E. J. León, N. A. Wilson, H. Piontkivska, A. L. Hughes, J. Sidney, A. Sette, and D. I. Watkins. 2008. Comprehensive immunological evaluation reveals surprisingly few differences between elite controller and progressor Mamu-B*17-positive Simian immunodeficiency virus-infected rhesus macaques. *J. Virol.* 82(11):5245–5254.
 170. Mansfield, K., S. M. Lang, M. C. Gauduin, H. B. Sanford, J. D. Lifson, R. P. Johnson, and R. C. Desrosiers. 2008. Vaccine protection by live, attenuated simian immunodeficiency virus in the absence of high-titer antibody responses and high-frequency cellular immune responses measurable in the periphery. *J. Virol.* 82(8):4135–4148.
 171. Mansfield, K. G., A. Carville, L. Wachtman, B. R. Goldin, J. Yearley, W. Li, M. Woods, L. Gualtieri, R. Shannon, and C. Wanke. 2007. A diet high in saturated fat and cholesterol accelerates simian immunodeficiency virus disease progression. *J. Infect. Dis.* 196(8):1202–1210.
 172. Mansfield, K. G., N. W. Lerch, M. B. Gardner, and A. A. Lackner. 1995. Origins of simian immunodeficiency virus infection in macaques at the New England Regional Primate Research Center. *J. Med. Primatol.* 24(3):116–122.
 173. Mansfield, K. G., S. V. Westmoreland, C. D. DeBakker, S. Czajak, A. A. Lackner, and R. C. Desrosiers. 1999. Experimental infection of rhesus and pig-tailed macaques with macaque rhadinoviruses. *J. Virol.* 73(12):10320–10328.
 174. Marcondes, M. C., C. M. Lanigan, T. H. Burdo, D. D. Watry, and H. S. Fox. 2008. Increased expression of monocyte CD44v6 correlates with the development of encephalitis in rhesus macaques infected with simian immunodeficiency virus. *J. Infect. Dis.* 197:1567–1576.
 175. Martin Amedee, A., N. Lacour, L. N. Martin, J. E. Clements, R. B. Bohm Jr., B. Davison, R. Harrison, and M. Murphey-Corb. 1996. Genotypic analysis of infant macaques infected transplacentally and orally. *J. Med. Primatol.* 25(3):225–235.
 176. Martin, L. N., B. J. Gormus, R. H. Wolf, P. J. Gerone, W. M. Meyers, G. P. Walsh, C. H. Binford, T. L. Hadfield, and C. J. Schlagel. 1985. Depression of lymphocyte responses to mitogens in mangabeys with disseminated experimental leprosy. *Cell Immunol.* 90(1):115–130.
 177. Martinon, F., P. Brochard, M. Ripaux, B. Delache, G. Aurégan, B. Vaslin, and R. Le Grand. 2008. Improved protection against simian immunodeficiency virus mucosal challenge in macaques primed with a DNA vaccine and boosted with the recombinant modified vaccinia virus Ankara and recombinant Semliki forest virus. *Vaccine* 26(4):532–545.
 178. Marx, P. A., Y. Li, N. W. Lerche, S. Sutjipto, A. Gettie, J. Yee, B. H. Brotman, A. M. Prince, A. Hanson, R. G. Webster, and R. C. Desrosiers. 1991. Isolation of a simian immunodeficiency virus related to human immunodeficiency virus Type 2 from a West African pet sooty mangabey. *J. Virol.* 65:4480–4485.
 179. Marx, P. A., A. I. Spira, A. Gettie, P. J. Dailey, R. S. Veazey, A. A. Lackner, C. J. Mahoney, C. J. Miller, L. E. Claypool, D. D. Ho, and N. J. Alexander. 1996. Progesterone implants enhance SIV vaginal transmission and early virus load. *Nat. Med.* 2(10):1084–1089.
 180. Mascola, J. R., G. Stiegler, T. C. VanCott, H. Katinger, C. B. Carpenter, C. E. Hanson, H. Beary, D. Hayes, S. S. Frankel, D. L. Birx, and M. G. Lewis. 2000. Protection of macaques against vaginal

- transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nat. Med.* 6(2):207–210.
181. Masters, C. L., M. P. Alpers, D. C. Gajdusek, C. J. Gibbs Jr., and B. A. Kakulas. 1976. Experimental kuru in the gibbon and sooty mangabey and Creutzfeldt–Jakob disease in the pigtailed macaque. With a summary of the host range of the subacute spongiform virus encephalopathies. *J. Med. Primatol.* 5:205–209.
 182. Matano, T., R. Shibata, C. Siemon, M. Connors, H. C. Lane, and M. A. Martin. 1998. Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. *J. Virol.* 72:164–169.
 183. McChesney, M. B., J. R. Collins, D. Lu, X. Lu, J. Torten, R. L. Ashley, M. W. Cloyd, and C. J. Miller. 1998. Occult systemic infection and persistent simian immunodeficiency virus (SIV)-specific CD4(+) T-cell proliferative responses in rhesus macaques that were transiently viremic after intravaginal inoculation of SIV. *J. Virol.* 72(12):10029–10035.
 184. McClure, H. M., D. C. Anderson, T. P. Gordon, A. A. Ansari, P. N. Fultz, S. A. Klumpp, P. Emau, and M. Isahakia. 1992. Natural simian immunodeficiency virus infections in nonhuman primates. In: Matano, S. R., H. Tuttle, H. Ishida, and M. Goodman (eds), *Evolutionary Biology, Reproductive Endocrinology and Virology. Topics in Primatology*, Vol. 3. Tokyo, Japan: University of Tokyo Press, pp. 425–438.
 185. McMichael, A. J. 2006. HIV vaccines. *Annu. Rev. Immunol.* 24:227–255.
 186. Means, R. E., T. Greenough, and R. C. Desrosiers. 1997. Neutralization sensitivity of cell culture-passaged simian immunodeficiency virus. *J. Virol.* 71:7895–7902.
 187. Méderlé, I., R. Le Grand, B. Vaslin, E. Badell, B. Vingert, D. Dormont, B. Gicquel, and N. Winter. 2003. Mucosal administration of three recombinant *Mycobacterium bovis* BCG-SIVmac251 strains to cynomolgus macaques induces rectal IgAs and boosts systemic cellular immune responses that are primed by intradermal vaccination. *Vaccine* 21(27–30):4153–4166.
 188. Miller, C. J., N. J. Alexander, A. Gettie, A. G. Hendrickx, and P. A. Marx. 1992. The effect of contraceptives containing nonoxynol-9 on the genital transmission of simian immunodeficiency virus in rhesus macaques. *Fertil. Steril.* 57(5):126–128.
 189. Miller, C. J., N. J. Alexander, S. Sutjipto, A. A. Lakcner, A. Gettie, A. G. Hendrickx, L. J. Lowenstein, M. Jennings, and P. A. Marx. 1989. Genital mucosal transmission of simian immunodeficiency virus: animal model for heterosexual transmission of human immunodeficiency virus. *J. Virol.* 63(10):1277–1284.
 190. Miller, C. J., N. J. Alexander, P. Vogel, J. Anderson, and P. A. Marx. 1992. Mechanism of genital transmission of SIV: a hypothesis based on transmission studies and the location of SIV in the genital tract of chronically infected female rhesus macaques. *J. Med. Primatol.* 21(2–3):64–68.
 191. Miller, C. J. and J. Hu. 1999. T cell-tropic simian immunodeficiency virus (SIV) and simian–human immunodeficiency viruses are readily transmitted by vaginal inoculation of rhesus macaques, and Langerhans' cells of the female genital tract are infected with SIV. *J. Infect. Dis.* 179(3):S413–S417.
 192. Miller, C. J., M. Marthas, J. Greenier, D. Lu, P. J. Dailey, and Y. Lu. 1998. In vivo replication capacity rather than in vitro macrophage tropism predicts efficiency of vaginal transmission of simian immunodeficiency virus or simian/human immunodeficiency virus in rhesus macaques. *J. Virol.* 72:3248–3258.
 193. Miller, C. J., M. Marthas, J. Torten, N. J. Alexander, J. P. Moore, G. F. Doncel, and A. G. Hendrickx. 1994. Intravaginal inoculation of rhesus macaques with cell-free simian immunodeficiency virus results in persistent or transient viremia. *J. Virol.* 68(10):6391–6400.
 194. Miller, C. J., M. B. McChesney, X. Lü, P. J. Dailey, C. Chutkowski, D. Lu, P. Brosio, B. Roberts, and Y. Lu. 1997. Rhesus macaques previously infected with simian/human immunodeficiency virus are protected from vaginal challenge with pathogenic SIVmac239. *J. Virol.* 71(3):1911–1921.
 195. Miller, C. J., P. Vogel, N. J. Alexander, S. Dandekar, A. G. Hendrickx, and P. A. Marx. 1994. Pathology and localization of simian immunodeficiency virus in the reproductive tract of chronically infected male rhesus macaques. *Lab. Invest.* 70(2):255–262.
 196. Miller, C. J., P. Vogel, N. J. Alexander, S. Sutjipto, A. G. Hendrickx, and P. A. Marx. 1992. Localization of SIV in the genital tract of chronically infected female rhesus macaques. *Am. J. Pathol.* 141(3):655–660.
 197. Milush, J. M., K. Stefano-Cole, K. Schmidt, A. Durudas, I. Pandrea, and D. L. Sodora. 2007. Mucosal innate immune response associated with a timely humoral immune response and slower disease progression after oral transmission of simian immunodeficiency virus to rhesus macaques. *J. Virol.* 81:6175–6186.
 198. Monceaux, V., J. Estaquier, M. Février, M. C. Cumont, Y. Rivière, A. M. Aubertin, J. C. Ameisen, and B. Hurtrel. 2003. Extensive apoptosis in lymphoid organs during primary SIV infection predicts rapid progression towards AIDS. *AIDS* 17(11):1585–1596.

199. Montefiori, D. C. 2005. Evaluating neutralizing antibodies against HIV, SIV, and SHIV in luciferase reporter gene assays. *Curr. Protoc. Immunol.* Chapter 12:Unit 12.11, pp. 1–15.
200. Mori, K., Y. Yasutomi, S. Ohgimoto, T. Nakasone, S. Takamura, T. Shioda, and Y. Nagai. 2001. Quintuple deglycosylation mutant of simian immunodeficiency virus SIVmac239 in rhesus macaques: robust primary replication, tightly contained chronic infection, and elicitation of potent immunity against the parental wild-type strain. *J. Virol.* 75:4023–4028.
201. Mori, K., Y. Yasutomi, S. Sawada, F. Villinger, K. Sugama, B. Rosenwith, J. L. Heeney, K. Überla, S. Yamazaki, A. A. Ansari, and H. Rübsamen-Waigmann. 2000. Suppression of acute viremia by short-term postexposure prophylaxis of simian/human immunodeficiency virus SHIV-RT-infected monkeys with a novel reverse transcriptase inhibitor (GW420867) allows for development of potent antiviral immune responses resulting in efficient containment of infection. *J. Virol.* 74(13):5747–5753.
202. Morrow, C. D., D. C. Porter, D. C. Ansardi, Z. Moldoveanu, and P. N. Fultz. 1994. New approaches for mucosal vaccines for AIDS: encapsidation and serial passages of poliovirus replicons that express HIV-1 proteins on infection. *AIDS Res. Hum. Retroviruses* 10(2):S61–S66.
203. Morrow, W. J., M. Wharton, D. Lau, and J. A. Levy. 1987. Small animals are not susceptible to human immunodeficiency virus infection. *J. Gen. Virol.* 68(Pt 8):2253–2257.
204. Morton, W. R., L. Kuller, R. E. Benveniste, E. A. Clark, C. C. Tsai, M. J. Gale, M. E. Thouless, J. Overbaugh, and M. G. Katze. 1989. Transmission of the simian immunodeficiency virus SIVmne in macaques and baboons. *J. Med. Primatol.* 18(3–4):237–245.
205. Murphey-Corb, M., L. N. Martin, B. Davison-Fairburn, R. C. Montelaro, M. Miller, M. West, S. Ohkawa, G. B. Baskin, J. Y. Zhang, S. D. Putney, A. C. Allison, and D. A. Eppstein. 1989. A formalin-inactivated whole SIV vaccine confers protection in macaques. *Science* 246(4935):1293–1297.
206. Murphey-Corb, M., L. N. Martin, S. R. Rangan, G. B. Baskin, B. J. Gormus, R. H. Wolf, W. A. Andes, M. West, and R. C. Montelaro. 1986. Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. *Nature* 321(6068):435–437.
207. Muthumani, K., M. Bagarazzi, D. Conway, D. S. Hwang, K. Manson, R. Ciccarelli, Z. Israel, D. C. Montefiori, K. Ugen, N. Miller, J. Kim, J. Boyer, and D. B. Weiner. 2003. A Gag-Pol/Env-Rev SIV239 DNA vaccine improves CD4 counts, and reduce viral loads after pathogenic intrarectal SIV(mac)251 challenge in rhesus Macaques. *Vaccine* 21(7–8):629–637.
208. Naidu, Y. M., H. W. Kestler 3rd, Y. Li, C. V. Butler, D. P. Silva, D. K. Schmidt, C. D. Troup, P. K. Sehgal, P. Sonigo, M. D. Daniel, and R. C. Desrosiers. 1988. Characterization of infectious molecular clones of simian immunodeficiency virus (SIVmac) and human immunodeficiency virus type 2: persistent infection of rhesus monkeys with molecularly cloned SIVmac. *J. Virol.* 62(12):4691–4696.
209. [No authors listed]. 2003. VaxGen vaccine trial fails the test but may offer insights. *AIDS Alert* 18(41):43–45.
210. North, T. W., K. K. Van Rompay, J. Higgins, T. B. Matthews, D. A. Wadford, N. C. Pedersen, and R. F. Schinazi. 2005. Suppression of virus load by highly active antiretroviral therapy in rhesus macaques infected with a recombinant simian immunodeficiency virus containing reverse transcriptase from human immunodeficiency virus type 1. *J. Virol.* 79:7349–7354.
211. Norton, R. *The Concise Encyclopedia of Economics*, 2nd edn. Indianapolis, IN: Library Fund Incorporated.
212. Notka, F., C. Stahl-Hennig, U. Dittmer, H. Wolf, and R. Wagner. 1999. Accelerated clearance of SHIV in rhesus monkeys by virus-like particle vaccines is dependent on induction of neutralizing antibodies. *Vaccine* 18(3–4):291–301.
213. Novembre, F. J., M. Saucier, D. C. Anderson, S. A. Klumpp, S. P. O’Neil, C. R. Brown 2nd, C. E. Hart, P. C. Guenthner, R. B. Swenson, and H. M. McClure. 1997. Development of AIDS in a chimpanzee infected with human immunodeficiency virus type 1. *J. Virol.* 71(5):4086–4091.
214. O’Neill, E., V. Bostik, D. C. Montefiori, E. Kraiselburg, and F. Villinger. 2003. IL-12/GM-CSF co-administration in an SIV DNA prime/protein boost protocol enhances Gag-specific T cells but not virus-specific neutralizing antibodies in rhesus macaques. *AIDS Res. Hum. Retroviruses* 19:883–890.
215. Ourmanov, I., M. Bilska, V. H. Hirsch, and D. C. Montefiori. 2000. Recombinant modified vaccinia virus Ankara expressing the surface gp120 of simian immunodeficiency virus (SIV) primes for a rapid neutralizing antibody response to SIV infection in macaques. *J. Virol.* 74(6):2960–2965.
216. Palacios, E., L. Digilio, H. M. McClure, Z. Chen, P. A. Marx, M. A. Goldsmith, and R. M. Grant. 1998. Parallel evolution of CCR5-null phenotypes in humans and in a natural host of simian immunodeficiency virus. *Curr. Biol.* 8(16):943–946.
217. Pandrea, I., G. Silvestri, R. Onanga, R. S. Veazey, P. A. Marx, V. Hirsch, and C. Apetrei. 2006. Simian immunodeficiency viruses replication dynamics in

- African non-human primate hosts: common patterns and species-specific differences [Abstract]. *J. Med. Primatol.* 35(4–5):194–201.
218. Pantaleo, G., C. Graziosi, and A. S. Fauci. 1993. New concepts in the immunopathogenesis of human immunodeficiency virus infection. *N. Engl. J. Med.* 328(5):327–335.
219. Parren, P. W., P. A. Marx, A. J. Hessell, A. Luckay, J. Harouse, C. Cheng-Mayer, J. P. Moore, and D. R. Burton. 2001. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. *J. Virol.* 75(17):8340–8347.
220. Patterson, L. J., J. Beal, T. Demberg, R. H. Florese, N. Malkevich, D. Venzon, K. Aldrich, E. Richardson, V. S. Kalyanaraman, I. Kalisz, E. M. Lee, D. C. Montefiori, F. A. Robey, and M. Robert-Guroff. 2008. Replicating adenovirus HIV/SIV recombinant priming alone or in combination with a gp140 protein boost results in significant control of viremia following a SHIV89.6P challenge in Mamu-A*01 negative rhesus macaques. *Virology* 374(2):322–337.
221. Patterson, L. J., N. Malkevitch, D. Venzon, J. Pinczewski, V. R. Gómez-Román, L. Wang, V. S. Kalyanaraman, P. D. Markham, F. A. Robey, and M. Robert-Guroff. 2004. Protection against mucosal simian immunodeficiency virus SIV_{mac251} challenge by using replicating adenovirus-SIV multigene vaccine priming and subunit boosting. *J. Virol.* 78(5):2212–2221.
222. Pauza, C. D., P. Emau, M. S. Salvato, P. Trivedi, D. MacKenzie, M. Malkovsky, H. Uno, and K. T. Schultz. 1993. Pathogenesis of SIV_{mac251} after atraumatic inoculation of the rectal mucosa in rhesus monkeys. *J. Med. Primatol.* 22(2–3):154–161.
223. Peeters, M., K. Fransen, E. Delaporte, M. Van den Haesevelde, G. M. Gershy-Damet, L. Kestens, G. van der Groen, and P. Piot. 1992. Isolation and characterization of a new chimpanzee lentivirus (simian immunodeficiency virus isolate cpz-ant) from a wild-captured chimpanzee *AIDS* 6(5):447–451.
224. Peng, B., L. R. Wang, V. R. Gómez-Román, A. Davis-Warren, D. C. Montefiori, V. S. Kalyanaraman, D. Venzon, J. Zhao, E. Kan, T. J. Rowell, K. K. Murthy, I. Srivastava, S. W. Barnett, and M. Robert-Guroff. 2005. Replicating rather than nonreplicating adenovirus-human immunodeficiency virus recombinant vaccines are better at eliciting potent cellular immunity and priming high-titer antibodies. *J. Virol.* 79(16):10200–10209.
225. Pitisuttithum, P., P. Gilbert, M. Gurwith, W. Heyward, M. Martin, F. van Griensven, D. Hu, J. W. Tappero, and K. Choopanya, for Bangkok Vaccine Evaluation Group. 2006. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J. Infect. Dis.* 194(12):1661–1671.
226. Popov, J., T. McGraw, B. Hofmann, B. Vowels, A. Shum, P. Nishanian, and J. L. Fahey. 1992. Acute lymphoid changes and ongoing immune activation in SIV infection. *J. Acquir. Immune Defic. Syndr.* 5(4):391–399.
227. Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo. 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 224(4648):497–500.
228. Prince, A. M., J. Allan, L. Andrus, B. Brotman, J. Eichber, R. Fouts, J. Goodall, P. Marx, K. K. Murthy, S. McGreal, and C. Noon. 1999. Virulent HIV strains, chimpanzees, and trial vaccines. *Science* 283(5405):1117–1118.
229. Prince, A. M., B. Horowitz, L. Baker, R. W. Shulman, H. Ralph, J. Valinsky, A. Cundell, B. Brotman, W. Boehle, and F. Rey. 1988. Failure of a human immunodeficiency virus (HIV) immune globulin to protect chimpanzees against experimental challenge with HIV. *PNAS* 85(18):6944–6948.
230. Prince, A. M., H. Reesink, D. Pascual, B. Horowitz, I. Hewlett, K. K. Murthy, K. E. Cobb, and J. W. Eichberg. 1991. Prevention of HIV infection by passive immunization with HIV immunoglobulin. *AIDS Res. Hum. Retroviruses* 7(12):971–973.
231. Putkonen, P., R. Thorstensson, J. Albert, K. Hild, E. Norrby, P. Biberfeld, and G. Biberfeld. 1990. Infection of cynomolgus monkeys with HIV-2 protects against pathogenic consequences of a subsequent simian immunodeficiency virus infection. *AIDS* 4(8):783–789.
232. Putkonen, P., R. Thorstensson, L. Ghavamzadeh, J. Albert, K. Hild, G. Biberfeld, and E. Norrby. 1991. Prevention of HIV-2 and SIVsm infection by passive immunization in cynomolgus monkeys. *Nature* 352(6334):436–438.
233. Putkonen, P., L. Walther, Y. J. Zhang, S. L. Li, C. Nilsson, J. Albert, P. Biberfeld, R. Thorstensson, and G. Biberfeld. 1995. Long-term protection against SIV-induced disease in macaques vaccinated with a live attenuated HIV-2 vaccine. *Nat. Med.* 1(9):914–918.
234. Ramsburg, E., N. F. Rose, P. A. Marx, M. Mefford, D. F. Nixon, W. J. Moretto, D. Montefiori, P. Earl, B. Moss, and J. K. Rose. 2004. Highly effective control of an AIDS virus challenge in macaques by using vesicular stomatitis virus and modified vaccinia virus

- Ankara vaccine vectors in a single-boost protocol. *J. Virol.* 78(8):3930–3940.
235. Ratner, L., A. Fisher, L. L. Jagodzinski, H. Mitsuya, R. S. Liou, R. C. Gallo, and F. Wong-Staal. 1987. Complete nucleotide sequences of functional clones of the AIDS virus. *AIDS Res. Hum. Retroviruses* 3(1):57–69.
236. Reimann, K. A., J. T. Li, R. Veazey, M. Halloran, I. W. Park, G. B. Karlsson, J. Sodroski, and N. L. Letvin. 1996. A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate env causes an AIDS-like disease after in vivo passage in rhesus monkeys. *J. Virol.* 70(10):6922–6928.
237. Robinson, T. M., M. K. Sidhu, G. N. Pavlakis, B. K. Felber, P. Silvera, M. G. Lewis, J. Eldridge, D. B. Weiner, and J. D. Boyer. 2007. Macaques co-immunized with SIVgag/pol-HIVenv and IL-12 plasmid have increased cellular responses. *J. Med. Primatol.* 36(4–5):276–284.
238. Rose, N. F., P. A. Marx, A. Luckay, D. F. Nixon, W. J. Moretto, S. M. Donahoe, D. Montefiori, A. Roberts, L. Buonocore, and J. K. Rose. 2001. An effective AIDS vaccine based on live attenuated vesicular stomatitis virus recombinants. *Cell* 106:539–549.
239. Rose, N. F., J. Publicover, A. Chattopadhyay, and J. K. Rose. 2008. Hybrid alphavirus–rhabdovirus propagating replicon particles are versatile and potent vaccine vectors. *Proc. Natl. Acad. Sci. U. S. A.* 105(15):5839–5843.
240. Rud, E. W., M. Cranage, J. Yon, J. Quirk, L. Ogilvie, N. Cook, S. Webster, M. Dennis, and B. E. Clarke. 1994. Molecular and biological characterization of simian immunodeficiency virus macaque strain 32H proviral clones containing nef size variants. *J. Gen. Virol.* 75(3):529–543.
241. Rychert, J., N. Lacour, and A. Martin Amedee. 2006. Genetic analysis of simian immunodeficiency virus expressed in milk and selectively transmitted through breastfeeding. *J. Virol.* 80(8):3721–3731.
242. Saksela, K., E. Muchmore, M. Girard, P. Fultz, and D. Baltimore. 1993. High viral load in lymph nodes and latent human immunodeficiency virus (HIV) in peripheral blood cells of HIV-1-infected chimpanzees. *J. Virol.* 67(12):7423–7427.
243. Schmitz, J. E., M. J. Kuroda, S. Santra, V. G. Sasseville, M. A. Simon, M. A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B. J. Scallan, J. Ghrayeb, M. A. Forman, D. C. Montefiori, E. P. Rieber, N. L. Letvin, and K. A. Reimann. 1999. Control of viremia in simian immunodeficiency virus infection by CD8⁺ lymphocytes. *Science* 283:857–860.
244. Schmitz, J. E., M. A. Simon, M. J. Kuroda, M. A. Lifton, M. W. Ollert, C. W. Vogel, P. Racz, K. Tenner-Racz, B. J. Scallan, M. Dalesandro, J. Ghrayeb, E. P. Rieber, V. G. Sasseville, and K. A. Reimann. 1999. A nonhuman primate model for the selective elimination of CD8⁺ lymphocytes using a mouse–human chimeric monoclonal antibody. *Am. J. Pathol.* 154:1923–1932.
245. Schwiebert, R. and P. N. Fultz. 1994. Immune activation and viral burden in acute disease induced by simian immunodeficiency virus SIVsmmPBj14: correlation between in vitro and in vivo events. *J. Virol.* 68(9):5538–5547.
246. Sharpe, S. A., A. Cope, S. Dowall, N. Berry, C. Ham, J. L. Heeney, D. Hopkins, L. Easterbrook, M. Dennis, N. Almond, and M. Cranage. 2004. Macaques infected long-term with attenuated simian immunodeficiency virus (SIVmac) remain resistant to wild-type challenge, despite declining cytotoxic T lymphocyte responses to an immunodominant epitope. *J. Gen. Virol.* 85(9):2591–2602.
247. Shen, A., M. C. Zink, J. L. Mankowski, K. Chadwick, J. B. Margolick, L. M. Carruth, M. Li, J. E. Clements, and R. F. Siliciano. 2003. Resting CD4⁺ T lymphocytes but not thymocytes provide a latent viral reservoir in a simian immunodeficiency virus—*Macaca nemestrina* model of human immunodeficiency virus type 1-infected patients on highly active antiretroviral therapy. *J. Virol.* 77:4938–4949.
248. Simon, F., S. Souquière, F. Damond, A. Kfutwah, M. Makuwa, E. Leroy, P. Rouquet, J. L. Berthier, J. Rigoulet, A. Lecu, P. T. Telfer, I. Pandrea, J. C. Plantier, F. Barré-Sinoussi, P. Roques, M. C. Müller-Trutwin, and C. Apetrei. 2001. Synthetic peptide strategy for the detection of and discrimination among highly divergent primate lentiviruses. *AIDS Res. Hum. Retroviruses* 17(10):937–952.
249. Smith, S. M., G. B. Baskin, and P. A. Marx. 2000. Estrogen protects against vaginal transmission of simian immunodeficiency virus. *J. Infect. Dis.* 182(3):708–715.
250. Smith, S. M., B. Holland, C. Russo, P. J. Dailey, P. A. Marx, and R. I. Connor. 1999. Retrospective analysis of viral load and SIV antibody responses in rhesus macaques infected with pathogenic SIV: predictive value for disease progression. *AIDS Res. Hum. Retroviruses* 15:1691–1701.
251. Smith, S. M., M. Mefford, D. Sodora, Z. Klase, M. Singh, N. Alexander, D. Hess, and P. A. Marx. 2004. Topical estrogen protects against SIV vaginal transmission without evidence of systemic effect. *AIDS* 18(12):1637–1643.

252. Soderberg, K., L. Denekamp, S. Nikiforow, K. Sautter, R. C. Desrosiers, and L. Alexander. 2002. A nucleotide substitution in the tRNA(Lys) primer binding site dramatically increases replication of recombinant simian immunodeficiency virus containing a human immunodeficiency virus type 1 reverse transcriptase. *J. Virol.* 76(11):5803–5806.
253. Solnick, D. 1981. Construction of an adenovirus SV40 recombinant producing SV40 T antigen from an adenovirus late promoter. *Cell* 24(1):135–143.
254. Song, R. J., A. L. Chenine, R. A. Rasmussen, C. R. Ruprecht, S. Mirshahidi, R. D. Grisson, W. Xu, J. B. Whitney, L. M. Goins, H. Ong, P. L. Li, E. Shai-Kobiler, T. Wang, C. M. McCann, H. Zhang, C. Wood, C. Kankasa, W. E. Secor, H. M. McClure, E. Strobert, J. G. Else, and R. M. Ruprecht. 2006. Molecularly cloned SHIV-1157ipd3N4: a highly replication-competent, mucosally transmissible R5 simian–human immunodeficiency virus encoding HIV clade C Env. *J. Virol.* 80(17):8729–8738.
255. Steger, K. K. and C. D. Pauza. 1997. Immunization of *Macaca mulatta* with aroA attenuated *Salmonella typhimurium* expressing the SIVp27 antigen. *J. Med. Primatol.* 26(1–2):44–50.
256. Steger, K. K., P. J. Valentine, F. Heffron, M. So, and C. D. Pauza. 1999. Recombinant, attenuated *Salmonella typhimurium* stimulate lymphoproliferative responses to SIV capsid antigen in rhesus macaques. *Vaccine* 17(7–8):923–932.
257. Steger, K. K., D. P. M. Waterman, and C. D. Pauza. 1999. Acute effects of pathogenic simian–human immunodeficiency virus challenge on vaccine-induced cellular and humoral immune responses to gag in rhesus macaques. *J. Virol.* 73:1853–1859.
258. Stott, E. J. 1991. Anti-cell antibody in macaques. *Nature* 353(6343):393.
259. Stott, E. J., W. L. Chan, K. H. Mills, M. Page, F. Taffs, M. Cranage, P. Greenaway, and P. Kitchin. 1990. Preliminary report: protection of cynomolgus macaques against simian immunodeficiency virus by fixed infected-cell vaccine. *Lancet* 336(8730):1538–1541.
260. Stowell, R. E., E. K. Smith, C. España, and V. G. Nelson. 1971. Outbreak of malignant lymphoma in rhesus monkeys. *Lab. Invest.* 25(5):476–479.
261. Sutjipto, S., N. C. Pedersen, C. J. Miller, M. B. Gardner, C. V. Hanson, A. Gettie, M. Jennings, J. Higgins, and P. A. Marx. 1990. Inactivated simian immunodeficiency virus vaccine failed to protect rhesus macaques from intravenous or genital mucosal infection but delayed disease in intravenously exposed animals. *J. Virol.* 64(5):2290–2297.
262. Tan, R. C., J. M. Harouse, A. Gettie, and C. Cheng-Mayer. 1999. In vivo adaptation of SHIV(SF162): chimeric virus expressing a NSI, CCR5-specific envelope protein. *J. Med. Primatol.* 28(4–5):164–168.
263. Tang, Y. and R. Swanstrom. 2008. Development and characterization of a new single cycle vaccine vector in the simian immunodeficiency virus model system. *Virology* 372(1):72–84.
264. Trichel, A. M., P. A. Rajakumar, and M. Murphey-Corb. 2002. Species-specific variation in SIV disease progression between Chinese and Indian subspecies of rhesus macaque [Abstract]. *J. Med. Primatol.* 31(4–5):171–178.
265. Trunova, N., L. Tsai, S. Tung, E. Schneider, J. Harouse, A. Gettie, V. Simon, J. Blanchard, and C. Cheng-Mayer. 2006. Progestin-based contraceptive suppresses cellular immune responses in SHIV-infected rhesus macaques. *Virology* 352(1):169–177.
266. Tsai, C. C., K. E. Follis, A. Sabo, R. F. Grant, C. Bartz, R. E. Nolte, R. E. Benveniste, and N. Bischofberger. 1994. Preexposure prophylaxis with 9-(2-phosphonylmethoxyethyl)adenine against simian immunodeficiency virus infection in macaques. *J. Infect. Dis.* 169(2):260–266.
267. Turville, S., J. Wilkinson, P. Cameron, J. Dable and A. L. Cunningham. 2003. The role of dendritic cell C-type lectin receptors in HIV pathogenesis. *J. Leukoc. Biol.* 74:710–718.
268. Van de Perre, P., A. Simonon, P. Msellati, D. G. Hitimana, D. Vaira, A. Bazubagira, C. Van Goethem, A. M. Stevens, E. Karita, D. Sondag-Thull, F. Dabis, and P. LePage. 1991. Postnatal transmission of human immunodeficiency virus type 1 from mother to infant. A prospective cohort study in Kigali, Rwanda. *N. Engl. J. Med.* 325:593–598.
269. Van Rompay, K. K. 2005. Antiretroviral drug studies in nonhuman primates: a valid animal model for innovative drug efficacy and pathogenesis experiments. *AIDS Rev.* 7(2):67–83.
270. Van Rompay, K. K., J. A. Johnson, E. J. Blackwood, R. P. Singh, J. Lipscomb, T. B. Matthews, M. L. Marthas, N. C. Pedersen, N. Bischofberger, W. Heneine, and T. W. North. 2007. Sequential emergence and clinical implications of viral mutants with K70E and K65R mutation in reverse transcriptase during prolonged tenofovir monotherapy in rhesus macaques with chronic RT-SHIV infection. *Retrovirology* 6(4):25.
271. Van Rompay, K. K., M. L. Marthas, R. A. Ramos, C. P. Mandell, E. K. McGowan, S. M. Joye, and N. C. Pedersen. 1992. Simian immunodeficiency virus (SIV) infection of infant rhesus macaques as a model to test antiretroviral drug prophylaxis and therapy: oral 3'-azido-3'-deoxythymidine prevents SIV infection. *Antimicrob. Agents Chemother.* 36(11):2381–2386.

272. Veazey, R. S., M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner. 1998. Gastrointestinal tract as a major site of CD4⁺ T cell depletion and viral replication in SIV infection. *Science* 280(5362):427–431.
273. Veazey, R. S., P. J. Klasse, S. M. Schader, Q. Hu, T. J. Ketas, M. Lu, P. A. Marx, J. Dufour, R. J. Colonna, R. J. Shattock, M. S. Springer, and J. P. Moore. 2005. Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion [Abstract]. *Nature* 438(7064):99–102.
274. Veazey, R. S. and A. A. Lackner. 2005. HIV swiftly guts the immune system. *Nat. Med.* 11(5):469–470.
275. Veazey, R. S., R. J. Shattock, M. Pope, J. C. Kirijan, J. Jones, Q. Hu, T. Ketas, P. A. Marx, P. J. Klasse, D. R. Burton, and J. P. Moore. 2003. Abstract prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. *Nat. Med.* 9(3):343–346.
276. Wainberg, M. A. 2004. HIV-1 subtype distribution and the problem of drug resistance. *AIDS* 18(Suppl 3):S63–S68.
277. Walther-Jallow, L., C. Nilsson, J. Söderlund, P. ten Haaf, B. Mäkitalo, P. Biberfeld, P. Böttiger, J. Heeney, G. Biberfeld, and R. Thorsten. 2001. Cross-protection against mucosal simian immunodeficiency virus (SIVsm) challenge in human immunodeficiency virus type 2-vaccinated cynomolgus monkeys. *J. Gen. Virol.* 82:1601–1612.
278. Watkins, D. I., D. R. Burton, E. G. Kallas, J. P. Moore, and W. C. Koff. 2008. Nonhuman primate models and the failure of the Merck HIV-1 vaccine in humans. *Nat. Med.* 14(6):617–621.
279. Weiler, A., G. E. May, Y. Qi, N. Wilson, and D. I. Watkins. 2006. Polymorphisms in eight host genes associated with control of HIV replication do not mediate elite control of viral replication in SIV-infected Indian rhesus macaques. *Immunogenetics* 58:1003–1009.
280. Westmoreland, S. V., E. Halpern, and A. A. Lackner. 1998. Simian immunodeficiency virus encephalitis in rhesus macaques is associated with rapid disease progression. *J. Neurovirol.* 4(3):260–268.
281. Wolf, R. H., B. J. Gormus, L. N. Martin, G. B. Baskin, G. P. Walsh, W. M. Meyers, and C. H. Binford. 1985. Experimental leprosy in three species of monkeys. *Science* 227(4686):529–531.
282. Wyand, M. S., K. H. Manson, M. Garcia-Moll, D. Montefiori, and R. C. Desrosiers. 1996. Vaccine protection by a triple deletion mutant of simian immunodeficiency virus. *J. Virol.* 70(6):3724–3733.
283. Wyand, M. S., D. J. Ringler, Y. M. Naidu, M. Mattmuller, L. V. Chalifoux, P. K. Sehgal, M. D. Daniel, R. C. Desrosiers, and N. W. King. 1989. Cellular localization of simian immunodeficiency virus in lymphoid tissues. II. In situ hybridization. *Am. J. Pathol.* 134(2):385–393.
284. Xu, R., I. K. Srivastava, C. E. Greer, I. Zarkikh, Z. Kraft, L. Kuller, J. M. Polo, S. W. Barnett, and L. Stamatatos. 2006. Characterization of immune responses elicited in macaques immunized sequentially with chimeric VEE/SIN alphavirus replicon particles expressing SIVGag and/or HIVEnv and with recombinant HIVgp140Env protein. *AIDS Res. Hum. Retroviruses* 22(10):1022–1030.
285. Yasutomi, Y., S. Koenig, S. S. Haun, C. K. Stover, R. K. Jackson, P. Conard, A. J. Conley, E. A. Emini, T. R. Fuerst, and N. L. Letvin. 1993. Immunization with recombinant BCG-SIV elicits SIV-specific cytotoxic T lymphocytes in rhesus monkeys. *J. Immunol.* 150(7):3101–3107.
286. Yasutomi, Y., S. Koenig, R. M. Woods, J. Madsen, N. M. Wassef, C. R. Alving, H. J. Klein, T. E. Nolan, L. J. Boots, J. A. Kessler, E. A. Emini, A. J. Conley, and N. L. Letvin. 1995. A vaccine-elicited, single viral epitope-specific cytotoxic T lymphocyte response does not protect against intravenous, cell-free simian immunodeficiency virus challenge. *J. Virol.* 69(4):2279–2284.
287. Zhang, Z., T. Schuler, M. Zupancic, S. Wietgrefe, K. A. Staskus, K. A. Reimann, T. A. Reinhart, M. Rogan, W. Cavert, C. J. Miller, R. S. Veazey, D. Notermans, S. Little, S. A. Danner, D. D. Richman, D. Havlir, J. Wong, H. L. Jordan, T. W. Schacker, P. Racz, K. Tenner-Racz, N. L. Letvin, S. Wolinsky, and A. T. Haase. 1999. Sexual transmission and propagation of SIV and HIV in resting and activated CD4⁺ T cells. *Science* 286:1353–1357.
288. Zhou, Q., R. Hidajat, B. Peng, D. Venzon, M. K. Aldrich, E. Richardson, E. M. Lee, V. S. Kalyanaraman, G. Grimes, V. R. Gómez-Román, L. E. Summers, N. Malkevich, and M. Robert-Guroff. 2007. Comparative evaluation of oral and intranasal priming with replication-competent adenovirus 5 host range mutant (Ad5hr)-simian immunodeficiency virus (SIV) recombinant vaccines on immunogenicity and protective efficacy against SIV(mac251). *Vaccine* 25(47):8021–8035.
289. Ziegler, J. B., D. A. Cooper, R. O. Johnson, and J. Gold. 1985. Postnatal transmission of AIDS-associated retrovirus from mother to infant. *Lancet* 1(8434):896–898.
290. Zou, W., A. A. Lackner, M. Simon, I. Durand-Gasselin, P. Galanaud, R. C. Desrosiers, and

- D. Emilie. 1997. Early cytokine and chemokine gene expression in lymph nodes of macaques infected with simian immunodeficiency virus is predictive of disease outcome and vaccine efficacy. *J. Virol.* 71(2):1227–1236.
291. zur Megede, J., B. Sanders-Beer, P. Silvera, D. Golightly, A. Bowlsbey, D. Hebblewaite, D. Sites, L. Nieves-Duran, R. Srivastava, G. R. Otten, D. Rabusay, L. Zhang, J. B. Ulmer, S. W. Barnett, and J. J. Donnelly. 2008. A therapeutic SIV DNA vaccine elicits T-cell immune responses, but no sustained control of viremia in SIVmac239-infected rhesus macaques. *AIDS Res. Hum. Retroviruses* 24(8):1103–1116.

5

Origins of Epidemic Forms HIV-1 and HIV-2

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5.1. INTRODUCTION

Acquired immunodeficiency disease (AIDS) is the most significant new human viral disease to emerge since the 1918–1919 influenza pandemic. Deaths from this influenza pandemic are estimated at 50 million and some researchers claim higher numbers.¹⁷ Since 1981, 25 million deaths have occurred worldwide from AIDS. The World Health Organization estimates that there were over 30 million persons living with HIV in 2007.³¹ It seems likely, therefore, that AIDS will surpass the number of influenza deaths.

Evolution of a completely new virus from uninfected host cells must be rare and has not been shown. Therefore, new viral diseases must emerge from existing

reservoirs in nature. The study of the reservoirs of the ancestral viruses of HIV has yielded a partial answer to the question of the origins of AIDS after 25 years of research. The major questions are four: (a) what are the sources of the two epidemic HIV types 1 and 2, (b) when did these two viruses emerge, (c) what caused their emergence, and (d) is AIDS a truly new disease or just a newly discovered disease? These questions are especially important to simian virology because it was two nonhuman primate retroviruses that are the sources of the AIDS viruses.

5.2. TWO DIFFERENT SIMIAN SOURCES OF THE AIDS VIRUSES

All origin theories for AIDS must address these puzzling facts (a) AIDS is caused by two distinct viral species, HIV-1 and HIV-2, (b) these two different HIVs arose independently of each other, (c) emergence was at about the same time in the twentieth century, and (d) occurred in different parts of Africa from two different simian species.

Simian immunodeficiency virus (SIV) was discovered in macaque (mac) monkeys of Asian origin that had developed AIDS at the New England Primate Research Center.⁴ However, shortly thereafter, SIV was found at the Tulane National Primate Research Center in clinically healthy sooty mangabeys (sm) (*Cercocebus atys*),^{7,26} a monkey species of West African origin. SIVsm was then linked to HIV-2 when this SIV was sequenced and was shown to be closely related to HIV-2.¹⁴ The link between SIVsm and HIV-2 was firmly established in West Africa when SIVsm was

found in household pet sooty mangabees in Liberia and Sierra Leone.^{2,25} The occurrence of HIV-2 overlaps with the natural range of sooty mangabees in West Africa. The HIV-1 link was established in 1989 when an SIV was isolated from a household pet chimpanzee (cpz) in Gabon.^{27,28} This virus was shown to be closely related to HIV-1.¹⁵ Therefore, in only 6 years following the discovery of SIVmac, two different SIVs were separately linked to HIV-1 and HIV-2. Many details have since been established; however, these initial discoveries have been borne out. The fundamental issue raised was the finding that there were two different sources for HIV, a west African monkey^{14,25} and a central African chimpanzee.^{8,19,29} The ranges of these two simian species do not overlap. HIV emergence from two different geographical areas and from two different species in the twentieth century remains to be explained.

5.2.1. Eleven Separate SIV Transmissions to Humans

HIV-1 is further divided into three groups, M, N, and O, with M the “main” group, O the “outlier,” and, with subtle irony added, group N filling the alphabetical gap between the two.^{1,5,12,30} HIV-2 consists of eight subtypes, A–H, which are completely analogous to HIV-1 groups.^{2,3,9} The three HIV-1 groups and eight HIV-2 groups are sufficiently different from one another that

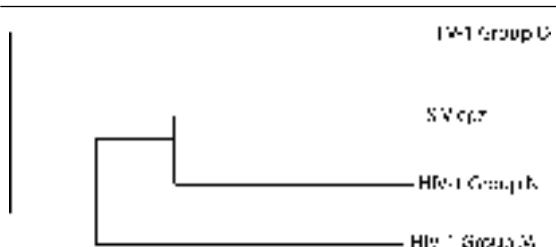


Figure 5.1. Phylogenetic tree of HIV-1. HIV exists as three groups, M, O, and N. These HIVs are highly disparate in the number of persons infected.

they represent 11 separate crossovers of SIV, suggesting that eight sooty mangabees and three chimpanzees were responsible for all known HIV infections among humans thus far. However, these 11 HIVs have remarkably different pathogenicity. Figure 5.1 shows the phylogenetic trees of HIV-1 and Figure 5.2 shows HIV-2.

HIV-1 group M is the pandemic strain of HIV infecting more than 30 million persons in 2007. However, HIV-1 group O infects approximately 100,000 persons and group N is known to infect only 7 persons in Cameroon. These statistics illustrate the disparity in HIV-1 pathogenesis. The question to address is why is there so much difference in transmission efficiency between these three HIVs. HIV-1 group M is highly

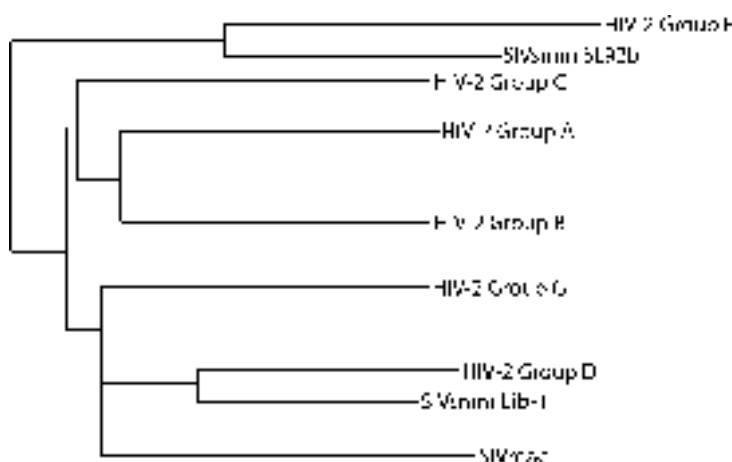


Figure 5.2. Phylogenetic tree of HIV-2. HIV-2 exists as at least eight groups³ and they are also highly disparate in the number of persons infected.

adapted to transmission between humans while HIV-1 group N must be a poorly adapted virus.

The same disparity exists for HIV-2. Groups A through H have been found. However, only groups A and B are epidemic. The remaining groups C through H are known only as single person infections. Therefore, all HIVs are not the same. One, group M, is highly adapted for transmission, but the majority of HIVs are poorly adapted to humans. The major question is what accounts for this difference. How did SIV become better adapted to humans?

5.3. EMERGENCE OF AIDS IN POSTCOLONIAL AFRICA—IS AIDS A NEW DISEASE IN AFRICA?

AIDS is new to Europe and the Americas, being discovered in Los Angeles in 1981.¹¹ The symptoms and signs of AIDS are dramatic and would not have been missed if AIDS was not new to the Americas and Europe. The situation on the African continent was different. Medical infrastructure remains poor in postcolonial Africa, such that AIDS could have gone unrecognized as a new disease. Wasting or “slim man” disease could be related to other infectious diseases or to malnutrition. The argument that AIDS is old in Africa relies on the movement of Africans to urban centers from the countryside. These movements may have brought a rare disease from rural areas to the cities, where the epidemic emerged in the mid-twentieth century. However, there are strong historical arguments against AIDS being an old or ancient disease in Africa. The strongest argument centers on slave trade that existed from about 1,600 to 1,830. During that time there was a great upheaval along the west coast of Africa when 20 million Africans were forcibly emigrated to the Americas. If AIDS were an old disease in Africa, it would have spread to Americas during slave trade.²² Virus evolution models done on computers calculate that HIV emerged in the twentieth century.²¹ By comparison human T-cell lymphoma/leukemia virus (HTLV-1), a less common human retrovirus, is found outside the African continent primarily along former slave trade routes in the Caribbean, the southern United States, and South America,³³ proving that a human retrovirus that was old in Africa would have spread during slave trade. The question of the timing of HIV emergence is important because it bears on the conditions that changed a stable viral ecology causing SIV to adapt to human transmission in postcolonial Africa and be able to cause disease.



Figure 5.3. A household pet sooty mangabey, *Cecocebus atys* in West Africa found to be naturally infected with SIVsm. The virus, SIVsmSL92b, is described in reference 2 and is closely related to HIV-2 group F. (Photo by Paul Telfer.) See color version page 11.

5.4. RISK OF SIV HUMAN INFECTION

5.4.1. SIV Acquisition

The two steps in assessing the risk of human SIV infection are acquisition and transmission. It must be established that humans are routinely exposed to SIV. The risk to humans for acquiring SIV occurs from exposure to SIV-infected household pets and exposure to simian bush meat. Figures 5.3 and 5.4 show an SIV-positive pet sooty mangabey and an arm for sale of a mandrill of unknown SIV status. Surveys of humans in areas where SIV is endemic show that SIV-like human infections can be found in both the west and central Africa.^{2,9,18} Acquisition of SIV by humans is a well-established phenomenon.

5.4.2. Assessment of the Risk of SIV Human-to-Human Transmission—Is AIDS a Zoonosis?

Human SIV infections are defined as an infection in which the infecting virus is more closely related to SIV than it is to known HIV strains. This type of infection is an SIV cross-species transmission to humans from an infected monkey or ape. Cross-species SIV transmission is demonstrated for HIV-2 group E/PA (Figure 5.2). This



Figure 5.4. Bush meat for sale at a road stand. Arm of a mandrill next to vegetables. Mandrills are naturally infected with one of two simian immunodeficiency viruses (SIVs), SIVmnd-1 and SIVmnd-2. (Photo by Preston A. Marx.) See color version page 11.

virus is most closely related to SIVsmSL92b, an isolate from a household pet sooty mangabey in Sierra Leone. SL92 means Sierra Leone 1992, the place and date of sampling this mangabey (Figure 5.3 shows this particular sooty mangabey). The final proof is that the HIV-2 E/PA-infected person was from Sierra Leone. Therefore, HIV-2 E/PA was derived from SIVsm circulating in Sierra Leone. Equally interesting, HIV-2 E/PA was nonpathogenic. This individual lived for 20 years without evidence of AIDS.² Repeated attempts to isolate the

virus from peripheral blood were negative showing that this individual's immune system had controlled SIV. Finally, HIV-2 E/PA is only known from this single person infection.² It was not transmitted from person to person. This is true for most HIV-2 groups. Only groups A and B have spread from human to human. Therefore, SIV is not directly pathogenic in humans.

Although the simian origins of HIV and other human retroviruses such as human T-cell lymphoma/leukemia virus and foamy viruses are not in dispute, the hypothesis that simian retroviral infections are zoonoses is not directly supported by research findings and has been seriously questioned.²⁴ Nevertheless, the issue of AIDS as a zoonosis has played prominently in both the lay and scientific press.¹³ Use of bush meat has been described as a public health threat. The term *zoonosis* is often misused and is even misunderstood. The definition of a zoonosis is very specific and is a disease transmitted directly from animals to humans, like Herpes B encephalitis transmission to persons occupationally exposed to macaques¹⁶ (see Chapter 12). The evidence is that SIV does not cause AIDS directly and therefore, it cannot be a zoonosis. The central meaning of zoonosis is that the disease, and not just the infection, is acquired from an animal. Any infection and disease acquired from another human being is therefore not zoonosis. AIDS does not fulfill the criteria for a zoonosis. Bush meat has been used for centuries in Africa, but AIDS only emerged in the mid-twentieth century. The missing knowledge is the process that adapted SIVsm and SIVcpz to humans in the twentieth century and not before. It is worthy of mention that although simian bush meat carries no known risk for AIDS, it is a source of other potentially dangerous viruses such as the Ebola virus.¹⁰

5.5. ACCIDENTAL TRANSMISSION OF SIVB670 TO A LABORATORY WORKER

5.5.1. Lack of Pathogenesis of an SIV Human (SIVhu) Infection

Another key to understanding SIV pathogenesis in humans is the study of an accidental infection of a laboratory worker with SIVb670.²⁰ SIVb670 is highly pathogenic for rhesus macaques of Indian origin.²⁶ Most inoculated macaques develop AIDS in less than 2 years, some developing the disease in as little as 3 months. This infection was apparently transmitted through the skin of a researcher who had dermatitis on the hands. The infection was followed closely for 3½ years. SIVb670

infection was effectively controlled and AIDS did not develop in this individual. The SIV was reisolated from the researcher and is referred to as SIVhu for SIV from a human. SIVb670 accumulated deletions and was not pathogenic upon reinoculation into macaques.³²

5.6. ORIGIN THEORIES—CIA CONSPIRACY, ZOONOSIS, OR SERIAL ADAPTATION

The question of how animal viruses adapt to humans is critically important for SIV and other viruses having animal reservoirs. In all areas of the world, AIDS is being confronted along with avian flu, Ebola, and SARS. Yet the question of what launches new epidemics and pandemics is a black box. While the origins of AIDS and avian influenza are known, and good evidence is available for Ebola origins,¹⁰ nothing about the factors that launch animal viruses into epidemics, pandemics, or dead ends is understood. The ecological events that are rooted in the adaptive changes that resulted in SIV becoming pandemic HIV remain unknown.

Several origin theories have emerged. All theories must include a unifying hypothesis to explain the unlikely coincidence of two different HIVs emerging in the mid-twentieth century and not before. Anthropologists document 50,000 years of human inhabitation of the west and central Africa, yet AIDS emerged in the twentieth century. Two theories are currently being considered. The first invokes the unprecedented deforestation in the twentieth century. It further states that certain strains of SIVcpz and SIVsm exist that are much more pathogenic than the strains identified thus far. Because of deforestation and increased hunting, the odds increased significantly that humans would finally be exposed to these pathogenic SIV strains.⁶ This is the “modern cut hunter hypothesis.” The second theory is that HIV/AIDS arose from an increase in the use of unsafe injections and transfusions that occurred in postcolonial Africa. This last factor might have significantly promoted SIV adaptation through serial passage.^{6,22–24}

5.7. SUMMARY

The most perplexing issue concerning the origin of AIDS is that HIV has two separate origins. HIV-1 is derived from an SIV found in chimpanzees in Central Africa and HIV-2 is derived from sooty mangabeys in West Africa. All theories, therefore, must explain origins in two species that occur about 1,000 miles apart. The

mystery is further compounded because SIVs are ancient in Africa and AIDS is a truly new disease worldwide, including Africa. AIDS developed in the mid-twentieth century, yet contact between humans and SIV is ancient. The question as to why HIV emerged in twentieth century and not earlier remains unanswered. A few SIV infections have been found in humans living in areas where SIV is endemic. However, these infections are dead end infections and transmission between humans has not been observed. Theories to explain how an apparently benign simian virus transformed itself into a virus capable of a pandemic are¹: deforestation has led to more intense and frequent exposure to SIV in modern times, and SIV was inadvertently adapted to human beings by serial transmission from unsterilized needles during vaccine campaigns and extensive transfusion practices in postcolonial Africa.²

REFERENCES

1. Barré-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruet, C. Dauguet, C. Axler-Blin, F. Vézinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 220(4599):868–871.
2. Chen, Z., P. Telfier, A. Gettie, P. Reed, L. Zhang, D. D. Ho, and P. A. Marx. 1996. Genetic characterization of new West African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop. *J. Virol.* 70(6):3617–3627.
3. Damond, F., M. Worobey, P. Campa, I. Farfara, G. Colin, S. Matheron, F. Brun-Vézinet, D. L. Robertson, and F. Simon. 2004. Identification of a highly divergent HIV type 2 and proposal for a change in HIV type 2 classification. *AIDS Res. Hum. Retroviruses* 20(6):666–672.
4. Daniel, M. D., N. L. Letvin, N. W. King, M. Kanagai, P. K. Sehgal, R. D. Hunt, P. J. Kanki, M. Essex, and R. C. Desrosiers. 1985. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* 228(4704):1201–1204.
5. De Leys, R., B. Vanderborght, M. Vanden Haesevelde, L. Heyndrickx, A. van Geel, C. Wauters, R. Bernaerts, E. Saman, P. Nijs, B. Willems, H. Taelman, G. Van Der Groen, P. Piot, T. Tersmette, J. D. Huisman, and H. Van Heuverswyn. 1990. Isolation and partial characterization of an unusual human immunodeficiency

- retrovirus from two persons of west-central African origin. *J. Virol.* 64(3):1207–1216.
6. Drucker, E., P. G. Alcabes, and P. A. Marx. 2001. The injection century: consequences of massive unsterile injecting for the emergence of human pathogens. *Lancet* 358:1989.
 7. Fultz, P. N., H. M. McClure, D. C. Anderson, R. B. Swenson, R. Anand, and A. Srinivasan. 1986. Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (*Cercocebus atys*). *Proc. Natl. Acad. Sci. U. S. A.* 83(14):5286–5290.
 8. Gao, F., E. Bailes, D. L. Robertson, Y. Chen, C. M. Rodenburg, S. F. Michael, L. B. Cummins, L. O. Arthur, M. Peeters, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 1999. Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. *Nature* 397(6718):436–441.
 9. Gao, F., L. Yue, A. T. White, P. G. Pappas, J. Barchue, A. P. Hanson, B. M. Greene, P. M. Sharp, G. M. Shaw, and B. H. Hahn. 1992. Human infection by genetically diverse SIVSM-related HIV-2 in west Africa. *Nature* 358(6386):495–499.
 10. Gonzalez, J. P., X. Pourrut, and E. Leroy. 2007. Ebolavirus and other filoviruses. *Curr. Top. Microbiol. Immunol.* 315:363–387.
 11. Gottlieb, M. S., R. Schroff, H. M. Schanker, J. D. Weisman, P. T. Fan, R. A. Wolf, and A. Saxon. 1981. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N. Engl. J. Med.* 305(24):1425–1431.
 12. Gurtler, L. G., P. H. Hauser, J. Eberle, A. Von Brunn, S. Knapp, L. Zekeng, J. M. Tsague, and L. Kaptue. 1994. A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon. *J. Virol.* 68(3):1581–1585.
 13. Hahn, B. H., G. M. Shaw, K. M. De Cock, and P. M. Sharp. 2000. AIDS as a zoonosis: scientific and public health implications. *Science* 287(5453):607–614.
 14. Hirsch, V. M., R. A. Olmsted, M. Murphey-Corb, R. H. Purcell, and P. R. Johnson. 1989. An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature* 339(6223):389–392.
 15. Huet, T., R. Cheynier, A. Meyerhans, G. Roelants, and S. Wain-Hobson. 1990. Genetic organization of a chimpanzee lentivirus related to HIV-1. *Nature* 345:356–359. Comment in: *Nature* 24(345):288–289.
 16. Jainkittivong, A. and R. P. Langlais. 1998. Herpes B virus infection. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 85(4):399–403.
 17. Johnson, N. P. and J. Mueller. 2002. Updating the accounts: global mortality of the 1918–1920 “Spanish” influenza pandemic. *Bull. Hist. Med.* 76(1):105–115.
 18. Kalish, M. L., N. D. Wolfe, C. B. Ndongmo, J. McNicholl, K. E. Robbins, M. Aidoo, P. N. Fonjungo, G. Alemnji, C. Zeh, C. F. Djoko, E. Mpoudi-Ngole, D. S. Burke, and T. M. Folks. 2005. Central African hunters exposed to simian immunodeficiency virus. *Emerg. Infect. Dis.* 11(12):1928–1930.
 19. Keele, B. F., F. Van Heuverswyn, Y. Li, E. Bailes, J. Takehisa, M. L. Santiago, F. Bibollet-Ruche, Y. Chen, L. V. Wain, F. Liegeois, S. Loul, E. M. Ngole, Y. Bienvenue, E. Delaporte, J. F. Brookfield, P. M. Sharp, G. M. Shaw, M. Peeters, and B. H. Hahn. 2006. Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. *Science* 313(5786):523–526.
 20. Khabbaz, R. F., W. Heneine, J. R. George, B. Parekh, T. Rowe, T. Woods, W. M. Switzer, H. M. McClure, M. Murphey-Corb, and T. M. Folks. 1994. Brief report: infection of a laboratory worker with simian immunodeficiency virus. *N. Engl. J. Med.* 330(3):172–177.
 21. Korber, B., M. Muldoon, J. Theiler, F. Gao, R. Gupta, A. Lapedes, B. H. Hahn, S. Wolinsky, and T. Bhattacharya. 2000. Timing the ancestor of the HIV-1 pandemic strains. *Science* 288(5472):1789–1796.
 22. Marx, P. A. 2005. Unsolved questions over the origin of HIV and AIDS. *ASM News* 17:15–20.
 23. Marx, P. A., P.G. Alcabes, and E. Drucker. 2001. Serial human passage of SIV by unsterile injecting and the emergence of epidemic HIV in Africa. *Phil. Trans. R. Soc. Ser. B* 356:911–920.
 24. Marx, P. A., C. Apetrei, and E. Drucker. 2004. AIDS as a zoonosis? Confusion over the origin of the virus and the origin of the epidemics. *J. Med. Primatol.* 33(5–6):220–226.
 25. Marx, P. A., Y. Li, N. W. Lerche, S. Sutjipto, A. Gettie, J. A. Yee, B. H. Brotman, A. M. Prince, A. Hanson, R. G. Webster, and R. C. Desrosiers. 1991. Isolation of a simian immunodeficiency virus related to human immunodeficiency virus type 2 from a west African pet sooty mangabey. *J. Virol.* 65(8):4480–4485.
 26. Murphey-Corb, M., L. N. Martin, S. R. Rangan, G. B. Baskin, B. J. Gormus, R. H. Wolf, W. A. Andes, M. West, and R. C. Montelaro. 1986. Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. *Nature* 321:435–437.
 27. Peeters, M. 2004. Cross-species transmissions of simian retroviruses in Africa and risk for human health. *Lancet* 363(9413):911–912.
 28. Peeters, M., C. Honoré, T. Huet, L. Bedjabaga, S. Os-sari, P. Bussi, R. W. Cooper, and E. Delaporte. 1989. Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon. *AIDS* 3(10):625–630.

29. Prince, A. M., B. Brotman, D. H. Lee, L. Andrus, J. Valinsky, and P. Marx. 2002. Lack of evidence for HIV type 1-related SIVcpz infection in captive and wild chimpanzees (*Pan troglodytes verus*) in West Africa. *AIDS Res. Hum. Retroviruses* 18(9):657–660.
30. Simon, F., P. Mauclère, P. Roques, I. Loussert-Ajaka, M. C. Müller-Trutwin, S. Saragosti, M. C. Georges-Courbot, F. Barré-Sinoussi, and F. Brun-Vézinet. 1998. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. *Nat. Med.* 4(9):1032–1037.
31. www.unaids.org/en/KnowledgeCentre/HIVData/Epi-Update/EpiUpdArchive/2007/default.asp. 2007. AIDS Epidemic Update no. 7:3–15.
32. Villinger, F., W. M. Switzer, B. S. Parekh, R. A. Otten, D. Adams, V. Shanmugam, P. Bostik, A. E. Mayne, N. F. Chikkala, H. M. McClure, F. Novembre, Q. Yao, W. Heneine, T. M. Folks, and A. A. Ansari. 2000. Induction of long-term protective effects against heterologous challenge in SIVhu-infected macaques. *Virology* 278(1):194–206.
33. Williams, A. E., C. T. Fang, D. J. Slamon, B. J. Poiesz, S. G. Sandler, W. F. Darr 2nd, G. Shulman, E. I. McGowan, D. K. Douglas, R. J. Bowman, F. Peetoom, H. K. Steven, B. Lenes, and R. Y. Dodd. 1988. Seroprevalence and epidemiological correlates of HTLV-I infection in U.S. blood donors. *Science* 240(4852):643–646.

6

Betaretroviruses

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6.1. INTRODUCTION

The genus *Betaretrovirus* includes the closely related group of exogenous simian retroviruses that cause immunodeficiency disease in macaques as well as endogenous simian retroviruses that have not been associated with any known disease. The genus also contains the mouse mammary tumor virus, which is the best-studied virus in the group.¹²

The older nomenclature classifies these viruses as type D retroviruses on the basis of morphology (Figure 6.1). The morphological classification that divides retroviruses into morphological types A, B, C, and D

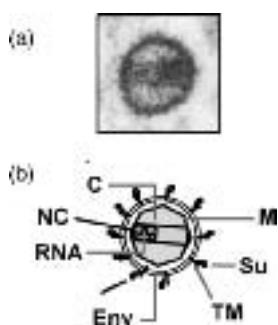


Figure 6.1. Betaretrovirus morphology. (a) Electron micrograph of simian retrovirus serotype 1 (SRV-1) isolated from a rhesus macaque at the California National Primate Research Center. (b) Diagram showing structures of the SRV virion. C, capsid is the internal protein shell structure in betaretroviruses that encloses the RNA genome; NC, nucleocapsid encloses the capsid plus the RNA genome; M, matrix encloses the nucleocapsid; env, envelope is the outer lipid-containing bilayer that surrounds the virion and anchors the glycoprotein spikes; Su, surface external spikes that bind susceptible cells; TM, transmembrane protein that anchors the spikes.

is still commonly used. For a general discussion of retroviral morphology, see Introduction to Retroviruses; Figure RI.1). The first type D retrovirus was isolated in 1970 from a rhesus monkey with a mammary carcinoma.¹⁸ This virus is the prototype of the genus and bears the somewhat unusual name, Mason-Pfizer monkey virus (MPMV). The virus was named for the Mason Institute in Worcester, Massachusetts, where the monkey was housed, and for the Pfizer pharmaceutical company which had supported the research using this monkey. The finding of MPMV in cancerous breast tissue led to much interest and a hypothesis that MPMV or a related virus was involved in human breast cancer. Although an association between MPMV and human breast cancer tissue was reported,²⁰ no role for a betaretrovirus was ever confirmed or established.

In the 1980s, the type D retrovirus field experienced a resurgence due to the association of these viruses with immunosuppressive disease. The association between

retroviruses and immunosuppression was discovered by Dr. Herman Friedman in 1968 using a mouse model.¹³ Soon after that report, the immunosuppressive activity of feline retroviruses was reported.⁷⁰ Fine and colleagues were the first to demonstrate retrovirus-associated immune suppression in a nonhuman primate (NHP) model. In 1975, they reported immune impairment in MPMV-infected newborn rhesus monkeys.²³ Although this study raised some interest in type D retrovirus animal models, the full impact was not realized until the discovery of acquired immune deficiency syndrome (AIDS) in humans in 1981.²⁷ The gradual recognition of the potential of type D retroviruses to cause a disease similar to AIDS ushered in the modern era of the study of retrovirus-induced immune disorders.^{60,87}

6.2. ICTV AND “SUPPLEMENTARY” CLASSIFICATIONS AND NOMENCLATURE

Three type-D retroviruses infecting NHPs are recognized as species by the International Committee on Taxonomy of Viruses (ICTV). The first MPMV of which simian retrovirus 1 and 2 (SRV-1, SRV-2) are listed as isolates within the MPMV species. The name SRV has undergone revision. SRV was first defined⁶⁰ as simian acquired immunodeficiency (SAIDS) retrovirus and abbreviated SRV. The S in SRV was an abbreviation for the abbreviation SAIDS, resulting in a nested abbreviation. SRV was simplified in later publications to simian retrovirus. In some publications SDRV has been used for simian D retrovirus.

Seven distinct types of SRV isolates known as SRV-1 through SRV-7 can be distinguished by neutralization tests, competitive radioimmunoassay,⁶⁰ and various molecular assays including sequence analysis.^{32,59,66,73,81,86} The types or more accurately serotypes 1 through 5 were initially defined based on their cross-neutralization pattern. These groupings are fully supported by the results of phylogenetic analysis and immunochemical comparison of virus proteins. MPMV is assigned to serotype 3.^{11,54,91} Types 6 and 7 (SRV-6 and SRV-7) are defined based on the results of phylogenetic analysis only. They are distinct but closely related.^{32,66,91}

Endogenous D retroviruses are common among NHPs and other species, even including marsupials¹ indicating that it is an ancient retroviral lineage. Endogenous D retroviruses in NHPs are simian endogenous retrovirus (SERV) of *Papio cynocephalus*,⁹⁴ squirrel

monkey retrovirus (SMRV) of *Saimiri scurius*,⁸² and langur virus (LNEV or Po-1-Lu) of *Presbytis obscurus*, the spectacled langur,⁹⁸ now classified as *Trachypithecus obscurus*, the dusky leaf-monkey.^{84,92} Each is present in the genome of the host species and many more such viruses undoubtedly exist.

A nomenclature has been devised for SRVs that is similar to that used for influenza viruses and is an aid for tracking isolates and their origins.⁵⁸ For example, D1/rhe/CA/84 is the serotype 1 D retrovirus that was isolated at the California National Primate Research Center (CNPRC) from a rhesus macaque with an AIDS-like disease and was reported in 1984.⁶⁰ D2/Cel/OR/85 is the 1985 SRV-2 isolate from a Celebes black macaque with retroperitoneal fibromatosis at the Oregon National Primate Research Center.⁵⁹ MPMV is the first isolate and is the prototype for the virus species. MPMV retains its original name for these historical reasons.

6.3. PHYLOGENETIC CLASSIFICATION OF SIMIAN BETARETROVIRUSES

As early 1977, DNA hybridization studies suggested that Po-1-lu and MPMV shared a common ancestry.⁴ This was an attractive hypothesis since macaque and langur monkey ranges overlap extensively in southern and southeast Asia. Although a limited number of detailed phylogenetic analyses of simian betaretroviruses have been done^{11,54,64,66,71,73,84,86,91,94} compared to other simian retroviruses, sufficient information exists to show the genetic relationships among the common SRV/MPMV isolates, langur and squirrel monkey endogenous viruses, and the baboon endogenous viruses.

Phylogenetic analysis of exogenous SRVs, MPMV, and endogenous betaretroviruses are shown in Figure 6.2. The simian viruses in the tree segregate into three distinct groups, the SMRV group, the SERV group from baboons, and the SRV/langur group.⁸⁴

6.3.1. SMRV Cluster

SMRV is in the genome of the South American squirrel monkey, *S. sciureus*, and forms a group with the endogenous virus of an Australian marsupial¹ (Figure 6.2). This unusual relationship may be due to only two viruses being known for this cluster. Other closely related viruses undoubtedly exist and a clearer picture of the group will await characterization of more viruses.

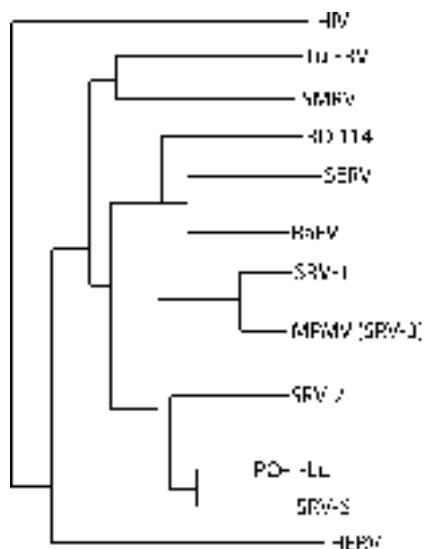


Figure 6.2. Unrooted tree of type C and type D endogenous and exogenous retroviruses. Simian retroviruses (SRVs) serotypes 1 and 2 and Mason–Pfizer monkey virus (MPMV), a.k.a. SRV-3, are pathogenic exogenous retroviruses of macaques; SRV-6 is from a langur monkey. Type D endogenous retroviruses are from the squirrel monkey (SMRV), bush-tailed possum (TvRVD), Po-1-Lu from the langur monkey. Human endogenous retrovirus (HERV), simian endogenous retrovirus (SERV), and baboon endogenous retrovirus (BaEV) are also related. RD114 is endogenous virus of cats. (Adapted from Nandi *et al.*⁶⁵ with permission.)

6.3.2. SERV Baboon Cluster

The endogenous betaretroviruses of baboons form a cluster with RD114, an endogenous virus of cats (Figure 6.2). RD114 is a recombinant virus, its *env* gene being derived from a virus in the SERV group, proving the promiscuity of retroviruses in general. The type C retrovirus, baboon endogenous virus (BaEV) is also in the cluster. BaEV was initially described in the 1970s^{3,37} and is likely to have arisen through recombination of the *gag-pol* region of BaEV and the SERV *env* region. SERV sequences are common in many Old World monkey species and this virus may be ancestral to other retroviruses in African monkeys.⁹⁴

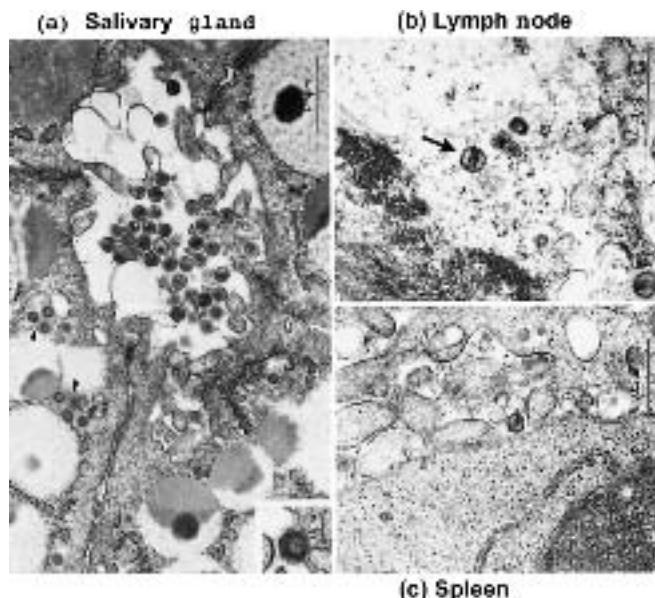


Figure 6.3. Electron micrograph of SRV-1 in rhesus macaque tissues displaying various stages of virus development.⁴² (a) Retroviral particles are shown in (a) salivary gland (arrowheads), (b) germinal center of a lymph node (arrow), and (c) spleen of rhesus monkeys with fatal simian AIDS. In the salivary gland (a), numerous mature extracellular particles in a small acinar lumen, as well as immature intracytoplasmic A particles (arrowheads) and a budding particle (lower right inset) are seen. In the lymph node germinal center (b), a single mature virion (arrow) particle adjacent to a lymphoid cell and numerous cellular processes possibly belonging to follicular dendritic cells are seen. A mature virion in tissue culture is shown for comparison (lower right inset). (c) In the spleen, a single mature particle (center of image) is seen in the extracellular space.

6.3.3. Macaque and Langur Viruses Group

The macaque–langur cluster consists of the common SRV isolates (SRV-1, -2, and MPMV/SRV-3), the langur endogenous virus, Po-1-LU, and the exogenous langur virus SRV-6. This grouping strongly points to an ancestral relationship between langur and macaque betaretroviruses. The clustering of SRV-6 with Po-1-Lu is particularly interesting in that SRV-6 is an exogenous virus of feral langur monkeys in India.⁶⁴ Thus, endogenous langur monkey viruses were the likely ancestors of the exogenous langur viruses, SRV-6, which in turn may have infected Asian macaques through cross-species transmission. This scenario is a model for emergence new retroviruses.

The common SRV isolates, SRV-1, -2 and MPMV, cluster in two separate groups. SRV-1/MPMV viruses form one cluster and SRV-2 isolates form the other.

SRV-2 isolates are known to be relatively stable.^{25,77} Therefore, relatively small differences among SRVs may be significant in that they represent separate introductions of Asian macaque SRVs into American macaque colonies.

A fourth group consists of reticuloendotheliosis virus and spleen necrosis virus (not shown). No simian viruses are known to cluster in this lineage.

6.4. MORPHOLOGY

All members of the betaretrovirus group have type D morphology as described in Introduction to Retroviruses. Figure 6.3 shows the morphology of SRV-1 *in vivo* in rhesus macaque tissues. Figure 6.1 shows a diagrammatic representation of the virion of betaretroviruses and an electron micrograph of SRV-1 grown in cell culture for comparison.

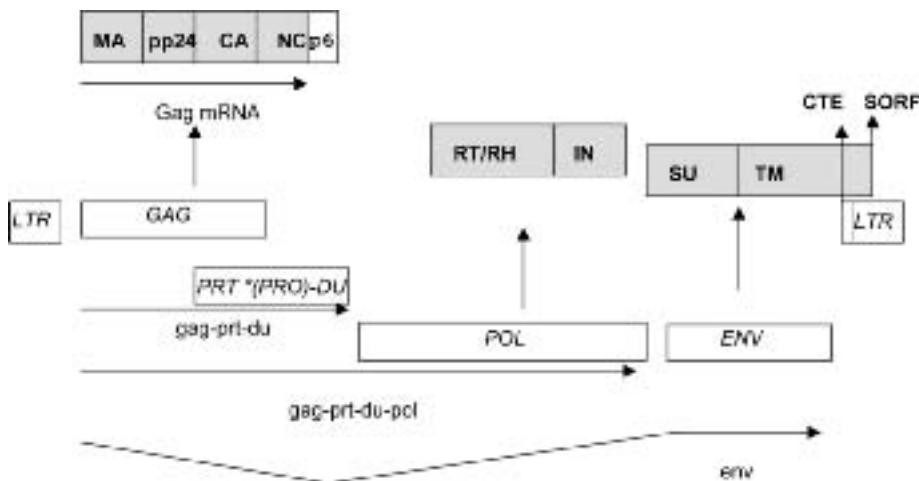


Figure 6.4. Genome map of simian betaretrovirus. Rectangles show LTR, CTE, and genes. Shaded boxes show protein products. LTR, long terminal repeat; CTE, cytoplasmic transport element. Genes for betaretroviruses are *gag*, *pol* polymerase, and *env*. SORF (short open reading frame) has no known protein. Viral proteins are MA matrix, pp24 phosphoprotein, NC nucleocapsid, p6, RT reverse transcriptase, RH ribonuclease H, IN integrase, SU surface glycoprotein, TM transmembrane protein. Arrows represent mRNA with V indicating splicing of mRNA.

6.5. GENOMIC ORGANIZATION

Betaretrovirus genomes are characterized by a simple structure consisting of four genes, *gag*, *pro* (*prt*), *pol*, and *env* (Figure 6.4). There is an open reading frame (ORF) in SRV-1 that was named the short open reading frame (SORF).⁷³ SORF potentially encodes a protein of 109 amino acids,⁷³ but no function is known, nor is it known if SORF is expressed.

SRV-1,⁷³ SRV-2,⁹¹ MPMV/SRV-3,⁸⁶ SRV-5,⁵⁴ SRV-6, and SRV-7⁶⁶ have the same genomic organization. The differences between the MPMV species and other simple retroviruses are relatively minor. The first three genes, *gag*, *pro*, and *pol*, each has different reading frames (Figure 6.4), like their nearest relative, the murine mammary tumor virus.⁶⁸ The *env* gene is expressed as a monocistronic message spliced from genomic size RNA.

6.6. OVERVIEW OF REPLICATION CYCLE

As with all retroviruses, the synthesis of new D-type progeny virus begins with the DNA provirus integrated into a host DNA chromosome in the nucleus. Overall, MPMV species follow the general retroviral replication

scheme described in Introduction to Retroviruses. Thus, only the aspects of replication particular to D retroviruses are presented.

Betaretroviruses, like all retroviruses, must export the unspliced full-length genome from the nucleus to the cytoplasm for translation and encapsulation. Since newly made retroviral genomes have the same features as pre-mRNA (+ polarity and poly A tail), the cellular splicing mechanism that normally acts on RNA transcripts to process them into monocistronic RNA must be bypassed or suppressed. Specific data on splice regulation by betaretroviruses are lacking, but by analogy with other simple retroviruses, processing of genome transcripts to monocistronic Env mRNA is likely to be an inefficient process resulting in a pool of unprocessed genomic RNA.⁹⁷

However, the mechanism that allows full-length D virus RNA genome to be exported unspliced from the nucleus to the cytoplasm is known.^{8,88,89,95,100} MPMV, SRV-1, and SRV-2, and presumably the other less well-characterized SRVs, employ a constitutive transport element (CTE), a string of 173 untranslated nucleotides located between the *env* gene and the beginning of the 3'-LTR (Figure 6.4). The CTE allows unspliced RNA

to be transported to the cytoplasm through the binding of the host cell proteins Tap and NXT1. Normally, Tap and NXT1³⁰ function in the nuclear pore complex to transport sliced mRNAs from the nucleus.⁹⁵ The CTE is unique among retroviruses. Complex retroviruses, like HIV, code for regulatory genes (*rev* in the case of HIV) that function to transport the genome from the nucleus. This fact was exploited to prove the function of the CTE by substituting the MPMV CTE for the *rev* gene in HIV-1. *Rev* defective HIV-1 genomes were exported normally from the nucleus when the MPMV CTE was incorporated into the HIV genome.⁸

All retroviruses package-specific tRNAs into the virion serve as primers for proviral DNA synthesis. Betaretroviruses use tRNAlys1-3 for this purpose.^{86,91} The mechanism of binding and use of tRNAlys1-3 have been well studied in other retroviruses.⁶⁹

6.7. VIRAL PROTEINS

6.7.1. Gag—Capsid Protein

The *gag* gene encodes for 654 amino acids synthesized as a 78-kDa Gag precursor polyprotein (Pr78) that is cleaved to form the smaller functional virion proteins. Pr78 undergoes proteolytic cleavage by the *pro/prt* gene product, the viral protease, to yield the virion proteins p10(MA), pp24, p12, p24(CA), p14(NC), and p4. pp24 is further cleaved from the C-terminal end to pp16 and to Np24 from the N-terminal end. pp16 is required for budding from the cell membrane and genome packaging.⁶ Np24 is essential for replication and is involved in packaging of the genome into the virion.⁶ This packaging function has been traced to the amino acid motif KKPKR.⁶ The protease enzyme is encoded by the *pro* gene, which had been named *prt* in early publications.⁷³ The remaining *gag*-encoded proteins are the p10 matrix protein (MA), p24 major capsid protein (C), p14 nucleocapsid protein (NC), and p4.³⁴ The p24 protein (C), also named p27 in some publications,³⁴ is the most abundant protein in the mature virion (Figure 6.1). The p10 protein, MA, resides just under the envelope of the virion (Figure 6.1). p12, NC, forms a tightly associated complex with the RNA genome.

Unlike other retroviruses, betaretroviral immature capsids are preassembled in the cytoplasm (Figure 6.3a, arrowheads). Upon translation, Pr78 is targeted to the pericentriolar region in the cytoplasm, near the nuclear membrane where it forms procapsids. Cytoplasmic assembly is a function of the Gag MA protein.

An 18-amino acid sequence, the cytoplasmic targeting/retention signal or CTRS, targets Pr78 to the pericentriolar region.⁸⁰ The functions were shown by a single amino acid change in MA converted assembly for D-type to C-type budding.⁷⁴ Myristylation and specific sequences in Pr78 provide specific targeting of the outer cytoplasmic membrane for release.

6.7.2. Pro—Protease

The *pro* gene overlaps the *gag* region by 61 codons. The *pro* gene is highly conserved across the genus with >95% identity between SRV-1 and MPMV. SRV-2 shows >80% homology with SRV-1 and MPMV. The protease itself is translated from the genome length mRNA as two polyproteins: Gag-Pro and Gag-Pro-Pol.⁸⁶ Because the *pro* gene is out of frame with respect to *gag*, the polyprotein that contains the protease results from a ribosomal frame shifting mechanism. The final protease protein is produced by two autocatalytic cuts, one at the N-terminus removing the translated gene from the Gag-Pro and Gag-Pro-Pol precursors as a 17-kDa product and a second cut at the carboxyl end producing the final 13-kDa protease enzyme.⁹⁹ An active 12-kDa product has also been described. The protease is incorporated into the immature capsids in the cytoplasm (Figure 6.3a, arrowheads). It functions to cleave the *gag*-coded polyprotein into smaller proteins that make up the internal capsid structure of the virion as described above. The protease gene contains an UTPase domain that is also present, somewhat unexpectedly, in nonprimate lentiviruses, including feline immunodeficiency virus, equine infectious anemia virus, visna virus, and caprine encephalitis virus.²² Studies to identify the function of the UTPase-domain in betaretroviruses are limited;² however, studies of nonprimate lentiviruses suggest that dUTPase-deleted viruses are less pathogenic in vivo and less fit for replication in nondividing cells.⁷²

6.7.3. Pol—Multifunctional Enzyme

A second ribosomal frame shift within the *pro-pol* overlap region generates a larger polyprotein precursor containing the Pol proteins (Figure 6.4). The *pol* gene encodes the reverse transcriptase (RT), ribonuclease H, and integrase proteins. Like other retroviruses, the gene encoding the integrase of MPMV is located at the 3'-end of the *pol* ORF and the RT is at the opposite 5'-end of the gene.⁸³ The Pol polyprotein is believed

to undergo post-translational protease-mediated processing to produce two separate proteins, the RT and endonuclease/integrase.

6.7.4. Env—Envelope Protein

The *env* gene is located in the 3'-part of the genome (Figure 6.4). The *env* encodes two proteins, the external envelope spike protein (SU) and the transmembrane protein (TM) (Figure 6.1). Like all retroviruses, the mRNA coding the Env proteins is spliced in the nucleus to remove the *gag* and *pol* coding regions, thus forming the only known monocistronic mRNA in SRV and MPMV virus replication.^{71,86} The mRNA is exported to the cytoplasm and is processed like any other monocistronic mRNA. The Env precursor protein is cleaved into the SU or (gp70) and the TM (gp20) (Figure 6.4) by cellular protease.^{54,86} Both the SU gp70 and TM gp20 proteins are glycosylated. Ten putative N-linked glycosylation sites are present in SRV-1, -2, and MPMV SU protein and one is found in the TM.⁷¹ A 25-mer immunosuppressive peptide is present within the TM proteins of SRV-1, -2, and MPMV. This protein motif is highly homologous with that of other immunosuppressive retroviruses such as feline leukemia virus (FeLV).^{71,86} A monoclonal antibody is available against the TM-gp20³⁹ and this antibody is useful for diagnostic testing. The gp70(SU) is the least conserved among the SRVs. The differences in the *env* gene between SRV-1, SRV-2, MPMV/SRV-3, SRV-4, and SRV-5 are responsible for the different neutralization serotypes, SRV-1, SRV-2, and so on.^{11,54,59} MPMV and SRV-1 Env proteins are 83% identical and SRV-1 and SRV-2 are 66% identical.⁹¹ Sera from antibody-positive–virus-negative monkeys, i.e., monkeys that suppressed viremia, have serotype-specific antibody.¹¹

The immature capsids (arrowheads in Figure 6.3) are transported to the cytoplasmic membrane where they bud through the membrane and acquire the Env proteins. The completed D type virion consists of an envelope and a cylindrical or rod-shaped core (Figure 6.1).

The extracellular infectious virion initiates a new round of replication by attaching to the virus-specific receptor on the surface membrane of a susceptible cell. All SRVs and MPMV use a common receptor.⁸⁵ The cell surface receptor has been identified as an amino acid transport protein (Bo) that is present in human cells including white blood cells and permanent cell lines.⁹⁰ The cell receptor for BaEV, the endogenous betaretro-

viruses, is an amino acid transporter protein as its cell surface receptor.⁵⁷

6.8. HOST RANGE IN VITRO

SRV/MPMV has a broad cellular tropism and infects lymphoid, monocytes,^{44,62} and epithelial cells in vitro.⁶⁰ SRV readily infects human T-cell lines, HuT-78, CEM-SS, MT-4, and SubT-1. SRV induces syncytia in the human B-cell line, Raji and these cells are commonly used for SRV isolation, cultivation, and neutralization assays.^{21,59}

6.9. NATURAL HOSTS AND PREVALENCE OF INFECTION

The natural hosts of MPMV and the SRV isolates are macaques. The infection is common in rhesus monkeys in laboratory colonies, especially among the primate centers in the United States. SRV isolates and MPMV infect *M. mulatta*,⁶⁰ *M. nemestrina*,⁹³ *M. fascicularis*,^{11,93} *M. nigra*,⁵⁹ *M. fuscata*,⁹³ *M. radiata*,⁵⁹ and *M. cyclopis*.²¹ Knowledge of natural SRV infections in Asia is limited. Rhesus monkeys and common langur monkeys, *Semnopithecus entellus*, are infected in portions of their natural range in India.^{65,66} An SRV, named SRV/D-T, is prevalent in a captive colony of *M. fascicularis*, the cynomolgus macaque, in Japan.³²

Type D retroviruses are a significant problem for macaque research C, since infected animals may develop an AIDS-like disease during the experiment. Potentially, SRV infection is a confounding variable in unrelated experiments.⁴⁹ The most common SRV isolate in American primate centers is SRV-2, followed by SRV-1, SRV-5, and MPMV. SRV-4 is only known from a single isolate from cynomolgus macaques at the public health laboratory in Berkeley, California.

6.10. MODES OF TRANSMISSION

6.10.1. Simian to Simian

With the possible exception of brain tissue, infectious SRV is found throughout the body. The virus is present in blood, urine, saliva, lymphoid, and nonlymphoid tissues of infected Indian-origin rhesus macaques (Figure 6.3).^{31,42} Inoculation of any of these fluids or tissues into rhesus monkeys will transmit the infection.^{25,29,48} Typically, a third of the infected rhesus monkeys will develop type D virus-associated SAIDS (SAIDS-D).^{35,50}



Figure 6.5. Classic experiment in epidemiology to examine natural SRV-1 transmission in outdoor groups of rhesus monkeys at the California National Primate Research Center.⁴⁸ This outdoor enclosure was divided into three zones, left zone animals naturally affected with simian AIDS, center 10-foot open air barrier, and, right empty, no animals. Juvenile rhesus macaques (shown in figure) were introduced in the left and right zones. Nineteen of 23 rhesus juveniles in the left enclosure developed simian AIDS in 1 year or less. In contrast, all 21 juveniles rhesus in the right zone were not affected after 5 years of observation. This experiment excluded vectors (mosquitoes, rodents) and fomites, such as rainwater flowing between the cages, from a role in AIDS transmission. Physical contact was required. This experiment occurred before the cause of known of AIDS was known. Many theories were in circulation, including environmental factors. This experiment showed the cause was an infectious agent requiring close physical contact, a result that still stands. SRV-1 was isolated from an animal in the left enclosure, cloned and sequenced, and shown to be the etiologic agent of naturally occurring simian AIDS in Asian macaques. (Photo by Preston Marx.) See color version page 12.

Inoculation of blood from SRV-2-infected rhesus induced fatal SAIDS-D in juvenile rhesus.

A classic experiment to examine natural SRV transmission was conducted in outdoor groups of rhesus monkeys at the CNPRC (Figure 6.5).⁴⁸ In this experiment, it was proven that uninfected monkeys must have physical contact with infected monkeys for transmission of SRV to occur, and strongly pointed to natural transmission by bite from infected saliva. The need for contact was shown by keeping 21 healthy monkeys in the same enclosure with SRV-infected monkeys but separated by two chain link fences creating a 10-ft/(3-m) open-air barrier (Figure 6.5). The barrier prevented monkey-to-monkey physical contact but allowed birds, rodents, and insects, including mosquitoes, to move freely between

the enclosures. Rainwater could also flow through the enclosures. Of the 23 uninfected monkeys placed in physical contact with the infected monkeys, 19 (83%) became infected and developed SAIDS within 9 months. All monkeys kept in the “double-fenced” enclosure remained SRV-negative and healthy until the end of experiment.

Monkeys that are healthy carriers of SRV may fuel SRV transmission in group housing. An epidemiologic study of groups of rhesus monkeys at the CNPRC implicated one particular female healthy carrier that occupied a socially dominant role in an outdoor enclosure. This healthy carrier was infected for as long as 10 years without developing the AIDS-like disease. Repeated testing of her saliva showed >1 million infectious

units of SRV per mL—hence the likely link between saliva and transmission.⁵⁰ Of critical importance is the fact that healthy carriers may remain antibody-negative, making their identification problematic. Only PCR or direct virus isolation can readily identify these carriers. Healthy carriers present a potential problem for macaque laboratory colonies because they will spread SRV-associated immune deficiency and remain themselves undetected.⁴⁹ The SRV problem is controlled by screening for SRV infection by ELISA and PCR testing.^{45,56} Antibody-positive or PCR-positive monkeys are removed from contact with the rest of the laboratory colony. Test and removal programs are a highly successful approach to developing specific pathogen-free colonies for research.^{47,78} However, the prevalence of SRV healthy carriers remains unknown. Therefore, if SRV antibody-positive monkeys are removed from a closed colony, but new SRV infections appear, a healthy carrier is likely. Thus, the surveillance and SPF colony establishment programs must take into account the possibility of healthy carriers. In groups without healthy carriers, sequential antibody testing alone will remove SRV from the group.⁵²

6.10.2. Simian to Human

The zoonotic potential of SRV was investigated in general populations, patients with different diseases, as well as in occupational exposure settings.

6.10.2.1. INFECTION OF OCCUPATIONALLY EXPOSED WORKERS

Simian-to-human transmission of SRV and related viruses is very rare. The strongest evidence to date for SRV human infections comes from a serosurvey of workers occupationally exposed to SRV at primate centers in the United States.⁵¹ Occupational exposure could come from contact with infected monkeys during routine care such as cage cleaning or accidental exposure by a contaminated needle stick. Two of 231 persons tested were strongly positive by Western blot. Each of the two persons displayed antibody to more than one gene of SRV (Figure 6.6). Lanes 1 and 2 show a reactivity with the gp70, p31, p24, and p20 bands indicated reaction with the *pol*, *env*, and *gag* gene products. One subject had neutralizing antibody which provided strong evidence of an SRV infection in the past. Repeated attempts to amplify genomic DNA specific for SRV from whole blood as well as attempts to recover infectious

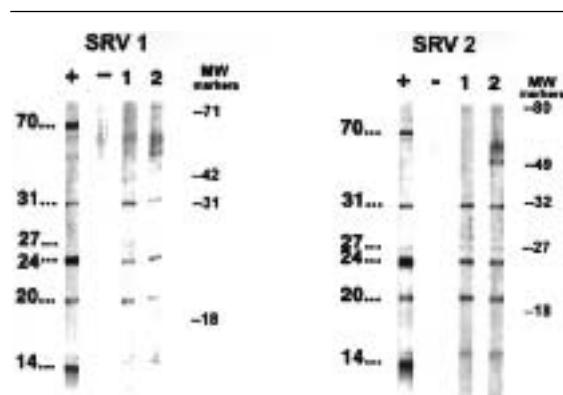


Figure 6.6. Western blot (WB) reactivity against SRV-1 and SRV-2 on initial screening in sera from two persons occupationally exposed to nonhuman primates. Lanes: (+) known SRV-positive serum from a rhesus monkey; (-) SRV-negative serum from a rhesus monkey; lane 1, subject 1; lane 2, subject 2; MW, molecular weights of viral proteins are in kilodaltons shown to the left of the + lane, 70, 31, 27, 24, 20, and 14, the surface spike protein of SRV. (Adapted from Lerche *et al.*⁵¹ with permission.)

virus were not successful. Both workers had evidence of SRV-2 infection, based on SRV-2 neutralization antibody that was detected. They reported contact with SRV-2-positive *M. nemestrina*. The infections were transient. The precise mode of transmission is unknown and there was no evidence of persistent infection.

These findings are nevertheless important for understanding zoonotic infections and the related but more dangerous outcome of zoonosis. Zoonotic infections are infections transmitted from animals without disease. Zoonotic infections may be relatively common under the right exposure conditions and the only evidence of a past infection may be the presence of specific antibody. Zoonosis, a zoonotic infection resulting in disease, is generally a rare outcome. Even though SRV may readily infect human blood cells in vitro and grow transiently in the human host, an intact immune system of the non-natural human host is apparently capable of eliminating the zoonotic infection. Past infections will be evident from trace amounts of specific antibody that were induced during the transient infections. Rare infections of

humans with SRV and other simian retroviruses, such as simian immunodeficiency virus (SIV) and simian foamy viruses, are well documented, but evidence of zoonosis has not been found thus far.

6.10.2.2. SEARCH FOR SRV IN GENERAL HUMAN POPULATION AND PATIENTS WITH DIFFERENT DISEASES

The markers of SRV or MPMV have been reported in humans with widely different diseases such as cancer⁶⁷ and schizophrenia,⁵⁵ Burkett's lymphoma,⁴⁰ B-cell lymphoma,²⁴ and breast cancer.^{5,20,67} However, direct evidence of SRV as an etiologic agent of human disease is unproven and likely to be related to laboratory contamination or spurious laboratory results.¹⁴

An extensive study of over 1,000 persons with various diseases failed to find evidence of SRV-associated human disease.⁴⁶ Sera were tested from lymphoproliferative diseases patients, human immunodeficiency virus (HIV)-1-infected persons, persons with unexplained low CD4 lymphocyte counts, blood donors, and intravenous drug users from the United States and Thailand. Serum samples were screened for antibodies against SRV by an ELISA, and reactive samples were retested by Western blot. None of the samples were seropositive.

An MPMV serological survey of European and African blood donors in Guinea-Bissau revealed 1 of 61 to be weakly reactive with the MPMV p27 protein using Western blots.⁶³ SMRV-specific Western blots also revealed a few additional reactive samples. This type of reaction with a single p27 viral protein in a Western blot is interpreted as an indeterminant result and most likely is a false-positive reaction. In spite of repeated reports of D retrovirus infection of humans, direct evidence of an active infection is still lacking.

6.10.2.3. THE PROBLEM OF TISSUE CULTURE CONTAMINATION

D retrovirus readily infects tissue culture cells derived from human beings. Consequentially, several research groups have reported contamination of human tissue cell lines, in particular derivatives of HeLa cells.²⁶ How the HeLa cells became infected with SRV is not known. In any case, there is a strong risk of laboratory contamination of cell cultures with SRVs and some contaminations have been described.⁷⁵ Therefore, evidence of human infection that is based only on isolation of a D

retrovirus from cultured human cells must be viewed with a skeptical eye.

6.11. PATHOGENESIS AND CHARACTERISTICS OF INFECTION

6.11.1. Infection of Target Cells In Vivo

In vivo studies carried out on SRV-1-infected rhesus macaques showed widespread infection of epithelial and lymphoid cells (Figure 6.3).^{42,43} The infection of epithelial cells in vivo is striking in its widespread nature.^{42,43} Mucosal epithelial cells were heavily infected as early as 1 month postinoculation.⁴³ Infected Langerhans cells were found using immunohistochemical techniques, but they were rare in tissues.⁴³ Higher virus load was found in salivary glands and lymphoid tissue to be more heavily infected than brain tissue.

6.11.2. Immunosuppression by MPMV and SRV Isolates

Inoculation of SRV-1, MPMV, or SRV-2 isolates grown in cells of rhesus monkey origin will induce immune deficiency disease in most of the infected macaques.^{10,39,60,87,93} Pathogenic studies are best carried out using virus grown in cells of the original host and animals free of SIV infection. Cultivation of the virus in human cells, in particular Raji cells, may attenuate the virus.⁵³

The clinical signs of the disease, SAIDS-D, and the case definition are shown in Table 6.1.^{29,35,60} The clinical and pathologic features of SAIDS-D are well established and correlate closely with immunologic and histopathologic abnormalities.²⁵

Generalized lymphadenopathy is an essential feature of the disease. It is often accompanied by splenomegaly, fever, diarrhea, opportunistic infections, and wasting. Another major feature is a striking neutropenia, seen early in the course of the disease that may be followed by lymphopenia and a marked anemia. An abnormal monocytosis is often detected in peripheral blood smears, but the bone marrow remains hypercellular, with a left shift in myeloid elements throughout the course of the disease. Chronic bacterial infection, unresponsive to treatment, and opportunistic viral, fungal, and protozoan infections develop and usually signal the terminal phase of the disease. Most common among the secondary pathogens are disseminated simian cytomegalovirus infection, oral and esophageal candidiasis, and intestinal cryptosporidiosis. Fibrosarcoma and retroperitoneal

Table 6.1. Clinical Signs of Simian Acquired Immunodeficiency Disease (SAIDS) in SRV-1-Infected Rhesus Monkeys Inoculated with Tissue Culture-Grown SRV-1 or Blood, Tissue, Plasma, Saliva from SRV-1-Positive Monkeys

SAIDS*	Typical Onset after Infection in Weeks
Splenomegaly	1–2
Neutropenia <1,700	10
Lymphopenia <1,600 or histologic lymphoid depletion	15–17
Abnormal peripheral blood monocytes	2
Weight loss >10%	15
Persistent diarrhea	12
Anemia <30% PCV	7
Fibrosarcoma, retroperitoneal fibromatosis†	>1 year
Bone marrow hyperplasia	17
Opportunistic infections‡	8

Case definition is generalized lymphadenopathy plus 4 of the 10 clinical signs/diseases listed above.

*Prodromal signs are generalized lymphadenopathy, splenomegaly, fever, anorexia, diarrhea, and weight loss.

†Pathognomonic of SRV infection, not seen in SIV-infected macaques.

‡Including but not limited to disseminated cytomegalovirus infection, NOMA, intestinal cryptosporidiosis.

Pneumocystis carinii pneumonia and B-cell lymphomas are not seen in SRV-1-induced SAIDS. These diseases are associated with simian immunodeficiency virus infections such as SIVmac (see Chapter 5).

fibromatosis (RF) are associated with SRV infections in macaques. Initially, it was thought that B-cell lymphomas and *Pneumocystis carinii* pneumonia were associated with SRV infections. However, this is not the case and these diseases are associated with SIV-induced AIDS. Little is known about the interplay between different retroviruses and host immunity. Why are some retroviruses linked with specific opportunistic tumors. New approaches involving genomics may shed light on this question.³⁸

In the Indian-origin rhesus monkeys experimentally infected with SRV-1, isolate D1/rhe/CA/84, the typical clinical course for SRV-1 is 1/3 developing disease in

less than 6 months, 1/3 developing diseases in 6 months to 3 years, and a third recover but remain antibody-positive for SRV.^{33,60} SRV-1-infected Rh that develop SAIDS-D within 6 months of infection are rapid progressors and may not have detectable antibody during the clinical course.³⁹ Recovered animals may develop AIDS after a long clinically latent period. Clinical outcomes with other SRV isolates in other macaque species will vary. For example, SRV-2 infection of *M. nigra* may result SAIDS D disease plus retroperitoneal fibromatosis.⁵⁹

In contrast to the findings of immunosuppression, the central nervous system does not display pathologic changes after SRV infection. No differences in CSF parameters between infected and uninfected animals were detected.⁴¹ Infectious SRV-1 was isolated from the CSF from 13 out of 19 (68%) viremic rhesus monkeys. The probable source of the virus was the choroid plexus, where approximately 1 in 1,000 surface epithelial cells were found to contain viral antigen.⁴¹ Antibodies against SRV-1 were not detected in the CSF even when present in the serum. Neither infectious virus nor viral antigen was found in the brain tissue of any animal examined. Therefore, infection of the CNS by SRV-1 is subclinical without an intrathecal immune response. This may be related to the apparent restriction of productive infection in the CNS to cells of the choroid plexus.

The disease induced by SRV is AIDS-like, but SRV should not be confused with SIVmac/SIVsm, lentiviruses related to HIV-2 that also causes AIDS-like disease in rhesus macaques. Macaques are not the natural hosts of SIV. SIV naturally occurs only in sub-Saharan African cercopithecine monkeys, chimpanzees, and gorillas (see Chapter 3). The species naturally infected with SRV are all Asian-origin species. These two different immunosuppressive infections can be easily distinguished by immunological and molecular tests.

6.11.3. Retroperitoneal Fibromatosis and Fibrosarcoma

A tumor that occurs in the space behind the abdominal cavity (retroperitoneum) is associated with SRV-2 infections and the retroperitoneal fibromatosis-associated herpesvirus (RFHV).^{7,35,76} RFHV is the macaque homolog of the human virus, Kaposi's sarcoma herpesvirus (KSHV). This monkey tumor is, therefore, an excellent model for Kaposi sarcoma, one of the tumors that commonly occurs in AIDS patients. DNA sequence data

identified KSHV-related herpesviruses in the RF tissue of pig-tailed and rhesus macaques. The basic fibroblast growth factor was found to be associated with RF tissues in SRV-2-infected macaques.¹⁹ It is believed that SRV-2 induces immunosuppression that predisposes for RF. However, the mechanisms of interplay between RFHV and SRV-2 infections underplaying RF development are not known. The fibrosarcoma associated with SRV-1-induced SAIDS-D is also a Kaposi-like tumor.^{25,35}

6.11.4. Induction of Disease with an Infectious Molecular Clone of SRV

The first retroviral molecular clone to induce disease in monkeys was derived from SRV-1. Infection with this clone of SRV-1 ruled out adventitious agents as being involved in SAIDS and proved conclusively that SRV-1 can cause the disease.³³

Six juvenile rhesus macaques were infected with the SRV-1 clone derived from transfected D17 canine osteosarcoma cells. Three of the six were euthanized with terminal SAIDS within 2–3 months of infection. A rapid progression to SAIDS is typical of natural transmission of SRV-1 in Indian-origin rhesus monkeys.⁴⁸ One of the six had a transient infection with early prodromal signs of SAIDS and the remaining two had persistent infections for 2 years with mild to moderate signs of SAIDS. These clinical outcomes are typical of biological isolates of SRV-1.^{33,48,60}

6.12. IMMUNE RESPONSES

6.12.1. Humoral Responses

The humoral immune response to SRV varies depending on the clinical progression.^{39,96} Animals that progress rapidly to disease will have weak to undetectable antibody responses. Animals with slow progression to SAIDS-D or those that suppress viremia have specific antibody detectable by ELISA or Western blot.^{39,52}

6.12.1.1. NEUTRALIZATION

SRV-1, SRV-2, and MPMV/SRV-3 induce significant levels of neutralizing antibody and each isolate can be distinguished by in vitro neutralization.¹⁰ Macaque sera from animals with a past history of SRV infection and no current viremia will display high titers of neutralization antibody ranging from 1:320 to 1:1,280. This in vitro assay employs Raji cells inoculated with the SRV isolate to be tested.

6.12.2. Cellular Responses

Knowledge of simian betaretrovirus replication and pathogenesis is in stark contrast to the lack of knowledge of cell-mediated immune responses to SRV/MPMV. One study examined T-cell proliferation in a vaccine experiment in which a recombinant-vaccinia vector expressing SRV-2 Env protein was used to immunize and protect pig-tailed macaques.³⁶ Purified SRV-2 virions were added to peripheral blood mononuclear cell (PBMC) cultures from macaques immunized with either vaccinia-SRV-2 env hybrid or vaccinia alone. PBMCs from macaques receiving the vaccinia-SRV-2 hybrid virus were stimulated by SRV-2. Macaques receiving vaccinia without the SRV env responded to vaccinia but not to SRV, showing the specificity of the proliferation responses.

6.12.2.1. ANTIBODY-DEPENDENT CELL CYTOTOXICITY

Findings of antibody-dependent cell cytotoxicity (ADCC) are limited to a single study.³⁶ A recombinant-vaccinia vector expressing SRV Env protein was used to immunize and protect pig-tailed macaques. Plasma from immunized monkeys was shown to have ADCC activity. PBMCs from healthy normal macaques were used as the effector cells. Only plasma from the immunized macaques had conferred significant ADCC activity to the PBMCs. The same vectors also induced neutralizing antibody and CMI. Therefore, the role of ADCC could not be specially evaluated in efficacy of the vaccine.

6.13. DIAGNOSIS STRATEGIES

Diagnostic strategies for identifying past or current MPMV/SRV infections employ a three-tiered algorithm.⁵² When a more rigorous analysis is required, such as testing of suspected SRV human infections, a four-tiered algorithm is used. Both approaches begin with initially screening of sera by ELISA. Samples scoring positive are confirmed by Western blots. Confirmed samples are tested further by PCR or by direct virus isolation by cocultivation of human or monkey PBMCs with Raji cells.^{21,52} The third tier of PCR testing is necessary in macaques because inapparent carriers of SRV may be antibody-negative,^{50,52} but will have high levels of virus in plasma or PBMCs. The fourth tier is inoculation of an Indian-origin rhesus macaque with whole blood confirmed as positive by PCR or Western blot. The four-tiered approach was used in the case of a

laboratory worker who had SRV neutralizing antibody, and was Western immunoblot (WB)-positive for SRV.⁵¹ The inoculated monkey did not show evidence of SRV infection leading to the conclusion that the human infection had been resolved without disease.

6.13.1. Virus Isolation

Virus isolation from clinical specimens is routinely carried by cocultivation of mitogen-stimulated PBMCs with Raji cells, a human B-cell line that is very sensitive to infection by exogenous macaque betaretroviruses.^{21,51,66} Raji cells infected with SRV will develop syncytia that are easily identified by ordinary light microscopy.²¹

6.13.2. Antibody Assays

Several in-house SRV ELISAs have been described.^{47,50,51,66,79} All employ gradient purified SRV. Unfortunately, there are, as yet, no commercial ready-to-use assays. However, commercially prepared virus is available at <http://www.bioreliance.com/simian-assays.html> and may be used to construct in-house assays. The number of false-positives obtained by such ELISA can be quite high, particularly when human sera are tested. Of 231 sera of occupationally exposed workers, 60 were ELISA-positive.⁵¹ Yet only in two cases anti-SRV-positivity was confirmed by WB. The majority of false-positives in this assay are caused by reaction with a single SRV protein, p27. Such reactivity is reported as indeterminant result (Ind p27). ELISA false-positives and Ind p27 reactions are a significant problem.^{46,51} Multiplex microbead immunoassay has been employed for SRV testing.^{38a} This test detects antibodies against multiple simian viruses in a single assay.

WB is essential for confirmation sero-surveys for SRV done by other methods.^{45–48,50–52} Ready-to-use SRV WB is not available commercially. In-house SRV WB tests use either commercially available or in-house made sucrose gradient purified SRV. The purity of different lots varies significantly and SRV assays are not standardized. Figure 6.6 shows a WB that was used to confirm infections of persons occupationally exposed to SRV-infected monkeys and laboratory specimens.⁵¹ The criteria for WB-positivity are reaction with at least one *gag*-coded antigen and one *env* gene product (gp20 or gp70).

A monoclonal antibody against the gp20 transmembrane protein was developed³⁹ and is used in immunofluorescence assays to confirm infection of Raji cells³⁹ and for detection of SRV in tissues by immunohistochemistry.⁴²

6.13.3. Molecular Assays

Polymerase chain reaction (PCR) can be used to distinguish SRV infections by various serotypes.^{45,56} PCR can be used alone, since results are conclusive, or in combination with serological assays. PCR is necessary because it will detect SRV-infection in antibody-negative monkeys such as healthy carriers, late seroconvertors, and nonseroconverting rapid progressors to SAIDS.^{47,50}

6.14. VACCINES

Two types of vaccines have been successfully tested in the SRV SAIDS model: a killed virus formulation and vaccinia virus vector expressing the envelope proteins (gp70 and gp20) of SRV-1 and MPMV.

6.14.1. Killed Whole-Virus Vaccines

SRV-1 was inactivated with formalin and injected intramuscularly to induce antibody. Protection was attained and was associated with neutralizing antibody.⁶¹ Importantly, the SRV challenge virus used to test the efficacy of the vaccine was prepared in isogenic rhesus monkey kidney cells, therefore ruling out induction of anti-cellular antibody as the protective mechanism. This formalin-killed vaccine was the first vaccine shown to be effective against a primate retrovirus.

6.14.2. Vaccinia-Based Vaccines

The second vaccine approach uses recombinant vaccinia virus vectors.^{9,36} The vaccinia virus vector expressed the envelope proteins (gp70 and gp20) of SRV-1 and MPMV. Upon challenge with live SRV-1 by the intravenous route, both MPMV- and SRV-1-immunized animals were protected. The vaccine therefore, conferred cross-protection for SRV-types 1 and MPMV, demonstrating their close relationship. The neutralizing antibody induced by the vaccine did not cross-react with the more distant SRV-2.⁹ Using a similar approach, vaccinia vectors expressing SRV-2 (isolate W) Env protected pigtailed macaques from a lethal i.v. challenge with SRV-2W. The vaccinia vector induced neutralizing antibody, cell-mediated immunity, and antibody-dependent cell-mediated cytotoxicity.³⁶

6.15. BIOLOGY OF ENDOGENOUS D RETROVIRUSES

Endogenous retroviruses are retroviruses inherited as proviruses passed to offspring in the germ line of species. They are often defective. If infectious they usually do not replicate in cells of the host species of origin (see Introduction to Retroviruses for details). Their isolation, therefore, involves cocultivation of NHP host cells with cells from an unrelated species. Three groups of NHP endogenous betaretroviruses are known: squirrel monkey endogenous D retrovirus, langur monkey endogenous D retrovirus, and baboon endogenous D retrovirus. None of these viruses are known to cause diseases in the host species, although it is likely that pathogenic exogenous retroviruses originated from endogenous D retroviruses through cross-species transmission. As a group, endogenous D retroviruses are ancient and ubiquitous in NHP genomes.

6.15.1. Squirrel Monkey Endogenous D Retrovirus

Squirrel monkey retrovirus (SMRV) is the first endogenous betaretrovirus to be characterized.⁸² The virus was isolated by cocultivation of placenta cells with mink lung cells. SMRV is part of *Samiri sciureus* genome and related endogenous viruses have been described in animals other than primates.¹ There are 10–15 copies of the SMRV genome in the *S. sciureus* genome. SMRV can be propagated in a variety of permanent cell lines of primate and other vertebrate species.^{15–17,90}

6.15.2. Langur Monkey Endogenous D Retrovirus

The langur monkey endogenous D retrovirus derives its unusual name, PO-1-LU, from its species of origin, *Presbytis obscurus* (PO). It was obtained by cocultivation of iododeoxyuridine-treated langur lung tissue with bat lung cells.⁹² This endogenous virus is closely related to SRV-6, an exogenous betaretrovirus that naturally infects hanuman langurs (*Semnopithecus entellus*) in India.^{64,65} The close similarity between SRV-6 and MPMV and the SRV isolates SRV-1 and SRV-2 (Figure 6.2) suggest that SRV-6 arose from the langur endogenous virus group and spread into rhesus macaques in the region.⁸⁴

6.15.3. Simian Endogenous Retrovirus

SERV was discovered in the genome of *Papio cynocephalus*. SERV is closely related to SRVpc, isolated from a baboon at the Washington National Primate

Research Center.²⁸ Close relatives of these two isolates are present in the genome of 44 species of the subfamily *Cercopithecinae* but not in apes or humans. The phylogenetic tree of these viruses indicates that SERV-related viruses are ancient and ubiquitous in Old World monkeys and that they coevolved with their host species.⁹⁴ The genetic organization SERV is typical of the betaretrovirus group and is closely related to SRV-1, SRV-2, and MPMV in gag.⁹⁴ SERV is also related to the type C Baboon endogenous retrovirus (BaEV)³ in the 3'-end of the genome.^{3,94}

6.16. SUMMARY

Exogenous simian type D retrovirus (SRV) isolates and MPMV, a.k.a. SRV-3, are members of the genus *Betaretrovirus* of the *Retroviridae* family. MPMV is the prototype for this group. All simian betaretroviruses are simple retroviruses encoding *gag*, *pol*, *pro*, and *env* genes. They naturally infect macaques and langurs. SRV appears to be specific for these two monkey genera, although the virus will replicate in vitro in the cells of other species including human. SRV infections are a significant problem for laboratory macaque colonies. Infected macaques may develop a fatal disease with many of the characteristics of AIDS. SRV-infected monkeys may also develop retroperitoneal fibromatosis or fibrosarcoma, Kaposi sarcoma-like cancers that provide a model for Kaposi sarcoma in AIDS patients. Markers of SRV or MPMV infection, mainly serological, have been reported in healthy humans and patients with wide range of diseases cancers, including breast cancer, Burkitt's lymphoma, B-cell lymphoma, human AIDS, and even schizophrenia. However, direct evidence of SRV as an etiologic agent of any human disease is lacking. Moreover, there is no solid evidence that SRV can infect humans in settings other than occupational exposure. The most likely explanation for such persistent reports is laboratory contaminations of human tissue culture or indeterminant serological reactions. However, a strong case has been made for rare transient SRV infections through occupational exposure to infected macaques. Killed whole virus and vaccinia-recombinant SRV vaccines expressing Env have been tested experimentally and are effective. Vaccination of laboratory workers is not recommended, because containment measures have been effective in preventing infection of the vast majority of laboratory workers. Using a test-and-removal program, exogenous betaretroviruses, such as SRV,

can be successfully eliminated from NHP research facilities.

REFERENCES

1. Baillie, G. J. and R. J. Wilki. 2001. Endogenous type D retrovirus in a marsupial, the common brushtail possum (*Trichosurus vulpecula*). *J. Virol.* 75(5):2499–2507.
2. Barabás, O., M. Rumlová, A. Erdei, V. Pongrácz, I. Pichová, and B. G. Vértess. 2003. dUTPase and nucleocapsid polypeptides of the mason-pfizer monkey virus form a fusion protein in the virion with homotrimeric organization and low catalytic efficiency. *J. Biol. Chem.* 278(40):38803–38812.
3. Benveniste, R. E., R. Heinemann, G. L. Wilson, R. Callahan, and G. J. Todaro. 1974. Detection of baboon type C viral sequences in various primate tissues by molecular hybridization. *J. Virol.* 14(1): 56–67.
4. Benveniste, R. E. and G. J. Todaro. 1977. Evolution of primate oncornaviruses: an endogenous virus from langurs (*Presbytis* spp.) with related virogene sequences in other Old World monkeys. *Proc. Natl. Acad. Sci. U. S. A.* 74(10):4557–4561.
5. Bindra, A., S. Muradrasoli, R. Kisekka, H. Nordgren, F. Wärnberg, and J. Blomberg. 2007. Search for DNA of exogenous mouse mammary tumor virus-related virus in human breast cancer samples. *J. Gen. Virol.* 88(6):1806–1809.
6. Bohl, C. R., S. M. Brown, and R. A. Weldon Jr. 2005. The pp24 phosphoprotein of Mason-Pfizer monkey virus contributes to viral genome packaging. *Retrovirology* 7(2):68.
7. Bosch, M. L., E. Harper, A. Schmidt, K. B. Strand, S. Thormahlen, M. E. Thouless, and Y. Wang. 1999. Activation in vivo of retroperitoneal fibromatosis-associated herpesvirus, a simian homologue of human herpesvirus-8. *J. Gen. Virol.* 80(Pt 2):467–475.
8. Bray, M., S. Prasad, J. W. Dubay, E. Hunter, K. Jeang, D. Rekosh, and M. Hammarskjold. 1994. A small element from the Mason-Pfizer monkey virus genome makes human immunodeficiency virus type 1 expression and replication rev-independent. *Proc. Natl. Acad. Sci.* 91(4):1256–1260.
9. Brody, B. A., E. Hunter, J. D. Kluge, R. Lasarow, M. Gardner, and P. A. Marx. 1992. Protection of macaques against infection with simian type D retrovirus (SRV-1) by immunization with recombinant vaccinia virus expressing the envelope glycoproteins of either SRV-1 or Mason-Pfizer monkey virus (SRV-3). *J. Virol.* 66(6):3950–3954.
10. Bryant, M. L., M. B. Gardner, P. A. Marx, D. H. Maul, N. W. Lerche, K. G. Osborn, L. J. Lowenstein, A. Bodgen, L. O. Arthur, and E. Hunter. 1986. Immunodeficiency in rhesus monkeys associated with the original Mason-Pfizer monkey virus. *J. Natl. Cancer Inst.* 77(4):957–965.
11. Bryant, M. L., P. A. Marx, S. M. Shiigi, B. J. Wilson, W. P. McNulty, and M. B. Gardner. 1986. Distribution of type D retrovirus sequences in tissues of macaques with simian acquired immune deficiency and retroperitoneal fibromatosis. *Virology* 150(1):149–160.
12. Cardiff, R. D. and N. Kenney. 2007. Mouse mammary tumor biology: a short history. *Adv Cancer Res.* 98:53–116.
13. Chan, G., M. W. Rancourt, W. S. Ceglopski, and H. Friedman. 1968. Leukemia virus suppression of antibody-forming cells: ultrastructure of infected spleens. *Science* 159(813):437–439.
14. Charman, H. P., R. Rahman, M. H. White, N. Kim, and R. V. Gilden. 1977. Radioimmunoassay for the major structural protein of Mason-Pfizer monkey virus: attempts to detect the presence of antigen or antibody in humans. *Int. J. Cancer* 19(4): 498–504.
15. Chiu, I. M., R. Callahan, S. R. Tronick, J. Scholm, and S. A. Aaronson. 1984. Major *pol* gene progenitors in the evolution of oncoviruses. *Science* 223(4634):364–370.
16. Chiu, I. M., R. C. Huang, and S. A. Aaronson. 1985. Genetic relatedness between intracisternal A particles and other major oncovirus genera. *Virus Res.* 3(1):1–11.
17. Chiu, I. M. and S. F. Skuntz. 1986. Nucleotide sequence analysis of squirrel monkey retrovirus reveals a novel primer-binding site for tRNALys 1,2. *J. Virol.* 58(3):983–987.
18. Chopra, H. C. and M. M. Mason. 1970. A new virus in a spontaneous mammary tumor of a rhesus monkey. *Cancer Res.* 30(8):2081–2086.
19. Chung, C. H., J. Chiang, C. M. Jiang, Y. Y. Chen, C. Y. Huang, P. G. Chen, and Y. J. Chen. 2001. Basic fibroblast growth factor as a growth factor for SRV-2-infected simian retroperitoneal fibromatosis cells, an animal model for AIDS related Kaposi's sarcoma. *Neoplasma* 48(3):192–199.
20. Colcher, D., S. Spiegelman, and J. Schlom. 1974. Sequence homology between the RNA of Mason-Pfizer monkey virus and the RNA of human malignant breast tumors. *Proc. Natl. Acad. Sci. U. S. A.* 71(12):4975–4979.
21. Daniel, M. D., N. W. King, N. L. Letvin, R. D. Hunt, P. K. Sehgal, and R. C. Desrosiers. 1984. A new type

- D retrovirus isolated from macaques with an immunodeficiency syndrome. *Science* 223(4636):602–605.
22. Elder, J. H., D. L. Lerner, C. S. Hasselkus-Light, D. J. Fontenot, E. Hunter, P. A. Luciw, R. C. Montelaro, and T. R. Phillips. 1992. Distinct subsets of retroviruses encode dUTPase. *J. Virol.* 66(3):1791–1794.
 23. Fine, D. L., J. C. Landon, R. J. Pienta, M. T. Kubicek, M. G. Valerio, W. F. Loeb, and H. C. Chopra. 1975. Responses of infant rhesus monkeys to inoculation with Mason-Pfizer monkey virus materials. *J. Natl. Cancer Inst.* 54(3):651–658.
 24. Ford, R. J., L. A. Donehower, and R. C. Bohannon. 1992. Studies on a type D retrovirus isolated from an AIDS patient lymphoma. *AIDS Res. Hum. Retroviruses* 8(5):742–751.
 25. Gardner, M. B. and P. A. Marx. 1985. Simian acquired immunodeficiency syndrome. *Adv. Viral Oncol.* 5:57–81.
 26. Gelderblom, H. and H. Schwarz. 1976. Relationship between the Mason-Pfizer monkey virus and HeLa virus: immunoelectron microscope. *J. Natl. Cancer Inst.* 56(3):635–637.
 27. Gottlieb, M. S., R. Schröff, H. M. Schanker, J. D. Weisman, P. T. Fan, R. A. Wolf, and A. Saxon. 1981. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N. Engl. J. Med.* 305(24):1425–1431.
 28. Grant, R. F., S. K. Windsor, C. J. Malinak, C. R. Bartz, A. Sabo, R. E. Benveniste, and C. C. Tsai. 1995. Characterization of infectious type D retrovirus from Baboons. *Virology* 20(207):292–296.
 29. Gravell, M., W. T. London, S. A. Houff, D. L. Madden, M. C. Dalakas, J. L. Sever, K. G. Osborn, D. H. Maul, R. V. Henrickson, P. A. Marx, N. W. Lerche, S. Prahalada, and M. B. Gardner. 1984. Transmission of simian acquired immunodeficiency syndrome (SAIDS) with blood or filtered plasma. *Science* 223(4631):74–76.
 30. Grüter, P., C. Tabernero, C. von Kobbe, C. Schmitt, C. Saavedra, A. Bach, M. Wilms, B. K. Felber, and E. Izaurralde. 1998. TAP, the human homolog of Mex67p, mediates CTE-dependent RNA export from the nucleus. *Mol. Cell* 1(5):649–659.
 31. Hara, M., T. Kikuchi, T. Sata, N. Nakajima, Y. Ami, Y. Sato, K. Tanaka, T. Narita, F. Ono, H. Akari, K. Terao, and R. Mukai. 2007. Detection of SRV/D shedding in body fluids of cynomolgus macaques and comparison of partial gp70 sequences in SRV/D-T isolates. *Virus Genes* 35(2):281–288.
 32. Hara, M., T. Sata, T. Kikuchi, N. Nakajima, A. Uda, K. Fujimoto, T. Baba, and R. Muka. 2005. Isolation and characterization of a new simian retrovirus type D subtype from monkeys at the Tsukuba Primate Center, Japan. *Microbes Infect.* 7(1):126–131.
 33. Heidecker, G., N. W. Lerche, L. J. Lowenstein, A. A. Lackner, K. G. Osborn, M. B. Gardner, and P. A. Marx. 1987. Induction of simian acquired immune deficiency syndrome (SAIDS) with a molecular clone of a type D SAIDS retrovirus. *J. Virol.* 61(10):3066–3071.
 34. Henderson, L. E., R. Sowder, G. Smythers, R. E. Benveniste, and S. Oroszlan. 1985. Purification and N-terminal amino acid sequence comparisons of structural proteins from retrovirus-D/Washington and Mason-Pfizer monkey virus. *J. Virol.* 55(3):778–787.
 35. Henrickson, R. V., D. H. Maul, K. G. Osborn, J. L. Sever, D. L. Madden, L. R. Ellingsworth, J. H. Anderson, L. J. Lowenstein, and M. B. Gardner. 1983. Epidemic of acquired immunodeficiency in rhesus monkeys. *Lancet* (8321):388–390.
 36. Hu, S. L., J. M. Zarling, J. Chinn, B. M. Travis, P. A. Moran, J. Sias, L. Kuller, W. R. Morton, G. Heidecker, and R. E. Benveniste. 1989. Protection of macaques against simian AIDS by immunization with a recombinant vaccinia virus expressing the envelope glycoproteins of simian type D retrovirus. *Proc. Natl. Acad. Sci. U. S. A.* 86(18):7213–7217.
 37. Kato, S., K. Matsuo, N. Nishimura, N. Takahashi, and T. Takano. 1987. The entire nucleotide sequence of baboon endogenous virus DNA: a chimeric genome structure of murine type C and type D retroviruses. *Jpn. J. Genet.* 62:127–137.
 38. Katze, M. G., J. L. Fornek, R. E. Palermo, K. A. Walters, and M. J. Korth. 2008. Innate immune modulation by RNA viruses: emerging insights from functional genomics. *Nat. Rev. Immunol.* 8(8):644–654.
 39. Kwang, H. S., N. C. Pedersen, N. W. Lerche, K. G. Osborn, P. A. Marx, and M. B. Gardner. 1987. Viremia, antigenemia, and serum antibodies in rhesus macaques infected with simian retrovirus type 1 and their relationship to disease course. *Lab. Invest.* 56(6):591–597.
 - 39a. Khan IH, Mendoza S, Yee J, Deane M, Venkateswaran K, Zhou SS, Barry PA, Lerche NVV, Luciw PA. 2006. Simultaneous detection of antibodies to six nonhuman-primate viruses by multiplex microbead immunoassay. *Clin Vaccine Immunol.* 13(1):45–52.
 40. Kzhyshkowska, J. G., A. V. Kiselev, G. A. Gordina, V. I. Kurmashev, N. M. Portjanko, A. S. Ostashkin, and K. V. Ilyin. 1996. Markers of type D retroviruses in children with Burkitt's-type lymphoma. *Immunol. Lett.* 53(2–3):101–104.
 41. Lackner, A. A., P. A. Marx, N. W. Lerche, M. B. Gardner, J. D. Kluge, A. Spinner, H. S. Kwangat, and L. J. Lowenstein. 1989. Asymptomatic infection of

- the central nervous system by the macaque immunosuppressive type D retrovirus, SRV-1. *J. Gen. Virol.* 70(Pt 7):1641–1651.
42. Lackner, A. A., M. H. Rodriguez, C. E. Bush, R. J. Munn, H. S. Kwang, P. F. Moore, K. G. Osborn, P. A. Marx, M. B. Gardner, and L. J. Lowenstein. 1988. Distribution of a macaque immunosuppressive type D retrovirus in neural, lymphoid, and salivary tissues. *J. Virol.* 62(6):2134–2142.
 43. Lackner, A. A., M. Schiodt, G. C. Armitage, P. F. Moore, R. J. Munn, P. A. Marx, M. B. Gardner, and L. J. Lowenstein. 1989. Mucosal epithelial cells and langerhans cells are targets for infection by the immunosuppressive Type D retrovirus simian AIDS retrovirus serotype 1. *J. Med. Primatol.* 18(3–4):195–207.
 44. LeGrand, E. K., R. M. Donovan, P. A. Marx, J. E. Moulton, A. T. Cheung, A. E. Lewis, and M. B. Gardner. 1985. Monocyte function in rhesus monkeys with simian acquired immune deficiency syndrome. *Vet. Immunol. Immunopathol.* 10(2–3):131–146.
 45. Lerche, N. W., R. F. Cotterman, M. D. Dobson, J. L. Yee, A. N. Rosenthal, and W. M. Heneine. 1997. Screening for simian type-D retrovirus infection in macaques, using nested polymerase chain reaction. *Lab. Anim. Sci.* 47(3):263–268.
 46. Lerche, N. W., W. Heneine, J. E. Kaplan, T. Spira, J. L. Yee, and R. F. Khabbaz. 1995. An expanded search for human infection with simian type D retrovirus. *AIDS Res. Hum. Retroviruses* 11(4):527–529.
 47. Lerche, N. W., P. A. Marx, and M. B. Gardner. 1991. Elimination of type D retrovirus infection from group-housed rhesus monkeys using serial test and removal. *Lab. Anim. Sci.* 41(2):123–127.
 48. Lerche, N. W., P. A. Marx, K. G. Osborn, D. H. Maul, L. J. Lowenstein, M. L. Bleviss, P. Moody, R. V. Henrickson, and M. B. Gardner. 1987. Natural history of endemic type D retrovirus infection and acquired immune deficiency syndrome in group-housed rhesus monkeys. *J. Natl. Cancer Inst.* 79(4):847–854.
 49. Lerche, N. W. and K. G. Osborn. 2003. Simian retrovirus infections: potential confounding variables in primate toxicology studies. *Toxicol. Pathol.* 31(Suppl):103–110.
 50. Lerche, N. W., K. G. Osborn, P. A. Marx, S. Prahalada, D. H. Maul, L. J. Lowenstein, R. J. Munn, M. L. Bryant, R. V. Henrickson, L. O. Arthur, R. V. Gilden, C. S. Barker, E. Hunter, and M. B. Gardner. 1986. Inapparent carriers of simian acquired immune deficiency syndrome type D retrovirus and disease transmission with saliva. *J. Natl. Cancer Inst.* 77(2):489–495.
 51. Lerche, N. W., W. M. Switzer, J. L. Yee, V. Shanmugam, A. N. Rosenthal, L. E. Chapman, T. M. Folks, and W. Heneine. 2001. Evidence of infection with simian type D retrovirus in persons occupationally exposed to nonhuman primates. *J. Virol.* 75(4):1783–1789.
 52. Lerche, N. W., J. L. Yee, and M. B. Jennings. 1994. Establishing specific retrovirus-free breeding colonies of macaques: an approach to primary screening and surveillance. *Lab. Anim. Sci.* 44(3):217–221.
 53. Letvin, N. L., M. D. Daniel, P. K. Sehgal, L. V. Chalifoux, N. W. King, R. D. Hunt, W. R. Aldrich, K. Holley, D. K. Schmidit, and R. C. Desrosiers. 1984. Experimental infection of rhesus monkeys with type D retrovirus. *J. Virol.* 52:683–686.
 54. Li, B., M. K. Axthelm, and C. A. Machida. 2000. Simian retrovirus serogroup 5: partial gag-prt sequence and viral RNA distribution in an infected rhesus macaque. *Virus Genes* 21(3):241–248.
 55. Lillehoj, E. P., G. M. Ford, S. Bachmann, J. Schröder, E. F. Torrey, and R. H. Yolken. 2000. Serum antibodies reactive with non-human primate retroviruses identified in acute onset schizophrenia. *J. Neurovirol.* 6(6):492–497.
 56. Liska, V., N. W. Lerche, and R. M. Ruprecht. 1997. Simultaneous detection of simian retrovirus type D serotypes 1, 2, and 3 by polymerase chain reaction. *AIDS Res. Hum. Retroviruses* 13(5):433–437.
 57. Marin, M., C. S. Tailor, A. Nouri, and D. Kabat. 2000. Sodium-dependent neutral amino acid transporter type 1 is an auxiliary receptor for baboon endogenous retrovirus. *J. Virol.* 74(17):8085–8093.
 58. Marracci, G. H., N. A. Avery, S. M. Shiigi, G. Couch, H. Palmer, K. Y. Pilcher, H. Nichols, L. M. Hallick, M. K. Axthelm, and C. A. Machida. 1999. Molecular cloning and cell-specific growth characterization of polymorphic variants of type D serogroup 2 simian retroviruses. *Virology* 261(1):43–58.
 59. Marx, P. A., M. L. Bryant, K. G. Osborn, D. H. Maul, N. W. Lerche, L. J. Lowenstein, J. D. Kluge, C. Zaiss, R. V. Henrickson, S. M. Shiigi, B. J. Wilson, A. Malley, L. C. Olson, W. P. McNulty, L. O. Arthur, R. V. Gilden, C. S. Barker, E. Hunter, R. J. Munn, G. Heidecker, and M. B. Gardner. 1985. Isolation of a new serotype of simian acquired immune deficiency syndrome type D retrovirus from Celebes black macaques (*Macaca nigra*) with immune deficiency and retroperitoneal fibromatosis. *J. Virol.* 56(2):571–578.
 60. Marx, P. A., D. H. Maul, K. G. Osborn, N. W. Lerche, P. Moody, L. J. Lowenstein, R. V. Henrickson, L. O. Arthur, R. V. Gilden, M. Gravell, W. T. London, J. L. Sever, J. A. Levy, J. Munn, and M. B. Gardner. 1984.

- Simian AIDS: isolation of a type D retrovirus and transmission of the disease. *Science* 223(4640):1083–1086.
61. Marx, P. A., N. C. Pedersen, N. W. Lerche, K. G. Osborn, L. J. Lowenstein, A. A. Lackner, D. H. Maul, H. S. Kwang, J. D. Kluge, C. P. Zaiss, V. Sharpe, A. P. Spinner, A. C. Allison, and M. B. Gardner. 1986. Prevention of simian acquired immune deficiency syndrome with a formalin-inactivated type D retrovirus vaccine. *J. Virol.* 60(2):431–435.
 62. Maul, D. H., C. P. Zaiss, M. R. MacKenzie, S. M. Shiigi, P. A. Marx, and M. B. Gardner. 1988. Simian retrovirus D serogroup 1 has a broad cellular tropism for lymphoid and nonlymphoid cells. *J. Virol.* 62(5):1768–1773.
 63. Morozov, V. A., F. Saal, A. Gessain, A. Terrinha, and J. Périès. 1991. Antibodies to gag gene coded polypeptides of type D retroviruses in healthy people from Guinea-Bissau. *Intervirology* 32(4):253–257.
 64. Nandi, J. S., V. Bhavalkar-Potdar, S. Tikute, and C. G. Raut. 2000. A novel type D simian retrovirus naturally infecting the Indian Hanuman langur (*Semnopithecus entellus*). *Virology* 277(1):6–13.
 65. Nandi, J. S., S. A. Tikute, A. K. Chhangani, V. A. Potdar, M. Tiwari-Mishra, R. A. Ashtekar, J. Kumari, A. Walimbe, and S. M. Mohnot. 2003. Natural infection by simian retrovirus-6 (SRV-6) in Hanuman langurs (*Semnopithecus entellus*) from two different geographical regions of India. *Virology* 311(1):192–201.
 66. Nandi, J. S., S. Van Dooren, A. K. Chhangani, and S. M. Mohnot. 2006. Virus genes new simian beta retroviruses from rhesus monkeys (*Macaca mulatta*) and langurs (*Semnopithecus entellus*) from Rajasthan, India. *Virus Genes* 33(1):107–116.
 67. Ohno, T., R. W. Sweet, D. Dejak, and S. Spiegelman. 1977. Purification and characterization of the DNA polymerase of human breast cancer particles. *Proc. Natl. Acad. Sci. U. S. A.* 74(2):764–768.
 68. Ono, M., H. Toh, T. Miyata, and T. Awaya. 1985. Nucleotide sequence of the Syrian Hamster intracisternal A-particle gene: close evolutionary relationship of type A particle gene to types B and D oncovirus genes. *J. Virol.* 55(2):387–394.
 69. Oude Essink, B. B., A. T. Das, and B. Berkhouit. 1995. Structural requirements for the binding of tRNA to reverse transcriptase of the human immunodeficiency virus type 1. *Am. Soc. Biochem. Mol. Biol. Inc.* 270(40):23867–23874.
 70. Perryman, L. E., E. A. Hoover, and D. S. Yohn. 1972. Immunologic reactivity of the cat: immunosuppression in experimental feline leukemia. *J. Natl. Cancer Inst.* 49(5):1357–1365.
 71. Philipp-Staheli, J., T. Marquardt, M. E. Thouless, A. G. Bruce, R. F. Grant, C. C. Tsai, and T. M. Rose1. 2006. Genetic variability of the envelope gene of Type D simian retrovirus-2 (SRV-2) subtypes associated with SAIDS-related retroperitoneal fibromatosis in different macaque species. *J. Virol.* 80(3):1111–1119.
 72. Phillips, T. R., O. Prospero-Garcia, D. W. Wheeler, P. C. Wagaman, D. L. Lerner, H. S. Fox, L. R. Whalen, F. E. Bloom, J. H. Elder, and S. J. Henriksen. 1996. Neurological dysfunctions caused by a molecular clone of feline immunodeficiency virus, FIV-PPR. *J. Neurovirol.* 2(6):388–396.
 73. Power, M. D., P. A. Marx, M. L. Bryant, M. B. Gardner, P. J. Barr, and P. A. Luciw. 1986. Nucleotide sequence of SRV-1, a type D simian acquired immune deficiency syndrome retrovirus. *Science* 231(4745):1567–1572.
 74. Rhee, S. S. and E. Hunter. 1990. A single amino acid substitution within the matrix protein of a type D retrovirus converts its morphogenesis to that of a type C retrovirus. *Cell* 63(1):77–86.
 75. Robert-Guroff, M., T. L. Stern, E. S. Richardson, B. C. Giovanella, and F. H. Michaels. 1996. Presence of Mason-Pfizer monkey virus in some stocks of the human HBL-100 mammary epithelial cell line. *J. Natl. Cancer Inst.* 88(6):372–374.
 76. Rose, T. M., K. B. Strand, E. R. Schultz, G. Schaefer, G. W. Rankin Jr., M. E. Thouless, C. C. Tsai, and M. L. Bosch. 1997. Identification of two homologs of the Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in retroperitoneal fibromatosis of different macaque species. *J. Virol.* 71(5):4138–4144.
 77. Rosenblum, L. L., R. A. Weiss, and M. O. McClure. 2000. Virus load and sequence variation in simian retrovirus type 2 infection. *J. Virol.* 74(8):3449–3454.
 78. Schroder, M. A., S. K. Fisk, and N. W. Lerche. 2000. Eradication of simian retrovirus type D from a colony of cynomolgus, rhesus, and stump-tailed macaques by using serial testing and removal. *Contemp. Top. Lab. Anim. Sci.* 39(4):16–23.
 79. Schultz, K. T., C. Thomas, K. Toohey, and T. Curro. 1989. AELISA for detection of antibodies to a type D retrovirus, SRV-W Viral. *Immunology* 2(1):47–55.
 80. Sfakianos, J. N., R. A. LaCasse, and E. Hunter. 2003. The M-PMV cytoplasmic targeting-retention signal directs nascent Gag polypeptides to a pericentriolar region of the cell. *Traffic* 4(10):660–670.
 81. Shiigi, S., B. Wilson, G. Leo, N. MacDonald, D. Toyooka, L. Hallick, R. Karty, M. L. Belozer, W. McNulty, J. Wolff, A. van Bueren, C. Howard Jr., and M. Axthelm. 1989. Serologic and virologic analysis of type D simian retrovirus infection in a colony

- of Celebes black macaques (*Macaca nigra*). *J. Med. Primatol.* 18(3–4):185–193.
82. Smith, G. C., R. L. Heberling, R. J. Helmke, S. T. Barker, and S. S. Kalter. 1977. Oncornavirus-like particles in squirrel monkey (*Saimiri sciureus*) placenta and placenta culture. *Science* 195(4275):289–292.
83. Snasel, J., Z. Krejcik, V. Jencová, I. Rosenberg, T. Rumí, J. Alexandratos, A. Gustchina, and I. Pichová. 2005. Integrase of Mason–Pfizer monkey virus. *FEBS J.* 272(1):203–216.
84. Sommerfelt, M. A., N. Harkestad, and E. Hunter. 2003. The endogenous langur type D retrovirus PO-1-Lu and its exogenous counterparts in macaque and langur monkeys. *Virology* 315(2):275–282.
85. Sommerfelt, M. A. and R. A. Weiss. 1990. Receptor interference groups of 20 retroviruses plating on human cells. *Virology* 176(1):58–69.
86. Sonigo, P., C. Barker, E. Hunter, and S. Wain-Hobson. 1986. Nucleotide sequence of Mason-Pfizer monkey virus: an immunosuppressive D-type retrovirus. *Cell* 45(3):375–385.
87. Stromberg, K., R. E. Benveniste, L. O. Arthur, H. Rabin, W. E. Giddens Jr., H. D. Ochs, W. R. Morton, and C. C. Tsai. 1984. Characterization of exogenous type D retrovirus from a fibroma of a macaque with simian AIDS and fibromatosis. *Science* 224(4646):289–292.
88. Tabernero, C., A. S. Zolotukhin, J. Bear, R. Schneider, G. Karsenty, and B. K. Felber. 1997. Identification of an RNA sequence within an intracisternal-A particle element able to replace Rev-mediated posttranscriptional regulation of human immunodeficiency virus type 1. *J. Virol.* 71(1):95–101.
89. Tabernero, C., A. S. Zolotukhin, A. Valentin, G. N. Pavlakis, and B. K. Felber. 1996. The posttranscriptional control element of the simian retrovirus type 1 forms an extensive RNA secondary structure necessary for its function. *J. Virol.* 70(9):5998–6011.
90. Tailor, C. S., A. Nouri, Y. Zhao, Y. Takeuchi, and D. Kabat. 1999. A sodium-dependent neutral-amino-acid transporter mediates infections of feline and baboon endogenous retroviruses and simian type D retroviruses. *J. Virol.* 73(5):4470–4474.
91. Thayer, R. M., M. D. Power, M. L. Bryant, M. B. Gardner, P. J. Barr, and P. A. Luciw. 1987. Sequence relationships of type D retroviruses which cause simian acquired immunodeficiency syndrome. *Virology* 157(2):317–329.
92. Todaro, G. J., R. E. Benveniste, C. J. Sherr, J. Schlam, G. Schidlovsky, and J. R. Stephenson. 1978. Isolation and characterisation of a new type D retrovirus from the Asian primate, *Presbytis obscurus* (Spectacled Langur). *Virology* 84(1):189–194.
93. Tsai, C. C., W. E. Giddens Jr., W. R. Morton, S. L. Rosenkranz, H. D. Ochs, and R. E. Benveniste. 1985. Retroperitoneal fibromatosis and acquired immunodeficiency syndrome in macaques: epidemiologic studies. *Lab. Anim. Sci.* 35(5):460–464.
94. van der Kuyl, A. C., R. Mang, J. T. Dekker, and J. Goudsmit. 1997. Complete nucleotide sequence of simian endogenous type D retrovirus with intact genome organization: evidence for ancestry to simian retrovirus and baboon endogenous virus. *J. Virol.* 71(5):3666–3676.
95. Wiegand, H. L., G. A. Coburn, Y. Zeng, Y. Kang, H. P. Bogerd, and B. R. Cullen. 2002. Formation of Tap/NXT1 heterodimers activates Tap-dependent nuclear mRNA export by enhancing recruitment to nuclear pore complexes. *Mol. Cell. Biol.* 22(1):245–256.
96. Wilson, B. J., S. M. Shiigi, J. L. Zeigler, L. C. Olson, A. Malley, and C. F. Howard Jr. 1986. Transmission of simian acquired immunodeficiency syndrome with a type D retrovirus: immunological aspects. *Clin. Immunol. Immunopathol.* 41(3):453–460.
97. Wilusz, J. E. and K. L. Beemo. 2006. The negative regulator of splicing element of Rous sarcoma virus promotes polyadenylation. *J. Virol.* 80(19):9634–9640.
98. Wolfheim, J. H. (ed.) 1983. *Primates of the World*. Washington: University of Washington Press, pp. 611–614.
99. Zábranský, A., M. Andreánsky, O. Hruková-Heidingsfeldová, V. Havlík, E. Hunter, T. Rumí, and I. Pichová. 1998. Three active forms of aspartic proteinase from Mason–Pfizer monkey virus. *Virology* 245(2):250–256.
100. Zolotukhin, A. S., A. Valentin, G. N. Pavlakis, and B. K. Felber. 1994. Continuous propagation of RRE(–) and Rev(–)RRE(–) human immunodeficiency virus type 1 molecular clones containing a cis-acting element of simian retrovirus type 1 in human peripheral blood lymphocytes. *J. Virol.* 68(12):7944–7952.

7 Gammaretroviruses

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7.1. INTRODUCTION

Two simian gammaretroviruses are known: gibbon ape leukemia virus (GALV) and simian sarcoma virus type 1 (SSV-1). Both viruses are oncogenic. GALV/SSV research was at the forefront of virology in the 1970s and early 1980s, but after the discovery of HIV-1 the interest in these viruses waned. The state of knowledge in the GALV/SSV field has remained almost “frozen” since the late 1980s. The only progress that has been made is the identification of the GALV receptor³¹ and the intriguing finding of a close genetic relationship between GALV and koala retrovirus (KoRV).¹⁴ Meanwhile, many interesting questions remain unanswered and the research potential in the GALV/SSV field is far from being exhausted. Moreover, some of the gaps in our knowledge

of GALV/SSV can be easily filled with the use of modern methodologies.

7.2. CLASSIFICATION AND NOMENCLATURE

The official, International Committee on Taxonomy of Viruses (ICTV)-recognized names of GALV and SSV are *gibbon ape leukemia virus* (GALV) and *woolly monkey sarcoma virus* (WMSV). Both viruses are recognized as species within the *Gammaretrovirus* family. GALV is included in the mammalian gammaretroviruses group; WMSV is included in the replication-defective gammaretroviruses group. The WMSV name is rarely used; it became the official name of this viral species due to the priority rule in the naming of organisms. The alternative name of WMSV, simian sarcoma virus type 1 (SSV-1), is also recognized by the ICTV as the designation for WMSM isolates/strains, but not as a species name.

GALV and WMSV have the same genomic organization and type-C morphology as murine leukemia and sarcoma viruses, which are prototypic for the *Gammaretrovirus* family, hence, their classification as gammaretroviruses. In terms of the genomic sequence similarity, the closest to GALV/WMSV is the KoRV.¹⁴

Although they are not included in the ICTV classification, several strains of GALV are known: GALV-SF,²⁵ GALV-SEATO,²³ GALV-Br,⁴⁹ GALV-H,^{13,35} and simian sarcoma-associated virus (SSAV).⁵³

Only one isolate of WMSV/SSV-1 is known.⁴⁶ The stock of this virus contains two viruses: the replication-defective, acutely-transforming sarcoma virus (SSV-1 per se) and replication-competent, helper virus (a variant of GALV).⁵³

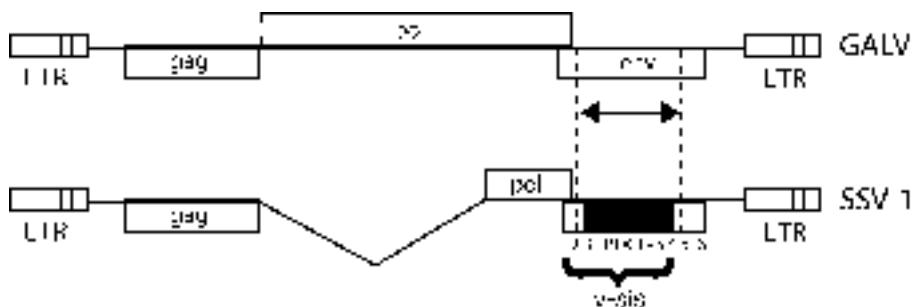


Figure 7.1. GALV and SSV-1 genome maps. GALV and SSV-1 genomes are collinear except for two regions. SSV has large deletions in *pol* and *env* genes. The deletion in the *env* gene is replaced with cell-derived sequences (double arrow). The structure of the rearranged *env* region is as follows: 1–2: 5'-end of the *env* gene (encodes 45 aa); 2–3: cellular sequence of unknown origin (encodes 6aa); 3–4: cellular sequence encoding PDGF-B (227 aa); 4–5: cellular sequences of unknown origin (untranslated); 5–6: 3'-end of the *env* gene [encodes almost the entire p15E(TM)]; p28^{Env-sis} protein is translated from the *v-sis* oncogene (1–4).

7.3. GIBBON APE LEUKEMIA VIRUS

7.3.1. Genomic Organization and Gene Products

The organization of the GALV genome is typical for the simple retroviruses. There are only four genes, *gag*, *pro*, *pol*, and *env*, which in the provirus are flanked by the identical LTRs (Figure 7.1).

The *gag* gene encodes the inner structural proteins p30, p12, p15, p10. The *pro* gene encodes viral protease. In the GALV/SSV literature, this gene is considered as a part of the *pol* gene. The *pol* gene proper encodes enzymes engaged in “manipulating” viral nucleic acids: reverse transcriptase, RnaseH, and integrase. The *env* gene encodes two envelope glycoproteins: the external (gp70) and transmembrane (p15E).

The length of the GALV provirus is 8,515 bb. The complete genomic sequence of two GALV isolates is known: GALV-SEATO (GenBank Acc. No. NC_001885) and GALV-X (GenBank Acc. No. U60065). It has to be mentioned that the first of these sequences⁵ is composite; that is, it is not entirely derived from the GALV-SEATO strain. A piece of this sequence at the 3'-end of *pol* gene (positions 4518–4926) originates from the GALV-SF strain. There is also a technicality which causes confusion: the GALV-SEATO sequence represents the RNA genome, not the provirus; that is, its structure is R-U5-gag-pro-pol-env-U3-R; hence, the difference between the size of this sequence (8,088 nt) and the size of the provirus. The sec-

ond full-length sequence belongs to the isolate GALV-X from a human cell line co-infected with HIV-1.³³ This GALV is most probably a laboratory contaminant originating from GALV-SF.

The GALV genome appears to be relatively stable. No changes, detectable by molecular hybridization of cDNA probes with the viral genomic RNA, are found in the virus serially passaged through three unrelated gibbons as well in virus spread horizontally in a gibbon colony.⁴³

The length of GALV-SEATO LTR is 496 bp (U3: 370 bp; R: 69 bp; U5: 57 bp). Immediately downstream to the 5'LTR (positions 126–142) there is the primer-binding site (PBS) which is complementary to tRNA_{Pro}. The *gag* gene of GALV-SEATO (positions 631–2190) encodes Gag polyprotein. The segments of this gene encoding mature Gag proteins are aligned as follows: 5'-p15(MA)-p12-p30(CA)-p10(NC)-3'. The *pro-pol* gene is located “head-in-tail” with the *gag* gene (positions 2194–5691). The *pro* segment encoding protease (PR) is located next to the *gag* gene. *Pro* is followed by the genes encoding reverse transcriptase/RnaseH and integrase, the *pol* gene per se. The start of the *env* gene (positions 5552–7555) is “encroached” by 140 bp into the 3'-end of the *pol*. Both LTRs are separated from the neighboring genes by stretches of nontranslated sequences (Figure 7.1).

The *gag* gene is translated as the Gag precursor protein Pr68 and as a part of the larger Gag-Pro-Pol

precursor Pr180. The latter is synthesized through a translational read-through mechanism. Viral protease cleaves the Gag precursor into four mature structural proteins: p30(CA), p15(MA), p10(NC), and p12; the predicted numbers of amino acids for these proteins are 259, 125, 66, and 70, respectively. The location in the virion and function of CA, MA, and NC proteins are presumed to be the same as in other retroviruses. The p12 protein is a peculiarity of gammaretroviruses. There are no data on function of this protein specifically in GALV. However, by the analogy with the better-studied murine leukemia virus (MuLV), the GALV-p12 may be involved in uncoating and release. The sequence motif important for the MuLV release (PPPY) is also present in GALV-p12. The antigenicity of p12 protein is type-specific, which is used for the immunochemical discrimination between different strains of GALV.⁵⁰ In contrast, the major structural protein GALV-p30 is group-specific antigenically for GALV and SSV-1.³⁴

The Pro-Pol precursor is autocleaved into p14(PR), p80(RT/RH), and p46(IN); the predicted numbers of amino acids for these proteins are 120, 658, and 387, respectively.

The primary translation product of the *env* gene is the Env precursor protein (Pr80). The Pr80 is cleaved by the cellular protease into gp70(SU) and p15E(TM) protein; the predicted numbers of amino acids for these proteins are 489 and 178, respectively. In a similar manner to p12, the antigenicity of gp70-GALV is type-specific.^{16,29} Neutralizing antibodies against GALV are directed against gp70. However, the major neutralization epitopes of this protein have not been mapped.

7.3.2. Overview of Replication Cycle In Vitro

The GALV replication cycle is not cytopidal and is presumed to be similar to that of simple nontransforming retroviruses. Data specific for GALV are available only for the virus receptor.^{12,17,31} The GALV receptor Pit1 (formerly named GLVR1) is a cellular protein, a sodium-dependent phosphate transporter. The Pit1 molecule has 10 transmembrane regions, 5 extracellular loops, and a large intracellular terminal domain. Receptor activity is mapped to the fourth extracellular loop. The residues 550 and 551, apparently, are critical for GALV binding.¹⁸ Interestingly, Pit1 is also used as a receptor by the feline leukemia virus, subtype B (FeLV-B).⁴⁴ The binding sites for GALV and FeLV-B are located in the same region of the receptor, but are not identical.

The host range of GALV in vitro is extremely broad. Cells of many species (human, rhesus, feline, canine, rabbit, mink, bat) can be infected. The exceptions are mouse cells which are nonpermissive for GALV.³⁰

7.3.3. Mode of Transmission and Prevalence of Infection

GALV infection is reported only in captive gibbons. For some reasons it is presumed that the virus is absent in wild gibbons. However, there are no relevant published data. The transmission of GALV is predominantly horizontal. The GALV viremic gibbons shed virus into urine and feces.²⁷ However, prenatal transmission also occurs.²⁸

Little is known about the prevalence of GALV and factors influencing the spread of the virus. GALV infection is recorded in several gibbon colonies in the United States, Thailand, and Bermuda.^{13,23,25,41,49} In one case, the virus was isolated from materials obtained from wild-caught gibbons 4 months after capture.⁴⁹ The prevalence of persistent GALV infection in diverse group of captive gibbons ($n = 53$) held in primate centers, zoos, and as pets in the United States was 11%. Another 26% (14/53) of animals in this sample showed evidence of prior exposure to the virus; that is, they were antibody-positive.²⁷

Whether or not species other than the gibbons can be infected with GALV is unclear. GALV infection is described only in gibbons. However, there are no published GALV surveys in other animals which share a habitat with gibbons. The virus has been inoculated into rhesus and squirrel monkeys, marmosets, pigs, dogs, rabbits, guinea pigs, rats, and mice. None of these animals developed leukemia; however, whether or not some of these animals became infected with GALV, at least transiently, was not reported.²⁶

No case of simian-to-human transmission of GALV is known,⁴² including those individuals exposed to GALV-positive gibbons.²⁹

7.3.4. Immune Responses

Apparently, antibody immune response against GALV in gibbons is efficient in clearing the virus: the virus cannot be recovered from blood of anti-GALV-positive gibbons ($n = 14$), whereas persistently viremic gibbons ($n = 6$) are antibody-negative.²⁷ Thus, it appears that viremia and antibody-positivity are mutually exclusive. Leukemia and other hematological malignancies

develop only in those animals which are viremic and antibody-negative.^{26,29}

No data are available on cellular and innate immunity against GALV.

7.3.5. Diagnostic Assays

The diagnosis of ongoing GALV infection can be achieved by the isolation of virus or detection of viral antigens or nucleic acids.

The presence of antibodies against GALV antigens indicates previous exposure and, possibly, protective immunity against GALV. Immunofluorescence and radioimmunoassay are used.^{23,24,29,34,50} Western blot and ELISA for GALV have not been described.

Isolation of GALV is relatively straightforward because there are many permanent cell lines which are highly permissive for the virus. An incomplete list of such cell lines includes human (NC-37, A204), rhesus (DBS-FRhL-1), mink (MvILu, 64J1), feline (FEC), canine (FCf2Th), rabbit (SIRC), bat (TBILu) cells, and many others.

Diagnostic polymerase chain reaction (PCR) for GALV would be the method of choice for detection of the virus. No such test has been described, but obviously such a PCR could be quickly developed if the need arises.

7.3.6. Oncogenicity

In the 1970s, a number of cases of hematological malignancies were described in gibbons.^{4,13,19,21,25,41} The largest outbreak was recorded in the SEATO Laboratory in Bangkok, where nine cases of chronic granulocytic leukemia were observed in a colony of 195 gibbons.^{4,25} All cases were shown to be positive for GALV.^{25,41} The cellular material from one of the gibbons with GALV-positive chronic granulocytic leukemia was serially passed in two juvenile gibbons. The chronic granulocytic leukemia developed in both animals after an incubation period of 14 months.^{4,26}

These data clearly suggested that GALV is oncogenic; however, this could not be considered as formal proof because the materials used for the inoculations were not cell-free. The only direct proof of GALV oncogenicity is the experimental induction of leukemia by tissue-culture grown, cell-free virus.²⁶ The virus (GALV-SEATO) was inoculated into two GALV-negative colony-born young gibbons (8 and 9 months old) by the intraperitoneal (IP) route. The animals received 10^5 and 10^3 TCID of the

virus, respectively. Five months after the first inoculation the animal that received the 10^3 TCID dose was reinoculated, this time intramedullary, with the same dose of the virus. Both inoculated animals developed persistent GALV viremia and the disease. In the animal inoculated with the larger dose of GALV, viremia was detected 1.5 months after the inoculation and persisted thereafter. The disease in this animal, described as "myeloproliferative," developed 11 months after the inoculation. The second animal became viremic 5 months after inoculation and developed chronic granulocytic leukemia, also 11 months after the first inoculation. Interestingly, similar bone proliferative lesions developed in both animals.

Two older gibbons (both 14 months old) were inoculated with the same stock of virus, one IP and the other, IP and intramedullary. The same dose (10^3 TCID) was used. These animals developed vigorous antibody responses against GALV. Anti-GALV antibodies were first detected 2 weeks after inoculation and persisted thereafter. No viremia was observed in these gibbons during 3 years of observations. Apparently, the age at which the virus is acquired is critical for determining the outcome. However, the number of observations is too small to draw definitive conclusions.

Unfortunately, since 1980, no attempt has been made to explore this interesting model of leukemia. The mechanism of GALV oncogenicity and its apparent "tropism" to the granulocytic lineage remain unknown.

7.3.7. Origin of GALV

The origin of GALV is enigmatic. Whether or not the virus is present in wild gibbons is unknown. The virus is clearly exogenous for gibbons and the infection is prevalent in various unlinked groups of captive gibbons. Several unequivocally distinguishable strains of GALV have been isolated independently.^{13,23,25,35,49} In each GALV-positive gibbon colony the animals are infected with a different strain of GALV. The closest "relative" of GALV is the virus from the koala, the animal extremely distant from gibbons and separated geographically.¹⁴ The entire genome similarity of GALV and KoRV is approximately 80%, which is just slightly less than the genomic similarity between different strains of GALV. Interestingly, the KoRV is endogenous in some natural koala populations, whereas other populations are either virus-free or only partially infected.⁴⁵ Thus, apparently, the "endogenization" of KoRV is an ongoing process.

The origin of GALV and KoRV is unknown. Three South Asian rodents (*Mus caroli*, *M. cervicolor*, and *Vandeleuria oleracea*) were considered as the candidates, based on the isolation of GALV-related endogenous xenotropic retroviruses.^{1,3,30} However, the exact degree of homology between these viruses and GALV/KoRV is unknown; their genomic sequences are not available. Judging by the molecular cross-hybridization data, the similarity between these endogenous rodent viruses and GALV is substantially less than would be expected in the case of recent interspecies transmission. It is also difficult to explain how these endogenous viruses, which were recovered in the infectious form by an artificial procedure involving chemical induction, can be transmitted to other species under natural conditions. The interspecies transmission of these viruses to gibbons seems particularly unrealistic because it should have happened at least several times.

7.3.8. GALV as a Vector for Gene Therapy

GALV is potentially useful for construction of retroviral vectors, mainly because of its wide host range.¹¹ However, GALV-based vectors are not being considered for use in clinical trials.

7.4. WOOLLY MONKEY SARCOMA VIRUS OR SIMIAN SARCOMA VIRUS TYPE 1

This virus was isolated from the woolly monkey (*Lagothrix* spp.) fibrosarcoma.^{46,52,53} The tumor contained type-C viral particles and could be transmitted to marmosets by cell-free extracts.^{46,47,52} Similarly to the murine and feline sarcoma viruses, two agents are found in the woolly monkey sarcoma virus stock.⁵³ The first is sarcoma virus per se, most commonly named SSV-1. SSV-1 is an acutely transforming virus; it transforms *in vitro* fibroblasts from various primate species, including humans. The transforming activity of SSV-1 can be quantitatively measured by the focus transformation assay.⁵³ The transforming activity of SSV-1 directly correlates with the ability of the virus to cause sarcomas *in vivo*. SSV-1 is replication-defective. It can be transmitted serially only together with the helper virus, the simian sarcoma-associated virus 1 (SSAV-1). SSAV is present in excess in SSV-1 stocks and can be considered as one of the GALV strains. SSAV envelope antigens are distinct from those of all known GALV strains, although in the case of GALV-S, cross-reactivity is substantial.⁴⁸ The comparison of SSV-1 and GALV genomes leaves

no doubt that SSV-1 originated from GALV (Sections 7.4.1 and 7.4.2). An obvious question is: how did GALV infect the woolly monkey in which SSV-1-positive sarcoma developed? Apparently, the explanation is simple: the gibbon and woolly monkey were kept as pets in the same household. Presumably, this gibbon was GALV-positive, although direct evidence is absent.

7.4.1. Genomic Organization

The genome of SSV-1 spans 5,157 bp (GenBank Acc. No. NC_009424). The SSV-1 genome is collinear with the GALV genome, except for two regions (Figure 7.1).^{6-8,37,38} The 5'-parts of the SSV-1 and GALV genomes (LTR-gag) are structurally identical. The first difference is a large deletion in the *pro-pol* region. The *pro* gene and most of the *pol* gene are deleted; the deletion is about 2,600 bp. The only part of the GALV *pol* gene retained in the SSV-1 genome is the truncated integrase gene. Most of the *env* gene is also deleted in the SSV-1 genome. In contrast to the *pol* deletion, the *env* deletion is substituted with a cell-derived sequence, named *v-sis* (Section 7.4.2). This cell-derived sequence is responsible for the transforming and sarcomogenic activity of this virus; that is, *v-sis* is an oncogene. The *v-sis* sequence is framed by the “remains” of the *env* gene, “left” (positions 3220–3354) and “right” (positions 4380–4613), at the 5'- and 3'-end of the *v-sis*, respectively. The left *env* piece, encoding 45 amino acids, is in frame with the *v-sis*. It is transcribed and translated together with *v-sis* as a fusion protein p28^{Env-Sis}. The right *env* piece encodes almost the entire p15E(TM). This *env* sequence does not contribute to the coding sequence of p28^{Env-Sis} and it is not clear whether it is expressed as a separate gene. The parts of the SSV-1 and GALV genomes located downstream to *v-sis* are structurally identical.

7.4.2. Oncogene *v-sis* and the Origin of simian sarcoma virus type 1

The hallmark of *v-sis* as an oncogene is the homology with platelet-derived growth factor (PDGF). The discovery of this homology attracted a great deal of publicity in the early 1980s as the first direct link between oncogenes and growth factors.^{10,36,51} This property of *v-sis* became a textbook fact. At the same time many details of the protein structure, mechanisms of synthesis, and action remain unknown.

The product of *v-sis*, the v-Sis protein (p28^{Env-sis}), is as its name suggests, composed of two parts, Env (45 aa) and Sis (227 aa).^{7,9,39} A common misinterpretation is that the whole Sis part of p28^{Env-sis} is homologous to PDGF. In fact, the PDGF homologous sequence (positions 3374–4035) is framed by two non-PDGF cellular sequences (positions 3355–3373 and 4036–4379, at the 3'- and 5'-end, respectively). Six amino acids encoded by the shorter non-PDGF cellular sequence are included in the p28^{Env-sis}. The longer cell-derived sequence located after the stop codon of PDGF is, presumably, not expressed.

After translation, p28^{Env-sis} is dimerized and glycosylated.^{15,32,39} The dimerization is required for its transforming activity, apparently.

The PDGF moiety in v-Sis is equivalent to the PDGF B chain.^{10,20,22,51} Through this moiety, v-Sis binds to the PDGF receptor and triggers the PDGF mitogenic pathway. Ultimately, it leads to autocrine stimulation of host cell proliferation. Cells lacking a PDGF receptor are not transformed by SSV-1. Interestingly, the entire PDGF moiety is not required for transformation. The “minimal oncogene” PDGF is mapped to the region between residues 127-214 in v-Sis.⁴⁰

V-sis was presumably acquired from the genome of the woolly monkey (*Lagothrix* spp.). Interestingly, this “textbook fact” has not been proved formally: no direct comparison of woolly monkey PDGF and *v-sis* sequences has been done. The closest homolog of *v-sis* is a fragment of rhesus monkey PDGF B-chain gene (94% similarity). The homology of *v-sis* to other known primate PDGF sequences, human and chimpanzee, is 93%. The exact origin of the non-PDGF cell-derived sequences within the SSV-1 genome is not known.

The PDGF-derived viral oncogene is not unique for SSV-1. A similar oncogene is present in the genome of Parodi-Irgens feline sarcoma virus. Presumably, it was acquired from the cat genome.²

7.5. SUMMARY

There are two simian gammaretroviruses—GALV and SSV-1, also named WMSV. The distinguishable strains of GALV circulated in different groups of captive gibbons, but there is no evidence that the virus is present in wild gibbons. GALV infection is clearly exogenous in gibbons. Hence, it is believed that captive gibbons acquired GALV from some as yet unidentified species. The candidate natural hosts of GALV are Asian rodents car-

rying endogenous retroviruses related to GALV. However, this hypothesis is still far from being proved and the origin of GALV and its closest relative, the KoRV, remains enigmatic. GALV infection in the gibbons has alternative outcomes: clearance of the virus or establishment of persistent infection. The animals that clear the virus remain anti-GALV-positive and, presumably, immune. Some gibbons persistently infected with GALV develop hematological malignancies, most commonly myelogenous leukemia. How, and from which source, the unrelated groups of captive gibbons became infected with GALV remains unknown. Also unknown is the mechanism of GALV-induced oncogenesis and the factors underlying the apparently unique susceptibility of gibbons to GALV infection.

SSV-1 is presumed to have originated through the unrecognized infection of a woolly monkey with GALV, resulting in the recombination of GALV and cellular genomes. GALV lost its *pro* gene and most of the *pol* and *env* gene and acquired a cellular gene, the B subunit of PDGF. The PDGF sequence incorporated into the viral genome is named *v-sis*. *V-sis* is an oncogene responsible for the acutely transforming and sarcomogenic activity of SSV-1. Due to the absence of the *env*, *pro*, and *pol* genes, SSV-1 is a replication-defective virus. Its replication is possible only in the presence of helper virus, the “parent” GALV variant, called SSAV-1. Whether the “creation” of SSV-1 is a truly stochastic event, or GALV is prone to recombine with PDGF, is not known.

REFERENCES

1. Benveniste, R. E., R. Callahan, C. J. Sherr, V. Chapman, and G. J. Todaro. 1977. Two distinct endogenous type C viruses isolated from the Asian rodent *Mus cervicolor*: conservation of virogene sequences in related rodent species. *J. Virol.* 21(3):849–862.
2. Besmer, P., H. W. Snyder Jr., J. E. Murphy, W. D. Hardy Jr., and A. Parodi. 1983. The Parodi-Irgens feline sarcoma virus and simian sarcoma virus have homologous oncogenes, but in different contexts of the viral genomes. *J. Virol.* 46(2):606–613.
3. Callahan, R., C. Meade, and G. J. Todaro. 1979. Isolation of an endogenous type C virus related to the infectious primate type C viruses from the Asian rodent *Vandeleuria oleracea*. *J. Virol.* 30(1):124–131.
4. De Paoli, A. and D. O. Johnsen. 1973. Granulocytic leukemia in whitehanded gibbons. *J. Am. Vet. Med. Assoc.* 163(6):624–628.

5. Delassus, S., P. Sonigo, and S. Wain-Hobson. 1989. Genetic organization of gibbon ape leukemia virus. *Virology* 173(1):205–213.
6. Devare, S. G., E. P. Reddy, J. D. Law, and S. A. Aaronson. 1982. Nucleotide sequence analysis of the long terminal repeat of integrated simian sarcoma virus: evolutionary relationship with other mammalian retroviral long terminal repeats. *J. Virol.* 42(3):1108–1113.
7. Devare, S. G., E. P. Reddy, J. D. Law, K. C. Robbins, and S. A. Aaronson. 1983. Nucleotide sequence of the simian sarcoma virus genome: demonstration that its acquired cellular sequences encode the transforming gene product p28sis. *Proc. Natl. Acad. Sci. U. S. A.* 80(3):731–735.
8. Devare, S. G., E. P. Reddy, K. C. Robbins, P. R. Andersen, S. R. Tronick, and S. A. Aaronson. 1982. Nucleotide sequence of the transforming gene of simian sarcoma virus. *Proc. Natl. Acad. Sci. U. S. A.* 79(10):3179–3182.
9. Devare, S. G., A. Shatzman, K. C. Robbins, M. Rosenberg, and S. A. Aaronson. 1984. Expression of the PDGF-related transforming protein of simian sarcoma virus in *E. coli*. *Cell* 36(1):43–49.
10. Doolittle, R. F., M. W. Hunkapiller, L. E. Hood, S. G. Devare, K. C. Robbins, S. A. Aaronson, and H. N. Antoniades. 1983. Simian sarcoma virus onc gene, v-sis, is derived from the gene (or genes) encoding a platelet-derived growth factor. *Science* 221(4607):275–277.
11. Eglitis, M. A., R. D. Schneiderman, P. M. Rice, and M. V. Eiden. 1995. Evaluation of retroviral vectors based on the gibbon ape leukemia virus. *Gene Ther.* 2(7):486–492.
12. Farrell, K. B. and M. V. Eiden. 2005. Dissection of gammaretroviral receptor function by using type III phosphate transporters as models. *J. Virol.* 79(14):9332–9336.
13. Gallo, R. C., R. E. Gallagher, F. Wong-Staal, T. Aoki, P. D. Markham, H. Schetters, F. Ruscetti, M. Valevrio, M. J. Walling, R. T. O'Keeffe, W. C. Saxinger, R. G. Smith, D. H. Gillespie, and M. S. Reitz Jr. 1978. Isolation and tissue distribution of type-C virus and viral components from a gibbon ape (*Hylobates lar*) with lymphocytic leukemia. *Virology* 84(2):359–373.
14. Hanger, J. J., L. D. Bromham, J. J. McKee, T. M. O'Brien, and W. F. Robinson. 2000. The nucleotide sequence of koala (*Phascolarctos cinereus*) retrovirus: a novel type C endogenous virus related to gibbon ape leukemia virus. *J. Virol.* 74(9):4264–4272.
15. Hannink, M., M. K. Sauer, and D. J. Donoghue. 1986. Deletions in the C-terminal coding region of the v-sis gene: dimerization is required for transformation. *Mol. Cell Biol.* 6(4):1304–1314.
16. Hino, S., J. R. Stephenson, and S. A. Aaronson. 1975. Antigenic determinants of the 70,000 molecular weight glycoprotein of woolly monkey type C RNA virus. *J. Immunol.* 115(4):922–927.
17. Johann, S. V., J. J. Gibbons, and B. O'Hara. 1992. GLVR1, a receptor for gibbon ape leukemia virus, is homologous to a phosphate permease of *Neurospora crassa* and is expressed at high levels in the brain and thymus. *J. Virol.* 66(3):1635–1640.
18. Johann, S. V., M. van Zeijl, J. Cekleniak, and B. O'Hara. 1993. Definition of a domain of GLVR1 which is necessary for infection by gibbon ape leukemia virus and which is highly polymorphic between species. *J. Virol.* 67(11):6733–6736.
19. Johnsen, D. O., W. L. Wooding, P. Tantcharoeynos, and C. H. Bourgeois Jr. 1971. Malignant lymphoma in the gibbon. *J. Am. Vet. Med. Assoc.* 159(5):563–566.
20. Johnsson, A., C. H. Heldin, A. Wasteson, B. Westermark, T. F. Deuel, J. S. Huang, P. H. Seeburg, A. Gray, A. Ullrich, G. Scrace, P. Stroobant, and M. D. Waterfield. 1984. The c-sis gene encodes a precursor of the B chain of platelet-derived growth factor. *EMBO J.* 3(5):921–928.
21. Jones, M. D., D. T. Lau, and J. Warthen. 1972. Lymphoblastic lymphosarcoma in two white-handed gibbons, *Hylobates lar*. *J. Natl. Cancer Inst.* 49(2):599–601.
22. Josephs, S. F., C. Guo, L. Ratner, and F. Wong-Staal. 1984. Human-proto-oncogene nucleotide sequences corresponding to the transforming region of simian sarcoma virus. *Science* 223(4635):487–491.
23. Kawakami, T. G. and P. M. Buckley. 1974. Antigenic studies on gibbon type-C viruses. *Transplant. Proc.* 6(2):193–196.
24. Kawakami, T. G., P. M. Buckley, A. dePaoli, W. Noll, and L. K. Bustad. 1975. Studies on the prevalence of type C virus associated with gibbon hematopoietic neoplasms. *Bibl. Haematol.* 40:385–389.
25. Kawakami, T. G., S. D. Huff, P. M. Buckley, D. L. Dungworth, S. P. Synder, and R. V. Gilden. 1972. C-type virus associated with gibbon lymphosarcoma. *Nat. New Biol.* 235(58):170–171.
26. Kawakami, T. G., G. V. Kollias Jr., and C. Holmberg. 1980. Oncogenicity of gibbon type-C myelogenous leukemia virus. *Int. J. Cancer* 25(5):641–646.
27. Kawakami, T. G., L. Sun, and T. S. McDowell. 1977. Infectious primate type-C virus shed by healthy gibbons. *Nature* 268(5619):448–450.

28. Kawakami, T. G., L. Sun, and T. S. McDowell. 1978. Natural transmission of gibbon leukemia virus. *J. Natl. Cancer Inst.* 61(4):1113–1115.
29. Krakower, J. M., S. R. Tronick, R. E. Gallagher, R. C. Gallo, and S. A. Aaronson. 1978. Antigenic characterization of a new gibbon ape leukemia virus isolate: seroepidemiologic assessment of an outbreak of gibbon leukemia. *Int. J. Cancer* 22(6):715–720.
30. Lieber, M. M., C. J. Sherr, G. J. Todaro, R. E. Benveniste, R. Callahan, and H. G. Coon. 1975. Isolation from the Asian mouse *Mus caroli* of an endogenous type C virus related to infectious primate type C viruses. *Proc. Natl. Acad. Sci. U. S. A.* 72(6):2315–2319.
31. O'Hara, B., S. V. Johann, H. P. Klinger, D. G. Blair, H. Robinson, K. J. Dunn, P. Sass, S. M. Vitek, and T. Robins. 1990. Characterization of a human gene conferring sensitivity to infection by gibbon ape leukemia virus. *Cell Growth Differ.* 1(3):119–127.
32. Owen, A. J., P. Pantazis, and H. N. Antoniades. 1984. Simian sarcoma virus-transformed cells secrete a mitogen identical to platelet-derived growth factor. *Science* 225(4657):54–56.
33. Parent, I., Y. Qin, A. T. Vandenbroucke, C. Walon, N. Delferriere, E. Godfroid, and G. Burtonboy. 1998. Characterization of a C-type retrovirus isolated from an HIV infected cell line: complete nucleotide sequence. *Arch. Virol.* 143(6):1077–1092.
34. Parks, W. P., E. M. Scolnick, M. C. Noon, C. J. Watson, and T. G. Kawakami. 1973. Radioimmunoassay of mammalian type-C polypeptides. IV. Characterization of woolly monkey and gibbon viral antigens. *Int. J. Cancer* 12(1):129–137.
35. Reitz, M. S., Jr., F. Wong-Staal, W. A. Haseltine, D. G. Kleid, C. D. Trainor, R. E. Gallagher, and R. C. Gallo. 1979. Gibbon ape leukemia virus-Hall's Island: new strain of gibbon ape leukemia virus. *J. Virol.* 29(1):395–400.
36. Robbins, K. C., H. N. Antoniades, S. G. Devare, M. W. Hunkapiller, and S. A. Aaronson. 1983. Structural and immunological similarities between simian sarcoma virus gene product(s) and human platelet-derived growth factor. *Nature* 305(5935):605–608.
37. Robbins, K. C., S. G. Devare, and S. A. Aaronson. 1981. Molecular cloning of integrated simian sarcoma virus: genome organization of infectious DNA clones. *Proc. Natl. Acad. Sci. U. S. A.* 78(5):2918–2922.
38. Robbins, K. C., S. G. Devare, E. P. Reddy, and S. A. Aaronson. 1982. In vivo identification of the transforming gene product of simian sarcoma virus. *Science* 218(4577):1131–1133.
39. Sauer, M. K. and D. J. Donoghue. 1988. Identification of nonessential disulfide bonds and altered conformations in the v-sis protein, a homolog of the B chain of platelet-derived growth factor. *Mol. Cell Biol.* 8(3):1011–1018.
40. Sauer, M. K., M. Hannink, and D. J. Donoghue. 1986. Deletions in the N-terminal coding region of the v-sis gene: determination of the minimal transforming region. *J. Virol.* 59(2):292–300.
41. Snyder, S. P., D. L. Dungworth, T. G. Kawakami, E. Callaway, and D. T. Lau. 1973. Lymphosarcomas in two gibbons (*Hylobates lar*) with associated C-type virus. *J. Natl. Cancer Inst.* 51(1):89–94.
42. Stephenson, J. R. and S. A. Aaronson. 1976. Search for antigens and antibodies crossreactive with type C viruses of the woolly monkeys and gibbon ape in animal models and in humans. *Proc. Natl. Acad. Sci. U. S. A.* 73(5):1725–1729.
43. Sun, L., T. G. Kawakami, and S. I. Matoba. 1978. Genomic stability of gibbon oncornaovirus. *J. Virol.* 28(3):767–771.
44. Takeuchi, Y., R. G. Vile, G. Simpson, B. O'Hara, M. K. Collins, and R. A. Weiss. 1992. Feline leukemia virus subgroup B uses the same cell surface receptor as gibbon ape leukemia virus. *J. Virol.* 66(2):1219–1222.
45. Tarlinton, R. E., J. Meers, and P. R. Young. 2006. Retroviral invasion of the koala genome. *Nature* 442(7098):79–81.
46. Theilen, G. H., D. Gould, M. Fowler, and D. L. Dungworth. 1971. C-type virus in tumor tissue of a woolly monkey (*Lagothrix* spp.) with fibrosarcoma. *J. Natl. Cancer Inst.* 47(4):881–889.
47. Theilen, G. H., L. G. Wolfe, H. Rabin, F. Deinhardt, D. L. Dungworth, M. E. Fowler, D. Gould, and R. Cooper. 1973. Biological studies in four species of nonhuman primates with simian sarcoma virus (*Lagothrix*). *Bibl. Haematol.* 39:251–257.
48. Ting, Y. T., C. A. Wilson, K. B. Farrell, G. J. Chaudry, and M. V. Eiden. 1998. Simian sarcoma-associated virus fails to infect Chinese hamster cells despite the presence of functional gibbon ape leukemia virus receptors. *J. Virol.* 72(12):9453–9458.
49. Todaro, G. J., M. M. Lieber, R. E. Benveniste, and C. J. Sherr. 1975. Infectious primate type C viruses: three isolates belonging to a new subgroup from the brains of normal gibbons. *Virology* 67(2):335–343.
50. Tronick, S. R., J. R. Stephenson, S. A. Aaronson, and T. G. Kawakami. 1975. Antigenic characterization of type C RNA virus isolates of gibbon apes. *J. Virol.* 15(1):115–120.
51. Waterfield, M. D., G. T. Scrase, N. Whittle, P. Stroobant, A. Johnsson, A. Wasteson, B. Westermark, C. H. Heldin, J. S. Huang, and T. F. Deuel. 1983.

- Platelet-derived growth factor is structurally related to the putative transforming protein p28sis of simian sarcoma virus. *Nature* 304(5921):35–39.
52. Wolfe, L. G., F. Deinhardt, G. H. Theilen, H. Rabin, T. Kawakami, and L. K. Bustad. 1971. Induction of tumors in marmoset monkeys by simian sarcoma virus, type 1 (Lagothrix): a preliminary report. *J. Natl. Cancer Inst.* 47(5):1115–1120.
53. Wolfe, L. G., R. K. Smith, and F. Deinhardt. 1972. Simian sarcoma virus, type 1 (Lagothrix): focus assay and demonstration of nontransforming associated virus. *J. Natl. Cancer Inst.* 48(6):1905–1908.

8

Deltaretroviruses

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- 8.9 Summary

8.1. INTRODUCTION

The search for a human oncogenic retrovirus was a dominant theme in virological research in the 1970s. The discovery of human T-cell lymphoma/leukemia virus type 1 (HTLV-1) in 1980 was the culmination of this search.¹⁰⁶ HTLV-1, as its name suggests, is a cause of the malignancy named adult T-cell lymphoma/leukemia (ATL or ATLL). The virus also causes inflammatory syndromes, most notably, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and some other diseases (reviewed in Verdonck *et al.*¹⁴⁶).

Shortly after discovery of HTLV-1, closely related viruses were isolated from Asian^{95–97} and African monkeys.¹⁶¹ These viruses were named simian T-lymphotropic virus type 1 (STLV-1) by analogy with the human prototype. Subsequently two other simian deltaretroviruses were discovered, simian lymphotoic virus types 2 and 3 (STLV-2 and STLV-3). Both have human counterparts, HTLV-2 and HTLV-3, respectively. HTLVs and STLVs share many properties—hence their designation as primate T-lymphotropic viruses (PTLVs). The most extensively studied simian PTLV is STLV-1. This virus is harbored by many Old World monkeys and ape species. Perhaps the most interesting feature of STLV-1 is its oncogenicity. STLV-1 is associated with simian T-cell lymphomas in a way that is strikingly similar to the association between HTLV-1 and adult T-cell lymphomaleukemia in humans.^{133,153,154,163}

The most actively developing area of STLV research is analysis of phylogenetic relationships among STLVs and HTLVs. The close phylogenetic relationship between HTLVs and STLVs strongly points to the simian origin of HTLVs.^{21,61,116,141,152}

This chapter consists of two parts. Properties which are presumably common to human and simian viruses are described in the first part (Sections 8.2–8.4). The second part is devoted to the specific characteristics of each STLV type (Sections 8.5–8.8).

8.2. CLASSIFICATION AND NOMENCLATURE

Deltaretroviruses belong to the genus *Deltaretrovirus*, subfamily *Orthoretrovirinae*, family *Retroviridae*. In the latest International Committee on Taxonomy of Viruses (ICTV) classification (8th Report), this genus includes three primate viral species: *Primate T-lymphotropic virus types 1, 2, and 3* (PTLV-1, PTLV-2, and PTLV-3).

Each PTLV species includes both human and simian viruses which are classified as isolates/strains, HTLV-1, HTLV-2, HTLV-3 and STLV-1, STLV-2, STLV-3, respectively. Morphologically PTLVs are indistinguishable; they are classified as type C.

The nomenclature of STLVs is not formalized. Most of the designations currently in use have an ad hoc basis. As a rule, the isolate name includes information on the host species of origin, usually as a three-letter abbreviation. The abbreviations of both scientific and common names of simian species are used. For example, STLV-1-PtrA204 is an isolate from a chimpanzee (*Pan troglodytes*; Ptr) whereas STLV-1-Agm1968 is an isolate from an African green monkey (Agm).

8.3. GENERAL CHARACTERISTICS

This section describes properties of PTLVs which are assumed to be common for both human and simian viruses, with the caveat that most of these properties were established in HTLV-1 studies and direct proof of their veracity for other HTLV types, as well as for STLV, is frequently lacking.

8.3.1. Genomic Organization and Gene Products

The size of the PTLV genome is about 9,000 bp. The organization of the genome is similar for all PTLVs (Figure 8.1).

The coding part of the PTLV genome is flanked by identical long terminal repeats (LTRs). The boundaries between the U3, R, and U5 regions of the LTR are well

conserved in all PTLVs. There are a number of functionally important sequence elements within the PTLV LTR. Some of them are common with other retroviruses, the others, such as the Tax response elements (TRE) and the Rex response elements (RxRE), are specific to PTLVs.

The *gag* gene encodes the core proteins CA, MA, and NC whereas the *env* gene encodes the two envelope proteins SU and TM. The *pro* gene encodes the viral protease (PR). The *pol* gene encodes three enzymes: reverse transcriptase (RT), Ribonuclease H (RH), and integrase (IN).

Mature Gag proteins p24(CA), p19(MA), and p15(NC) are derived from three precursors: Gag (Pr55), Gag-Pro (Pr76), and Gag-Pro-Pol (Pr180). Each of the individual Gag proteins is required for assembly of infectious virions. The precursors are translated from the genomic RNA by the use of one and two ribosomal frameshifts for Gag-Pro and Gag-Pro-Pol, respectively. The molar ratio of Pr55:Pr76:Pr180 is estimated for HTLV-1 at 100:10:1.⁷⁸ The order of the individual Gag proteins is NH₂-MA-CA-NC-COOH. The N-terminal p19(MA) protein is localized at the inner surface of the virion envelope. Its role in the formation of virions is not clear. The structural protein p24(CA) is the major building block of the nucleocapsid.⁵⁵ The C-terminal p15(NC) protein is a nucleic acid-binding protein. In virions, NC is attached to the genomic RNA.

The protease p14(PR) is derived from the Gag-Pro and Pro-Pol precursors. The Leu/Pro cleavage site separating PR and RT is conserved in all PTLVs. The Pol precursor is cleaved into two proteins, the RTR/Nase

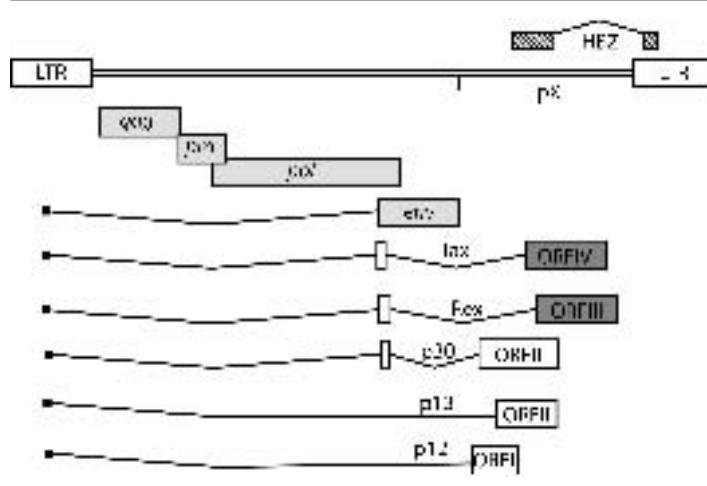


Figure 8.1. Map of PTLV genome and major spliced transcripts. *gag*, *pro*, *pol*, and *env*, structural genes; pX, genomic region containing major exons of regulatory auxiliary proteins; HBZ, exons in anti-sense strand encoding HBZ protein; Env protein is encoded by a single-spliced transcript; Tax, Rex, and p30, regulatory proteins encoded by double-spliced transcripts; p13 and p12, regulatory proteins encoded by single-spliced transcripts.

Table 8.1. Nomenclature and Major Characteristics of PTLV Genes Located in the pX Region and Their Products

ORF	Splicing Pattern	Products*	Major Function
ORF I	Single	p12 ^I	?
	Double	p12 ^{I-2}	
ORF II	Single	p13 ^{II}	?
	Double	p30 ^{II}	
ORF III/ rex	Double	p27 ^{Rex}	Inhibiting double splicing; transporting genomic and singly-spliced RNA to cytoplasm ?
	Single	Rex p21 ^{Rex}	
ORF IV/tax	Double	p40 ^{Tax} Tax	Trans-activating of viral and some cellular genes expression
HBZ	Unspliced	HBZ [†] , p31	Suppression of Tax expression
	Single	HBZ-SI [‡]	

? means “function is unknown.”

*Proteins identified in HTLV-1-infected/transformed cells.

[†]HTLV-1 bZIP factor.

[‡]Spliced isoforms of HBZ.

H complex p95(RT/RH) and the integrase p15(IN). The Env precursor is translated from a singly-spliced mRNA. After glycosylation it is cleaved by a cellular protease into two mature envelope glycoproteins, gp46(SU) and gp21(TM). In the virion, envelope proteins gp46 and gp21 are noncovalently linked and the gp46+21 glycoprotein complex is arranged as a trimeric structure in which gp46 is facing outwards and gp21 spans the envelope lipid bilayer. The receptor-binding site (RBS) is located within the N-terminal part of gp46.⁵⁹ Although the sequence of the RBS is quite stable, some variation in this domain is reported.⁵⁸

The genomic region located between the *env* gene and 3'-LTR contains several overlapping open reading frames (ORFs) encoding nonstructural regulatory proteins (Figure 8.1). Initially, this part of the PTLV genome was named pX, and this designation is still sometimes used. The nomenclature of pX ORFs and their products is complicated and can be confusing (Table 8.1). Four ORFs (I through IV) are present in all PTLV genomes.^{37,115,126,130} The most important pX genes, *tax* (ORF IV) and *rex* (ORF III), encode the Tax (p40^{Tax}) and Rex (p27^{Rex}) proteins, respectively. More precisely, the sequences within ORF IV and ORF III make up the major (second) exons of the *tax* and *rex* genes, respectively. The first exon of both of these genes is located within the *env* gene. ORF III is located entirely within ORF IV, but utilizes a different reading frame. Hence the alternative name for ORF IV: the *tax/rex* region.

In addition to the full-length Rex protein, ORF III also encodes a truncated form of the Rex protein (p21^{Rex}).

8.3.2. Overview of Replication Cycle

Little if any infectious cell-free PTLV is produced *in vivo*. *In vitro* these viruses are also highly cell-associated and cell-to-cell contact is required for transmission of the infection. It has been shown that HTLV-1 moves from an infected cell to an uninfected cell through a special junction structure designated the “virological synapse” (VS).^{5,50} This feature of HTLV-1 is probably shared by other PTLVs, although direct proof is lacking. Exactly how the VS operates is largely hypothetical. However, it is clear that the cytoskeleton structure “the microtubule organizing center” (MTOC) is involved in some way.

Identification of the receptor for PTLV was elusive for many years. The HTLV-1 receptor was recently identified as the glucose transporter protein GLUT1.^{85,86} It is likely that the same molecule functions as the receptor for STLV-1. Detailed mechanisms of PTLV uncoating, reverse transcription, and transport of the provirus into the nucleus are unknown. By default they are presumed to be typical for the retroviruses.

PTLV proviruses integrate at random chromosomal sites.²⁴ The transcription pattern of PTLV provirus includes complex splicing that takes place in the nucleus. Viral transcripts fall into three categories: unspliced, singly-, and doubly-spliced. Unspliced

full-length genome transcripts include RNA molecules which are (1) packaged into virions as genomic RNA and (2) mRNAs which serve as templates for translation of the precursor polyproteins Gag, Gag-Pro, and Gag-Pro-Pol. The Gag-Pro and Gag-Pro-Pol polyproteins are translated using ribosomal frameshifts.

The major singly-spliced transcript is the mRNA encoding the Env precursor protein. Some pX HTLV-1 transcripts are also singly-spliced (Table 8.1). At least some of these pX transcripts are presumed to be shared by STLVs. However, direct evidence is largely absent.

The most important of the double-spliced transcripts encode Tax and Rex proteins. The first of these proteins activates *in-trans* the promoter controlling expression of the PTLV structural genes as well as expression of some cellular genes. The Rex protein shifts the splicing balance toward formation of unspliced and single-spliced mRNAs as well as facilitating transport of such RNAs from the nucleus to the cytoplasm. Ultimately, this activity of Rex results in the preferential synthesis of the structural proteins required for assembly of virions.

Very little is known about the assembly and egress of PTLVs. However, it is clear that cleavage of the Gag and Env precursor proteins is required for formation of infectious virions. The cleavage of these two precursor proteins is performed by viral and cellular proteases, respectively. The exact timing of these events and how they are coordinated are not known. The assembly of HTLV-1 virions starts by accumulation of Gag and Env proteins in the vicinity of cell-to-cell contact points and is coordinated with formation of the VS. Egress and entry are accompanied by reorganization of the cytoskeleton in and near the VS.^{5,50}

PTLVs rarely go through a complete replication cycle *in vivo*. Rather, PTLVs use an alternative replication strategy of copying the provirus by cellular DNA polymerase complexes together with the cellular chromosomal DNA before each cell division.^{33,155} Such cell-associated replication ensures the remarkable stability of PTLV genomes because cellular DNA polymerases are much less error-prone than is the viral reverse transcriptase.

8.3.3. Transforming Capacity

The term *transformation* in the context of PTLV describes infinite growth of PTLV-positive T-cells *in vitro*. There are two types of PTLV-transformed T-cell lines: IL-2-dependent and IL-2-independent. Some

authors distinguish between “transformation” (IL-2-independent growth) and “immortalization” (IL-2-dependent growth). However, more often these terms are used interchangeably.

The transformation of T-cells *in vitro* can only be achieved if PTLV-producing cells are cocultured with susceptible lymphocytes; cell-free virus does not transform T-lymphocytes. It is generally accepted that PTLV-induced transformation is caused and maintained by autostimulation of cell proliferation, a process triggered by the virus. The stimulatory loop, also referred to as an autocrine stimulation, includes IL-2 and the IL-2 receptor (both of which are overexpressed in PTLV-positive cells) and possibly other molecules. The main viral factor involved in the induction of IL-2 and IL-2 receptor overexpression is the Tax protein acting “*in-trans*.” However, despite the fact that the concept of PTLV oncogenesis through virus-induced autocrine stimulation of cell proliferation is included in textbooks, the details of this mechanism are not clear.

8.3.4. Phylogeny

The genomic diversity of PTLV genomes is profound. At the same time the genomes of individual PTLV isolates are remarkably stable over directly observable periods of time. This combination of properties provides fertile ground for evolutionary reconstructions.^{21,56,61,70,71,74,75,79–84,91,101,102,113,114,116,117,121,122,124,129,138,141,143,144,149,150,152} However, a note of caution is required regarding some shortcomings associated with such analysis. First, most of STLV genomic sequences currently available originate from captive animals having close contact with other simian species. Interspecies transmission of STLV-1 in captivity has been documented.^{28,140,151} Although as a rule the natural host can be identified, the possibility of interspecies transmission of STLV introduces some uncertainty into the interpretation of the results. Second, the taxonomic identification of simian hosts of STLV is not always accurate and this uncertainty can blur the clustering of STLVs or be wrongly interpreted as evidence of interspecies transmission. Third, almost all inferences of PTLV phylogeny are based on the analysis of relatively short genomic sequences which limits the resolution of the analysis.

There are four major lineages in the PTLV phylogeny, namely, PTLV-1, HTLV-2, STLV-2, and PTLV-3 (Figure 8.2).

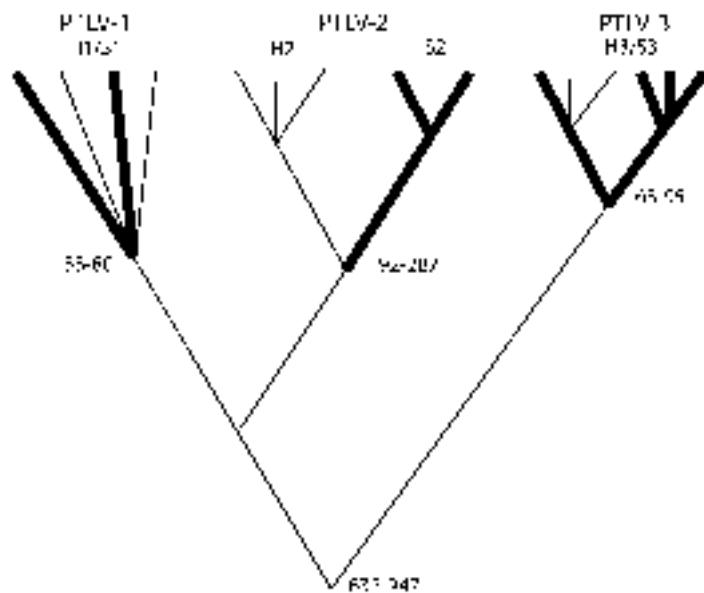


Figure 8.2. Major lineages in PTLV phylogeny. Cladogram of major PTLV lineages. STLV branches are shown in bold. Divergence time estimates in thousands of years¹²⁴ are shown near major nodes. Note that HTLV-1 and STLV-1 lineages are intermingled. So are STLV-3 and HTLV-3 lineages. In contrast, HTLV-2 and STLV-2 lineages are clearly separated. H1, HTLV1; S1, STLV1; H2, HTLV2; S2, STLV2; H3, HTLV3; S3, STLV3.

Human and simian viruses within PTLV-1 and PTLV-3 lineages are intermingled, whereas HTLV-2 and STLV-2 lineages are clearly separated. Thus, combining HTLV-2s and STLV-2s in one species (PTLV-2) is disputable. Dating of major nodes in the PTLV phylogeny, based on the analysis of complete genomes, provides the following divergence time estimates: 632–947 thousand years ago (TYA) for the divergence of the PTLV-3 and PTLV1/PTLV-2 lineages; 579–867 TYA for the divergence of PTLV-1 and PTLV-2 lineages; 192–287 TYA for the divergence of HTLV-2 and STLV-2 lineages.¹²⁴ Although the assumptions used in this analysis may or may not be true, the estimates provide a useful temporal framework for understanding the evolution of PTLV.

8.4. DIAGNOSIS OF DELTARETROVIRAL INFECTIONS

8.4.1. Virus Isolation

PTLVs cannot be isolated using classical procedures, that is, the infection of a permanent cell line with the cell-free material containing virus. Isolation of PTLV is achieved by establishing virus-producing cell lines directly from explanted tissues or cells from PTLV-infected hosts. The success rate of this procedure is low and it is not suitable for diagnostic purpose.

8.4.2. Antibody Assays

The presence of specific antibodies is a reliable marker of PTLV infections. The antigens of HTLVs and STLVs are cross-reactive. The degree of cross-reactivity is sufficient for the detection of anti-STLV antibodies in nonhuman primates (NHPs) using commercial antibody assays for HTLV-1/2. The standard algorithm for such testing includes consequential testing by two HTLV-1/2 ELISAs, preferably based on the different types of antigens, followed by confirmatory testing of ELISA-positive sera by HTLV Western blot or line assays.^{20,80,109,113,114,147} A highly specific confirmatory test for anti-STLV-1 antibodies is the radioimmunoprecipitation assay (RIPA).^{98,150,162} However, commercial HTLV-RIPA kits are not available, the assay is cumbersome and for this reason it is no longer used for diagnosis of STLV infections.

A common problem in HTLV immunoblot-based STLV serodiagnostics is difficulty in the interpretation of incomplete serological profiles when NHP sera react with only a subset of HTLV proteins or peptides. In HTLV serodiagnostics such results can be scored as “positive” or “indeterminate” according to the criteria as they are defined in commercial kits. Positive result criteria generally work well for identifying STLV-infected animals. However, the significance of indeterminate results is different in human and

simian PTLV diagnostics. In human diagnostics the indeterminate result almost always is due to nonspecific reactivity. In simian diagnostics the indeterminate result commonly is indicative of infection with STLV-3.⁷²

The indirect immunofluorescence assay (IFA) for anti-STLV antibody using fixed HTLV-1-producing cells is an alternative to ELISA.^{43,54,93,133,154} IFA for PTLVs is quite specific and has been widely used, but commercial IFA kits are not available. The reliability of diagnosing STLV infections by IFA depends on the propensity of sera to bind nonspecifically to the fixed cells. When sera are “sticky,” the resulting nonspecific staining can make interpretation of IFA difficult, if not impossible.

Antibodies against STLV-1 can be detected in urine; a urine-based test is very useful for field studies.⁶⁹

Detection of antibodies against STLV-1 can be integrated into multiplexed assays aiming at simultaneous detection of antibodies to multiple simian viruses.^{57,62} However, these tests are not widely used as yet.

8.4.3. Molecular Assays

STLV infection can be considered as unequivocally confirmed if STLV-specific nucleic acid sequences are detected. Molecular STLV diagnostics are primarily based on the detection of proviral DNA by polymerase chain reaction (PCR). Cellular genomic DNA from PBMCs (peripheral blood mononuclear cells) is usually used as a sample. Viral DNA may be extracted from cell-free body fluids including serum, plasma, CSF. However, these materials are mainly used as a last resort option when cellular DNA samples are not available. Successful amplification of STLV-1 DNA extracted from a museum sample stored for almost 100 years has been described.¹²

Many PCR tests have been described for detection of STLVs.^{23,25,61,73,114,145} Two fragments of the STLV-1 genome are particularly frequently targeted: a 522-bp *env* fragment⁶¹ and a 118-bp *pol* fragment.¹¹⁴ Nested and semi-nested PTLV PCRs are widely used. In general, the concordance of serological and molecular assays is good, although both antibody-negative PCR-positive and antibody-positive PCR-negative results have been observed.^{73,150} The latest versions of STLV PCR tests are more reliable in diagnosing STLV-1 infection than the antibody assays.²³

When molecular testing is aimed at detecting previously unknown STLVs, the most broadly reactive assay, usually a generic PCR targeting the PTLV *tax/rex* region

is used first, followed by STLV-1, STLV-2, and STLV-3-specific PCRs.^{20,72,145} Sequencing of PCR amplified genomic fragments followed by phylogenetic analysis is now considered to be a required confirmatory element of STLV diagnostic protocols.

8.5. SIMIAN T-LYMPHOTROPIC VIRUS TYPE 1

8.5.1. Genomic Organization

Three STLV-1 genomes have been completely sequenced: STLV-1-Mto-TE (GenBank Acc. No. Z46900), STLV-1-Mar-marc (GenBank Acc. No. AY590142), and STLV-1-Cta-Tan90 (GenBank Acc. No. AF074966/NC_000858).^{48,114,138} The length of STLV-1 LTR is in the range 753–765 bp. The U3 region of LTR spans about 350 bp. It contains elements responsible for the activation of transcription [transcription start site (TATA box), Tax response elements (TRE), Rex response element (RxRE)], polyadenylation site (AATAAA), and some other functionally important sequence motifs. Three imperfect 21 bp repeats comprising TREs are positioned head-to-tail and spaced by short GC-rich sequences. The first TRE is similar to the cAMP-responsive element (CRE) present in the promoter of many cellular genes—hence its second name—“CRE-like element.” The R region of the LTR spans about 230 bp and contains a splice donor site for all spliced viral RNAs as well as the polyadenylation signal (not to be confused with the polyadenylation site in the U3 region).

Structural genes of STLV-1 are positioned between the LTRs in the following order 5' to 3': *gag-pro-pol-env*. The composition of the pX region is complex.¹¹⁵ At least five ORFs have been identified in this region of the HTLV-1 genome. These ORFs, singly or in concert with the second exon located in *pol-env* region, encode eight HTLV-1 proteins (Figure 8.1, Table 8.1). Homologous ORFs are also present in all STLV-1 genomes. Whether or not all pX-encoded products expressed by HTLV-1 are also expressed by STLV-1 is not known. The “classical” pX proteins are Tax (p40^{Tax}) and Rex (p27^{Rex}). Both proteins are translated from double-spliced mRNAs. The first, smaller exons encoding the Tax and Rex proteins are located in the genomic region where the *pol* and *env* genes overlap. The second larger exons of the *tax* and *rex* genes coincide with ORF IV and ORF III in the pX region, respectively. Two pX proteins p12^{I-II} and p30^{II} are translated similarly, that is, from the double-spliced transcripts. The first exons encoding

these proteins are also located in the *pol/env* region, whereas the second exon lies in the pX region, ORF I and ORF II for p12^{I–2} and p30^{II}, respectively. Three other pX proteins (p12^I, p13^{II}, and p21^{Rex}) are translated from a single-spliced mRNA. The genes encoding these proteins are located within ORF I, ORF II, and ORF III, respectively. The latest addition to the list of pX-encoded products is the HBZ protein (*HTLV-1 basic region zipper factor*).³⁴ The HBZ ORF is located on the opposite strand from all other ORFs (anti-sense strand). Multiple isoforms of HBZ protein, translated from unspliced and singly-spliced transcripts, have been identified.¹³ The splice donor for spliced isoform (HBZ-SI) is located in the 3'-LTR.

8.5.2. Phylogeny

The origin of the common ancestor of PTLV-1s is estimated to be about 53–80 TYA. The split between Asian STLV-1s and the Melanesian/Australian lineage of HTLV-1 occurred first. The common ancestor of African STLV-1s and HTLV-1s dated within a 19–29 TYA time frame.¹²⁴ The diversity of African PTLV-1 isolates is greater than that of Asian PTLV-1 isolates. There are four African HTLV-1 subtypes: central African (B, D, and E) and west African or Gabonese (F) that are clearly distinguished by phylogenetic analysis.¹⁴⁶ Some African STLV-1 genomes fit into these HTLV-1 subtypes whereas others form separate clades in the PTLV-1 phylogeny (Table 8.2). Phylogenetic associations among those African STLV-1 isolates which cannot be assigned to an HTLV-1 subtype are ambiguous.¹⁴¹ The major HTLV-1 subtype, the Cosmopolitan (A), is presumed to be of African origin. Interestingly, no STLV-1 isolates cluster with this HTLV-1 subtype.

8.5.3. Infection in the Wild

STLV-1 infects many species of NHPs in the wild, both African and Asian, but no STLV has been detected in any South American primate species. The absence of infection in the Old World NHPs is exceptional if a sufficiently large sample is tested.^{7,51} In general, STLV-1 infection is more common in African than in Asian NHPs, although the difference may be due to a sampling bias (Table 8.3). The evidence that STLV-1 infection is present in the wild is mainly serological; that is, anti-STLV antibody is present in sera drawn from wild or recently caught (less than 2 weeks in captivity) monkeys and apes.^{27,43,44,47,52,54,93,108} In relatively

few cases, infection has been confirmed by detection of STLV-1 genomic sequences by PCR.^{20,72,108,113,150}

8.5.4. Modes of Transmission

The mode of STLV-1 transmission has been studied mainly in captive baboon colonies. STLV-1 infection is transmitted predominantly among sexually mature animals.^{23,29,35,98,105,154} The simplest explanation for this observation would be sexual transmission. This mode of transmission is possible;^{8,30,67} however, recent data indicate that sexual transmission of STLV-1 is inefficient, at least in captive olive baboons.²³ Sexual transmission of STLV-1 was also found to be rare in a breeding colony of common chimpanzees.¹⁰³

Tracing new STLV infections in a captive olive baboon colony using molecular markers gave unexpected results: about 70% of the new infections were found to be female-to-female transmissions.²³ This pattern of STLV-1 transmission can be explained by fighting among females in establishing and defending their social ranking. Such fights are frequently associated with bleeding in the anogenital area and the virus could be transmitted by contaminated blood from STLV-1-positive older females to uninfected younger females. Male-to-male transmission of STLV-1 as a result of bloody fights may be an important route of transmission in captive conditions when many males are kept together, for example, in gang cages.¹⁰⁰

Iatrogenic transmission of STLV-1 in captivity is also possible. Transmission by blood transfusion has been documented.⁹⁴ The transmission through cross-immunization with white or red blood cells or tissues passaged from animal to animal possibly was common in the 1960s–1980s when such procedures were widely used.

8.5.5. Biological Properties In Vitro

Surprisingly little is known about the biological properties of STLV-1 in vitro. Most permanent STLV-producing cell lines were established in the 1980s and early 1990s and it is not clear which of those are available.

As a rule, STLV-producing cell lines are of T-cell origin. Early passages of these cell lines require IL-2, whereas after transformation they may become IL-2-independent. IL-2-independent growth of STLV-1-positive T cells is usually associated with constitutive activation of the Jak/STAT signaling pathway and

Table 8.2. PTLV-1/HTLV-1 Subtypes and Distinct STLV-1 Lineages*

PTLV-1 Subtypes [†] HTLV-1 subtypes [‡] STLV-1 clades [§]	Representative (HTLV-1 Isolate)	Representative (STLV-1 Isolate or Frag-Virus)	STLV-1 Host Species
Central African (B)	H24	Ptr-A256	<i>Pan troglodytes</i>
Central African (D)	H23	Mnd13	<i>Mandrillus sphinx</i>
Central African (E)	Efe1	Agm-6242	<i>Chlorocebus pygerythrus</i>
West African (Gabonese) (F) <i>Cosmopolitan (A)</i> <i>Melanesian (Austronesian) (C)</i>	Lib2 ATK-1 MEL-5	Pan-X4258	<i>Papio anubis</i>
Asian STLV-1 clades			
<i>Rhesus monkey</i>		MmuR25	<i>Macaca mulatta</i>
<i>Stump-tailed macaque</i>		Marc1	<i>M. arctoides</i>
<i>Tonkeana macaque</i>		TE4	<i>M. tonkeana</i>
<i>Maura nigra macaques</i>		Mm98	<i>M. maura</i>
<i>Cynomolgus macaque</i>		Mfac194	<i>M. fascicularis</i>
<i>Japanese macaque</i>		Mfucla	<i>M. fuscata</i>
<i>Orangutan</i>		OU-Ka	<i>Pongo pygmaeus</i>
<i>Siamang</i>		Hsy31	<i>Sympalangus syndactylus</i>
African STLV-1 clades			
<i>Barbary macaque</i>		Msy92	<i>M. sylvanus</i>
<i>Drill-mangobey</i>		SL135	<i>Cercopithecus torquatus</i>
<i>Red-tailed guenon</i>		Cas-Tembla	<i>Cercopithecus ascanius</i>
<i>Yellow baboon</i>		Pcy5101	<i>Papio cynocephalus</i>
<i>Vervet</i>		Agm-1968	<i>Chlorocebus pygerythrus</i>
<i>Tantalus monkey</i>		Tan90	<i>Chlorocebus tantalus</i>
<i>Mustached guenon</i>		Cce1374	<i>Cercopithecus cephus</i>
<i>Olive baboon</i>		Pan622	<i>P. anubis</i>

* Many more distinct STLV-1 lineages clearly exist but cannot be unequivocally identified because the sequence data available are too fragmentary.

[†]Each PTLV-1 subtype includes both HTLV-1s and STLV-1s.

[‡]Each HTLV-1 subtype includes only HTLV-1s.

[§]List of clades is not complete; clades identified by the phylogenetic analysis of various genomic fragments may differ.

release of IL-6, bFGF, and GM-CSF. Those cell lines which are higher virus producers also release TNF-beta and PDGF.⁶⁶ All STLV-1-positive simian T-cell lines express the IL-2 receptor, whereas the expression of CD4 is detected only in some cell lines.⁴² The STLV-1 Tax protein is presumed to play a key role in mediating

STLV-1-induced cell transformation by analogy with HTLV-1; however, direct proof of this is lacking.

Spontaneous STLV-1-producing transformed cell lines of B-cell origin from African green monkeys, baboons, and stump-tailed macaques have also been reported.^{2,119,162} These cells are immortalized by

Table 8.3. Simian Species Harboring STLV-1

Scientific Name	Evidence*	References†
Asian NHPs		
<i>Macaca cyclopis</i>	V	131
<i>Macaca fascicularis</i>	f-V, Ab, V	52
<i>Macaca fuscata</i>	Ab, f-V, V	41, 44, 96
<i>Macaca maura</i>	Ab, V	52, 138
<i>Macaca mulatta</i>	Ab, f-V	61, 110, 151
<i>Macaca nemestrina</i>	f-V, V	41, 108
<i>Macaca nigra</i>	f-V	138
<i>Macaca nigrescens</i>	Ab	52
<i>Macaca radiata</i>	V	41, 131
<i>Macaca arctoides</i>	Ab, f-V, V	41, 52, 82, 138
<i>Macaca sinica</i>	Ab	52
<i>Macaca sylvanus</i>	Ab, f-V	138
<i>Macaca tonkeana</i>	Ab, V	48, 52
<i>Sympalangus syndactylus</i>	V	54
<i>Pongo pygmaeus</i>	f-V	49, 141, 147
African NHPs		
<i>Allenopithecus nigroviridis</i>	Ab, f-V	91
<i>Cercocebus agilis</i>	Ab, f-V	72, 102
<i>Cercocebus atys</i>	Ab, f-V	30, 31, 129
<i>Cercocebus torquatus</i>	Ab, f-V	72, 89
<i>Lophocebus albigena</i>	Ab, f-V	72
<i>Cercopithecus ascanius</i>	f-V	138
<i>Cercopithecus cephus</i>	Ab, f-V	20, 72
<i>Cercopithecus mitis</i>	F-V	41, 61, 142
<i>Cercopithecus mona</i>	Ab, f-V	89
<i>Cercopithecus nictitans</i>	Ab, f-V	72, 84, 89
<i>Cercopithecus pogonias</i>	Ab, f-V	20, 72
<i>Cercopithecus preussi</i>	Ab	89
<i>Chlorocebus aethiops</i>	Ab, f-V	61, 89
<i>Chlorocebus pygerythrus</i>	Ab, f-V	61
<i>Chlorocebus sabaeus</i>	Ab, f-V	61
<i>Chlorocebus tantalus</i>	Ab, f-V, V	61
<i>Erythrocebus patas</i>	Ab, f-V	112
<i>Miopithecus ogouensis</i>	Ab, f-V	20
<i>Miopithecus talapoin</i>	Ab	77
<i>Papio hamadryas</i>	Ab, f-V, V	1, 53, 149, 154
<i>Papio anubis</i>	Ab, f-V, V	53, 81, 89, 113, 148, 142
<i>Papio cynocephalus</i>	Ab, f-V	80, 89, 150
<i>Papio papio</i>	Ab, f-V	61
<i>Papio ursinus</i>	Ab, f-V	81
<i>Theropithecus gelada</i>	Ab, f-V	53
<i>Mandrillus sphinx</i>	Ab, f-V	20, 102
<i>Gorilla gorilla</i>	Ab, f-V	6, 68, 54, 123, 101
<i>Pan troglodytes</i>	Ab, f-V, V	41, 60, 61, 131, 152

*Ab, antibodies against STLV-1; f-V, frag-viruses; V, infectious virus.

†Representative references.

coinfection with lymphocryptovirus (see Chapter 15). Whether or not the biological properties of STLV isolates derived from T- and B-cells differ is not known. A T-cell line coinfecting with STLV-1 and simian immunodeficiency virus (SIV) has also been described.³²

There are only a few reports describing in vitro transformation of uninfected, nonautologous (usually homologous) simian cells by STLV-1.^{162,164} Human peripheral T-cells can also be transformed in vitro by STLV-1.¹⁶²

8.5.6. Lymphoma Association

Lymphoma is the most common malignancy in monkeys and apes with hundreds of cases being described.^{19,63,64,65} A relatively small proportion of these cases were tested for the presence of potentially oncogenic viruses and the most common finding was an association with STLV-1.

High titer antibodies against STLV-1 are detected in several species of lymphomatous NHPs including baboons,^{4,46,87,98,154} African green monkeys,^{3,104,111,118,128,132,133} and gorillas.^{68,107,123} However, because STLV-1 infection is quite prevalent in many simian species the development of lymphoma in an anti-STLV-positive monkey or ape *per se* cannot be considered as sufficient evidence for a causal role for the virus. STLV-1-positive lymphomas develop in a few percent of virus carriers. The vast majority of STLV-1-infected NHPs appear to be healthy and immunologically competent.⁹ An association of nonmalignant fatal diseases with STLV-1 infection has been described.^{6,88} However, the causal role of STLV-1 in these cases has not been proven. SIV immunosuppression-associated B-cell lymphomas in macaques may develop in STLV-1 antibody-positive monkeys.^{14,45} These tumors are STLV-1-negative¹⁴ and they are likely caused by a lymphocryptovirus (see Chapter 15). These two completely different types of simian lymphomas should not be confused. Much more convincing is the evidence of monoclonally integrated provirus in malignant cells, as has been shown for baboon^{22,153} and African green monkey lymphomas.^{132,133}

All STLV-1-positive simian lymphomas reported in the literature are “spontaneous.” There are no publications describing controlled experiments in which induction of lymphomas in NHPs has been achieved by experimental inoculation with STLV-1. The development of a reproducible simian model of STLV-1 lymphoma is hampered by several obstacles. The biological activity of

the virus cannot be easily standardized and no infectious molecular clones of STLV-1 are available. Additionally, the incubation period is long and, like in humans, lymphomas develop in a relatively small proportion of the infected animals.

Two major outbreaks of STLV-1-positive malignant lymphomas have been reported. Both outbreaks occurred in large captive baboon colonies, one at the Institute of Experimental Pathology and Therapy of the USSR Academy of Medical Sciences (also known as the Sukhumi Primate Center) and another at the Southwest National Primate Research Center (SWNPRC), San Antonio, Texas. The Sukhumi outbreak is the largest and most extensively studied.^{63–65,151,153,154,157–159} The Sukhumi hamadryas baboon colony where the outbreak occurred was established in 1927. The first cases of malignant lymphomas were observed in the late 1960s.⁶³ Over the next 30 years more than 300 cases of malignant lymphoma were recorded in this high-lymphoma colony whose size ranged from 1,000 to 1,500 animals in the 1970s–1980s. About half of the baboons from the Sukhumi high-lymphoma colony were shown to be STLV-1 antibody-positive, whereas all but one lymphomatous baboon were seropositive for STLV-1.¹⁵⁴ Several dozens lymphomas from baboons of this colony were analyzed for the presence of integrated STLV-1 provirus and all were found to be positive; the integration was monoclonal or, in some cases, oligoclonal.^{18,22,119,120,153}

An intriguing finding related to the Sukhumi lymphoma outbreak is the non-baboon origin of the associated STLV-1. The proviral STLV-1 sequences present in Sukhumi baboon lymphomas are closely related to those of macaque STLV-1s and quite distant from homologous sequences of baboon STLV-1 isolates.¹⁵¹ Further evidence for a macaque origin of STLV-1 virus as a causative agent for this outbreak is the finding that the strain of STLV-1 (991-ICC) isolated from a yellow baboon, which was inoculated in the United States with materials from a Sukhumi hamadryas baboon,⁴⁰ is also a macaque virus.^{61,75} These data are consistent with the hypothesis that the lymphoma outbreak in the Sukhumi baboon colony was triggered by the introduction of a macaque STLV-1 into the colony followed by rapid spread of the virus in a nonnatural and quite distinct host. Such a scenario is known to be frequently associated with pathological consequences. Why baboon-to-baboon transmission of a macaque STLV-1 became so efficient is unclear.

The baboon colony at the SWNPRC was established in 1958. Most of the colony founders (yellow, olive, and hamadryas baboons) were imported from Kenya and Tanzania in the 1960s–1970s. A few Guinea baboons from different zoos were also introduced. The baboons of different species in the SWNPRC colony were kept and bred together. The current size of the colony is about 4,000 baboons. The first cases of lymphoma at the SWNPRC occurred in 1977–1982,³⁸ and 27 additional cases were reported in the early 1990s.⁴⁶ During the last 15 years, 98 cases of malignant lymphoma were observed,¹⁹ which is by far the most prevalent malignancy in the SWNPRC baboons.^{4,19} The average prevalence of anti-STLV-1 in the SWNPRC baboon colony is about 40%, similar to the seroprevalence of STLV-1 in the Sukhumi high lymphoma colony.^{4,98} Antibodies against STLV-1 have been detected in all lymphomatous baboons tested ($n = 20$).⁴⁶ In one case, the monoclonal integration of STLV-1 provirus in a SWNPRC baboon lymphoma was also documented.^{4,46,98}

Inoculation of rabbits with either baboon and macaque lymphoblastoid cell lines, dually infected with STLV-1 and an EBV-like lymphocryptovirus, results in the development of lymphoma 1–2 months after inoculation.^{119,127,160} However, due to the presence of several viruses in the inoculum, these results cannot be interpreted unambiguously.

8.6. SIMIAN T-LYMPHOTROPIC VIRUS TYPE 2

Simian counterparts of HTLV-2 have been isolated almost simultaneously and independently from captive pygmy chimpanzees (*Pan paniscus*) housed in the Antwerpen Zoo⁷⁶ and the Yerkes National Primate Research Center, USA.³⁶ Isolation was done by coculturing chimpanzee PBMCs with human cord blood lymphocytes. Initially, these viruses were referred to as STLV-PP1664⁷⁶ and STLV_{pan-p}.³⁶ The designation was subsequently changed to STLV-2.^{136,137} The original isolates remain the only known STLV-2s.

Although it is presumed that wild pygmy chimpanzees from the Democratic Republic of Congo (former Zaire) harbor STLV-2, this has not been proven. STLV-2 infection has not been found in other NHP species with the exception of one description of an “STLV-2” in a South American spider monkey (*Ateles geoffroyi*).¹⁵ However, this claim was not confirmed and no other STLVs have ever been identified in New World monkeys.

The complete genome sequence is known for both STLV-2 isolates.^{26,136} The size of the STLV-2 genome is about 8,850 bp. The genomic sequences of both isolates are 93% homologous and approximately equidistant from the PTLV-1 and PTLV-3 genomes; sequence similarity is in the range 60–65%. The nucleotide sequence identity between STLV-2 and HTLV-2 genomes is about 75%, much smaller than in HTLV-1/STLV-1 and HTLV-3/STLV-3 pairs. The most conserved regions of STLV-2 genomes are segments of the *gag* and *env* genes encoding proteins p15 and gp21, respectively. The least conserved, in terms of amino acid identity of the predicted products, are the *pol* and *tax/rex* genes.¹³⁶ This is unusual, as a rule the *pol* gene in retroviruses is well conserved. The STLV-2 LTR is shorter than the LTR of PTLV-1 and HTLV-2 isolates (712 bp versus 755 bp). The difference is mainly due to a reduction in the number of TRE elements (also known as 21 bp repeats). There is only one TRE element in the STLV-2 LTR. The composition of the pX region, particular in the part related to ORFs other than *tax* and *rex*, is rearranged as compared to the pX region of PTLV-1 and HTLV-2.¹³⁶

STLV-2 transforms simian and human T-cells in vitro. Transformation requires cocultivation of virus-positive and virus-negative cells. Both IL-2-dependent and IL-2-independent STLV-2-producing cell lines have been established from pygmy chimpanzees and humans.^{36,76,99} The transformed cells have a mature T-cell phenotype and usually are positive for both CD4 and CD8.³⁶ A double-negative (CD4–CD8–), IL-2-independent T-cell line infected with STLV-2 (L9379B-) has also been established.⁹⁹

Little is known about the mechanism of STLV-2-induced transformation: it has been suggested that STLV-2 transforms T-cell independently of the Jak/STAT pathway activation.⁹⁹ However, no new data on STLV-2-induced transformation has been reported since the late 1980s, and it is unclear to what degree T-cell transforming activity of STLV-2 is reproducible and quantifiable.

STLV-2_{pan-p} can be transmitted to New Zealand White rabbits as well as pig-tailed and rhesus macaques by inoculation with STLV-2-positive cells (L93-79B and L93-79C; 4×10^6 cells i.v.). The inoculated animals seroconvert within 1 month and become persistently infected, but remain disease-free.²⁶

The divergence of HTLV-2 and STLV-2 from a common ancestor is estimated at 192–287 TYA. The radiation of extant HTLV-2 and STLV-2 isolates from their

common ancestors is dated to approximately the same period: 28–43 and 27–41 TYA, respectively.¹²⁴ As it was mentioned combining HTLV-2s and STLV-2s into one species is not supported by phylogenetic analyses; however, only two STLV-2 isolates have been analyzed, clearly too small a sample for making far reaching conclusions.

8.7. SIMIAN T-LYMPHOTROPIC VIRUS TYPE 3

The virus currently known as STLV-3 was first isolated from a captive Eritrean hamadryas baboon (*Papio hamadryas*) in 1994.³⁹ Two permanent T-cell lines were established by coculturing the animal's PBMCs with human cord blood lymphocytes (PH969) and lymphocytes from another baboon (PH1824). Both cell lines contained retroviruses related to but clearly different from STLV-1.^{74,134,135} Initially, the virus was named PTLV-L. The name was later changed to STLV-3 when similar agents were found in various simian species from both Eastern and Western Central Africa.^{72,89,90,92,125,139,140} The PH969 and PH1824 cell lines remain the only source of infectious STLV-3. An infectious molecular clone of STLV-3 was constructed from the genomic fragments of a virus (Ppa-F3) from a Guinea baboon (*P. papio*).¹⁷ All other known STLV-3s are frag-viruses. Two frag-viruses closely related to STLV-3 (HTLV-3-Py143 and HTLV-3-2026ND) have been identified in humans.^{10,11,124,156} One of these viruses (HTLV-3-Py143) is almost identical to STLV-3-Cto604 from Cameroonian *Cercopithecus torquatus*.¹⁰

The complete genome sequence is known for three STLV-3 isolates [Cto-604,⁹⁰ Tge-2117,¹⁴⁰ and Ppa-F3⁸⁹] and for both HTLV-3 isolates (Py143 and 2026ND).^{10,124} The STLV-3 and HTLV-3 genomes are very similar. Their length is about 8,900 bp. The HTLV-3-Py143 genome is exceptional in being shorter (8,553 bp) due to a 366-bp deletion in the proximal pX region.¹⁰ The overall sequence identity of PTLV-3 genomes is in the range of 87–99%; the genomes of HTLV-3-Py143 and STLV-3-Cto604 are almost identical (99.1% nucleotide identity, excluding the deletion). The PTLV-3 isolates are equally related to PTLV-1 and PTLV-2 with sequence identities in the range of 62–63%. The most conserved regions of the PTLV-3 genome are those encoding p24^{Gag} and gp21^{Env} (98–99% of amino acid identity), and the least conserved regions are the LTR (86–91% of nucleotide identity) and the *pro* gene (87–91% of amino acid identity). The PTLV-3 LTR is shorter

than that of PTLV-1 and PTLV-2, 695 bp versus approximately 750 bp. The difference in the length of the LTRs is mainly due to a reduction in number of TRE elements: two TREs in PTLV-3 versus three TREs in PTLV-1 and PTLV-2; the TRE element most distant from the U3R boundary being absent in PTLV-3. The functional significance of the TRE deletion is not known. Other functional elements in the LTR are conserved. The organization of *gag-pro-pol-env* region of the PTLV-3 genome is basically the same as in the PTLV-1 and PTLV-2 genomes. Two classical genes located in the pX region, *tax* (ORF IV) and *rex* (ORF III), are constructed similarly to their PTLV-1 and PTLV-2 counterparts. Homologues of pX-ORF I, pX-ORF II, and the newest pX ORF (HBZ) are also present in the PTLV-3 genome; however, it is not clear if these ORFs are expressed.

Very little is known about the biological properties of PTLV-3 isolates. The original isolate immortalized both human and baboon T-lymphocytes in vitro.³⁹ However, no attempts to reproduce transformation of T cells by PTLV-3 have been described. By analogy with HTLV-1, the Tax-3 protein encoded by the PTLV-3 *tax* gene is believed to be responsible for T-cell transformation. Functional properties of Tax-3 are more similar to those of HTLV-1 Tax-1 than HTLV-2 Tax-2. The Tax-3 protein is expressed in vivo as indicated by the detection of antibodies against this protein in PTLV-3-infected humans and monkeys.^{10,16} These data suggest that PTLV-3s may be oncogenic. However, direct proof of this is absent.

The start of radiation of extent PTLV-3s is dated at 63–95 TYA. The most ancient split separated the East African lineage STLV-3s (viruses from gelada and hamadryas baboon; TGE2117 and PH969, respectively) from HTLV-3 and other STLV-3 isolates. The next split, dated at 55–83 TYA, separated the lineage leading to the Cameroonian red-capped mangabey STLV-3 isolate (CTO-604). The closest relatives of HTLV-3 (2026ND) are STLV-3 (PPAF-3) from a Guinea baboon and STLV-3 (CTO-NG409) from a Nigerian red-capped mangabey. The common ancestor for this mixed HTLV/STLV-3 clade (2026ND/PPAF-3/CTO-NG409) is dated into 36–54 TYA interval.¹²⁴

STLV-3 is harbored by wild monkeys in East, Central, and West Africa (Table 8.4).

There are no reports of STLV-3 infection in Asian NHPs. STLV-3 infection is less common, apparently, than STLV-1 infection, although much less data are available for the former. Surveys for STLV-3 in wild NHPs have been performed in animals from Ethiopia¹²⁵

Table 8.4. Simian Species Harboring STLV-3

Scientific Name	Evidence*	References
East African NHP		
<i>Papio hamadryas</i>	Ab, f-V, V	39, 125
<i>P. hamadryas × P. anubis</i>	Ab, f-V	125
<i>Theropithecus gelada</i>	Ab, f-V	140
West African NHP		
<i>Papio papio</i>	Ab, f-V, G	17, 89
<i>Cercocebus torquatus</i>	Ab, f-V, G	72, 89, 90, 92
Central Africa		
<i>Cercocebus agilis</i>	Ab, f-V	20, 72
<i>Lophocebus albigena</i>	Ab, f-V	72
<i>Cercopithecus cephus</i>	Ab, f-V	72
<i>Cercopithecus nictitans</i>	Ab, f-V	20, 139

*Ab, suggestive serology; f-V, frag-viruses; V, infectious virus; G, complete genome sequence.

and Cameroon.^{20,72} The algorithm for the detection of STLV-3 infection includes testing for antibodies cross-reacting with HTLV-1/2 antigens using commercially available assays followed by STLV-3-specific PCR on DNA samples from the seropositive animals. Among three patterns of serological reactivity in “human” assays (HTLV-1+, HTLV-2+, and HTLV-1&2+), HTLV-2+ and HTLV-1&2+ reactivities are suggestive of STLV-3 infection. Importantly, a substantial propor-

tion of cases with HTLV indeterminate reactivity have proved to be STLV-3-positive.⁷²

In Ethiopia, STLV-3 was found in 50% of hamadryas baboons (*P. hamadryas*; $n = 40$) and 38% of hybrid baboons (*P. hamadryas × P. anubis*; $n = 50$). At the same time all olive baboons (*P. anubis*; $n = 96$) from southwest Ethiopia were negative for STLV-3 as were wild geladas (*Theropithecus gelada*; $n = 156$) and grivets (presumably *Chlorocebus aethiops*; $n = 177$) sampled in regions neighboring the habitat of the hamadryas baboons.¹²⁵

Wild NHPs from Cameroon have been surveyed for STLV-3 infection the most extensively.^{20,72} The results of these studies are summarized in Table 8.5. Apparently, STLV-3 infection is most prevalent in some mangabey species, such as *Cercocebus agilis* (7.7%) and *Lophocebus albigena* (13.9%). In some STLV-3-positive species the infection is quite rare, for instance in *Cercopithecus cephus* (0.4%). The available data do not allow a conclusion as to whether or not there are STLV-3-free NHP species in Cameroon.

8.8. PRIMATE T-LYMPHOTROPIC VIRUS

TYPE 4

PTLV-4 is a hypothetical deltaretrovirus species. At the time of writing (spring 2008) only one frag-virus, a PTLV-4 prototype is known. This frag-virus, designated as HTLV-4 (1863LE), has been detected in a hunter from a remote village in southeastern Cameroon.¹⁵⁶ This

Table 8.5. Prevalence of STLV-3 Infection in Wild NHPs Surveyed in Cameroon

Scientific Name	Common Name	Survey 1 ²⁰	Survey 2 ⁷²	Total
<i>Cercopithecus cephus</i>	Mustached guenon	0/104	2/411	2/515 (3.9%)
<i>C. nictitans</i>	Greater spot-nosed monkey	2/80	2/307	4/387 (1%)
<i>C. pogonias</i>	Crested mona monkey	0/35	0/202	0/237
<i>C. mona</i>	Mona monkey	0/1		0/1
<i>C. neglectus</i>	De Brazza's monkey	0/8		0/8
<i>Lophocebus albigena</i>	Gray-cheeked mangabey	0/12	16/103	16/115 (13.9%)
<i>Cercocebus agilis</i>	Agile mangabey	7/48	3/81	10/129 (7.7%)
<i>Cercocebus torquatus</i>	Collared mangabey	0/1	1/2	1/3
<i>Colobus guereza</i>	Mantled guereza	0/18	0/7	0/25
<i>Colobus satanus</i>	Black colobus		0/8	0/8
<i>Mandrillus sphinx</i>	Mandrill	0/1	0/7	0/8
<i>Miopithecus ogouensis</i>	Gabon talapoin	0/11	0/8	0/19
<i>Pan troglodytes</i>	Common chimpanzee	0/6	0/5	0/11
<i>Gorilla gorilla</i>	Western gorilla	0/3	0/1	0/4

individual was positive for anti-HTLV antibody; the reactivity pattern was HTLV-2-like. Two virus-specific DNA fragments have been amplified from his DNA that was extracted from uncultured PBLs: 275 bp of *tax* and 662 bp of *pol*. The recovered sequences are approximately equidistant from the homologous PTLV-1, PTLV-2, and PTLV-3 sequences; nucleotide identity is in the range of 65–75% and 75–80% for *pol* and *tax*, respectively. The person from whom the HTLV-4 frag-virus was isolated had multiple occupational exposures to the blood of monkeys and apes. Thus, it is conceivable that HTLV-4 may be a simian virus. However, so far no STLV-4 has been detected in NHP species, despite substantial efforts.⁷²

8.9. SUMMARY

Three primate deltaretrovirus species are currently recognized: *Primate T-lymphotropic virus types 1, 2, and 3* (PTLV-1, PTLV-2, and PTLV-3). Each species includes both human (HTLV-1, HTLV-2, and HTLV-3) and simian (STLV-1, STLV-2, and STLV-3) strains/isolates. The degree of relatedness of isolates within each PTLV species is not uniform, being significantly greater for PTLV-2 isolates than for the other PTLV species. The evolution and phylogenetic relationships of STLVs and HTLVs are tightly interlaced. Apparently, multiple transmissions of STLV-1 to humans as well as between sympatric NHP species have occurred. Some of these transmissions are dated to prehistoric periods, whereas others are relatively recent events. STLVs are complex retroviruses; that is, their genomes include the essential retroviral genes *gag*, *pol*, *pro*, and *env* as well as auxiliary genes, the most important of which are *tax* and *rex*. The main biological property of STLVs *in vitro* is their ability to transform or immortalize T-lymphocytes. The transforming activity of STLVs requires cell-to-cell contact. Pathological consequences of STLV infections *in vivo* are known only for STLV-1. This virus is oncogenic, although its oncogenic potential is realized in only a small fraction of infected animals. Both sporadic and epizootic STLV-1-positive T-lymphomas have been described in several simian species, mostly in baboons. This malignancy closely resembles HTLV-1-positive adult T-cell lymphoma/leukemia in humans. The presence of a monoclonally integrated STLV-1 genome in baboon and African green monkey T-lymphoma cells is considered as strong evidence for a causal role of STLV-1 in the development of such lymphomas. Apparently, the onco-

genicity of STLV-1 may be enhanced if the virus infects a nonnatural host. No data are available on the experimental induction of lymphoma in NHPs by inoculation of purified or cloned STLV-1.

REFERENCES

1. Agrba, V., V. Timanovskaya, V. Kakubava, L. Indzhiia, D. Aravashvili, M. Chikobava, and B. Lapin. 1994. Establishment and characteristics of an interleukin-2-dependent STLV-1-producing lymphoid cell line, SPH-7(T), obtained from Papio hamadryas with malignant lymphoma. *In Vitro Cell Dev. Biol. Anim.* 30A(10):637–639.
2. Agrba, V. Z., B. A. Lapin, N. M. Medvedeva, M. G. Chikobava, L. A. Iakovleva, and D. D. Karal-ogly. 2005. [The virogenic status of cultured continuous simian lymphoid cells and their immunophenotypical characteristics]. *Vopr. Virusol.* 50(5):44–49.
3. Akari, H., F. Ono, I. Sakakibara, H. Takahashi, Y. Murayama, A. Hiyaoka, K. Terao, I. Otani, R. Mukai, A. Adachi, and Y. Yoshikawa. 1998. Simian T cell leukemia virus type I-induced malignant adult T cell leukemia-like disease in a naturally infected African green monkey: implication of CD8+ T cell leukemia. *AIDS Res. Hum. Retroviruses* 14(4):367–371.
4. Allan, J. S., M. Leland, S. Broussard, J. Mone, and G. Hubbard. 2001. Simian T-cell lymphotropic Viruses (STLVs) and lymphomas in African nonhuman primates. *Cancer Invest.* 19(4):383–395.
5. Barnard, A. L., T. Igakura, Y. Tanaka, G. P. Taylor, and C. R. Bangham. 2005. Engagement of specific T-cell surface molecules regulates cytoskeletal polarization in HTLV-1-infected lymphocytes. *Blood* 106(3):988–995.
6. Blakeslee, J. R., Jr., H. M. McClure, D. C. Anderson, R. M. Bauer, L. Y. Huff, and R. G. Olsen. 1987. Chronic fatal disease in gorillas seropositive for simian T-lymphotropic virus I antibodies. *Cancer Lett.* 37:1–6.
7. Blakeslee, J. R., Jr., W. G. Sowder, and J. Baulu. 1985. Wild African green monkeys of Barbados are HTLV negative [Letter]. *Lancet* 1:525.
8. Botha, M. C., M. Jones, W. A. de Klerk, and N. Yamamoto. 1985. Spread and distribution of human T-cell leukaemia virus type I- reactive antibody among baboons and monkeys in the northern and eastern Transvaal. *S. Afr. Med. J.* 67:665–668.
9. Brignolo, L., A. Spinner, J. L. Yee, and N. W. Lerche. 2004. Subsets of T cells in healthy rhesus macaques (*Macaca mulatta*) infected with simian T-lymphotropic virus type 1. *Comp. Med.* 54(3):271–274.

10. Calattini, S., S. A. Chevalier, R. Duprez, P. Afonso, A. Froment, A. Gessain, and R. Mahieux. 2006. Human T-cell lymphotropic virus type 3: complete nucleotide sequence and characterization of the human tax3 protein. *J. Virol.* 80(19):9876–9888.
11. Calattini, S., S. A. Chevalier, R. Duprez, S. Bassot, A. Froment, R. Mahieux, and A. Gessain. 2005. Discovery of a new human T-cell lymphotropic virus (HTLV-3) in Central Africa. *Retrovirology* 2(1):30.
12. Calvignac, S., J. M. Terme, S. M. Hensley, P. Jalinot, A. D. Greenwood, and C. Hanni. 2008. Ancient DNA identification of early 20th century simian T-cell leukemia virus type 1. *Mol. Biol. Evol.* 25(6):1093–1098.
13. Cavanagh, M. H., S. Landry, B. Audet, C. Arpin-Andre, P. Hivin, M. E. Pare, J. Thete, E. Wattel, S. J. Marriott, J. M. Mesnard, and B. Barbeau. 2006. HTLV-I antisense transcripts initiating in the 3'LTR are alternatively spliced and polyadenylated. *Retrovirology* 3:15.
14. Chalifoux, L. V., N. W. King, M. D. Daniel, M. Kannagi, R. C. Desrosiers, P. K. Sehgal, L. M. Waldron, R. D. Hunt, and N. L. Letvin. 1986. Lymphoproliferative syndrome in an immunodeficient rhesus monkey naturally infected with an HTLV-III-like virus (STLV-III). *Lab. Invest.* 55(1):43–50.
15. Chen, Y. M., Y. J. Jang, P. J. Kanki, Q. C. Yu, J. J. Wang, R. J. Montali, K. P. Samuel, and T. S. Papas. 1994. Isolation and characterization of simian T-cell leukemia virus type II from New World monkeys. *J. Virol.* 68:1149–1157.
16. Chevalier, S. A., L. Meertens, C. Pise-Masison, S. Calattini, H. Park, A. A. Alhaj, M. Zhou, A. Gessain, F. Kashanchi, J. N. Brady, and R. Mahieux. 2006. The tax protein from the primate T-cell lymphotropic virus type 3 is expressed in vivo and is functionally related to HTLV-1 Tax rather than HTLV-2 Tax. *Oncogene* 25(32):4470–4482.
17. Chevalier, S. A., M. Walic, S. Calattini, A. Mallet, M. C. Prevost, A. Gessain, and R. Mahieux. 2007. Construction and characterization of a full length infectious Simian T-cell Lymphotropic Virus Type-3 molecular clone. *J. Virol.* 81(12):6276–6285.
18. Chikobaeva, M. G., H. Schatzl, D. Rose, U. Bush, L. A. Iakovleva, F. Deinhardt, K. Helm, and B. A. Lapin. 1993. [A new variant of the simian T-lymphotropic retrovirus type I (STLV-IF) in the Sukhumi colony of hamadryas baboons] Novyi variant obez'ian'ego T-lymphotropnogo retrovirusa per vogu tipa (STLV-1F) v Sukhumskoi kolonii pavianov gamadrilov. *Vopr. Virusol.* 38(6):249–253.
19. Cianciolo, R. E., S. D. Butler, J. S. Eggers, E. J. Dick Jr., M. M. Leland, M. de la Garza, K. M. Brasky, L. B. Cummins, and G. B. Hubbard. 2007. Spontaneous neoplasia in the baboon (*Papio* spp.). *J. Med. Primatol.* 36(2):61–79.
20. Courgnaud, V., S. Van Dooren, F. Liegeois, X. Pourrut, B. Abela, S. Loul, E. Mpoudi-Ngole, A. Vandamme, E. Delaporte, and M. Peeters. 2004. Simian T-cell leukemia virus (STLV) infection in wild primate populations in Cameroon: evidence for dual STLV type 1 and type 3 infection in agile mangabeys (*Cercocebus agilis*). *J. Virol.* 78(9):4700–4709.
21. Crandall, K. A. 1996. Multiple interspecies transmissions of human and simian T-cell leukemialymphoma virus type I sequences. *Mol. Biol. Evol.* 13(1):115–131.
22. D'iachenko, A. G., I. G. Kondzharia, E. V. Indzhia, B. A. Lapin, L. A. Iakovleva, L. D. Rudaia, and B. L. Dzhalagonia. 1990. [Monoclonal integration of simian T-cell leukemia virus in hamadryas with malignant lymphoma]. *Eksp. Onkol.* 12:15–18.
23. d'Offay, J. M., R. Eberle, Y. Sucol, L. Schoelkopf, M. A. White, B. D. Valentine, G. L. White, and N. W. Lerche. 2007. Transmission dynamics of simian T-lymphotropic virus type 1 (STLV1) in a baboon breeding colony: predominance of female-to-female transmission. *Comp. Med.* 57(1):105–114.
24. Derse, D., B. Crise, Y. Li, G. Princler, N. Lum, C. Stewart, C. F. McGrath, S. H. Hughes, D. J. Munroe, and X. Wu. 2007. Human T-cell leukemia virus type 1 integration target sites in the human genome: comparison with those of other retroviruses. *J. Virol.* 81(12):6731–6741.
25. Dezzutti, C. S., A. Lazo, J. Y. Yee, J. R. Blakeslee, L. E. Mathes, B. G. Brown, and M. D. Lairmore. 1992. Detection of simian T-lymphotropic virus type I using the polymerase chain reaction. *Int. J. Cancer* 50:805–810.
26. Digilio, L., A. Giri, N. Cho, J. Slattery, P. Markham, and G. Franchini. 1997. The simian T-lymphotropicleukemia virus from Pan paniscus belongs to the type 2 family and infects Asian macaques. *J. Virol.* 71(5):3684–3692.
27. Dracopoli, N. C., T. R. Turner, J. G. Else, C. J. Jolly, R. Anthony, R. C. Gallo, and W. C. Saxinger. 1986. STLV-I antibodies in feral populations of East African vervet monkeys (*Cercopithecus aethiops*). *Int. J. Cancer* 38:523–529.
28. Englebrecht, S., E. J. van Rensburg, and B. A. Robinson. 1996. Sequence variation and subtyping of human and simian T-cell lymphotropic virus type I strains from South Africa. *J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol.* 12(3):298–302.
29. Estaquier, J., M. Peeters, L. Bedjabaga, C. Honore, P. Bussi, A. Dixson, and E. Delaporte. 1991. Prevalence

- and transmission of simian immunodeficiency virus and simian T-cell leukemia virus in a semi-free-range breeding colony of mandrills in Gabon [Letter]. *AIDS* 5:1385–1386.
30. Fultz, P. N., T. P. Gordon, D. C. Anderson, and H. M. McClure. 1990. Prevalence of natural infection with simian immunodeficiency virus and simian T-cell leukemia virus type I in a breeding colony of sooty mangabey monkeys. *AIDS* 4:619–625.
 31. Fultz, P. N., H. M. McClure, D. C. Anderson, R. B. Swenson, R. Anand, and A. Srinivasan. 1986. Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (*Cercocebus atys*). *Proc. Natl. Acad. Sci. U. S. A.* 83(14):5286–5290.
 32. Fultz, P. N., L. Su, P. May, and J. T. West. 1997. Isolation of sooty mangabey simian T-cell leukemia virus type I [STLV-I(sm)] and characterization of a mangabey T-cell line coinfecting with STLV-I(sm) and simian immunodeficiency virus SIVsmmpBj14. *Virology* 235(2):271–285.
 33. Gabet, A. S., A. Gessain, and E. Wattel. 2003. High simian T-cell leukemia virus type 1 proviral loads combined with genetic stability as a result of cell-associated provirus replication in naturally infected, asymptomatic monkeys. *Int. J. Cancer* 107(1):74–83.
 34. Gaudray, G., F. Gachon, J. Basbous, M. Biard-Piechaczyk, C. Devaux, and J. M. Mesnard. 2002. The complementary strand of the human T-cell leukemia virus type 1 RNA genome encodes a bZIP transcription factor that down-regulates viral transcription. *J. Virol.* 76(24):12813–12822.
 35. Georges-Courbot, M. C., P. Moisson, E. Leroy, A. M. Pingard, E. Nerrienet, G. Dubreuil, E. J. Wickings, F. Debels, I. Bedjabaga, V. Poaty-Mavoungou, N. T. Hahn, and A. J. Georges. 1996. Occurrence and frequency of transmission of naturally occurring simian retroviral infections (SIV, STLV, and SRV) at the CIRMF Primate Center, Gabon. *J. Med. Primatol.* 25(5):313–326.
 36. Giri, A., P. Markham, L. Digilio, G. Hurteau, R. C. Gallo, and G. Franchini. 1994. Isolation of a novel simian T-cell lymphotropic virus from Pan paniscus that is distantly related to the human T-cell leukemialymphotropic virus types I and II. *J. Virol.* 68(12):8392–8395.
 37. Giri, A., J. P. Slattery, W. Heneine, A. Gessain, E. Rivadeneira, R. C. Desrosiers, L. Rosen, R. Anthony, J. Pamungkas, D. Iskandriati, A. L. Richards, V. Herve, H. McClure, S. J. O'Brien, and G. Franchini. 1997. The tax gene sequences form two divergent monophyletic lineages corresponding to types I and II of simian and human T-cell leukemialymphotropic viruses. *Virology* 231(1):96–104.
 38. Gleiser, C. A., K. D. Carey, and R. L. Heberling. 1984. Malignant lymphoma and Hodgkin's disease in baboons (*Papio* sp.). *Lab. Anim. Sci.* 34(3):286–289.
 39. Goubau, P., M. Van Brussel, A. M. Vandamme, H. F. Liu, and J. Desmyter. 1994. A primate T-lymphotropic virus, PTLV-L, different from human T-lymphotropic viruses types I and II, in a wild-caught baboon (*Papio hamadryas*). *Proc. Natl. Acad. Sci. U. S. A.* 91(7):2848–2852.
 40. Guo, H. G., F. Wong-Stall, and R. C. Gallo. 1984. Novel viral sequences related to human T-cell leukemia virus in T cells of a seropositive baboon. *Science* 223:1195–1197.
 41. Haga, S., H. Tanaka, H. Tsujimoto, and M. Hayami. 1986. Conventional and immunocolloidal gold electron microscopy of eight simian retroviruses closely related to human T-cell leukemia virus type I. *Cancer Res.* 46:293–299.
 42. Hayami, M. 1986. Simian T-cell leukemia viruses, STLV (HTLV-I-related simian retroviruses). *Cancer Rev.* 1:35–63.
 43. Hayami, M., K. Ishikawa, A. Komuro, Y. Kawamoto, K. Nozawa, K. Yamamoto, T. Ishida, and Y. Hinuma. 1983. ATLV antibody in cynomolgus monkeys in the wild [Letter]. *Lancet* 2:620.
 44. Hayami, M., A. Komuro, K. Nozawa, T. Shotake, K. Ishikawa, K. Yamamoto, T. Ishida, S. Honjo, and Y. Hinuma. 1984. Prevalence of antibody to adult T-cell leukemia virus-associated antigens (ATLA) in Japanese monkeys and other non-human primates. *Int. J. Cancer* 33:179–183.
 45. Homma, T., P. J. Kanki, N. W. King, Jr., R. D. Hunt, M. J. O'Connell, N. L. Letvin, M. D. Daniel, R. C. Desrosiers, C. S. Yang, and M. Essex. 1984. Lymphoma in macaques: association with virus of human T lymphotropic family. *Science* 225:716–718.
 46. Hubbard, G. B., J. P. Mone, J. S. Allan, K. J. Davis, M. M. Leland, P. M. Banks, and B. Smir. 1993. Spontaneously generated non-Hodgkin's lymphoma in twenty-seven simian T-cell leukemia virus type 1 antibody-positive baboons (*Papio* species). *Lab. Anim. Sci.* 43:301–309.
 47. Hunsmann, G., J. Schneider, J. Schmitt, and N. Yamamoto. 1983. Detection of serum antibodies to adult T-cell leukemia virus in non-human primates and in people from Africa. *Int. J. Cancer* 32:329–332.
 48. Ibrahim, F., G. De The, and A. Gessain. 1995. Isolation and characterization of a new simian T-cell leukemia virus type 1 from naturally infected celebes macaques (*Macaca tonkeana*): complete nucleotide sequence and phylogenetic relationship with

- the Australo-Melanesian human T-cell leukemia virus type 1. *J. Virol.* 69(11):6980–6993.
49. Ibuki, K., E. Ido, S. Setiyaningsih, M. Yamashita, L. R. Agus, J. Takehisa, T. Miura, S. Dondin, and M. Hayami. 1997. Isolation of STLV-I from orangutan, a great ape species in Southeast Asia, and its relation to other HTLV-Is/STLV-Is. *Jpn. J. Cancer Res.* 88(1): 1–4.
50. Igakura, T., J. C. Stinchcombe, P. K. Goon, G. P. Taylor, J. N. Weber, G. M. Griffiths, Y. Tanaka, M. Osame, and C. R. Bangham. 2003. Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. *Science* 299(5613):1713–1716.
51. Ishida, T. and P. Varavudhi. 1992. Wild long-tailed macaques (*Macaca fascicularis*) in Thailand are highly infected with gamma herpes virus but not with simian T-lymphotropic retrovirus of type 1. *Folia Primatol.(Basel)* 59:163–168.
52. Ishida, T., K. Yamamoto, G. Ishimoto, T. Shotake, O. Takenaka, K. Nozawa, M. Hayami, and Y. Hinuma. 1985. A field study of infection with human T-cell leukemia virus among Asian primates. *Microbiol. Immunol.* 29:839–846.
53. Ishida, T., K. Yamamoto, T. Shotake, K. Nozawa, M. Hayami, and Y. Hinuma. 1986. A field study of infection with human T-cell leukemia virus among African primates. *Microbiol. Immunol.* 30(4):315–321.
54. Ishikawa, K., M. Fukasawa, H. Tsujimoto, J. G. Else, M. Isahakia, N. K. Ubhi, T. Ishida, O. Takenaka, Y. Kawamoto, and T. Shotake. 1987. Serological survey and virus isolation of simian T-cell leukemia/T-lymphotropic virus type I (STLV-I) in non-human primates in their native countries. *Int. J. Cancer* 40:233–239.
55. Jurkiewicz, E., H. Nakamura, J. Schneider, N. Yamamoto, M. Hayami, and G. Hunsmann. 1986. Structural analysis of p19 and p24 core polypeptides of primate lymphotropic retroviruses (PLRV). *Virology* 150:291–298.
56. Kelsey, C. R., K. A. Crandall, and A. F. Voevodin. 1999. Different models, different trees: the geographic origin of PTLV-I. *Mol. Phylogenet. Evol.* 13(2):336–347.
57. Khan, I. H., S. Mendoza, J. Yee, M. Deane, K. Venkateswaran, S. S. Zhou, P. A. Barry, N. W. Lerche, and P. A. Luciw. 2006. Simultaneous detection of antibodies to six nonhuman-primate viruses by multiplex microbead immunoassay. *Clin. Vaccine Immunol.* 13(1):45–52.
58. Kim, F. J., M. Lavanya, A. Gessain, S. Gallego, J. L. Battini, M. Sitbon, and V. Courgnaud. 2006. Intra-host variations in the envelope receptor-binding domain (RBD) of HTLV-1 and STLV-1 primary isolates. *Retrovirology* 3:29.
59. Kim, F. J., N. Manel, E. N. Garrido, C. Valle, M. Sitbon, and J. L. Battini. 2004. HTLV-1 and -2 envelope SU subdomains and critical determinants in receptor binding. *Retrovirology* 1:41.
60. Komuro, A., T. Watanabe, I. Miyoshi, M. Hayami, H. Tsujimoto, M. Seiki, and M. Yoshida. 1984. Detection and characterization of simian retroviruses homologous to human T-cell leukemia virus type I. *Virology* 138:373–378.
61. Koralnik, I. J., E. Boeri, W. C. Saxinger, A. L. Monico, J. Fullen, A. Gessain, H. G. Guo, R. C. Gallo, P. Markham, V. Kalyanaraman, V. Hirsch, J. Allan, K. Murthy, P. Alford, J. P. Slattery, S. J. O'Brien, and G. Franchini. 1994. Phylogenetic associations of human and simian T-cell leukemia/lymphotropic virus type I strains: evidence for interspecies transmission. *J. Virol.* 68(4):2693–2707.
62. Kuller, L., R. Watanabe, D. Anderson, and R. Grant. 2005. Development of a whole-virus multiplex flow cytometric assay for antibody screening of a specific pathogen-free primate colony. *Diagn. Microbiol. Infect. Dis.* 53(3):185–193.
63. Lapin, B. A. 1973. The epidemiologic and genetic aspects of an outbreak of leukemia among Hamadryas baboons of the Sukhumi monkey colony. *Bibl. Haematol.* 39:263–268.
64. Lapin, B. A., L. A. Iakovleva, A. F. Voevodin, L. V. Indzhiia, and V. Z. Agrba. 1983. [Study of virus-associated lymphomas in primates using a model of malignant lymphoma of the baboon]. *Vopr. Onkol.* 29(12):61–66.
65. Lapin, B. A., L. A. Yakovleva, L. V. Indzhiia, V. Z. Agrba, G. S. Tsiripova, A. F. Voevodin, M. T. Ivanov, and A. G. Djatchenko. 1975. Transmission of human leukaemia to nonhuman primates. *Proc. R. Soc. Med.* 68(3):141–145.
66. Lazo, A. and R. T. Bailer. 1996. Constitutive cytokine release by simian T-cell lymphotrophic virus type I (STLV-I) and human T-cell lymphotrophic virus types III (HTLV-III) transformed cell lines. *J. Med. Primatol.* 25(4):257–266.
67. Lazo, A., R. T. Bailer, M. D. Lairmore, J. A. Yee, J. Andrews, V. C. Stevens, and J. R. Blakeslee. 1994. Sexual transmission of simian T-lymphotropic virus type I: a model of human T-lymphotropic virus type I infection. *Leukemia* 8(Suppl 1):S222–S226.
68. Lee, R. V., A. W. Prown, S. K. Satchidanand, and B. I. Srivastava. 1985. Non-Hodgkin's lymphoma and HTLV-1 antibodies in a gorilla [Letter]. *N. Engl. J. Med.* 312:118–119.

69. Leendertz, F. H., C. Boesch, H. Ellerbrok, W. Retschel, E. Couacy-Hymann, and G. Pauli. 2004. Non-invasive testing reveals a high prevalence of simian T-lymphotropic virus type 1 antibodies in wild adult chimpanzees of the Tai National Park, Cote d'Ivoire. *J. Gen. Virol.* 85(Pt 11):3305–3312.
70. Leendertz, F. H., C. Boesch, S. Junglen, G. Pauli, and H. Ellerbrok. 2003. Characterization of a new simian T-lymphocyte virus type 1 (STLV-1) in a wild living chimpanzee (*Pan troglodytes verus*) from Ivory Coast: evidence of a new STLV-1 group? *AIDS Res. Hum. Retroviruses* 19(3):255–258.
71. Leendertz, F. H., S. Junglen, C. Boesch, P. Formenty, E. Couacy-Hymann, V. Courgaud, G. Pauli, and H. Ellerbrok. 2004. High variety of different simian T-cell leukemia virus type 1 strains in chimpanzees (*Pan troglodytes verus*) of the Tai National Park, Cote d'Ivoire. *J. Virol.* 78(8):4352–4356.
72. Liegeois, F., B. Lafay, W. M. Switzer, S. Locatelli, E. Mpoudi-Ngole, S. Loul, W. Heneine, E. Delaporte, and M. Peeters. 2008. Identification and molecular characterization of new STLV-1 and STLV-3 strains in wild-caught nonhuman primates in Cameroon. *Virology* 371(2):405–417.
73. Liska, V., P. N. Fultz, L. Su, and R. M. Ruprecht. 1997. Detection of simian T cell leukemia virus type I infection in seronegative macaques. *AIDS Res. Hum. Retroviruses* 13(13):1147–1153.
74. Liu, H. F., P. Goubaud, M. Van Brussel, J. Desmyter, and A. M. Vandamme. 1997. Phylogenetic analysis of a simian T lymphotropic virus type I from a hamadryas baboon. *AIDS Res. Hum. Retroviruses* 13(17):1545–1548.
75. Liu, H. F., P. Goubaud, M. Van Brussel, K. Van Laethem, Y. C. Chen, J. Desmyter, and A. M. Vandamme. 1996. The three human T-lymphotropic virus type I subtypes arose from three geographically distinct simian reservoirs. *J. Gen. Virol.* 77(Pt 2):359–368.
76. Liu, H. F., A. M. Vandamme, M. Van Brussel, J. Desmyter, and P. Goubaud. 1994. New retroviruses in human and simian T-lymphotropic viruses. *Lancet* 344(8917):265–266.
77. Lowenstein, L. J., N. C. Pedersen, J. Higgins, K. C. Pallis, A. Uyeda, P. Marx, N. W. Lerche, R. J. Munn, and M. B. Gardner. 1986. Seroepidemiologic survey of captive old-world primates for antibodies to human and simian retroviruses, and isolation of a lentivirus from sooty mangabeys (*Cercocebus atys*). *Int. J. Cancer* 38:563–574.
78. Mador, N., A. Panet, and A. Honigman. 1989. Translation of gag, pro, and pol gene products of human T-cell leukemia virus type 2. *J. Virol.* 63(5):2400–2404.
79. Mahieux, R., C. Chappey, M. C. Georges-Courbot, G. Dubreuil, P. Mauclere, A. Georges, and A. Gessain. 1998. Simian T-cell lymphotropic virus type 1 from *Mandrillus sphinx* as a simian counterpart of human T-cell lymphotropic virus type 1 subtype D. *J. Virol.* 72(12):10316–10322.
80. Mahieux, R., C. Chappey, L. Meertens, P. Mauclere, J. Lewis, and A. Gessain. 2000. Molecular characterization and phylogenetic analyses of a new simian T cell lymphotropic virus type 1 in a wild-caught African baboon (*Papio anubis*) with an indeterminate STLV type 2-like serology. *AIDS Res. Hum. Retroviruses* 16(18):2043–2048.
81. Mahieux, R., J. Pecon-Slattery, G. M. Chen, and A. Gessain. 1998. Evolutionary inferences of novel simian T lymphotropic virus type 1 from wild-caught chacma (*Papio ursinus*) and olive baboons (*Papio anubis*). *Virology* 251(1):71–84.
82. Mahieux, R., J. Pecon-Slattery, and A. Gessain. 1997. Molecular characterization and phylogenetic analyses of a new, highly divergent simian T-cell lymphotropic virus type 1 (STLV-1marc1) in *Macaca arctoides*. *J. Virol.* 71(8):6253–6258.
83. Makuwa, M., S. Souquiere, S. L. Clifford, P. T. Telfer, B. Salle, O. Bourry, R. Onanga, A. Mouinga-Ondeme, E. J. Wickings, K. A. Abernethy, P. Rouquet, F. Simon, and P. Roques. 2004. Two distinct STLV-1 subtypes infecting *Mandrillus sphinx* follow the geographic distribution of their hosts. *AIDS Res. Hum. Retroviruses* 20(10):1137–1143.
84. Makuwa, M., S. Souquiere, P. Telfer, A. Mouinga-Ondeme, O. Bourry, and P. Roques. 2004. A new STLV-1 in a household pet *Cercopithecus nictitans* from Gabon. *AIDS Res. Hum. Retroviruses* 20(6):679–683.
85. Manel, N., J. L. Battini, N. Taylor, and M. Sitbon. 2005. HTLV-1 tropism and envelope receptor. *Oncogene* 24(39):6016–6025.
86. Manel, N., F. J. Kim, S. Kinet, N. Taylor, M. Sitbon, and J. L. Battini. 2003. The ubiquitous glucose transporter GLUT-1 is a receptor for HTLV. *Cell* 115(4):449–459.
87. McCarthy, T. J., J. L. Kennedy, J. R. Blakeslee, and B. T. Bennett. 1990. Spontaneous malignant lymphoma and leukemia in a simian T-lymphotropic virus type I (STLV-I) antibody positive olive baboon. *Lab. Anim. Sci.* 40:79–81.
88. McGinn, T. M., B. Tao, S. Cartner, T. Schoeb, I. Davis, L. Ratner, and P. N. Fultz. 2002. Association of primate T-cell lymphotropic virus infection of pig-tailed macaques with high mortality. *Virology* 304(2):364–378.

89. Meertens, L. and A. Gessain. 2003. Divergent simian T-cell lymphotropic virus type 3 (STLV-3) in wild-caught *Papio hamadryas papio* from Senegal: widespread distribution of STLV-3 in Africa. *J. Virol.* 77(1):782–789.
90. Meertens, L., R. Mahieux, P. Mauclere, J. Lewis, and A. Gessain. 2002. Complete sequence of a novel highly divergent simian T-cell lymphotropic virus from wild-caught red-capped mangabeys (*Cercocebus torquatus*) from Cameroon: a new primate T-lymphotropic virus type 3 subtype. *J. Virol.* 76(1):259–268.
91. Meertens, L., J. Rigoulet, P. Mauclere, M. Van Beveren, G. M. Chen, O. Diop, G. Dubreuil, M. C. Georges-Goubot, J. L. Berthier, J. Lewis, and A. Gessain. 2001. Molecular and phylogenetic analyses of 16 novel simian T cell leukemia virus type 1 from Africa: close relationship of STLV-1 from *Allochetinus nigroviridis* to HTLV-1 subtype B strains. *Virology* 287(2):275–285.
92. Meertens, L., V. Shanmugam, A. Gessain, B. E. Beer, Z. Tooze, W. Heneine, and W. M. Switzer. 2003. A novel, divergent simian T-cell lymphotropic virus type 3 in a wild-caught red-capped mangabey (*Cercocebus torquatus torquatus*) from Nigeria. *J. Gen. Virol.* 84(Pt 10):2723–2727.
93. Miyoshi, I., M. Fujishita, H. Taguchi, K. Matsubayashi, N. Miwa, and Y. Tanioka. 1983. Natural infection in non-human primates with adult T-cell leukemia virus or a closely related agent. *Int. J. Cancer* 32:333–336.
94. Miyoshi, I., M. Fujishita, S. Yoshimoto, I. Kubonishi, H. Taguchi, Y. Ohtsuki, and Y. Tanioka. 1984. Transmission of monkey retrovirus similar to human T-cell leukemia virus by blood transfusion. *Gann.* 75:479–481.
95. Miyoshi, I., Y. Ohtsuki, M. Fujishita, S. Yoshimoto, I. Kubonishi, and M. Minezawa. 1982. Detection of type C virus particles in Japanese monkeys seropositive to adult T-cell leukemia-associated antigens. *Gann.* 73:848–849.
96. Miyoshi, I., S. Yoshimoto, M. Fujishita, Y. Ohtsuki, H. Taguchi, Y. Shiraishi, T. Akagi, and M. Minezawa. 1983. Isolation in culture of a type C virus from a Japanese monkey seropositive to adult T-cell leukemia-associated antigens. *Gann.* 74:323–326.
97. Miyoshi, I., S. Yoshimoto, M. Fujishita, H. Taguchi, I. Kubonishi, K. Niiya, and M. Minezawa. 1982. Natural adult T-cell leukemia virus infection in Japanese monkeys [Letter]. *Lancet* 2:658–658.
98. Mone, J., E. Whitehead, M. Leland, G. Hubbard, and J. S. Allan. 1992. Simian T-cell leukemia virus type I infection in captive baboons. *AIDS Res. Hum. Retroviruses* 8:1653–1661.
99. Mulloy, J. C., T. S. Migone, T. M. Ross, N. Ton, P. L. Green, W. J. Leonard, and G. Franchini. 1998. Human and simian T-cell leukemia viruses type 2 (HTLV-2 and STLV-2(pan-p)) transform T cells independently of Jak/STAT activation. *J. Virol.* 72(5):4408–4412.
100. Nerrienet, E., X. Amouretti, M. C. Muller-Trutwin, V. Poaty-Mavoungou, I. Bedjebaga, H. T. Nguyen, G. Dubreuil, S. Corbet, E. J. Wickings, F. Barre-Sinoussi, A. J. Georges, and M. C. Georges-Courbot. 1998. Phylogenetic analysis of SIV and STLV type I in mandrills (*Mandrillus sphinx*): indications that intracolony transmissions are predominantly the result of male-to-male aggressive contacts. *AIDS Res. Hum. Retroviruses* 14(9):785–796.
101. Nerrienet, E., L. Meertens, A. Kfutwah, Y. Fouopouapouognigni, A. Ayouba, and A. Gessain. 2004. Simian T cell leukaemia virus type I subtype B in a wild-caught gorilla (*Gorilla gorilla gorilla*) and chimpanzee (*Pan troglodytes vellerosus*) from Cameroon. *J. Gen. Virol.* 85(Pt 1):25–29.
102. Nerrienet, E., L. Meertens, A. Kfutwah, Y. Fouopouapouognigni, and A. Gessain. 2001. Molecular epidemiology of simian T-lymphotropic virus (STLV) in wild-caught monkeys and apes from Cameroon: a new STLV-1, related to human T-lymphotropic virus subtype F, in a *Cercocetus agilis*. *J. Gen. Virol.* 82(Pt 12):2973–2977.
103. Niphuis, H., E. J. Verschoor, I. Bontjer, M. Peeters, and J. L. Heeney. 2003. Reduced transmission and prevalence of simian T-cell lymphotropic virus in a closed breeding colony of chimpanzees (*Pan troglodytes verus*). *J. Gen. Virol.* 84(Pt 3):615–620.
104. Noda, Y., K. Ishikawa, A. Sasagawa, S. Honjo, S. Mori, H. Tsujimoto, and M. Hayami. 1986. Hematologic abnormalities similar to the preleukemic state of adult T-cell leukemia in African green monkeys naturally infected with simian T-cell leukemia virus. *Jpn. J. Cancer Res.* 77:1227–1234.
105. Parrish, S. W., A. E. Brown, P. Chanbancherd, M. Gettayacamin, and J. H. Parrish. 2004. Transmission of STLV in a closed colony of macaques. *Am. J. Primatol.* 63(2):103–109.
106. Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. U. S. A.* 77(12):7415–7419.
107. Prowten, A. W., R. V. Lee, R. M. Krishnamsetty, S. K. Satchidanand, and B. I. Srivastava. 1985. T-cell lymphoma associated with immunologic evidence of

- retrovirus infection in a lowland gorilla. *J. Am. Vet. Med. Assoc.* 187:1280–1282.
108. Richards, A. L., A. Giri, D. Iskandriati, J. Pamungkas, A. Sie, L. Rosen, R. L. Anthony, and G. Franchini. 1998. Simian T-lymphotropic virus type I infection among wild-caught Indonesian pig-tailed macaques (*Macaca nemestrina*). *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 19(5):542–545.
 109. Rudolph, D. L., J. Yee, J. Mone, S. K. Foung, J. J. Lipka, G. R. Reyes, K. Hadlock, L. Chan, F. Villinger, and M. D. Lairmore. 1992. Serologic confirmation of simian T-lymphotropic virus type I infection by using immunoassays developed for human T-lymphotropic virus antibody detection. *J. Clin. Microbiol.* 30:858–861.
 110. Rudolph, D. L., J. Yee, T. Parker, J. E. Coligan, and R. B. Lal. 1993. Antibody responses to the env epitopes of human T-lymphotropic virus type I in rhesus macaques naturally infected with simian T-lymphotropic virus type I. *Res. Virol.* 144:193–199.
 111. Sakakibara, I., Y. Sugimoto, A. Sasagawa, S. Honjo, H. Tsujimoto, H. Nakamura, and M. Hayami. 1986. Spontaneous malignant lymphoma in an African green monkey naturally infected with simian T-lymphotropic virus (STLV). *J. Med. Primatol.* 15:311–318.
 112. Saksena, N., Y. C. Ge, V. Herve, O. Diop, M. Miranda-Saksena, C. Mathiot, and J. P. Digoutte. 1995. Sequence analysis of simian T cell lymphoma/leukemia virus type 1 from naturally infected monkeys from central and west Africa reveals evolutionary conservation of immunogenic and neutralizing domains of gp46. *AIDS Res. Hum. Retroviruses* 11(10):1261–1263.
 113. Saksena, N. K., V. Herve, J. P. Durand, B. Leguenno, O. M. Diop, J. P. Digouette, C. Mathiot, M. C. Muller, J. L. Love, and S. Dube. 1994. Seroepidemiologic, molecular, and phylogenetic analyses of simian T-cell leukemia viruses (STLV-I) from various naturally infected monkey species from central and western Africa. *Virology* 198:297–310.
 114. Saksena, N. K., V. Herve, M. P. Sherman, J. P. Durand, C. Mathiot, M. Muller, J. L. Love, B. Leguenno, F. B. Sinoussi, and D. K. Dube. 1993. Sequence and phylogenetic analyses of a new STLV-I from a naturally infected tantalus monkey from Central Africa. *Virology* 192:312–320.
 115. Saksena, N. K., A. Srinivasan, Y. C. Ge, S. H. Xiang, A. Azad, W. Bolton, V. Herve, S. Reddy, O. Diop, M. Miranda-Saksena, W. D. Rawlinson, A. M. Vandamme, and F. Barre-Sinoussi. 1997. Simian T cell leukemia virus type I from naturally infected feral monkeys from central and west Africa encodes a 91-
 - amino acid p12 (ORF-I) protein as opposed to a 99-amino acid protein encoded by HTLV type I from humans. *AIDS Res. Hum. Retroviruses* 13(5):425–432.
 116. Salemi, M., J. Desmyter, and A. M. Vandamme. 2000. Tempo and mode of human and simian T-lymphotropic virus (HTLV/STLV) evolution revealed by analyses of full-genome sequences. *Mol. Biol. Evol.* 17(3):374–386.
 117. Salemi, M., S. Van Dooren, E. Audenaert, E. Delaporte, P. Goubau, J. Desmyter, and A. M. Vandamme. 1998. Two new human T-lymphotropic virus type I phylogenetic subtypes in seroindeterminates, a Mbuti pygmy and a Gabonese, have closest relatives among African STLV-I strains. *Virology* 246(2):277–287.
 118. Sato, Y., S. Matsuura, K. Kadota, and I. Miyazawa. 1999. T-cell lymphoma in a savanna monkey (*Cercopithecus aethiops*) probably related to simian T-cell leukemia virus infection. *J. Vet. Med. Sci.* 61(1):49–52.
 119. Schatzl, H., M. Tschikobava, D. Rose, A. Voevodin, H. Nitschko, E. Sieger, U. Busch, K. von der Helm, and B. Lapin. 1993. The Sukhumi primate monkey model for viral lymphomagenesis: high incidence of lymphomas with presence of STLV-I and EBV-like virus. *Leukemia* 7(Suppl 2):S86–S92.
 120. Schatzl, H., L. Yakovleva, B. Lapin, D. Rose, L. Inzhiia, K. Gaedigk-Nitschko, F. Deinhardt, and K. von der Helm. 1992. Detection and characterization of T-cell leukemia virus-like proviral sequences in PBL and tissues of baboons by PCR. *Leukemia* 6(Suppl 3):158S–160S.
 121. Slattery, J. P., G. Franchini, and A. Gessain. 1999. Genomic evolution, patterns of global dissemination, and interspecies transmission of human and simian T-cell leukemialymphotropic viruses. *Genome Res.* 9(6):525–540.
 122. Song, K. J., V. R. Nerurkar, N. Saitou, A. Lazo, J. R. Blakeslee, I. Miyoshi, and R. Yanagihara. 1994. Genetic analysis and molecular phylogeny of simian T-cell lymphotropic virus type I: evidence for independent virus evolution in Asia and Africa. *Virology* 199(1):56–66.
 123. Srivastava, B. I., F. Wong-Staal, and J. P. Getchell. 1986. Human T-cell leukemia virus I provirus and antibodies in a captive gorilla with non-Hodgkin's lymphoma. *Cancer Res.* 46:4756–4758.
 124. Switzer, W. M., S. H. Qari, N. D. Wolfe, D. S. Burke, T. M. Folks, and W. Heneine. 2006. Ancient origin and molecular features of the novel human T-lymphotropic virus type 3 revealed by complete genome analysis. *J. Virol.* 80(15):7427–7438.
 125. Takemura, T., M. Yamashita, M. K. Shimada, S. Ohkura, T. Shotake, M. Ikeda, T. Miura, and

- M. Hayami. 2002. High prevalence of simian T-lymphotropic virus type L in wild ethiopian baboons. *J. Virol.* 76(4):1642–1648.
126. Tanaka, Y., A. Yoshida, Y. Takayama, H. Tsujimoto, A. Tsujimoto, M. Hayami, and H. Tozawa. 1990. Heterogeneity of antigen molecules recognized by anti-tax1 monoclonal antibody Lt-4 in cell lines bearing human T cell leukemia virus type I and related retroviruses. *Jpn. J. Cancer Res.* 81:225–231.
127. Timanovskaia, V. V., A. F. Voevodin, L. A. Iakovleva, D. S. Markarian, and M. T. Ivanov. 1988. [Malignant lymphoma in rabbits induced by the administration of herpes virus-containing material from brown macaques]. *Eksp. Onkol.* 10(3):47–51.
128. Traina-Dorge, V., J. Blanchard, L. Martin, and M. Murphey-Corb. 1992. Immunodeficiency and lymphoproliferative disease in an African green monkey dually infected with SIV and STLV-I. *AIDS Res. Hum. Retroviruses* 8:97–100.
129. Traina-Dorge, V. L., R. Lorino, B. J. Gormus, M. Metzger, P. Telfer, D. Richardson, D. L. Robertson, P. A. Marx, and C. Apetrei. 2005. Molecular epidemiology of simian T-cell lymphotropic virus type 1 in wild and captive sooty mangabeys. *J. Virol.* 79(4):2541–2548.
130. Tsujimoto, A., H. Tsujimoto, N. Yanaihara, K. Abe, M. Hayami, M. Miwa, and K. Shimotohno. 1986. Detection of the X gene product of simian T-cell leukemia virus. *FEBS Lett.* 196:301–304.
131. Tsujimoto, H., A. Komuro, K. Iijima, J. Miyamoto, K. Ishikawa, and M. Hayami. 1985. Isolation of simian retroviruses closely related to human T-cell leukemia virus by establishment of lymphoid cell lines from various non-human primates. *Int. J. Cancer* 35:377–384.
132. Tsujimoto, H., Y. Noda, K. Ishikawa, H. Nakamura, M. Fukasawa, I. Sakakibara, A. Sasagawa, S. Honjo, and M. Hayami. 1987. Development of adult T-cell leukemia-like disease in African green monkey associated with clonal integration of simian T-cell leukemia virus type I. *Cancer Res.* 47:269–274.
133. Tsujimoto, H., M. Seiki, H. Nakamura, T. Watanabe, I. Sakakibara, A. Sasagawa, S. Honjo, M. Hayami, and M. Yoshida. 1985. Adult T-cell leukemia-like disease in monkey naturally infected with simian retrovirus related to human T-cell leukemia virus type I. *Jpn. J. Cancer Res.* 76:911–914.
134. Van Brussel, M., P. Goubaud, R. Rousseau, J. Desmyter, and A. M. Vandamme. 1996. The genomic structure of a new simian T-lymphotropic virus, STLV- PH969, differs from that of human T-lymphotropic virus types I and II. *J. Gen. Virol.* 77(Pt 2):347–358.
135. Van Brussel, M., P. Goubaud, R. Rousseau, J. Desmyter, and A. M. Vandamme. 1997. Complete nucleotide sequence of the new simian T-lymphotropic virus, STLV-PH969 from a Hamadryas baboon, and unusual features of its long terminal repeat. *J. Virol.* 71(7):5464–5472.
136. Van Brussel, M., M. Salemi, H. F. Liu, J. Gabriels, P. Goubaud, J. Desmyter, and A. M. Vandamme. 1998. The simian T-lymphotropic virus STLV-PP1664 from Pan paniscus is distinctly related to HTLV-2 but differs in genomic organization. *Virology* 243(2):366–379.
137. Van Brussel, M., M. Salemi, H. F. Liu, P. Goubaud, J. Desmyter, and A. M. Vandamme. 1999. The discovery of two new divergent STLVs has implications for the evolution and epidemiology of HTLVs. *Rev. Med. Virol.* 9(3):155–170.
138. Van Dooren, S., L. Meertens, P. Lemey, A. Gessain, and A. M. Vandamme. 2005. Full-genome analysis of a highly divergent simian T-cell lymphotropic virus type 1 strain in Macaca arctoides. *J. Gen. Virol.* 86(Pt 7):1953–1959.
139. Van Dooren, S., M. Salemi, X. Pourrut, M. Peeters, E. Delaporte, M. Van Ranst, and A. M. Vandamme. 2001. Evidence for a second simian T-cell lymphotropic virus type 3 in Cercopithecus nictitans from Cameroon. *J. Virol.* 75(23):11939–11941.
140. Van Dooren, S., V. Shanmugam, V. Bhullar, B. Parekh, A. M. Vandamme, W. Heneine, and W. M. Switzer. 2004. Identification in gelada baboons (*Theropithecus gelada*) of a distinct simian T-cell lymphotropic virus type 3 with a broad range of Western blot reactivity. *J. Gen. Virol.* 85(Pt 2):507–519.
141. Van Dooren, S., E. J. Verschoor, Z. Fagrouch, and A. M. Vandamme. 2007. Phylogeny of primate T lymphotropic virus type 1 (PTLV-1) including various new Asian and African non-human primate strains. *Infect. Genet. Evol.* 7(3):374–381.
142. van Rensburg, E., S. Engelbrecht, B. Robson, D. Langat, M. Isahakia, and J. Mwenda. 1999. Phylogenetic analysis of simian T lymphotropic virus type I from Kenyan olive baboons (*Papio anubis*), lowland Sykes monkeys (*Cercopithecus mitis*), and vervet monkeys (*Cercopithecus aethiops pygerythrurus*). *AIDS Res. Hum. Retroviruses* 15(8):781–784.
143. Vandamme, A. M., H. F. Liu, P. Goubaud, and J. Desmyter. 1994. Primate T-lymphotropic virus type I LTR sequence variation and its phylogenetic analysis: compatibility with an African origin of PTLV-I. *Virology* 202:212–223.
144. Vandamme, A. M., H. F. Liu, M. Van Brussel, W. De Meurichy, J. Desmyter, and P. Goubaud. 1996. The presence of a divergent T-lymphotropic virus in a

- wild-caught pygmy chimpanzee (*Pan paniscus*) supports an African origin for the human T-lymphotropic simian T-lymphotropic group of viruses. *J. Gen. Virol.* 77(Pt 5):1089–1099.
145. Vandamme, A. M., K. Van Laethem, H. F. Liu, M. Van Brussel, E. Delaporte, C. M. Castro Costa, C. Fleischer, G. Taylor, U. Bertazzoni, J. Desmyter, and P. Goubau. 1997. Use of a generic polymerase chain reaction assay detecting human T-lymphotropic virus (HTLV) types I, II and divergent simian strains in the evaluation of individuals with indeterminate HTLV serology. *J. Med. Virol.* 52(1):1–7.
 146. Verdonck, K., E. Gonzalez, S. Van Dooren, A. M. Vandamme, G. Vanham, and E. Gotuzzo. 2007. Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect. Dis.* 7(4):266–281.
 147. Verschoor, E. J., K. S. Warren, H. Niphuis, Heriyanto, R. A. Swan, and J. L. Heeney. 1998. Characterization of a simian T-lymphotropic virus from a wild-caught orang-utan (*Pongo pygmaeus*) from Kalimantan, Indonesia. *J. Gen. Virol.* 79(Pt 1):51–55.
 148. Vincent, M. J., F. J. Novembre, V. F. Yamshchikov, H. M. McClure, and R. W. Compans. 1996. Characterization of a novel baboon virus closely resembling human T-cell leukemia virus. *Virology* 226(1):57–65.
 149. Voevodin, A., T. Miura, E. Samilchuk, and H. Schatzl. 1996. Phylogenetic characterization of simian T-lymphotropic virus type I (STLV-I) from the Ethiopian sacred baboon (*Papio hamadryas*). *AIDS Res. Hum. Retroviruses* 12(3):255–258.
 150. Voevodin, A., E. Samilchuk, J. Allan, J. Rogers, and S. Broussard. 1997. Simian T-lymphotropic virus type 1 (STLV-1) infection in wild yellow baboons (*Papio hamadryas cynocephalus*) from Mikumi National Park, Tanzania. *Virology* 228(2):350–359.
 151. Voevodin, A., E. Samilchuk, H. Schatzl, E. Boeri, and G. Franchini. 1996. Interspecies transmission of macaque simian T-cell leukemia/lymphoma virus type 1 in baboons resulted in an outbreak of malignant lymphoma. *J. Virol.* 70(3):1633–1639.
 152. Voevodin, A. F., B. K. Johnson, E. I. Samilchuk, G. A. Stone, R. Druilhet, W. J. Greer, and C. J. Gibbs, Jr. 1997. Phylogenetic analysis of simian T-lymphotropic virus Type I (STLV-I) in common chimpanzees (*Pan troglodytes*): evidence for interspecies transmission of the virus between chimpanzees and humans in Central Africa. *Virology* 238(2):212–220.
 153. Voevodin, A. F., B. A. Lapin, A. G. Tatosyan, and I. Hirsch. 1987. Markers of HTLV-I-related virus in hamadryas baboon lymphoma. *Haematol. Blood Transfus.* 31:392–394.
 154. Voevodin, A. F., B. A. Lapin, L. A. Yakovleva, T. I. Ponomaryeva, T. E. Oganyan, and E. N. Razmadze. 1985. Antibodies reacting with human T-lymphotropic retrovirus (HTLV-I) or related antigens in lymphomatous and healthy hamadryas baboons. *Int. J. Cancer* 36:579–584.
 155. Wattel, E., J. P. Vartanian, C. Pannetier, and S. Wain-Hobson. 1995. Clonal expansion of human T-cell leukemia virus type I-infected cells in asymptomatic and symptomatic carriers without malignancy. *J. Virol.* 69(5):2863–2868.
 156. Wolfe, N. D., W. Heneine, J. K. Carr, A. D. Garcia, V. Shanmugam, U. Tamoufe, J. N. Torimiro, A. T. Prosser, M. Lebreton, E. Mpoudi-Ngole, F. E. McCutchan, D. L. Birx, T. M. Folks, D. S. Burke, and W. M. Switzer. 2005. Emergence of unique primate T-lymphotropic viruses among central African bushmeat hunters. *Proc. Natl. Acad. Sci. U. S. A.* 102(22):7994–7999.
 157. Yakovleva, L. A., L. V. Indzhiia, M. G. Chikobava, N. P. Demenkova, I. N. Klots, G. Shetts'l', F. Deinkhardt, and B. A. Lapin. 1995. [Large-cell anaplastic malignant CD30/Ki-1-positive T-type lymphoma, associated with STLV-1 virus (baboon malignant lymphoma model) Krupokletochnye anaplasticheskie zлокачественные CD30/Ki-1-позитивные лимфомы Т-типа, ассоциированные с вирусом STLV-1 (на модели злокачественных лимфом павианов)]. *Gematol. Transfuziol.* 40(1):10–16.
 158. Yakovleva, L. A., L. V. Indzhiia, M. G. Chikobaeva, G. Shats'l', and B. A. Lapin. 1997. [Morphoimmunologic phenotypes of T-cell non-Hodgkin's malignant lymphoma in baboons and their connection with T-lymphotropic retrovirus STLV-1]. *Arkh. Patol.* 59(1):19–25.
 159. Yakovleva, L. A., K. Lennert, M. G. Chikobava, L. V. Indzhiia, I. N. Klotz, and B. A. Lapin. 1993. Morphological characteristics of malignant T-cell lymphomas in baboons. *Virchows Arch. A. Pathol. Anat. Histopathol.* 422:109–120.
 160. Yakovleva, L. A., V. V. Timanovskaya, L. V. Indzhiia, B. A. Lapin, and A. F. Voevodin. 1987. [Modelling of malignant lymphoma in rabbits using primate oncogenic viruses. Preliminary report]. *Biull. Eksp. Biol. Med.* 103(3):336–338.
 161. Yamamoto, N., Y. Hinuma, H. zur Hausen, J. Schneider, and G. Hunsmann. 1983. African green monkeys are infected with adult T-cell leukaemia virus or closely related agent [Letter]. *Lancet* 1:240–241.
 162. Yamamoto, N., N. Kobayashi, K. Takeuchi, Y. Koyanagi, M. Hatanaka, Y. Hinuma, T. Chosa, J. Schneider, and G. Hunsmann. 1984. Characterization of African green monkey B-cell lines releasing an adult T-cell leukemia-virus-related agent. *Int. J. Cancer* 34:77–82.

163. Yoshida, M., M. Seiki, K. Yamaguchi, and K. Takatsuki. 1984. Monoclonal integration of human T-cell leukemia provirus in all primary tumors of adult T-cell leukemia suggests causative role of human T-cell leukemia virus in the disease. *Proc. Natl. Acad. Sci. U. S. A.* 81(8):2534–2537.
164. Yoshimura, N., H. Nakamura, K. Ishikawa, Y. Noda, S. Honjo, and M. Hayami. 1990. Simian T cell leukemia virus type-1-specific killer T cells in naturally infected African green monkey carriers. *J. Immunol.* 144:2173–2178.

9

Spumaviruses

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9.1. INTRODUCTION

Spumaviruses (Latin *spuma* = foam) are commonly referred to as “foamy” viruses because of the dramatic vacuolizing (foam-like) cytopathic effects (CPEs) they induce in tissue culture (Figure 9.1).

Paradoxically, these viruses are not known to cause disease. Being “medically insignificant,” foamy viruses have attracted little interest, except a short-lived spike of attention when it was shown they contain the reverse transcriptase. To an extant the situation has changed in the mid-1990s, when it became known that spumaviruses are fundamentally different from other retroviruses.^{31,70,75,82,121} A striking feature of spumaviruses is the unusual timing of the reverse transcription, which occurs inside virions. As a result, some virions acquire a complete DNA genome before in-

festing a susceptible cell. Moreover, only the DNA-containing virions are infectious. Spumaviruses occupy a unique position in the viral world, being a link between the reverse transcriptase-containing viruses with RNA genome (all *Retroviridae*, except spumaviruses) and DNA genome (*Hepadnaviridae*).

Spumavirus infection is common in nonhuman primates (NHPs) and can be transmitted from monkeys and apes to humans. At the same time no human spumavirus is known, which is unexpected considering that virtually all Old World simian species harbor spumaviruses.

9.2. CLASSIFICATION AND NOMENCLATURE

Spumaviruses are grouped in the subfamily *Spumaretrovirinae* within the *Retroviridae* family. There is only one genus in this subfamily—*Spumavirus*. Three species of simian spumaviruses are currently recognized by the International Committee on Taxonomy of Viruses (ICTV): *African green monkey simian foamy virus* (SFVagm), *Macaque simian foamy virus* (SFVmac), and prototypic *Simian foamy virus*. The prototype SFV was isolated from human material.¹ The virus was believed to be of human origin and named accordingly as human foamy virus (HFV). Most of the strains used in spumavirus research are derived from this isolate. By the mid-1990s, it became clear that “HFV” was not a human virus. Its natural host is the common chimpanzee,⁴⁴ more specifically, the East African chimpanzee subspecies (*Pan troglodytes schweinfurthii*).¹¹³ The controversy regarding the origin of “HFV” is reflected in the usage of various names for this isolate [HFV, SFVhu, SFVcpz(hu), CFV/hu]. These names are superseded by the ICTV-recommended designation “prototype foamy virus” (PFV).

Table 9.1. Infectious SFV Isolates from Primate Species

Abbreviated Name	Serotypes	Host Species	References
Old World monkeys			
SFVmac*	SFV-1	<i>Macaca mulatta</i>	99
SFVmac	SFV-2	<i>M. cyclopis</i>	49
SFVagm*	SFV-3	<i>Chlorocebus aethiops</i>	110
SFVbab	SFV-10	<i>Papio cynocephalus</i>	94
SFVtfr	?	<i>Trachypithecus francoisi</i>	48
SFVag16	?	<i>Cercopithecus</i> spp.	14
Apes			
PFV*	?	<i>P. t. schweinfurthii</i>	1, 44
SFVcpz	SFV-6	<i>Pan troglodytes</i>	96
SFVcpz	SFV-7	<i>Pan troglodytes</i>	96
SFVora	SFV-11	<i>Pongo pygmaeus</i>	73
SFVgor	?	<i>Gorilla gorilla</i>	6
SFVhpi	?	<i>Hylobates pileatus</i>	48
SFVhle	?	<i>Hylobates leucogenys</i>	48
New World monkeys			
SFVsqu	SFV-4	<i>Saimiri sciureus</i>	50
SFVspm	SFV-8	<i>Ateles</i> sp.	47
SFVcap	SFV-9	<i>Cebus</i> sp.	46
SFVmar	?	<i>Callithrix jacchus</i>	72
Prosimians			
SFVgal	SFV-5	<i>Galago</i> spp.	50

*ICTV-recognized species.

?, Serotype not known.

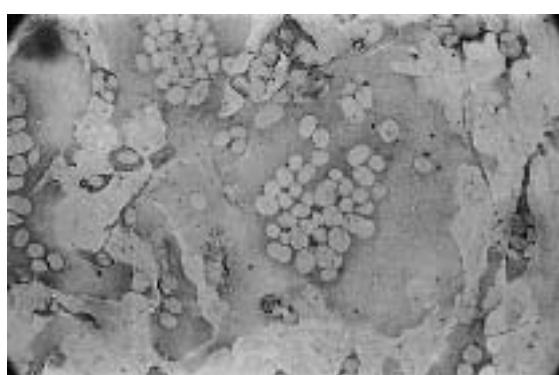


Figure 9.1. Cytopathic effects (CPEs) caused by SFV in vitro. Syncytia-formation caused by SFV in human fibrosarcoma cells HT1080. (Image is kindly provided by Prof. Myra McClure and Dr. Gillian Wills.)

Historically, the first was the classification of spumaviruses based on the pattern of cross-neutralization.⁵⁰ Eleven serotypes of simian foamy viruses (SFV-1 through SFV-11) have been described (Table 9.1). Although this classification is largely abandoned, its “relics” are still found in the literature. The number of known SFVs is greater than it is reflected in the ICTV classification (Table 9.1). In addition, SFV genomic fragments (“frag-viruses”) have been detected in virtually every Old World NHP species sufficiently well studied in this respect (Table 9.2).

Names of SFV isolates and frag-viruses include information on the species of origin, usually as three-letter subscript, for example, SFVcpz (chimpanzee) and SFVmac (macaque). These subscripts may be confusing due to the lack of standardization in abbreviating species names. For example, SFVora, SFVpon, and SFVppy, where “ora,” “pon,” and “ppy” mean orangutan, *Pongo*,

Table 9.2. SFV Frag-Viruses

Abbreviated Name	Host Species	GenBank Access Numbers
Old World monkeys		
SFVmne	<i>M. nemestrina</i>	AY686196*
SFVmar	<i>M. arctoides</i>	AY686198*
SFVmto	<i>M. tonkeana</i>	DQ354089*
SFVmfa	<i>M. fascicularis</i>	AY686197*
SFVmra	<i>M. radiata</i>	AY686199
SFVmfu	<i>M. fuscata</i>	AY686200
SFVmsi	<i>M. silenus</i>	AY686201*
SFVmsy	<i>M. sylvanus</i>	AY686190*
SFVmsp	<i>Mandrillus sphinx</i>	AY278790*
SFVmle	<i>M. leucophaeus</i>	AY686181*
SFVpha	<i>Papio hamadryas</i>	AY686178
SFVppa	<i>P. papio</i>	AY686177
SFVpan	<i>P. anubis</i>	AY686175*
SFVpcy	<i>P. cynocephalus</i>	AY686176
SFVpur	<i>P. ursinus</i>	AY686179*
SFVtge	<i>T. gelada</i>	AY686180
SFVcto	<i>Cercocebus torquatus</i>	AY686183
SFVcat	<i>C. atys</i>	AY686184
SFVcag	<i>C. agilis</i>	AY278786
SFVcae	<i>Chlorocebus aethiops</i>	AY686187*
SFVcpy	<i>C. pygerythrus</i>	AY686185
SFVcsa	<i>C. sabaeus</i>	AY686186
SFVcne	<i>Cercopithecus neglectus</i>	AY686188
SFVcmo	<i>C. mona</i>	AY686189
SFVcal	<i>C. albogularis</i>	AY278778
SFVelh	<i>C. l'hoesti</i>	AY278781
SFVepa	<i>Erythrocebus patas</i>	AY278780
Apes		
SFFpab	<i>Pongo pygmaeus abelii</i>	AY686205*
SFVhle	<i>Hylobates leucogenys</i>	AF516487
SFVhpi	<i>H. pileatus</i>	AF516486
SFVptt	<i>Pan troglodytes troglodytes</i>	AY639140*
SFVpvr	<i>P. t. verus</i>	AY195685*
SFVpve	<i>P. t. vellerosus</i>	AY639141*
SFVpsc	<i>P. t. schweinfurthii</i>	AJ627553*
SFVppn	<i>P. paniscus</i>	AY686195*
SFVggo	<i>Gorilla gorilla gorilla</i>	AJ627549*
SFVgsp	<i>Gorilla spp.</i>	AY603410*

*A representative frag-virus from this species or subspecies.

and *Pongo pygmaeus*, respectively, are used for the same orangutan foamy virus.

The morphology of spumaviruses is distinct and does not fit into type C or type D, although there is some resemblance with “immature” type-C particles: the core of spumaviruses is ring-like. Prominent surface spikes and the wide space between the envelope and the core distinguish spumaviruses from typical immature type-C particles (Figure 9.2).

The phylogeny of SFV is characterized by nearly perfect correspondence between viral groupings and host taxonomy¹¹³ indicating long co-evolution of SFV and their NHP host species. The congruence of viral and host phylogenies allows accurate identification of the naturally infected host species, sometimes even the subspecies. For example, the great apes, such as Bornean and Sumatran orangutans (*Pongo pygmaeus pygmaeus* and *P. p. abelii*)¹¹⁷ and the common chimpanzee from West, Central, and Eastern African regions (*Pan troglodytes verus*, *P. t. vellerosus*, *P. t. troglodytes*, *P. t. schweinfurthii*) are each naturally infected with host-specific variants of SFV.^{16,113}

The genomic fragment commonly used in the phylogenetic analysis of SFVs is a 425-bp fragment of the integrase (*int*) region in the *pol* gene. The primers used for amplification of this fragment are generic for almost all SFVs¹⁰⁵ and the 425-bp *pol* sequence is available for virtually all known SFVs. In many cases, the sequence of this fragment is the only evidence that a particular simian species/subspecies harbors SFV (frag-viruses in Table 9.2).

9.3. GENOMIC ORGANIZATION

The spumavirus genome is about 13,000 bp, and is therefore larger than all other retroviral genomes. Five complete SFV genome sequences are available: PFV (GenBank Acc. No. Y07725), SFVchz (GenBank Acc. No. NC_001364), SFVmac (GenBank Acc. No. X54482), SFVora (GenBank Acc. No. AJ544579), and SFVspm (GenBank Acc. No. EU010385).^{44,63,92,114,118} As expected, the New World monkey SFV genome (SFVspm) is the most divergent among these genomes.¹¹⁴ However, all open reading frames (ORFs) and other major genomic features are conserved among simian SFV genomes.

The genome of individual SFV is remarkably stable.¹⁰⁶ In this respect SFVs are similar to primate deltaretroviruses. It was suggested, by analogy with

deltaretroviruses, that the reason for the stability of SFV genome is a low viral replication rate in vivo. It is presumed that most of the spumavirus genome copying in vivo is done by cellular DNA polymerase which copies the integrated provirus during the S phase of the cell cycle. The cellular DNA polymerase is less error-prone than the reverse transcriptase—hence the stability of the SFV genome. The SFV genome is structured like that of other complex retroviruses:

5'-LTR-gag-pol-env-auxiliary genes-3'-LTR

There are two auxiliary genes: *tas* (previously called *bel-1*) and *bel-2*.³³ A schematic map of spumavirus genome is presented in Figure 9.3. An additional ORF named *bel-3* is present in the PFV genome. However, no product encoded by *bel-3* has been found in PFV-infected cells and this ORF is absent in the genomes of other SFVs.

The LTR of spumaviruses is the largest among all retroviruses. The length varies from 1,760 bp in SFVcpz to 1,621 bp in SFVora. Apparently, SFVs from African NHPs have longer LTRs. The mutants containing large, multiple LTR deletions retain infectivity.¹⁰³ Despite significant variability, all functionally important elements in SFV LTRs are well conserved.^{103,114,118} The longest LTR region is U3 (1,286 bp and 1,423 bp in SFVora and SFVcpz, respectively). The U3 sequence is the least conserved in SFV LTR, both in terms of the number and length of indels and the number of substitutions.¹⁰³ At the same time, many functionally important sites are located in the U3: the transcription factor recognition sites (Est-1, Est-2, and Ap-1) in the 5'-part of the U3 and the transcription initiation signal (TATAA box) in the vicinity of the U3/R boundary. The prototypical retroviral promoter (P) is also located in the U3. Spumaviruses, in contrast to other retroviruses, also have an internal promoter (IP) located in the *env* gene. The length of the R region is about 180 bp. The major functional element located in the R region is the polyadenylation site. The cap site sequence, which starts at the end of the U3, extends 6 bp into the R region. The smallest LTR region is U5; its length is about 155 bp. The primer-binding site (PBS) for tRNAlys_{1,2}, which serves as the primer for SFV reverse transcriptase, is located in U5. There are also two sites in U5 (SI and SII) that are involved in the dimerization of genomic RNA, a feature distinguishing spumaviruses from orthoretroviruses.^{13,29}

In contrast to other retroviruses the packaging sequence, psi (Ψ), is split: the generic part of Ψ is located



Figure 9.2. Morphology of SFV in thin section EM. Multiple budding viral particles with characteristic spumavirus features (protruding surface spikes, ring-shaped core, and wide space between the core and envelope) are clearly visible. (Image is kindly provided by Prof. Hans R. Gelderblom.)

in the vicinity of the genome's 5'-end, while the second part, unique to the spumaviruses, is located within the *pol* gene.^{28,89}

9.4. VIRAL PROTEINS

Like other retroviruses, spumaviruses have structural proteins that are the products of the *gag*, *env*, and *pol* genes, and nonstructural proteins that are the products of the *tas* and *bel-2* genes.

Gag polyprotein is the major building block of the virus capsid. In contrast to other retroviruses, it is not cleaved into individual MA, CA, and NP proteins after release of progeny virions, and its complete processing before initiation of a new round of infection is not required for the infectivity. The Gag polyprotein consists of approximately 650 amino acids and its molecular mass is close to 71 kDa. During the assembly/release of virions, a short (25–30 aa) peptide is cleaved by a viral protease from the carboxyl terminus of the Gag precursor. This cleavage occurs in approximately 50% of Gag molecules composing the virion. Thus, two forms of the protein (p68 and p71), the so-called “Gag doublet,” are included in the virions in approximately equal proportion. The cleavage is required for production of infectious virus.²⁶ The Gag doublet is the most immunogenic component of SFVs.

The secondary cleavages of the Gag protein occur shortly after entry into a newly infected cell. The major secondary cut is located within the CA portion of Gag. It is required for the uncoating and subsequent

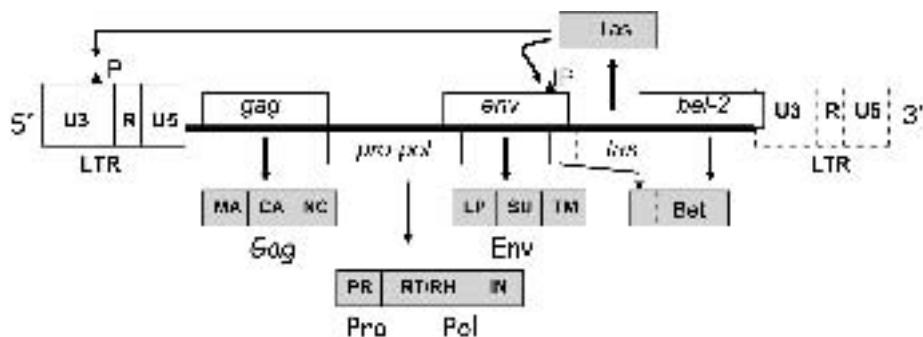


Figure 9.3. SFV genome map and gene products. LTR, long terminal repeat; viral genes, *gag*, *pro-pol*, *env*, *tas*, and *bel-2*; P, promoter; IP, internal promoter; structural proteins, Gag, Env, Pro, Pol; regulatory proteins, Tas and Bet—Bet protein is encoded by two exons located within *tas* and *bel-2* genes. Tas protein interacts with P and IP and activates these promoters.

integration.⁹⁰ Cleavages at other sites may also occur, but their functional significance, if any, is unknown.^{66,90}

It is assumed that the amino-terminal, central, and carboxyl-terminal parts of SFV Gag polyprotein are functionally analogous to MA, CA, and NC proteins of orthoretroviruses. However, there is a significant difference between these parts of SFV Gag polyprotein and their orthoretroviral analogues. Spumaviral Gag does not have the major homology region (MHR) in the CA-like part and cystidine-histidine (Cys-His) motifs in the NC-like part. Instead of Cys-His motifs, C-terminal region of Gag contains three glycine-arginine rich sequences named GR boxes (GR1, GR2, and GR3). All three GR boxes are required for efficient packaging of PFV genomic RNA. The GR1 box, also named nucleic acid-binding domain (NAB) plays a major role in this process.¹²³ The GR2 box contains a nuclear localization signal (NLS). In PFV-infected fibroblasts, newly synthesized Gag protein is transported transiently to the nucleus.¹⁰² Functional significance of this “nuclear excursion” is unclear. However, it is known that the mutants lacking NLS are infectious.¹²³

Spumaviruses are assembled in the cytoplasm. The sequence responsible for the choice of this site of assembly is the “cytoplasmic targeting and retention signal” (CTRS). It is located in the MA-like domain of Gag polyprotein.¹⁸ The CTRS-mediated cytoplasmic assembly is a feature shared by spumaviruses and betaretroviruses. However, the deletion of CTRS completely blocks spumavirus assembly, while in betaretroviruses the assembly is shifted to the plasma membrane. This distinction is due to the absence of the myristylation signal that targets protein to the plasma membrane in the spumavirus Gag.²³ The Gag functional domains M and I, important for the assembly of the orthoretroviruses, are not clearly defined in the spumaviruses, while the L domain has been recently identified.^{88,109} In contrast to orthoretroviruses, the cleavage of SFV Gag protein occurs during entry/uncoating in newly infected cell.

Similar to other retroviruses, the Pol precursor polyprotein (molecular mass 127 kDa) includes protease (PR), reverse transcriptase/RNaseH (RT/RH or simply RT), and integrase (IN) domains. However, in contrast to other retroviruses only the IN moiety (molecular mass 40 kDa) is cleaved from the Pol polyprotein and functions as an individual protein.⁹⁸ The PR and RT/RH domains remain linked, constituting a multifunctional enzyme (molecular mass 85 kDa). Active PR is required for SFV infectivity.⁶¹

The spumavirus RT is more active than RTs of orthoretroviruses.^{9,10} Interestingly, the substitution of V for M in the YVDD catalytic domain of spumavirus RT completely abolishes infectivity,⁹⁵ whereas the RTs of most orthoretroviruses have a YMDD catalytic domain. The reason for YMDD inactivity in spumavirus RT is not clear. The fidelity of spumavirus RT is approximately the same as in other retroviruses.¹⁰ Activity of SFV RT is inhibited by the azidothymidine (AZT). The SFVmac mutants resistant to AZT have been described.^{37,62} In contrast to the orthoretroviruses, spumavirus Pol polyprotein does not have a Gag domain. Thus, it cannot be encapsidated through the mechanism operating in orthoretroviruses.^{61,70} The mechanism of spumavirus Pol encapsidation is poorly understood. Apparently, the specific cis-acting RNA sequence(s) and the Gag GR2/GR3 are required.⁸⁹

The spumavirus IN protein is similar to this enzyme from other retroviruses. The integration of the provirus mediated by IN is essential for spumavirus replication. As in other retroviruses the activity of IN is destroyed by mutations in the DD₃₅ E motif.^{27,74} The integration is accompanied by the formation of 4 nt cellular repeats at each chromosomal-proviral DNA junctions.⁸⁵

The envelope precursor protein whose molecular mass is approximately 130 kDa (gp130) consists of three moieties: amino-terminal leader peptide (LP, gp18), central surface subunit (SU, gp80), and carboxyl-terminal transmembrane subunit (TM, gp48). The peculiarity of spumavirus envelope is the presence of gp18(LP). Cleavage of this peptide is mediated by a furin-like cellular protease²² and is required for the exit of spumavirus virions from the cell. Mutations changing two tryptophan residues in the amino-terminus of gp18 block the release of spumavirus virions.⁶⁹ After cleavage, which occurs during transport of precursor protein to the site of virus assembly, all three spumavirus glycoproteins are incorporated into the virion envelope as trimeric complexes, each subunit of which is composed from gp80, gp48, and gp18.¹¹⁹

The receptor-binding domain (RBD) of PFV is located in gp80(SU). Two regions in this glycoprotein (aa225-396 and aa484-555), which participate in the formation of the RBD and the N-glycosylation site at position 391, are critical for binding to the receptor.²¹

Several properties of spumavirus glycoproteins are reminiscent of those in hepadnaviruses: the dependence of egress on the presence of processed Env proteins in virions; the ability to form extracellular virus-like

particles;¹⁰⁸ and the presence of an endoplasmic reticulum (ER) retrieval signal at the carboxyl-terminus of the TM glycoprotein.³⁵

In contrast to other retroviruses, spumaviruses cannot be pseudotyped; that is, their envelope cannot be replaced with the envelope proteins of other retroviruses or by the widely used vesicular stomatitis virus envelope.⁹¹ Similarly, orthoretroviruses cannot be pseudotyped with intact spumavirus envelope. However, this can be achieved using engineered chimeric envelope consisting of SU and TM proteins from spumavirus and the cytoplasmic domain from murine leukemia virus.⁶⁸

The Tas protein is a potent transcriptional transactivator of viral expression. Tas is unique to spumaviruses.⁵ Two functional motifs are known in this protein: a DNA-binding domain (DB), located in the central part, and an activation domain (AD), located in the carboxyl-terminal part.³⁸ Tas-mediated activation of expression is triggered by Tas binding to the P and IP promoters. The structural mechanism of this binding is not clear. Both promoters are approximately 25 nt long but their sequences are highly divergent. Tas has a higher affinity for IP than for P.⁵⁷ In contrast to P, the IP has basal transcriptional activity, which allows initiation of Tas synthesis. Binding of Tas switches IP from basal to active transcriptional mode. As a result, transcription and translation are accelerated and Tas protein is accumulated to a quantity sufficient for activation of the P promoter. When P is active, the *gag*, *pol*, and *env* genes are transcribed and translated. Expression of the spumavirus genome in vitro is inhibited by microRNAs. Tas abrogates this inhibition and triggers a lytic replication cycle.⁶⁴ To what extent this is relevant to SFV infection in vivo is not clear. The promyelocytic leukemia protein (PML) binds to Tas and inhibits replication of SFV in vitro. However, this interaction does not contribute to establishing latency in vitro.⁷⁷ The significance of PML–Tas interaction in vivo is unknown.

Bet protein (p60) is encoded by part of the *tas* gene and the entire *bel-2* gene (88 and 322–457 aa, respectively).⁸¹ Although the Bet is not essential for spumavirus replication^{5,124} it may have an important function. Indeed, Bet is expressed at much higher level than Tas and other viral proteins, both in vivo and in vitro^{36,78} and the sequence of *bel-2* is well conserved in all spumaviruses. However, the function of Bet remains illusive.

It is hypothesized that Bet may play a role in establishing in vivo latency, but supporting experimental evi-

dence is lacking. Interestingly, Bet of feline spumavirus can inactivate APOBEC3 deaminase by a mechanism different from that utilized by HIV. However, SFV Bet apparently does not have this property.²⁰

9.5. OVERVIEW OF REPLICATION CYCLE IN VITRO

Two major distinguishing features of spumavirus replication cycle are early completion of the reverse transcription and delayed cleavage of the Gag polyprotein.

The spumavirus receptor remains unknown, as are the details of entry and uncoating. However, one early event, cleavage of the Gag precursor protein, is known to be critical for replication.

The genomic DNA (not RNA!) released after completion of uncoating is believed to be full-length. However, it is possible that at least some genomic DNA molecules are incomplete and require repair by cellular mechanisms. The proviral DNA is transported into the nucleus and integrated into the host chromosomal DNA. The integration is mediated by the integrase (IN) and its mechanism is similar to that for other retroviruses.

The transcription of the spumavirus genome proceeds in two stages. During the first stage, *tas* and *bel-1* are transcribed under control of the minimally active IP promoter, and small amounts of Tas and Bet proteins are translated. Tas protein then activates the IP. As a result, larger amounts of Tas are synthesized and the P promoter, which controls the transcription of *gag*, *pol*, and *env* genes, becomes activated. Gag, Pol, and Env precursor proteins are translated from corresponding mRNAs. In contrast to orthoretroviruses, Pol polyprotein is translated from a separate transcript. The full-length genomic RNA is also transcribed during this stage.

The assembly of nucleocapsids occurs in the cytoplasm, in the pericentriolar site. The nucleocapsids are transported to the trans-Golgi network, where they are coated with the envelope.¹²² The interaction between Gag and Env proteins is required for the egress of virions by budding from intracellular and plasma membranes. Cleavage of the viral Gag polyprotein into MA, CA, and NC proteins, typical of other retroviruses, does not occur in the released spumavirus virions.

Reverse transcription of the viral genome takes place during the assembly of virions or shortly thereafter.^{80,125} As a result of this process, a proportion of virions (approximately 20%) acquire a full-length dsDNA genome. The remaining virions contain incomplete viral RNA

and possibly some DNA. Importantly, only full-length DNA-containing virions are infectious.

The “unconventional” features of the spumavirus replication mechanism (timing of reverse transcription, separate Pol transcript, and cleavage of the Gag polyprotein) are also characteristic of hepadnaviruses.¹²¹

9.6. HOST RANGE AND CYTOPATHOGENICITY IN VITRO

The host range of SFVs in vitro is extremely broad. Various cell types (fibroblastoid, epithelioid, and lymphoid) from all vertebrate species tested support replication of foamy viruses.⁴⁵ This property of foamy viruses is very useful in the context of gene therapy. On the other hand, the lack of cell lines completely resistant to SFV infection hampers the identification of the spumavirus receptor.

SFV infection in vitro is usually lytic, manifested as formation of large syncytia followed by the cell death within a few days. Syncytial SFV infection is characteristic for epithelioid and fibroblastoid cells.

Persistent noncytopathic SFV infection, when no cytopathic effect is observed and growth kinetics of infected cells remains normal, is less common.⁷⁹ In this case, the virus is produced in amounts of about 100 times less than in lytic infections. This type of SFV infection is characteristic of lymphoid cells. Treatment of persistently infected cells with mitogens or receptor ligands activates lytic infection.⁷⁶

Simian spumaviruses frequently contaminate and destroy tissue cultures from NHPs, which is a nuisance because they hamper the establishment of permanent simian cell lines.

9.7. NATURAL HOSTS AND PREVALENCE OF INFECTION

The data on the prevalence of SFV infection in the wild are available only for common chimpanzees.⁷¹ The samples for this extensive survey were collected from 724 wild chimpanzees of various subspecies at 25 sites throughout equatorial Africa. Noninvasive methodology was used for identifying SFV-positive animals by detection of antibodies against SFV and viral RNA in the fecal samples collected in the field. The prevalence of SFV in the sample populations/groups varied from 44 to 100%. Phylogenetic analysis of genomic sequences from wild chimpanzee SFVs showed full concordance of viral and host species phylogenies. Viral sequences

segregated into four clades, each corresponding to the chimpanzee subspecies *P. t. troglodytes*, *P. t. verus*, *P. t. vellerosus*, and *P. t. schieinfurthii* and the relatedness of these clades was congruent with the relatedness between host subspecies. Of note, several apparently recombinant SFV frag-viruses have been identified in the wild chimpanzees. Whether or not they represent circulating recombinant forms (CRFs) is to be determined.

Surveys of recently caught monkeys and apes kept in conditions minimizing SFV transmission (individual cages) provide an estimate, although less stringent than that based on the sampling of wild animals. Antibodies against SFV, as determined by Western blot (WB) assay have been found in gorillas (5/27—19%), chimpanzees (13/31—42%), mandrills (7/11—64%), and drills (2/6—33%) from Cameroon and Gabon.^{15,16} SFV infection in most of the serologically positive animals was confirmed by polymerase chain reaction (PCR); however, WB-positive/PCR-negative results were observed in gorillas (1/5)¹⁵ and chimpanzees (4/13).¹⁶ SFV infection, as determined by the WB serology only, is common also in wild-caught Mona monkeys (5/14—36%), red-capped mangabeys (8/13—62%), and Bornean orangutans (20/20—100%).⁴⁸ SFV infection is very common (88%) in a free-ranging population of barbary macaques (*M. silvanus*) in Gibraltar, a population free from several other ubiquitous simian viruses.²⁵

SFV infection is not found in several guenon species (spot-nosed, red-eared, crowned, Selater's, and Preuss's monkey).⁴⁸ However, samples from these species are very small and whether or not SFV-free simian species exist in nature remains an open question.

9.8. MODE OF TRANSMISSION

9.8.1. Simian-to-Simian

The age-dependence of SFV seropositivity rate in captive NHPs is typical for an infection transmitted horizontally by nonsexual routes; that is, the prevalence increases with age and the majority of animals seroconvert by the age of 2–5 years.⁷

Transmission of SFV likely occurs through saliva, although the virus is not produced in the salivary glands.⁸⁴ Productive SFV infection, as indicated by the presence of viral RNA, takes place in the oral mucosa and is restricted to the superficial epithelium.^{30,83}

Close contact between animals is required for SFV transmission. The type of contact responsible for transmission may differ depending on the species, behavioral

characteristics and conditions in which the animals are kept. In a breeding colony of Tonkeana macaques a significant increase in SFV prevalence was observed in the adult males competing for females.¹⁷ In this case, severe biting during male fighting may be the major mode of transmission. On the other hand, in a juvenile baboon colony, in which most of the animals seroconverted by the age of 2 years, licking is likely responsible for the transmission of SFV.⁷

Transmission of SFV by sexual route cannot be excluded, but it has not been documented.

Interspecies transmission of SFV from colobus monkeys (*Pilocolobus badius*) to common chimpanzee (*Pan troglodytes verus*) in a natural hunter–prey system between the chimpanzees (hunter) and colobus monkeys (prey) has been described.⁶⁵

9.8.2. Simian-to-Human

Spumavirus infection is extremely rare in humans and all confirmed human cases can be attributed to a zoonotic transmission. The common denominator in these cases is extensive exposure to NHPs. Zoonotic transmission from apes (chimpanzee, gorilla) and monkeys (mandrill, baboon, African green monkey, de Brazza's guenon, gorilla, and macaques) has been documented.^{3,12,14,19,44,42,52,53,100,104,107,111,112,120}

The rate of simian-to-human transmission of SFV is estimated in two risk groups. It is about 2% for zoo workers and animal caretakers in primate research centers.^{43,100} Approximately the same is the prevalence of SFV in persons in South and Southeast Asia who live and work in the proximity of NHP natural habitats.⁵³ About 1% of individuals from rural areas of southern Cameroon highly exposed to the NHPs and bushmeat are SFV-positive.¹²⁰ Transmission of SFV from NHPs to hunters living in remote areas of southwest and southern Cameroon is shown to be clearly associated with severe bites mostly by the great apes.¹⁴ Among 13 cases of human SFV infection reported in this study, 8 individuals acquired the virus from the gorillas and 3 from common chimpanzees (*P. t. troglodytes*); only in 2 cases, the virus was acquired from monkeys (mandrill and yet to be identified guenon species). The risk of interspecies transmission of SFV to hunters severely bitten by a gorilla or chimpanzee is estimated to be around 35%.¹⁴ All SFV-infected individuals are persistently infected with the virus. On average viral load in the infected humans is low although the range of variation is wide (1–1,000

copies in 500 ng of PBL DNA). The infection appears to be asymptomatic even in those individuals which have high viral load.¹⁴ No secondary human-to-human transmissions of SFV have been documented in the spouses and children of the infected individuals.^{8,14}

A very large human population is exposed to urban, temple, and pet monkeys in Asia, and potentially at risk of acquiring SFV.^{24,34,51,101} However, only one case of SFV infection is documented in a monkey temple worker.⁵²

SFV DNA was detected in tissues of two human recipients of baboon liver transplants.⁴ However, it is not clear whether the virus was confined only to donor cells or spread into recipient cells.

Although no human-to-human transmission of SFV has been identified, the number of cases that have been followed for evidence of secondary transmission is too small to exclude such a possibility.

9.9. DISEASE ASSOCIATION

As mentioned, no pathology is associated with SFV infection, both in natural and nonnatural hosts. The puzzling question is what mechanism is preventing pathogenic consequences of SFV infection *in vivo*. The answer to this question is not known.

Reports in the literature claimed an association between SFV and autoimmune diseases (Graves' disease, thyroiditis de Quervain, multiple sclerosis, and myasthenia gravis) in humans (reviewed in Meiering and Linial⁷⁵). However, when this possibility was reexamined using more stringent serological and molecular assays, these claims were not confirmed.^{3,41,97,107} Therefore, there is no evidence of spumavirus infection in humans with the exception of the “dead-end” zoonotic transmissions described above.

9.10. IMMUNE RESPONSE

SFV infection is accompanied by antibody response. There is no confirmed antibody-negative, virus-positive case.^{17,48}

The dominant component of anti-SFV antibody response is anti-Gag IgG that is a reliable marker of ongoing SFV infection. The next most frequent antibodies are anti-Bet IgG. Neutralizing antibodies, presumably anti-Env, are also common; however, anti-Env antibodies are not easily detected in tests other than neutralization assays.

Very little is known about the dynamics of the antibody response against SFV in NHPs. Only one seroconversion was reported in a setting allowing determination of the exact timing of seroconversion.¹¹ In this case, the seroconversion was detected 9 weeks after transfusion of blood from SFV-positive macaque to another monkey of the same species. The timing of seroconversion for SFV infection acquired by natural routes remains unknown.

Data on mucosal immunity against SFV are limited to a few cases when anti-SFV-specific IgA was tested in SFV-positive chimpanzees: these antibodies were not found in urine and saliva.¹⁹

Cell-mediated immunity against SFV has not been studied directly. The only relevant published study investigated the replication of SFV in simian immunodeficiency virus (SIV)-immunosuppressed macaques.⁸⁴ Contrary to expectation, the SIV-induced depletion of CD4⁺ cells does not lead to the burst of SFV in blood as indicated by the absence of SFV RNA. Paradoxically, the SFV viral load is decreased in the oral tissues of SIV-immunosuppressed macaques. Thus, T-helper-dependent immunity seemingly is not responsible for the suppression of SFV replication in the blood compartment. The decrease in SFV replication in oral tissues might be due to the induction of interferons (IFN) by SIV. Indeed, SIV is known to induce INFs,² whereas replication of SFV in vitro is inhibited by IFNs, particularly IFN- γ .³² However, this explanation remains to be proven.

Interestingly, despite the absence of systemic activation of SFV replication in SIV-immunosuppressed macaques, productive SFV infection is activated in the small intestine, specifically in the jejunum.⁸⁴ Why this happens remains unknown.

9.11. DIAGNOSIS

9.11.1. Virus Isolation

SFV isolation is relatively straightforward due to the prominent nature of the CPE. A wide variety of animal and human permanent cell lines can be used. The cell lines most commonly used are Cf2Th (canine), Vero (African green monkey), BHK-21 (hamster), and *Mus dunni* (mouse). The latter is particular susceptible to SFV.⁵⁹ Another cell line highly susceptible for SFV is TF, rhesus macaque fibroblasts immortalized through expression of human telomerase.^{54,60} Two cell lines, the FAB and SFAB cells, were engineered for the titration of live PFV and Asian SFVs, respectively.^{54,124} Both

cell lines are derived from the BHK-21 cells and contain LacZ reporter gene under the control of spumavirus LTR (derived from PFV and SFVmac in FAB and SFAB cells, respectively). When SFV infects FAB or SFAB cells, the viral Tas protein activates the P promoter controlling the expression of the LacZ gene. As a result, the synthesis of β -galactosidase is switched on and the enzyme is detected in the virus-infected cells. SFV-infected cells (β -galactosidase-positive) can be easily stained and counted. The SFAB cells are suitable for the titration of SFVmac, SFVmμ, SFVmfa, and SFVmne,⁵⁴ but not PFV. It is quite possible that FAB cells can be used not only for the titration of PFV but also for SFVcpz and related African SFVs. However, this has not yet been tested.

The isolation of SFV for diagnostic purposes is now replaced by the detection of viral DNA. Nevertheless, the virus isolation is still commonly used as the “gold standard” for evaluation of other diagnostic tests. However, whereas the specificity of virus isolation is excellent, its sensitivity may be lower than that of molecular tests.

9.11.2. Antibody Assays

In the early years of spumavirus research the main serological test for SFV infection was the neutralization assay.^{46,47,49,50,96,110} This test is highly specific and allows determination of the serotype. Any cell line susceptible to SFV can be used as the indicator cells. Neutralization is usually detected as abrogation of the CPE; however, any other marker of ongoing SFV infection could be used. One of the best options currently available is the use of SFAB and similar cell lines.⁵⁴ The neutralization test is laborious and time-consuming and its use for identification of SFVs has been discontinued. At the same time, the test is irreplaceable for measuring the protective component of the humoral immune response against SFV.

The WB assay for anti-SFV antibodies is the most commonly used serological test for SFV. Because SFV is highly cell-associated, a lysate of infected cells rather than virions purified from culture medium is used as an antigen. The most reliable version of WB assay currently available includes SFV antigens from both monkey (SF-Vagm) and ape (SFVcpz) viruses.⁴⁸ The criterion for diagnosing SFV infection by WB is the reactivity with the Gag precursor proteins manifested as a “doublet” of bands in the 70 kDa region (named gp71/68 or gp71/74). Reactivity with a single band in the 68–74 kDa range

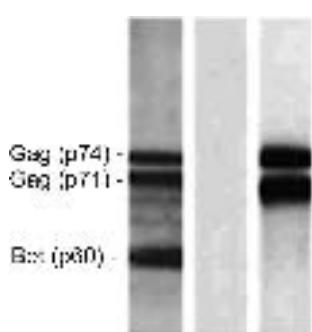


Figure 9.4. Western blot (WB) reactivity diagnostic for SFV infection. “Doublet” bands (gp71/74) are diagnostic for SFV infection by WB (right and left lanes). Anti-p60 reactivity (anti-Bet) is common in “doublet”-positive sera (left lane). Negative result (central lane). (Image is adapted from Liu *et al.*⁷¹)

is interpreted as indeterminate; whereas the lack of reactivity with gp71/74 doublet indicates the absence of infection (more cautious interpretation is “seronegative” status). Reactivity with a p60 band (anti-Bet) is quite common in “doublet”-positive sera (Figure 9.4).

The WB based on SFV_{agm}/SFV_{cpz} antigens has excellent sensitivity and specificity for diagnosis of infection with most known SFVs. The exception is orangutan SFV (SFV_{pon}). Inclusion of homologous antigen is required for diagnosis of SFV infection by WB in orangutans. The concordance of WB and PCR results in general is very good. However, some WB-positive/PCR-negative results are observed.^{16,48} If such cases are not resolved by the result of virus isolation, their unequivocal interpretation is not possible.

All currently used WB tests for anti-SFV are “in-house” assays and there is no comparative data on the specificity and sensitivity of WB assays used in different laboratories.

Immunofluorescence and radioimmunoprecipitation (RIPA) assays for anti-SFV were also used,^{36,59} but have largely been discontinued.

Until recently, crude antigens consisting of extracts of infected cells were used in ELISAs for anti-SFV. Such tests usually have low specificity due to reactivity with cellular components.^{7,75,120} The improved version of ELISA, based on the recombinant Gag protein derived from SFV_{mac} (193 N-terminal amino acids of Gag), has been described.⁵⁴ This test is highly sensitive

and specific for the diagnosis of SFV infection in various macaque species and at least in one langur species (*Presbytis obscura*).

Another recent addition to the tests for the serological diagnosis of SFV infection is the multiplex microbead immunoassay (MMIA), which allows simultaneous detection of antibodies against six common simian viruses, including SFV.⁵⁸ This test is well suited for screening. Sucrose-density purified SFV of unspecified origin has been used as the antigen. MMIA is slightly more sensitive than regular SFV ELISA based on the same antigen. Data on the comparison of MMIA with WB and molecular assays are not available.

9.11.3. Molecular Assays

The most commonly used generic PCR test for SFV is targeting the well-conserved region of the *pol* gene (425 bp fragment of integrase).¹⁰⁵ The analytical sensitivity of this test is 1–10 viral DNA copies in 500 ng of genomic DNA. The additional advantage of this SFV *pol* PCR is the possibility of comparing the sequence of a newly amplified fragment with the sequences of virtually all known SFVs. Another commonly used SFV PCR test is semi-nested PCR targeting the 109 bp fragment of the LTR.⁷³ This test is less sensitive than *pol* PCR.¹⁶ Nested PCR for amplification of the 660 bp fragment of the *gag* gene has been described,¹¹¹ but its performance as a diagnostic test has not been evaluated.

9.12. SPUMAVIRUSES AS VECTORS FOR GENE THERAPY

Spumaviruses lack pathogenicity and have an extremely broad host range. The size of their genome is larger than that of other retroviruses used as vectors for gene therapy. The frequency of integration within transcriptionally active regions of human genome is lower for spumaviruses than for other retroviral vectors.¹¹⁶ Preexisting antibodies against spumaviruses are extremely rare in humans. This combination of properties makes spumaviruses very promising as vectors for gene therapy.⁹³

There are two types of spumavirus-based vectors: replication incompetent vectors capable of only a single round of replication and replication competent vectors capable of multiple rounds of replication. The replication incompetent vectors are produced by cotransfection of four expression plasmids, separately producing Gag, Pol, Env, and the product of interest into a T-cell line.^{39,87,115} In the replication competent vectors,

the gene of interest replaces the *Bet* gene. Such vectors can be potentially used as live vaccines, but this field is still at an early stage.

Spumavirus vectors are capable of infecting hematopoietic progenitor cells with efficiency comparable to that of lentivirus-based vectors and better than that of murine leukemia virus-based vectors.^{55,56,67}

Spumavirus-based vectors are used in studies aimed at the inhibition of SIV replication through targeting SIV *rev/env* by siRNAs. The construct suppresses expression of *rev/env* and inhibits, though incompletely, SIV replication in vitro.⁸⁶

The SFV-based replication-competent vectors are used for delivery of “suicide” genes to solid tumors in a nude mouse model.⁴⁰ Unexpectedly, the oncolytic effect is observed both in the experimental and control groups. This intriguing observation deserves further exploration.

9.13. SUMMARY

Spumavirus infection is very common in NHPs. It is transmitted horizontally, most likely via saliva. There are no clinical signs associated with SFV infection of NHPs and the infection is life-long. Lack of pathogenicity in vivo contrasts with a profound cytopathic effect induced by spumaviruses in vitro. The explanation for this paradox is lacking.

Simian spumaviruses can infect humans, but such cases are rare and have no known pathological consequence. Secondary human-to-human transmissions are not known.

Spumavirus virions may contain both genomic RNA and DNA, but only DNA-containing virions are infectious. This and some other features link spumaviruses to seemingly unrelated hepadnaviruses.

The lack of pathogenicity, extremely broad host range, large size of genome, low frequency of integration within transcriptionally active genomic regions of human genome, and the virtual absence of preexisting antibodies in humans make spumaviruses very promising as vectors for gene therapy.

REFERENCES

- Achong, B. G., P. W. Mansell, M. A. Epstein, and P. Clifford. 1971. An unusual virus in cultures from a human nasopharyngeal carcinoma. *J. Natl. Cancer Inst.* 46(2):299–307.
- Ahmed, R. K., G. Biberfeld, and R. Thorstensson. 2005. Innate immunity in experimental SIV infection and vaccination. *Mol. Immunol.* 42(2):251–258.
- Ali, M., G. P. Taylor, R. J. Pitman, D. Parker, A. Rethwilm, R. Cheingsong-Popov, J. N. Weber, P. D. Bieniasz, J. Bradley, and M. O. McClure. 1996. No evidence of antibody to human foamy virus in widespread human populations. *AIDS Res. Hum. Retroviruses* 12(15):1473–1483.
- Allan, J. S., S. R. Broussard, M. G. Michaels, T. E. Starzl, K. L. Leighton, E. M. Whitehead, A. G. Comuzzie, R. E. Lanford, M. M. Leland, W. M. Switzer, and W. Heneine. 1998. Amplification of simian retroviral sequences from human recipients of baboon liver transplants. *AIDS Res. Hum. Retroviruses* 14(10):821–824.
- Baunach, G., B. Maurer, H. Hahn, M. Kranz, and A. Rethwilm. 1993. Functional analysis of human foamy virus accessory reading frames. *J. Virol.* 67(9):5411–5418.
- Bieniasz, P. D., A. Rethwilm, R. Pitman, M. D. Daniel, I. Chrystie, and M. O. McClure. 1995. A comparative study of higher primate foamy viruses, including a new virus from a gorilla. *Virology* 207(1):217–228.
- Blewett, E. L., D. H. Black, N. W. Lerche, G. White, and R. Eberle. 2000. Simian foamy virus infections in a baboon breeding colony. *Virology* 278(1):183–193.
- Boneva, R. S., W. M. Switzer, T. J. Spira, V. B. Bhullar, V. Shanmugam, M. E. Cong, L. Lam, W. Heneine, T. M. Folks, and L. E. Chapman. 2007. Clinical and virological characterization of persistent human infection with simian foamy viruses. *AIDS Res. Hum. Retroviruses* 23(11):1330–1337.
- Boyer, P. L., C. R. Stenbak, P. K. Clark, M. L. Linial, and S. H. Hughes. 2004. Characterization of the polymerase and RNase H activities of human foamy virus reverse transcriptase. *J. Virol.* 78(12):6112–6121.
- Boyer, P. L., C. R. Stenbak, D. Hoberman, M. L. Linial, and S. H. Hughes. 2007. In vitro fidelity of the prototype primate foamy virus (PFV) RT compared to HIV-1 RT. *Virology* 367(2):253–264.
- Brooks, J. I., H. W. Merks, J. Fournier, R. S. Boneva, and P. A. Sandstrom. 2007. Characterization of blood-borne transmission of simian foamy virus. *Transfusion* 47(1):162–170.
- Brooks, J. I., E. W. Rud, R. G. Pilon, J. M. Smith, W. M. Switzer, and P. A. Sandstrom. 2002. Cross-species retroviral transmission from macaques to human beings. *Lancet* 360(9330):387–388.
- Cain, D., O. Erlwein, A. Grigg, R. A. Russell, and M. O. McClure. 2001. Palindromic sequence plays

- a critical role in human foamy virus dimerization. *J. Virol.* 75(8):3731–3739.
- 14. Calattini, S., E. B. Betsem, A. Froment, P. Mauclere, P. Tortevoye, C. Schmitt, R. Njouom, A. Saib, and A. Gessain. 2007. Simian foamy virus transmission from apes to humans, rural Cameroon. *Emerg. Infect. Dis.* 13(9):1314–1320.
 - 15. Calattini, S., E. Nerrienet, P. Mauclere, M. C. Georges-Courbot, A. Saib, and A. Gessain. 2004. Natural simian foamy virus infection in wild-caught gorillas, mandrills and drills from Cameroon and Gabon. *J. Gen. Virol.* 85(Pt 11):3313–3317.
 - 16. Calattini, S., E. Nerrienet, P. Mauclere, M. C. Georges-Courbot, A. Saib, and A. Gessain. 2006. Detection and molecular characterization of foamy viruses in Central African chimpanzees of the *Pan troglodytes troglodytes* and *Pan troglodytes vellerosus* subspecies. *J. Med. Primatol.* 35(2):59–66.
 - 17. Calattini, S., F. Wanert, B. Thierry, C. Schmitt, S. Bassot, A. Saib, N. Herrenschmidt, and A. Gessain. 2006. Modes of transmission and genetic diversity of foamy viruses in a *Macaca tonkeana* colony. *Retrovirology* 3:23.
 - 18. Cartellieri, M., O. Herchenroder, W. Rudolph, M. Heinkelein, D. Lindemann, H. Zentgraf, and A. Rethwilm. 2005. N-terminal Gag domain required for foamy virus particle assembly and export. *J. Virol.* 79(19):12464–12476.
 - 19. Cummins, J. E., Jr., R. S. Boneva, W. M. Switzer, L. L. Christensen, P. Sandstrom, W. Heneine, L. E. Chapman, and C. S. Dezzutti. 2005. Mucosal and systemic antibody responses in humans infected with simian foamy virus. *J. Virol.* 79(20):13186–13189.
 - 20. Delebecque, F., R. Suspene, S. Calattini, N. Casartelli, A. Saib, A. Froment, S. Wain-Hobson, A. Gessain, J. P. Vartanian, and O. Schwartz. 2006. Restriction of foamy viruses by APOBEC cytidine deaminases. *J. Virol.* 80(2):605–614.
 - 21. Duda, A., D. Luftenegger, T. Pietschmann, and D. Lindemann. 2006. Characterization of the prototype foamy virus envelope glycoprotein receptor-binding domain. *J. Virol.* 80(16):8158–8167.
 - 22. Duda, A., A. Stange, D. Luftenegger, N. Stanke, D. Westphal, T. Pietschmann, S. W. Eastman, M. L. Linial, A. Rethwilm, and D. Lindemann. 2004. Prototype foamy virus envelope glycoprotein leader peptide processing is mediated by a furin-like cellular protease, but cleavage is not essential for viral infectivity. *J. Virol.* 78(24):13865–13870.
 - 23. Eastman, S. W. and M. L. Linial. 2001. Identification of a conserved residue of foamy virus Gag required for intracellular capsid assembly. *J. Virol.* 75(15):6857–6864.
 - 24. Engel, G., L. L. Hungerford, L. Jones-Engel, D. Travis, R. Eberle, A. Fuentes, R. Grant, R. Kyes, and M. Schillaci. 2006. Risk assessment: a model for predicting cross-species transmission of simian foamy virus from macaques (*M. fascicularis*) to humans at a monkey temple in Bali, Indonesia. *Am. J. Primatol.* 68(9):934–948.
 - 25. Engel, G. A., M. Pizarro, E. Shaw, J. Cortes, A. Fuentes, P. Barry, N. Lerche, R. Grant, D. Cohn, and L. Jones-Engel. 2008. Unique pattern of enzootic primate viruses in Gibraltar macaques. *Emerg. Infect. Dis.* 14(7):1112–1115.
 - 26. Enssle, J., N. Fischer, A. Moebes, B. Mauer, U. Smola, and A. Rethwilm. 1997. Carboxy-terminal cleavage of the human foamy virus Gag precursor molecule is an essential step in the viral life cycle. *J. Virol.* 71(10):7312–7317.
 - 27. Enssle, J., A. Moebes, M. Heinkelein, M. Panhuysen, B. Mauer, M. Schweizer, D. Neumann-Haefelin, and A. Rethwilm. 1999. An active foamy virus integrase is required for virus replication. *J. Gen. Virol.* 80(Pt 6):1445–1452.
 - 28. Erlwein, O., P. D. Bieniasz, and M. O. McClure. 1998. Sequences in pol are required for transfer of human foamy virus-based vectors. *J. Virol.* 72(7):5510–5516.
 - 29. Erlwein, O., D. Cain, N. Fischer, A. Rethwilm, and M. O. McClure. 1997. Identification of sites that act together to direct dimerization of human foamy virus RNA in vitro. *Virology* 229(1):251–258.
 - 30. Falcone, V., J. Leupold, J. Clotten, E. Urbanyi, O. Herchenroder, W. Spatz, B. Volk, N. Bohm, A. Toniolo, D. Neumann-Haefelin, and M. Schweizer. 1999. Sites of simian foamy virus persistence in naturally infected African green monkeys: latent provirus is ubiquitous, whereas viral replication is restricted to the oral mucosa. *Virology* 257(1):7–14.
 - 31. Falcone, V., M. Schweizer, and D. Neumann-Haefelin. 2003. Replication of primate foamy viruses in natural and experimental hosts. *Curr. Top. Microbiol. Immunol.* 277:161–180.
 - 32. Falcone, V., M. Schweizer, A. Toniolo, D. Neumann-Haefelin, and A. Meyerhans. 1999. Gamma interferon is a major suppressive factor produced by activated human peripheral blood lymphocytes that is able to inhibit foamy virus-induced cytopathic effects. *J. Virol.* 73(2):1724–1728.
 - 33. Flugel, R. M., A. Rethwilm, B. Maurer, and G. Darai. 1987. Nucleotide sequence analysis of the env gene and its flanking regions of the human spumaretrovirus reveals two novel genes. *EMBO J.* 6(7):2077–2084.
 - 34. Fuentes, A. 2006. Human culture and monkey behavior: assessing the contexts of potential pathogen

- transmission between macaques and humans. *Am. J. Primatol.* 68(9):880–896.
35. Goepfert, P. A., K. L. Shaw, G. D. Ritter Jr., and M. J. Mulligan. 1997. A sorting motif localizes the foamy virus glycoprotein to the endoplasmic reticulum. *J. Virol.* 71(1):778–784.
 36. Hahn, H., G. Baunach, S. Brautigam, A. Mergia, D. Neumann-Haefelin, M. D. Daniel, M. O. McClure, and A. Rethwilm. 1994. Reactivity of primate sera to foamy virus Gag and Bet proteins. *J. Gen. Virol.* 75(Pt 10):2635–2644.
 37. Hartl, M. J., B. Kretzschmar, A. Frohn, A. Nowrouzi, A. Rethwilm, and B. M. Wohrl. 2008. AZT resistance of simian foamy virus reverse transcriptase is based on the excision of AZTMP in the presence of ATP. *Nucleic Acids Res.* 36(3):1009–1016.
 38. He, F., J. D. Sun, E. D. Garrett, and B. R. Cullen. 1993. Functional organization of the Bel-1 trans activator of human foamy virus. *J. Virol.* 67(4):1896–1904.
 39. Heinkelein, M., M. Dressler, G. Jarmy, M. Rammeling, H. Imrich, J. Thurow, D. Lindemann, and A. Rethwilm. 2002. Improved primate foamy virus vectors and packaging constructs. *J. Virol.* 76(8):3774–3783.
 40. Heinkelein, M., U. Hoffmann, M. Lucke, H. Imrich, J. G. Muller, J. Meixensberger, M. Westphahl, A. Kretschmer, and A. Rethwilm. 2005. Experimental therapy of allogeneic solid tumors induced in athymic mice with suicide gene-transducing replication-competent foamy virus vectors. *Cancer Gene Ther.* 12(12):947–953.
 41. Heneine, W., V. C. Musey, S. D. Sinha, A. Landay, G. Northrup, R. Khabbaz, and J. E. Kaplan. 1995. Absence of evidence for human spumaretrovirus sequences in patients with Graves' disease. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 9(1):99–101.
 42. Heneine, W., M. Schweizer, P. Sandstrom, and T. Folks. 2003. Human infection with foamy viruses. *Curr. Top. Microbiol. Immunol.* 277:181–196.
 43. Heneine, W., W. M. Switzer, P. Sandstrom, J. Brown, S. Vedapuri, C. A. Schable, A. S. Khan, N. W. Lerche, M. Schweizer, D. Neumann-Haefelin, L. E. Chapman, and T. M. Folks. 1998. Identification of a human population infected with simian foamy viruses. *Nat. Med.* 4(4):403–407.
 44. Herchenroder, O., R. Renne, D. Loncar, E. K. Cobb, K. K. Murthy, J. Schneider, A. Mergia, and P. A. Luciw. 1994. Isolation, cloning, and sequencing of simian foamy viruses from chimpanzees (SFVcpz): high homology to human foamy virus (HFV). *Virol.* 201(2):187–199.
 45. Hill, C. L., P. D. Bieniasz, and M. O. McClure. 1999. Properties of human foamy virus relevant to its development as a vector for gene therapy. *J. Gen. Virol.* 80(Pt 8):2003–2009.
 46. Hooks, J. J. and C. J. Gibbs Jr. 1975. The foamy viruses. *Bacteriol. Rev.* 39(3):169–185.
 47. Hooks, J. J., C. J. Gibbs Jr., S. Chou, R. Howk, M. Lewis, and D. C. Gajdusek. 1973. Isolation of a new simian foamy virus from a spider monkey brain culture. *Infect. Immun.* 8(5):804–813.
 48. Hussain, A. I., V. Shanmugam, V. B. Bhullar, B. E. Beer, D. Vallet, A. Gautier-Hion, N. D. Wolfe, W. B. Karesh, A. M. Kilbourn, Z. Tooze, W. Heneine, and W. M. Switzer. 2003. Screening for simian foamy virus infection by using a combined antigen Western blot assay: evidence for a wide distribution among Old World primates and identification of four new divergent viruses. *Virology* 309(2):248–257.
 49. Johnston, P. B. 1961. A second immunologic type of simian foamy virus: monkey throat infections and unmasking by both types. *J. Infect. Dis.* 109: 1–9.
 50. Johnston, P. B. 1971. Taxonomic features of seven serotypes of simian and ape foamy viruses. *Infect. Immun.* 3(6):793–799.
 51. Jones-Engel, L., G. A. Engel, J. Heidrich, M. Chalise, N. Poudel, R. Viscidi, P. A. Barry, J. S. Allan, R. Grant, and R. Kyes. 2006. Temple monkeys and health implications of commensalism, Kathmandu, Nepal. *Emerg. Infect. Dis.* 12(6):900–906.
 52. Jones-Engel, L., G. A. Engel, M. A. Schillaci, A. Rompis, A. Putra, K. G. Suaryana, A. Fuentes, B. Beer, S. Hicks, R. White, B. Wilson, and J. S. Allan. 2005. Primate-to-human retroviral transmission in Asia. *Emerg. Infect. Dis.* 11(7):1028–1035.
 53. Jones-Engel, L., C. C. May, G. A. Engel, K. A. Steinkraus, M. A. Schillaci, A. Fuentes, A. Rompis, M. K. Chalise, N. Aggimaranangsee, M. M. Feeroz, R. Grant, J. S. Allan, A. Putra, I. N. Wandia, R. Watanabe, L. Kuller, S. Thongsawat, R. Chaiwarith, R. C. Kyes, and M. L. Linial. 2008. Diverse contexts of zoonotic transmission of simian foamy viruses in Asia. *Emerg. Infect. Dis.* 14(8):1200–1208.
 54. Jones-Engel, L., K. A. Steinkraus, S. M. Murray, G. A. Engel, R. Grant, N. Aggimaranangsee, B. P. Lee, C. May, M. A. Schillaci, C. Somgird, T. Suttipat, L. Vojtech, J. Zhao, and M. L. Linial. 2007. Sensitive assays for simian foamy viruses reveal a high prevalence of infection in commensal, free-ranging Asian monkeys. *J. Virol.* 81(14):7330–7337.
 55. Josephson, N. C., G. Trobridge, and D. W. Russell. 2004. Transduction of long-term and mobilized peripheral blood-derived NOD/SCID repopulating cells by foamy virus vectors. *Hum. Gene Ther.* 15(1):87–92.

56. Josephson, N. C., G. Vassilopoulos, G. D. Trobridge, G. V. Priestley, B. L. Wood, T. Papayannopoulou, and D. W. Russell. 2002. Transduction of human NOD/SCID-repopulating cells with both lymphoid and myeloid potential by foamy virus vectors. *Proc. Natl. Acad. Sci. U. S. A.* 99(12):8295–8300.
57. Kang, Y., W. S. Blair, and B. R. Cullen. 1998. Identification and functional characterization of a high-affinity Bel-1 DNA binding site located in the human foamy virus internal promoter. *J. Virol.* 72(1):504–511.
58. Khan, I. H., S. Mendoza, J. Yee, M. Deane, K. Venkateswaran, S. S. Zhou, P. A. Barry, N. W. Lerche, and P. A. Luciw. 2006. Simultaneous detection of antibodies to six nonhuman-primate viruses by multiplex microbead immunoassay. *Clin. Vaccine Immunol.* 13(1):45–52.
59. Khan, A. S., J. F. Sears, J. Muller, T. A. Galvin, and M. Shahabuddin. 1999. Sensitive assays for isolation and detection of simian foamy retroviruses. *J. Clin. Microbiol.* 37(8):2678–2686.
60. Kirchoff, V., S. Wong, J. S. St, and G. S. Pari. 2002. Generation of a life-expanded rhesus monkey fibroblast cell line for the growth of rhesus rhadinovirus (RRV). *Arch. Virol.* 147(2):321–333.
61. Konvalinka, J., M. Lochelt, H. Zentgraf, R. M. Flugel, and H. G. Krausslich. 1995. Active foamy virus proteinase is essential for virus infectivity but not for formation of a Pol polyprotein. *J. Virol.* 69(11):7264–7268.
62. Kretzschmar, B., A. Nowrouzi, M. J. Hartl, K. Gartner, T. Wiktorowicz, O. Herchenroder, S. Kanzler, W. Rudolph, A. Mergia, B. Wohrl, and A. Rethwilm. 2008. AZT-resistant foamy virus. *Virology* 370(1):151–157.
63. Kupiec, J. J., A. Kay, M. Hayat, R. Ravier, J. Peries, and F. Galibert. 1991. Sequence analysis of the simian foamy virus type 1 genome. *Gene* 101(2):185–194.
64. Lecellier, C. H., P. Dunoyer, K. Arar, J. Lehmann-Che, S. Eyquem, C. Himber, A. Saib, and O. Voinnet. 2005. A cellular microRNA mediates antiviral defense in human cells. *Science* 308(5721):557–560.
65. Leendertz, F. H., F. Zirkel, E. Couacy-Hymann, H. Ellerbrok, V. A. Morozov, G. Pauli, C. Hedemann, P. Formenty, S. A. Jensen, C. Boesch, and S. Junglen. 2008. Interspecies transmission of simian foamy virus in a natural predator-prey system. *J. Virol.* 82(15):7741–7744.
66. Lehmann-Che, J., M. L. Giron, O. Delelis, M. Lochelt, P. Bittoun, J. Tobaly-Tapiero, H. de The, and A. Saib. 2005. Protease-dependent uncoating of a complex retrovirus. *J. Virol.* 79(14):9244–9253.
67. Leurs, C., M. Jansen, K. E. Pollok, M. Heinkelein, M. Schmidt, M. Wissler, D. Lindemann, C. Von Kalle, A. Rethwilm, D. A. Williams, and H. Hanenberg. 2003. Comparison of three retroviral vector systems for transduction of nonobese diabetic/severe combined immunodeficiency mice repopulating human CD34+ cord blood cells. *Hum. Gene Ther.* 14(6):509–519.
68. Lindemann, D., M. Bock, M. Schweizer, and A. Rethwilm. 1997. Efficient pseudotyping of murine leukemia virus particles with chimeric human foamy virus envelope proteins. *J. Virol.* 71(6):4815–4820.
69. Lindemann, D., T. Pietschmann, M. Picard-Maureau, A. Berg, M. Heinkelein, J. Thurow, P. Knaus, H. Zentgraf, and A. Rethwilm. 2001. A particle-associated glycoprotein signal peptide essential for virus maturation and infectivity. *J. Virol.* 75(13):5762–5771.
70. Linial, M. L. 1999. Foamy viruses are unconventional retroviruses. *J. Virol.* 73(3):1747–1755.
71. Liu, W., M. Worobey, Y. Li, B. F. Keele, F. Bibollet-Ruche, Y. Guo, P. A. Goepfert, M. L. Santiago, J. B. Ndjango, C. Neel, S. L. Clifford, C. Sanz, S. Kamanya, M. L. Wilson, A. E. Pusey, N. Gross-Camp, C. Boesch, V. Smith, K. Zamma, M. A. Huffman, J. C. Mitani, D. P. Watts, M. Peeters, G. M. Shaw, W. M. Switzer, P. M. Sharp, and B. H. Hahn. 2008. Molecular ecology and natural history of simian foamy virus infection in wild-living chimpanzees. *PLoS Pathog.* 4(7):e1000097.
72. Marczynska, B., C. J. Jones, and L. G. Wolfe. 1981. Syncytium-forming virus of common marmosets (*Callithrix jacchus jacchus*). *Infect. Immun.* 31(3):1261–1269.
73. McClure, M. O., P. D. Bieniasz, T. F. Schulz, I. L. Chrystie, G. Simpson, A. Aguzzi, J. G. Hoad, A. Cunningham, J. Kirkwood, and R. A. Weiss. 1994. Isolation of a new foamy retrovirus from orangutans. *J. Virol.* 68(11):7124–7130.
74. Meiering, C. D., K. E. Comstock, and M. L. Linial. 2000. Multiple integrations of human foamy virus in persistently infected human erythroleukemia cells. *J. Virol.* 74(4):1718–1726.
75. Meiering, C. D. and M. L. Linial. 2001. Historical perspective of foamy virus epidemiology and infection. *Clin. Microbiol. Rev.* 14(1):165–176.
76. Meiering, C. D. and M. L. Linial. 2002. Reactivation of a complex retrovirus is controlled by a molecular switch and is inhibited by a viral protein. *Proc. Natl. Acad. Sci. U. S. A.* 99(23):15130–15135.
77. Meiering, C. D. and M. L. Linial. 2003. The promyelocytic leukemia protein does not mediate foamy virus latency in vitro. *J. Virol.* 77(3):2207–2213.

78. Meiering, C. D., C. Rubio, C. May, and M. L. Linial. 2001. Cell-type-specific regulation of the two foamy virus promoters. *J. Virol.* 75(14):6547–6557.
79. Mikovits, J. A., P. M. Hoffman, A. Rethwilm, and F. W. Ruscetti. 1996. In vitro infection of primary and retrovirus-infected human leukocytes by human foamy virus. *J. Virol.* 70(5):2774–2780.
80. Moebes, A., J. Enssle, P. D. Bieniasz, M. Heinkelein, D. Lindemann, M. Bock, M. O. McClure, and A. Rethwilm. 1997. Human foamy virus reverse transcription that occurs late in the viral replication cycle. *J. Virol.* 71(10):7305–7311.
81. Muranyi, W. and R. M. Flugel. 1991. Analysis of splicing patterns of human spumaretrovirus by polymerase chain reaction reveals complex RNA structures. *J. Virol.* 65(2):727–735.
82. Murray, S. M. and M. L. Linial. 2006. Foamy virus infection in primates. *J. Med. Primatol.* 35(4–5):225–235.
83. Murray, S. M., L. J. Picker, M. K. Axthelm, K. Hudkins, C. E. Alpers, and M. L. Linial. 2008. Replication in a superficial epithelial cell niche explains the lack of pathogenicity of primate foamy virus infections. *J. Virol.* 82(12):5981–5985.
84. Murray, S. M., L. J. Picker, M. K. Axthelm, and M. L. Linial. 2006. Expanded tissue targets for foamy virus replication with simian immunodeficiency virus-induced immunosuppression. *J. Virol.* 80(2):663–670.
85. Neves, M., J. Peries, and A. Saib. 1998. Study of human foamy virus proviral integration in chronically infected murine cells. *Res. Virol.* 149(6):393–401.
86. Park, J., P. Nadeau, J. R. Zucali, C. M. Johnson, and A. Mergia. 2005. Inhibition of simian immunodeficiency virus by foamy virus vectors expressing siRNAs. *Virology* 343(2):275–282.
87. Park, J., P. E. Nadeau, and A. Mergia. 2002. A minimal genome simian foamy virus type 1 vector system with efficient gene transfer. *Virology* 302(2):236–244.
88. Patton, G. S., S. A. Morris, W. Chung, P. D. Bieniasz, and M. O. McClure. 2005. Identification of domains in gag important for prototypic foamy virus egress. *J. Virol.* 79(10):6392–6399.
89. Peters, K., T. Wiktorowicz, M. Heinkelein, and A. Rethwilm. 2005. RNA and protein requirements for incorporation of the Pol protein into foamy virus particles. *J. Virol.* 79(11):7005–7013.
90. Pfrepper, K. I., M. Lochelt, H. R. Rackwitz, M. Schnolzer, H. Heid, and R. M. Flugel. 1999. Molecular characterization of proteolytic processing of the Gag proteins of human spumavirus. *J. Virol.* 73(9):7907–7911.
91. Pietschmann, T., M. Heinkelein, M. Heldmann, H. Zentgraf, A. Rethwilm, and D. Lindemann. 1999. Foamy virus capsids require the cognate envelope protein for particle export. *J. Virol.* 73(4):2613–2621.
92. Renne, R., E. Friedl, M. Schweizer, U. Fleps, R. Turek, and D. Neumann-Haefelin. 1992. Genomic organization and expression of simian foamy virus type 3 (SFV-3). *Virology* 186(2):597–608.
93. Rethwilm, A. 2007. Foamy virus vectors: an awaited alternative to gammar. *Curr. Gene Ther.* 7(4):261–271.
94. Rhodes-Feuillette, A., F. Saal, J. Lasneret, P. Dubouch, and J. Peries. 1979. Isolation and characterization of a new simian foamy virus serotype from lymphocytes of a Papio cynocephalus baboon. *J. Med. Primatol.* 8(5):308–320.
95. Rinke, C. S., P. L. Boyer, M. D. Sullivan, S. H. Hughes, and M. L. Linial. 2002. Mutation of the catalytic domain of the foamy virus reverse transcriptase leads to loss of processivity and infectivity. *J. Virol.* 76(15):7560–7570.
96. Rogers, N. G., M. Basnight, C. J. Gibbs, and D. C. Gajdusek. 1967. Latent viruses in chimpanzees with experimental kuru. *Nature* 216(5114):446–449.
97. Rosener, M., H. Hahn, M. Kranz, J. Heeney, and A. Rethwilm. 1996. Absence of serological evidence for foamy virus infection in patients with amyotrophic lateral sclerosis. *J. Med. Virol.* 48(3):222–226.
98. Roy, J. and M. L. Linial. 2007. Role of the foamy virus pol cleavage site in viral replication. *J. Virol.* 81(10):4956–4962.
99. Rustigian, R., P. Johnston, and H. Reihart. 1955. Infection of monkey kidney tissue cultures with virus-like agents. *Proc. Soc. Exp. Biol. Med.* 88(1):8–16.
100. Sandstrom, P. A., K. O. Phan, W. M. Switzer, T. Fredeking, L. Chapman, W. Heneine, and T. M. Folks. 2000. Simian foamy virus infection among zoo keepers. *Lancet* 355(9203):551–552.
101. Schillaci, M. A., L. Jones-Engel, G. A. Engel, Y. Paramastri, E. Iskandar, B. Wilson, J. S. Allan, R. C. Kyes, R. Watanabe, and R. Grant. 2005. Prevalence of enzootic simian viruses among urban performance monkeys in Indonesia. *Trop. Med. Int. Health* 10(12):1305–1314.
102. Schliephake, A. W. and A. Rethwilm. 1994. Nuclear localization of foamy virus Gag precursor protein. *J. Virol.* 68(8):4946–4954.
103. Schmidt, M., O. Herchenroder, J. Heeney, and A. Rethwilm. 1997. Long terminal repeat U3 length polymorphism of human foamy virus. *Virology* 230(2):167–178.
104. Schweizer, M., V. Falcone, J. Gange, R. Turek, and D. Neumann-Haefelin. 1997. Simian foamy virus

- isolated from an accidentally infected human individual. *J. Virol.* 71(6):4821–4824.
105. Schweizer, M. and D. Neumann-Haefelin. 1995. Phylogenetic analysis of primate foamy viruses by comparison of pol sequences. *Virology* 207(2):577–582.
 106. Schweizer, M., H. Schleer, M. Pietrek, J. Liegibel, V. Falcone, and D. Neumann-Haefelin. 1999. Genetic stability of foamy viruses: long-term study in an African green monkey population. *J. Virol.* 73(11):9256–9265.
 107. Schweizer, M., R. Turek, H. Hahn, A. Schliephake, K. O. Netzer, G. Eder, M. Reinhardt, A. Rethwilm, and D. Neumann-Haefelin. 1995. Markers of foamy virus infections in monkeys, apes, and accidentally infected humans: appropriate testing fails to confirm suspected foamy virus prevalence in humans. *AIDS Res. Hum. Retroviruses* 11(1):161–170.
 108. Shaw, K. L., D. Lindemann, M. J. Mulligan, and P. A. Goepfert. 2003. Foamy virus envelope glycoprotein is sufficient for particle budding and release. *J. Virol.* 77(4):2338–2348.
 109. Stange, A., I. Mannigel, K. Peters, M. Heinkelein, N. Stanke, M. Cartellieri, H. Gottlinger, A. Rethwilm, H. Zentgraf, and D. Lindemann. 2005. Characterization of prototype foamy virus gag late assembly domain motifs and their role in particle egress and infectivity. *J. Virol.* 79(9):5466–5476.
 110. Stiles, G. E., J. L. Bittle, and V. J. Cabasso. 1964. Comparison of simian foamy virus strains including a new serological type. *Nature* 201:1350–1351.
 111. Switzer, W. M., V. Bhullar, V. Shanmugam, M. E. Cong, B. Parekh, N. W. Lerche, J. L. Yee, J. J. Ely, R. Boneva, L. E. Chapman, T. M. Folks, and W. Heneine. 2004. Frequent simian foamy virus infection in persons occupationally exposed to nonhuman primates. *J. Virol.* 78(6):2780–2789.
 112. Switzer, W. M., A. D. Garcia, C. Yang, A. Wright, M. L. Kalish, T. M. Folks, and W. Heneine. 2008. Coinfection with HIV-1 and simian foamy virus in West Central Africans. *J. Infect. Dis.* 197(10):1389–1393.
 113. Switzer, W. M., M. Salemi, V. Shanmugam, F. Gao, M. E. Cong, C. Kuiken, V. Bhullar, B. E. Beer, D. Vallet, A. Gautier-Hion, Z. Tooze, F. Villinger, E. C. Holmes, and W. Heneine. 2005. Ancient co-speciation of simian foamy viruses and primates. *Nature* 434(7031):376–380.
 114. Thumer, L., A. Rethwilm, E. C. Holmes, and J. Boden. 2007. The complete nucleotide sequence of a New World simian foamy virus. *Virology* 369(1):191–197.
 115. Trobridge, G. and D. W. Russell. 2004. Cell cycle requirements for transduction by foamy virus vectors compared to those of oncovirus and lentivirus vectors. *J. Virol.* 78(5):2327–2335.
 116. Trobridge, G. D., D. G. Miller, M. A. Jacobs, J. M. Allen, H. P. Kiem, R. Kaul, and D. W. Russell. 2006. Foamy virus vector integration sites in normal human cells. *Proc. Natl. Acad. Sci. U. S. A.* 103(5):1498–1503.
 117. Verschoor, E. J., S. Langenhuijzen, I. Bontjer, Z. Fagrouch, H. Niphuis, K. S. Warren, K. Eulenberger, and J. L. Heeney. 2004. The phylogeography of orangutan foamy viruses supports the theory of ancient re-population of Sumatra. *J. Virol.* 78(22):12712–12716.
 118. Verschoor, E. J., S. Langenhuijzen, S. van den Engel, H. Niphuis, K. S. Warren, and J. L. Heeney. 2003. Structural and evolutionary analysis of an orangutan foamy virus. *J. Virol.* 77(15):8584–8587.
 119. Wilk, T., F. de Haas, A. Wagner, T. Rutten, S. Fuller, R. M. Flugel, and M. Lochelt. 2000. The intact retroviral Env glycoprotein of human foamy virus is a trimer. *J. Virol.* 74(6):2885–2887.
 120. Wolfe, N. D., W. M. Switzer, J. K. Carr, V. B. Bhullar, V. Shanmugam, U. Tamoufe, A. T. Prosser, J. N. Torimiro, A. Wright, E. Mpoudi-Ngole, F. E. McCutchan, D. L. Birx, T. M. Folks, D. S. Burke, and W. Heneine. 2004. Naturally acquired simian retrovirus infections in central African hunters. *Lancet* 363(9413):932–937.
 121. Yu, S. F., D. N. Baldwin, S. R. Gwynn, S. Yendapalli, and M. L. Linial. 1996. Human foamy virus replication: a pathway distinct from that of retroviruses and hepadnaviruses. *Science* 271(5255):1579–1582.
 122. Yu, S. F., S. W. Eastman, and M. L. Linial. 2006. Foamy virus capsid assembly occurs at a pericentriolar region through a cytoplasmic targeting/retention signal in Gag. *Traffic* 7(8):966–977.
 123. Yu, S. F., K. Edelmann, R. K. Strong, A. Moebes, A. Rethwilm, and M. L. Linial. 1996. The carboxyl terminus of the human foamy virus Gag protein contains separable nucleic acid binding and nuclear transport domains. *J. Virol.* 70(12):8255–8262.
 124. Yu, S. F. and M. L. Linial. 1993. Analysis of the role of the bel and bet open reading frames of human foamy virus by using a new quantitative assay. *J. Virol.* 67(11):6618–6624.
 125. Yu, S. F., M. D. Sullivan, and M. L. Linial. 1999. Evidence that the human foamy virus genome is DNA. *J. Virol.* 73(2):1565–1572.

Section 1.2:

Other Simian RNA Viruses

10

Picornaviruses

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10.1. INTRODUCTION

Picornaviruses occupy a special place in the history of virology and simian virology in particular. The first animal virus discovered by Loeffler and Frosch in 1898 was a picornavirus, the foot-and-mouth disease virus. Perhaps, the most “famous” picornavirus is poliovirus, the causative agent of poliomyelitis. The first Nobel Prize in medicine given for research in virology was awarded in 1954 to John Enders, Thomas Weller, and Frederick Robbins for the development of a method for the propagation of poliovirus in tissue culture. This methodology paved the way for the development of effective polio vaccines and revolutionized virology by providing a universal approach for propagating viruses *in vitro*. The inception of simian virology is also “linked” to poliovirus—in 1908, Carl Landsteiner and Erwin Popper transmitted poliomyelitis from a human to a rhesus monkey with cell-free material. This experiment not only proved the viral etiology of poliomyelitis, but it also provided the first nonhuman primate (NHP) model of human viral disease. A number of picornaviruses are important human pathogens, causing a wide range of

diseases, from the common cold to life-threatening CNS infections, hepatitis, myocarditis, and others.

Most of the simian picornaviruses were discovered in the 1950s and 1960s, a period of explosive growth of simian virology during the quest for a vaccine against poliomyelitis. By the 1970s, the “golden age” of poliovirus research ended and the main interest in the picornavirus field has shifted to another important human pathogen, human hepatitis A virus. A “by-product” of this research was identification of the simian counterpart of human hepatitis A virus.

This chapter contains information on the picornaviruses harbored by NHPs. Data on the experimental infection of NHPs with human picornaviruses as well as on the susceptibility of NHP to a nonprimate picornavirus (encephalomyocarditis virus) are covered in Chapter 24.

10.2. CLASSIFICATION AND NOMENCLATURE

As their name suggests (Latin *pico* = small; *rna* = RNA) picornaviruses are small (27–30 nm in diameter) RNA viruses. The picornaviruses are nonenveloped—hence their resistance to environmental factors. Their virions are icosahedral, consisting of 60 capsomers (Figure 10.1). Genomic RNA is a positive-sense single-stranded RNA molecule about 7,500 nt in length.

Picornaviruses comprise the *Picornaviridae* family. This family includes nine genera. Medically important picornaviruses belong to four of these genera, namely, *Enterovirus*, *Hepatovirus*, *Rhinovirus*, and *Parechovirus*. All known simian picornaviruses belong to either the *Enterovirus* or *Hepatovirus* genera. Surprisingly, despite the existence of more than 100 types

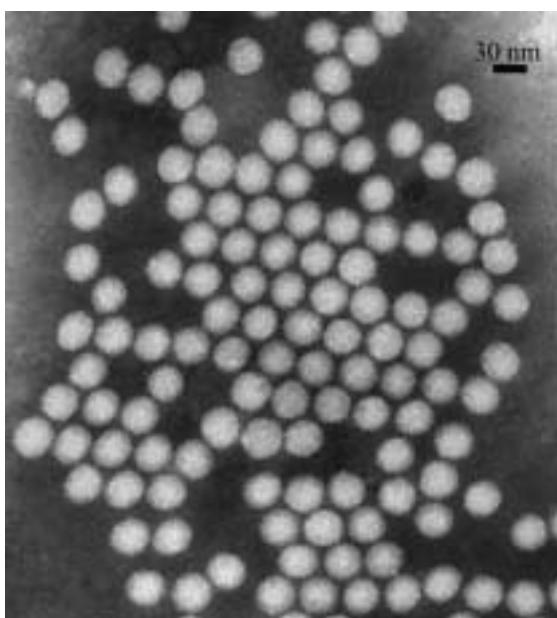


Figure 10.1. Electron micrograph of poliovirus. Negatively-stained poliovirus virions appear as uniform spherical particles (30 nm in diameter). (Image is kindly provided by Prof. Hans R. Gelderblom.)

of human rhinoviruses, no simian rhinovirus has been described.

In the latest International Committee on Taxonomy of Viruses (ICTV) classification (8th Report), the taxonomic rank of simian enteroviruses (SEVs) is defined as a tentative species. Two nomenclatures of SEVs are in use. The first, historical nomenclature includes designations assigned to the viruses at the time of their isolation. These names start with SV or SA for the viruses isolated from Asian and African species, respectively. SV and SA are followed by numerical identifiers which may or may not be consecutive, for example, SV4, SA4. The ICTV-approved names of SEVs include the abbreviation SEV and the consecutive numerical identifiers (SEV-1 through SEV-18). The SEV number, in general, corresponds to the chronology of virus isolation. There are two exceptions: SEV-N125 and SEV-N203. The SEV and SV/SA numerical identifiers are different, for example, SEV-1 and SV-2 are the names of the same virus. Basic information on the origin and naming of SEVs is presented in Table 10.1.

Many SEVs are related to the human enteroviruses (HEVs). The nomenclature of HEV is perplexing. Two naming schemes are in use. Traditional naming is based on the old classifications of enteroviruses into the polioviruses (PV), coxsackieviruses A (CVA), coxsackieviruses B (CVB), echoviruses (E), and “other” enteroviruses (EV). Coxsackie is a town in the United States where the patient from whom the first coxsackievirus was isolated lived. “Echo” is an acronym from *enteric cytopathogenic human orphan virus*, where orphan means not linked to a known disease. Despite the unsystematic, ad hoc nature of these designations they are widely used. Within each of the traditional groups the viruses are consecutively numbered: PV types 1 to 3, CVA types 1 to 24, CVB1-6, E1-34, EV68-71. The ICTV classification divides HEVs into four species: HEV-A, -B, -C, and -D. These species do not correlate with the traditional groups, for example, the most numerous enteroviral species, HEV-B, includes coxsackieviruses B, echoviruses, and some “numbered enteroviruses.”

In the latest ICTV classification (8th Report), there is only one species in the *Hepatovirus* genus: *hepatitis A virus* (HAV). This viral species includes human hepatitis A virus (HHAV) and simian hepatitis A virus (SHAV) which are considered as strains within HAV species. The abbreviation HHAV is very rarely used. Human hepatitis A virus is usually abbreviated as HAV, although, strictly speaking, HAV is a generic designation for all hepatitis A viruses, both human and simian. In this chapter, the abbreviation HAV is used for the designation of human hepatitis A viruses only.

10.3. SIMIAN ENTEROVIRUSES

As was mentioned above, all SEVs were isolated many years ago, the “youngest” among them has been known since 1977 (Table 10.1).^{16,17,21,23–25,29,45} Many more SEVs likely exist, but this field is only marginally explored using modern methodologies. The first such attempt using enterovirus generic polymerase chain reaction has resulted in the detection of two new simian viruses, provisionally named enterovirus 92 and 103 (EV92 and EV103).³⁴ These newly described SEVs are frag-viruses, yet to be isolated as infectious agents.

By analogy with HEVs, SEVs are presumed to be transmitted by the fecal–oral route. This is supported by studies showing that SEV is excreted in feces. In fact, about half of the known SEVs were isolated from feces or rectal swabs, mostly from monkeys with

Table 10.1. Simian Enteroviruses

Tentative Species	Traditional Name/Isolate	Species of Origin/Source of Specimen	Original References
<i>Simian enterovirus 1</i> (SEV-1)	SV2/2383	<i>Macaca mulatta</i> /kidney culture	24
<i>Simian enterovirus 2</i> (SEV-2)	SV6/1631	<i>M. mulatta</i> /kidney culture	24
<i>Simian enterovirus 3</i> (SEV-3)	SV16/2450 SD	<i>M. mulatta</i> /kidney culture	23
<i>Simian enterovirus 4</i> (SEV-4)	SV18/2481 B2	<i>M. mulatta</i> /kidney culture	23
<i>Simian enterovirus 5</i> (SEV-5)*	SV19/M19s-P2	<i>M. fascicularis</i> /rectal swab	21
<i>Simian enterovirus 6</i> (SEV-6)*	SV26/3163	<i>M. mulatta</i> /CNS	23
<i>Simian enterovirus 7</i> (SEV-7)	SV28/9128†	<i>M. mulatta</i> /CNS	
	SV4/1715 UWB†	<i>M. mulatta</i> /kidney culture	23
	SV35/A7987	<i>M. mulatta</i> /CNS	23
<i>Simian enterovirus 8</i> (SEV-8)*	SV42/M9-P1	<i>M. fascicularis</i> /rectal swab	21
<i>Simian enterovirus 9</i> (SEV-9)	SV43/OM112t-P12	<i>M. fascicularis</i> /rectal swab	21
<i>Simian enterovirus 10</i> (SEV-10)	SV44/OM114s-P13	<i>M. fascicularis</i> /rectal swab	21
<i>Simian enterovirus 11</i> (SEV-11)	SV45/M19h-P14	<i>M. mulatta</i> /rectal swab	17
<i>Simian enterovirus 12</i> (SEV-12)	SV46/OM22-P15	<i>Macaca</i> spp./rectal swab	17
<i>Simian enterovirus 13</i> (SEV-13)	SV47/P16	<i>M. fascicularis</i> ? <i>M. fascicularis</i> ?	17
<i>Simian enterovirus 14</i> (SEV-14)	SV49/2600/P19	<i>M. mulatta</i> /rectal swab	17
<i>Simian enterovirus 15</i> (SEV-15)	SA4/L79C3	<i>Cercopithecus aethiops</i> /kidney culture	28
<i>Simian enterovirus 16</i> (SEV-16)	SA5/B165	<i>C. aethiops</i> /rectal swab	28
<i>Simian enterovirus 17</i> (SEV-17)	BaEV/BA13, A13	<i>Papio anubis</i> /feces	16
<i>Simian enterovirus 18</i> (SEV-18)	N125	<i>P. cynocephalus</i> ? <i>P. cynocephalus</i> /throat swab	44
<i>Simian enterovirus N125</i> (SEV-N125)‡	N203		44
<i>Simian enterovirus N203</i> (SEV-N203)‡			

?, source of specimen is unknown.

*SEV-5 (SV-19), SEV-6 (SV-26), and SEV-8 (SV-35) belong to the same neutralization serotype.

†SV-4 and SV-28 (SEV-7) belong to the same neutralization serotype.

‡SEV-N125 and SEV-N203 belong to the same neutralization serotype.

diarrhea.^{16,17,21} However, there is no experimental proof supporting the role of SEVs in the etiology of diarrhea. This is also true for the much better studied HEVs. SEVs preferentially replicate in the intestine; however, they can also replicate in oropharyngeal tissues, the kidneys, and the CNS. Enteroviruses do not establish latency either in vitro or in vivo. Naturally occurring simian analogs of human enteroviral diseases have not been described. It is likely that some of them may develop in monkeys, but this field has not been explored.

SEVs are presumed to be prevalent in NHPs but available data are sparse and limited to infections of captive monkeys.⁵² At the Yerkes National Primate Research Center (YNPRC), SEV has been detected in 30 of 40 rhesus monkeys, 5 of 11 pig-tailed macaques, and 2 of 4 mangabeys tested. Five SEV strains have been found to circulate in this primate center: SV6, SV19, SV46, and previously unknown EV92 and EV103. Interestingly, EV92 was the most frequent finding.³⁴

10.3.1. Genome Organization and Gene Products

The organization of the enteroviral genome is prototypic for all picornaviruses.⁴¹ The genomic RNA is of positive polarity and, therefore, can be translated directly.

It is also infectious, that is produces infectious virion progeny, if delivered in the cell. The genomic RNA has a poly-A tail at the 3'-end, which is characteristic of cellular mRNA, but no "cap" structure at the 5'-end. Instead, a small basic protein of about 20 amino acids, the viral protein genome-linked (VPg), is covalently linked to the 5'-end of the genomic RNA. The genome includes two terminal untranslated regions (UTRs) and a protein-encoding region located between the UTRs. The genomic map and gene products are depicted in Figure 10.2.

The 5'-UTR is quite long (744–666 nt). It contains the sequence motifs important for translation and encapsulation of virion RNA. One of them, the internal ribosome entry site (IRES), is essential for the translation of viral "cap-less" RNA. The 3'-UTR spans 50–100 nt. It contains sequences important for the synthesis of the negative-sense copy of the genomic RNA.

The genome encodes one large polyprotein (2,100–2,400 amino acids), which is posttranslationally cleaved by virus-coded proteases into structural and nonstructural proteins (Figure 10.2). The coding region is divided into three parts, named P1, P2, and P3. P1 contains four genes encoding structural proteins VP4, VP2, VP3, and

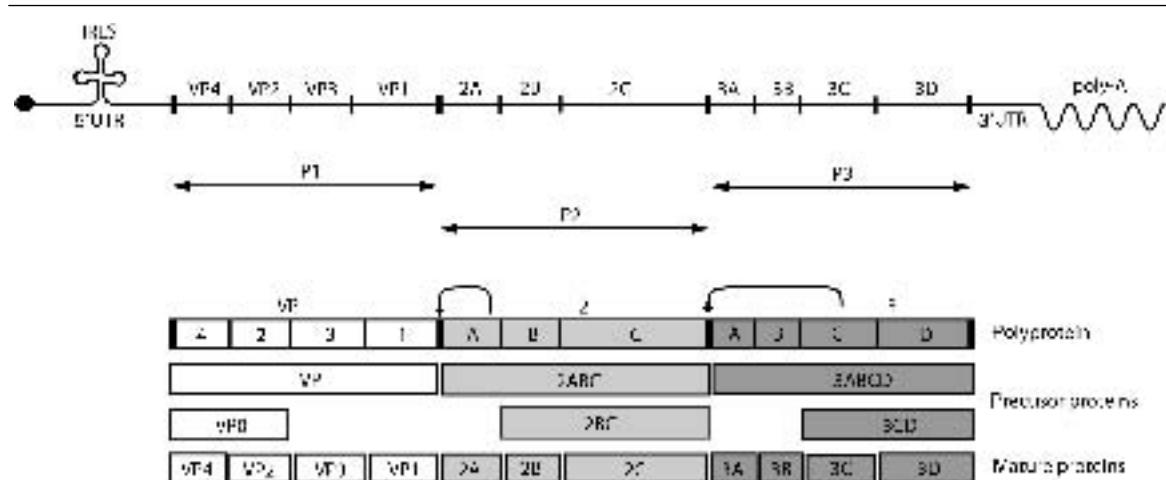


Figure 10.2. Schematic map of a picornavirus genome and processing of viral proteins. (Top) RNA genome. •, VPg protein; UTR, untranslated regions; IRES, internal ribosome entry site; VP1, VP2, VP3, and VP4, genes encoding structural viral proteins; 2A, 2B, 2C, 3A, 3B, 3C, 3D, genes encoding nonstructural proteins; P1, P2, and P3, major coding regions encoding precursor proteins VP, 2ABC, and 3ABCD, respectively. (Bottom) Processing of viral proteins. Mature viral proteins give rise through multiple cleavages of primary translation product (polyprotein) and intermediaries (precursor proteins).

VP1 (in 5'-3'-order). The P2 region contains three genes, named 2A, 2B, and 2C. The product of the 2A gene is the 2A^{Pro} protease, which cleaves between P1 and P2. The products of the 2B and 2C genes are required for the replication of genomic RNA. The P3 region contains four genes, named 3A, 3B, 3C, and 3D. The product of the 3C gene is the 3C^{Pro} protease, which cleaves between P2 and P3. Viral RNA-dependent RNA polymerase 3D^{Pol} is encoded by the 3D gene. The 3B gene encodes the VPg protein. The product of the 3A gene is a hydrophobic protein which is required for replication and determines the host range of the virus.

10.3.2. Overview of Replication Cycle

The enteroviral replication cycle is mostly known from poliovirus studies. However, the major mechanisms of replication are likely to be common for all enteroviruses. Replication of enteroviruses occurs entirely in the cytoplasm of the host cell; their replication is possible even in enucleated cells. Replication starts by the binding of the virus to the receptor. The receptors for SEVs are not known. The analogy with HEVs is not useful for predicting SEV receptors because different HEVs use distinct molecules as receptors. For example, CD-155 is a receptor for polioviruses, whereas ICAM-1 is used as a receptor by coxsackie A viruses.

After binding to the receptor, the VP4 protein is cleaved from the virion. The capsid and the underlying cellular membrane undergo conformational changes that result in the formation of a “pit” containing the virion. A fraction of these “trapped” virions establish a connection with the cytoplasm and release their genomic RNA into the cytoplasm. The remaining virions, which did not manage to “inject” their RNA, are released from the cell membrane. These VP4-stripped virions, named “A” particles, are not infectious. The empty capsids are also detached from the receptor after the release of RNA.

Translation of genomic RNA starts almost immediately after entry. A key role in this process is played by the IRES, a functional substitute for the cap, normally required for ribosomal binding by mRNA. The ribosomes bind to the IRES and are delivered directly to the initiation codon of the primary translation product, the polyprotein.

A characteristic feature of enterovirus replication is the “shut off” of cellular protein synthesis which occurs simultaneously with the beginning of viral RNA transcription. This cellular “shut off” is a consequence of

the inactivation of the cellular protein eIF-4G, an important component of the cap-dependent translation mechanism. Viral protease 2A^{Pro} is presumably responsible for this inactivation. The “shut off” allows full engagement of the cellular protein synthesis machinery in the production of viral components. The profound cytopathic effect (CPE) that is characteristic of enteroviral infections in vitro is also a consequence of the enterovirus-induced “shut off.”

Post-translational processing of the polyprotein is quite complex (Figure 10.2). The polyprotein itself does not accumulate in the cytoplasm. Self-cleavage starts as soon as the nascent polypeptide chain passes the 2A coding sequence. The primary cleavage mediated by 2A^{Pro} separates P1(VPs) and P2-P3 (2ABC-3ABCD) precursor proteins. A series of subsequent cleavages cleave P2 and P3 and, through the intermediate precursors (VP0, 2BC, 3AB, and 3CD), produce the mature structural proteins (VP1, VP2, VP3, and VP4), the nonstructural proteins (2A^{Pro}, 2B, 2C, 3A, 3C^{Pro}, and 3D^{Pol}), and RNA-linked VPg(3B). These cleavages are mediated by the 3C^{Pro} protein and its precursor 3CD.

Replication of genomic RNA is mediated by the RNA-dependent RNA polymerase (3D^{Pol} protein). VPg, 2B, 2C, and the precursor 3AB also participate in genome replication. Copying of genomic RNA is “asymmetrical”; that is, it is 30–70 times more efficient in producing the positive-sense molecules than the negative-sense molecules. The mechanism of the asymmetrical RNA synthesis is not clear. Newly produced positive-sense viral RNAs are used as mRNA templates for the synthesis of viral proteins and as genomic RNA for the newly assembled virions. The only difference between these two is the presence of covalently bound VPg at the 5'-end of the genomic RNA. VPg acts as a primer for viral RNA synthesis, which is rather unusual. Normally nucleic acid synthesis is primed by oligonucleotides.

Capsid assembly is a multi-stage process. The assembly starts by the formation of the “protomers” (also called 5S units). Five protomers associate and form “pentamers” (also called 14S units). The pentamers are self-assembled into empty capsids (80S particles). Genomic RNA is inserted in the capsids. “Filled” capsids (150S particles) in which VP0 is not yet cleaved are called provirions. Cleavage of the VP0 to VP2 and VP4 heralds the formation of the mature virions (160S particles). The release of newly formed virions occurs through the rupture of the host cell membrane.

Table 10.2. Phylogeny-Based Classification of Simian Enteroviruses

Phylogenetic Groupings	Old Name/ICTV Name	Closest Nonsimian Relatives
“Simian enterovirus A” (SEV-A)*	SV4-SV28/SEV-7	Enteroviruses 68, 70 (EV68, 70)
“Simian enterovirus B” (SEV-B)*	SV6/SEV-2, SEV-N125/N203	HEV-A viruses
“Human enterovirus A” (HEV-A)	SV19-26-35/SEV-5-6-8, SV43/SEV-10, SV46/SEV-13, SA4/SEV-16, BaEV/SEV-18	Enteroviruses 91, 90, 76, 89 (EV91, 90, 76, 89)
“Human enterovirus B” (HEV-B)	SA5/SEV-17	HEV-B viruses
<i>Sapelovirus</i> [†]	SV2/SEV-1, SV16/SEV-3, SV18/SEV-4, SV42/SEV-9, SV44/SEV-11, SV45/SEV-12, SV49/SEV-15	Porcine enterovirus 8 (PEV8)

*Suggested new enterovirus species.

[†]Suggested new picornavirus genus.

10.3.3. Phylogeny-Based Classification

Current SEV research is mainly focused on unraveling the relationships within the *Enterovirus* genus using phylogenetic analysis.^{34–36,38,40} Formally, all SEVs are “equal,” in the sense that all of them are listed in the ICTV classification as “tentative species.” However, with the accumulation of SEV genomic sequences, it has become clear that these viruses are not equidistant from each other and from their closest relatives, mostly HEVs. Some SEVs, for example, SV19, SV26, and SV35, turned out to be so similar in terms of the sequence homology that they can be considered as different isolates of the same virus. Thus, SEVs may not be as diverse as they appear from the numerical identifiers.

Phylogenetic analysis of different regions across SEV genomes produces results which are not universally concordant. Sequence variation in various genomic regions is constrained differently and, as a result, some regions are more conserved than the others. This may partly explain the inconsistencies in the results of the phylogenetic analysis. In addition, the data available for HEV reveals that recombination of enteroviral genomes is a common phenomenon³⁷ and therefore some SEV may also be recombinant viruses.

The relationships between SEV and other enteroviruses, established by phylogenetic analyses, are summarized in Table 10.2.

SEV strains fall into the following categories:

1. isolates belonging to HEV species;
2. strains comprising a new enteroviral species;
3. strains which do not belong to any *Enterovirus* species and comprise a new genus within the *Picornaviridae* family.

The first group includes SEV isolates clustered with HEV-A (SV19, 26, 35, 43, 46, SA4, and BaEV) and HEV-B (SA5). The frag-SEV EV92, apparently, belongs to HEV-A species.³⁴

The second group is represented by the *simian enterovirus A* (SEV-A) and *simian enterovirus B* (SEV-B) species.^{35,38} SEV-A species includes closely related SV4 and SV28 isolates as well as the A2-plaque enterovirus. The latter was isolated from a hepatitis B patient using African green monkey kidney cell culture, which was “presumed” to be virus-free.⁴⁷ Phylogenetic analysis indicates that the A2-plaque virus is of simian origin. Most likely, it originated from African green monkey kidney cell culture. A more remote possibility is a zoonotic infection of the index patient. SEV-B species includes SV6, N125, and N203 isolates. The frag-SEV EV103, apparently, belongs to SEV-B species.³⁴

The third group includes SV2, 16, 18, 42, 44, 45, and 48. These closely related viruses comprise a yet to be formally approved picornaviral genus, the suggested name of which is *Sapelovirus*.³⁶

10.4. SIMIAN HEPATITIS A VIRUS

The disease currently named viral hepatitis A has been known since ancient times and described under various names which included the word “jaundice.” The disease was associated with poor hygienic conditions. By the late 1940s, it was established that this form of hepatitis was transmitted by the oral–fecal route and was clearly distinguishable from the parenterally transmitted infectious hepatitis, the “serum” hepatitis. These two forms of infectious hepatitis were named viral hepatitis A and hepatitis B, respectively, and in 1973 the WHO

legitimized these terms. In the same year, the virus causing hepatitis A (HAV) was unequivocally identified.¹⁵ Initially, HAV was classified as enterovirus 72. This name had been rarely used and was discontinued after the introduction of the *Hepatovirus* genus within the *Picornaviridae* family.

Studies suggesting the existence of simian analogs of HAV were published in the late 1950s. Several outbreaks of “infectious hepatitis” in animal caretakers who had close contact with newly captured infant chimpanzees were described.^{18–20,26,31,32,46} These cases have been diagnosed retrospectively as viral hepatitis A.¹³ It remains unknown whether the virus transmitted from chimpanzees to humans was of simian or human origin.⁴³ The latter possibility could not be excluded because administration of human blood or serum to newly captured apes was a common practice at that time.⁴³ The procedure was aimed at protecting the animals against human pathogens, but could have unintentionally transmitted human blood-borne viruses. Interestingly, passive immunization with human immunoglobulin was found to be effective in preventing chimpanzee-to-human transmission of hepatitis A.²⁶ However, these data cannot be considered as conclusive proof that the virus transmitted from chimpanzees to humans was HAV, due to the extensive antigenic cross-reactivity between human and simian hepatitis viruses.

By the early 1980s, assays for the detection of antibodies against HAV became widely available. Using these tests it has been repeatedly demonstrated that some sera from the wild African (*Chlorocebus aethiops*, *Erythrocebus patas*, *Papio ursinus*) and Asian monkeys (*Macaca fascicularis*) are positive for “anti-HAV” antibodies.^{9–11,28,50} Hepatitis A has also been described in newly captured macaques and African green monkeys.^{1,5,27,49} Outbreaks of anti-HAV-positive hepatitis have been reported in captive colonies of macaques (*M. mulatta*, *M. fascicularis*, *M. arctoides*) and African green monkeys (*C. aethiops*).^{2,5,6,48} These data, however, were not sufficient to prove the existence of a “simian HAV” because the serology and electron microscopy, which were used, did not distinguish between human and SHAVs.

SHAVs were unequivocally identified around 1990 when it was shown that genomic sequences of simian and human HAVs were clearly distinct. Three SHAV isolates, AGM-27 from the African green monkey (*Chlorocebus aethiops*), CY-145 from the cynomolgus macaque (*Macaca fascicularis*), and JM-55 from the Japanese macaque (*M. fuscata*), have been characterized the

best.^{3,4,7,33,51} These isolates are likely to be genuine simian viruses. The isolate PA21, from a New World monkey, initially reported as SHAV⁸ was later shown to be a human virus.⁴⁴

SHAVs are indistinguishable from HAV by routine serological tests that are used in the diagnosis of hepatitis A. There are some HAV-specific antibodies which do not react with SHAV.¹⁴ However, these antigenic differences are negligible in the context of protective immunity: immunization of chimpanzees with AGM-27-based vaccine protects against a challenge with virulent human HAV.¹⁴

10.4.1. Genome Organization and Gene Products

Genome composition is virtually identical for the hepat- and enteroviruses and the same nomenclature is used for the designation of the genes and gene products.^{12,22} The major regions in the hepatoviral genome are:

**5' – NTR – (VP4 – VP2 – VP3 – VP1) –
(2A – 2B – 2C) – (3A – 3B – 3C – 3D) – 3' – NTR**

The VPg protein is bound to the 5'-end of the genomic RNA. The 5'-NTR contains the internal ribosomal entry site IRES. The 3'-NTR is flanked by the poly-A tail. The number of structural and nonstructural proteins is the same as in the enteroviruses. However, there are some features that distinguish hepatoviruses from enteroviruses, mainly in the mechanisms of polyprotein processing. The main difference is the absence of cleavage at the P1/P2 junction. Cleavage occurs, instead, at the 2A/2B junction and it is mediated by the 3C protease. Also, the VP4 protein of hepatoviruses is smaller than in other picornaviruses and contains no myristylation signal. This may be the reason why HAV/SHAV virions do not form the “canyon,” a characteristic surface structure at the receptor-binding site of other picornaviruses.

10.4.2. Overview of Replication Cycle

In contrast to the enteroviruses, HAV and SHAV are fastidious growers in vitro. Usually, the primary isolates replicate in vitro very inefficiently. Adaptation to in vitro growth can be achieved, although not consistently, by blind passage of the virus. Such adaptation is accompanied by the accumulation of mutations throughout the viral genome. The major mutation underlying in vitro growth competence is an Ala-to-Val substitution at position 216 in the 2B protein of HAV. In contrast to enteroviruses, tissue culture-adapted HAV and SHAV

do not cause profound CPE and “shut off” of cellular protein synthesis. In other respects, the replication mechanisms of hepato- and enteroviruses are very similar.

10.4.3. Phylogeny-Based Classification

The first genotypic classification of hepatoviruses was based on the phylogenetic analysis of the putative VP1-2A region (168 nt fragment).⁴⁴ Seven HAV genotypes designated by Roman numerals from I to VII were identified. Three of the genotypes (IV, V, and VI) include simian hepatoviruses only, whereas the remaining genotypes (I, II, III, and VII) include only human hepatoviruses. Three known strains of simian hepatitis A virus, SHAV (Cy-145), SHAV (AGM-27), and SHAV (JM-55) belong to the genotypes IV, V, and VI, respectively. The distinction of SHAV from HAV is also supported by the presence of SHAV-specific “signature” sequences in the VP3/VP1 junction sequence.^{8,33,51}

Subsequently, genotypic classification of hepatoviruses was revised.¹² The new classification is based on phylogenetic analysis of the full-length VP1 sequence. However, the only difference between the old and new classifications is the absence of genotype VII which is now included in genotype II. The “simian genotypes” IV, V, and VI in both classifications are fully concordant.

The closest relative of hepatoviruses among known picornaviruses is avian encephalomyelitis virus (AEV).³⁰ Formerly, this virus was included in the *Hepatovirus* genus, as a tentative species. However, AEV is quite distant from hepatitis A viruses and a proposal to classify this virus as the prototype of a new picornavirus genus is under consideration by the ICTV.

10.4.4. Pathogenicity

Experimental inoculation of SHAV (strains AGM-27 and JM-55) causes hepatitis similar to human hepatitis A in Old World monkeys (*C. aethiops*, *M. fascicularis*, *M. arctoides*, *M. mulatta*) and New World monkeys (*S. mystax*).^{1,14,39,53} Interestingly, SHAV (AGM-27) is nonpathogenic for the common chimpanzee (*P. troglodytes*).¹⁴ Whether or not this is also true for other SHAV strains is not known.

Little is known about factors responsible for the pathogenicity of SHAV. It has been shown that the 2C gene of SHAV (AGM-27) confers virulence to non-pathogenic HAV. This was demonstrated by the inoc-

ulation of tamarins with SHAV-HAV chimeric viruses which had different combinations of SHAV and HAV genes. It has also been shown that the amino acid residues required for the virulence phenotypes were located in both terminal parts of the 2C protein.⁴²

10.5. SUMMARY

Picornaviruses are small, nonenveloped RNA viruses with a positive polarity genome (mRNA) comprising the family *Picornaviridae*. The picornavirus genome encodes only 11 proteins, only 5 of which are included in virions, 4 structural proteins (VP1–VP4) and 1 (VPg) covalently bound to the 5'-end of the genomic RNA. The coding region of the genome is flanked by the noncoding or UTRs. The 5'-UTR contains a complex secondary structure, the internal ribosome-binding site (IRES), which is a functional substitute for the “cap” structure normally present at the 5'-ends of mRNA molecules. Replication of picornaviruses takes place entirely in the cytoplasm and is accompanied by a “shut off” of cellular protein synthesis, although the degree of shut off varies among the different genera of the enteroviruses. Simian picornaviruses belong to two genera, the *Enterovirus* genus (20 tentative species) and the *Hepatovirus* genus (1 species). SEV can be classified as:

- (a) belonging to the known HEV species;
- (b) comprising a new enteroviral species;
- (c) comprising the new genus within the *Picornaviridae* family (suggested name *Sapelovirus*).

The pathogenic potential of SEV remains unknown.

Simian hepatitis A viruses (SHAVs) are closely related to human hepatitis virus (HAV). Moreover, in some classifications SHAVs are considered as different genotypes of HAV. SHAV may cause disease in monkeys similar to that caused in humans by human hepatitis A.

REFERENCES

1. Andzhaparidze, A. G., M. S. Balaian, A. P. Savinov, IuA Kazachkov, and I. P. Titova. 1987. [Spontaneous hepatitis similar to hepatitis A in African green monkeys]. *Vopr. Virusol.* 32(6):681–686.
2. Andzhaparidze, A. G., IuV Karetnyi, L. I. Korzaia, M. S. Balaian, and I. P. Titova. 1989. [Epizootic hepatitis A among African green monkeys kept in a vivarium]. *Vopr. Virusol.* 34(3):292–296.
3. Andzhaparidze, A. G., IuA Kazachkov, M. S. Balaian, IuIu Kusov, and V. F. Poleshchuk. 1987. [Hepatitis A in *Macaca fascicularis* and *M. arctoides* infected by

- the Java monkey-55 strain of hepatitis A virus]. *Vopr. Virusol.* 32(4):440–448.
- 4. Andzhaparidze, A. G., T. M. Orlova, M. S. Balaian, and Iulu Kusov. 1987. [Adaptation of hepatitis A virus strain (JaM-55) originally pathogenic for monkeys to a cell culture]. *Mol. Gen. Mikrobiol. Virusol.* 5:40–44.
 - 5. Andzhaparidze, A. G., V. F. Poleshchuk, N. A. Zamiatina, A. P. Savinov, and I. N. Gavrilovskaia. 1985. [Spontaneous hepatitis in the crab-eating macaque exposed to immunodepressants]. *Vopr. Virusol.* 30(4):468–474.
 - 6. Andzhaparidze, A. G., Z. V. Shevtsov, L. I. Korzaia, IuV Karetnyi, and M. S. Balaian. 1987. [Signs of natural infection with hepatitis A in brown macaques (*Macaca arctoides*)]. *Vopr. Virusol.* 32(5):541–544.
 - 7. Balayan, M. S., YuYu Kusov, A. G. Andjaparidze, S. A. Tsarev, E. D. Sverdlov, V. E. Chizhikov, V. M. Blinov, and S. K. Vasilenko. 1989. Variations in genome fragments coding for RNA polymerase in human and simian hepatitis A viruses. *FEBS Lett.* 247(2):425–428.
 - 8. Brown, E. A., R. W. Jansen, and S. M. Lemon. 1989. Characterization of a simian hepatitis A virus (HAV): antigenic and genetic comparison with human HAV. *J. Virol.* 63(11):4932–4937.
 - 9. Burke, D. S., R. R. Graham, and G. B. Heisey. 1981. Hepatitis A virus in primates outside captivity. *Lancet* 318(October 24):928.
 - 10. Burke, D. S. and G. B. Heisey. 1984. Wild Malaysian cynomolgus monkeys are exposed to hepatitis A virus. *Am. J. Trop. Med. Hyg.* 33(5):940–944.
 - 11. Coursaget, P., B. Levesque, E. Getillat, M. Eyraud, L. Ferrara, and M. Germain. 1981. Hepatitis A virus in primates outside captivity. *Lancet* 318(October 24):929.
 - 12. Cristina, J. and M. Costa-Mattioli. 2007. Genetic variability and molecular evolution of Hepatitis A virus. *Virus Res.* 127(2): 151–157.
 - 13. Dienstag, J. L., F. M. Davenport, R. W. McCollum, A. V. Hennessy, G. Klatskin, and R. H. Purcell. 1976. Nonhuman primate-associated viral hepatitis type A. Serologic evidence of hepatitis A virus infection. *JAMA* 236(5):462–464.
 - 14. Emerson, S. U., S. A. Tsarev, S. Govindarajan, M. Shapiro, and R. H. Purcell. 1996. A simian strain of hepatitis A virus, AGM-27, functions as an attenuated vaccine for chimpanzees. *J. Infect. Dis.* 173(3):592–597.
 - 15. Feinstone, S. M., A. Z. Kapikian, and R. H. Purcell. 1973. Hepatitis A: detection by immune electron microscopy of a viruslike antigen associated with acute illness. *Science* 182(116):1026–1028.
 - 16. Fuentes-Marins, R., A. R. Rodriguez, S. S. Kalter, A. Hellman, and R. A. Crandell. 1963. Isolation of enteroviruses from the “normal” baboon (*Papio doguera*). *J. Bacteriol.* 85:1045–1050.
 - 17. Heberling, R. L. and F. S. Cheever. 1965. Some characteristics of the simian enteroviruses. *Am. J. Epidemiol.* 81:106–123.
 - 18. Hillis, W.D. 1961. An outbreak of infectious hepatitis among chimpanzee handlers at a United States Air Force Base. *Am. J. Hyg.* 73:316–328.
 - 19. Hillis, W. D. 1963. Viral hepatitis associated with sub-human primates. *Transfusion* 3:445–454.
 - 20. Hinthon, D. R., M. T. Foster Jr., H. L. Bruce, and R. D. Aach. 1974. An outbreak of chimpanzee associated hepatitis. *J. Occup. Med.* 16(6):388–391.
 - 21. Hoffert, W. R., M. E. Bates, and F. S. Cheever. 1958. Study of enteric viruses of simian origin. *Am. J. Hyg.* 68(1):15–30.
 - 22. Hollinger, F. B. and S. U. Emerson. 2007. Hepatitis A virus. In: Knipe, D. M. and P. M. Howley (eds), *Fields Virology*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, Wolters Kluwer Business, pp. 911–947.
 - 23. Hull, R. N., J. R. Minner, and C. C. Mascoli. 1958. New viral agents recovered from tissue cultures of monkey kidney cells. III. Recovery of additional agents both from cultures of monkey tissues and directly from tissues and excreta. *Am. J. Hyg.* 68(1):31–44.
 - 24. Hull, R. N., J. R. Minner, and J. W. Smith. 1956. New viral agents recovered from tissue cultures of monkey kidney cells. I. Origin and properties of cytopathogenic agents S.V.1, S.V.2, S.V.4, S.V.5, S.V.6, S.V.11, S.V.12 and S.V.15. *Am. J. Hyg.* 63(2):204–215.
 - 25. Kalter, S. S., C. S. Kim, and E. A. Sueltenfuss. 1967. Further characterization of agents isolated from normal baboon (*Papio* sp.). *J. Infect. Dis.* 117(4):301–306.
 - 26. Krushak, D. H. 1970. Application of preventive health measures to curtail chimpanzee-associated infectious hepatitis in handlers. *Lab. Anim. Care* 20(1):52–56.
 - 27. Lankas, G. R. and R. D. Jensen. 1987. Evidence of hepatitis A infection in immature rhesus monkeys. *Vet. Pathol.* 24(4):340–344.
 - 28. Le Bras, J., B. Larouze, M. Geniteau, B. Andrieu, M. C. Dazza, and F. Rodhain. 1984. Malaria, arbovirus and hepatitis infections in *Macaca fascicularis* from Malaysia. *Lab. Anim.* 18(1):61–64.
 - 29. Malherbe, H. and R. Harwin. 1963. The cytopathic effects of vervet monkey viruses. *S. Afr. Med. J.* 37:407–411.
 - 30. Marvil, P., N. J. Knowles, A. P. Mockett, P. Britton, T. D. Brown, and D. Cavanagh. 1999. Avian encephalomyelitis virus is a picornavirus and is most closely related to hepatitis A virus. *J. Gen. Virol.* 80(Pt 3):653–662.

31. Maynard, J. E., W. V. Hartwell, and K. R. Berquist. 1971. Hepatitis-associated antigen in chimpanzees. *J. Infect. Dis.* 123(6):660–664.
32. Mosley, J. W., H. P. Reinhardt, and F. R. Hassler. 1967. Chimpanzee-associated hepatitis. An outbreak in Oklahoma. *JAMA* 199(10):695–697.
33. Nainan, O. V., H. S. Margolis, B. H. Robertson, M. Balayan, and M. A. Brinton. 1991. Sequence analysis of a new hepatitis A virus naturally infecting cynomolgus macaques (*Macaca fascicularis*). *J. Gen. Virol.* 72(Pt 7):1685–1689.
34. Nix, W. A., B. Jiang, K. Maher, E. Strobert, and M. S. Oberste. 2008. Identification of enteroviruses in naturally infected captive primates. *J. Clin. Microbiol.* 46(9):2874–2878.
35. Oberste, M. S., K. Maher, and M. A. Pallansch. 2002. Molecular phylogeny and proposed classification of the simian picornaviruses. *J. Virol.* 76(3):1244–1251.
36. Oberste, M. S., K. Maher, and M. A. Pallansch. 2003. Genomic evidence that simian virus 2 and six other simian picornaviruses represent a new genus in Picornaviridae. *Virology* 314(1):283–293.
37. Oberste, M. S., K. Maher, and M. A. Pallansch. 2004. Evidence for frequent recombination within species human enterovirus B based on complete genomic sequences of all thirty-seven serotypes. *J. Virol.* 78(2):855–867.
38. Oberste, M. S., K. Maher, and M. A. Pallansch. 2007. Complete genome sequences for nine simian enteroviruses. *J. Gen. Virol.* 88(Pt 12):3360–3372.
39. Poleshchuk, V. F., M. S. Balaian, A. G. Andzharidze, A. V. Sobol', V. P. Dokin, T. V. Guljaeva, and I. P. Titova. 1990. [The modelling of hepatitis A and of enterally transmitted non-A, non-B hepatitis (hepatitis E) in *Saguinus mystax tamarins*]. *Vopr. Virusol.* 35(5):379–382.
40. Poyry, T., L. Kinnunen, T. Hovi, and T. Hyypia. 1999. Relationships between simian and human enteroviruses. *J. Gen. Virol.* 80(Pt 3):635–638.
41. Racaniello, V. R. 2007. *Picornaviridae*: the viruses and their replication. In: Knipe, D. M. and P. M. Howley (eds), *Fields Virology*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, Wolters Kluwer Business, pp. 796–838.
42. Raychaudhuri, G., S. Govindarajan, M. Shapiro, R. H. Purcell, and S. U. Emerson. 1998. Utilization of chimeras between human (HM-175) and simian (AGM-27) strains of hepatitis A virus to study the molecular basis of virulence. *J. Virol.* 72(9):7467–7475.
43. Robertson, B. H. 2001. Viral hepatitis and primates: historical and molecular analysis of human and nonhuman primate hepatitis A, B, and the GB-related viruses. *J. Viral Hepat.* 8(4):233–242.
44. Robertson, B. H., R. W. Jansen, B. Khanna, A. Totsuka, O. V. Nainan, G. Siegl, A. Widell, H. S. Margolis, S. Isomura, K. Ito, T. Ishizu, Y. Moritsugu, and S. M. Lemon. 1992. Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. *J. Gen. Virol.* 73(Pt 6):1365–1377.
45. Rodriguez, A. R., S. S. Kalter, R. L. Heberling, R. J. Helmke, and J. E. Guajardo. 1977. Viral infections of the captive Kenya baboon (*Papio cynocephalus*): a five-year epidemiologic study of an outdoor colony. *Lab. Anim. Sci.* 27(3):356–371.
46. Ruddy, S. J., J. W. Mosley, and J. R. Held. 1967. Chimpanzee-associated viral hepatitis in 1963. *Am. J. Epidemiol.* 86(3):634–640.
47. Shaw, E. D., A. P. McKee, M. Rancourt, and L. Hollenbeck. 1973. Induction of hepatitis B antibody in experimental animals by immunization with A-2 plaque virus. *J. Virol.* 12(6):1598–1607.
48. Shevtsova, Z. V., R. I. Krylova, E. G. Belova, L. I. Korzaia, and A. G. Andzharidze. 1987. [Spontaneous hepatitis A with a fatal outcome in rhesus monkeys]. *Vopr. Virusol.* 32(6):686–690.
49. Slighter, R. G., J. P. Kimball, T. A. Barbolt, A. D. Sherer, and H. P. Drobek. 2008. Enzootic hepatitis A infection in cynomolgus monkeys (*Macaca fascicularis*). *Am. J. Primatol.* 14:73–81.
50. Smith, M. S., P. J. Swanepoel, and M. Bootsma. 1980. Hepatitis A in non-human primates in nature. *Lancet* 2(8206):1241–1242.
51. Tsarev, S. A., S. U. Emerson, M. S. Balayan, J. Ticehurst, and R. H. Purcell. 1991. Simian hepatitis A virus (HAV) strain AGM-27: comparison of genome structure and growth in cell culture with other HAV strains. *J. Gen. Virol.* 72(Pt 7):1677–1683.
52. Wang, Y., X. Tu, C. Humphrey, H. McClure, X. Jiang, C. Qin, R. I. Glass, and B. Jiang. 2007. Detection of viral agents in fecal specimens of monkeys with diarrhea. *J. Med. Primatol.* 36(2):101–107.
53. Zamiatina, N. A., A. G. Andzharidze, M. S. Balaian, A. V. Sobol', and I. P. Titova. 1990. [Susceptibility of *Macaca rhesus* to infection with hepatitis A virus strains isolated from man and monkeys]. *Vopr. Virusol.* 35(1):30–33.

11

Arteriviruses

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11.1. INTRODUCTION

Arteriviruses are a relatively recently established viral taxon named after its prototype virus, the equine arteritis virus (EAV). Only three other arteriviruses are currently known: simian hemorrhagic fever virus (SHFV), porcine reproductive/respiratory syndrome virus (PRRSV), and murine lactate dehydrogenase elevating virus (LDV).

SHFV is extremely virulent for macaques. The virus has caused at least nine fatal outbreaks of hemorrhagic fever involving hundreds of captive monkeys.^{3,4,15,20} The first outbreaks occurred in the early and mid-1960s in the Sukhumi Primate Center, in the former USSR (1963), and in two American primate facilities at the NIH (1964) and the California National Primate Research Center (1967).^{2,17,25,27,28} A causal agent of the disease was isolated independently by Soviet and American researchers.^{17,27,32} Soon after the initial reports it was shown that both isolates were very similar, if not identical.^{18,29,32}

Interest in the arteriviruses has been driven mainly by the economical importance of EAV and PRRSV, particularly the latter. Interest in SHFV has largely waned, partly because no large outbreaks of SHFV-induced hemorrhagic fever have been reported since the early 1990s. However, the potential danger of SHFV outbreaks for primate facilities does exist and should not be underestimated. Therefore, implementation of quarantine rules requiring separate housing of monkeys belonging to different species must be strictly followed. A gloomy scenario, fortunately purely theoretical, would be the possible emergence of a highly contagious SHFV mutant that could spread easily among monkeys and to humans.

11.2. CLASSIFICATION AND PHYLOGENY

The road to the current taxonomy of SHFV was bumpy. Initial studies showed that SHFV was an enveloped RNA virus.³² However, the virus did not fit into any viral taxon known at that time. In the mid-1970s, SHFV was placed in the *Togaviridae* family, first as a flavivirus³³ than as a pestivirus.¹¹

By the mid-1990s, it was established that genomic features, as well as molecular mechanisms of replication and gene expression, were shared by SHFV and three other animal viruses, EAV, PRRSV, and LDV. These four viruses were clearly distinct from all other animal RNA viruses, although they share some features with coronaviruses.³¹ In 1996, the International Committee on Taxonomy of Viruses (ICTV) legitimized this “gang of four” by formally establishing the family *Arteriviridae* within a new viral order, *Nidovirales*. The *Arteriviridae* family includes a single genus, the *Arterivirus*. SHFV, EAV, PRRSV, and LDV are classified as species within this genus.

The name nidovirus comes from the Latin *nidus* meaning nest, which emphasizes the gene expression pattern characteristic of all nidoviruses: synthesis of their mRNAs as a “nested set” of subgenomic transcripts. In addition to the *Arteriviridae*, there are two other families in the *Nidovirales* order which include viruses from mammalian species: the *Coronaviridae* and *Toroviridae*. The arteriviruses have a common ancestor with the coronaviruses.⁸ The best known coronavirus is the SARS virus (see Chapter 23). Within the *Arteriviridae* the closest relative of SHFV is EAV. Within-species phylogeny of SHFV is not known because the genomic sequence is available only for one SHFV isolate. However, SHFV, as is typical of RNA viruses in general, has an error-prone genome replication mechanism. Therefore, it is likely that different SHFV genotypes exist. Available biological data^{10,11} are consistent with this suggestion (Section 11.5.1).

11.2.1. Morphology

SHFV virions are enveloped spherical particles with a diameter of 45–50 nm. Their envelope is relatively smooth, without clearly visible spikes or projections. The icosahedral nucleocapsid (25–35 nm) is located symmetrically inside the envelope. The central part of the nucleocapsid containing the genomic RNA looks less electron-dense than the nucleocapsid “shell.” The space between the nucleocapsid and envelope is filled with material of medium electron density, approximately the same density as the nucleocapsid center.

Nucleocapsids are assembled in the cytoplasm and acquire an envelope by budding into the lumen of the smooth endoplasmic reticulum (ER) and Golgi complex. Enveloped virions accumulate in double-membrane vesicles (Figure 11.1). The vesicles are transported to the plasma membrane and progeny virions are released by fusion of the vesicle and plasma membranes.

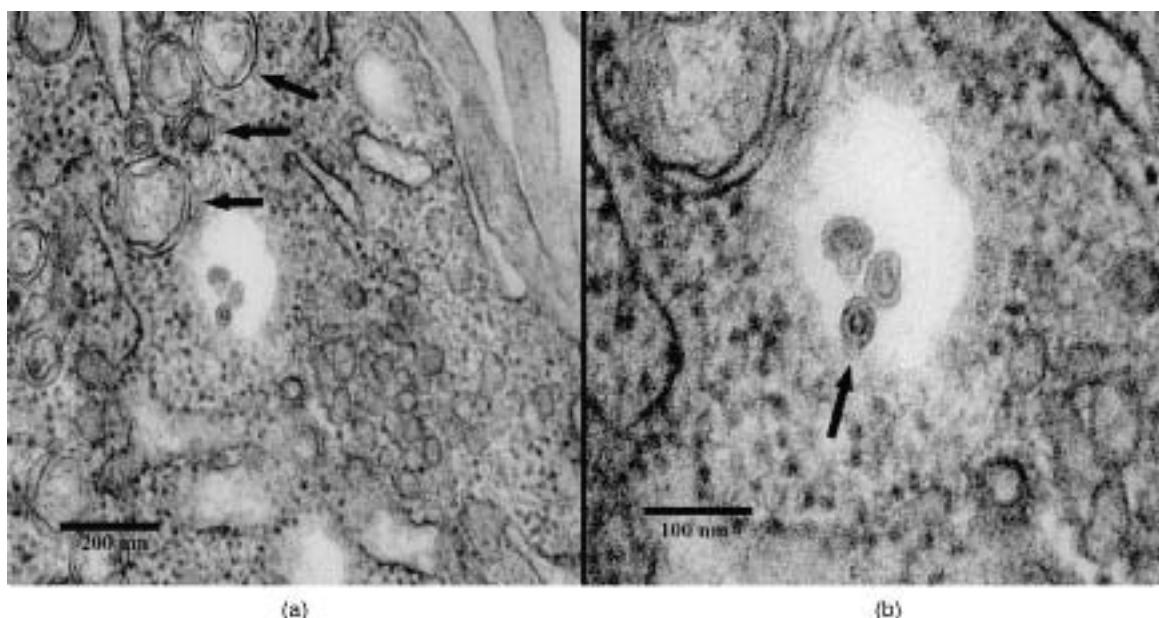


Figure 11.1. Morphology of SHFV in the thin sections. (a) Electron micrograph of double-membrane cytoplasmic vesicles (DMVs) characteristic of cells infected with SHFV and other arteriviruses. The function of the DMVs is not known. The MA104 cell shown was fixed 26 h after infection. Arrows indicated three DMVs. Bar is 200 nm. (b) Enlargement of the cytoplasmic vesicle in (a) that contains two mature virions in its lumen and a budding virion. The arrow indicates the stalk connecting the virion with the vesicle membrane. Bar is 100 nm. (Images are kindly provided by Dr. Margo A. Brinton and Dr. Robert Simmons.)

11.3. GENOMIC ORGANIZATION AND GENE PRODUCTS

SHFV genomic RNA is a single-stranded molecule of positive polarity with a cap at the 3'-end and a poly-A tail at the 5'-end.^{6,7,26,30,35,36} The length of the SHFV genome, excluding the poly-A tail, is 15,706 nt. The exact length of poly-A tail is not known. The complete genome sequence is available only for one SHFV strain (LVR 42-0/M6941) (GenBank Acc. No. NC_003092). A map of the SHFV genome is presented in Figure 11.2.

The coding part of the genome containing the nonstructural and structural genes is flanked by nontranslated regions (NTRs). The 5'-NTR and 3'-NTR span 209 nt and 76 nt, respectively. Both NTRs contain elements required for virus replication. There are multiple secondary structures in the 5'-NTR. One of those, “leader transcription-regulating sequence (TRS) hairpin” (LTH), is crucial for the production of mRNA encoding structural viral proteins. The 3'-NTR contains binding sites for cellular proteins.^{13,14,22,23} Two of these proteins, p56 and p42, have been identified as

the polypyrimidine tract-binding protein and fructose-bisphosphate aldolase A, respectively.²³

The major SHFV ORFs and their predicted products are summarized in Table 11.1.

ORF1a and ORF1b encode the nonstructural proteins which comprise the replication enzymatic complex, the replicase. Together, these two ORFs occupy approximately two-thirds of the genome; the remaining one-third is tightly packed with overlapping genes that encode the structural proteins, mostly glycoproteins (GPs). A distinguishing feature of the SHFV genome as compared to other arteriviruses is an insertion located between ORF1b and the N gene. The origin and function of this insertion are not known.

11.4. OVERVIEW OF REPLICATION CYCLE

Very little is known about the replication of SHFV. However, reasonable presumptions can be made based on the data available for other better studied arteriviruses.

The receptor for SHFV is not known. SHFV, as well as other arteriviruses, is macrophage-tropic.¹² However,

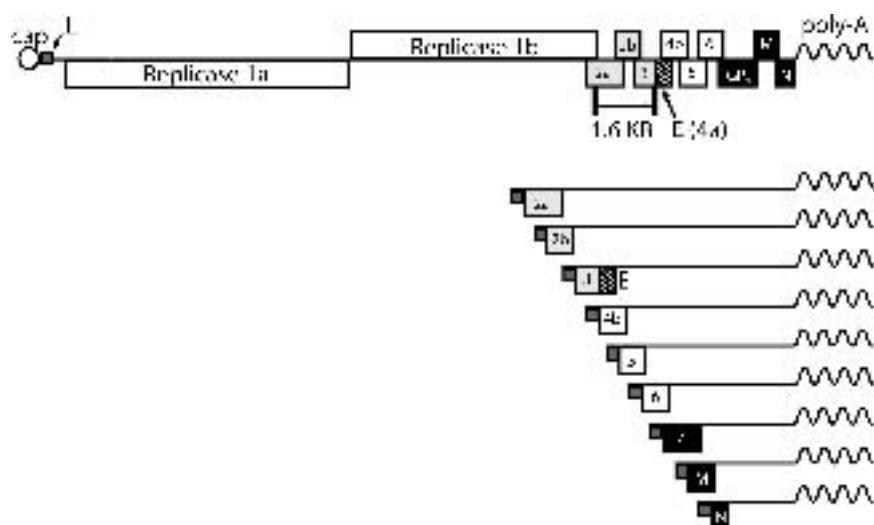


Figure 11.2. Schematic map of SHFV genome and subgenomic RNA transcripts (sgRNAs). Genes encoding major structural proteins are shown in black. Genes shown in light gray (2a, 2b, and 3) are SHFV-specific; that is, they are absent in the genomes of other arteriviruses. Replicase 1a and 1b genes encode nonstructural proteins comprising the viral replication complex. Nested sgRNAs encode structural proteins. These transcripts are 5'- and 3'-co-terminal. Leader sequence (L) at the 5'-end of each sgRNA is derived from the 5'-terminus of the viral genome (shown as small dark gray box).

Table 11.1. Basic Characteristics of SHFV Structural Proteins

Gene/Protein	Protein Size (aa)	Localization	Comments
ORF2a/GP _{2a}	281	Envelope	Minor protein, absent in other arteriviruses
ORF2b/GP _{2b}	204	Envelope	Minor protein, absent in other arteriviruses
ORF3/GP ₃	205	Envelope	Minor protein, absent in other arteriviruses
ORF4a/E	80	Envelope	Minor protein, ion-channel properties
ORF4b/GP ₄	214	Envelope	Minor protein
ORF5/GP ₅	179	Envelope	Minor protein
ORF6/GP ₆	182	Envelope	Minor protein
ORF7/GP ₇	278	Envelope	Major protein, external, p54
ORF8/M	162	Envelope	Major protein, transmembrane; the most conserved among arterivirus proteins
ORF9/N	111	Capsid	Major protein, basic, phosphorylated, RNA-binding

it is not known whether this tropism is mediated by a specific receptor or by a post entry mechanism. Presumably, cellular entry is achieved through the fusion of the viral envelope with the plasma membrane. The mechanism of uncoating is unknown.

The entire SHFV replication process takes place in the cytoplasm. All the viral building blocks, RNA, and proteins are produced in the cytoplasm and nucleocapsids are assembled there. The replicase proteins are translated directly from genomic RNA. Despite the significant distance (209 nt) between the genome cap and the ORF1a initiation codon, translation does not require the internal ribosomal entry site (IRES) and is initiated after conventional ribosomal scanning of the 5'-NTR. Two large precursor polyproteins are synthesized. One polypeptide (pp1a, 2,105 aa) is translated from ORF1a while the second polypeptide (pp1ab, 3,594 aa) is translated from ORFs 1a and 1b using -1 ribosomal frameshift within a slippery sequence located at the junction of ORF1a and ORF1b. These precursor polyproteins undergo autocleavage into individual proteins that comprise the functional replicase/transcriptase complex as well as additional nonstructural proteins—12 or 13 proteins in total. The replicase complex mediates viral RNA synthesis that is operationally divided into “replication” and “transcription,” that is, production of full-length copies of the genomic RNA and subgenomic RNAs (sgRNAs) encoding structural viral proteins.

Copying of the genomic RNA is asymmetrical in that the process is about 100 times more efficient in producing positive-sense, than negative-sense, molecules.

The mechanism of this asymmetrical RNA synthesis is not clear. Newly synthesized full-length positive-sense RNAs are encapsulated in the nucleocapsids as genomic RNAs.

As a result of the subgenomic transcription, nine species of mRNAs encoding structural proteins are produced (Figure 11.2). These transcripts, named subgenomic RNA (sgRNA) are “nested,” in the sense that they include overlapping sequences ending at the same position immediately downstream to ORF-N and differ by the 5'-terminal ORF. All but one of the sgRNAs are structurally polycistronic; however, only the 5'-terminal ORF in each transcript is translated. The mechanism of such selective translation is not clear. The ends of sgRNAs are co-terminal. For the 3'-ends it is obvious, whereas how identical 5'-ends of sgRNAs are formed is not completely clear. Whatever the mechanism, each positive-sense subgenomic RNA contains a 5'-terminal identical sequence named “leader” that is linked upstream to the translationally active ORF named “body.” The leader sequence is copied from the 5'-NTR. The junction of the leader and body of sgRNA includes two short conserved transcription-regulated sequences (TRS), the leader-TRS and the body-TRS. These sequences are required for translation of the structural proteins.

The mechanisms of virion assembly, envelopment, and egress are not known. Envelopment takes place at the membranes of the smooth ER and Golgi apparatus. It is presumed, by analogy with other arteriviruses, that formation of the GP7 and M protein dimers plays a key role in this process.

11.5. INFECTION IN THE NATURAL HOST

SHFV infection is not found in Asian simian species under natural conditions. The main natural host of the virus is the patas monkey (*Erythrocebus patas*). About 10% of recently imported patas monkeys are subclinically infected.²⁰ Data on SHFV infection in other African simian species are fragmentary. Inapparent infection has been detected in about 1% of captive baboons (*Papio anubis*, *P. cynocephalus*) and African green monkeys (*Chlorocebus aethiops*), but not in the common chimpanzee (*Pan troglodytes*).²⁰ However, the possibility of inapparent SHFV infections in other African simian species cannot be excluded.

11.5.1. Variability of SHFV

SHFV strains infecting patas monkeys *in vivo* can be placed into two groups: (a) those causing persistent subclinical infections (strains P-248, P-741); and (b) those causing acute self-limiting infections (strains LVR, P-180).^{9–11}

The biological characteristics of the persistent and acute-type SHFV strains *in vitro* are also different. Persistent strains establish a nonlytic persistent infection in African green monkey MA104 cells and their derivative lines. In contrast, acute strains replicate lytically in MA104 cells. Interestingly, in primary rhesus monkey macrophage cultures, all SHFV strains replicate lytically and their CPE is indistinguishable.^{10,11} Consistent with this observation, in terms of their pathogenicity for rhesus macaques, all SHFV strains are indistinguishable; they all cause fatal hemorrhagic fever.¹⁰

Antigenic differences between SHFV isolates are significant.^{10,21} There is no cross-neutralization between isolates, even those having similar biological properties. Antigens prepared from different isolates react differently with the same anti-SHFV positive sera in ELISA.¹⁰ The genomic sequence variability underlying the differences in biological and antigenic properties of SHFV isolates is not known.

11.6. MODE OF TRANSMISSION

The mode of SHFV transmission in the wild is not known. How SHFV spreads from the patas to rhesus monkeys in captivity is also not completely clear. Transmission does not consistently occur even when patas and rhesus monkeys are housed together in the same cage.²⁰ The SHFV outbreaks in the 1960s–1970s apparently occurred through parenteral exposure of macaques

to SHFV. How SHFV was transmitted to cynomolgus macaques (*M. fascicularis*) imported to the United States from the Philippines in 1989 remains a mystery.¹⁵ These monkeys were kept in the United States in facilities where no African monkeys had been housed for several years. The only logical explanation is that transmission occurred through contact with patas or other African species harboring SHFV en route from the Philippines to the United States (there was a “stop-over” at the Amsterdam airport). However, direct contact could not be documented. It is worth mentioning that the same group of monkeys was coinfected with filovirus (Ebola–Reston strain) (see Chapter 23).

Once transmitted to macaques, SHFV spreads very rapidly in the contaminated colony (within 1–2 days). Transmission occurs by direct or indirect contact. Fortunately, SHFV is not transmitted by the airborne route.

11.7. DISEASE PRESENTATION AND PATHOGENESIS

SHFV is highly pathogenic for macaques of different species (*M. mulatta*, *M. fascicularis*, *M. assamensis*, and *M. nemestrina*), the infection invariably results in a fatal hemorrhagic fever.^{2,16,17,20,25,27–29}

The incubation period of experimentally induced disease is 3–7 days²⁰ and the onset of clinical disease is rapid. Infected macaques stop eating and become depressed and lethargic. Within hours of fever, facial edema, and cyanosis development, the animals become too weak to stand. Severe dehydration, multiple skin hemorrhages, nosebleeds, and bloody diarrhea are the most typical clinical manifestations. Retrobulbar hemorrhage is also common.^{2,17,20,25,27,28} Routine clinical pathology tests show profound thrombocytopenia and a proteinuria.^{1,34} Most deaths occur 7–13 days after the onset of the disease; however, fatalities are observed starting from the third day of the illness. Multiple external and internal hemorrhages, hemorrhagic necrosis of proximal small intestine, intravascular coagulation, and generalized necrosis of lymphoid tissues are observed on autopsy.¹⁶

Little is known about the pathogenesis of SHFV-induced hemorrhagic fever. The main target for the virus is macrophages.¹² Presumably, a major factor in the disease pathogenesis is a massive release of cytokines from infected macrophages, the “cytokine storm.” However, no concrete data regarding this pathogenic mechanism are available.

11.7.1. Nonfatal SHFV Disease in Patas Monkeys

The vast majority of SHFV-positive patas monkeys do not exhibit any clinical manifestations. However, some SHFV strains, such as LVR and P-180, induce an acute, nonfatal disease in patas monkeys. The disease is manifested by mild subcutaneous hemorrhages, facial edema, and dehydration. However, in contrast to the full-blown SHFV hemorrhagic fever observed in macaques, patas monkeys clear the virus and their clinical recovery occurs by day 21 after inoculation.⁹

11.8. IMMUNE RESPONSE

The course of the disease in macaques is so rapid that there is no time for the development of a protective adaptive immune response.

Infection of patas monkeys with SHFV strains that induce subclinical infections is not accompanied by the production of neutralizing antibodies. Many SHFV-infected patas monkeys do not even have detectable levels of anti-SHFV antibodies.^{9,12}

In patas monkeys experimentally infected with the patas-pathogenic SHFV strains, neutralizing antibodies become detectable at day 7 after inoculation. The increase in titer of neutralizing antibodies correlates well with clinical recovery and clearance of the virus.

Nothing is known about cellular immune responses to SHFV. As mentioned, the proinflammatory cytokines, interleukins, and other factors of innate immunity are likely to be engaged in the pathogenesis of this hemorrhagic fever. However, relevant experimental data are absent.

11.9. DIAGNOSIS

There is no simple method for identifying SHFV carriers. The most reliable test is inoculation of macaques with sera from a presumptive carrier. Obviously, this test is not practical.

A positive anti-SHFV antibody test in an African monkey that was not experimentally infected with SHFV should be interpreted as evidence that the animal is persistently infected. It is not known how long monkeys remain seropositive after clearing the virus. Even if a test for anti-SHFV antibodies is negative, a firm conclusion regarding SHFV-status is not possible because many inapparent carriers of the virus are seronegative.

Virus isolation from an SHFV carrier is also informative only if the result is positive. Unfortunately, negative results for virus isolation in tissue culture from sam-

ples proven to be positive for infectious and pathogenic SHFV, by in vivo testing, are not uncommon.²⁴

Isolation of SHFV is usually performed on MA-104 cells. These cells are permissive for most but not all SHFV strains.¹⁰ Primary rhesus monkey macrophage cultures derived from peritoneal macrophages are permissive for all known SHFV strains. However, establishing such cultures is a laborious process and the cultures can be maintained for a short time only.

SHFV is highly cytocidal in permissive cells. Viral CPE is manifested as rounding of the cells followed by their detachment within 2 days after inoculation. Virus titer in permissive cell cultures reaches 10^8 PFU/mL. SHFV virions are very labile and the virus quickly loses infectivity at temperatures of +4°C or higher.

Although RT-PCR tests have been used extensively for studying SHFV genome structure and gene expression, the diagnostic utility of these tests is not known. It remains to be determined how early during the course of SHFV infection that viral RNA becomes detectable, and which regions of the SHFV genome are sufficiently conserved to ensure detection of all SHFV strains.

The most commonly used test for detection of anti-SHFV antibodies is the immunofluorescence assay.¹² The problem of high background plagued attempts to develop an ELISA for SHFV. This problem was solved by using fish gelatin instead of milk proteins as a blocking agent.⁵ The latest version of ELISA for detection of anti-SHFV antibodies uses a lysate of virus purified by isopycnic ultracentrifugation. This assay is as specific as the immunofluorescence test and, most probably, more sensitive. However, it is not known how this test performs in detecting antibodies against different SHFV strains. Standardization may also pose a problem due to significant batch-to-batch variability of the gradient-purified virus preparations.

11.10. PREVENTION AND TREATMENT

The main prophylactic measure is prevention of contact between patas monkeys and macaques. Isolation of macaques from all African nonhuman primates (NHPs) is essential.

Intriguing results showing the possibility of eradicating SHFV from persistently infected patas monkeys have been reported.⁹ The virus was completely cleared in 20 SHFV carriers within 3 months after superinfection with SHFV strains that cause acute non-fatal SHFV disease in patas monkeys (Section 11.7.1).

Unfortunately, this interesting finding has not been explored further.

Animals recently exposed to SHFV can be prophylactically treated with interferon inducers. Poly-riI/poly-rC containing poly-L-lysine and carboxymethylcellulose has been shown to be effective in preventing disease in macaques, if administered before the onset of clinical signs. However, the drug is ineffective if the disease is already manifested.¹⁹ No specific treatment for SHFV-induced hemorrhagic fever is available.

11.11. SUMMARY

SHFV is an enveloped RNA virus with a single-stranded, nonsegmented genome of positive polarity. It is the only known primate arterivirus. SHFV replicates in vitro and is cytocidal for primary macrophage cultures of susceptible simian species and some permanent cell lines of NHP origin.

The primary natural host of SHFV is the patas monkey (*Erythrocebus patas*), although the virus may also be present in other African simian species. Natural SHFV infection in African NHPs is generally subclinical.

Transmission of SHFV to macaques, either experimental or accidental, results in development of acute hemorrhagic fever which is invariably fatal. A number of outbreaks of this disease in captive macaque colonies have been reported. There is no specific treatment for SHFV hemorrhagic fever. However, interferon inducers are effective in prevention of SHFV hemorrhagic fever if a prophylactic course is started before the onset of clinical manifestations. The main prophylactic measure against SHFV hemorrhagic fever is preventing contact between macaques and patas monkeys.

REFERENCES

1. Abildgaard, C., J. Harrison, C. Espana, W. Spangler, and D. Gribble. 1975. Simian hemorrhagic fever: studies of coagulation and pathology. *Am. J. Trop. Med. Hyg.* 24(3):537–544.
2. Allen, A. M., A. E. Palmer, N. M. Tauraso, and A. Shelokov. 1968. Simian hemorrhagic fever. II. Studies in pathology. *Am. J. Trop. Med. Hyg.* 17(3):413–421.
3. Dalgard, D. W., R. J. Hardy, S. L. Pearson, G. J. Pucak, R. V. Quander, P. M. Zack, C. J. Peters, and P. B. Jahrling. 1992. Combined simian hemorrhagic fever and Ebola virus infection in cynomolgus monkeys. *Lab. Anim. Sci.* 42(2):152–157.
4. Espana, C. 1971. Review of some outbreaks of viral disease in captive nonhuman primates. *Lab. Anim. Sci.* 21(6):1023–1031.
5. Godeny, E. K. 2002. Enzyme-linked immunosorbent assay for detection of antibodies against simian hemorrhagic fever virus. *Comp. Med.* 52(3):229–232.
6. Godeny, E. K., A. A. de Vries, X. C. Wang, S. L. Smith, and R. J. de Groot. 1998. Identification of the leader-body junctions for the viral subgenomic mRNAs and organization of the simian hemorrhagic fever virus genome: evidence for gene duplication during arterivirus evolution. *J. Virol.* 72(1):862–867.
7. Godeny, E. K., L. Zeng, S. L. Smith, and M. A. Brinton. 1995. Molecular characterization of the 3' terminus of the simian hemorrhagic fever virus genome. *J. Virol.* 69(4):2679–2683.
8. Gorbalenya, A. E., L. Enjuanes, J. Ziebuhr, and E. J. Snijder. 2006. Nidovirales: evolving the largest RNA virus genome. *Virus Res.* 117(1):17–37.
9. Gravell, M., W. T. London, M. Leon, A. E. Palmer, and R. S. Hamilton. 1986. Elimination of persistent simian hemorrhagic fever (SHF) virus infection in patas monkeys. *Proc. Soc. Exp. Biol. Med.* 181(2):219–225.
10. Gravell, M., W. T. London, M. E. Leon, A. E. Palmer, and R. S. Hamilton. 1986. Differences among isolates of simian hemorrhagic fever (SHF) virus. *Proc. Soc. Exp. Biol. Med.* 181(1):112–119.
11. Gravell, M., W. T. London, M. Rodriguez, A. E. Palmer, and R. S. Hamilton. 1980. Simian haemorrhagic fever (SHF): new virus isolate from a chronically infected patas monkey. *J. Gen. Virol.* 51(Pt 1):99–106.
12. Gravell, M., A. E. Palmer, M. Rodriguez, W. T. London, and R. S. Hamilton. 1980. Method to detect asymptomatic carriers of simian hemorrhagic fever virus. *Lab. Anim. Sci.* 30(6):988–991.
13. Hwang, Y. K. and M. A. Brinton. 1998. Cell proteins bind to a 67 nucleotide sequence within the 3' noncoding region (NCR) of simian hemorrhagic fever virus (SHFV) negative-strand RNA. *Adv. Exp. Med. Biol.* 440:235–240.
14. Hwang, Y. K. and M. A. Brinton. 1998. A 68-nucleotide sequence within the 3' noncoding region of simian hemorrhagic fever virus negative-strand RNA binds to four MA104 cell proteins. *J. Virol.* 72(5):4341–4351.
15. Jahrling, P. B., T. W. Geisbert, D. W. Dalgard, E. D. Johnson, T. G. Ksiazek, W. C. Hall, and C. J. Peters. 1990. Preliminary report: isolation of Ebola virus from monkeys imported to USA. *Lancet* 335(8688):502–505.

16. Krylova, R. I. and Z. V. Shevtsova. 1969. [Pathomorphology of experimental hemorrhagic fever in monkeys]. *Arkh. Patol.* 31(8):65–69.
17. Lapin, B. A., S. M. Pekerman, L. A. Iakovleva, E. K. Dzhikidze, Z. V. Shevtsova, M. I. Kuksova, L. V. Dan'ko, R. I. Krylova, E. I. Akbroit, and V. Z. Agrba. 1967. [Hemorrhagic fever in monkeys]. *Vopr. Virusol.* 12(2):168–173.
18. Lapin, B. A. and Z. V. Shevtsova. 1971. On the identity of two simian hemorrhagic fever virus strains (Sukhumi and NIH). *Z. Versuchstierkd.* 13(1):21–23.
19. Levy, H. B., W. London, D. A. Fuccillo, S. Baron, and J. Rice. 1976. Prophylactic control of simian hemorrhagic fever in monkeys by an interferon inducer, polyriboinosinic-polyribocytidylic acid-poly-L-lysine. *J. Infect. Dis.* 133(Suppl):A256–A259.
20. London, W. T. 1977. Epizootiology, transmission and approach to prevention of fatal simian haemorrhagic fever in rhesus monkeys. *Nature* 268(5618):344–345.
21. Madden, D. L., D. A. Fuccillo, J. A. Dorosz, W. T. London, A. E. Palmer, and G. A. Castellano. 1978. Antigenic relationship of two strains of simian hemorrhagic fever virus. *Lab. Anim. Sci.* 28(4):422–427.
22. Maines, T. R. and M. A. Brinton. 2001. Identification of cell proteins that bind to the SHFV 3' (+)NCR. *Adv. Exp. Med. Biol.* 494:647–653.
23. Maines, T. R., M. Young, N. N. Dinh, and M. A. Brinton. 2005. Two cellular proteins that interact with a stem loop in the simian hemorrhagic fever virus 3' (+)NCR RNA. *Virus Res.* 109(2):109–124.
24. Myers, M. G., M. M. Vincent, S. A. Hensen, and N. M. Tauraso. 1972. Problems in the laboratory isolation of simian hemorrhagic fever viruses and isolate metabolite enterobacteriaceae classification enterobacteriaceae enzymology hydrogen peroxide metabolism enterobacteriaceae metabolism of the agent responsible for the Sussex-69 epizootic. *Appl. Microbiol.* 24(1):62–69.
25. Palmer, A. E., A. M. Allen, N. M. Tauraso, and A. Shelokov. 1968. Simian hemorrhagic fever. I. Clinical and epizootiologic aspects of an outbreak among quarantined monkeys. *Am. J. Trop. Med. Hyg.* 17(3):404–412.
26. Sagripanti, J. L., R. O. Zandomeni, and R. Weinmann. 1986. The cap structure of simian hemorrhagic fever virion RNA. *Virology* 151(1):146–150.
27. Shevtsova, Z. V. 1967. [Study of the etiology of hemorrhagic fever in monkeys]. *Vopr. Virusol.* 12(1):47–51.
28. Shevtsova, Z. V. 1969. [A further study of simian hemorrhagic fever virus]. *Vopr. Virusol.* 14(5):604–607.
29. Shevtsova, Z. V. and R. I. Krylova. 1971. [A comparative study of 2 strains of simian hemorrhagic fever virus]. *Vopr. Virusol.* 16(6):686–688.
30. Smith, S. L., X. Wang, and E. K. Godeny. 1997. Sequence of the 3' end of the simian hemorrhagic fever virus genome. *Gene* 191(2):205–210.
31. Snijder, E. J. and J. J. Meulenberg. 1998. The molecular biology of arteriviruses. *J. Gen. Virol.* 79(Pt 5):961–979.
32. Tauraso, N. M., A. Shelokov, A. E. Palmer, and A. M. Allen. 1968. Simian hemorrhagic fever. 3. Isolation and characterization of a viral agent. *Am. J. Trop. Med. Hyg.* 17(3):422–431.
33. Trousdale, M. D., D. W. Trent, and A. Shelokov. 1975. Simian hemorrhagic fever virus: a new togavirus. *Proc. Soc. Exp. Biol. Med.* 150(3):707–711.
34. Uvarova, V. I. and Z. V. Shevtsova. 1976. [Study of the hemostasis system in hemorrhagic fever of macaques]. *Patol. Fiziol. Eksp. Ter.* 1:64–66.
35. Wang, X. C., S. L. Smith, and E. K. Godeny. 1998. Organization of the simian hemorrhagic fever virus genome and identification of the sgRNA junction sequences. *Adv. Exp. Med. Biol.* 440:281–287.
36. Zeng, L., E. K. Godeny, S. L. Methven, and M. A. Brinton. 1995. Analysis of simian hemorrhagic fever virus (SHFV) subgenomic RNAs, junction sequences, and 5' leader. *Virology* 207(2):543–548.

Section 2:

DNA Viruses

Section 2.1:

Simian Herpesviruses

Introduction to Herpesviruses

VIRION ARCHITECTURE

Characteristic morphology is the defining feature of herpesviruses; they are virtually indistinguishable under the electron microscope. Three major components of herpes virions are the capsid, tegument, and envelope (Figure HI.1). The icosahedral capsid is made up of 162 structural units (capsomers) which are clearly visible in negatively stained preparations. There are two types of capsomers: pentamers and hexamers. The pentamers are composed of five molecules of the major capsid protein and are surrounded by five neighbor capsomers—hence their name. Each of 12 vertices of the icosahedral capsid is occupied by a pentamer. Eleven pentamers are identical; a unique pentamer comprises the portal complex, a machinery presumably involved in the loading/unloading of genomic DNA. The hexamers, as their name suggests, are composed of six molecules of the major capsid protein, and each hexamer is surrounded by six neighbor capsomers. There are 150 hexamers in the herpesviral capsid. Genomic DNA and associated viral proteins are packed inside the capsid, forming an electron-dense structure of toroid shape named the core. Genome-loaded capsids are named nucleocapsids.

The nucleocapsid is surrounded by the tegument, a protein layer that looks “amorphous” under the electron microscope. The “tegumented” nucleocapsid is enclosed in the envelope, a lipid bilayer membrane with the viral glycoproteins embedded in it.

CLASSIFICATION AND NOMENCLATURE

Until recently, all herpesviruses were included in the family *Herpesviridae*. In May 2008, the International Committee on Taxonomy of Viruses (ICTV) approved a proposal to elevate the highest herpesviral taxon to the order level. The order *Herpesvirales* includes several herpesvirus families; the *Herpesviridae* is one of them. However, the taxonomic position of the mam-

malian herpesviruses did not change; as before they are still included in the *Herpesviridae* family.

The *Herpesviridae* family is divided into three subfamilies: *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*. Remarkably, this α - β - γ trichotomy, which was introduced in 1981 based on in vivo biological properties of the viruses, has withstood scrutiny based on genomic sequence comparisons. Phylogenetic analysis of herpesviral genomes confirmed the existence of three monophyletic groups of herpesviruses corresponding to the classical alpha-, beta-, and gamma-herpesviruses (Figure HI.2). These major groups are not equidistant; beta- and gamma-herpesviruses are closer to each other than to the alpha-herpesviruses.

Known simian herpesviruses belong to five genera: *Simplexvirus* and *Varicellavirus* (*Alphaherpesvirinae*), *Cytomegalovirus* (*Betaherpesvirinae*), *Lymphocryptovirus*, and *Radinovirus* (*Gammaherpesvirinae*). The list of the ICTV-recognized simian herpesvirus species and their human prototypes is presented in Table HI.1.

The nomenclature of simian herpesvirus is perplexing. Names of simian herpesviruses which had been official before May 2008 included the name of the host subfamily, or genus, written as an adjective followed by the word “herpesvirus” and the number. For instance, herpesviruses from monkeys belonging to the *Cercopithecinae* subfamily had been named *Cercopithecine herpesvirus* 1, 2, and so on (CeHV-1, CeHV-2, etc.). When this nomenclature was introduced, it was presumed that the numbering of simian herpesvirus species would be consecutive in order of their discovery and description. However, over the years some simian herpesvirus species turned out to be redundant and were “declassified.” As a result, gaps appeared in the numbering of the simian herpesviruses, for example, CeHV-3, -4, -6, -7, and -11 are absent in the latest version of the ICTV classification (8th Report). In addition, host taxonomic affiliation in some designations was mistaken, for example, chimpanzee viruses (host genus *Pan*) were

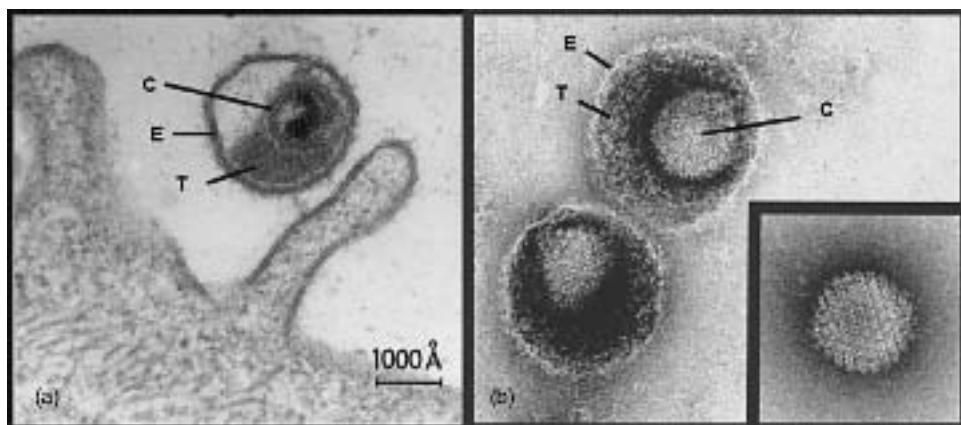


Figure HI.1. Major components of a herpesvirus virion. E, envelope, T, tegument, C, capsid. (a) Thin section of herpesvirus virion; the electron-dense (dark) structure inside the capsid is the core. The core contains the genomic DNA. The capsid “loaded” with the core is called the nucleocapsid. (Image of herpes simplex virus type 1 is kindly provided by Prof. Hans R. Gelderblom.). (b) Herpesvirus virion; inset—naked herpesvirus capsid or nucleocapsid (negative staining). The capsid structural units, the capsomers, are clearly visible. Stain does not penetrate the capsids and their inner structure is not revealed. These capsids may contain genomic DNA and would be named nucleocapsids. (Image of herpes simplex virus type 1 is kindly provided by Prof. Guy A. Cabral.)

designated as *Pongine herpesvirus* 1, 4 (the *Pongo* genus includes orangutan species).

The 2008 overhaul of the classification of herpesviruses includes changes in the names of simian herpesviruses. The inconsistency and mistakes in host designations have been corrected: new names indicate the natural host at the level of the genus. For example, baboon herpesviruses (host genus *Papio*) are now named *Papiine herpesvirus*; the viruses from macaques (host genus *Macaca*) are named *Macacine herpesvirus* and so forth. New nomenclature makes virus names more informative. Also, the “numerical row” of CeHV-names is now substantially shortened. For example, the old ICTV name *Cercopithecine herpesvirus* 16 (CeHV-16) is replaced with the name *Papiine herpesvirus* 2 (PaHV-2). However, new ICTV names of simian herpesviruses still lack information on the viral genus, for example, simplexviruses, cytomegalovirus, lymphocryptoviruses, as well as on the host species of origin. Transition from old to new ICTV names is also unlikely to be smooth, with older names persisting in the literature for some time to come.

“Unofficial” names of simian herpesviruses are more commonly used than the official ICTV designations. To an extent this is a tribute to tradition. Perhaps more importantly, common names are usually more informative than the official names [compare: Rhesus lymphocryptovirus (Rh-LCV) and *Cercopithecine herpesvirus* 15 (CeHV-15)]. In this book, we have tried to follow the ICTV nomenclature as much as possible. However, for the sake of clarity, common names are used in covering cytomegaloviruses, lymphocryptoviruses, and rhadinoviruses (see Chapters 16, 17, and 18).

GENOME, GENE PRODUCTS, AND EXPRESSION PATTERNS

All herpesviruses have a large (110–240 kb, 70–165 genes) double-stranded DNA genome which is linear in the virions. There are three types of “macro” elements in the genomes of herpesviruses: the unique (i.e., nonrepeated) sequences (U), internal repeats (IRs), and terminal repeats (TRs). The genome structure is similar for herpesviruses belonging to the same genus, but

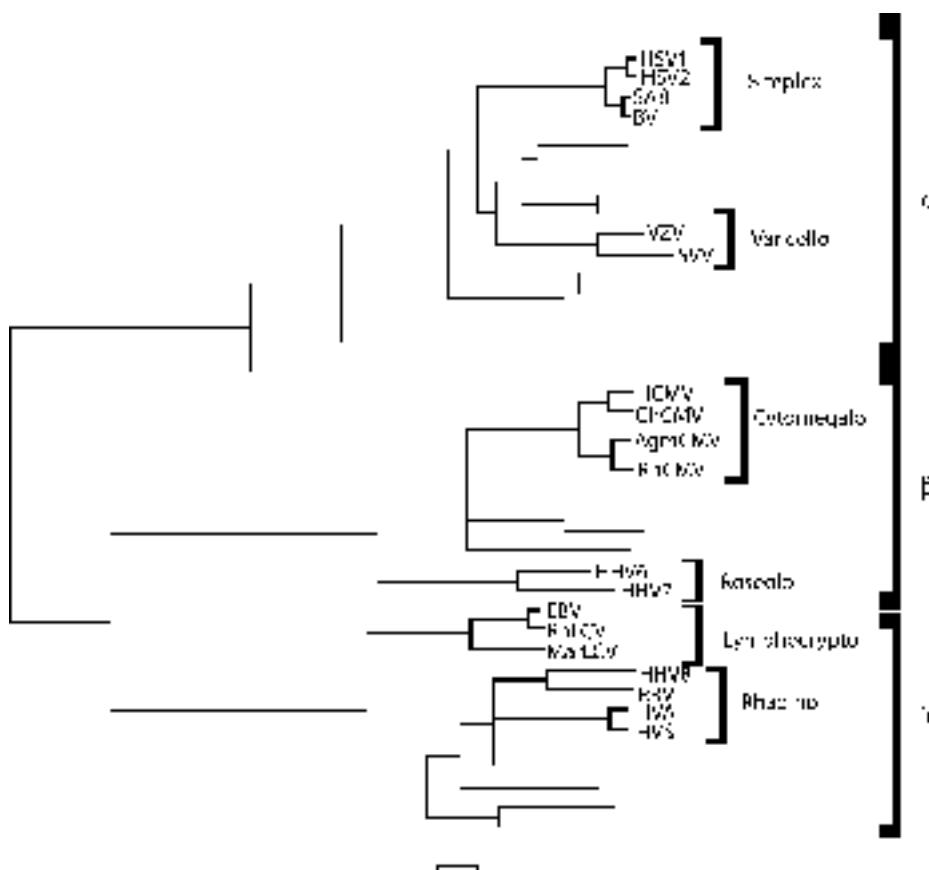


Figure HI.2. Phylogeny of herpesviruses. Phylogenetic tree was constructed based on concatenated sequences of six genes from 40 herpesvirus genomes. Only primate herpesviruses are shown; branches of human and simian viruses are shown bold. ICTV species names of simian herpesviruses are in brackets. α , β , γ —subfamilies; simplex, varicello, cytomegalo, roseolo, lymphocrypto, rhadino—genera. Simplexviruses: HSV1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; SA8, Simian agent 8 (*Cercopithecine herpesvirus* 2); BV, B virus (*Macacine herpesvirus* 1). Varicelloviruses: VZV, varicella-zoster virus; SVV, simian varicella virus (*Cercopithecine herpesvirus* 9). Cytomegaloviruses: HCMV, human cytomegalovirus (CMV); ChCMV, chimpanzee CMV (*Panine herpesvirus* 2); AgmCMV, African green monkey CMV (*Cercopithecine herpesvirus* 5); RhCMV, rhesus monkey CMV (*Macacine herpesvirus* 3). Roseoloviruses: HHV6, human herpesvirus type 6; HHV7, human herpesvirus type 7. Lymphocryptoviruses: EBV, Epstein-Barr virus; RhLCV, rhesus lymphocryptovirus (*Macacine herpesvirus* 4); MarLCV, marmoset lymphocryptovirus (*Callitrichine herpesvirus* 3). Rhadinoviruses: HHV8, human herpesvirus type 8; RRV, rhesus rhadinovirus (*Macacine herpesvirus* 5); HVA, herpesvirus atelis (*Ateline herpesvirus* 2 and *Ateline herpesvirus* 3); HVS, herpesvirus saimiri (*Saimiriine herpesvirus* 2). (Adapted from McGeoch *et al.* *Virus Research* 117, pp. 90–104, 2006, with permission.)

Table HI.1 Simian Herpesvirus Species and Their Human Prototypes

Genus	Human Prototype	Old ICTV Name	New ICTV Name	Most Frequently Used Common Name
Simplexvirus				
Herpes simplex virus 1 and 2 (HSV-1 and HSV-2)	<i>Cercopithecine herpesvirus 1</i> (CeHV-1)	<i>Macacine herpesvirus 1</i> (McHV-1)		B virus (BV)
	<i>Cercopithecine herpesvirus 2</i> (CeHV-2)	<i>Cercopithecine herpesvirus 2</i> (CeHV-2)		Simian agent 8 (SA8)
	<i>Cercopithecine herpesvirus 16</i> (CeHV-16)	<i>Papiine herpesvirus 2</i> (PaHV-2)		Herpesvirus papio 2 (HVP-2)
	<i>Saimiriine herpesvirus 1</i> (SaHV-1)	<i>Saimiriine herpesvirus 1</i> (SaHV-1)		Herpesvirus saimiri 1 (HVS-1)
	<i>Ateline herpesvirus 1</i> (AtHV-1)	<i>Ateline herpesvirus 1</i> (AtHV-1)		Herpesvirus atelles 1 (HVA-1)
Varicellovirus				
Varicella-zoster virus (VZV)	<i>Cercopithecine herpesvirus 9</i> (CeHV-9)	<i>Cercopithecine herpesvirus 9</i> (CeHV-9)		Simian varicella virus (SVV)
Cytomegalovirus				
Human cytomegalovirus (CMV or HCMV)	<i>Cercopithecine herpesvirus 8</i> (CeHV-8)	<i>Macacine herpesvirus 3</i> (McHV-3)		Rhesus monkey cytomegalovirus (RhCMV)
	<i>Cercopithecine herpesvirus 5</i> (CeHV-5)	<i>Cercopithecine herpesvirus 5</i> (CeHV-5)		African green monkey cytomegalovirus (AgmCMV)
	<i>Pongine herpesvirus 4</i> (PoHV-4)	<i>Panine herpesvirus 2</i> (PnHV-2)		Chimpanzee cytomegalovirus (ChCMV or CCMV)
Lymphocryptovirus				
Epstein-Barr virus (EBV)	<i>Cercopithecine herpesvirus 12</i> (CeHV-12)	<i>Papiine herpesvirus 1</i> (PaHV-1)		Herpesvirus papio (HVP)
	<i>Cercopithecine herpesvirus 14</i> (CeHV-14)	<i>Cercopithecine herpesvirus 14</i> (CeHV-14)		African green monkey LCV
	<i>Cercopithecine herpesvirus 15</i> (CeHV-15)	<i>Macacine herpesvirus 4</i> (McHV-4)		Rhesus LCV
	<i>Pongine herpesvirus 1</i> (PoHV-1)	<i>Panine herpesvirus 1</i> (PnHV-1)		Chimpanzee LCV
	<i>Pongine herpesvirus 2</i> (PoHV-2)	<i>Pongine herpesvirus 2</i> (PoHV-2)		Orangutan LCV
	<i>Pongine herpesvirus 3</i> (PoHV-3)	<i>Gorilline herpesvirus 1</i> (GoHV-3)		Gorilla LCV
	<i>Callitrichine herpesvirus 3</i> (Ca1HV-3)	<i>Callitrichine herpesvirus 3</i> (Ca1HV-3)		Marmoset LCV
Rhadinovirus				
Human herpesvirus 8 (HHV-8) or Kaposi sarcoma herpesvirus (KSHV)	<i>Cercopithecine herpesvirus 17</i> (CeHV-17)	<i>Macacine herpesvirus 5</i> (McHV-5)		Rhesus rhadinovirus (RRV)
	<i>Saimiriine herpesvirus 2</i> (SaHV-2)	<i>Saimiriine herpesvirus 2</i> (SaHV-2)		Herpesvirus saimiri (HVS)
	<i>Ateline herpesvirus 2</i> (AtHV-2)	<i>Ateline herpesvirus 2</i> (AtHV-2)		Herpesvirus atelles (HVA)
		<i>Ateline herpesvirus 3*</i> (AtHV-3)		Herpesvirus atelles (HVA)

*New simian rhadinovirus species.

quite different between the genera. Both strands of the genomic DNA are coding; however, in each strand the coding sequences are not continuous over the whole length. Genomic regions where both strands are coding are common. Most genes consist of a single open reading frame (ORF); the proportion of spliced genes, that is, those which have several exons, is relatively small (from a few to 10%). Most herpesvirus genes have a promoter and a transcription initiation site located 50–200 bp upstream and 20–25 bp downstream of a TATA box, respectively. Apart from the protein-coding genes there are also genes encoding RNAs which are not translated. The most notable examples of such RNAs are the latency-associated transcripts (LATs) of the simplexviruses and EBER-RNAs of the lymphocryptoviruses. MicroRNA species are also encoded at least in some simian herpesviral genomes.

About 40 genes are well conserved in all herpesviruses (Table HI.2). These “core genes” are largely located in the central part of the genome and are arranged in blocks. Within each block the order of genes and their polarity are conserved. However, the arrangement of the core gene blocks is similar only in viruses belonging to the same genus. Most of the core genes encode proteins essential for lytic replication.

A characteristic feature of herpesvirus genomes is the presence of genes acquired from the host. Such genes are particularly numerous in the genome of rhabdoviruses. The acquired genes tend to be located in the terminal regions of the genome. The proteins encoded by these genes may mimic function of their cellular counterparts and facilitate viral evasion from the immune response or other host defenses.

Herpesvirus genes are transcribed by RNA polymerase II. Transcription by RNA polymerase III is exceptional (e.g., the transcription of EBER RNAs). Transcription of some genes is regulated by the use of different promoters.

The patterns of gene expression are different in productively and latently infected cells. Depending on the stage of the viral replicative cycle and type of the virus-host interaction, various subsets of genes are expressed in a sophisticatedly coordinated manner. During lytic infection, genes are expressed in consecutive cascades: the immediate-early, early, and late. The expression of immediate-early and early genes precedes replication of genomic DNA. Protein products of the early genes are primarily nonstructural proteins required for DNA replication and activation of the late gene expression.

The products of late genes, mostly structural proteins, are required for assembly of virions. The assembly of capsids and nucleocapsids as well as coating with the tegument takes place in the nucleus. The acquisition of the envelope is a complex process which is not fully understood. Available data appear to be best explained by the “double envelopment” model. According to this model the nucleocapsids first acquire a “temporary” envelope by budding at the inner nuclear membrane. This provisional envelope is “stripped off” during egress of the “previrions” from the nucleus, while the tegument is retained. Then these “partly stripped” (tegumented) nucleocapsids are transported into the Golgi and endosome compartments. During entry into these compartments they acquire their final envelope by budding at the Golgi apparatus and endosomal membranes (Figure HI.3).

LATENCY

Herpesviruses have developed a very successful survival strategy: long-term “peaceful coexistence” with their natural host. A key component of this strategy is the ability to establish latency and to reactivate from latency. True latency, that is, almost complete and reversible suppression of viral genome expression, exists in individual cells. The type of cells in which the virus stays “dormant,” and the route used by the virus to reach the site of latency, are characteristic of different groups of herpesviruses. For example, the alpha-herpesviruses travel through axons to neuronal bodies and stay latent in the neurons of sensory ganglia.

Once the virus is “seeded” in the latency site the infection is lifelong. However, it is rare that an infected carrier of a herpesvirus has only latently infected cells. Reactivation and subsequent lytic replication is always occurring in a proportion of latently infected cells. Normally, the balance between reactivation and latency is shifted toward dominance of the latent infection. Usually, such persistent infection is inapparent. However, it is not uncommon that reactivation occurs simultaneously in a significantly larger than normal proportion of latently infected cells. On the one hand, such “synchronized” reactivations are beneficial for survival of the virus as species because it increases the probability of host-to-host transmission of the virus. On the other hand, reactivation may tip the balance between the virus and host defenses toward the development of disease.

Table HI.2 Core Genes of Herpesviruses and Their Products

Gene	Product/Location/Function
DNA replication machinery	
<i>UL30</i>	Catalytic subunit of DNA polymerase complex
<i>UL42</i>	Processivity subunit of DNA polymerase complex
<i>UL9</i>	Origin-binding protein; helicase*
<i>UL5</i>	Component of DNA helicase–primase complex; helicase
<i>UL8</i>	Component of DNA helicase–primase complex
<i>UL52</i>	Component of DNA helicase–primase complex; primase
<i>UL29</i>	Single-stranded DNA-binding protein
Enzyme peripheral to DNA replication	
<i>UL23</i>	Thymidine (or pyrimidine deoxynucleoside) kinase†
<i>UL39</i>	Large subunit of ribonucleotide reductase‡
<i>UL40</i>	Small subunit of ribonucleotide reductase§
<i>UL50</i>	Deoxyuridine triphosphatase¶
<i>UL2</i>	Uracil–DNA glycosylase
Processing and packaging of DNA	
<i>UL12</i>	Deoxyribonuclease; role in DNA maturation and recombination
<i>UL15</i>	Putative ATPase subunit of terminase; capsid-associated
<i>UL28</i>	Putative subunit of terminase; capsid-associated
<i>UL6</i>	Portal protein; forms dodecameric ring at capsid vertex
Complexed with terminase	
<i>UL25</i>	Possibly caps the portal after DNA packaging is complete
<i>UL32</i>	Involved in capsid localization in the nucleus
<i>UL33</i>	Interacts with terminase
<i>UL17</i>	Involved in capsid localization in the nucleus
Egress of capsids from nucleus	
<i>UL31</i>	Nuclear matrix protein; component of capsid docking complex on nuclear membrane
<i>UL34</i>	Inner nuclear membrane protein; component of capsid docking complex on nuclear lamina
Control of gene expression	
<i>UL54</i>	Multifunctional regulator of gene expression
Capsid assembly and structure	
<i>UL19</i>	Major capsid protein; components of hexons and pentons
<i>UL18</i>	Component of intercapsomeric triplex between hexons and pentons
<i>UL38</i>	Component of intercapsomeric triplex between hexons and pentons
<i>UL35</i>	Small capsid protein located on tips of hexons
<i>UL26</i>	Maturation protease; generates mature forms of scaffolding proteins
<i>UL26.5</i>	Scaffolding protein removed from capsid during DNA packaging
Tegument	
<i>UL7</i>	Unknown
<i>UL11</i>	Interacts with UL16 protein; role in virion egress and secondary envelopment in the cytoplasm; myristylated and palmitylated protein
<i>UL13</i>	Serine–threonine protein kinase
<i>UL14</i>	Interacts with UL11 protein
<i>UL16</i>	Interacts with UL11 protein
<i>UL36</i>	The largest virion protein; interacts with UL37 protein; influences release of DNA from capsids during entry
<i>UL37</i>	Interacts with UL36 protein
<i>UL51</i>	Unknown
<i>UL24</i>	Unknown**

Table HI.2 Core Genes of Herpesviruses and Their Products

Gene	Product/Location/Function
Envelope	
<i>UL27</i>	Glycoprotein B
<i>UL1</i>	Glycoprotein L; complexed with glycoprotein H
<i>UL22</i>	Glycoprotein H; complexed with glycoprotein L
<i>UL10</i>	Glycoprotein M; complexed with glycoprotein N
<i>UL49 A</i>	Glycoprotein N; complexed with glycoprotein M ^{††}

*Present in subfamily *Alphaherpesvirinae* and genus *Roseolovirus* of subfamily *Betaherpesvirinae*.

†Present in subfamilies *Alpha-* and *Gammaherpesvirinae*.

‡Probably not an active enzyme in subfamily *Betaherpesvirinae*.

§Present in subfamilies *Alpha-* and *Gammaherpesvirinae*.

¶Not an active enzyme in subfamily *Betaherpesvirinae*.

**Presumptive tegument protein.

††Not glycosylated in some herpesviruses.

Genes shown in italic are essential for growth in cell culture. Adapted from McGeoch *et al.*, *Virus Research* 117, pp. 90–104, 2006, with permission.

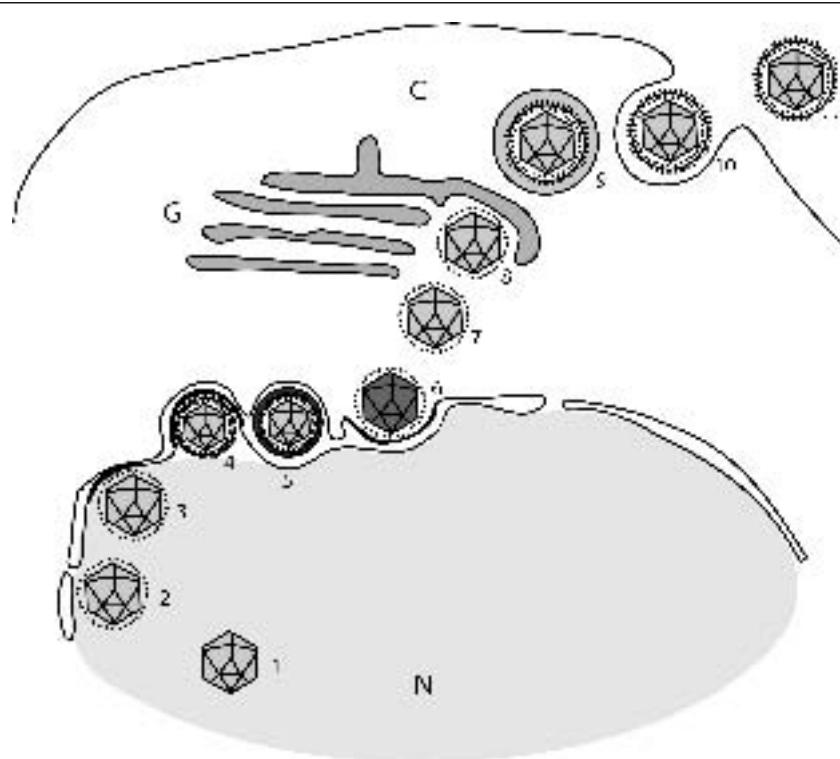


Figure HI.3. Scheme of herpes double envelopment. N, nucleus; C, cytoplasm; G, Golgi apparatus. 1, nucleocapsids are assembled in the nucleus; 2, nucleocapsids are tegumented, that is, covered with the tegument proteins and are transported to the nuclear membrane; 3–5, tegumented nucleocapsids acquire “temporary” envelope by budding at the inner nuclear membrane; 6, during egress from the nucleus, the temporary envelope is “stripped off” and tegumented nucleocapsids are released in the cytoplasm; 7, tegumented nucleocapsids are transported to the Golgi apparatus; 8, tegumented nucleocapsids acquire a final envelope at the Golgi membranes; 9, vesicles with the enveloped virions are transported to plasma membrane; 10 and 11, enveloped virions are released from the cell.

TRANSMISSION

Herpesviruses are transmitted horizontally mainly from healthy virus carriers. The virus is excreted in bodily fluids such as saliva (typical of lymphocryptoviruses) and urine (typical of cytomegaloviruses). The kinetics of spread in simian populations differs for various herpesviruses. It also depends on housing conditions, behavioral characteristics of host species, and other factors. However, it is not uncommon that by adulthood the vast majority of animals, particularly in captive populations, are infected with multiple simian herpesviruses.

PATHOGENICITY

Primary infection with herpesviruses in the natural hosts is usually inapparent. The likelihood of disease development can increase dramatically when herpesviruses are transmitted to a nonnatural host. In immunocompromised nonhuman primates (NHPs), herpesviruses cause significant morbidity and mortality. The spectrum of herpesviral diseases is broad, ranging from mild eruptions to fatal CNS infections and lymphomas. The specifics of the pathogenicity of different simian herpesviruses are covered in Chapters 12 through 16.

12

Simplexviruses

- 12.1 Introduction
- 12.2 Classification and nomenclature
- 12.3 General properties
 - 12.3.1 Genomic organization and gene products
 - 12.3.2 Overview of replication cycle
 - 12.3.3 Latency
- 12.4 B virus (*Macacine herpesvirus 1*, former *Cercopithecine herpesvirus 1*)
 - 12.4.1 Prevalence of infection
 - 12.4.2 Modes of transmission
 - 12.4.3 Variability
 - 12.4.4 Immune response
 - 12.4.5 Pathogenicity in simian hosts
 - 12.4.6 Diagnosis
 - 12.4.6.1 Virus isolation
 - 12.4.6.2 Antibody detection
 - 12.4.6.3 Viral DNA detection
 - 12.4.7 SPF colonies
 - 12.4.8 Monkey-to-human transmission
 - 12.4.8.1 Epidemiology
 - 12.4.8.2 Disease presentation
 - 12.4.8.3 Laboratory diagnosis
 - 12.4.8.4 Post-exposure prophylaxis and treatment
 - 12.4.9 Prevention
 - 12.4.9.1 Vaccines
- 12.5 Simian agent 8 (*Cercopithecine herpesvirus 2*)
- 12.6 Herpesvirus papio 2 (*Papiine herpesvirus 2*, former *Cercopithecine herpesvirus 16*)
- 12.7 Chimpanzee herpesvirus
- 12.8 New World monkey simplexviruses
 - 12.8.1 *Saimiriine herpesvirus 1*
 - 12.8.2 *Ateline herpesvirus 1*
- 12.9 Summary

12.1. INTRODUCTION

The first simian simplexvirus was discovered in the early 1930s. Tragic circumstances preceded isolation of the virus. A researcher whose initials were W.B. was bitten on the hand by a seemingly healthy rhesus monkey. Shortly after the injury occurred, the researcher died of acute encephalomyelitis. The first viral isolate from this patient was named “W virus.”³² Soon after the first report, another isolate from the same patient was described and designated “B virus” (BV).⁹⁰ The name “B virus” (and related designations including “herpes B” and “herpesvirus B”) prevailed and continue to be widely used today. The International Committee on Taxonomy of Viruses (ICTV) name for this virus is *Macacine herpesvirus 1* (McHV-1).

Among known simian viruses, BV is probably the most dangerous for human beings. If transmitted to humans, this virus causes a severe acute disease involving the central nervous system (CNS). The fatality rate for the untreated disease is 70–80%. Fortunately, simian-to-human transmissions of BV are very rare: only about 50 cases have been reported. However, it is impossible to predict the outcome of human exposure to BV. Therefore, any unprotected exposure to BV-infected macaques, particularly rhesus monkeys, carries a risk of contracting this lethal virus.

Starting from the late 1950s, several other human herpes simplex virus (HSV)/BV-related simian viruses were identified in Old and New World monkeys and apes (Sections 12.5–12.9). Fortunately, none of these viruses are known to be dangerous for humans.

Simian simplexviruses have many properties in common with their human counterparts, HSVs. The first part of this chapter (Sections 12.2–12.3) describes the shared properties of the primate simplexviruses. The second

part (Sections 12.4–12.9) is devoted to the specifics of individual simian simplexviruses.

12.2. CLASSIFICATION AND NOMENCLATURE

HSVs and related simian viruses comprise the primate simplexvirus group within the genus *Simplexvirus*. HSV type 1 (HSV-1) and type 2 (HSV-2) are the prototypes of the *Simplexvirus* genus. The ICTV-approved designations of these viruses are *human herpesvirus* 1 (HHV-1) and *human herpesvirus* 2 (HHV-2). However, these names are rarely used.

Five simian simplexviruses are currently recognized by the ICTV as viral species: *Macacine herpesvirus* 1 (McHV-1), *Cercopithecine herpesvirus* (CeHV-2), *Papiine herpesvirus* 2 (PaHV-2), *Saimiriine herpesvirus* 1 (SaHV-1), and *Ateline herpesvirus* 1 (AtHV-2). All

ICTV and synonymous names of these viruses are presented in Table 12.1. ICTV names of two simian simplexviruses (McHV-1 and PaHV-2) were recently changed (May 2008). To minimize confusion during the transition to the new names, in this chapter we use the trivial names of the renamed viruses that are most commonly used (shown in bold in Table 12.1).

Two additional simian simplexviruses have not yet been included in the ICTV classification. These are chimpanzee herpesvirus (ChHV)⁵⁸ and langur herpesvirus (HVL).^{50,51} ChHV is well characterized and there is little doubt that it will be recognized as a simplexvirus species in the future. As to the langur virus, published information is too scarce to make a conclusion regarding the taxonomic rank of this isolate.

Serological evidence suggests the existence of simian simplexviruses naturally infecting other nonhuman

Table 12.1. Simian Simplexviruses

ICTV Name*	Old ICTV Name*	Synonymous Names†	Natural Host	Original References
<i>Macacine herpesvirus</i> (McHV-1)	<i>Cercopithecine herpesvirus</i> 1 (CeHV-1)	B virus (BV) , herpes B, Herpesvirus simiae	<i>Macaca</i> spp.	32, 90
<i>Cercopithecine herpesvirus</i> (CeHV-2)	<i>Cercopithecine herpesvirus</i> 2 (CeHV-2)	Simian agent 8 (SA8)	<i>Chlorocebus</i> spp.	59
<i>Papiine herpesvirus</i> (PaHV-2)	<i>Cercopithecine herpesvirus</i> 16 (CeHV-16)	Herpesvirus papio 2 (HVP-2)	<i>Papio</i> spp.	22
<i>Saimiriine herpesvirus</i> (SaHV-1)	<i>Saimiriine herpesvirus</i> 1 (SaHV-1)	Herpesvirus tamarinus (HVT), Herpes T Marmoset herpesvirus (MHV) Herpesvirus saimiri 1 (HVS-1)	<i>Saimiri</i> spp.	40, 65
<i>Ateline herpesvirus</i> 1 (AtHV-1)	<i>Ateline herpesvirus</i> 1 (AtHV-1)	Spider monkey herpesvirus (SMHV) Herpesvirus atelis 1 (HVA-1)	<i>Ateles geoffroyi</i>	45
NOR‡		Chimpanzee herpesvirus (ChHV)	<i>Pan troglodytes</i>	58
NOR‡		Langur herpesvirus (HVL)	<i>Presbytis</i> spp.	50

* ICTV-recognized viral species in italics.

† Most commonly used names in bold.

‡ Not officially recognized.

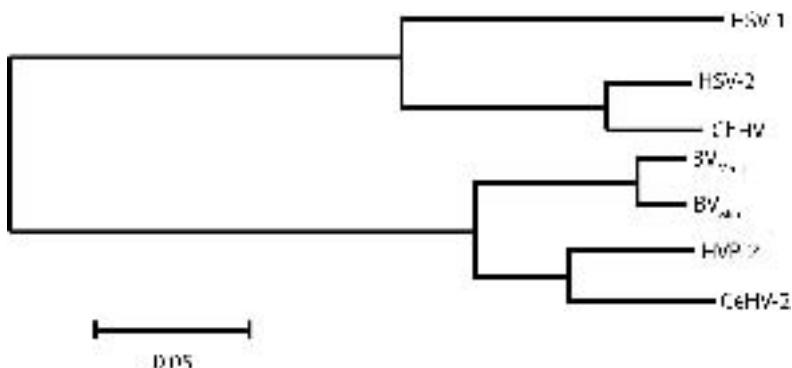


Figure 12.1. Simplexvirus phylogeny. Phylogenetic tree is constructed using neighbor-joining analysis of concatenated sequences derived from 11 different genes (UL13, UL14, UL23, UL27, UL28, UL44, UL47-UL49, US6, and US7) of primate simplexviruses. Bootstrap support values for all nodes of this tree are 100%. HSV-1 and HSV-2, human herpes simplex viruses, type 1 and type 2; ChHV, chimpanzee herpesvirus; *BV_{Mmu}* and *BV_{Mfa}*, B virus isolates from rhesus (Mmu) and cynomolgus macaques (Mfa); HVP-2, herpesvirus papio 2; CeHV-2, *Cercopithecine herpesvirus* 2. (Adapted from Luebcke *et al.*⁵⁸ with permission.)

primate (NHP) species,^{18,35,69} so it is likely that there are many simian simplexviruses awaiting identification.

There are three major groupings coinciding with the taxonomy of the hosts in the simplexvirus phylogeny: simplexviruses of humans and great apes, Old World monkeys, and New World monkeys. An intriguing feature of primate simplexvirus phylogeny is the closer relationship between HSV-2 and ChHV than between HSV-1 and HSV-2 (Figure 12.1).

12.3. GENERAL PROPERTIES

12.3.1. Genomic Organization and Gene Products

The genomes of all primate simplexviruses are collinear; that is, the genes are positioned in the same order for all viral species.^{72,82,105,106} The only difference in the gene repertoire of simian and human simplexviruses is the absence of the RL1 gene in the simian viral genomes. The product of the RL1 gene, the $\gamma_134.5$ protein, is a neurovirulence factor of HSVs. The absence of RL1 suggests a different mechanism for neurovirulence in BV.

The complete genomic sequences are known for the following simian simplexviruses: *BV*⁸² (GenBank Acc. No. NC_004812), CeHV-2¹⁰⁵ (GenBank Acc.

No. NC_006560), and HVP-2¹⁰⁶ (GenBank Acc. No. NC_007653). Although only the relatively short fragments of other simian simplexvirus genomes have been sequenced, it is most likely that the major features of genomic organization of these viruses are the same as those of BV, CeHV-2, and HVP-2.^{20,58,70} The size of the linear dsDNA genomes of the simian viruses is in the range 150–157 kb (156,789 bp, 150,715 bp, and 156,487 bp for BV, CeHV-2, and HVP-2, respectively). The “macro” features of the genomes are presented in Figure 12.2.

The simplexvirus genome consists of two covalently linked components, named *U_L* (unique long) and *U_S* (unique short), each “bracketed” by blocks of inverted repeat sequences. The orientation of the *U_L* and *U_S* regions in individual genome molecules is not identical. There are four genomic isomers: P (prototype), I_S (inverted *U_S*), I_L (inverted *U_L*), and I_{S+L} (inverted *U_L* and *U_S*) (Figure 12.2). Interestingly, virions with different genomic isomers are functionally indistinguishable.

The inverted repeats flanking the *U_L* each consist of two units designated *a* and *b* (or *a'* and *b'* in the inverted copy). The number of repeats in the *a* unit is variable. The *U_S* region is also flanked by repeats which are designated *c* and *c'*. One copy of the *a* sequence is also present at the end of the *c* repeat at the *U_S* terminus of

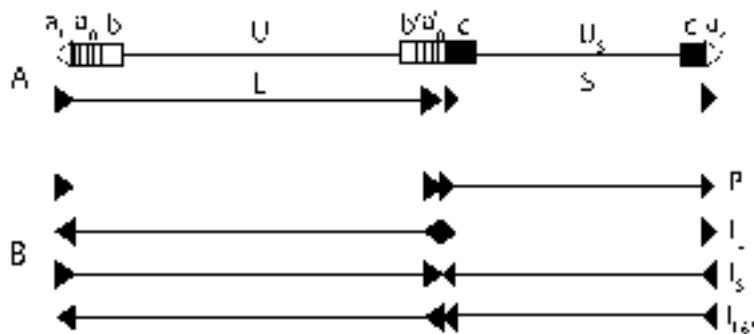


Figure 12.2. “Macro” map of a simplexvirus genome and scheme of genomic isomers. **A**, “Macro” map of simplexvirus genome; L and S, long and short genome segments; U_L and U_S, long and short unique regions; a_L and a_S, terminal sequences flanking U_L and U_S, respectively; a and b, repeats flanking U_L region; c, repeats flanking U_S region; **B**, genomic isomers; P, prototype; I_L, inverted L segment; I_S, inverted S segment; I_{L&S}, inverted L and S segments.

the genome. The structure of simplexvirus genome in “P” configuration can be depicted as:

$$a_L[(a)_n - b] - U_L - [b'(a')_m]\{c'\} - U_S - \{c\}a_S$$

The terminal *a* sequences (*a*_L and *a*_S) are not identical, and they are also different from the nonterminal *a* repeats. Importantly, the *a*_L and *a*_S sequences are required for circularization and packaging of viral DNA.⁷⁸

There are about 70 protein-coding genes in the simplexvirus genome as well as a number of nonprotein-coding sequence elements. All completely sequenced simian simplexviruses have exactly the same set of genes. Open reading frames (ORFs) and other features of primate simplexvirus genomes (as exemplified by the HVP-2) are summarized in Table 12.2. Regions of the genomes of incompletely sequenced primate viruses are also strictly collinear with the homologous regions of completely sequenced simplexvirus genomes.

Nomenclature of simplexvirus proteins is not formalized and is quite confusing. The names of many proteins are derived from purely technical characteristics and have little or no biological meaning. A list of predicted viral proteins and information on their presumptive functions (based on known functions of homologous HSV proteins) is presented in Table 12.2.

12.3.2. Overview of Replication Cycle

Knowledge of the simplexvirus replication cycle is mostly derived from studies on HSV-1 and, to a lesser

extent, HSV-2.⁷⁸ It is presumed that the major steps in the replication cycle of simian viruses are the same, although some exceptions in the replication of individual simian simplexviruses cannot be excluded.

The attachment of simplexviruses to a target cell occurs as a double-binding event: the envelope glycoproteins gB and gC bind to cell surface proteoglycans, heparin or chondroitin sulphates. This step is followed by the binding of the glycoprotein gD to a second receptor.

These binding events, together with the contribution of the fourth envelope glycoprotein gL, initiate fusion of the viral envelope and cellular membrane. Fusion results in entry of the virus into the cell. Internalized components of the virion include both the nucleocapsid and tegument proteins. The nucleocapsids as well as the tegument protein VP16 are transported to the nuclear membrane. Microtubules play an active role in this translocation. The nucleocapsid docks at the cytoplasmic opening of a nuclear pore and releases genomic DNA through the pore into the nucleus. VP16 is also transported into the nucleus where, in concert with host transcription factors, it switches on transcription of the “immediate early” or α -genes. The α -genes are transcribed by the cellular RNA polymerase II. Primary α -transcripts are all spliced. They are transported to the cytoplasm and translated into α -proteins. The α -proteins are required for switching on transcription of another set of genes, the “early” or β -genes. In order to trigger transcription of β -genes, the α -proteins have to

Table 12.2. Major Characteristics of Open Reading Frames and Predicted Proteins of Simian Simplexviruses (Percent Amino Acid Identity for HPV-2)

ORF Feature	Expression Group	% aa Identity CeHV-2	% aa Identity CeHV-1	% aa Identity HSV-1	% aa Identity HSV-2	Predicted Gene Product	Function Feature
<i>a</i> sequence TRL							
RL2	α	79	69	42	43	ICP0	Terminal direct repeat region
exon 1							Multifunctional regulatory protein
exon 2							
exon 3							
UL1	γ	85	67	49	47	Virion membrane glycoprotein L	Complex with gH; membrane fusion
UL2	$\beta\gamma$	93	86	75	74	Uracil-DNA glycosylase	DNA repair
UL3	γ	86	77	55	56		Colocalization with ICP22 and UL4 in small, dense nuclear bodies
UL4	γ	90	89	55	57		Colocalization with ICP22 and UL3 in small, dense nuclear bodies
UL5	β	96	94	82	83	Component of helicase-primase complex	
UL6	γ	95	92	67	69	Capsid protein	DNA cleavage/packaging

(Continued)

Table 12.2. (Continued)

ORF Feature	Expression Group	% aa Identity CeHV-2	% aa Identity CeHV-1	% aa Identity HSV-1	% aa Identity HSV-2	Predicted Gene Product	Function Feature
UL7	γ	94	87	65	64	Capsid protein	DNA cleavage/ packaging
UL8	β	93	79	57	60	Component of helicase-primease complex	
UL9	β	95	90	73	76	<i>ori</i> binding protein	Helicase activity
UL10	γ	94	88	66	65	Virion membrane glycoprotein M	Capsid development
UL11	γ	82	71	46	47	Mystified tegument protein	Capsid development
UL12	β	90	81	61	63	DNase; endoexonuclease	Processing of DNA replication intermediates
UL13	γ	96	92	65	67	Virion protein kinase	
UL14	γ	96	91	67	70	Minor tegument protein	
UL15							
exon 1	γ	98	97	88	88		
exon 2							
UL16	γ	96	91	64	65	Capsid associated	DNA cleavage/ packaging; transiently associated with maturing capsids
UL17	γ	92	88	67	69	Tegument protein	DNA cleavage/ packaging

UL18	γ	98	94	81	81	Capsid protein VP23	Forms triplexes with VP19C that connect pentons and hexons in capsids
UL19	γ	98	96	86	86	Major capsid protein VP5	Forms pentons and hexons of capsid shell
UL20	γ	95	92	65	67	Virion membrane protein	Virion egress; <i>syn5</i> locus
UL21	γ	94	86	64	66	Nucleotidylated phosphoprotein	Interact with microtubules; intracellular transport of the virus
UL22	γ	90	83	57	59	Virion membrane glycoprotein H	Complex with gL; membrane fusion, entry, cell-to-cell spread
UL23	β	88	85	55	57	Thymidine kinase	
UL24	γ	89	84	63	62	Nonglycosylated membrane-associated protein	<i>syn5</i> locus
UL25	γ	96	91	79	81	Minor capsid protein	DNA packaging; DNA anchoring?
UL26	γ	88	82	62	62	Capsid maturation protease	
UL26.5	γ	82	74	54	53	Scaffolding protein	

(Continued)

Table 12.2 (Continued)

ORF Feature	Expression Group	% aa Identity CeHV-2	% aa Identity CeHV-1	% aa Identity HSV-1	% aa Identity HSV-2	Predicted Gene Product	Function Feature
UL27	γ	94	92	79	79	Virion membrane glycoprotein B	Cell entry; <i>syn3</i> locus
UL28	γ	94	93	82	82	DNA cleavage packaging	Transiently associated with maturing capsids
UL29	β	97	95	83	82	ssDNA-binding protein	Assembly of DNA replication complex
oriL						Inverted repeat region of origin <i>oriL</i>	
UL30	β	93	91	77	78	DNA polymerase catalytic subunit	
UL31	γ	95	92	78	77	Nuclear phosphoprotein	Interacts with UL34; capsid egress from nucleus
UL32	γ	93	90	74	74		DNA packaging; not associated with capsids
UL33	γ	91	84	70	90		DNA packaging; not associated with capsids

UL34	γ	90	90	68	70	Type II nuclear membrane-associated phosphoprotein Interacts with UL31 phosphoprotein; interacts with UL31
UL35	γ	97	82	58	53	Basic phosphorylated capsid protein VP26 Very large tegument protein
UL36	γ	82	83	57	58	Interacts with UL19 and UL37
UL37	γ	93	87	66	65	Minor tegument protein
UL38	γ	94	90	70	68	Capsid protein VP19C Forms triplexes with VP23 connecting pentons and hexons
UL39	β	91	85	68	67	Large subunit of ribonucleotide reductase
UL40	β	92	89	80	80	Small subunit of ribonucleotide reductase
UL41	γ	95	93	71	72	Tegument phosphoprotein Virion-associated host shutoff (vhs) protein
UL42	β	66	72	45	45	dsDNA-binding protein
UL43	γ	74	80	45	45	DNA polymerase subunit Membrane-associated protein

(Continued)

Table 12.2 (Continued)

ORF Feature	Expression Group	% aa Identity CeHV-2	% aa Identity CeHV-1	% aa Identity HSV-1	% aa Identity HSV-2	Predicted Gene Product	Function Feature
UL44	γ	79	81	47	48	Virion membrane glycoprotein C	Cell attachment; blocking immune response Cell fusion?
UL45	γ	85	85	61	57	Type II membrane protein	
UL46	γ	89	82	52	53	Tegument phosphoprotein VP11/12	Modulates alpha <i>trans</i> -inducing factor activity
UL47	γ	90	85	60	58	Tegument phosphoprotein VP13/14	Modulate TIF activity; RNA binding
UL48	γ	93	92	69	69	Major tegument protein VP16	<i>Trans</i> -activator of gene expression
UL49	γ	88	80	47	46	Major tegument protein VP22	RNA binding; carrying mRNA from infected to uninfected cells
UL49A	γ	82	65	37	42	Envelope protein	
UL50	β	93	84	56	53	Deoxyuridine triphosphatase	
UL51	γ	87	80	66	69	Capsid tegument-associated phosphoprotein	

UL52	β	96	90	72	72	Component of helicase-primase complex
UL53	γ	92	90	67	70	Membrane glycoprotein K Virion egress; contains <i>syn1</i> locus
UL54	α	88	76	54	56	Immediate-early protein ICP27 Regulates some early and all late gene expression
UL55	γ	95	85	60	61	Nuclear matrix-associated protein
UL56	γ	77	59	38	39	Type II membrane protein Pathogenicity involvement
IRL						Internal copy of large inverted repeat region
RL2	α	79	69	42	43	Immediate-early protein ICP0 Multifunctional regulatory protein
exon 3						Inverted copy of a sequence
exon 2						Internal copy of small inverted repeat region
exon 1						Immediate-early protein ICP4 Regulator of gene expression
α' sequence IRS						
RS1	α	86	79	63	64	

(Continued)

Table 12.2 (Continued)

ORF Feature	Expression Group	% aa Identity CeHV-2	% aa Identity CeHV-1	% aa Identity HSV-1	% aa Identity HSV-2	Predicted Gene Product	Function Feature
OriS							Inverted repeat region of replication origin <i>oriS</i>
US1	α	80	68	39	41	Immediate-early protein ICP22	Required for optimal ICP0 expression
US2	γ	88	79	53	51	Tegument protein	Anti-apoptotic activity
US3	γ	91	83	62	62	Protein kinase	Entry into polarized cells
US4	γ	72	61	11	37	Virion membrane glycoprotein G	Blocking apoptosis
US5	γ	73	64	27	21	Glycoprotein J	Interaction with cellular receptors
US6	γ	89	86	56	57	Virion membrane glycoprotein D	Complex with gE; basolateral viral spread
US7	γ	85	73	47	47	Virion membrane glycoprotein I	Complex with gI; basolateral viral spread
US8	γ	87	76	46	48	Virion membrane glycoprotein E	Nucleolar phosphoprotein
US8A	γ	75	70	44	43	Tegument protein	Tegument protein
US9	γ	91	81	54	56		

US10	γ	68	62	39	40	Tegument protein
US11	γ	45	49	37	34	RNA-binding tegument protein
US12	α	85	58	36	33	Immediate–early protein ICP4/7
TRS						Terminal copy of small repeat region
oriS						Inverted repeat region of replication origin <i>oriS</i>
RS1	86	79	63	64		Immediate–early protein ICP4
<i>a sequence</i>						Regulator of gene expression
						Terminal direct repeat

be transported back to the nucleus. β -gene transcripts are mostly unspliced. They are transported to the cytoplasm where they are translated. Most β -proteins are involved in DNA replication and related pathways. Viral DNA replication is initiated at the genomic sites which are designated as “origin of replication” (*ori*) sequences. The end-result of DNA replication is the formation of long, “head-in-tail” concatenated viral genomic dsDNA. This concatenated genomic DNA serves as a template for the third wave of genomic expression, the transcription, and translation of the “late” or γ -genes. Most of the late transcripts are unspliced. The γ -mRNAs are transported to the cytoplasm where they are translated into the γ -proteins, which as a rule are structural proteins. However, there are also late nonstructural proteins which are required for assembly and egress of progeny virions. Assembly of nucleocapsids and their coating with the tegument occurs in the nucleus. Tegumented nucleocapsids acquire envelope through the double envelopment process described in Introduction to Herpesviruses.

12.3.3. Latency

Simplexvirus infection *in vitro* is productive. However, *in vivo* infections can be either productive (lytic) or latent. When the virus infects a new host it replicates in epithelial cells at the site of entry. The primary infection is usually unapparent or clinically mild. Typically, primary infections are manifested as vesicular lesions on oral or genital mucosa or skin, which heal within 7–10 days. Importantly, during and after healing, the virus is not cleared from the body. During the primary infection the virus enters sensory nerve endings present in the epithelium at the site of infection and “travels” inside nerve cell axons toward the neuronal bodies located in sensory ganglia. For example, if the primary infection occurs in the orofacial area, the virus infects sensory neurons comprising the trigeminal ganglia. Two outcomes are possible in infected neurons: death of the nerve cell due to productive infection or establishment of latency, a kind of “peaceful coexistence” of the virus and the cell.

Latently infected neurons contain a complete simplexvirus genome but do not express viral proteins. Gene expression is restricted to a small region of the genome located in the repeat sequences flanking the UL region. These transcripts are the latency-associated transcripts (*LAT*). The primary *LAT* transcript spans about 8 kb. Part of this transcript is spliced out and remains in the

nucleus as the relatively stable 2-kb *LAT* RNA. The remaining *LAT* transcript is transported to the cytoplasm. However, data on the translation of this RNA are controversial and it is not clear whether or not *LAT* proteins are actually synthesized.

The suggested function of the *LAT* “gene” is to promote survival of the latently infected neuron. There is evidence that *LAT* encoded microRNAs are involved in this process by promoting degradation of cellular mRNAs coding for transforming growth factor- β (TGF- β) and the mediator of its signaling.³³

Simplexvirus DNA does not integrate into the chromosomal DNA during latency. Instead, the viral DNA exists as an episome in latently infected cells. Once latency is established, simplexviruses are not eliminated from the host. In some latently infected neurons the virus can be reactivated from its dormant state. Reactivation may be triggered by many external or internal factors, such as overexposure to sun or cold temperature, stress, and others. The presumptive common denominator for the reactivation triggers is immunosuppression. However, the exact mechanism of switching from latency to productive infection is not known. When reactivation occurs in the neurons, the newly produced virions undertake a descending “return journey” through the axon toward the mucous membranes and skin. Clinically, the end-result of such reactivation is shedding of infectious virus. Recurrent infections can be asymptomatic or manifested as recurrent lesions, usually in the orofacial or genital areas.

12.4. B VIRUS (MACACINE HERPESVIRUS 1, FORMER CERCOPITHECINE HERPESVIRUS 1)

BV is undoubtedly the most medically important of the simian simplexviruses. The virus is found in various macaque species including *M. mulatta*, *M. fascicularis*, *M. arctoides*, *M. nemestrina*, *M. fuscata*, *M. radiata*, *M. silenus*, and *M. cyclopis*.^{52,92,95,102,111} Although not formally proven, it should be presumed that no macaque species is free from BV infection and all macaques should be treated as if they are infected.

12.4.1. Prevalence of Infection

Most data on the prevalence of BV infection have been reported for captive animals. Prevalence estimates vary widely, from 5 to 100%, depending on the type of housing, extent and type of contact with other macaques, sampling, and techniques used for diagnosing infection.

In most captive macaque colonies, the majority of adult animals are infected, as determined by detection of anti-BV IgG antibodies.^{53,91,92,110,111} High prevalence of BV infection has been reported in the free-ranging rhesus monkey colony on the island of Cayo Santiago, Puerto Rico⁵³ and also in “semi-wild” temple cynomolgus macaques in Bali, Indonesia.²⁸ A relatively low BV prevalence of about 5% is reported for urban street-performing cynomolgus macaques in Jakarta, Indonesia.⁹³

Surprisingly little is known about the prevalence of BV infection in the wild. A high prevalence of anti-BV antibodies has been reported in Indian rhesus monkeys.^{75,95} Among monkeys trapped in various fields in Jammu and Kashmir, 80–90% were infected and seroprevalence was age-dependent: 12%, 37%, and 73% in juveniles, young adults, and adults, respectively.⁷⁵

The prevalence of BV in Japanese macaques varies from 0 to 90% in different wild troops.⁶⁷ Four out of 11 troops studied were BV-free; the prevalence of anti-BV in the remaining 7 troops was in the range 30–90%.

The largest known BV-free wild macaque population is on the island of Mauritius. The founders of this cynomolgus macaque “colony” were introduced to the island several hundred years ago. During the last 20 years, thousands of serum samples from Mauritian cynomolgus monkeys have been tested for anti-BV antibodies and all have been seronegative.⁶³ Mauritian cynomolgus macaques originate from a few founders brought to the island by sailors 400 years ago.¹⁰³ It happened that the founder animals were BV-negative, which is not surprising if it is assumed that pet monkeys on ships were collected, typically, when they were very young.

12.4.2. Modes of Transmission

Infectious BV can be detected with approximately the same frequency in oral, conjunctival, and genital secretions. The virus is transmitted among juvenile rhesus monkeys by direct contact. Most transmission occurs in the age group of 2–3 years and by the age of 3, nearly all animals are infected.¹¹⁰ Sexual contact is an important means of virus spread, but is not the predominant mode of transmission of BV. Rather, transmission to infant and juvenile monkeys as oral infections seems to be the major means.¹¹²

Interestingly, in colonies of rhesus monkeys systematically monitored for the spread of BV, all transmissions

occurred during the breeding season.^{43,110} Possibly, this is explained by the increased frequency of virus reactivation caused by hormonal changes and stress associated with breeding activity.

12.4.3. Variability

BV strains from various macaque species are distinguishable by genomic sequence polymorphisms^{73,97–99,102} and, to a lesser degree, by antigenic differences.⁹⁹

Initially, three genotypes of BV were unequivocally identified: genotype I in rhesus and Japanese macaques, genotype II in cynomolgus macaques, and genotype III in pig-tailed macaques.⁹⁹ More recently it has been shown that BV from Japanese macaques can be distinguished from rhesus BV.⁷³ The most divergence among CeHV genotypes occurs between variants from rhesus and cynomolgus macaques and variants from pig-tailed and lion-tailed macaques. Because a relatively small number of BV isolates have been investigated for genotypic variability, it is likely that BV genotypic diversity is greater than is currently known. There are no data suggesting biological differences between BV genotypes, but this possibility cannot be excluded.

12.4.4. Immune Response

An antibody response against primary BV infection becomes detectable starting about 7–10 days after infection. As expected, antibodies belonging to the IgM class appear first. IgG antibodies against BV become detectable 10–14 days postinfection.⁵⁴ Antibodies against viral glycoproteins appear first and are followed by antibodies against other structural proteins. After seroconversion, infected animals continue to be seropositive lifelong, although the level of anti-BV may fluctuate.⁶⁸

Neutralizing antibodies are detectable in nearly all seropositive animals.⁷ Macaque neutralizing anti-BV antibodies cross-neutralize all other primate simplexviruses, whereas human HSV neutralizing antibodies do not neutralize BV.¹¹³ Virtually nothing is known about cell-mediated and innate immune responses against BV.

12.4.5. Pathogenicity in Simian Hosts

The pathogenic potential of BV in NHPs is different in natural (macaques) and nonnatural (African and New World monkeys) hosts.

BV infection in macaques is usually subclinical. Primary infection can be accompanied by the development of erythema and vesicles at the site of virus entry. From the epithelium, the virus travels through nerves innervating the site of virus replication to the sensory ganglia (trigeminal or dorsal root) and establishes latency. Latent BV can be recovered from ganglial tissues by cocultivation with permissive cell lines. Reactivation of latent infections occurs intermittently, but is not usually manifested clinically. Anti-BV IgG titers in latently infected animals fluctuate, presumably increasing following reactivation events.⁶⁸ During reactivation, BV can be readily isolated from mucosal, buccal, ophthalmic, and genital sites as well as from urine and some internal organs (liver, kidney). However, viremia is rare. Generalized disease due to BV can develop in immunosuppressed macaques.^{12,96}

Transmission of BV to non-macaque species has been observed in captivity, although rarely. BV infection in nonnatural hosts usually results in a fatal disease. However, a subclinical course is also possible, as has been observed in capuchin monkeys (*Cebus apella*).¹⁴ Fatal BV disease has been described in patas,¹¹⁴ colobus,⁵⁶ and DeBrazza's monkeys.¹⁰² The largest known outbreak caused by interspecies transmission of BV occurred in 1981 in a colony of DeBrazza's monkeys (*Cercopithecus neglectus*). The disease was manifested as vesicular and ulcerative lesions on the lips, tongue, and palate. Seven of eight affected animals died from the infection. The probable source of the infection was lion-tailed macaques (*M. silenus*) housed in an adjacent cage.¹⁰²

12.4.6. Diagnosis

BV infection is diagnosed by virus isolation or detection of antiviral antibodies or viral DNA. There are some peculiarities in diagnosing BV infection in human beings which are discussed in Section 12.4.6.3.

12.4.6.1. VIRUS ISOLATION

BV replicates well in monolayer cell cultures of simian origin as well as in rabbit kidney cells. The Vero cell line (of African green monkey origin) appears to be the most sensitive for isolation and propagation of the virus. In susceptible cell cultures BV causes CPE, usually syncytial; however, some primary isolates may induce cell rounding followed by ballooning. Eosinophilic intranuclear inclusions (Cowdry type A) are observed in fixed and stained infected cell cultures. Virus isolation can be confirmed by detection of BV antigens or DNA.

Specimens for virus isolation can be obtained from the buccal mucosa, the conjunctiva, and the urogenital area. A positive result of virus isolation from simian material unequivocally confirms that the animal is infected with BV. A negative result should be interpreted with caution because shedding of the virus is intermittent.

The main difficulty in BV isolation is the biohazard associated with working with infectious BV. According to current US regulations, any manipulation of diagnostic samples suspected of containing live BV must be performed under Biosafety Level 3 containment. In the United States, further restrictions (the Select Agent Program mandated under the Patriot Act) come into effect once a diagnostic sample is known to contain infectious BV.

12.4.6.2. ANTIBODY DETECTION

Detection of antibodies against BV is the most commonly used approach for diagnosing this infection in monkeys. The presence of anti-BV IgG is a reliable marker of infection. However, a seropositive result does not predict the risk of virus transmission. A negative antibody test also does not completely exclude BV infection because there is a lag in the appearance of detectable antibodies after virus infection; furthermore, antibody levels in persistently infected animals can fall below the threshold limit of the test, producing a negative result.

The virus neutralization test is the gold standard for specificity of detection of anti-BV antibodies. However, this test is time- and labor-consuming and, more importantly, it can be performed only in Biohazard Level 3 or Level 4 facilities. For these reasons the neutralization test is rarely used.

Other methods for detection of anti-BV antibodies are immunofluorescence, ELISA, radioimmunoassay, dot-blot, and Western blot (WB).^{5,34,49,68,71} Either homologous (BV) or heterologous (HSV-1/HSV-2, HVP-2, and CeHV-2) antigens can be used in these assays.^{5,74,100,117}

ELISA and the conceptually similar dot-blot assay are the most common methods for the detection of anti-BV antibodies.^{34,49,74,116,117} Most BV ELISAs are based on crude antigen extracts of virus-infected cells, extracts of uninfected cells being used as a control antigen. A problem frequently encountered with this type of ELISA is the nonspecific reactivity of monkey sera. This problem can be minimized by preabsorption of monkey sera with the control antigen extract.⁹¹ Diagnostic specificity of crude antigen-based BV ELISAs is close to 100%, whereas their diagnostic sensitivity is markedly lower (70–80%).

The alternative to the crude antigens is purified recombinant BV proteins or peptides. However, ELISAs based on such antigens are not yet developed to the stage where they can be used as routine diagnostic tests.^{79,83,101}

The most commonly used type of BV ELISA assay, the indirect ELISA, does not allow reliable discrimination between BV and other simian simplexviruses. Promising in this context is the fact that a recombinant membrane-associated form of the BV gG protein is antigenically virus-specific.⁷⁹ However, a diagnostic ELISA based on this antigen has yet to be developed. Some discrimination of antibodies against individual simian simplexviruses has been achieved using competitive ELISA assays.⁵

WB is used in some laboratories for diagnosing BV infections rather than ELISAs. However, correlation between WB and ELISA results is poor. In addition, the criteria for discrimination of WB-positive and indeterminate results as well as interpretation of the indeterminate results are not well established.⁹¹

12.4.6.3. VIRAL DNA DETECTION

Detection of BV DNA by polymerase chain reaction (PCR) is a versatile and increasingly commonly used tool for detection and identification of this virus.^{4,38,39,48,66,76,80,94,97} BV PCR is gradually replacing classical virus isolation because it is more sensitive, quicker, less laborious, and less hazardous. PCR also can be optimized for use in various contexts, such as:

- (a) detection of BV in human materials;^{66,80}
- (b) detection of BV in simian materials containing other simplexviruses;^{4,48}
- (c) quantitation of BV viral load;^{43,80}
- (d) distinguishing various BV genotypes;^{73,97}
- (e) simultaneous detection of BV and other primate herpesviruses.⁴⁸

Sequencing of PCR-amplified fragments is becoming widely available and could provide important information on sequence polymorphisms that may modify virulence of BV, or contribute to resistance to antiherpetic drugs.

For the purpose of BV detection and quantitation the quantitative real-time PCR assay targeting nonconserved region of the gG gene appears to be optimal.⁸⁰ This test is specific for BV and is significantly more sensitive than virus isolation. It also has excellent reproducibility (intra- and inter-assay coefficients of variation

of 0.6% and 2.4%, respectively), and a very wide quantitation range covering 6 logs. This assay is a good candidate for use as the gold standard for the detection of BV in simian and human materials. However, it requires further validation, particularly with respect of detecting different BV genotypes.

Interpretation of positive results of BV PCR is straightforward—it confirms infection. A negative result for BV PCR performed on simian samples cannot be considered as conclusively proving that the animal is virus-free, because the presence of viral DNA is intermittent in host sites that are readily accessible for sampling. In other words, PCR cannot detect infection in latently infected monkeys that are not shedding virus into the body fluids and secretions.

12.4.7. SPF Colonies

Health risks associated with BV infection have prompted the establishment of specific pathogen-free (SPF) macaque colonies in which all animals are free of BV. Beginning in 1989, breeding programs in the United States aimed at establishing such colonies were funded by the US National Institutes of Health and are being implemented in several US National Primate Research Centers and commercial breeding ventures.^{36,91,107–109}

Conceptually the establishment of BV-free macaque colonies is simple: virus-negative animals are identified by serial antibody testing and bred separately in strict isolation from BV-positive animals. However, practical implementation of this algorithm presents a challenge. The main issue is the reliability of determining a monkey's virus status by antibody assay. Conversions for anti-BV antibody positivity have been observed in previously seronegative macaques kept isolated from BV-positive animals for over 4 years.^{36,108,109} Tests used for the detection of anti-BV are not standardized, and their diagnostic sensitivity and specificity can vary significantly when testing different monkey populations.

These problems are dealt with by using several antibody tests based on different principles, repetitive testing, and immediate removal of animals that have seroconverted and close monitoring of their contacts.

12.4.8. Monkey-to-Human Transmission

12.4.8.1. EPIDEMIOLOGY

The transmission of BV to humans is a rare event. All known cases have occurred in primate facility settings. About 50 such cases are known, but only half of them

are sufficiently well documented in terms of the type of exposure. The most dangerous are monkey bites. However, less invasive exposures such as scratch, splash of feces, urine, or saliva onto mucosal surfaces, and even possible aerosol transmission have been reported.¹³

The transmission of BV to humans apparently always results in disease development. The search for asymptomatic BV carriers among animal caretakers, including those who experienced monkey bites or scratches has not revealed any seropositive cases.³⁰ Moreover, no evidence of asymptomatic BV infection has been found among household contacts or hospital workers exposed to patients with BV encephalitis.^{16,42} Thus, the likelihood of human-to-human transmission is low. Only one such case has been reported. In this case, the virus was transmitted to the wife of a veterinary technician who later died of BV encephalitis. She was apparently infected by application of hydrocortisone cream to treat dermatitis of her finger after applying the same cream to lesions on her husband. After the husband was diagnosed with BV encephalitis, a sample taken from the wife's finger lesion tested positive for BV by virus isolation. She was immediately treated with intravenous acyclovir. Her dermatitis cleared, and the diseases did not progress further.

Patients who have survived BV encephalitis should, theoretically, be latently infected with the virus. It is not known if reactivation of BV will occur in these individuals. However, no cases of BV disease have been traced to contact with a disease survivor. Also puzzling is the lack of any reports of human BV disease resulting from exposure to pet, "semi-wild," or wild macaques. Equally puzzling is the lack of reported human BV infections in any primate facilities outside the United States.

12.4.8.2. DISEASE PRESENTATION

The incubation period for documented human BV infections ranges from 2 to 30 days; in most cases, it is from a few days to one week before symptoms appear. Vesicular lesions usually develop at the site of entry. The generalized disease starts with influenza-like symptoms such as fever, muscle aches, headache, and fatigue. Progressive neurological deterioration consistent with the clinical picture of encephalitis or encephalomyelitis follows. Patients are confused or comatose. Various signs and symptoms suggesting generalized brain damage are present.

Historically, the case fatality rate is about 70%. Antiviral therapy reduces fatality, if initiated before devel-

opment of full-blown encephalitis. If treatment is started later, most patients die even when supportive care is provided. Death is usually caused by ascending paralysis leading to respiratory failure. Survivors usually have serious neurological sequelae, but cases of complete recovery are also known.

12.4.8.3. LABORATORY DIAGNOSIS

Samples taken from infected humans for virus isolation or viral DNA detection must be obtained *after* thorough wound or exposure site cleansing. This procedure reduces the amount of virus, particularly infectious virus. As a result, the diagnostic sensitivity of virus isolation from human samples obtained shortly after exposure is low.¹³ Thus, a negative result is not conclusive.

A positive result of BV isolation from a human specimen obtained within several hours of exposure does not confirm that this person is persistently infected. However, such a result indicates that a "high-risk exposure" has occurred and justifies an extended course of chemoprophylaxis. Virus isolation from BV-exposed individuals who have signs and symptoms consistent with BV encephalitis days to weeks after the exposure will confirm BV disease.

The main problem in serological diagnosis of BV infection in exposed individuals is the presence of cross-reacting anti-HSV antibodies in nearly all humans. A recently described ELISA based on the truncated recombinant glycoprotein gD, lacking the transmembrane domain and cytoplasmic tail of this protein, allows discrimination between anti-CeHV and anti-HSV antibody responses.³¹ This assay may be helpful for diagnosing human BV infection. Quantitative BV PCR can be used for monitoring treatment of proven BV cases.

12.4.8.4. POST-EXPOSURE PROPHYLAXIS AND TREATMENT

The most important component of post-exposure prophylaxis in cases of potential exposure to BV is extensive washing of the site of exposure, which must be initiated as soon as possible and last for at least 15 min. Bite wounds, skin punctures, and scratches must be cleansed with soap or detergent solution. Exposed mucosal surfaces must be rinsed with saline or running water.

Chemoprophylaxis and treatment of BV infection in humans remains largely empiric. Prophylactic treatment is not unconditionally recommended. However, taking into consideration that antiherpetic drugs such as

acyclovir (ACV), valacyclovir (VACV), and famciclovir (FCV) are safe and well tolerated, the trend is toward the use of post-exposure chemoprophylaxis rather than not. Although ACV remains the most commonly used drug, the newer drugs VACV and FCV may be at least as effective and their administration schedules are more convenient.

Symptomatic BV infections require more intensive treatment. According to the latest recommendations, intravenous ACV and ganciclovir (GCV) should be used. GCV is 3–8 times more potent against BV than ACV²⁹ but it is a very toxic drug. ACV is recommended for cases when CNS symptoms are absent, whereas GCV is recommended if CNS symptoms are present.¹³

The profile of in vitro sensitivity of BV to antiherpetic drugs is significantly different from that of HSVs. From the practical point of view, the most important difference is lesser sensitivity of BV to ACV. The difference between BV and HSV-1/HSV-2 in sensitivity to ACV varies 2.5- to 10-fold. The most potent inhibitors of BV growth in vitro are 5'-trifluoromethyl-2'-deoxyuridine (TFT) and 5-iododeoxyuridine (IUdR). They are 15–65 and 10–15 times more potent than ACV, respectively.²⁹ Unfortunately, TFT and IUdR are too toxic for systemic use. Two other drugs tested in vitro against BV, penciclovir (PCV) and 5-Ethyl-2'-deoxyuridine (Et-dU), are as potent as GCV but much less toxic.²⁹ Et-dU is not approved for human use. However, the data on PCV might be clinically relevant because therapeutic concentrations of PCV in vivo can be achieved by the administration of FCV (FCV is the pro-drug of PCV).

12.4.9. Prevention

The main preventive measure is wearing protective gear when working with monkeys, particularly macaques, and prevention of bites, scratches, and other injuries. The availability of BV-free macaques from SPF colonies (Section 12.4.3) also reduces the risk of simian-to-human transmission of BV.

12.4.9.1. VACCINES

Theoretically, the risk of BV transmission to humans can be reduced or even eliminated by vaccination of individuals having extensive contact with macaques. Also, vaccination of BV-positive macaques may reduce the shedding of the virus and decrease the risk of transmission.

The first attempts to develop a BV vaccine were undertaken in the 1960s.^{44,46,47} The vaccine, a formalin-inactivated virus, induced a short-lived specific antibody response in human volunteers which could be sustained by multiple and frequent boosters. This vaccine was clearly not suitable for human use and the possibility of its use in monkeys was not explored.

Research aiming at the development of a BV vaccine resumed in the late 1990s. Two approaches have been used: a recombinant vaccinia-based vector expressing the BV gD glycoprotein¹ and DNA-based vaccines that express the gB or gD glycoprotein genes of BV.^{37,57,81}

The recombinant vaccinia-based vaccine has been shown to be protective in rabbits: 10 out of 11 animals challenged with a lethal dose of BV survived and BV DNA was not detected in the dorsal root ganglia of the immunized animals.¹

Both recombinant and DNA vaccines induce specific antibodies in rabbits, mice, and monkeys, including neutralizing antibodies. Cell-mediated immune responses have also been recorded.³⁷ However, the protective efficacy of these vaccines in terms of preventing infection and/or disease in monkeys has not been evaluated. It also remains unknown whether or not vaccination of BV-positive macaques will reduce shedding of the virus.

12.5. SIMIAN AGENT 8 (*CERCOPITHECINE HERPESVIRUS 2*)

CeHV-2 was first isolated from an African green monkey⁵⁹ and then repeatedly from baboons.^{60,61} Initially, this virus was named simian agent 8 (SA-8) and this name is still used. Originally, CeHV-2 was presumed to infect all species of African monkeys including baboons. However, recent studies have demonstrated that baboons carry a distinct, albeit very closely related virus (HVP-2; see Section 12.6). It now appears that African green monkeys are the natural host of CeHV-2.

Morphologically, CeHV-2 is a typical herpesvirus.⁶ It shares major properties with other primate simplexviruses.^{2,19,22,23,25} The complete genome sequence of CeHV-2 is available.¹⁰⁵ Its genome structure is collinear with other primate simplexvirus genomes. Each CeHV-2 ORF has a homolog in the genomes of BV and HVP-2. The major distinction between the BV and CeHV-2 genomes and the genomes of HSVs is the absence of an RL1 (γ_1 34.5) homologue.

The closest relative of CeHV-2 is HVP-2. Phylogenetic analysis of individual ORFs shows that all but a

few ORFs of BV and CeHV-2 cluster together. The exceptions are UL41-44 and UL36 (Section 12.6).

Virtually nothing is known regarding infections caused by CeHV-2. Most African green monkey colonies are negative for the virus, and no major outbreaks of disease associated with CeHV-2 infection have ever been reported in colonies that do have the virus.

12.6. HERPESVIRUS PAPIO 2 (PAPIINE HERPESVIRUS 2, FORMER CERCOPITHECINE HERPESVIRUS 16)

HVP-2 was isolated from the genital and oral ulcerous lesions of baboons during the investigation of an outbreak that occurred in the mixed baboon colony at the Southwest National Primate Research Center (SWN-PRC), San Antonio, USA. Initially, this virus was identified as SA-8, that is, CeHV-2.⁵⁵ However, HVP-2 was distinguished from CeHV-2 in the mid-1990s and the virus has been named Herpesvirus papio 2 (HVP-2).²² This name is still in use although it has been gradually replaced by the ICTV name *Cercopithecine herpesvirus 16* (CeHV-16), but recently (May 2008) this name was replaced with the name *Papiine herpesvirus 2* (PaHV-2).

HVP-2 infection as detected by the presence of specific antibodies is common in captive baboons: 90% of adults are anti-HVP-2-positive. Baboons are presumed to be the natural host of HVP-2, but no data on the presence of this virus in wild baboons has been published. Majority of captive baboons acquire HVP-2 before sexual maturity which is consistent with oral transmission in infants and juveniles, rather than sexual transmission of the virus in adults.^{21,77}

Disease caused by HVP-2 in baboons can take several forms. HVP-2 can cause oral and genital lesions in baboons similar to those caused by HSV in humans.^{55,62,98} HVP-2 can also cause severe neonatal herpes infection resembling this disease in humans.^{8,26,115} There does not appear to be any difference in the HVP-2 susceptibility of baboons belonging to different species. During a disease outbreak in a large baboon colony (at the SWNPRC) yellow, olive, and hamadryas baboons were all affected. Why the HVP-2-induced outbreak occurred only in the SWNPRC colony is not clear.

As described earlier, the closest relative of HVP-2 is CeHV-2.^{3,106} However, two genomic regions, UL41 to UL44 and UL36 are exceptional. These gene sequences are more similar to their BV homologs,¹⁰⁶ suggesting that recombination between progenitors of HVP-2 and

BV may have occurred sometime during the evolution of these simian simplexviruses.

Most strains of HVP-2 are highly neuropathogenic for mice whereas others are avirulent.^{86–89} The pathology caused in mice by the neuropathogenic HVP-2 strains is virtually indistinguishable from that caused by strongly neuropathogenic BV strains.^{84,85,88} However, in contrast to BV in which the variation of neuropathogenicity for mice of different strains is continuous, all HVP-2 strains fall into two distinct categories: highly neurovirulent and avirulent. Interestingly, there is no difference in the disease produced in baboons by neurovirulent or avirulent HVP-2 strains.⁸⁹

HVP-2 has been isolated from a fatal case involving the CNS in a colobus monkey¹⁰⁴ indicating that this virus does have the ability to infect primate species other than baboons. However, HVP-2 infection has not been reported in humans.

The antigenic cross-reactivity of HVP-2 and BV is sufficient to allow the use of HVP-2 for detection of antibodies against BV.^{74,117} ELISAs based on crude HVP-2 antigen have similar sensitivity and specificity for the detection of anti-BV as a BV-based ELISA. The advantage of using HVP-2 antigen-based ELISA is the lower risk to humans during the preparation of the antigen.

12.7. CHIMPANZEE HERPESVIRUS

The existence of a chimpanzee simplexvirus had been suggested by serological studies reported in the late 1980s.^{24,64} However, a virus that appears to be a chimpanzee virus (named ChHV) was isolated only recently.⁵⁸ The isolate was obtained from an oral lesion of a captive common chimpanzee (*Pan troglodytes*). The animal, a 5-year-old female, was a member of a group that was housed together. Five chimpanzees in this group, both males and females, developed oral and pharyngeal ulcers. Aside from the lesion, the affected animals remained clinically normal and showed no signs of discomfort. In 2–3 weeks the ulcers resolved and no additional cases were observed.

Isolation of ChHV was achieved by inoculation of Vero cells with biopsy material obtained from an ulcerative lesion of the tongue. Clumps of rounded-up cells, CPE typical of HSV-1, appeared within 48 h. No giant cells or syncytia that are typical of HSV-2 and the simian herpesviruses were observed. ChHV does not spread readily within the monolayer cell culture and forms well-defined foci of infected cells. Infectious titers

of ChHV obtainable from Vero cells are not high, rarely exceeding 10⁷ PFU/mL, indicating that ChHV is highly cell-associated.

The complete genome sequence of ChHV is not available. However, the sequences of many genes located in various regions (total length is about 35,000 bp) have been determined and no deviation from collinearity with other primate simplexvirus genomes has been found. Phylogenetic analysis of ChHV sequences did not reveal any evidence of recombination, as observed for HVP-2.⁵⁸ Overall tree topology was the same regardless of which gene, or combination of genes, was used in the analyses. The intriguing feature of the phylogenetic relationship of ChHV to other primate simplexviruses is its closeness to HSV-2: the chimpanzee virus clusters together with HSV-2, not HSV-1. Assuming the same pace of the molecular clock along different lineages of primate simplexviruses, the timing of HSV-2/ChHV split has been estimated at 5 Mya, much later than the estimated time for HSV-1/HSV-2 divergence (13–14 Mya).

Interestingly, wild mountain gorillas (*Gorilla beringei beringei*) and a few captive western lowland gorillas (*G. gorilla gorilla*) have antibodies which cross-react more strongly with HSV-2 than with HSV-1.¹⁸ The presumptive gorilla simplexvirus has not yet been isolated; however, these serological data suggest that it is also more closely related to HSV-2 than to HSV-1. In addition to phylogenetic analyses based on gene sequence data, there are also several genomic features characteristic of HSV-1 which are absent in the genomes of all known simian simplexviruses, such as: the existence of a UL20.5 ORF located between UL20 and UL21 and the comparatively small size of the HSV-1 US4 (gG) gene. This raises the possibility that HSV-1 may represent a distinct simplexvirus lineage. If true, it appears that simplexviruses belonging to the HSV-1 lineage are absent in NHPs. It is not clear why the HSV-1 lineage has survived only in humans.

12.8. NEW WORLD MONKEY SIMPLEXVIRUSES

12.8.1. *Saimiriine Herpesvirus 1*

The first isolates of simplexvirus from New World monkeys, currently known as SaHV-1, were reported in the mid-1960s. A presumably identical herpesvirus was isolated from dead and moribund tamarins and marmosets.^{40,65} Several years later the same virus was

isolated from an owl monkey (*Aotus trivirgatus*)²⁷ and squirrel monkeys (*Saimiri sciureus*).¹⁵ Most squirrel monkeys have antibodies against SaHV-1⁴¹ which suggests that *Saimiri sciureus* is the natural host of SaHV-1.

The history of SaHV-1 nomenclature is quite confusing. Initially, names reflecting the species or origin were used: Herpesvirus tamarinus (HVT), Herpesvirus T (HT), and marmoset herpesvirus (MHV). Once it was realized that squirrel monkeys were the natural host for the virus, HVS (Herpesvirus saimiri) replaced the other names. It happened that approximately at the same time another herpesvirus, an oncogenic T-lymphotropic virus, was isolated from the squirrel monkey and was also named Herpesvirus saimiri. This second virus is a rhadinovirus (see Chapter 16). To distinguish the two different HVSs, the simplexvirus has been designated HVS-1 and the rhadinovirus as HVS-2. Subsequently ICTV classified HVS-1 as *Saimiriine herpesvirus 1* (SaHV-1) and HVS-2 as *Saimiriine herpesvirus 2* (SaHV-2).

Genomic variability of SaHV-2 is low as indicated by nearly identical restriction endonuclease cleavage patterns of virion DNA isolated from independent SaHV-2 isolates.¹⁷ The sequence of a relatively short fragment (6,751 bp) of the SaHV-1 genome has been reported²⁰ (GenBank Acc. No. AY095366). SaHV-1 genes located in this fragment are collinear with the homologous genes of other primate simplexviruses.

Interest in SaHV-1 has recently reemerged.¹⁰ A recombinant strain of SaHV-1 which expresses enhanced green fluorescent protein has been constructed.⁹ The recombinant virus is comparable to wild-type SaHV-1 in its capacity to induce disease in mice and can be used for tracking the spread of the virus in experimentally infected mice.^{9,11}

12.8.2. *Ateline Herpesvirus 1*

Another New World monkey simplexvirus was isolated from oral lesions and nervous tissue of a young captive spider monkey in the United States (*Atelopus geoffroyi*).⁴⁵ Initially this virus was named *Herpesvirus atelopus* (HVA). The prototype isolate (strain Lennette) remained the only known isolate until 1986 when an apparently identical virus was isolated from vesicular lesions of another young spider monkey in France.⁷⁰ *A. geoffroyi* is considered to be the natural host of this virus although the evidence is circumstantial. The ICTV designation for this virus is AtHV-1.

The history of the naming of AtHV-1 is surprisingly similar to that of its “cousin” SaHV-1. Almost simultaneous with the isolation of AtHV-1, another herpesvirus was isolated from a spider monkey and was given the name Herpesvirus atelis. This second HVA was a T-lymphotropic oncogenic virus, also a rhadinovirus like SaHV-2. The simplex-HVA and rhadino-HVA were designated as HVA-1 and HVA-2, respectively. The ICTV has since classified them as simplexvirus species *Ateline herpesvirus 1* (AtHV-1) and rhadinovirus species *Ateline herpesvirus 2* (AtHV-2), respectively.

AtHV-1 infection in spider monkeys is usually subclinical. Experimental infection of marmosets with AtHV-1 is uniformly lethal. However, no outbreak due to the interspecies transmission of AtHV-1 has been reported. Rhesus monkeys and vervet African green monkeys are completely resistant to AtHV-1; probably, all Old World monkeys are resistant to this virus.

AtHV-1 can induce localized skin lesions in rabbits, guinea pigs, and mice when administered intradermally or subcutaneously. However, AtHV-1 does not induce a fatal generalized disease in these species.

Very little is known about the molecular characteristics of AtHV-1. The only genomic sequence available is a relatively short fragment (5,154 bp, GenBank Acc. No. M95785). AtHV-1 genes located in this fragment are collinear with the homologous genes of other primate simplexviruses and are most closely related to SaHV-1 (81% of nucleotide identity).

12.9. SUMMARY

The prototypes for the *Simplexvirus* genus of the *Alphaherpesvirinae* subfamily are human herpes simplex viruses 1 and 2. Several HSV-related simian viruses are currently known, namely, *Macacine herpesvirus 1* or B virus (former *Cercopithecine herpesvirus 1*), *Cercopithecine herpesvirus 2*, *Papiine herpesvirus 2* or Herpesvirus papio 2 (former *Cercopithecine herpesvirus 16*), Chimpanzee herpesvirus, *Saimiriine herpesvirus 1*, *Ateline herpesvirus 1*. Likely, there are many more simplexviruses harbored by NHP species.

The genomes of all primate simian viruses are collinear and highly homologous. Only one gene of human simplexviruses (RL1) is absent in simplexviruses of Old World monkeys. The genome expression pattern, features of their replication cycle, and mechanism of latency also appear very similar for human and simian simplexviruses.

Simian simplexviruses do not typically cause severe morbidity in their natural host species. However, they may be lethal for nonnatural hosts.

Most of the interest in simian simplexviruses is focused on B virus. This virus can be transmitted to humans from subclinically infected macaques, primarily rhesus monkeys. Although simian-to-human transmission of B virus is rare, the consequences of such inter-species transmission are very serious. The fatality rate for untreated B virus encephalitis in humans is 70–80%, and currently available treatments are not uniformly effective.

REFERENCES

1. Bennett, A. M., M. J. Slomka, D. W. Brown, G. Lloyd, and M. Mackett. 1999. Protection against herpes B virus infection in rabbits with a recombinant vaccinia virus expressing glycoprotein D. *J. Med. Virol.* 57(1):47–56.
2. Bigger, J. E. and D. W. Martin. 2004. Identification of an ICP47 homologue in simian agent 8 (SA8). *Virus Genes* 28(2):223–225.
3. Bigger, J. E. and D. W. Martin. 2006. Herpesvirus papio 2 (HVP2): sequence analysis of the unique short (US) region. *Virus Genes* 32(2):211–212.
4. Black, D. H. and R. Eberle. 1997. Detection and differentiation of primate alpha-herpesviruses by PCR. *J. Vet. Diagn. Invest.* 9(3):225–231.
5. Blewett, E. L., J. T. Saliki, and R. Eberle. 1999. Development of a competitive ELISA for detection of primates infected with monkey B virus (Herpesvirus simiae). *J. Virol. Methods* 77(1):59–67.
6. Borchers, K. and M. Ozel. 1993. Simian agent 8 (SA8): morphogenesis and ultrastructure. *Zentralbl. Bakteriol.* 279(4):526–536.
7. Boulter, E. A., S. S. Kalter, R. L. Heberling, J. E. Guajardo, and T. L. Lester. 1982. A comparison of neutralization tests for the detection of antibodies to Herpesvirus simiae (monkey B virus). *Lab. Anim. Sci.* 32(2):150–152.
8. Brack, M., J. W. Eichberg, R. L. Heberling, and S. S. Kalter. 1985. Experimental Herpes neonatalis in SA 8-infected baboons (*Papio cynocephalus*). *Lab. Anim.* 19(2):125–131.
9. Breshears, M. A., D. H. Black, J. W. Ritchey, and R. Eberle. 2003. Construction and in vivo detection of an enhanced green fluorescent protein-expressing strain of Saimiriine herpesvirus 1 (SaHV-1). *Arch. Virol.* 148(2):311–327.
10. Breshears, M. A., R. Eberle, and J. W. Ritchey. 2001. Characterization of gross and histological lesions in

- Balb/c mice experimentally infected with herpesvirus saimiri 1 (HVS1). *J. Comp Pathol.* 125(1):25–33.
11. Breshears, M. A., R. Eberle, and J. W. Ritchey. 2005. Temporal progression of viral replication and gross and histological lesions in Balb c mice inoculated epidermally with Saimiriine herpesvirus 1 (SaHV-1). *J. Comp. Pathol.* 133(2–3):103–113.
 12. Carlson, C. S., M. G. O'Sullivan, M. J. Jayo, D. K. Anderson, E. S. Harber, W. G. Jerome, B. C. Bullock, and R. L. Heberling. 1997. Fatal disseminated cercopithecine herpesvirus 1 (herpes B infection in cynomolgus monkeys (*Macaca fascicularis*). *Vet. Pathol.* 34(5):405–414.
 13. Cohen, J. I., D. S. Davenport, J. A. Stewart, S. Deitchman, J. K. Hilliard, and L. E. Chapman. 2002. Recommendations for prevention of and therapy for exposure to B virus (cercopithecine herpesvirus 1). *Clin. Infect. Dis.* 35(10):1191–1203.
 14. Coulibaly, C., R. Hack, J. Seidl, M. Chudy, G. Itter, and R. Plesker. 2004. A natural asymptomatic herpes B virus infection in a colony of laboratory brown capuchin monkeys (*Cebus apella*). *Lab. Anim.* 38(4):432–438.
 15. Daniel, M. D., A. Karpas, L. V. Melendez, N. W. King, and R. D. Hunt. 1967. Isolation of herpes-T virus from a spontaneous disease in squirrel monkeys (*Saimiri sciureus*). *Arch. Gesamte Virusforsch.* 22(3):324–331.
 16. Davenport, D. S., D. R. Johnson, G. P. Holmes, D. A. Jewett, S. C. Ross, and J. K. Hilliard. 1994. Diagnosis and management of human B virus (Herpesvirus simiae) infections in Michigan. *Clin. Infect. Dis.* 19(1):33–41.
 17. Desrosiers, R. C. and L. A. Falk Jr. 1981. Herpesvirus tamarinus and its relation to herpes simplex virus. *J. Gen. Virol.* 56(Pt 1):119–130.
 18. Eberle, R. 1992. Evidence for an alpha-herpesvirus indigenous to mountain gorillas. *J. Med. Primatol.* 21(5):246–251.
 19. Eberle, R. and D. Black. 1991. The simian herpesvirus SA8 homologue of the herpes simplex virus gB gene: mapping, sequencing, and comparison to the HSV gB. *Arch. Virol.* 118(1–2):67–86.
 20. Eberle, R. and D. Black. 1993. Sequence analysis of herpes simplex virus gB gene homologs of two platyrhine monkey alpha-herpesviruses. *Arch. Virol.* 129(1–4):167–182.
 21. Eberle, R., D. H. Black, T. W. Lehenbauer, and G. L. White. 1998. Shedding and transmission of baboon Herpesvirus papio 2 (HVP2) in a breeding colony. *Lab. Anim. Sci.* 48(1):23–28.
 22. Eberle, R., D. H. Black, S. Lipper, and J. K. Hilliard. 1995. Herpesvirus papio 2, an SA8-like alpha-herpesvirus of baboons. *Arch. Virol.* 140(3):529–545.
 23. Eberle, R. and J. K. Hilliard. 1984. Replication of simian herpesvirus SA8 and identification of viral polypeptides in infected cells. *J. Virol.* 50(2):316–324.
 24. Eberle, R. and J. K. Hilliard. 1989. Serological evidence for variation in the incidence of herpesvirus infections in different species of apes. *J. Clin. Microbiol.* 27(6):1357–1366.
 25. Eberle, R., M. Zhang, and D. H. Black. 1993. Gene mapping and sequence analysis of the unique short region of the simian herpesvirus SA 8 genome. *Arch. Virol.* 130(3–4):391–411.
 26. Eichberg, J. W., B. McCullough, S. S. Kalter, D. E. Thor, and A. R. Rodriguez. 1976. Clinical, virological, and pathological features of herpesvirus SA8 infection in conventional and gnotobiotic infant baboons (*Papio cynocephalus*). *Arch. Virol.* 50(4):255–270.
 27. Emmons, R. W., D. H. Gribble, and E. H. Lennette. 1968. Natural fatal infection of an owl monkey (*Aotus trivirgatus*) with Herpes T virus. *J. Infect. Dis.* 118(2):153–159.
 28. Engel, G. A., L. Jones-Engel, M. A. Schillaci, K. G. Suaryana, A. Putra, A. Fuentes, and R. Henkel. 2002. Human exposure to herpesvirus B-seropositive macaques, Bali, Indonesia. *Emerg. Infect. Dis.* 8(8):789–795.
 29. Focher, F., A. Lossani, A. Verri, S. Spadari, A. Maioli, J. J. Gambino, G. E. Wright, R. Eberle, D. H. Black, P. Medveczky, M. Medveczky, and D. Shugar. 2007. Sensitivity of monkey B virus (Cercopithecine herpesvirus 1) to antiviral drugs: role of thymidine kinase in antiviral activities of substrate analogs and acyclonucleosides. *Antimicrob. Agents Chemother.* 51(6):2028–2034.
 30. Freifeld, A. G., J. Hilliard, J. Southers, M. Murray, B. Savarese, J. M. Schmitt, and S. E. Straus. 1995. A controlled seroprevalence survey of primate handlers for evidence of asymptomatic herpes B virus infection. *J. Infect. Dis.* 171(4):1031–1034.
 31. Fujima, A., Y. Ochiai, A. Saito, Y. Omori, A. Noda, Y. Kazuyama, H. Shoji, K. Tanabayashi, F. Ueda, Y. Yoshikawa, and R. Hondo. 2008. Discrimination of antibody to herpes B virus from antibody to herpes simplex virus types 1 and 2 in human and macaque sera. *J. Clin. Microbiol.* 46(1):56–61.
 32. Gay, F. P. and M. Holden. 1933. The herpes encephalitis problem. *J. Infect. Dis.* 53:287–303.
 33. Gupta, A., J. J. Gartner, P. Sethupathy, A. G. Hatziogiorgiou, and N. W. Fraser. 2006. Anti-apoptotic function of a microRNA encoded by the HSV-1

- latency-associated transcript. *Nature* 442(7098): 82–85.
34. Heberling, R. L. and S. S. Kalter. 1987. A dot-immunobinding assay on nitrocellulose with psoralen inactivated Herpesvirus simiae (B virus). *Lab. Anim. Sci.* 37(3):304–308.
 35. Henkel, R. D., H. M. McClure, P. Krug, D. Katz, and J. K. Hilliard. 2002. Serological evidence of alpha herpesvirus infection in sooty mangabeys. *J. Med. Primatol.* 31(3):120–128.
 36. Hilliard, J. K. and J. A. Ward. 1999. B-virus specific-pathogen-free breeding colonies of macaques (*Macaca mulatta*): retrospective study of seven years of testing. *Lab. Anim. Sci.* 49(2):144–148.
 37. Hirano, M., S. Nakamura, F. Mitsunaga, M. Okada, K. Shimizu, M. Ueda, A. Bennett, and R. Eberle. 2002. Efficacy of a B virus gDNA vaccine for induction of humoral and cellular immune responses in Japanese macaques. *Vaccine* 20(19–20):2523–2532.
 38. Hirano, M., S. Nakamura, F. Mitsunaga, M. Okada, S. Shirahama, and R. Eberle. 2002. One-step PCR to distinguish B virus from related primate alphaherpesviruses. *Clin. Diagn. Lab. Immunol.* 9(3):716–719.
 39. Hirano, M., S. Nakamura, M. Okada, M. Ueda, and R. Mukai. 2000. Rapid discrimination of monkey B virus from human herpes simplex viruses by PCR in the presence of betaine. *J. Clin. Microbiol.* 38(3):1255–1257.
 40. Holmes, A. W., R. G. Cadwell, R. E. Dedmon, and F. Deinhardt. 1964. Isolation and characterization of a new herpes virus. *J. Immunol.* 92:602–610.
 41. Holmes, A. W., J. A. Devine, E. Nowakowski, and F. Deinhardt. 1966. The epidemiology of a herpes virus infection of New World monkeys. *J. Immunol.* 96(4):668–671.
 42. Holmes, G. P., J. K. Hilliard, K. C. Klontz, A. H. Rupert, C. M. Schindler, E. Parrish, D. G. Griffin, G. S. Ward, N. D. Bernstein, T. W. Bean. 1990. B virus (Herpesvirus simiae) infection in humans: epidemiologic investigation of a cluster. *Ann. Intern. Med.* 112(11):833–839.
 43. Huff, J. L., R. Eberle, J. Capitanio, S. S. Zhou, and P. A. Barry. 2003. Differential detection of B virus and rhesus cytomegalovirus in rhesus macaques. *J. Gen. Virol.* 84(Pt 1):83–92.
 44. Hull, R. N. 1971. B virus vaccine. *Lab. Anim. Sci.* 21(6):1068–1071.
 45. Hull, R. N., A. C. Dwyer, A. W. Holmes, E. Nowakowski, F. Deinhardt, E. H. Lennette, and R. W. Emmons. 1972. Recovery and characterization of a new simian herpesvirus from a fatally infected spider monkey. *J. Natl. Cancer Inst.* 49(1):225–231.
 46. Hull, R. N. and J. C. Nash. 1960. Immunization against B virus infection. I. Preparation of an experimental vaccine. *Am. J. Hyg.* 71:15–28.
 47. Hull, R. N., F. B. Peck Jr., T. G. Ward, and J. C. Nash. 1962. Immunization against B virus infection. II. Further laboratory and clinical studies with an experimental vaccine. *Am. J. Hyg.* 76:239–251.
 48. Johnson, G., D. Dick, M. Ayers, M. Petric, and R. Tellier. 2003. Detection and species-level identification of primate herpesviruses with a comprehensive PCR test for human herpesviruses. *J. Clin. Microbiol.* 41(3):1256–1258.
 49. Katz, D., J. K. Hilliard, R. Eberle, and S. L. Lipper. 1986. ELISA for detection of group-common and virus-specific antibodies in human and simian sera induced by herpes simplex and related simian viruses. *J. Virol. Methods* 14(2):99–109.
 50. Katz, D., W. Shi, P. W. Krug, R. Henkel, H. McClure, and J. K. Hilliard. 2002. Antibody cross-reactivity of alphaherpesviruses as mirrored in naturally infected primates. *Arch. Virol.* 147(5):929–941.
 51. Katz, D., W. Shi, P. W. Krug, and J. K. Hilliard. 2002. Alphaherpesvirus antigen quantitation to optimize the diagnosis of herpes B virus infection. *J. Virol. Methods* 103(1):15–25.
 52. Keeble, S. A., G. J. Christofinis, and W. Wood. 1958. Natural virus-B infection in rhesus monkeys. *J. Pathol. Bacteriol.* 76(1):189–199.
 53. Kessler, M. J., W. T. London, D. L. Madden, J. M. Dambrosia, J. K. Hilliard, K. F. Soike, and R. G. Rawlins. 1989. Serological survey for viral diseases in the Cayo Santiago rhesus macaque population. *P. R. Health Sci. J.* 8(1):95–97.
 54. Lees, D. N., A. Baskerville, L. M. Cropper, and D. W. Brown. 1991. Herpesvirus simiae (B virus) antibody response and virus shedding in experimental primary infection of cynomolgus monkeys. *Lab. Anim. Sci.* 41(4):360–364.
 55. Levin, J. L., J. K. Hilliard, S. L. Lipper, T. M. Butler, and W. J. Goodwin. 1988. A naturally occurring epizootic of simian agent 8 in the baboon. *Lab. Anim. Sci.* 38(4):394–397.
 56. Loomis, M. R., T. O'Neill, M. Bush, and R. J. Montali. 1981. Fatal herpesvirus infection in patas monkeys and a black and white colobus monkey. *J. Am. Vet. Med. Assoc.* 179(11):1236–1239.
 57. Loomis-Huff, J. E., R. Eberle, K. M. Lockridge, G. Rhodes, and P. A. Barry. 2001. Immunogenicity of a DNA vaccine against herpes B virus in mice and rhesus macaques. *Vaccine* 19(32):4865–4873.
 58. Luebcke, E., E. Dubovi, D. Black, K. Ohsawa, and R. Eberle. 2006. Isolation and characterization of a

- chimpanzee alphaherpesvirus. *J. Gen. Virol.* 87(Pt 1):11–19.
59. Malherbe, H. and R. Harwin. 1958. Neurotropic virus in African monkeys. *Lancet* 2:530.
60. Malherbe, H. and M. Strickland-Cholmley. 1969. Simian herpesvirus SA8 from a baboon. *Lancet* 2(7635):1427.
61. Malherbe, H. and M. Strickland-Cholmley. 1969. Virus from baboons. *Lancet* 2(7633):1300.
62. Martino, M. A., G. B. Hubbard, T. M. Butler, and J. K. Hilliard. 1998. Clinical disease associated with simian agent 8 infection in the baboon. *Lab. Anim. Sci.* 48(1):18–22.
63. Matsubayashi, K., S. Goton, Y. Kawamoto, T. Watanabe, K. Nozawa, M. Takasaka, T. Narita, O. Griffiths, and M.-A. Stankey. 1992. Clinical examinations on crab-eating macaques in Mauritius. *Primates* 33(2):281–288.
64. McClure, H. M., R. B. Swenson, S. S. Kalter, and T. L. Lester. 1980. Natural genital herpesvirus hominis infection in chimpanzees (*Pan troglodytes* and *Pan paniscus*). *Lab. Anim. Sci.* 30(5):895–901.
65. Melnick, J. L., M. Midulla, I. Wimberly, J. G. Barrera-Oro, and B. M. Levy. 1964. A new member of the herpesvirus group isolated from South American marmosets. *J. Immunol.* 92:596–601.
66. Miranda, M. B., M. Handermann, and G. Darai. 2005. DNA polymerase gene locus of Cercopithecine herpesvirus 1 is a suitable target for specific and rapid identification of viral infection by PCR technology. *Virus Genes* 30(3):307–322.
67. Mitsunaga, F. and S. Nakamura. 2006. Prevalence of B Virus and Cytomegalovirus infections among Japanese macaque troops. *Exp. Anim.* 55(3):233.
68. Mitsunaga, F., S. Nakamura, T. Hayashi, and R. Eberle. 2007. Changes in the titer of anti-B virus antibody in captive macaques (*Macaca fuscata*, *M. mulatta*, *M. fascicularis*). *Comp. Med.* 57(1):120–124.
69. Mootnick, A. R., M. Reingold, H. J. Holshuh, and R. R. Mirkovic. 1998. Isolation of a herpes simplex virus type 1-like agent from the brain of a mountain agile gibbon (*Hylobates agilis agilis*) with encephalitis. *J. Zoo. Wildl. Med.* 29(1):61–64.
70. Mou, S. W., J. K. Hilliard, C. H. Song, and R. Eberle. 1986. Comparison of the primate alphaherpesviruses. I. Characterization of two herpesviruses from spider monkeys and squirrel monkeys and viral polypeptides synthesized in infected cells. *Arch. Virol.* 91(1–2):117–133.
71. Norcott, J. P. and D. W. Brown. 1993. Competitive radioimmunoassay to detect antibodies to herpes B virus and SA8 virus. *J. Clin. Microbiol.* 31(4):931–935.
72. Ohsawa, K., D. H. Black, H. Sato, K. Rogers, and R. Eberle. 2003. Sequence and genetic arrangement of the UL region of the monkey B virus (Cercopithecine herpesvirus 1) genome and comparison with the UL region of other primate herpesviruses. *Arch. Virol.* 148(5):989–997.
73. Ohsawa, K., D. H. Black, R. Torii, H. Sato, and R. Eberle. 2002. Detection of a unique genotype of monkey B virus (Cercopithecine herpesvirus 1) indigenous to native Japanese macaques (*Macaca fuscata*). *Comp. Med.* 52(6):555–559.
74. Ohsawa, K., T. W. Lehenbauer, and R. Eberle. 1999. Herpesvirus papio 2: alternative antigen for use in monkey B virus diagnostic assays. *Lab. Anim. Sci.* 49(6):605–616.
75. Orcutt, R. P., G. J. Pucak, H. L. Foster, J. T. Kilcourse, and T. Ferrell. 1976. Multiple testing for the detection of B virus antibody in specially handled rhesus monkeys after capture from virgin trapping grounds. *Lab. Anim. Sci.* 26(1):70–74.
76. Oya, C., Y. Ochiai, Y. Taniuchi, T. Takano, F. Ueda, Y. Yoshikawa, and R. Hondo. 2004. Specific detection and identification of herpes B virus by a PCR-microplate hybridization assay. *J. Clin. Microbiol.* 42(5):1869–1874.
77. Payton, M. E., J. M. d'Offay, M. E. Prado, D. H. Black, B. Damania, G. L. White, and R. Eberle. 2004. Comparative transmission of multiple herpesviruses and simian virus 40 in a baboon breeding colony. *Comp. Med.* 54(6):695–704.
78. Pellett, P. E. and B. Roizman. 2007. The family *Herpesviridae*: a brief introduction. In: Knipe, D. M. and P. M. Howley (eds), *Fields Virology*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, Wolters Kluwer Business, pp. 2480–2499.
79. Perelygina, L., I. Patrusheva, S. Hombaiah, H. Zurkuhlen, M. J. Wildes, N. Patrushev, and J. K. Hilliard. 2005. Production of herpes B virus recombinant glycoproteins and evaluation of their diagnostic potential. *J. Clin. Microbiol.* 43(2):620–628.
80. Perelygina, L., I. Patrusheva, N. Manes, M. J. Wildes, P. Krug, and J. K. Hilliard. 2003. Quantitative real-time PCR for detection of monkey B virus (Cercopithecine herpesvirus 1) in clinical samples. *J. Virol. Methods* 109(2):245–251.
81. Perelygina, L., I. Patrusheva, H. Zurkuhlen, and J. K. Hilliard. 2002. Characterization of B virus glycoprotein antibodies induced by DNA immunization. *Arch. Virol.* 147(11):2057–2073.
82. Perelygina, L., L. Zhu, H. Zurkuhlen, R. Mills, M. Borodovsky, and J. K. Hilliard. 2003. Complete sequence and comparative analysis of the genome of

- herpes B virus (Cercopithecine herpesvirus 1) from a rhesus monkey. *J. Virol.* 77(11):6167–6177.
83. Perelygina, L., H. Zurkuhlen, I. Patrusheva, and J. K. Hilliard. 2002. Identification of a herpes B virus-specific glycoprotein D immunodominant epitope recognized by natural and foreign hosts. *J. Infect. Dis.* 186(4):453–461.
 84. Ritchey, J. W., K. A. Ealey, M. E. Payton, and R. Eberle. 2002. Comparative pathology of infections with baboon and African green monkey alpha-herpesviruses in mice. *J. Comp. Pathol.* 127(2–3):150–161.
 85. Ritchey, J. W., M. E. Payton, and R. Eberle. 2005. Clinicopathological characterization of monkey B virus (Cercopithecine herpesvirus 1) infection in mice. *J. Comp. Pathol.* 132(2–3):202–217.
 86. Rogers, K. M., D. H. Black, and R. Eberle. 2007. Primary mouse dermal fibroblast cell cultures as an in vitro model system for the differential pathogenicity of cross-species herpesvirus papio 2 infections. *Arch. Virol.* 152(3):543–552.
 87. Rogers, K. M., K. A. Ealey, J. W. Ritchey, D. H. Black, and R. Eberle. 2003. Pathogenicity of different baboon herpesvirus papio 2 isolates is characterized by either extreme neurovirulence or complete apathogenicity. *J. Virol.* 77(20):10731–10739.
 88. Rogers, K. M., J. W. Ritchey, M. Payton, D. H. Black, and R. Eberle. 2006. Neuropathogenesis of herpesvirus papio 2 in mice parallels infection with Cercopithecine herpesvirus 1 (B virus) in humans. *J. Gen. Virol.* 87(Pt 2):267–276.
 89. Rogers, K. M., R. F. Wolf, G. L. White, and R. Eberle. 2005. Experimental infection of baboons (*Papio cynocephalus anubis*) with apathogenic and neurovirulent subtypes of herpesvirus papio 2. *Comp. Med.* 55(5):425–430.
 90. Sabin, A. R. and W. M. Wright. 1934. Acute ascending myelitis following a monkey bite, with isolation of a virus capable of reproducing the disease. *J. Exp. Med.* 59:115–136.
 91. Sariol, C. A., T. Arana, E. Maldonado, M. Gerald, J. Gonzalez-Martinez, M. Rodriguez, and E. N. Kraiselburd. 2005. Herpes B-virus seroreactivity in a colony of *Macaca mulatta*: data from the Sabana Seca Field Station, a new specific pathogen-free program. *J. Med. Primatol.* 34(1):13–19.
 92. Sato, H., J. Arikawa, M. Furuya, J. Kitoh, K. Mannen, Y. Nishimune, K. Ohsawa, T. Serikawa, T. Shiba-hara, Y. Watanabe, K. Yagami, H. Yamamoto, and Y. Yoshikawa. 1998. Prevalence of herpes B virus antibody in nonhuman primates reared at the National University of Japan. *Exp. Anim.* 47(3):199–202.
 93. Schillaci, M. A., L. Jones-Engel, G. A. Engel, Y. Paramastri, E. Iskandar, B. Wilson, J. S. Allan, R. C. Kyes, R. Watanabe, and R. Grant. 2005. Prevalence of enzootic simian viruses among urban performance monkeys in Indonesia. *Trop. Med. Int. Health* 10(12):1305–1314.
 94. Scinicariello, F., R. Eberle, and J. K. Hilliard. 1993. Rapid detection of B virus (herpesvirus simiae) DNA by polymerase chain reaction. *J. Infect. Dis.* 168(3):747–750.
 95. Shah, K. V. and C. H. Southwick. 1965. Prevalence of antibodies to certain viruses in sera of free-living rhesus and of captive monkeys. *Indian J. Med. Res.* 53:488–500.
 96. Simon, M. A., M. D. Daniel, D. Lee-Parritz, N. W. King, and D. J. Ringler. 1993. Disseminated B virus infection in a cynomolgus monkey. *Lab. Anim. Sci.* 43(6):545–550.
 97. Slomka, M. J., D. W. Brown, J. P. Clewley, A. M. Bennett, L. Harrington, and D. C. Kelly. 1993. Polymerase chain reaction for detection of herpesvirus simiae (B virus) in clinical specimens. *Arch. Virol.* 131(1–2):89–99.
 98. Slomka, M. J., L. Harrington, C. Arnold, J. P. Norcott, and D. W. Brown. 1995. Complete nucleotide sequence of the herpesvirus simiae glycoprotein G gene and its expression as an immunogenic fusion protein in bacteria. *J. Gen. Virol.* 76(Pt 9):2161–2168.
 99. Smith, A. L., D. H. Black, and R. Eberle. 1998. Molecular evidence for distinct genotypes of monkey B virus (herpesvirus simiae) which are related to the macaque host species. *J. Virol.* 72(11):9224–9232.
 100. Takano, J., T. Narita, K. Fujimoto, R. Mukai, and A. Yamada. 2001. Detection of B virus infection in cynomolgus monkeys by ELISA using simian agent 8 as alternative antigen. *Exp. Anim.* 50(4):345–347.
 101. Tanabayashi, K., R. Mukai, and A. Yamada. 2001. Detection of B virus antibody in monkey sera using glycoprotein D expressed in mammalian cells. *J. Clin. Microbiol.* 39(9):3025–3030.
 102. Thompson, S. A., J. K. Hilliard, D. Kittel, S. Lipper, W. E. Giddens Jr., D. H. Black, and R. Eberle. 2000. Retrospective analysis of an outbreak of B virus infection in a colony of DeBrazza's monkeys (Cercopithecus neglectus). *Comp. Med.* 50(6):649–657.
 103. Tosi, A. J. and C. S. Coke. 2007. Comparative phylogenetics offer new insights into the biogeographic history of *Macaca fascicularis* and the origin of the Mauritian macaques. *Mol. Phylogenet. Evol.* 42(2):498–504.
 104. Troan, B. V., L. Perelygina, I. Patrusheva, A. J. Wettere, J. K. Hilliard, M. R. Loomis, and R. S. Voe. 2007. Naturally transmitted herpesvirus papio-2

- infection in a black and white colobus monkey. *J. Am. Vet. Med. Assoc.* 231(12):1878–1883.
105. Tyler, S. D., G. A. Peters, and A. Severini. 2005. Complete genome sequence of cercopithecine herpesvirus 2 (SA8) and comparison with other simplexviruses. *Virology* 331(2):429–440.
106. Tyler, S. D. and A. Severini. 2006. The complete genome sequence of herpesvirus papio 2 (Cercopithecine herpesvirus 16) shows evidence of recombination events among various progenitor herpesviruses. *J. Virol.* 80(3):1214–1221.
107. Ward, J. A. and J. K. Hilliard. 1994. B virus-specific pathogen-free (SPF) breeding colonies of macaques: issues, surveillance, and results in 1992. *Lab. Anim. Sci.* 44(3):222–228.
108. Ward, J. A. and J. K. Hilliard. 2002. Herpes B virus-specific pathogen-free breeding colonies of macaques: serologic test results and the B-virus status of the macaque. *Contemp. Top. Lab. Anim. Sci.* 41(4):36–41.
109. Ward, J. A., J. K. Hilliard, and S. Pearson. 2000. Herpes B-virus specific-pathogen-free breeding colonies of macaques (*Macaca mulatta*): diagnostic testing before and after elimination of the infection. *Comp. Med.* 50(3):317–322.
110. Weigler, B. J., D. W. Hird, J. K. Hilliard, N. W. Lerche, J. A. Roberts, and L. M. Scott. 1993. Epidemiology of cercopithecine herpesvirus 1 (B virus) infection and shedding in a large breeding cohort of rhesus macaques. *J. Infect. Dis.* 167(2):257–263.
111. Weigler, B. J., J. A. Roberts, D. W. Hird, N. W. Lerche, and J. K. Hilliard. 1990. A cross sectional survey for B virus antibody in a colony of group housed rhesus macaques. *Lab. Anim. Sci.* 40(3):257–261.
112. Weigler, B. J., F. Scinicariello, and J. K. Hilliard. 1995. Risk of venereal B virus (cercopithecine herpesvirus 1) transmission in rhesus monkeys using molecular epidemiology. *J. Infect. Dis.* 171(5):1139–1143.
113. Whitley, R. J. and J. Hilliard. 2007. Cercopithecine herpes virus 1 (B virus). In: Knipe, D. M. and P. M. Howley (eds), *Fields Virology*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, Wolters Kluwer Business, pp. 2889–2903.
114. Wilson, R. B., M. A. Holscher, T. Chang, and J. R. Hodges. 1990. Fatal Herpesvirus simiae (B virus) infection in a patas monkey (*Erythrocebus patas*). *J. Vet. Diagn. Invest.* 2(3):242–244.
115. Wolf, R. F., K. M. Rogers, E. L. Blewett, D. P. Dittmer, F. D. Fakhari, C. A. Hill, S. D. Kosanke, G. L. White, and R. Eberle. 2006. A naturally occurring fatal case of Herpesvirus papio 2 pneumonia in an infant baboon (*Papio hamadryas anubis*). *J. Am. Assoc. Lab. Anim. Sci.* 45(1):64–68.
116. Wolfensohn, S. E. and R. Gopal. 2001. Interpretation of serological test results for simian herpes B virus. *Lab. Anim.* 35(4):315–320.
117. Yamamoto, H., K. Ohsawa, S. E. Walz, J. L. Mitchen, Y. Watanabe, R. Eberle, H. Origasa, and H. Sato. 2005. Validation of an enzyme-linked immunosorbent assay kit using herpesvirus papio 2 (HVP2) antigen for detection of herpesvirus simiae (B virus) infection in rhesus monkeys. *Comp. Med.* 55(3):244–248.

13

Varicelloviruses

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13.1. INTRODUCTION

Chicken pox or varicella, one of the most common childhood diseases, is caused by a herpesvirus named varicella-zoster virus (VZV). The virus name emphasizes the “dual” pathogenicity of VZV; it causes two diseases, varicella and herpes zoster. The pathogenesis of these diseases is quite different, as are the susceptible age groups. Varicella is a clinical outcome of a primary VZV infection which usually occurs in children, and is manifested by characteristic skin eruptions. Uncomplicated varicella is a self-limited disease. However, recovery from varicella is not accompanied by clearance of the virus. During primary infection, VZV “travels” from the skin through sensory nerve axons to neural ganglia where it establishes a latent infection. Latent VZV may remain dormant throughout the rest of the host’s life or

the virus can reactivate. Reactivation is triggered by immunosuppression of different kinds, including natural waning of immunity associated with the aging. Clinically, the reactivation of latent VZV is manifested as “herpes zoster,” very painful skin lesions localized in areas innervated by the ganglion in which the reactivation occurred.

The simian counterpart of VZV, commonly referred to as simian varicella virus (SVV), was originally isolated in 1967 from the African green monkey (*Chlorocebus aethiops*).⁶ SVV induces disease in both Asian and African nonhuman primate (NHP) species and the mortality rate can be as high as 75%. Many properties of SVV, including its pathogenicity, mirror those of VZV, which makes SVV infection an excellent model of human VZV infection and its associated diseases (reviewed in Gray¹⁴).

13.2. CLASSIFICATION AND NOMENCLATURE

SVV is classified by the International Committee for Taxonomy of Viruses (ICTV) as *Cercopithecine herpesvirus 9* (CeHV-9), a species belonging to the genus *Varicellavirus* within the *Alphaherpesvirinae* subfamily of the family *Herpesviridae*. Human VZV (ICTV-approved name *Human herpesvirus 3*) is the prototype species for this genus. The official name of the virus is rarely used; the designation SVV continues to prevail and this will probably not change in the foreseeable future. In this chapter, we have opted to use the SVV designation.

Many SVV isolates have been described. Initially each was considered to be a unique virus and was given its own name (Table 13.1). With the accumulation of data it became clear that all of these isolates are closely

Table 13.1. Simian Varicella Virus Isolates

Isolate	Host Species	References
Liverpool vervet virus (LVV)	<i>Chlocebus aethiops</i>	6
Patas herpesvirus (PHV)	<i>Erythrocebus patas</i>	37
Delta herpesvirus (DHV-1, DHV-2)	<i>E. patas</i>	1
Medical Lake macaque virus (MLMV)	<i>Macaca nemestrina, M. fuscata, M. fascicularis</i>	4
Litton herpesvirus	<i>E. patas, C. aethiops</i>	44
Hazelton herpesvirus (HAZV)	<i>M. mulatta, M. fascicularis, C. aethiops</i>	14
Tsukuba herpesvirus	<i>M. fascicularis</i>	54

related, being either identical or differing only to the degree typical of different isolates of a single virus.^{10,11,17}

The morphology of CeHV-1 virions and nucleocapsids is typical for herpesviruses. The abundance of degraded virions among extracellular virus particles in cytoplasmic vacuoles is quite characteristic for SVV-infected cells. However, morphology alone cannot be used for reliable identification of SVV.

13.3. GENOMIC ORGANIZATION AND GENE PRODUCTS

The complete genome of SVV (strain Delta) has been sequenced¹⁹ (GenBank Acc. No. AF275348); its length is 124,784 bp. The “macro” structure of the SVV and VZV genomes is virtually identical (Figure 13.1).

The genomes are collinear; that is, the homologous genes are in the same order. The gene repertoire is also almost the same; a few exceptions are described below. The sequence homology is high: 89% on average in the most similar areas covering 45% of the genome, and slightly lower in the rest of the genome. The genome of SVV can be viewed as consisting of two major linear components: the long (L) and short (S), about 100 kb

and 20 kb, respectively. The S component has a 4,904 bp central unique region (US) which is flanked by inverted repeats of 7,557 bp. In the prototype arrangement of the genome, the left (upstream of the L region) and right (downstream of the L region) inverted repeats are designated IRS (internal repeat S) and TRS (terminal repeat S), respectively.¹⁸ The unique sequence part of the L component (UL) covers almost the entire length of the L region (104,120 bp). UL is flanked by two inverted repeats (TRL and URL) which in contrast to TRS and URS, are very short, just 8 bp in length. The L and S regions are covalently linked, but the orientation of the L region can be inverted relative to the S component. Accordingly, there are two isomeric forms of the SVV genome. It is believed that these genomic isomers are biologically indistinguishable, although direct experimental evidence supporting this is absent.

Sixty-nine open reading frames (ORFs) have been identified in the SVV genome (Table 13.2).

Transcripts corresponding to all these ORFs have been identified in the productively infected Vero cells.⁷ One ORF located within IRS/TRS is repeated three times (ORF-69, -70, -71). All but one of the SVV ORFs have homologs in the VZV genome, and the SVV



Figure 13.1. “Macro” map of SVV genome. U_L , long unique region; U_S , short unique region; TRL, terminal repeats flanking U_L ; IRS, internal repeat sequence; TRS, terminal repeat sequence. IRS and TRS flank U_S . R1, R2, R3, short tandem repeat sequences within U_L ; R4, short tandem repeat sequence within IRS and TRS. Macro maps of SVV and VZV genomes are collinear, except the presence of additional short tandem repeat R5 (not shown) in the VZV genome.

Table 13.2. Characteristics of CeHV-9 Open Reading Frames (ORFs) and Their Products²³

ORF	% Homology with VZV	Product Size CeHV-9 (aa)	Product Size VZV (aa)	HSV-1 Homologous ORF	Product/Presumptive Function
A	30	293			
B		114	157–224	UL54	Truncated homolog of ORF 4 ³⁶ Homolog of VZV ORF S/L ³⁶
1	27	101	108		Membrane protein
2			238		
3	64	183	179	UL55	Virion assembly
4	43	470	452	UL54	Transcriptional activator, immediate early protein 2
5	60	337	340	UL53	Glycoprotein K
6	38	1,081	1,083	UL52	Component of DNA helicase–primase complex
7	73	231	259	UL51	Virion phosphoprotein
8	38	395	396	UL50	DeoxyUTPase
9A	69	87	87	UL49A	Glycoprotein N
9	60	301	302	UL49	Tegument protein
10	62	406	410	UL48	Tegument protein, transcriptional activator
11	51	642	819	UL47	Tegument protein
12	60	655	661	UL46	Tegument protein
13	71	295	301		Thymidylate synthetase
14	44	540	560	UL44	Glycoprotein C ¹⁵
15	36	421	406	UL43	Membrane protein

Table 13.2. (Continued)

ORF	% Homology with VZV	Product Size CeHV-9 (aa)	Product Size VZV (aa)	HSV-1 Homologous ORF	Product/Presumptive Function
16	47	385	408	UL42	Associated with DNA polymerase
17	55	471	456	UL41	Host shutoff protein
18	73	311	306	UL40	Ribonucleotide reductase, small subunit
19	689	783	775	UL39	Ribonucleotide reductase, large subunit
20	60	467	483	UL38	Capsid protein
21	52	1,041	1,038	UL37	Tegument protein ⁵
22	49	2,653	2,763	UL36	Tegument protein
23	47	228	235	UL35	Capsid protein
24	61	214	269	UL34	Membrane phosphoprotein
25	45	153	156	UL33	Viral DNA cleavage/packaging
26	61	572	585	UL32	DNA cleavage/packaging
27	74	310	333	UL31	Nuclear phosphoprotein
28	66	1,172	1,194	UL30	DNA polymerase ³⁹
29	72	1,194	1,204	UL29	SsDNA-binding protein ³⁹
30	61	760	770	UL28	Viral DNA cleavage/packaging
31	75	916	868	UL27	Glycoprotein B
32	50	135	143		Phosphoprotein
33	64	588	605		Protease, capsid assembly protein
34	61	579	579		Viral DNA cleavage/packaging
35	50	248	258	UL25	Membrane protein
36	52	337	341	UL24	Thymidine kinase ⁴²
37	55	852	841	UL23	Glycoprotein H ^{3,41}
38	60	533	541	UL22	Virion protein
39	53	223	240	UL21	Envelope protein, viral egress
40	73	1,392	1,396	UL20	Major capsid protein
41	70	315	316	UL19	Viral terminase
42/45	68	744	747	UL18	Viral DNA cleavage/packaging
43	47	678	676	UL17	Virion protein
44	69	360	363	UL16	Tegument protein
46	58	199	199	UL14	Protein kinase
47	65	507	510	UL13	Deoxyribonuclease
48	56	509	551	UL12	

49	50	82	81	UL11
50	57	439	435	UL10
51	54	816	836	UL9
52	50	765	771	UL8
53	57	304	331	UL7
54	59	735	769	UL6
55	75	869	881	UL5
56	38	187	244	UL4
57	39	72	71	
58	42	203	221	UL3
59	55	300	305	UL2
60	43	175	159	UL1
61	43	503	467	RL2
62	58	1,279	1,310	RSI
63	52	261	278	US1
64	56	187	180	US10
65	49	77	102	US9
66	66	345	393	US3
67	37	353	354	US7
68	47	604	623	US8
69	56	187	180	US10
70	52	261	278	US1
71	58	1,279	1,310	ICP4

ORFs are numbered according to their VZV counterparts. ORFs unique to SVV or VZV are located in the terminal part of the L region: ORF A is present only in the SVV genome whereas ORF-2 is present only in the VZV genome. SVV ORF-A apparently is a truncated version of ORF-4, which is present in both viral genomes. The only sequence that is truly unique to SVV is located at the left terminus of the genome. It consists of 507 bp of unique sequence flanked by 79 bp inverted repeats. Interestingly, a short fragment of this sequence (64 bp) separates the L and S genomic segments.³² The functional significance of these sequences, unique to SVV, is unknown.

Both the SVV and VZV genomes have several direct tandem repeat sequences. There are four such repeats in the SVV genome that have been designated R1–R4 (Figure 13.1). The repeats are either located in noncoding regions between ORFs (R1 between ORF-7 and ORF-8; R4 between ORF-62 and ORF-63 within IRS as well as between ORF-70 and ORF-71 within TRS) or are “embedded” within coding sequences (R2 in ORF-14 and R3 in ORF-22). The R5 repeat present in the VZV genome between ORF-60 and ORF-61 is absent in the SVV genome.

The predicted properties of SVV proteins are listed in Table 13.2. The putative functions of these proteins are assumed to be the same as those of the better studied homologs of VZV and HSV. Relatively few SVV proteins have been characterized experimentally.

The most detailed studies have focused on the SVV glycoproteins gB, gC, gE, gH, and gL.^{3,15,21,41} Viral enzymes involved in viral DNA synthesis, including thymidine kinase (TK) and uracil DNA polymerase, have also been characterized.^{2,42} The product of ORF-62, in line with predictions based on homology with HSV and VZV counterparts of this protein, is the transcriptional trans-activator of SVV immediate early genes.

SVV genes are presumed to be expressed similarly to the genomes of the better studied alphaherpesviruses, HSV and VZV.⁷ In these viruses there are three cascades of transcription translation: immediate early, early, and late.

13.4. OVERVIEW OF REPLICATION CYCLE

The main features of the SVV cycle in vitro are presumed to follow the much better studied simplex viruses.¹⁴ However, very little is known beyond the

general alphaherpesvirus replication scheme about the specifics of SVV replication.

A peculiar feature of the in vitro replication of varicelloviruses, including SVV, is the high degree of cell association of newly produced virions. As a result, titers of infectious SVV produced in permissive cell cultures are relatively low (10^2 – 10^4 pfu/mL). This makes synchronous infection of permissive cells at high multiplicity difficult to achieve, hampering investigation of molecular aspects of the virus replicative cycle.

13.5. HOST RANGE IN VIVO AND PREVALENCE OF INFECTION

Outbreaks and sporadic cases of simian varicella have been observed in both African monkeys (*Chlorocebus aethiops*, *Erythrocebus patas*) and Asian monkeys (*Macaca nemestrina*, *M. fascicularis*, *M. fuscata*, *M. mulatta*). Among macaques, rhesus monkeys appear to be least susceptible to SVV.¹⁴ The natural host of SVV is not known. There are no published reports on the prevalence of this infection in feral monkeys and only one study has reported on the prevalence of antibodies against SVV in newly captured Malaysian cynomolgus macaques.⁴ In this study, the seroprevalence of SVV in the first days after capture was rather low at 0.8%, but it increased dramatically to 4% at 1 month and 40% at 3 months after capture. This observation indicates that SVV spreads very rapidly in crowded conditions such as those usually experienced by recently captured monkeys. The stress associated with capture likely contributes to such an explosive spread of the virus by stimulating reactivation of virus in latently infected monkeys.

13.6. MODE OF TRANSMISSION

Natural transmission of SVV occurs by inhalation of aerosols containing the virus. Transmission by direct contact with infected skin lesions is also possible.^{29,60} The virus is present in large quantities in skin and mucosal lesions that develop during both primary and reactivated infections. SVV can be readily isolated from the oral secretions of seropositive rhesus monkeys. Whether or not SVV is also excreted in urine and feces is not clear.

13.7. PATHOGENICITY

Natural or experimental infection of captive monkeys with SVV usually leads to the development of simian varicella, although subclinical infections also occur even after experimental inoculation.^{56,57} The incubation



Figure 13.2. Cutaneous manifestation of simian varicella. Rash on the torso of an African green monkey 10 days PI with SVV; maculopapular and vesicular eruptions are present simultaneously. (Image is kindly provided by Dr. Vicki L. Traina-Dorge.) See color version page 12.

period of acute simian varicella is 10–15 days. The clinical presentation of simian varicella is similar to that of human chicken pox.

The kinetics of the clinical presentation of varicella is known from experimental infection studies in African green monkeys utilizing intratracheal inoculation.^{13,24} The hallmark of the acute disease is the rash that appears on day 10 after inoculation (Figure 13.2).

The first eruptions occur in the inguinal area. Within the next 2 days, the rash becomes generalized, covering most of the body, except the palms and soles of the feet. Eruptions progress from macules to papules and then to vesicles. Skin eruptions appear in successive crops, which is also typical of human varicella. The simultaneous presence of all three types of lesions is a characteristic sign of varicella. Lesions may also appear in the oral cavity, on mucous membranes, and on the tongue. The severity of skin lesions reaches a maximum around day 12 postinoculation (PI) and lesions begin to heal around day 14 PI. Lesions progress to crusted scabs and then heal completely. General clinical signs of simian varicella include mild fever, anorexia, and lethargy. The elevation of serum aminotransferase, indicative of mild hepatitis, is also common.

The severity of simian varicella varies from a disease sufficiently mild to be unnoticeable without careful clinical examination to a severe disease with a high fatality

rate. However, in immunosuppressed monkeys primary infection with SVV may result in the development of fatal disseminated disease.²⁸ A poor prognostic sign in simian varicella, as in human varicella, is a hemorrhagic skin rash. The life-threatening complications associated with this form of varicella are pneumonia and severe hepatitis.

SVV infections are initiated in the epithelium of the upper respiratory tract. The virus quickly enters the blood stream, with viremia being readily detectable by day 3 PI. During the viremic stage, the virus can be isolated from both T- and B-lymphocytes, but is absent in monocytes. However, the preferential replication in T-lymphocytes that is characteristic of VZV is not mirrored in SVV infection. Viremia is transient and cleared by day 10–11 after inoculation when active replication of the virus begins in the skin, liver, lung, and lymphoid tissue. In severe cases other organs such as the esophagus, gastrointestinal tract, kidney, adrenal glands, and bone marrow can be affected as well.^{8,21,22}

Usually the central nervous system (CNS) is not affected during acute simian varicella. However, as the virus replicates, it enters sensory neurons and is transported intra-axonally to the neuron's body in sensory ganglia where it establishes a latent infection. While infectious virus cannot be recovered from ganglia at any stage of experimental simian varicella, the presence of viral transcripts in ganglia indicative of expression of the viral genome has been extensively documented.^{21,22,26,30,35,59} SVV DNA is also detectable in T-lymphocytes (both CD⁺ and CD8⁺), liver, and lung tissues of experimentally infected African green monkeys (intratracheal inoculation) nearly 2 years PI.^{58,59} However, in the African green monkeys naturally infected with SVV viral DNA was persistently detected only in the neural ganglia.³⁴

Latent SVV infection is presumed to persist lifelong. Reactivation of latent infection with SVV occurs in monkeys. Moreover, outbreaks of simian varicella in patas monkeys, which occurred at the Tulane National Primate Research Center in 1968 and 1974, were attributed to the spread of SVV from latently infected monkeys.^{10,37,45}

Clinical manifestations of reactivated SVV infection in monkeys are different from human herpes zoster. In the human disease lesions are usually confined to 1–3 dermatomes (an area of skin innervated by one ganglion), whereas the simian analog of "herpes zoster" is manifested as a whole-body rash. In contrast to human herpes zoster where lesions typically are very painful,

lesions in monkeys appear to be less disturbing and frequently can be overlooked.

Study of SVV reactivation is hampered by the lack of a suitable experimental model. One attempt to develop such a model has been recently reported.³³ SVV seronegative cynomolgus macaques were exposed to the virus by caging them together with experimentally infected monkeys. The seronegative monkeys contracted SVV, developed mild varicella, and became latently infected after the resolution of the disease.^{27,34} Seven months after the initial exposure, four of the newly infected monkeys were subjected to immunosuppressive treatment by total body irradiation plus administration of tacrolimus and prednisone. A zoster-like rash developed in one out of four monkeys 2 weeks after the start of immunosuppressive treatment. In all four monkeys SVV-specific RNA was detected in ganglia, and viral DNA and antigens were found in ganglia and other tissues. These findings indicated that subclinical reactivation of SVV occurred in the three immunosuppressed monkeys that did not develop clinically apparent zoster. Interestingly, similar evidence of subclinical reactivation of SVV was found in one nonimmunosuppressed monkey from the same group, which was attributed to the stress of transportation experienced by this monkey during the experiment.

13.7.1. Reverse Genetics

Research on SVV utilizing reverse genetics, which could greatly contribute to understanding the mechanisms of SVV latency and reactivation, is still in its infancy. However, an effective methodology for the generation of molecularly defined SVV mutants is available.²⁰ This is achieved by cotransfection of permissive Vero cells with four subgenomic overlapping cosmids (32–38 kb in length) covering the complete SVV genome. Infectious recombinant SVV is then recovered from the transfected cells. The desired genetic manipulations such as site-directed mutagenesis, deletions of genes or regulatory sequences, or insertion of new genes are performed on one of the cosmids before cotransfection.

Using this approach a variant of SVV has been produced which expresses the green fluorescent protein (GFP). This genetically manipulated virus replicates *in vitro* as efficiently as the wild-type virus. It can be used for the visualization of CeHV-1-infected cells *in vivo* via GFP expression and is very useful for tracking the virus during establishment of latency and reactivation.

Similar approaches can be used for investigating the role of different viral genes in the pathogenesis of varicella and herpes zoster by inactivating the selected genes and assessing the pathogenic consequences. The reverse genetics system for manipulating the SVV genome will also be useful for development of recombinant varicella viruses that express protective antigens of simian immunodeficiency virus (SIV) and respiratory syncytial virus (RSV).^{40,55}

13.8. IMMUNE RESPONSE

The kinetics of the antibody response against SVV antigens has been studied in experimentally infected monkeys.^{13,14,19,24} IgM antibodies against virus antigens are detected as early as day 5 PI, peak on days 12–13 PI, and then decline but are still detectable as late as day 40 PI. IgG antibodies against SVV become detectable about day 10 PI, reaching a peak on day 17 and persist at a relatively high level for at least 3 months. The kinetics of neutralizing antibody correlates with clearance of the virus. Neutralizing antibodies are first detected around day 10 PI, when infectious virus is cleared from the blood. The titer of the neutralizing antibodies increases 4- to 16-fold between day 14 (the late stage of acute disease) and day 21 PI (the early stage of convalescence), and neutralizing antibodies remain detectable for at least 4 months PI.

Using radioimmunoprecipitation assays (RIPAs) antibodies against at least 15 viral proteins have been detected.¹⁹ Particularly prominent during early stages of infection is antibody response against a 66-kDa protein, presumably glycoprotein B. Antibodies against glycoprotein E are also readily detected.²¹ The kinetics of development of anti-glycoprotein antibodies correlates well with that of neutralizing antibodies.

Cellular immunity is clearly important in controlling varicellovirus infection. It has been intensively studied in humans infected with VZV and is believed to be more important than humoral immunity in controlling the infection. However, no data on the cellular immune response against SVV have been published.

13.9. DIAGNOSIS

13.9.1. Virus Isolation

African green monkey kidney cells, such as Vero and BSC-1, are most frequently used for isolation and growth of SVV.⁴³ CPE caused by SVV is manifested as rounding and swelling of the cells and formation



Figure 13.3. SVV-induced cytopathic effects (CPE). SVV-induced plaque in the cell monolayer (Vero cells infected with SVV). (Image is kindly provided by Dr. Vicki L. Traina-Dorge.)

of syncytia. Eventually, infected cells detach and die (Figure 13.3).

It is likely that epithelioid cells of other NHP species as well as human cells could support SVV replication *in vitro*; however, this has not been tested. The practical consequence of the cell-associated nature of SVV is the use of cells rather than cell-free material for serial passages and cryopreservation of the virus.

13.9.2. Antibody Assays

The presence of antibodies against SVV antigens is considered to be a reliable marker of SVV infection, both primary and latent, although seronegative SVV infection is rarely observed. Various methods such as virus neutralization, RIPA, ELISA, and immunofluorescence are used for detection of anti-SVV IgG and IgM.^{11,19,44} Since there is no commercially available test for SVV, all such tests are developed “in-house” and no comparison of their diagnostic specificity and sensitivity has been reported. Standardization of SVV tests intended for routine use in the management of monkey colonies is clearly warranted.

13.9.3. Molecular Assays

Polymerase chain reaction (PCR) tests targeting various regions of the SVV genome are widely used in basic research. However, only one SVV PCR test has been designed specifically for use as a diagnostic test.²⁵

The development of more up-to-date diagnostic PCR tests such as quantitative and qualitative real-time PCR would be very useful. An SVV strain-specific PCR is not available. The development of such tests is hampered by the scarcity of sequence information on the genomic sequences of different SVV strains/isolates.

13.10. TREATMENT AND PREVENTION

13.10.1. Antiviral Drugs

A number of investigational anti-herpes drugs have been tested on SVV-infected monkeys in the 1970s–1980s.^{9,29,46–51,53} In some cases the antiviral drugs were effective in preventing varicella. An impressive reduction of mortality from 68 to 4% was achieved by i.m. administration of 1-b-D-arabinofuranosyl-E-2-bromovinil, (BV-ara-U) during the early stages of simian varicella infection.⁵⁴ However, all these regimens have been used ad hoc and none of them are accepted as an “industry standard” for the treatment and/or prevention of varicella in monkeys. In addition, none of these drugs are readily available.

The most widely used anti-herpes drug is acyclovir, and it is at least partially effective in the treatment of simian varicella.^{49,51} However, this drug has to be administered in doses significantly higher than those used in humans because SVV is approximately 10 times less sensitive to acyclovir than is VZV.⁴²

Thus, the prevention of simian varicella, including outbreak settings, is possible; however, preventive and therapeutic protocols employing modern and readily available drugs are not fully developed.

13.10.2. Vaccines

A vaccine against CeHV is not available. However, the possibility of prevention of varicella infection by vaccination is well established for VZV. Moreover, the attenuated VZV vaccine strain has been successfully used for immunization of patas monkeys against challenge with a high dose of infectious SVV.¹² Protection against acute simian varicella was also achieved by the immunization of African green monkeys with VZV glycoproteins (purified gB and gH, and a total glycoprotein preparation).⁵² This VZV vaccine is commercially available and widely used in medical practice. The prevention of simian varicella using this vaccine is theoretically possible.

There is little doubt that the development of a homologous SVV vaccine is possible. Different approaches can be used including the construction of attenuated SVV

strains using the recently developed methodology for manipulation in the SVV genome.²⁰

13.11. SVV AS AN EXPRESSION VECTOR

Potentially attenuated vaccine strains of VZV have been used as vectors for engineering recombinant live vaccines against other pathogens. SVV provides a useful model system for the exploration of this field. In this regard, recombinant SVV strains expressing SIV Gag and Env proteins (rSVV-SIVgag and rSVV-SIVenv) have been constructed.⁴⁰ Administration of these recombinant viruses intratracheally and subcutaneously demonstrated that they are infectious for African green monkeys. Transient viremia was detectable in all inoculated monkeys on day 6 PI. A vesicular rash typical of acute simian varicella developed in all inoculated monkeys 10–13 days after inoculation, and a mild hepatitis was observed in all but one rSVV-SIV-inoculated monkey. A neutralizing antibody response against SVV developed in all inoculated monkeys, with antibodies becoming detectable on day 14, and increased in titer 2- to 32-fold by day 56 after inoculation. A particularly steep increase in the titer of neutralizing antibodies was observed in monkeys inoculated by both rSVV-SIVgag and rSVV-SIVenv. Predictably, the rSVV-SIVgag and rSVV-SIVenv strains established latency in neural ganglia. The frequency of reactivation of these recombinant viruses from the latency is not known.

Importantly, SIV moieties of the rSVV-SIVgag and rSVV-SIVenv recombinants elicited both antibody and cell-mediated immune responses. The degree to which the immune response is protective against SIV is not yet known.

Recombinant SVV strains were also engineered to express RSV surface glycoprotein G and matrix protein M2.⁵⁵ The recombinant viruses, named rSVV/RSV-G and rSVV/RSV-M2, efficiently expressed RSV antigen while replicating in Vero cells. Rhesus monkeys inoculated with rSVV/RSV-G and rSVV/RSV-M2 produced antibodies against both SVV and RSV. Whether or not the immune response against such bivalent vaccine is protective is unknown. However, use of recombinant SVV and eventually VZV as bi- or poly-valent vaccines appears to be promising.

13.12. SUMMARY

The formal name for SVV is *Cercopithecine herpesvirus 9* (CeHV-9). This virus belongs to the *Var-*

cellovirus genus of the subfamily *Alphaherpesvirinae*, in the family *Herpesviridae*. SVV is the simian counterpart of human VZV/*Human herpesvirus 3*. SVV and VZV are closely related and share major biological and pathogenic properties. Their genomes are collinear and highly homologous, and most proteins of both viruses are antigenically cross-reactive. At the same time these viruses are clearly distinguishable.

The natural simian host of SVV is not known. The various/isolates strains of SVV apparently are either identical or very closely related. No strain-specific marker is currently known. Natural and experimental primary infection of monkeys (macaques, African green monkeys, and patas monkeys) results in the development of a varicella/chickenpox-like illness. Clinical and pathological features of simian varicella are remarkably similar to those of varicella in humans. However, in contrast to human varicella, simian varicella can have a very high mortality. A number of simian varicella outbreaks have been observed in monkey colonies and the mortality rate for most was in the 40–60% range. During simian varicella or subclinical primary infections, the virus “migrates” through the neurons to neural ganglia and establishes latent infection. Latent infections are clinically silent, but the virus can be reactivated. Reactivation of SVV results in development of a zoster-like illness or it may be subclinical. The reactivation of SVV in monkeys is uncommon and a reproducible NHP model for herpes zoster is not yet available. Methodologies for genetic manipulation of the SVV genome are well developed. Recombinant SVV strains with desired genetic features can be used as experimental vaccines and for gaining insights into the mechanisms of SVV pathogenicity and latency.

REFERENCES

1. Allen, W. P., A. D. Felsenfeld, R. H. Wolf, and H. F. Smetana. 1974. Recent studies on the isolation and characterization of Delta herpesvirus. *Lab. Anim. Sci.* 24(1):222–228.
2. Ashburn, C. V. and W. L. Gray. 1999. Identification and characterization of the simian varicella virus uracil DNA glycosylase. *Arch. Virol.* 144(11):2161–2172.
3. Ashburn, C. V. and W. L. Gray. 2002. Expression of the simian varicella virus glycoprotein L and H. *Arch. Virol.* 147(2):335–348.
4. Blakely, G. A., B. Lourie, W. G. Morton, H. H. Evans, and A. F. Kaufmann. 1973. A varicella-like disease in macaque monkeys. *J. Infect. Dis.* 127(6):617–625.

5. Clarke, P., W. L. Matlock, T. Beer, and D. H. Gilden. 1996. A simian varicella virus (SVV) homolog to varicella-zoster virus gene 21 is expressed in monkey ganglia latently infected with SVV. *J. Virol.* 70(8):5711–5715.
6. Clarkson, M. J., E. Thorpe, and K. McCarthy. 1967. A virus disease of captive vervet monkeys (*Cercopithecus aethiops*) caused by a new herpesvirus. *Arch. Gesamte Virusforsch.* 22(1):219–234.
7. Deitch, S. B., D. H. Gilden, M. Wellish, J. Smith, R. J. Cohrs, and R. Mahalingam. 2005. Array analysis of simian varicella virus gene transcription in productively infected cells in tissue culture. *J. Virol.* 79(9):5315–5325.
8. Dueland, A. N., J. R. Martin, M. E. Devlin, M. Wellish, R. Mahalingam, R. Cohrs, K. F. Soike, and D. H. Gilden. 1992. Acute simian varicella infection. Clinical, laboratory, pathologic, and virologic features. *Lab. Invest.* 66(6):762–773.
9. Felsenfeld, A. D., C. R. Abee, P. J. Gerone, K. F. Soike, and S. R. Williams. 1978. Phosphonoacetic acid in the treatment of simian varicella. *Antimicrob. Agents Chemother.* 14(3):331–335.
10. Felsenfeld, A. D. and N. J. Schmidt. 1975. Immunological relationship between delta herpesvirus of patas monkeys and varicella-zoster virus of humans. *Infect. Immun.* 12(2):261–266.
11. Felsenfeld, A. D. and N. J. Schmidt. 1977. Antigenic relationships among several simian varicella-like viruses and varicella-zoster virus. *Infect. Immun.* 15(3):807–812.
12. Felsenfeld, A. D. and N. J. Schmidt. 1979. Varicella-zoster virus immunizes patas monkeys against simian varicella-like disease. *J. Gen. Virol.* 42(1):171–178.
13. Gray, W. L. 2003. Pathogenesis of simian varicella virus. *J. Med. Virol.* 70(Suppl 1):S4–S8.
14. Gray, W. L. 2004. Simian varicella: a model for human varicella-zoster virus infections. *Rev. Med. Virol.* 14(6):363–381.
15. Gray, W. L. and B. H. Byrne. 2003. Characterization of the simian varicella virus glycoprotein C, which is nonessential for in vitro replication. *Arch. Virol.* 148(3):537–545.
16. Gray, W. L., K. Davis, Y. Ou, C. Ashburn, and T. M. Ward. 2007. Simian varicella virus gene 61 encodes a viral transactivator but is non-essential for in vitro replication. *Arch. Virol.* 152(3):553–563.
17. Gray, W. L. and N. J. Gusick. 1996. Viral isolates derived from simian varicella epizootics are genetically related but are distinct from other primate herpesviruses. *Virology* 224(1):161–166.
18. Gray, W. L., N. J. Gusick, C. Ek-Kommonen, S. E. Kempson, and T. M. Fletcher III. 1995. The inverted repeat regions of the simian varicella virus and varicella-zoster virus genomes have a similar genetic organization. *Virus Res.* 39(2–3):181–193.
19. Gray, W. L., N. J. Gusick, T. M. Fletcher, and K. F. Soike. 1995. Simian varicella virus antibody response in experimental infection of African green monkeys. *J. Med. Primatol.* 24(4):246–251.
20. Gray, W. L. and R. Mahalingam. 2005. A cosmid-based system for inserting mutations and foreign genes into the simian varicella virus genome. *J. Virol. Methods* 130(1–2):89–94.
21. Gray, W. L., L. B. Mullis, and K. F. Soike. 2001. Expression of the simian varicella virus glycoprotein E. *Virus Res.* 79(1–2):27–37.
22. Gray, W. L., L. Mullis, and K. F. Soike. 2002. Viral gene expression during acute simian varicella virus infection. *J. Gen. Virol.* 83(Pt 4):841–846.
23. Gray, W. L., B. Starnes, M. W. White, and R. Mahalingam. 2001. The DNA sequence of the simian varicella virus genome. *Virology* 284(1):123–130.
24. Gray, W. L., R. J. Williams, R. Chang, and K. F. Soike. 1998. Experimental simian varicella virus infection of St. Kitts vervet monkeys. *J. Med. Primatol.* 27(4):177–183.
25. Gray, W. L., R. J. Williams, and K. F. Soike. 1998. Rapid diagnosis of simian varicella using the polymerase chain reaction. *Lab. Anim. Sci.* 48(1):45–49.
26. Grinfeld, E. and P. G. Kennedy. 2007. The pattern of viral persistence in monkeys intra-tracheally infected with Simian varicella virus. *Virus Genes* 35(2):289–292.
27. Kennedy, P. G., E. Grinfeld, V. Traina-Dorge, D. H. Gilden, and R. Mahalingam. 2004. Neuronal localization of simian varicella virus DNA in ganglia of naturally infected African green monkeys. *Virus Genes* 28(3):273–276.
28. Kolappaswamy, K., R. Mahalingam, V. Traina-Dorge, S. T. Shipley, D. H. Gilden, B. K. Kleinschmidt-Demasters, C. G. McLeod Jr., L. L. Hungerford, and L. J. DeTolla. 2007. Disseminated simian varicella virus infection in an irradiated rhesus macaque (*Macaca mulatta*). *J. Virol.* 81(1):411–415.
29. Lehner, N. D., B. C. Bullock, and N. D. Jones. 1984. Simian varicella infection in the African green monkey (*Cercopithecus aethiops*). *Lab. Anim. Sci.* 34(3):281–285.
30. Mahalingam, R., P. Clarke, M. Wellish, A. N. Dueland, K. F. Soike, D. H. Gilden, and R. Cohrs. 1992. Prevalence and distribution of latent simian varicella virus DNA in monkey ganglia. *Virology* 188(1):193–197.
31. Mahalingam, R., D. H. Gilden, M. Wellish, and S. Pugazhenthi. 2006. Transactivation of the simian varicella virus (SVV) open reading frame (ORF) 21

- promoter by SVV ORF 62 is upregulated in neuronal cells but downregulated in non-neuronal cells by SVV ORF 63 protein. *Virology* 345(1):244–250.
32. Mahalingam, R. and W. L. Gray. 2007. The simian varicella virus genome contains an invertible 665 base pair terminal element that is absent in the varicella zoster virus genome. *Virology* 366(2):387–393.
 33. Mahalingam, R., V. Traina-Dorge, M. Wellish, R. Lorino, R. Sanford, E. P. Ribka, S. J. Alleman, E. Brazeau, and D. H. Gilden. 2007. Simian varicella virus reactivation in cynomolgus monkeys. *Virology* 368(1):50–59.
 34. Mahalingam, R., V. Traina-Dorge, M. Wellish, J. Smith, and D. H. Gilden. 2002. Naturally acquired simian varicella virus infection in African green monkeys. *J. Virol.* 76(17):8548–8550.
 35. Mahalingam, R., M. Wellish, K. Soike, T. White, B. K. Kleinschmidt-Demasters, and D. H. Gilden. 2001. Simian varicella virus infects ganglia before rash in experimentally infected monkeys. *Virology* 279(1):339–342.
 36. Mahalingam, R., T. White, M. Wellish, D. H. Gilden, K. Soike, and W. L. Gray. 2000. Sequence analysis of the leftward end of simian varicella virus (EcoRI-I fragment) reveals the presence of an 8-bp repeat flanking the unique long segment and an 881-bp open reading frame that is absent in the varicella zoster virus genome. *Virology* 274(2):420–428.
 37. McCarthy, K., E. Thorpe, A. C. Laursen, C. S. Heymann, and A. J. Beale. 1968. Exanthematous disease in patas monkeys caused by a herpes virus. *Lancet* 2(7573):856–857.
 38. Ou, Y., K. A. Davis, V. Traina-Dorge, and W. L. Gray. 2007. Simian varicella virus expresses a latency-associated transcript that is antisense to open reading frame 61 (ICP0) mRNA in neural ganglia of latently infected monkeys. *J. Virol.* 81(15):8149–8156.
 39. Ou, Y. and W. L. Gray. 2006. Simian varicella virus gene 28 and 29 promoters share a common upstream stimulatory factor-binding site and are induced by IE62 transactivation. *J. Gen. Virol.* 87(Pt 6):1501–1508.
 40. Ou, Y., V. Traina-Dorge, K. A. Davis, and W. L. Gray. 2007. Recombinant simian varicella viruses induce immune responses to simian immunodeficiency virus (SIV) antigens in immunized rhesus monkeys. *Virology* 364(2):291–300.
 41. Pumphrey, C. Y. and W. L. Gray. 1995. DNA sequence of the simian varicella virus (SVV) gH gene and analysis of the SVV and varicella zoster virus gH transcripts. *Virus Res.* 38(1):55–70.
 42. Pumphrey, C. Y. and W. L. Gray. 1996. Identification and analysis of the simian varicella virus thymidine kinase gene. *Arch. Virol.* 141(1):43–55.
 43. Schmidt, N. J. 1982. Improved yields and assay of simian varicella virus, and a comparison of certain biological properties of simian and human varicella viruses. *J. Virol. Methods* 5(3–4):229–241.
 44. Schmidt, N. J., A. M. Arvin, D. P. Martin, and E. A. Gard. 1983. Serological investigation of an outbreak of simian varicella in *Erythrocebus patas* monkeys. *J. Clin. Microbiol.* 18(4):901–904.
 45. Soike, K. F. 1992. Simian varicella virus infection in African and Asian monkeys. The potential for development of antivirals for animal diseases. *Ann. N. Y. Acad. Sci.* 653:323–333.
 46. Soike, K. F., G. Baskin, C. Cantrell, and P. Gerone. 1984. Investigation of antiviral activity of 1-beta-D-arabinofuranosylthymine (ara-T) and 1-beta-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil (BV-ara-U) in monkeys infected with simian varicella virus. *Antiviral Res.* 4(5):245–257.
 47. Soike, K. F., C. Cantrell, and P. J. Gerone. 1986. Activity of 1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-5-iodouracil against simian varicella virus infections in African green monkeys. *Antimicrob. Agents Chemother.* 29(1):20–25.
 48. Soike, K. F., D. A. Eppstein, C. A. Gloff, C. Cantrell, T. C. Chou, and P. J. Gerone. 1987. Effect of 9-(1,3-dihydroxy-2-propoxymethyl)guanine and recombinant human beta interferon alone and in combination on simian varicella virus infection in monkeys. *J. Infect. Dis.* 156(4):607–614.
 49. Soike, K. F., A. D. Felsenfeld, and P. J. Gerone. 1981. Acyclovir treatment of experimental simian varicella infection of monkeys. *Antimicrob. Agents Chemother.* 20(3):291–297.
 50. Soike, K. F., A. D. Felsenfeld, S. Gibson, and P. J. Gerone. 1980. Ineffectiveness of adenine arabinoside and adenine arabinoside 5'-monophosphate in simian varicella infection. *Antimicrob. Agents Chemother.* 18(1):142–147.
 51. Soike, K. F. and P. J. Gerone. 1982. Acyclovir in the treatment of simian varicella virus infection of the African green monkey. *Am. J. Med.* 73(1A):112–117.
 52. Soike, K. F., P. M. Keller, and R. W. Ellis. 1987. Immunization of monkeys with varicella-zoster virus glycoprotein antigens and their response to challenge with simian varicella virus. *J. Med. Virol.* 22(4):307–313.
 53. Soike, K. F., M. J. Kramer, and P. J. Gerone. 1983. In vivo antiviral activity of recombinant type alpha interferon A in monkeys with infections due to simian varicella virus. *J. Infect. Dis.* 147(5):933–938.
 54. Takasaki, M., I. Sakakibara, M. Suzuki, and R. Mukai. 1990. An outbreak of nonhuman primate varicella-like herpesvirus infection in the established

- breeding colony of cynomolgus monkeys. *Tsukuba Primate Center News* 9:5–11.
- 55. Ward, T. M., V. Traina-Dorge, K. A. Davis, and W. L. Gray. 2008. Recombinant simian varicella viruses expressing respiratory syncytial virus antigens are immunogenic. *J. Gen. Virol.* 89(Pt 3):741–750.
 - 56. Wenner, H. A., D. Abel, S. Barrick, and P. Seshumurty. 1977. Clinical and pathogenetic studies of Medical Lake macaque virus infections in cynomolgus monkeys (simian varicella). *J. Infect. Dis.* 135(4):611–622.
 - 57. Wenner, H. A., S. Barrick, D. Abel, and P. Seshumurty. 1975. The pathogenesis of simian varicella virus in cynomolgus monkeys. *Proc. Soc. Exp. Biol. Med.* 150(2):318–323.
 - 58. White, T. M., R. Mahalingam, V. Traina-Dorge, and D. H. Gilden. 2002. Persistence of simian varicella virus DNA in CD4(+) and CD8(+) blood mononuclear cells for years after intratracheal inoculation of African green monkeys. *Virology* 303(1):192–198.
 - 59. White, T. M., R. Mahalingam, V. Traina-Dorge, and D. H. Gilden. 2002. Simian varicella virus DNA is present and transcribed months after experimental infection of adult African green monkeys. *J. Neurovirol.* 8(3):191–203.
 - 60. Wolf, R. H., H. F. Smetana, W. P. Allen, and A. D. Felsenfeld. 1974. Pathology and clinical history of Delta herpesvirus infection in patas monkeys. *Lab. Anim. Sci.* 24(1):218–221.

14

Cytomegaloviruses

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14.1. INTRODUCTION

The prototype cytomegalovirus (CMV) is human cytomegalovirus (HCMV), commonly referred to in the medical literature as CMV (reviewed in Mocarski *et al.*⁵⁵). HCMV is normally acquired in early childhood or adolescence and the infection is lifelong. Most adults are asymptotically infected with the virus. HCMV is presumed to be an opportunistic pathogen; that is, it is harmless in immunocompetent individuals but may cause severe diseases in patients with various congenital or acquired immunodeficiencies, most notably in organ transplant and AIDS patients. HCMV may also be dangerous in the context of an immature immune system:

congenital infection with HCMV frequently results in severe pathology having irreversible sequelae such as bilateral sensorineural deafness. The congenital syndrome (generalized cytomegalic inclusion disease) was known long before the discovery of HCMV and, in fact, gave the name *cytomegalovirus* (reviewed in Ho²⁹). With the advent of HIV/AIDS era, CMV retinitis was recognized as one of the typical late clinical manifestations of AIDS. It has also been established that “CMV disease” is the most common infectious complication following organ and bone-marrow transplants. In addition, HCMV is recognized as the most important infectious cause of congenital abnormalities in developed countries. Given the medical importance of these diseases, it is not surprising that HCMV is one of the most extensively studied viruses.

The first simian CMV (SA-15) was isolated in 1957 from African green monkeys.⁴⁹ In 1963, a similar herpesvirus was described and designated SA-6.⁸ Although the state of knowledge regarding CMVs was at that time still in its infancy, both isolates were recognized as “relatives” of HCMV.⁵⁰ By the beginning of 1980s, it became clear that many Old World simian species and at least some New World monkeys harbor CMVs.^{2,18,19,23,54,75–77,82} The evidence for this was mainly serological; infectious isolates were reported only from the African green monkeys, chimpanzees, rhesus monkeys, and owl monkeys.^{2,18,19,82} At that time little attention was given to characterization of multiple “accidental” or “adventitious” simian CMV isolates because this virus, like foamy virus, was mostly regarded as a nuisance complicating isolation of “more important” viruses.

Currently simian CMVs attract interest primarily as models for HCMV infection since they are more

reflective of human CMV disease than are other non-primate laboratory models of CMV infection. Starting in the early 1990s this field, particularly rhesus CMV/rhesus monkey model, has been extensively explored (reviewed in Barry *et al.*⁵ and Powers and Fruh⁶³).

14.2. CLASSIFICATION AND NOMENCLATURE

CMVs (the genus *Cytomegalovirus*) belong to the *Beta-herpesvirinae* subfamily within the *Herpesviridae* family. Three simian CMVs are recognized as species in

the latest International Committee for Taxonomy of Viruses (ICTV) classification (8th Report): rhesus monkey CMV, chimpanzee CMV, and African green monkey CMV. Nomenclature of simian CMVs is perplexing. There are old and new ICTV designations of simian CMV species (Table 14.1).

The old ICTV names of the African green monkey CMV [*Cercopithecine herpesvirus 8* (CeHV-8)] and chimpanzee CMV [*Pongine herpesvirus 4* (PoHV-4)] are defunct as of May 2008 and have been replaced with the new names: *Macacine herpesvirus 3* (McHV-3) and *Panine herpesvirus 2* (PnHV-2) for former CeHV-8 and

Table 14.1. Simian Cytomegaloviruses

ICTV Name	Old ICTV Name	Synonymous Designations*	Isolates	Original References
<i>Macacine herpesvirus 3</i> (McHV-3)	<i>Cercopithecine herpesvirus 8</i> (CeHV-8)	Rhesus monkey cytomegalovirus (RhCMV)	CNPRC 68.1 180.92 22659	1, 2, 25, 59, 68
<i>Cercopithecine herpesvirus 5</i> (CeHV-5)	<i>Cercopithecine herpesvirus 5</i> (CeHV-5)	African green monkey cytomegalovirus (AgmCMV) , SA-6, SA-15, SCMV	SA-15 SA-6	8, 49
<i>Panine herpesvirus 2</i> (PnHV-2)	<i>Pongine herpesvirus 4</i> (PoHV-4)	Chimpanzee cytomegalovirus (ChCMV or CCMV)	4 × 148	20, 82
<i>Aotine herpesvirus 1</i> (AoHV-1) [†]	<i>Aotine herpesvirus 1</i> (AoHV-1) [†]	Herpesvirus aotus 1 (HVA-1)[‡]	S43E	19
<i>Aotine herpesvirus 3</i> (AoHV-3) [†]	<i>Aotine herpesvirus 3</i> (AoHV-3) [†]	Herpesvirus aotus 3 (HVA-3)		18
NOR [§]	NOR [§]	Baboon cytomegalovirus (BaCMV)	OCOM4-37 OCOM4-52	10
NOR [§]	NOR [§]	Drillcytomegalovirus (DrCMV)	OCOM6-2	9
NOR [§]	NOR [§]	Squirrel monkey cytomegalovirus (SquiCMV)	SqSHV	67

*Most commonly used designations are in bold.

[†]Tentative species.

[‡]Should not be confused with simplexvirus Herpesvirus atelus 1 (HVA-1)/*Ateline herpesvirus 1* (AtHV-1).

[§]Not officially recognized.

PoHV-4, respectively. However, the implementation of new ICTV names will take time. Also, the old ICTV names are imprinted in the scientific literature and genomic databases. On the other hand, unofficial names such as RhCMV, ChCMV, and AgmCMV for rhesus monkey, chimpanzee, and African green monkey CMVs, respectively, are self-explanatory. For this reason they are used more commonly than the official names.

Two simian CMVs are currently classified as “tentative” species within the *Cytomegalovirus* genus, namely *Aotine herpesvirus 1* (AoHV-1) and *Aotine herpesvirus 3* (AoHV-3). These viruses, isolated from owl monkeys (*Aotus trivirgatus*) in the early 1970s,^{18,19} have remained in oblivion. The only relevant data available aside from their original description is the sequence of a short fragment of the AoHV-1 DNA polymerase gene (GenBank Acc. No. U63456).

Several simian CMVs are not included in the ICTV classification. One of them is the virus isolated from a squirrel monkey (*Saimiri sciureus*).⁶⁷ This virus is also poorly characterized; its genomic sequence is not known. Two other simian CMVs isolated from baboons (BaCMV) and drills (DrCMV) are better characterized than the two tentative species (AoHV-1 and AoHV-3), but have yet to be recognized by the ICTV.^{9,10}

Although it is presumed that virtually all simian species harbor CMVs, data supporting this assumption are scarce. In addition to bona fide simian CMV isolates (Table 14.1), only a few “frag” CMVs have been described (Table 14.2).^{43,64}

Nevertheless, there is little doubt that many more simian CMVs exist than it is currently known. However, analysis of simian CMV diversity has not attracted sufficient attention and funding to move it forward. The

phylogeny of the few simian CMVs for which sequence data are available is characterized by a close parallelism with the phylogeny of the host species. Evidence of interspecies transmission of simian CMV has not been reported, although such a possibility cannot be excluded.

The closest relatives of CMVs are the roseoloviruses which also belong to the *Betaherpesvirinae* subfamily. The prototype of the *Roseolavirus* genus is *human herpesvirus 6* (HHV-6). Antibodies reacting with HHV-6 antigens have been detected in some monkey sera.²⁷ An HHV-6-related frag-virus has also been described in common chimpanzees.⁴⁴ However, replication-competent simian roseoloviruses have not yet been isolated.

14.3. GENERAL PROPERTIES

CMV virions are larger than virions of other herpesviruses (200–300 nm in diameter) and tend to be pleiomorphic due to the irregular shape of the tegument and envelope. Another peculiar morphological feature of CMVs is relative abundance of so-called “dense bodies,” virus-like particles containing unstructured electron-dense material enclosed in the envelope (Figure 14.1). Experienced electron microscopists can usually distinguish CMVs from other herpesviruses in thin sections.

Characteristic cytopathology, so-called “owl-eye” inclusion bodies, is considered by pathologists to be diagnostic for CMV disease (Figure 14.2).

Although these morphological characteristics are highly suggestive of CMV infection, reliable identification of CMVs can only be achieved by detection of CMV-specific viral antigens or more commonly DNA sequences that are homologous or identical to known CMVs.

Table 14.2. Simian CMV Frag-Viruses Detected in NHP Species from which Infectious CMVs have not been Isolated

Host Species	Genome Fragments*	GenBank Acc. No.
Mustached guenon (<i>Cercopithecus cephus</i>)	DNApol	AY728178
Mandrill (<i>Mandrillus sphinx</i>)	DNApol	AY129399
Mantled guereza (<i>Colobus guereza</i>)	MDBP, UL54/55/56/57	AY129397/EU118147
Agile mangabey (<i>Cercocebus agilis</i>)	DNApol	AY608713
Cynomolgus macaque (<i>Macaca fascicularis</i>)	DNApol	AY728171
Orangutan (<i>Pongo pygmaeus</i>)	DNApol	AY129396

*DNA polymerase (DNApol), major DNA-binding protein (MDBP), UL54/55/56/57 genes.

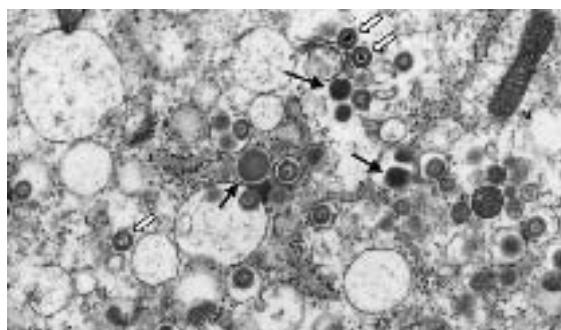


Figure 14.1. RhCMV virions in the cytoplasm of cultured human fibroblasts. Fully enveloped RhCMV virions (block arrows) are present in the cytoplasm of human fetal lung fibroblasts (MRC-5) infected with RhCMV variant 22659.^{1,59} Dense body-like structures are also present (black arrows) amidst cellular organelles, such as mitochondria (M) and ribosomes (R). (Image was kindly provided by Prof. Peter Barry.)

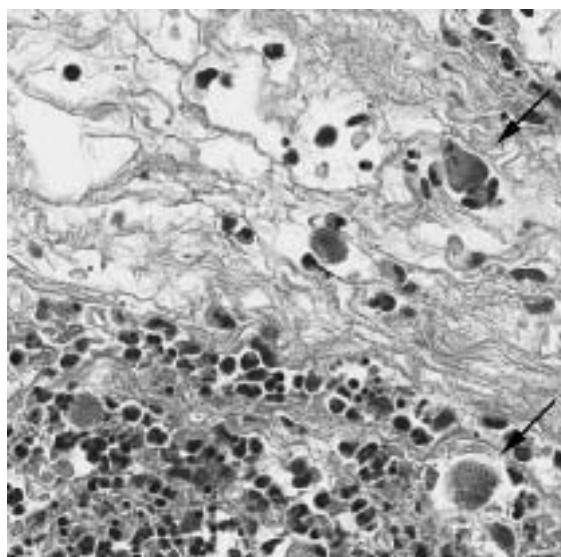


Figure 14.2. “Owl’s eye” cytopathology. A skin biopsy of a rhesus monkey inoculated subcutaneously with RhCMV (7 days PI). Two “owl’s eye” cells are noted by arrows. (Image was kindly provided by Prof. Peter Barry.)

Classical features of CMVs are their slow growth in vitro and ability to replicate only in homologous cells, that is, cells of the same species as the host of the virus. These properties initially defined beta-herpesviruses. However, it is now recognized that both features are not absolute. The kinetics of cytopathic effects (CPEs) induced by different CMV isolates varies significantly. Also, simian CMVs can replicate in some human cell lines.

CMV genomes are the largest among herpesviruses (215–230 kb). The genomic organization is similar in human and chimpanzee CMV genomes: they consist of two covalently linked subunits (U_L and U_S) flanked by repeats (TR_L/IR_L and IR_S/TR_S). Thus, the overall genomic configuration HCMV and ChCMV can be depicted as:

$$TR_L - U_L - IR_L - IR_S - U_S - TR_S \text{ (Figure 14.3).}$$

The U_L and U_S in the HCMV and ChCMV genomes can be present in either orientation, resulting in four isomeric forms of genomic DNA. Biological differences of virions containing different genomic isomers are not known. In contrast to HCMV and ChCMV, the genome of RhCMV does not have internal repeats separating the U_L and U_S segments, and the RhCMV genome therefore exists in a single form.

The sequence of the prototype HCMV genome was first reported in 1990.¹⁶ Nevertheless, even for HCMV, the best characterized CMV, not all genes have been identified unequivocally. The identification of bona fide CMV genes is complicated by several obstacles: (1) various protein-coding prediction algorithms produce different results;^{25,57,68} (2) identification of ORFs *in silico* is dependent on arbitrarily chosen parameters; (3) *in vitro*-adapted CMV strains have large deletions and other genomic alterations as compared to wild-type isolates;^{12,59,66} (4) *in silico* gene prediction must be verified by proving that a presumptive product is expressed and functionally active; it is difficult to do this for each of more than 200 predicted ORFs. As a result, the gene repertoires of HCMV, ChCMV, and RhCMV have been revised along with the accumulation of whole genome sequences of additional viral strains and functional characterization of viral proteins.^{45,56,57,59,68} More detailed information on the genomes of RhCMV and ChCMV is presented in Sections 14.4.1 and 14.5.

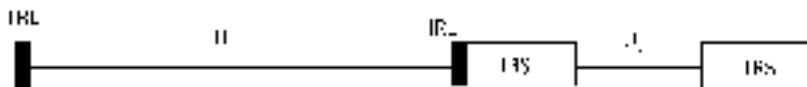


Figure 14.3. “Macro” map of CMV genome. U_L , long unique region; U_S , short unique region; TRL, IRL, terminal and internal repeats flanking U_L ; IRS TRS, internal and terminal repeats flanking U_S .

General features of the CMV replication cycle are consistent with the generic herpesvirus replication scheme described in Introduction to Herpesviruses. After entry, nucleocapsids are transported toward the cytoplasmic opening of nuclear pores and genomic DNA is delivered into the nucleolus. The expression of viral genome occurs in three successive waves, which in case of CMV are called immediate-early (IE) or α , delayed-early (DE) or β , and late (L) or γ . DE and L genes/transcripts/proteins are further divided into $\beta 1/\beta 2$ and $\gamma 1/\gamma 2$. All CMV genes are transcribed by host RNA polymerase II machinery. Newly synthesized structural proteins are transported to the nucleus where capsids are assembled and “loaded” with genomic DNA. Nucleocapsids undergo a complex “double envelopment” (see Introduction to Herpesviruses) and progeny virions are released from the host cell approximately 48–72 h after entry of the parental virion.

14.4. RHESUS MONKEY CMV

Rhesus monkey CMV (RhCMV) is the most extensively studied simian CMV. Major themes in RhCMV research are:

1. characterizing the virus, its genome, individual genes and their protein products;^{4,11,13,39,45,47,59,60,62,71}
2. characterizing the spectrum of pathogenicity of RhCMV in immunosuppressed rhesus monkeys;^{3,6,34,35,36,72}
3. modeling congenital CMV infection in rhesus monkeys;⁵
4. developing RhCMV-based experimental vaccines and testing them in rhesus monkeys.^{35,36,83,84,85,86}

14.4.1. Genomic Organization and Gene Products

Complete genome sequences are available for two RhCMV isolates, namely 68.1 and 180.92.^{25,68} The second of these sequences is better annotated due to the use of more powerful gene prediction algorithms and the availability of corrected data for the HCMV

genome.⁵⁶ RhCMV-68.1 is the prototypic RhCMV strain deposited in ATCC (ATTC VR-677). This virus was originally isolated from urine of rhesus monkey in 1968² and it has long, though poorly documented, in vitro passage history. RhCMV-180.92 originated from the lung of a simian immunodeficiency virus (SIV)-immunosuppressed rhesus monkey with SAIDS that developed CMV pneumonia. Its in vitro replication history prior to sequencing was relatively short: 6 passages on human fibroblasts (MRC-5) followed by 7 passages on primary rhesus monkey fibroblasts.³⁴

The length of RhCMV genome is about 220 kb (221,459 bp and 215,678 bp for 68.1 and 180.92 strains, respectively). The overall nucleotide sequence homology of these two genomes is 97%. About 260 ORFs have been identified in the RhCMV genome (260 and 258 ORFs in the 68.1 and 180.92 genomes, respectively).⁶⁸

Both completely sequenced RhCMV genomes are significantly altered in the region homologous to the $U_L b'$ segment of the HCMV genome.⁵⁹ HCMV strains adapted to growth in fibroblastoid cell lines in vitro are known to contain multiple rearrangements and deletions in this area of the genome.^{12,22,66} In contrast, wild-type RhCMV (at least the strain circulating in the rhesus monkey colony of California National Primate Research Center) has an intact $U_L b'$ genomic segment.⁵⁹ Thus, the genes located in the $U_L b'$ region are apparently dispensable for virus growth in vitro. Moreover, deletion of these genes provides a selective advantage to RhCMV and HCMV for replication in vitro. The mechanism underlying this phenomenon is unknown.

The products of RhCMV genes can provisionally be divided into two groups. The first and largest group includes putative viral proteins, that is, predicted gene products. The strength of evidence for the existence of proteins belonging to this group varies, being weakest for products without clear orthologs in the HCMV genome. The second still small but growing group consists of “confirmed” viral proteins. Their existence has been experimentally proven either directly

or indirectly through phenotypic changes conferred by mutations in the genes encoding these proteins.^{39,40,45,47,58,60,62,71,78,84}

14.4.2. Mode of Transmission and Prevalence of Infection

RhCMV is persistently excreted in the saliva, urine, and breast milk of seropositive monkeys.^{2,30} Transmission of the virus occurs horizontally through the direct contact between seropositive and seronegative animals. In breeding colony conditions almost 100% of infants acquire CMV by the age of 1 year.⁸¹ Thus, sexual transmission does not play significant role, at least in the captivity. Although concrete data on the RhCMV seroprevalence in wild rhesus monkeys are sparse, the infection appears to be ubiquitous in natural conditions as well.³² Whether the virus can be transmitted without direct contact between virus carrier and seronegative animals via contaminated objects is not known. However, it seems unlikely that this route of transmission is significant, particularly in the wild.

Interestingly, in contrast to HCMV, vertical transmission of RhCMV has not been documented in rhesus monkeys.^{5,81} Primary infections during pregnancy, which poses the greatest risk of congenital HCMV disease, is extremely rare in macaques; virtually all sexually mature females are seropositive for RhCMV. It is estimated that less than 1% of pregnancies in rhesus monkeys would result in the transplacental transmission of the virus, assuming that the rates of congenital CMV infection in humans and macaques are similar.⁵

14.4.3. Immune Responses

Infection with RhCMV elicits specific antibody and cell-mediated immune responses which are able to control the infection, maintaining it at a subclinical level in immunocompetent rhesus monkeys.

The antibody immune response against RhCMV is measured using neutralization assays or, more commonly, various antibody-binding assays.^{37,83,85,86} RhCMV-neutralizing antibodies are predominantly directed against glycoprotein B (gB). Immunodominant epitopes are located in the extracellular portion of this protein⁸⁶ and a truncated recombinant RhCMV gB protein-based ELISA is now used as a “surrogate” test for neutralizing antibodies.^{83,85} Dynamics of anti-gB and total anti-RhCMV antibody levels are well correlated.⁸⁶ Another strongly immunogenic RhCMV protein is the pp150 phosphoprotein (UL32 gene prod-

uct). In contrast to the gB antigen, pp150 is not used as a component of experimental vaccines. This allows for use of anti-pp150 antibodies as markers of a vaccination-independent immune response after challenge of vaccinated rhesus monkeys with live RhCMV.⁸³

Cellular immune responses against RhCMV are readily detectable in seropositive rhesus monkeys.^{35,36,65,84} RhCMV-specific T-lymphocytes are currently detected using IFN- γ ELISPOT assay. Stimulation of cells with whole virus antigen in this assay allows quantitation of CMV-specific CD4 $^{+}$ T-lymphocytes, whereas stimulation with vaccinia recombinants expressing several RhCMV proteins (IE1, pp65, vIL-10) is used for selective activation of CD8 $^{+}$ T-lymphocytes.^{35,36}

The cellular immune response is presumed to play a key role in keeping persistent CMV infections in the “subclinical framework.” HCMV phosphoprotein 65 (pp65) is one of the prime targets of the T-cell-mediated response. RhCMV has two homologous proteins (pp65-1 and pp65-2) encoded by ORF Rh111 and ORF Rh112, respectively. It has been shown that a T-cell-mediated immune response against pp65-2 in rhesus monkeys is elicited during primary infection and that this response subsequently persists at levels stable in individual animals but broadly variable between different monkeys. Interestingly, the cellular immune response against pp65-2 in rhesus monkeys appears to be more sustainable than the antibody-mediated response against the same protein.⁸⁴ The cytotoxic T-lymphocytes (CTL) response also targets the gB and gH glycoproteins. Interestingly, CTL epitopes recognized by human CD4 $^{+}$ lymphocytes are shared by HCMV and RhCMV as well as other simian CMVs (ChCMV and BaCMV).²⁴

Little is known about the innate immune responses triggered by RhCMV. Apparently, RhCMV virions contain an inhibitor of interferon-regulatory factor 3 (IRF-3). Infection of rhesus monkey fibroblasts with RhCMV does not induce expression of so-called interferon-stimulated genes, an innate response activated by IRF-3 upon infection with many viruses, including HCMV.²¹

14.4.4. Diagnosis of Infection

14.4.4.1. VIRUS ISOLATION

The classical cellular substrates for the isolation of infectious RhCMV are permanent human fibroblast cell lines such as MRC-5 or HFF. Primary rhesus monkey fibroblast cultures are highly permissive for replication

of RhCMV but they are less convenient than permanent cell lines and their properties are less reproducible.

Apparently, the best option currently available for the isolation of RhCMV is the use of engineered, telomerase-transformed rhesus monkey fibroblast cell line Telo-RF.^{14,38} The permissiveness of these cells for RhCMV is comparable to that of the primary rhesus monkey fibroblasts, and the robust replication of RhCMV in Telo-RF cells can be maintained permanently. The properties of RhCMV and the temporal pattern of viral gene expression are not altered when the virus is propagated in the Telo-RF cells, as compared to that in conventional cell lines.

Replication of RhCMV in susceptible cell cultures is accompanied by the development of CPE about a week after inoculation. CPE is manifested as clusters of rounded cells containing “owl-eyed” inclusion bodies (Figure 14.4).

14.4.4.2. ANTIBODY TESTS

All rhesus macaques that have antibodies against RhCMV are considered infected with the virus.

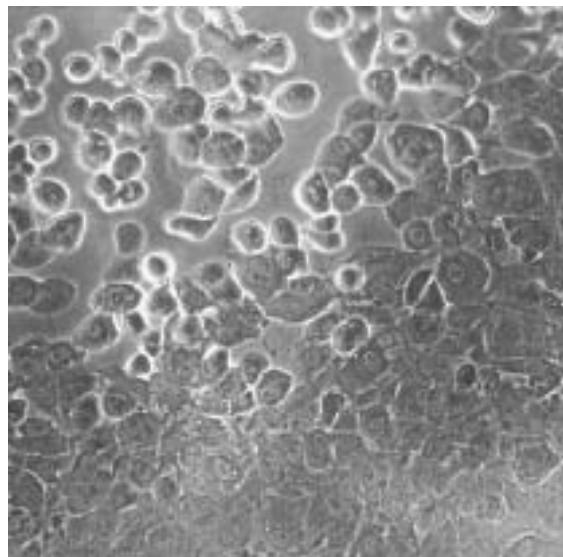


Figure 14.4. RhCMV-induced cytopathic effects (CPEs). RhCMV-induced CPE in the “telomerized” rhesus skin fibroblasts Telo-RF;¹⁴ a plaque (cluster of rounded cells). (Image was kindly provided by Prof. Peter Barry.)

Seronegative RhCMV infection has not been described although the possibility cannot be fully excluded. However, even if cases of seronegative RhCMV infection exist, they should be extremely rare.

Indirect ELISA is the standard methodology for detection of anti-RhCMV antibodies. The most commonly used version of RhCMV ELISA is based on the crude cell extracts prepared from infected cells. However, a current trend is to use defined RhCMV antigens, mostly recombinant proteins, such as RhCMV gB or its truncated derivative.⁸⁶ RhCMV antigen is included in the multiplex microbead assay that allows for simultaneous detection of antibodies to six simian viruses.³⁷

14.4.4.3. MOLECULAR TESTS

Detection of viral DNA in saliva, oral swabs, or urine confirms RhCMV infection. Viral DNA can be detected by a qualitative polymerase chain reaction (PCR) targeting any well-conserved region in the RhCMV genome. Such tests are relatively easy to establish. However, no systematic comparison of diagnostic RhCMV-PCRs with virus isolation and serology has been reported.

Quantitative PCR tests for RhCMV DNA are widely used. Several versions of RhCMV viral load tests have been described.^{30,35,65,72} A value equal to or greater than 1,000 copies of genomic DNA per mL of plasma has been used as a cut-off separating high and low RhCMV plasma viral load values.^{35,36} However, as for all cut-off values, this parameter is inherently arbitrary.

14.4.5. RhCMV in Immunosuppressed Macaques

The major setting in which RhCMV can reveal its pathogenic potential is simian AIDS (SAIDS). Although pathological findings attributable to RhCMV have been reported in SRV-induced SAIDS,²⁶ most data on RhCMV-induced pathology have been obtained in studies of SIV-induced immunosuppression.^{3,6,17,33,36,41,72,74} Disseminated RhCMV disease in rhesus monkeys infected with SIV affects multiple organs and tissues: the intestine, brain, lung, lymph nodes, liver, spleen, testicle, nerves, and arteries. Histologically confirmed end-organ RhCMV disease is observed in about 30–50% of macaques with SIV-induced SAIDS and is usually accompanied by other AIDS-defining illnesses. The disease is diagnosed by the presence of cytomegalic cells, amphophilic intranuclear inclusion bodies, and neutrophilic infiltrates positive for RhCMV

antigen (IE1 protein) in the affected organs. Macaques with RhCMV disease have plasma viral loads higher than 1,000 copies of RhCMV-DNA per mL.³⁶ The development of CMV disease in SIV-immunosuppressed macaques is accompanied by a decline of anti-RhCMV antibodies (twofold or greater) and RhCMV-specific CD8⁺ and CD4⁺ lymphocytes (fourfold and greater).³⁶ Ocular pathology attributable to RhCMV is very rare in SIV-immunosuppressed rhesus monkeys, although a few cases have been described.¹⁷ This is in contrast with human AIDS where CMV retinitis is a common manifestation. The discrepancy is possibly explained by the fact that macaques with AIDS are usually euthanized before SIV-induced immunosuppression becomes as profound as it is in the late stages of human AIDS when CMV retinitis develops.

The development of RhCMV-induced pathology has also been studied in the RhCMV-seronegative rhesus monkeys inoculated sequentially with RhCMV and SIV.⁷² The inoculations were given at 2 and 11 weeks intervals. The outcomes were significantly different depending of the interval between the inoculations. All four monkeys that received SIV during the initial stage of primary RhCMV infection had a significantly reduced antibody response against RhCMV and a high RhCMV viral load; all developed SAIDS and had multi-organ RhCMV histopathology. In contrast, none of four monkeys inoculated with SIV at the later stage of primary RhCMV infection developed histopathological changes characteristic for CMV disease. Anti-RhCMV antibody titers in these monkeys were high, whereas RhCMV viral load was low.

RhCMV-induced disease can also develop in rhesus monkeys subjected to pharmacological immunosuppression in the context of transplantation experiments. Different immunosuppressive regimens have not been compared in this respect. However, it has been shown that treatment with monoclonal anti-CD40 antibodies inhibited the primary immune response against RhCMV and led to the development of generalized CMV disease in all seronegative monkeys that received allografts from RhCMV-positive donors.⁶¹

14.4.6. Congenital Infection with RhCMV

Congenital CMV infection has been experimentally reproduced in rhesus monkeys by direct inoculation of fetuses with RhCMV at the various stages of pregnancy.^{5,15,48,80} In the original study, 25 fetuses were

inoculated with primary isolate of RhCMV (2×10^4 TCID₅₀). Eleven fetuses were inoculated by an intraamniotic route on day 50 of gestation and 14 were inoculated intracranially on day 80.⁴⁸ Various CNS abnormalities were detected in 16 inoculated fetuses (ventriculomegaly, leptomeningitis, and pathological changes in brain parenchyma). Placental abnormalities (deciduitis, infarction, calcification, hyalinization, and lymphocytic infiltrates) were also present in most cases.

All subsequent inoculations of rhesus monkey fetuses with RhCMV have been performed using strain 68-1 or its derivatives.^{5,15,80} These studies have confirmed that congenital infection with RhCMV results in the development of diverse, mainly CNS, abnormalities. It has also been shown that RhCMV replication in sensory and neural tissues of the developing inner ear leads to permanent damage.⁵ This is reminiscent of congenital HCMV infection which often results in deafness. However, hearing function is difficult to assess in monkeys.

Surprisingly, some fetuses inoculated with a high dose of RhCMV did not reveal any clinical or histopathological signs of disease. Although RhCMV is detectable in such fetuses, they remain visibly healthy. The mechanisms underlying such profound resistance are not known. Data on the protective effect of maternal anti-RhCMV antibodies on the outcome of fetal RhCMV infection are equivocal^{5,46} and it seems unlikely that resistance is mediated only by maternal antibodies.

14.4.7. RhCMV-Based Vaccines

Universal vaccination aiming at the prevention of HCMV infection is not considered, even theoretically. However, an HCMV vaccine could be useful for reduction of the risk of congenital CMV infection and CMV disease in immunocompromised individuals. The development of an HCMV vaccine and vaccination protocols, both prophylactic and therapeutic, is an extremely complex task due to the absence of an animal model for testing the protective efficiency of experimental HCMV-based vaccines.

The RhCMV-rhesus monkey model provides an excellent opportunity for testing various immunogens, adjuvants, and immunization protocols. RhCMV homologs of HCMV antigens that are considered most promising for vaccination purposes [such as gB, pp65 (RhCMV homolog is pp-65-2), and IE1] have been

used.^{83,85} Strong immunogenicity of these RhCMV antigens for rhesus monkeys when delivered as DNA vaccines or in a recombinant vaccinia Ankara virus (rMVA) vector has been demonstrated.^{83,85} Apparently, the best immunization protocol currently available includes priming with a DNA vaccine followed by boosting with rMVA vaccine, a so-called “prime-boost” immunization.⁸⁵ Immunized monkeys challenged with live RhCMV had lower peak values of acute infection-associated RhCMV viral load than did nonimmunized animals. This is indicative of a certain degree of protection having been achieved. Unfortunately, there is no straightforward way to test the protective efficacy of RhCMV vaccines in terms of prevention of pathology analogous to CMV disease in humans. Nevertheless, currently there is no alternative to the RhCMV-focused approach to development of efficient HCMV vaccines.

14.5. CHIMPANZEE CMV

The first description of CMV isolates from common chimpanzees (*Pan troglodytes*) was published in 1979.⁸² A few years later, several similar isolates were recovered from captive chimpanzees.⁷⁹ After these reports, research in this field paused for almost 20 years and it is not clear if the original “pioneer” isolates of ChCMV have survived.

The current ICTV designation of ChCMV is *Panine herpesvirus 2* (PnHV-2). This name has replaced the previous ICTV designation *Pongine herpesvirus 4* (PoHV-4). ChCMV is among the few CMVs for which the complete genome has been sequenced²⁰ (GenBank Acc. No. NC_003521). Moreover, the genomic sequence of ChCMV has been used as a kind of “Rosetta stone” for identification/verification of protein coding genes in the HCMV genome. However, beyond *in silico* studies ChCMV remains poorly characterized.

The ChCMV strain 4 × 148 (of which the genome has been sequenced) was isolated in the early 1980s by R. Heberling from a captive-born common chimpanzee housed at the Southwest National Primate Research Center in San Antonio, USA.²⁰ The virus was isolated in chimpanzee lung fibroblasts, and then grown to passage 16 in human fibroblast cell lines (HFF and MRC5). During 1983–2001, the virus was stored frozen. Thus, this isolate has a relatively short history of replication *in vitro* and genetic changes associated with *in vitro* adaptation are probably minimal.

The length of the ChCMV genome is 241,087 bp; the sizes of U_L and U_S genomic segments are 199,351 bp and 35,753 bp, respectively. The total length of the repeats flanking the U_L (TR_L and IR_L) is 11,247 bp; the repeats flanking the U_S (IR_S and TR_L) collectively are 2,254 bp long. One hundred and sixty-five ORFs presumably encoding viral proteins have been identified in the ChCMV genome using gene prediction algorithms.²⁰ The genomes of HCMV and ChCMV are collinear and homologous ORFs are named identically. Three HCMV ORFs ($UL1$, $UL111A$, and $UL3$) are absent in the ChCMV genome. It is of interest that one of these ORFs ($UL111A$) which encodes a viral interleukin-10 homolog is present in the genomes of other simian CMVs (RhCMV, AgmCMV, and BaCMV).⁴⁷ Four ChCMV ORFs ($UL146A$, $UL155$, $UL156$, and $UL157$) are absent in the HCMV genome. $UL146A$ and $UL157$ are closely related and presumably encode homologs of an α -chemokine.

The sequence similarity between ChCMV and its human counterpart is significantly greater than that of HCMV and any other known simian CMVs. The homology is higher in the central part of the U_L region where the core genes (shared by all herpesviruses) are located. However, even in this region there are multiple indels and the homology of even the most conserved genes (such as the DNA polymerase gene) is relatively low, around 80%. No obvious rearrangements, deletions, and other genetic changes associated with prolonged replication *in vitro* have been found in the ChCMV genome. The only possible exception is a frameshift in the first exon of $UL128$. A similar mutation is present in some laboratory strains of HCMV, but is absent in others.

The identification of ChCMV ORFs reported by Davidson *et al.*²⁰ is hardly final. At least four more ORFs predicted by using a newer gene prediction algorithm.⁵⁶ More importantly, *in silico* predictions have to be validated experimentally. Such experimental data are sparse for ChCMV; characterization of viral chemokine vCXCL-1 encoded by ChCMV $UL146$ being the only confirmed ORF. Although the amino acid sequence of this ChCMV protein is quite divergent from HCMV, the biological activities associated with both viral chemokines are similar.⁵³

Perhaps, the most unexpected result of *in silico* comparisons is a significant sequence similarity between prion protein PrP and ChCMV glycoprotein UL9.⁴² The biological meaning of this similarity is unclear.

14.6. AFRICAN GREEN MONKEY CMV

African green monkey or vervet CMV (AgmCMV) was the first simian CMV discovered. The official ICTV name of this virus is *Cercopithecine herpesvirus 5* (CeHV-5). AgmCMV was isolated in 1957 from a kidney culture from a vervet monkey (*Chlorocebus aethiops*) and provisionally identified as CMV in 1963.^{8,50} Despite its long presence on the virological scene, comparatively little is known about AgmCMV. The virus induces characteristic CPE in susceptible cell lines (such as MRC-5) which becomes visible approximately 2 weeks after inoculation. AgmCMV infection is apparently common in African green monkeys, although no systematic survey of wild monkeys for AgmCMV has been reported. The published data are limited to finding neutralizing antibodies against AgmCMV in 12 out of 12 vervets captured in the wild.⁵⁴

African green monkey kidney cultures are used for industrial propagation of poliovirus. It has been known since the late 1960s that such cultures are frequently contaminated with AgmCMV.⁷⁵ More recent PCR data show that about half of such cultures contain AgmCMV-specific DNA.^{7,31} However, the vast majority of the vaccine lots have been shown to be free of infectious AgmCMV.^{7,73}

14.7. BABOON CMV

CMVs have been isolated from several baboon species (*Papio anubis*, *P. ursinus*, and *P. cynocephalus*); all these isolates are generically named BaCMV.¹⁰ The isolation of BaCMV has been readily achieved by inoculation of human foreskin fibroblast (HFF) or human embryonic lung fibroblast (MRC-5) cell lines with baboon saliva or throat swabs. Genital swabs and urine were found to be a poor source of virus. BaCMV has also been isolated from a human recipient of a baboon liver xenotransplant.^{51,52}

In human fibroblasts, BaCMV induces a slowly developing CPE indistinguishable from that characteristic for the human and rhesus CMVs: foci of rounded cells with clearly visible inclusion bodies. Also typical for CMVs, BaCMV is highly cell-associated.

BaCMV infection can be diagnosed by detection of specific antibodies, virus isolation, or PCR.^{10,28,51,70} Antibodies against BaCMV are readily detectable by indirect ELISA using crude antigen prepared from virus-infected cultures at the peak of CPE. Antibodies against HCMV and RhCMV cross-react with such BaCMV anti-

gen and vice versa; in each case, however, the reactivity in homologous combinations is higher than that in heterologous combinations. More than 90% of captive and wild baboons, regardless of species, are seropositive for anti-BaCMV. Oral shedding is detectable by virus isolation in 25–50% of adult baboons, and immunosuppressed baboons exhibited increased shedding of virus.¹⁰ PCR tests specific for BaCMV isolates from yellow and chacma baboons are slightly less sensitive than the generic primate herpesvirus PCR.^{69,70} Genomic sequences are available for a few BaCMV genes [DNA polymerase (UL54 homolog), glycoprotein B (UL55 homolog), pUL56, and ssDNA bp (UL57 homolog)].

Most probably with the accumulation of data, particularly genomic sequences, species-specific characteristics of BaCMVs will be identified and designations indicating different baboon host species will be required.

14.8. DRILL CMV

The virus designated as DrCMV was isolated from an oropharyngeal swab obtained from a captive-born Nigerian drill (*Mandrillus leucophaeus*).⁹ Although more than 100 saliva and oral swab samples from 30 drills were tested by inoculation of human fibroblasts (MRC-5), only one isolate (OCOM6-2) was recovered. Whether this indicates a low prevalence of the virus in drills, a low incidence of viral shedding, or reflects technical difficulties is not clear. One possibility is that MRC-5 cells are not highly permissive for DrCMV and that isolate OCOM6-2 is either a mutant or originated in another species. The results of phylogenetic analysis of a partial DNA polymerase gene sequence suggest the possibility of a non-drill origin for DrCMV. Although DrCMV is closer related to BaCMV and AgmCMV than to RhCMV, it does not cluster with the frag-CMV from mandrill (*Mandrillus sphinx*), the species most closely related to drills.^{9,43} These data, clearly, are not conclusive and the analysis of a representative sample of CMVs from drills, mandrills, and simian CMVs from sympatric species is required to unequivocally establish the natural host of DrCMV (OCOM6-2).

14.9. SUMMARY

CMVs comprise the *Cytomegalovirus* genus within the *Betaherpesvirinae* subfamily of the *Herpesviridae* family. Three simian CMVs are recognized as species in the latest ICTV classification (8th Report); the viruses from rhesus monkey (RhCMV), chimpanzee (ChCMV),

and African green monkey (AgmCMV). CMV isolates from baboons, drills, owl, and squirrel monkeys have also been described. It is likely that many more simian species harbor their own CMVs. CMVs are larger than other herpesviruses (200–300 nm in diameter) and tend to be pleomorphic due to the irregular shape of the envelope. The CMV genome is also the largest among herpesvirus genomes. ChCMV is the closest relative of human CMV (HCMV). The genomes of HCMV and ChCMV are almost perfectly collinear. At the same time the sequence homology of orthologous genes in these genomes is on average moderate to low. Although RhCMV is clearly more distant from HCMV than ChCMV, many important features of HCMV infection are sufficiently closely mirrored in rhesus monkeys infected with RhCMV. The RhCMV/rhesus monkey model provides excellent opportunities for studying the pathogenesis of CMV disease in an immunocompromised host, most notably SIV-immunosuppressed macaques with SAIDS. Although congenital CMV disease is not observed in macaques, it can be experimentally induced by direct intrauterine inoculation of rhesus monkey fetuses with RhCMV. The development of effective prophylactic and therapeutic HCMV vaccines, a task of formidable complexity, can be facilitated by testing various immunization protocols using the RhCMV/rhesus monkey model.

REFERENCES

1. Alcendor, D. J., P. A. Barry, E. Pratt-Lowe, and P. A. Luciw. 1993. Analysis of the rhesus cytomegalovirus immediate-early gene promoter. *Virology* 194(2):815–821.
2. Asher, D. M., C. J. Gibbs Jr., D. J. Lang, D. C. Gajdusek, and R. M. Chanock. 1974. Persistent shedding of cytomegalovirus in the urine of healthy Rhesus monkeys. *Proc. Soc. Exp. Biol. Med.* 145(3):794–801.
3. Baroncelli, S., P. A. Barry, J. P. Capitanio, N. W. Lerche, M. Otsyula, and S. P. Mendoza. 1997. Cytomegalovirus and simian immunodeficiency virus coinfection: longitudinal study of antibody responses and disease progression. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 15(1):5–15.
4. Barry, P. A., D. J. Alcendor, M. D. Power, H. Kerr, and P. A. Luciw. 1996. Nucleotide sequence and molecular analysis of the rhesus cytomegalovirus immediate-early gene and the UL121-117 open reading frames. *Virology* 215(1):61–72.
5. Barry, P. A., K. M. Lockridge, S. Salamat, S. P. Tilling, Y. Yue, S. S. Zhou, S. M. Gospe Jr., W. J. Britt, and A. F. Tarantal. 2006. Nonhuman primate models of intrauterine cytomegalovirus infection. *ILAR J.* 47(1):49–64.
6. Baskin, G. B. 1987. Disseminated cytomegalovirus infection in immunodeficient rhesus monkeys. *Am. J. Pathol.* 129(2):345–352.
7. Baylis, S. A., N. Shah, A. Jenkins, N. J. Berry, and P. D. Minor. 2003. Simian cytomegalovirus and contamination of oral poliovirus vaccines. *Biologicals* 31(1):63–73.
8. Black, P. H., J. W. Hartley, and W. P. Rowe. 1963. Isolation of a cytomegalovirus from African green monkey. *Proc. Soc. Exp. Biol. Med.* 112:601–605.
9. Blewett, E. L., J. Lewis, E. L. Gadsby, S. R. Neubauer, and R. Eberle. 2003. Isolation of cytomegalovirus and foamy virus from the drill monkey (*Mandrillus leucophaeus*) and prevalence of antibodies to these viruses amongst wild-born and captive-bred individuals. *Arch. Virol.* 148(3):423–433.
10. Blewett, E. L., G. White, J. T. Saliki, and R. Eberle. 2001. Isolation and characterization of an endogenous cytomegalovirus (BaCMV) from baboons. *Arch. Virol.* 146(9):1723–1738.
11. Carlson, J. R., W. L. Chang, S. S. Zhou, A. F. Tarantal, and P. A. Barry. 2005. Rhesus brain microvascular endothelial cells are permissive for rhesus cytomegalovirus infection. *J. Gen. Virol.* 86(Pt 3):545–549.
12. Cha, T. A., E. Tom, G. W. Kemble, G. M. Duke, E. S. Mocarski, and R. R. Spaete. 1996. Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains. *J. Virol.* 70(1):78–83.
13. Chang, W. L. and P. A. Barry. 2003. Cloning of the full-length rhesus cytomegalovirus genome as an infectious and self-excisable bacterial artificial chromosome for analysis of viral pathogenesis. *J. Virol.* 77(9):5073–5083.
14. Chang, W. L., V. Kirchoff, G. S. Pari, and P. A. Barry. 2002. Replication of rhesus cytomegalovirus in life-expanded rhesus fibroblasts expressing human telomerase. *J. Virol. Methods* 104(2):135–146.
15. Chang, W. L., A. F. Tarantal, S. S. Zhou, A. D. Borowsky, and P. A. Barry. 2002. A recombinant rhesus cytomegalovirus expressing enhanced green fluorescent protein retains the wild-type phenotype and pathogenicity in fetal macaques. *J. Virol.* 76(18):9493–9504.
16. Chee, M. S., A. T. Bankier, S. Beck, R. Bohni, C. M. Brown, R. Cerny, T. Horsnell, C. A. Hutchison III, T. Kouzarides, J. A. Martignetti. 1990. Analysis of the protein-coding content of the sequence of human cytomegalovirus strain AD169. *Curr. Top. Microbiol. Immunol.* 154:125–169.

17. Conway, M. D., P. Didier, B. Fairburn, K. F. Soike, L. Martin, M. Murphey-Corb, N. Meiners, and M. S. Insler. 1990. Ocular manifestation of simian immunodeficiency syndrome (SAIDS). *Curr. Eye Res.* 9(8):759–770.
18. Daniel, M. D., L. V. Melendez, N. W. King, H. H. Barahona, C. E. Fraser, F. G. Garcia, and D. Silva. 1973. Isolation and characterization of a new virus from owl monkeys: herpesvirus aotus type 3. *Am. J. Phys. Anthropol.* 38(2):497–500.
19. Daniel, M. D., L. V. Melendez, N. W. King, C. E. Fraser, H. H. Barahona, R. D. Hunt, F. G. Garcia, and B. F. Trum. 1971. Herpes virus aotus: a latent herpesvirus from owl monkeys (*Aotus trivirgatus*) isolation and characterization. *Proc. Soc. Exp. Biol. Med.* 138(3):835–845.
20. Davison, A. J., A. Dolan, P. Akter, C. Addison, D. J. Dargan, D. J. Alcendor, D. J. McGeoch, and G. S. Hayward. 2003. The human cytomegalovirus genome revisited: comparison with the chimpanzee cytomegalovirus genome. *J. Gen. Virol.* 84(Pt 1):17–28.
21. DeFilippis, V. and K. Fruh. 2005. Rhesus cytomegalovirus particles prevent activation of interferon regulatory factor 3. *J. Virol.* 79(10):6419–6431.
22. Dolan, A., C. Cunningham, R. D. Hector, A. F. Hassan-Walker, L. Lee, C. Addison, D. J. Dargan, D. J. McGeoch, D. Gatherer, V. C. Emery, P. D. Griffiths, C. Sinzger, B. P. McSharry, G. W. Wilkinson, and A. J. Davison. 2004. Genetic content of wild-type human cytomegalovirus. *J. Gen. Virol.* 85(Pt 5):1301–1312.
23. Dreesman, G. R. and M. Benyesh-Melnick. 1967. Spectrum of human cytomegalovirus complement-fixing antigens. *J. Immunol.* 99(6):1106–1114.
24. Elkington, R., N. H. Shoukry, S. Walker, T. Crough, C. Fazou, A. Kaur, C. M. Walker, and R. Khanna. 2004. Cross-reactive recognition of human and primate cytomegalovirus sequences by human CD4 cytotoxic T lymphocytes specific for glycoprotein B and H. *Eur. J. Immunol.* 34(11):3216–3226.
25. Hansen, S. G., L. I. Strelow, D. C. Franchi, D. G. Anders, and S. W. Wong. 2003. Complete sequence and genomic analysis of rhesus cytomegalovirus. *J. Virol.* 77(12):6620–6636.
26. Henrickson, R. V., D. H. Maul, K. G. Osborn, J. L. Sever, D. L. Madden, L. R. Ellingsworth, J. H. Anderson, L. J. Lowenstein, and M. B. Gardner. 1983. Epidemic of acquired immunodeficiency in rhesus monkeys. *Lancet* 1(8321):388–390.
27. Higashi, K., H. Asada, T. Kurata, K. Ishikawa, M. Hayami, Y. Spratna, Y. Sutarmar, and K. Yamanishi. 1989. Presence of antibody to human herpesvirus 6 in monkeys. *J. Gen. Virol.* 70(Pt 12):3171–3176.
28. Hilliard, J., B. E. Lachmi, I. Soza, I. K. Brasky, and R. Mirkovic. 1996. PCR identification and differentiation of baboon cytomegalovirus from other human and nonhuman primate cytomegalovirus. *Mol. Diagn.* 1(3):267–273.
29. Ho, M. 2008. The history of cytomegalovirus and its diseases. *Med. Microbiol. Immunol.* 197(2):65–73.
30. Huff, J. L., R. Eberle, J. Capitanio, S. S. Zhou, and P. A. Barry. 2003. Differential detection of B virus and rhesus cytomegalovirus in rhesus macaques. *J. Gen. Virol.* 84(Pt 1):83–92.
31. Johnson, G., D. Dick, M. Ayers, M. Petric, and R. Tellier. 2003. Detection and species-level identification of primate herpesviruses with a comprehensive PCR test for human herpesviruses. *J. Clin. Microbiol.* 41(3):1256–1258.
32. Jones-Engel, L., G. A. Engel, J. Heidrich, M. Chalise, N. Poudel, R. Viscidi, P. A. Barry, J. S. Allan, R. Grant, and R. Kyes. 2006. Temple monkeys and health implications of commensalism, Kathmandu, Nepal. *Emerg. Infect. Dis.* 12(6):900–906.
33. Kaup, F., K. Matz-Rensing, E. Kuhn, P. Hunerbein, C. Stahl-Hennig, and G. Hunsmann. 1998. Gastrointestinal pathology in rhesus monkeys with experimental SIV infection. *Pathobiology* 66(3–4):159–164.
34. Kaur, A., M. D. Daniel, D. Hempel, D. Lee-Parritz, M. S. Hirsch, and R. P. Johnson. 1996. Cytotoxic T-lymphocyte responses to cytomegalovirus in normal and simian immunodeficiency virus-infected rhesus macaques. *J. Virol.* 70(11):7725–7733.
35. Kaur, A., C. L. Hale, B. Noren, N. Kassis, M. A. Simon, and R. P. Johnson. 2002. Decreased frequency of cytomegalovirus (CMV)-specific CD4⁺ T lymphocytes in simian immunodeficiency virus-infected rhesus macaques: inverse relationship with CMV viremia. *J. Virol.* 76(8):3646–3658.
36. Kaur, A., N. Kassis, C. L. Hale, M. Simon, M. Elliott, A. Gomez-Yafal, J. D. Lifson, R. C. Desrosiers, F. Wang, P. Barry, M. Mach, and R. P. Johnson. 2003. Direct relationship between suppression of virus-specific immunity and emergence of cytomegalovirus disease in simian AIDS. *J. Virol.* 77(10):5749–5758.
37. Khan, I. H., S. Mendoza, J. Yee, M. Deane, K. Venkateswaran, S. S. Zhou, P. A. Barry, N. W. Lerche, and P. A. Luciw. 2006. Simultaneous detection of antibodies to six nonhuman-primate viruses by multiplex microbead immunoassay. *Clin. Vaccine Immunol.* 13(1):45–52.
38. Kirchoff, V., S. Wong, J. S. St, and G. S. Pari. 2002. Generation of a life-expanded rhesus monkey fibroblast cell line for the growth of rhesus rhadinovirus (RRV). *Arch. Virol.* 147(2):321–333.
39. Kravitz, R. H., K. S. Sciabica, K. Cho, P. A. Luciw, and P. A. Barry. 1997. Cloning and characterization of rhesus cytomegalovirus glycoprotein B. *J. Gen. Virol.* 78(Pt 8):2009–2013.

40. Kropff, B. and M. Mach. 1997. Identification of the gene coding for rhesus cytomegalovirus glycoprotein B and immunological analysis of the protein. *J. Gen. Virol.* 78(Pt 8):1999–2007.
41. Kuhn, E. M., K. Matz-Rensing, C. Stahl-Hennig, B. Makoschey, G. Hunsmann, and F. J. Kaup. 1997. Intestinal manifestations of experimental SIV-infection in rhesus monkeys (*Macaca mulatta*): a histological and ultrastructural study. *Zentralbl. Veterinarmed. B* 44(8):501–512.
42. Kuznetsov, I. B. and S. Rackovsky. 2003. Similarity between the C-terminal domain of the prion protein and chimpanzee cytomegalovirus glycoprotein UL9. *Protein Eng.* 16(12):861–863.
43. Lacoste, V., P. Maclere, G. Dubreuil, J. Lewis, M. C. Georges-Courbot, J. Rigoulet, T. Petit, and A. Gessain. 2000. Simian homologues of human gamma-2 and betaherpesviruses in mandrill and drill monkeys. *J. Virol.* 74(24):11993–11999.
44. Lacoste, V., E. J. Verschoor, E. Nerrienet, and A. Gessain. 2005. A novel homologue of Human herpesvirus 6 in chimpanzees. *J. Gen. Virol.* 86(Pt 8):2135–2140.
45. Lilja, A. E., W. L. Chang, P. A. Barry, S. P. Becerra, and T. E. Shenk. 2008. Functional genetic analysis of rhesus cytomegalovirus: Rh01 is an epithelial cell tropism factor. *J. Virol.* 82(5):2170–2181.
46. Lockridge, K. M., G. Sequear, S. S. Zhou, Y. Yue, C. P. Mandell, and P. A. Barry. 1999. Pathogenesis of experimental rhesus cytomegalovirus infection. *J. Virol.* 73(11):9576–9583.
47. Lockridge, K. M., S. S. Zhou, R. H. Kravitz, J. L. Johnson, E. T. Sawai, E. L. Blewett, and P. A. Barry. 2000. Primate cytomegaloviruses encode and express an IL-10-like protein. *Virology* 268(2):272–280.
48. London, W. T., A. J. Martinez, S. A. Houff, W. C. Wallen, B. L. Curfman, R. G. Traub, and J. L. Sever. 1986. Experimental congenital disease with simian cytomegalovirus in rhesus monkeys. *Teratology* 33(3):323–331.
49. Malherbe, H. and R. Harwin. 1957. Seven viruses isolated from the vervet monkey. *Br. J. Exp. Pathol.* 38(5):539–541.
50. Malherbe, H. and R. Harwin. 1963. The cytopathic effects of vervet monkey viruses. *S. Afr. Med. J.* 37:407–411.
51. Michaels, M. G., D. J. Alcendor, K. St George, C. R. Rinaldo Jr., G. D. Ehrlich, M. J. Becich, and G. S. Hayward. 1997. Distinguishing baboon cytomegalovirus from human cytomegalovirus: importance for xenotransplantation. *J. Infect. Dis.* 176(6):1476–1483.
52. Michaels, M. G., F. J. Jenkins, K. St George, M. A. Nalesnik, T. E. Starzl, and C. R. Rinaldo Jr. 2001. Detection of infectious baboon cytomegalovirus after baboon-to-human liver xenotransplantation. *J. Virol.* 75(6):2825–2828.
53. Miller-Kittrell, M., J. Sai, M. Penfold, A. Richmonde, and T. E. Sparer. 2007. Functional characterization of chimpanzee cytomegalovirus chemokine, vCXCL-1(CCMV). *Virology* 364(2):454–465.
54. Minamishima, Y., B. J. Graham, and M. Benyesh-Melnick. 1971. Neutralizing antibodies to cytomegaloviruses in normal simian and human sera. *Infect. Immun.* 4(4):368–373.
55. Mocarski, E. S., T. Shenk, and R. F. Pass. 2007. Cytomegaloviruses. In: Knipe, D. M. and P. M. Howley (eds), *Fields Virology*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, Wolters Kluwer Business, pp. 2701–2772.
56. Murphy, E., I. Rigoutsos, T. Shibuya, and T. E. Shenk. 2003. Reevaluation of human cytomegalovirus coding potential. *Proc. Natl. Acad. Sci. U. S. A.* 100(23):13585–13590.
57. Murphy, E., D. Yu, J. Grimwood, J. Schmutz, M. Dickson, M. A. Jarvis, G. Hahn, J. A. Nelson, R. M. Myers, and T. E. Shenk. 2003. Coding potential of laboratory and clinical strains of human cytomegalovirus. *Proc. Natl. Acad. Sci. U. S. A.* 100(25):14976–14981.
58. North, T. W., G. Sequear, L. B. Townsend, J. C. Drach, and P. A. Barry. 2004. Rhesus cytomegalovirus is similar to human cytomegalovirus in susceptibility to benzimidazole nucleosides. *Antimicrob. Agents Chemother.* 48(7):2760–2765.
59. Oxford, K. L., M. K. Eberhardt, K. W. Yang, L. Strele, S. Kelly, S. S. Zhou, and P. A. Barry. 2008. Protein coding content of the UL)b' region of wild-type rhesus cytomegalovirus. *Virology* 373(1):181–188.
60. Pande, N. T., C. Powers, K. Ahn, and K. Fruh. 2005. Rhesus cytomegalovirus contains functional homologues of US2, US3, US6, and US11. *J. Virol.* 79(9):5786–5798.
61. Pearson, T. C., J. Trambley, K. Odom, D. C. Anderson, S. Cowan, R. Bray, A. Lin, D. Hollenbaugh, A. Aruffo, A. W. Siadak, E. Strobert, R. Hennigar, and C. P. Larsen. 2002. Anti-CD40 therapy extends renal allograft survival in rhesus macaques. *Transplantation* 74(7):933–940.
62. Penfold, M. E., T. L. Schmidt, D. J. Dairaghi, P. A. Barry, and T. J. Schall. 2003. Characterization of the rhesus cytomegalovirus US28 locus. *J. Virol.* 77(19):10404–10413.
63. Powers, C. and K. Fruh. 2008. Rhesus CMV: an emerging animal model for human CMV. *Med. Microbiol. Immunol.* 197(2):109–115.
64. Prepens, S., K. A. Kreuzer, F. Leendertz, A. Nitsche, and B. Ehlers. 2007. Discovery of herpesviruses in multi-infected primates using locked nucleic acids (LNA) and a bigenic PCR approach. *Virol. J.* 4:84.

65. Price, D. A., A. D. Bitmansour, J. B. Edgar, J. M. Walker, M. K. Axthelm, D. C. Douek, and L. J. Picker. 2008. Induction and evolution of cytomegalovirus-specific CD4⁺ T cell clonotypes in rhesus macaques. *J. Immunol.* 180(1):269–280.
66. Prichard, M. N., M. E. Penfold, G. M. Duke, R. R. Spaete, and G. W. Kemble. 2001. A review of genetic differences between limited and extensively passed human cytomegalovirus strains. *Rev. Med. Virol.* 11(3):191–200.
67. Rangan, S. R. and J. Chaiban. 1980. Isolation and characterization of a cytomegalovirus from the salivary gland of a squirrel monkey (*Saimiri sciureus*). *Lab. Anim. Sci.* 30(3):532–540.
68. Rivaillet, P., A. Kaur, R. P. Johnson, and F. Wang. 2006. Genomic sequence of rhesus cytomegalovirus 180.92: insights into the coding potential of rhesus cytomegalovirus. *J. Virol.* 80(8):4179–4182.
69. Ross, T. G., R. P. Rogers, N. Elfrink, N. Bray, and E. L. Blewett. 2005. Detection of baboon cytomegalovirus (BaCMV) by PCR using primers directed against the glycoprotein B gene. *J. Virol. Methods* 125(2):119–124.
70. Rose, T. M., K. B. Strand, E. R. Schultz, G. Schaefer, G. W. Rankin Jr., M. E. Thouless, C. C. Tsai, and M. L. Bosch. 1997. Identification of two homologs of the Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in retroperitoneal fibromatosis of different macaque species. *J. Virol.* 71(5):4138–4144.
71. Rue, C. A., M. A. Jarvis, A. J. Knoche, H. L. Meyers, V. R. DeFilippis, S. G. Hansen, M. Wagner, K. Fruh, D. G. Anders, S. W. Wong, P. A. Barry, and J. A. Nelson. 2004. A cyclooxygenase-2 homologue encoded by rhesus cytomegalovirus is a determinant for endothelial cell tropism. *J. Virol.* 78(22):12529–12536.
72. Se quar, G., W. J. Britt, F. D. Lakeman, K. M. Lockridge, R. P. Tarara, D. R. Canfield, S. S. Zhou, M. B. Gardner, and P. A. Barry. 2002. Experimental coinfection of rhesus macaques with rhesus cytomegalovirus and simian immunodeficiency virus: pathogenesis. *J. Virol.* 76(15):7661–7671.
73. Sierra-Honigmann, A. M. and P. R. Krause. 2002. Live oral poliovirus vaccines and simian cytomegalovirus. *Biologicals* 30(3):167–174.
74. Simon, M. A., L. V. Chalifoux, and D. J. Ringler. 1992. Pathologic features of SIV-induced disease and the association of macrophage infection with disease evolution. *AIDS Res. Hum. Retroviruses* 8(3):327–337.
75. Smith, K. O., J. F. Thiel, J. T. Newman, E. Harvey, M. D. Trousdale, W. D. Gehle, and G. Clark. 1969. Cytomegaloviruses as common adventitious contam inants in primary African green monkey kidney cell cultures. *J. Natl. Cancer Inst.* 42(3):489–496.
76. Swack, N. S. and G. D. Hsiung. 1982. Natural and experimental simian cytomegalovirus infections at a primate center. *J. Med. Primatol.* 11(3):169–177.
77. Swack, N. S., O. C. Liu, and G. D. Hsiung. 1971. Cytomegalovirus infections of monkeys and baboons. *Am. J. Epidemiol.* 94(4):397–402.
78. Swanson, R., E. Bergquam, and S. W. Wong. 1998. Characterization of rhesus cytomegalovirus genes associated with anti-viral susceptibility. *Virol.* 240(2):338–348.
79. Swinkels, B. W., J. L. Geelen, P. Wertheim-van Dillen, A. A. van Es, and J. van der Noordaa. 1984. Initial characterization of four cytomegalovirus strains isolated from chimpanzees. Brief report. *Arch. Virol.* 82(1–2):125–128.
80. Tarantal, A. F., M. S. Salamat, W. J. Britt, P. A. Luciw, A. G. Hendrickx, and P. A. Barry. 1998. Neuropathogenesis induced by rhesus cytomegalovirus in fetal rhesus monkeys (*Macaca mulatta*). *J. Infect. Dis.* 177(2):446–450.
81. Vogel, P., B. J. Weigler, H. Kerr, A. G. Hendrickx, and P. A. Barry. 1994. Seroepidemiologic studies of cytomegalovirus infection in a breeding population of rhesus macaques. *Lab. Anim. Sci.* 44(1):25–30.
82. Wroblewska, Z., D. Gilden, M. Devlin, E. S. Huang, L. B. Rorke, T. Hamada, T. Furukawa, L. Cummins, S. Kalter, and H. Koprowski. 1979. Cytomegalovirus isolation from a chimpanzee with acute demyelinating disease after inoculation of multiple sclerosis brain cells. *Infect. Immun.* 25(3):1008–1015.
83. Yue, Y., A. Kaur, M. K. Eberhardt, N. Kassis, S. S. Zhou, A. F. Tarantal, and P. A. Barry. 2007. Immunogenicity and protective efficacy of DNA vaccines expressing rhesus cytomegalovirus glycoprotein B, phosphoprotein 65-2, and viral interleukin-10 in rhesus macaques. *J. Virol.* 81(3):1095–1109.
84. Yue, Y., A. Kaur, S. S. Zhou, and P. A. Barry. 2006. Characterization and immunological analysis of the rhesus cytomegalovirus homologue (Rh112) of the human cytomegalovirus UL83 lower matrix phosphoprotein (pp65). *J. Gen. Virol.* 87(Pt 4):777–787.
85. Yue, Y., Z. Wang, K. Abel, J. Li, L. Strelow, A. Mandarino, M. K. Eberhardt, K. A. Schmidt, D. J. Diamond, and P. A. Barry. 2008. Evaluation of recombinant modified vaccinia Ankara virus-based rhesus cytomegalovirus vaccines in rhesus macaques. *Med. Microbiol. Immunol.* 197(2):117–123.
86. Yue, Y., S. S. Zhou, and P. A. Barry. 2003. Antibody responses to rhesus cytomegalovirus glycoprotein B in naturally infected rhesus macaques. *J. Gen. Virol.* 84(Pt 12):3371–3379.

15

Lymphocryptoviruses

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15.1. INTRODUCTION

The prototype primate lymphocryptovirus (LCV) is *Human herpesvirus 4* (HHV-4), much better known as Epstein–Barr virus (EBV). EBV is one of the most ex-

tensively studied viruses, perhaps second only to HIV in this respect.

EBV is ubiquitous in human populations. Generally the virus is acquired during childhood or adolescence and is carried by virtually every adult for life. Primary EBV infection is either asymptomatic or manifested as self-limiting lymphoproliferative disease, infectious mononucleosis (IM). Persistent EBV infection is asymptomatic in the vast majority of virus carriers. However, in a small fraction of infected individuals EBV causes malignancies, most notably Burkitt's lymphoma, nasopharyngeal carcinoma, and immunodeficiency-associated lymphoproliferative disease.

EBV-related LCVs have been found in virtually every Old World monkey and ape species that has been sufficiently well studied, as well as in some New World monkey species. Knowledge of simian LCVs in general is less comprehensive compared to EBV, and the degree of detail known about isolates from different simian species varies substantially. At the same time, it is clear that simian LCVs share many characteristics with EBV. For this reason the description of general properties of the primate LCVs is based mainly on EBV (Section 15.3). Specific characteristics of individual simian LCVs are described in Sections 15.4 and 15.5.

15.2. CLASSIFICATION AND NOMENCLATURE

Primate LCVs belong to the genus *Lymphocryptovirus*, within the subfamily *Gammaherpesvirinae*. Seven simian LCVs are currently recognized as species (Table 15.1). Several more simian LCVs (from stump-tailed macaques, cynomolgus macaques, and other species) have not been yet included in the International

Table 15.1. ICTV-Recognized Simian LCV Species

ICTV Old Name	ICTV New Name*	Common Names	Species of Origin	Original References
<i>Cercopithecine herpesvirus 12</i> (CeHV-12)	<i>Papiine herpesvirus 1</i> (PaHV-1)	Herpesvirus papio (HVP), Baboon herpesvirus, baboon EBV-like virus, baboon LCV, LCV _{Pha} †	<i>Papio</i> spp.	2
<i>Cercopithecine herpesvirus 14</i> (CeHV-14)	<i>Cercopithecine herpesvirus 14</i> (CeHV-14)	African green monkey EBV-like virus, Herpesvirus AGM, African green monkey LCV, LCV _{Cae} †	<i>Chlorocebus aethiops</i>	7
<i>Cercopithecine herpesvirus 15</i> (CeHV-15)	<i>Macacine herpesvirus 4</i> (McHV-4)	Rhesus EBV-like herpesvirus, Rhesus LCV, LCV _{Mmu} †	<i>Macaca mulatta</i>	3,95
<i>Pongine herpesvirus 1</i> (PoHV-1)	<i>Panine herpesvirus 1</i> (PnHV-1)	Herpesvirus pan, Chimpanzee EBV-like virus, Chimpanzee LCV, LCV _{Ptr} †	<i>Pan troglodytes</i>	50
<i>Pongine herpesvirus 2</i> (PoHV-2)	<i>Pongine herpesvirus 2</i> (PoHV-2)	Herpesvirus pongo Orangutan herpesvirus, Orangutan LCV, LCV _{Ppy} †	<i>Pongo pygmaeus</i>	97
<i>Pongine herpesvirus 3</i> (PoHV-3)	<i>Gorilline herpesvirus 1</i> (GoHV-3)	Gorilla herpesvirus Herpesvirus gorilla, gorilla LCV, LCV _{Ggo} †	<i>Gorilla gorilla</i>	80
<i>Callitrichine herpesvirus 3</i> (CalHV-3)	<i>Callitrichine herpesvirus 3</i> (CalHV-3)	Marmoset LCV, LCV _{Cja} †	<i>Callithrix jacchus</i>	10

*Formally approved in May 2008.

†Nomenclature used in this chapter.

Committee on Taxonomy of Viruses (ICTV) classification.

The official names of simian LCVs are rarely used. The main disadvantage of these names is the lack of information on the host species and viral genus. The situation with common names of simian LCVs is quite perplexing, particularly for the novice. Most of these names originated before introduction of the *Lymphocryptovirus* genus. Researchers active in this field tend to use the names for the isolates they first described. None of these ad hoc nomenclatures is fully satisfactory. Perhaps the most informative is the simian LCV naming scheme suggested by Wang *et al.*¹¹⁹ According to this scheme, a virus name includes the common name of the natural host plus the abbreviation LCV (e.g., rhesus LCV, baboon LCV). The drawback of this nomenclature is the ambiguity in information about the host

species because some common names like baboons and marmosets are generic for several species. In this chapter whenever possible, we use a three-letter subscript indicating the host species. For example, LCV_{Mmu} is a virus isolated from rhesus monkeys (*Macaca mulatta*).

With the advent of polymerase chain reaction (PCR)-based methods for detection of unknown herpesvirus sequences, multiple “frag-herpesviruses” have been identified in numerous nonhuman primate (NHP) species.^{18,19,90,104,113} Most of these “frag-LCVs” are known only from a short sequence within the highly conserved DNA polymerase gene.¹⁹ In a few cases, frag-LCVs are represented by a longer genomic sequence (about 3,300 bp).⁹⁰ Known frag-LCVs are listed in Table 15.2. Interestingly, more than one type of frag-LCV have been identified in some species such

Table 15.2. LCV Frag-Viruses

Host Species	Genomic Fragments*	References or GenBank Acc. No.
Apes		
<i>Hylobates agilis</i>	DNA pol	88
<i>H. lar</i>	DNA pol	19,88
<i>H. muelleri</i>	DNA pol	AY273184
<i>H. pileatus</i>	DNA pol	19,88
<i>Nomascus leucogenys</i>	DNA pol	AY425964
<i>Hylobates syndactylus</i>	DNA pol	AY608703
<i>Pongo pygmaeus</i>	DNA pol, gB	19,39
<i>Gorilla gorilla</i>	DNA pol, gB	19,39
<i>Pan troglodytes</i>	DNA pol, gB	19,39,90
<i>Pan paniscus</i>	DNA pol	19
Old World monkeys		
<i>Papio hamadryas</i>	DNA pol, gB	19,90
<i>Mandrillus sphinx</i>	DNA pol	19
<i>Erythrocebus patas</i>	DNA pol	19
<i>Cercopithecus neglectus</i>	DNA pol	AY608712
<i>C. cephus</i>	DNA pol	AY608711
<i>C. hamlyni</i>	DNA pol	AY608706
<i>C. nictitans</i>	DNA pol	AY608709
<i>Miopithecus talapoin</i>	DNA pol	AY608708
<i>Leontopithecus rosalia</i>	DNA pol	AY608705
<i>Chlorocebus aethiops</i>	DNA pol	AY608702
<i>Lophocebus albigena</i>	DNA pol	AY608710
<i>Colobus quereza</i>	DNA pol	19,90
<i>Piliocolobus badius</i>	DNA pol	19
<i>Cercocebus aterrimus</i>	DNA pol	19
<i>Macaca mulatta</i>	DNA pol	19
<i>M. fascicularis</i>	DNA pol, LMP-1, EBNA-1, gB	19,90
<i>M. fuscata</i>	DNA pol	19
<i>M. arctoides</i>	DNA pol	19
<i>M. sylvanus</i>	DNA pol	19
<i>M. silenus</i>	DNA pol	19
<i>M. tibetana</i>	DNA pol	19
<i>Semnopithecus entellus</i>	DNA pol	19
New World monkeys		
<i>Saimiri sciureus</i>	DNA pol	13,19
<i>Pithecia pithecia</i>	DNA pol	13,19
<i>Cebus albifrons</i>	DNA pol	19
<i>Ateles paniscus</i>	DNA pol	19
<i>Callithrix penicillata</i>	DNA pol	19
<i>C. jacchus</i>	DNA pol	19
<i>Saqinus midas</i>	DNA pol	13

*DNAPol, DNA polymerase gene; gB, glycoprotein B gene; LMP-1, latent membrane protein 1 gene; EBNA-1, EBV nuclear antigen 1 gene homolog.

as gorillas, hamadryas baboons, mandrills, Japanese macaques, and squirrel monkeys.^{19,90}

The phylogeny of primate LCVs is poorly resolved. Only two groups of LCVs have been reliably distinguished by phylogenetic analysis: (1) viruses from Old World simian species and humans and (2) viruses from New World monkeys.^{19,39,90} Statistical support for the lineages within these groups is low. The existence of two Old World LCV “genogroups” has been suggested.¹⁹ However, this is not supported by analysis of genomic regions other than the polymerase gene. Apparently, the main reason why LCV phylogeny is poorly resolved is the high degree of conservation within the genomic regions that have been analyzed; that is, the DNA polymerase and glycoprotein B genes.

15.3. GENERAL PROPERTIES

15.3.1. Genome Composition and Gene Products

Other than EBV, whole genome DNA sequences are available for two simian viruses: LCV_{Mmu} (GenBank Acc. No. AY037858) from rhesus monkeys and LCV_{Cja} (GenBank Acc. No. NC_004367) from common marmosets (*Callithrix jacchus*).^{101,102} The general composition of all three genomes is very similar. Sequence information on genomes of other simian LCVs is fragmentary.^{22,30–32,52,53,70,72,73,86–88,90,106,107,123} However, despite the incompleteness of these data, there is little doubt that all simian LCV genomes are structured similarly. The most general features of primate LCV genomes are presented in Figure 15.1.

The LCV genome is linear, being about 170,000 bp long (171,096 bp for LCV_{Mmu}). It contains two types of sequences: repeated and unique. The genome structure can be viewed as a string of alternating repeats and unique regions:

5'-TR-U1-IR1-U2-IR2
-U3-IR3-U4-IR4-U5-TR-3'

Terminal repeats (TRs) are located at both ends of the genome. There are four internal repeats (IR1–IR4). Each of the five unique sequence regions is bracketed by repeats. The length of the TR repeat unit in different LCV genomes varies; in EBV, it is almost half that in LCV_{Mmu} (538 bp versus 933 bp). The number of repeat unit copies in the TR is constant in clonal progeny derived from a single infectious virion. This marker is used for determining clonality of the virus.

The major internal repeat, IR1, contains 5–10 copies of a repeat unit. The length of this unit is approximately 3,000 bp. The IR1 repeat unit coincides with the BamH1 restriction fragment W in the EBV genome—hence the designation of this repeat as BamHI-W which is quite common in the literature.

There are about 80 open reading frames (ORFs) in the LCV genomes (Table 15.3). The genomes of EBV and LCV_{Mmu} are strictly collinear, in that every ORF is present in both genomes. The genetic repertoire of LCV_{Cja} is slightly different. However, the vast majority of LCV_{Cja} ORFs have homologs in EBV and LCV_{Mmu} genomes.¹⁰²

Nomenclature for simian LCV ORFs mimics that used for EBV. EBV ORFs were initially identified in BamH1 restriction fragments. These BamH1 fragments are designated A, B, C, and so forth, in order of their decreasing size. EBV ORFs are designated according to the location within a particular BamH1 fragment and the coding DNA strand. For example, BLRF1 and BLLF1 are ORFs located of BamH1 L fragment; these ORFs are transcribed in the rightward (R) and leftward (L) directions (in other words they are encoded by different strands of genomic DNA). The letter F which is present in all EBV ORF names means “frame.” If there is more than one ORF encoded by a strand within a particular BamH1 fragment they are numbered in the 5'-to-3' direction on the plus-strand. Those ORFs which encode the most extensively studied proteins are frequently referred to by their protein products. For example, BLRF1 and BLLF1 encode glycoprotein N (gN) and the major

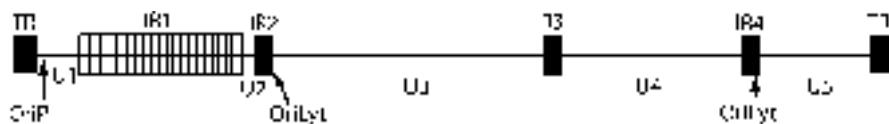


Figure 15.1. “Macro” map of primate lymphocryptovirus genome. TR, terminal repeats; U1, U2, U3, U4, U5, unique regions; IR1, IR2, IR3, IR4, internal repeats; OriLyt, origin of lytic replication; OriP, plasmid origin of replication.

Table 15.3. Homology between Orthologous ORFs in EBV, LCV_{Mmu}, and LCV_{Cja} Genomes^{101,102}

EBV ORFs	LCV _{Mmu} ORFs	% aa Homology EBV-LCV _{Mmu}	LCV _{Cja} ORFs	% aa Homology EBV-LCV _{Cja}	Putative Protein Products
LMP2A e2	LMP2A e2	66.2			
LMP2A e3	LMP2A e3	51.2			
LMP2A e4	LMP2A e4	67.5			
LMP2A e5	LMP2A e5	76.9			
LMP2A e6	LMP2A e6	50.9			
LMP2A e7	LMP2A e7	73.6			
LMP2A e8	LMP2A e8	45.7			
BNRF1	BNRF1	77.6	ORF65	40.9	Tegument protein
LMP2A e9	LMP2A e9				
EBER1	EBER1				RNA*
EBER2	EBER2				RNA*
BCRF1	BCRF1	84.1			IL-10 homologue
EBNA-LP	EBNA-LP	53.3			Nuclear protein
EBNA-LP C1	EBNA-LP C1				
EBNA-LP C2	EBNA-LP C2				
EBNA-LP W1	EBNA-LP W1	76.2			
EBNA-LP W2	EBNA-LP W2	52.3			
EBNA-LP Y1	EBNA-LP Y1	45.5			
EBNA-LP Y2	EBNA-LP Y2	44.1			
EBNA-2	EBNA2	29.8			Nuclear protein
BHLF1	BHLF1	63.6			
BHRF1	BHRF1	72.8	ORF64	20.5	bel-2 homologue
BFLF2	BFLF2	79.6			
BFLF1	BFLF1	85.5	ORF62	52.1	Glycoprotein
BFRF1	BFRF1	78.4	ORF61	45.2	Tegument protein
BFRF2	BFRF2	80.4	ORF60	34.5	
BFRF3	BFRF3	69.2	ORF59	38.3	Capsid protein
BPLF1	BPLF1	74.3	ORF58	45.8	Tegument protein
BORF1	BORF1	86.5	ORF56	46.8	Minor capsid protein
BOLF1	BOLF1	71.1	ORF57	37.5	Tegument protein
BORF2	BORF2	87.5	ORF55	55.5	Ribonucleotide reductase
BaRF1	BaRF1	96.0	ORF54	73.5	Ribonucleotide reductase
BMRF1	BMRF1	85.9	ORF53	40.4	DNA polymerase processivity factor
BMRF2	BMRF2	86.0	ORF52	46.2	Membrane protein
BMLF1	BMLF1	80.4	ORF51	38.8	Transactivator
BSLF1	BSLF1	86.4	ORF50	46.9	Helicase complex
BSRF1	BSRF1	89.4	ORF49	61.5	
BLLF3	BLLF3	87.3	ORF48	54.3	dUTPase
BLRF1	BLRF1	74.5	ORF47	44.1	Glycoprotein N, gp15
BLRF2	BLRF2	76.5	ORF46	40.3	
BLLF2	BLLF2	56.0			
gp350	gp350	49.3	ORF45	22.4	Glycoprotein, gp350

(Continued)

Table 15.3. (Continued)

EBV ORFs	LCV _{Mmu} ORFs	% aa Homology EBV-LCV _{Mmu}	LCV _{Cja} ORFs	% aa Homology EBV-LCV _{Cja}	Putative Protein Products
EBNA3A	EBNA3A	29.4			Nuclear protein
EBNA3A e1	EBNA3A e1	37.6			
EBNA3A e2	EBNA3A e2	28.0			
EBNA3B	EBNA3B	30.5			Nuclear protein
EBNA3B e1	EBNA3B e1	37.7			
EBNA3B e2	EBNA3B e2	31.8			
EBNA3C	EBNA3C	31.2			Nuclear protein
EBNA3C e1	EBNA3C e1	32.5			
EBNA3C e2	EBNA3C e2	30.1			
BZLF2	BZLF2	77.6	ORF44	33.6	Glycoprotein, gp42
BZLF1	BZLF1	71.3	ORF43	29.0	Transactivator
BZLF1 e3	BZLF1 e3	80.5			
BZLF1 e2	BZLF1 e2	88.9			
BZLF1 e1	BZLF1 e1	60.5			
BRLF1	BRLF1	76.3	ORF42	39.0	Transactivator
BRRF1	BRRF1	85.5	ORF41	46.7	
BRRF2	BRRF2	60.6	ORF40	22.4	
EBNA1(BKRF1)	EBNA1	46.3	ORF39	36.0	Nuclear protein
BKRF2	BKRF2	81.6	ORF38	50.4	Glycoprotein L, gp25
BKRF3	BKRF3	96.9	ORF37	74.9	Uracil-DNA glycosylase
BKRF4	BKRF4	69.0	ORF36	27.7	
BBRF1	BBRF1	92.5	ORF34	70.6	Capsid protein
BBLF4	BBLF4	93.4	ORF35	65.3	Helicase complex
BBRF2	BBRF2	91.4	ORF33	55.8	
BBLF3	BBLF3	73.6	ORF32	21.9	Helicase complex
BBLF2	BBLF2	72.8	ORF31	20.3	Helicase complex
BBRF3	BBRF3	90.4	ORF30	55.6	Glycoprotein M, gp84/1113
BBLF1	BBLF1	72.0	ORF29	38.7	Myristylated tegument protein
BGLF5	BGLF5	94.0	ORF28	69.6	Alkaline exonuclease
BGLF4	BGLF4	89.6	ORF27	59.9	Kinase
BGRF1	BDRF1e1	91.6	ORF24	63.7	Packaging protein
BGLF3	BGLF3	88.3	ORF25	48.2	
BGLF3.5			ORF26	35.6	
BGLF2	BGLF2	87.8	ORF23	57.4	
BGLF1	BGLF1	74.7	ORF22	30.4	
BDLF4	BDLF4	86.2	ORF21	51.9	
BDRF1	BDRF1 e2	94.7	ORF20	74.6	Packaging protein
BDLF3	BDLF3	46.6			Glycoprotein, gp150
BDLF2	BDLF2	68.5	ORF19	26.3	
BDLF1	BDLF1	96.7	ORF18	70.1	Capsid protein
BcLF1	BcLF1	95.8	ORF17	75.5	Capsid protein
BcRF1	BcRF1	83.0	ORF16	44.2	
BTRF1	BTRF1	86.6	ORF15	57.0	

Table 15.3. (Continued)

EBV ORFs	LCV _{Mmu} ORFs	% aa Homology EBV-LCV _{Mmu}	LCV _{Cja} ORFs	% aa Homology EBV-LCV _{Cja}	Putative Protein Products
BXLF2	BXLF2	85.1	ORF14	44.6	Glycoprotein H, gp85
BXLF1	BXLF1	89.1	ORF13	49.1	Thymidine kinase
BXRF1	BXRF1	80.6	ORF12	37.1	
BVRF1	BVRF1	84.6	ORF11	47.4	Tegument protein
BVRF2	BVRF2	66.4	ORF9	38.3	Capsid protein
BVLF1.5			ORF10	34.5	
BILF2	BILF2	75.0			Glycoprotein, gp78
LF3	LF3	42.7			
LF2	LF2	92.5	ORF8	63.6	
LF1	LF1	75.3	ORF7	24.0	
BILF1	BILF1	80.4	ORF6	38.2	Glycoprotein, gp64
ECRF4	ECRF4	79.3			
BALF5	BALF5	94.8	ORF5	73.5	DNA polymerase
BALF4	BALF4	85.6	ORF4	58.7	Glycoprotein B, gp110
BARF0	BARF0	77.0			Nuclear protein
BALF3	BALF3	85.7	ORF3	55.2	Transport protein
BALF2	BALF2	90.3	ORF2	68.3	DNA binding protein
BALF1	BALF1	84.1	ORF1	27.6	bcl-2 homologue
BARF1	BARF1	75.0			CSF1-R homologue
LMP2A	LMP2A	57.0			Latent membrane protein 2
LMP2A	LMP2A e1	31.9			
BNLF2b	BNLF2B	68.1			
BNLF2a	BNLF2A	51.7			
LMP1 e3	LMP1 e3	52.3			
LMP1 e2	LMP1 e2	39.3			
LMP1e1	LMP1 e1	30.7			
LMP1	LMP1	32.4			Latent membrane protein 1
LMP2B e1	LMP2B e1				

*Small RNA product.

envelope glycoprotein (gp350), respectively. Accordingly, their alternative designations are gN and gp350 genes.

According to their degree of conservation, primate LCV genes can be divided into three groups that are shared by all herpesviruses, shared by all primate LCVs, or specific for an LCV lineage. Within the common primate LCV gene set, two subsets can be distinguished: those which are clearly homologous based on their sequences and those which do not have significant sequence homology but are similarly positioned in the EBV and simian LCV genomes. In other words, these positional homologs have the same neighboring genes.

The genes that are expressed only during latent infection are called latent genes. In terms of se-

quence similarity, latent genes are among the least conserved. Simian LCVs have homologs of all EBV latent genes (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP-1, LMP-2A, LMP-2B, EBERs, and BARFO). However, the homology of EBV and simian LCV latent genes is positional and functional.^{6,11,22,30,31,101,102,105,111}

Genes expressed only during lytic infection are called lytic genes. These genes are much more numerous than the latent genes; there are about 70 lytic genes. Most LCV lytic genes are well conserved and have homologues in other herpesviruses. About 15 of the lytic genes are restricted to the LCVs. An interesting category of lytic genes are those that are homologues of cellular genes. Apparently, ancestors of these

genes were “hijacked” from the cellular genome. There are four such genes shared by EBV and LCV_{Mmu}, namely, viral interleukin-10 (vIL-10; BARF1), two *bel-2* homologues (BHRF1 and BALF1), and a homologue of colony-stimulating factor 1 receptor (vCSF-1R, BARF1).

15.3.2. Overview of Replication Cycle

All simian LCVs (with one possible exception; see Section 15.4.9) replicate *in vitro* only in lymphoid cells of B-lineage, excluding plasma cells; hence another name of these viruses, B-lymphotropic herpesviruses. The major EBV receptor is the CD21 molecule (the receptor for C3d component of complement). This receptor is a B-cell surface molecule, which at least partially explains the B-cell tropism of LCVs. A simian homologue of CD21 serves as the receptor for LCV_{Mmu}.⁷⁶ It is presumed that other simian LCVs use their host CD21 homologue as a receptor, but direct evidence for this is lacking.

The receptor-binding moiety in the viral envelope includes two related envelope glycoproteins, gp350 and gp220. These proteins are translated from an unspliced and a spliced mRNA, both encoded by BLLF1. Interestingly, the receptor binding sites of gp350/220 and the natural ligand for CD21 (C3d complement protein) are structurally similar. In addition to the receptor, coreceptors are required for LCV entry. MHC class II molecules encoded by the DR, DP, and some DQ alleles serve as coreceptors. Viral envelope glycoproteins gp42, gH, and gL are apparently engaged in interaction with coreceptors.⁸⁴ Binding of the virus to the receptors and coreceptors activates signal transduction mechanisms similar to activation caused by binding of antigens to immunoglobulin receptors.

Virus is internalized by endocytosis. Multiple viral glycoproteins (gH, gL, gp42, gM, gN, and gB) are actively involved in the internalization of LCVs; however, the key role in this process belongs to gB. Nucleocapsids are released from the endocytic vesicles in the cytoplasm and transported to the nuclear pores, presumably by the dynein and dinactin cellular “motors.” At the cytoplasmic opening of the nuclear pore, viral capsids are disassembled and genomic DNA is “injected” into the nucleus. The mechanistic details of this process are known mainly from studies on simplexviruses. Some relevant data are available for EBV, but simian LCVs are not well studied in this regard. However, the mech-

anism of nuclear entry is apparently conserved for all herpesviruses.

Once in the nucleus the linear LCV genome is circularized, forming the covalently closed episome (self-replicating circular genome). Gene expression from the episome follows one of two patterns: lytic or latent. If the program of lytic infection is enacted, gene expression involves all genes and occurs in three consecutive “waves”: immediate early, early, and late. A key role in the immediate early stage is played by the BZLF1 and BRLF1 genes and their products. These proteins act as trans-activators, initiating a program of viral DNA replication. Expression of the BZLF1 and BRLF1 genes continues during the following early stage, and is required for efficient DNA replication. During the early stage about 40 genes are expressed. The key event occurring at this stage is viral DNA replication. Completion of DNA synthesis demarcates the early and late stages. The major early genes and their products that are involved in DNA replication are BALF5 (DNA polymerase), BALF2 (major DNA-binding protein), BORF2 and BaRF1 (ribonucleotide reductase subunits), BXLF1 (thymidine kinase), BMRF1 (processivity factor), BSLF1 and BBLF4 (primase/helicase subunits), BKRF3 (uracil DNA glycosylase), and BGLF5 (alkaline exonuclease). It is worth mentioning that the DNA polymerase gene is the most popular target in LCV genomes for PCR amplification and sequencing.

The genes expressed during the late stage encode mainly structural proteins used for virion assembly. Little concrete information is known about the assembly and egress of LCVs. The fragmentary data available for EBV are generally consistent with the “double envelopment” model (see “General properties of herpesviruses”). The provisional and final envelopes differ in their quantity of gp350/220. These glycoproteins are more abundant in the final envelope. The end-result of lytic replication is rupture of the infected host cell and release of viral progeny, which in addition to infectious virions also includes the noninfectious virions, nucleocapsids, and empty capsids.

During latent infection, gene expression is restricted to a small set of genes, namely EBNA-1, -2, -3, LP, LMP-1 and -2, and the EBER RNAs and BARFs. Three patterns of latent gene expression are distinguished: latency types I, II, and III. The latency types I and II genes are characteristic of tumor cells. The latency type III genes are typical for normal B-cells latently infected with LCVs. Various types of latency are distinguished

by the expression of EBNA and LMPs. The most restricted in this respect is latency type I where only one type of EBNA (EBNA-1) is expressed. Latency type II is characterized by expression of EBNA-1 and both LMPs. All EBNA and LMPs are expressed in latency type III. Importantly, the latent LCV proteins collectively promote growth of B-lymphoid cells as well as a differentiation shift toward the plasma cell phenotype. In other words, B-cells latently infected with LCVs proliferate faster than virus-negative B-lymphocytes.

15.3.3. Immortalization of B-Lymphocytes

If maintained properly, B-lymphoblastoid cells persistently infected with LCVs can be grown indefinitely *in vitro*—hence the term “immortalized” cell line. Another name used for such cell lines is EBV- or LCV-transformed cells. At any given moment, LCV-transformed B-lymphoblastoid cultures include both lytically and latently infected cells. The proportion of lytically infected cells varies in different cultures, but remains relatively constant in each cell line. This implies that lytically infected cells are constantly being replenished from the pool of latently infected cells. Why and how the equilibrium between latently and lytically infected cells is maintained at different levels in individual LCV-transformed B-lymphoblastoid cell lines is not clear.

LCV-immortalized cell lines can be established “spontaneously” by explanting LCV-infected B-lymphocytes and culturing them without addition of virus. LCV-immortalized lines can also be “proactively” established by infecting B-lymphocytes *in vitro* with virus. B-lymphocytes latently infected with LCV are always present in the peripheral blood. In culture conditions they have a growth advantage over other types of lymphocytes. Indeed, T-lymphocytes and LCV-negative B-lymphocytes do not survive long *in vitro* without addition of special growth factors. In contrast, B-lymphocytes latently infected with LCV can actively proliferate *in vitro* in the absence of the growth factors other than those contained in fetal calf serum, a standard component of tissue culture media. Spontaneously transformed B-lymphoblastoid cell lines are the main source of infectious LCV and various viral components. Isolation of LCV usually is equivalent to the establishment of such cell lines.

Transformation of B-lymphocytes *in vitro* can be induced by infecting B-lymphocytes from an LCV-

negative donor with virus.^{1,21,76,91,118} This approach is usually used in two contexts: (1) when the host range of a simian LCV is tested; (2) when body secretions, usually oral, are tested for the presence of infectious LCV. The LCV-transformation assay requires use of LCV-negative target cells. Otherwise, positive result would be ambiguous due to the possibility of spontaneous transformation. Using T-cell-depleted lymphocytes as targets improves the efficiency of LCV-induced transformation assays.

15.3.4. Infection *In Vivo*

LCV infection *in vivo* is not restricted to lymphoid cells of the B-lineage. Some epithelial cells in the oral cavity are permissive for LCV replication. Moreover, production of infectious virus *in vivo* mostly, if not exclusively, occurs in epithelial cells located in the oral cavity. A probable site of LCV replication *in vivo* is the lingual epithelium. However, it is not known exactly where LCV-producing cells are located in the oral cavity or what distinguishes them from nonpermissive epithelial cells.

Primary infection with LCV occurs through exposure to virus in saliva. The likely primary targets are epithelial cells and B-cells in the so-called lymphoepithelium of the oropharynx. Ultimately LCVs infect B-lymphocytes. This is accompanied by a vigorous immune response, both antibody- and cell-mediated. The immune response eliminates most infected B-cells. However, a small fraction of latently infected B-lymphocytes survives and a latent reservoir of LCV is maintained in the B-cell compartment. Most B-cells latently infected with LCV pass the virus as the episome to progeny cells when they divide. At the same time, the activation of lytic infection occurs continually in a small fraction of latently-infected cells. Infectious, B-cell transformation-competent LCV is intermittently produced in the oral cavity of LCV carriers. Whether the virus is latently maintained in the oropharyngeal epithelium or if susceptible epithelial cells become infected by virus produced during reactivation of latent virus in B-cells is not clear.

15.3.5. Immune Response

Antibody responses against LCVs are readily detectable in infected animals. The most commonly used assay for detection of anti-LCV antibodies is the immunofluorescence assays, referred to as classical EBV serology (Section 15.3.7.2).^{12,20,33,40,54,59,60,64,80,93,108,115,116}

These antibodies are directed against intracellular antigens and therefore are unlikely to be protective.

Neutralizing anti-gp350 antibodies are detectable in all EBV carriers. The neutralizing antibody response against simian LCVs is presumed to be similar. Theoretically, persisting neutralizing antibodies should contribute to the control of LCV infection *in vivo* by minimizing spread of virus produced during reactivations and/or reducing the effective dose during reinfection from an exogenous source. However, the exact role of neutralizing anti-LCV antibodies *in vivo* is not clear.

Proliferation of B-cells latently infected with LCV *in vivo* is kept under control and the infection remains sub-clinical in the vast majority of the virus carriers. Cell-mediated immunity is believed to be a pivotal part of this control mechanism. T-cell responses against EBV are extensively studied in the context of primary infections manifested as IM. Particularly profound is the anti-EBV cytotoxic T cell (CD8⁺ CTL) immune response directed mainly against the EBNA-3 proteins (3A, 3B, and 3C) and to a lesser extent against LMP-2 and EBNA-1. The response against other latent antigens (ENBA-2, EBMA-LP, and LMP-1) is typically weak or absent.

Cellular immune responses against simian LCVs have been studied only in rhesus monkeys. The cellular response against latent proteins of LCV_{Mmu} is broad; it is directed against the homologs of EBNA-1, EBNA-3C, EBNA-LP, and LNP-2. The response against homologues of the EBNA-3 proteins is not dominant as in the case of EBV.²⁸ Interestingly, the mutant LCV_{Mmu} homolog of EBNA-1 lacking a glycine-alanine repeat (GAR) domain elicits a significantly stronger response than its wild-type counterpart.²⁸ Similar results have been reported for EBNA-1 of EBV.⁶⁹ GAR-mediated inhibition of the immune response may be a component of the immune evasion mechanism underlying virus persistence. However, data contradicting this hypothesis have also been reported: LCV_{Mmu} GAR did not inhibit antigen processing or presentation as determined by the *in vitro* CTL assays.⁵

The cellular response against a lytic protein, the BZLF1 homolog, is largely mediated by CD8⁺ cells. Using IFN-gamma ELISPOT assay a CTL response to this protein was detected in 63% of rhesus monkeys seropositive for LCV_{Mmu}. The epitopes eliciting this response have been mapped to the C-terminal part of LCV_{Mmu} BZLF1. The response is restricted by Mamu-A*01 and other yet unidentified Mamu Class I alleles.²⁷

The number of the natural killer (NK) CD56⁺ cells in the peripheral blood increases during primary EBV infection. Expression of HLA Class I is upregulated in EBV-transformed B-cells, an indication of evasion from the NK response. These observations are consistent with the hypothesis that innate immunity may contribute to control of EBV infections at very early stages of primary infections. Data on the role of innate immunity in controlling LCV infection in NHPs are absent.

15.3.6. Oncogenicity

There is a long list of diseases, mostly malignant, for which an association with EBV has been described. However, the strength of the evidence varies significantly and relatively few of these associations withstand critical analysis. A key piece of evidence for association is the presence of EBV DNA and latent antigen expression in the tumor cells, and virtually all tumor cells have to be positive. However, most claims of an association are based solely on serological data, that is, increased anti-EBV titers in the cases as compared with controls. A really strong association with EBV has been demonstrated for just a few malignancies: endemic Burkitt's lymphoma, undifferentiated nasopharyngeal carcinoma, immunodeficiency-associated B-lymphomas, and quite unexpectedly some T- and NK-cell lymphomas (hemophagocytic syndrome-associated and nasal) and smooth muscle cell tumors (leiomyomas and leiomyosarcomas). Interpretation of EBV disease association data is a challenging task. Virus apparently is necessary but insufficient for the development of these malignancies. Multiple poorly understood co-factors, both genetic and environmental, definitely contribute to the pathogenesis of EBV-associated diseases. The degree of association, measured as the percentage of EBV-positive tumors, varies significantly for the same malignancy type depending on the geographical area and ethnicity of patients.

Interpretation of data on the association of simian LCVs with malignancies is even more complicated because of the diversity of viruses and host species; the data are also quite fragmentary. Apparently, the only clear parallel between EBV and simian LCV-associated malignancies is B-cell lymphomas in immunosuppressed hosts. Such lymphomas positive for LCV DNA and latent antigens are observed in NHPs in a context of pharmacological (post-transplantation) and simian immunodeficiency virus (SIV)-induced

immunosuppression.^{3,6,23,41,48,58,71,89,98} Interestingly, the incidence of LCV-positive lymphomas is higher in cynomolgus than rhesus macaques.^{6,23} Whether this difference is due to viral or host factors is not clear.

A single case of LCV-positive spontaneous nasal cavity T/NK-cell lymphoma in Japanese macaque (*M. fuscata*) has been described.¹¹⁰

15.3.7. Diagnosis of LCV Infection

Diagnosis of simian LCV infections is based on methodologies developed for EBV diagnostics, although obviously with some modifications. Virus isolation and detection of antibodies are most commonly used. Detection of viral DNA by PCR and sequence analysis of amplified fragments has become increasingly common.

15.3.7.1. VIRUS ISOLATION

Simian LCVs are mainly isolated through spontaneous transformation of B-lymphocytes.^{2,7,34,80,95,97} The success rate in establishing such cell lines depends mainly on two factors: the number of latently infected B-cells and the number of T-lymphocytes committed against the latent viral antigens in the culture. The frequency of EBV-positive cells in human peripheral blood is 1 in 100,000–1,000,000. Although analogous data for simian LCVs are not available, culturing several million B-lymphocytes would guarantee the presence of LCV-positive cells. The greater the number of B-cells in culture, the better the chance for success in establishing a transformed B-lymphoblastoid cell line. Depleting T-lymphocytes from the peripheral blood mononuclear cell population before starting the culture significantly increases the chance of LCV-induced transformation because T-cell-mediated killing of LCV-infected B-cells during the first days in vitro would be minimized. Functional suppression of T-lymphocytes (such as use of cyclosporine A) may also be helpful.^{4,76,117}

15.3.7.2. CLASSICAL SEROLOGY

Classical EBV serology is based on the detection of antibodies against viral antigens present in the fixed cells of B-lymphoblastoid cultures using different versions of immunofluorescence. Three major classical EBV “antigens” are distinguished: virus capsid (VCA), early (EA), and nuclear (EBNA). Classical EBV antigens include many individual viral proteins; however, in the framework of classical serology each antigenic complex is considered as a single entity. VCA and EA are present

only in lytically infected cells; EBNA is present in both lytically and latently infected cells. Three major types of anti-EBV antibodies are detected that correspond to these antigens: anti-VCA, anti-EA, and anti-EBNA. Anti-VCA antibodies are those which react predominantly with cytoplasmic antigens in a low percent of cells in an EBV-positive B-lymphoblastoid cell line (cells actively producing virus). These lytically infected cells also contain EA. As for VCA, EA is predominantly a cytoplasmic antigen. Thus, to detect anti-EA antibodies, special cell targets free of VCA are required. This is achieved by using cells from EBV-positive cultures in which synthesis of VCA is inhibited either by defectiveness of the virus or use of chemical inhibitors. Both anti-VCA and anti-EA antibodies are detected using indirect immunofluorescence assay. Anti-EBNA antibodies, as the name suggests, react with nuclear antigen which is present in all cells of EBV-positive cultures. The detection of anti-EBNA antibodies requires a more sensitive version of the immunofluorescence assay, the so-called anti-complement immunofluorescence (ACIF) test. Certain rules have been established for interpretation of classical serology results. They are not free from ambiguity, but nevertheless are useful. Absence of anti-VCA antibody (VCA seronegativity) is considered to be reliable evidence of EBV-free status, whereas anti-VCA positivity confirms EBV infection. During the first few weeks after primary infection anti-VCA antibodies are undetectable. Anti-VCA IgM antibodies are considered diagnostic for a primary infection, although low titers of such antibodies may be detected in chronic EBV carriers.

The presence of anti-EA antibodies in addition to anti-VCA antibodies indicates an “active” EBV infection (anti-EA antibodies are never observed in the absence of anti-VCA). Exactly what constitutes an active infection is not clearly defined. It is presumed by default that the “activity of infection” correlates positively with the number of lytically infected cells in vivo.

Low titers of anti-EBNA antibodies can be detected in virtually all infected individuals. Whether or not titers of anti-EBNA antibodies are diagnostically informative is disputable. It is commonly presumed that there is a positive correlation between titers of anti-EBNA antibodies and the potency of anti-EBV cellular response. This assumption is based on the logical but simplistic assumption that when the CTL response is strong, more EBNA is released from lysed cells, resulting in the production of more anti-EBNA antibodies. Whether or not this supposition is correct has not been validated.

Classical serology remains the main approach for diagnosing LCV infections in NHPs, although newer methods for the detection of anti-LCV antibodies are now available. VCA of EBV, Old World monkey LCVs, and ape LCVs are strongly cross-reactive. Thus, any LCV-producing cell line can be used for the detection of anti-VCA in Old World NHP species.

The detection of anti-EA of simian LCV is more difficult and rarely used now. In contrast to EBV, no simian LCV-positive cell lines selectively expressing EA are available. The method for detection of antibodies against simian LCV EAs is based of selective inhibition of VCA by phosphonoacetic acid.⁷⁸ However, incomplete inhibition of VCA is commonly observed which compromises the specificity of this test.

In contrast to human anti-EBNA antibodies, simian anti-“EBNA” antibodies (referred to as anti-NA) are not readily detectable by the ACIF test.^{79,81–83,93} When the ACIF test for anti-NA does not work, simian LCV NA can be detected by the acid-fixed nuclear-binding technique (AFNB).^{81,93} This test requires purification of NA by DNA-affinity chromatography, preparation of cell targets by binding affinity purified NA to the acid-fixed nuclei, and use of these “reconstituted” cells for detection of anti-NA antibodies by ACIF. This AFNB technique is quite laborious and is no longer in use.

15.3.7.3. ANTIBODY DETECTION BY ELISA

Use of classical serology in the simian LCV field is decreasing. ELISAs based on recombinant or synthetic peptide LCV antigens are replacing immunofluorescence tests. ELISA tests for anti-VCA based on peptides mimicking immunodominant epitopes of small VCA (sVCA, BFRF3) are already sufficiently developed to replace the classical anti-VCA test in diagnosing LCV infection in the rhesus monkeys, baboons, and marmosets.^{26,85,96} An ELISA test for antibodies against the LCV_{Caj} EBNA-1 homolog has also been described.²⁶ If required, similar ELISAs can be developed for diagnosing infection with other simian LCVs.

15.3.7.4. MOLECULAR ASSAYS

The DNA polymerase gene is the most widely used target for PCR amplification of LCV sequences.^{19,107} Available LCV sequences provide almost limitless possibilities for design of PCR assays and a number of such tests have been used for studying simian LCVs. However, PCR tests for simian LCVs are not characterized

in terms of their diagnostic performance. Exceptional in this respect is RT-PCR for LCV_{Mmu} EBER RNA homologues in peripheral blood.⁹⁶ This test is highly sensitive and specific in diagnosing LCV_{Mmu} infection in rhesus monkeys. The test can be adapted to any simian LCV, assuming that the sequence of its EBER homologue is known. However, routine use EBER RT-PCR may be complicated because tests using RNA samples and RT-PCR are more technically demanding than regular PCR and serological assays. Real-time PCR for determination of viral load is currently available only for LCV_{Mmu}.¹⁰⁰

15.4. LCVs FROM OLD WORLD NHPs

In the late 1960s–early 1970s, multiple research groups reported the presence of “anti-EBV” antibodies in sera of Old World NHPs.^{12,17,35,38,40,59,64} The first simian lymphoblastoid cell lines infected with “EBV” were established from a chimpanzee in 1968.^{62,63} Although the suggestion was made that simian species may harbor their own “EBV-like” viruses, the alternative explanation that EBV infection was ubiquitous in higher primates remained dominant until the mid-1970s.

Instrumental in the recognition of simian LCVs was research by the team headed by B. Lapin in the former USSR and collaborating US laboratories.^{2,14–16,20,60,91,115,116} By the beginning of the 1980s almost all currently known LCVs from Old World monkeys had been isolated.^{2,7,37,48,80,97} Interestingly, the LCV from rhesus monkeys, the monkey species most commonly used in biomedical research, was isolated later, in 1986.^{3,95} Despite a late start, rhesus macaque LCV is currently the best characterized simian LCV.

15.4.1. Rhesus Macaque LCV

Rhesus monkey lymphocryptovirus (LCV_{Mmu}) infection is very common in rhesus monkeys kept in captivity; almost all adults are seropositive. Presumably, this infection is also common in wild rhesus monkeys; however, concrete data are absent.

LCV_{Mmu} is well characterized, particularly regarding its molecular properties.¹¹⁹ The most significant advance in this field was sequencing of the LCV_{Mmu} genome¹⁰² (GenBank Acc. No. AY037858). The length of the linear LCV_{Mmu} genome (strain LCV8664) is 171,096 bp (based on the presence of 5.7 copies of IR1 and 4 copies of TR). The lengths of the IR1 and TR are 3,072 bp and 933 bp, respectively. The overall

sequence homology between LCV_{Mmu} and EBV is 65%; in protein coding regions the homology is significantly higher (75%). Fifty-six lytic genes (1 immediate early, 32 early, and 24 late) have been identified in the LCV_{Mmu} genome. These genes are all well conserved: the average homology with corresponding EBV genes is 83%, and 14 lytic genes of LCV_{Mmu} and EBV are more than 90% homologous. However, it is worth mentioning that highly immunogenic component of the LCV_{Mmu} VCA, the small VCA (sVCA or BFRF3 homolog), is quite divergent from its EBV counterpart (only 69% of amino acid similarity). Most other components of the EBV and LCV_{Mmu} VCA are more than 90% homologous. The least conserved (49%) among lytic proteins is the major envelope glycoprotein gp350.⁷⁶ However, the receptor-binding site of LCV_{Mmu}-gp350 is sufficiently conserved to allow for binding of LCV_{Mmu} to the EBV receptor on human cells. Other viral glycoproteins participating in cell entry (gH, gL, gB, and gp42) are more conserved than gp350, particularly the gH glycoprotein (85% sequence similarity). The LCV_{Mmu} gH homolog is fully interchangeable with the EBV-gH in mediating viral fusion with human B-cells.¹²⁰

The sequences of LCV_{Mmu} latent genes and their products are less conserved: 46%, 30%, 30%, 53% for EBNA-1, EBNA2, EBNA-3, EBNA-LP homologs, respectively, and 32%, 57%, and 77% for LMP1, LMP2, and BATF0 homologs, respectively. However, LCV_{Mmu} and EBV latent proteins are clearly functionally homologous to a degree permitting mutual functional substitution.^{31,32,57,74,86,109,119,124} Similar to EBV, the LCV_{Mmu} homolog of EBNA-1 is required for episome maintenance in latently infected cells. This protein is smaller than EBNA-1; the difference is due almost entirely to a shorter glycine-alanine repeat (GAR). Different LCV_{Mmu} isolates have two variants of an EBNA-2 homolog; their sequence homology is just 41%.¹¹ Whether these polymorphic variants of LCV_{Mmu} EBNA-2 are functionally different is not known. Monoclonal antibodies against a conserved epitope of EBNA-2 (PE2) react with the LCV_{Mmu} EBNA-2 homolog and can be used for its detection and quantification. This monoclonal antibody reacts also with other simian LCV EBNA-2 homologs.

LCV_{Mmu} homologs of EBNA-3A, -3B, and -3C function as transcriptional trans-activators like their EBV counterparts. Specifically, they interact with transcription factor RBP-Jκ. The LCV_{Mmu} homolog of EBNA-LP is the most conserved among LCV_{Mmu} EBNA ho-

mologs; amino acid similarity of the protein domains encoded by different exons is in the range of 44–76%.^{86,102}

LMPs of LCV_{Mmu} and EBV are also functionally similar despite their low sequence homology, particularly in the C-terminal cytoplasmic domain.¹¹⁹ As in EBV, the LCV_{Mmu} LMP-1 can induce morphological transformation of rodent fibroblasts.¹¹⁹ This protein also induces nuclear factor-κB (NF-κB), adhesion molecule ICAM-1, and activator protein 1 (AP-1).^{10,31} These activities presumably contribute to cell growth promotion which is characteristic of latent LCV infections. The genes encoding the LCV_{Mmu} LMP-2 homologs (LMP-2A and 2B) are identical except for the first exon. The LMP-2s are better conserved than LMP-1. LCV_{Mmu} LMP-2s activate protein tyrosine kinases (PTK) and are themselves phosphorylated. They also have a characteristic “immunoreceptor tyrosine activated motif” (ITAM).^{30,103}

Experimental infection of rhesus monkeys with LCV_{Mmu} has been studied in both immunocompetent and immunosuppressed animals.^{77,100} However, this field remains relatively unexplored partly because seronegative monkeys are not readily available. In one experiment, two LCV_{Mmu}-seronegative rhesus monkeys were orally infected with 10⁶ transforming units of LCV_{Mmu}.⁷⁷ One of the animals developed axillary and inguinal lymphadenopathy 3–5 weeks after infection. Atypical lymphocytes (1–3%) were observed in the peripheral blood in both animals for about 10 weeks starting at day 7 after inoculation. Simultaneous with the appearance of atypical lymphocytes, the number of CD23⁺ cells in the peripheral blood increased dramatically from a baseline of 5% to 50–55%. Sixty percent of the CD23⁺ cells were activated B-lymphocytes while the remaining cells were monocytes. This response peaked between days 11 and 14 after inoculation and returned to baseline level 3–4 weeks after inoculation. Both animals developed high titer antibodies against LCV which reached a peak on day 14 after inoculation and remained at high for the whole period of observation (1.5 years after inoculation). Anti-VCA antibodies appeared first, becoming detectable at day 7 after inoculation. Antibodies against EBNA-2 became readily detectable at day 21 after inoculation. Interestingly, reinoculation of one of these monkeys (the same dose of the same virus stock) did not result in any detectable response. All hematological parameters as well as anti-LCV titers did not change significantly after reinoculation. Clinical manifestations and immune responses observed following experimentally induced primary LCV_{Mmu} infection of rhesus

monkeys are reminiscent of EBV-induced IM. However, in contrast to human IM pharyngitis and splenomegaly were absent. The rhesus model of IM has not been further explored.

In another set of experiments immunocompetent and SIV-immunosuppressed rhesus monkeys (actually SHIV-immunosuppressed) were inoculated with LCV_{Mmu}.¹⁰⁰ In the first experiment, eight monkeys, four in each group, were inoculated orally. All immunocompetent monkeys seroconverted, whereas the immunosuppressed animals failed to develop antibodies against LCV_{Mmu}. Both immunocompetent and immunosuppressed monkeys became persistently infected with LCV_{Mmu}; the infection was inapparent in all animals. In the second experiment four SHIV-immunosuppressed rhesus macaques were inoculated intravenously with a large dose of virus (10^8 autologous LCV_{Mmu}-transformed B-cells). Aggressive, LCV-positive B-cell lymphoma developed in one of the two immunosuppressed animals.

B-cell lymphomas are a common manifestation of simian AIDS. Approximately 90% of lymphomas in SIV-immunosuppressed rhesus monkeys are LCV_{Mmu}-positive.^{3,6,41,58,89} In many respects, simian AIDS-associated lymphomas resemble human disease, so the rhesus and can be considered a good model system. However, exploration of this model using “spontaneously” developed cases is complicated. Unfortunately, a model permitting reproducible induction of B-cell lymphomas by LCV_{Mmu} is not available.

Apart from B-cell lymphomas, LCV_{Mmu} can cause epithelial cell lesions in SIV-immunosuppressed macaques.⁶¹ These proliferative, hyperkeratotic lesions are reminiscent of oral hairy leukoplakia frequently observed in AIDS patients. In contrast to LCV-positive lymphomas where only latent products are expressed, the lytic LCV_{Mmu} products are abundantly expressed in epithelial lesions. LCV_{Mmu}-positive epithelial lesions are not restricted to oral cavity, and are also observed in the esophagus and on external surfaces (chest and hand skin, genitalia).

15.4.2. Baboon LCV

A baboon B-lymphoblastoid cell positive for LCV was first reported in 1968.⁶² However, baboon LCV was only conclusively identified in the mid-1970s.^{2,15,20,60} In the 1970s–1980s, this virus, originally named herpesvirus papio (HVP), was the most extensively studied simian LCV.^{1,14,16,21,49,51,52,66–68,73,79,87,91,114–116} Most of the

LCV-producing cell lines used at that time had been established from lymphomatous hamadryas baboons (*Papio hamadryas*) of “high lymphoma stock” at the Sukhumi Primate Center in the former USSR.^{1,2,91} One of these cell lines, named 594S,⁹¹ is a particular high virus producer; 594S cells and their derivative (594S-F9) have been widely used as a source of virus for immortalization of simian B-cells and for antigen in serological assays.

Apart from the basic characterization of baboon LCVs, the main focus of research in this field was the presumptive association of LCV_{Pha} with baboon malignant lymphoma. This association was prompted by the oncogenicity of LCV_{Pha} for marmosets.¹⁴ Intriguingly, lymphoproliferative disease in marmosets was induced only by LCV_{Pha} from a lymphomatous baboon; LCVs from healthy baboons were not oncogenic. However, this finding has not been fully explored and has not been independently confirmed. Association of LCV_{Pha} with lymphoma was also suggested by detection of viral DNA in lymphomatous baboon tissues^{16,107} and by the finding that titers of antibodies against LCV_{Pha} were elevated in sera of prelymphomatous baboons.¹¹⁶ However, these data can only be considered suggestive of a causal relationship between LCV_{Pha} and lymphoma development. In the mid-1980s, it became evident that malignant lymphoma in Sukhumi baboons was caused by the STLV-1 deltaretrovirus (see Chapter 8), and interest in LCV_{Pha} largely waned.

LCV infection is presumed to be prevalent in wild baboons; however, supporting data are absent. In captive baboon populations the prevalence of LCV infection (as determined by the serology) increases with age; 90% and more of adults (5 years and older) are positive.^{55,85,115,116} Most probably the virus is transmitted by saliva; it is readily recoverable by B-cell transformation assay from oral swabs.¹

There are no published data on the pathogenicity of baboon LCVs in baboons or other Old World monkeys in controlled experiments, that is, inoculation with well defined LCV preparations. At the same time, baboon LCV induces fatal lymphoproliferative disease in rabbits which resembles fatal IM in humans accompanied with a so-called viral hemophagocytic syndrome.^{46,47} The development of this disease is rapid (within 20–100 days after intravenous inoculation or oral administration of virus), and similar to human disease, it is accompanied by a hemophagocytic syndrome. This model is promising for studies on the pathogenesis of

hemophagocytic syndrome-associated lymphomas and its treatment modalities.^{44,45}

15.4.3. African Green Monkey LCV

The first infectious isolate of African green monkey LCV was described in 1980.⁷ A B-lymphoblastoid cell line producing this virus (LCV_{Cae} strain AGM-2206) was established from *Chlorocebus aethiops* by spontaneous transformation. LCV_{Cae} has been shown to be related to EBV antigenically. Very little, if anything, has been added to the knowledge of LCV_{Cae} and similar viruses from other African green monkey species after the original report.

15.4.4. Chimpanzee LCV

A chimpanzee LCV (LCV_{Ptr}) was described for the first time in 1968.⁶³ Almost a decade later in 1976, LCV_{Ptr} was rediscovered in several lymphoblastoid cell lines established from common chimpanzees (*Pan troglodytes*).^{36,37} Initial attempts to transform human or simian B-lymphocytes by LCV_{Ptr} failed. However, a B-cell transforming EBV-related virus was subsequently isolated from throat swabs obtained from immunosuppressed chimpanzees. LCV_{Ptr} transforms baboon and gibbon B-lymphocytes. Unexpectedly, baboon B-cells turned out to be more susceptible than gibbon cells. Baboon B-lymphoblastoid cell lines transformed by LCV_{Ptr} have been shown to be higher producers of virus; 5–10% of transformed cells are VCA-positive.³⁶ Surprisingly, there are no published data on the susceptibility of chimpanzee and human B-lymphocytes to transformation with LCV_{Ptr}.

The genomes of LCV_{Ptr} and EBV have been shown to be collinear,⁵⁰ however, only relatively short fragments of the LCV_{Ptr} genome have been sequenced.³⁹ In late 1970–early 1980, several B-lymphoblastoid cell lines producing LCV_{Ptr} were reported (chimpanzee cell lines chimp 8, chimp 9, Austin and baboon cell lines Ba65, Ba74). At least one of these cell lines, Austin, is apparently still available.³⁹ LCV_{Ptr} infection has been shown to be present in wild chimpanzees.¹⁹ Otherwise little progress has been made in this field since the early 1980s.

15.4.5. Orangutan LCV

Orangutan LCV (LCV_{Ppy}) was described in 1977 under the name *Herpesvirus pongo*.⁹⁷ The virus was detected in the B-lymphoblastoid cell line CP-81 estab-

lished from a leukemic *Pongo pygmaeus*. CP-81 cells were tumorigenic in *nude* mice, an indication that these cells likely originated from the malignancy.⁹² Based on antigenic cross-reactivity and DNA homology as determined by the DNA reassociation kinetics, LCV_{Ppy} is clearly related to EBV. The B-cell transforming activity of LCV_{Ppy} has been tested on human cord blood lymphocytes as well as hamadryas baboon, rhesus monkey, stump-tailed macaque, and gibbon lymphocytes. The virus immortalized only gibbon lymphocytes.^{92,93}

Phylogenetic analysis of the LCV_{Ppy} (CP-81) glycoprotein B gene produced a surprising result: the sequence is almost identical to the sequence of chimpanzee LCV_{Ptr} (Austin).³⁹ Sequencing of a larger genomic region, ideally the whole genome, is required to clarify whether this LCV is a recombinant virus or its true origin is from a chimpanzee. Regardless of the origin of this particular LCV strain, there are data showing that genuine LCV_{Ppy} exists. Identical LCV frag-viruses (polymerase gene fragment) have been detected in the noncultured specimens from five out of eight orangutans tested.¹⁹

15.4.6. Gorilla LCV

Gorilla LCV (LCV_{Gga}) was described in 1979 under the name *Herpes gorilla* or *H. gorilla*.⁸⁰ The virus has been detected in a B-cell lymphoblastoid cell line (designated Machi) that was established by spontaneous transformation of B-lymphocytes from a captive gorilla (*Gorilla gorilla*). The proportion of lytically LCV-infected cells in this culture was 1–2%; the rest of the cells were latently infected as indicated by the presence of LCV nuclear antigens. Treatment of Machi cells with iododeoxyuridine (IUDR) boosted the level of lytically infected cells to 10–15%.

LCV_{Gga} immortalizes gibbon B-lymphocytes. There are no published data on its transforming activity for B-cells of other species, including gorilla. Antigenically, LCV_{Gga} is cross-reactive with LCV_{Pha} and EBV, apparently being more closely related to EBV. For example, similar to EBV but in contrast to LCV_{Pha}, nuclear antigens of LCV_{Gga} can be detected by ACIF. All but one captive gorillas tested ($n = 12$) for antibodies against LCV_{Gga} were seropositive. Prevalence of LCV_{Gga} infection in wild gorillas is not known.

LCV_{Gga} genomic sequences are available only for relatively short fragments.^{19,39} Phylogenetic analysis of these sequences clearly places LCV_{Gga} in the same clade

with EBV. The closest relatives of LCV_{Gga} based on the analysis of gB-coding sequence are LCV_{Ptr} and LCV_{Ppy}.³⁹

Two types of frag-LCVs have been detected in gorillas using generic herpesvirus PCR.¹⁹ The sequence of the first variant designated GgorLCV1 was identical to the sequence of LCV_{Gga}; the sequence of the second variant GgorLCV2 was clearly different and more distant from EBV than LCV_{Gga}. The closest relatives of GgoLCV2 are frag-viruses detected in the orangutans and gibbons.

15.4.7. Cynomolgus Macaque LCV

The first LCV isolate from a cynomolgus macaque (*M. fascicularis*) was reported in 1982.⁴⁸ A B-lymphoblastoid cell line producing this virus (LCV_{Mfa}) was established from lymphomatous tissues of a monkey severely immunosuppressed after cardiac transplantation (total body irradiation plus cyclosporine A). LCV_{Mfa} has been shown to be transforming for cynomolgus monkey B-lymphocytes and, less efficiently, baboon B-lymphocytes. Apparently the original cell line producing LCV_{Mfa} has been lost.

In 1990, another LCV_{Mfa}-producing cell line (T_sB) was established from a healthy cynomolgus macaque.³⁴ An unusually high proportion (20–30%) of cells in this cell line was VCA-positive. LCV_{Mfa}(T_sB) has been shown to be transforming for macaque B-lymphocytes (cynomolgus, Japanese, and rhesus), while B-lymphocytes from humans, baboons, African green monkeys, as well as New World monkeys (squirrel monkey and mustached tamarin) are not susceptible.

LCV_{Mfa} (T_sB) is highly oncogenic in rabbits. LCV-positive malignant lymphomas develop in rabbits within 2–5 months after administration of the virus by either oral or parenteral routes.^{9,43,53} Surprisingly, these lymphomas are of T-cell origin.^{42,47} Whether T-lymphomagenicity is specific for particular strains of LCV_{Mfa} or if this is a generic property of cynomolgus macaque LCVs is not known.

LCV_{Mfa} may cause B-cell lymphomas in the natural host if the animal is profoundly immunosuppressed. B-cell lymphomas in SIV-immunosuppressed cynomolgus macaques are remarkably similar to EBV-positive AIDS-associated lymphomas.^{23,70,71,98}

15.4.8. Stump-Tailed Macaque LCV

Several permanent B-cell lymphoblastoid cell lines (MAL-1, -2, etc.) were established from *M. arctoides*

in the mid-1980s in the Sukhumi Primate Center. The virus produced by these cells (LCV_{Mar}, originally designated HVMA) has been shown to be related to EBV and LCV_{Pha}.^{65,107,112,122} The most interesting feature of LCV_{Mar} is its profound oncogenicity in rabbits. LCV_{Mar}-positive lymphomas develop rather quickly within 1–4 months after inoculation with virus from MAL-1 cells. Rabbits of different breeds are susceptible.^{112,121,122} LCV_{Mar} also efficiently transforms rabbit B-lymphocytes.⁷⁵

15.4.9. Pig-Tailed Macaque T-Lymphotropic LCV

Unexpectedly, pig-tailed macaque (*M. nemestrina*) LCV was originally isolated from an animal with cutaneous T-cell lymphoma/mycosis fungoides.^{24,25} This unusual T-tropic LCV was originally named Herpesvirus macaca nemestrina (HVmne). In this chapter, it is designated LCV_{Mne}T to emphasize its T-cell tropism and to distinguish it from B-tropic LCV_{Mne} which likely exists. Permanent T-cell lines producing LCV_{Mne}T were established from skin lesions and blood. Viral markers (EBER-like RNA and a fragment of the *pol* gene) have been detected in uncultured tumor cells. LCV_{Mne} transforms T-lymphocytes from pig-tailed macaques and mustached tamarins (*Saguinus mystax*) in vitro. Interestingly, LCV_{Mne}T-transformed cell lines from macaques are CD8⁺, whereas those from tamarins are CD4⁺. T-lymphocytes from humans and several species of New World monkey species (owl monkey, common marmoset, squirrel monkey, and black-capped capuchin) are not susceptible to transformation by LCV_{Mne}T. Some studies have investigated genomic sequences and antigenic properties of LCV_{Mne}T.^{24,25,99} Polymerase gene sequences of LCV_{Mne}T and EBV are 90% homologous. EBV-EBER probes readily detect homologous RNA species in LCV_{Mne}T-positive cells, and VCA of LCV_{Mne} and EBV are cross-reactive.

Inoculation of LCV_{Mne}T into New Zealand White rabbits consistently results in the development of T-cell lymphomas within 3–9 months after inoculation. Rabbit T-cell lymphomas can be induced by both irradiated virus-producing cells and cell-free virus. These tumors are LCV_{Mne}T-positive as determined by the presence of viral DNA and antigens. T-cell lines established from tumors are also LCV_{Mne}T-positive. Antibodies against VCA are detected only in rabbits with LCV_{Mne}T-induced lymphomas. Attempts to transmit the disease to pig-tailed macaques failed. However, it is not

clear whether or not the inoculated pig-tailed macaques were anti-LCV_{Mne} negative.

15.5. LCVs FROM NEW WORLD MONKEYS

Serological surveys performed in the 1970s generally failed to detect antibodies cross-reacting with EBV-VCA in New World monkeys.^{17,29} Although positive findings have been reported, the specificity of the assays used was doubtful and the view that EBV-like viruses are present only in the Old World NHPs became “dogmatized.”

This paradigm was broken in 2001 when a typical LCV-like virus was isolated from a common marmoset (*Callithrix jacchus*).¹⁰ Soon after the complete sequence of the LCV_{Cja} genome was determined¹⁰¹ and the presence LCV_{Cja} was convincingly demonstrated in both captive and wild New World monkeys.²⁶

15.5.1. Detection of New World Monkey LCVs

LCV infection in New World monkeys is diagnosed by detection of viral DNA by PCR and/or antibodies against viral antigens. The most sensitive PCR currently available for detection of New World monkey LCVs targets sequences located in the major internal repeat of the marmoset LCV genome.²⁶ Apparently the best serological tests for diagnosing LCV infection in New World monkeys are ELISAs based on a synthetic peptide or a recombinant protein derived from the proteins encoded by ORF-59 and ORF-39. ORF-59 is a homologue of EBV-BFRF3 and the product of this gene is a lytic protein, small viral capsid antigen (sVCA). ORF-39 is a homologue of EBV-BKRF1 (EBNA-1). Dual positivity for antibodies against these antigens is completely concordant with a positive PCR test, and dual negativity in antibody tests coincides with PCR negativity in the majority of cases. However, infrequent antibody-negative/PCR-positive results are observed.²⁶

Antibody reactivity against a single antigen is not uncommon. Such reactivity is suggestive of LCV infection. However, it is not yet clear whether or not such antibody reactivity patterns can be observed in the absence of LCV infection.

15.5.2. Prevalence of LCV Infection in New World Monkeys

LCV infection prevalence estimates in wild New World monkeys are available for common marmosets (*Callithrix jacchus*) and golden-handed tamarins (*Saguinus*

midas).¹³ Fifty percent of marmosets tested shortly after capture from the wild ($n = 24$) were seropositive using an assay highly specific for antibodies against LCV_{Caj}.²⁶ LCV frag-viruses have been detected in 57% (30/53) of wild golden-handed tamarins.¹³ LCV frag-viruses have also been detected in wild white-faced sakis (*Pithecia pithecia*) and squirrel monkeys (*Saimiri sciureus*).¹³ However, prevalence estimates for these species are not available.

The prevalence of LCV infection in three colonies of captive common marmosets is 54% ($n = 234$), 37% ($n = 165$), and 47% ($n = 126$).²⁶ This is different from what is observed in captive Old World monkeys where most adult animals are seropositive.^{55,96,115,116} Why a significant proportion of adult New World monkeys remains uninfected despite the exposure to LCV is not clear.

15.5.3. Marmoset LCV

Marmoset LCV was first detected as a frag-virus in the spontaneous B-cell lymphoma of a common marmoset (*Callithrix jacchus*) housed in the Wisconsin National Primate Research Center (WNPRC).⁹⁴ Although the official name of this virus is *Callitrichine herpesvirus 3* (CaHV-3), in this chapter it is designated LCV_{Cja}. The first B-lymphoblastoid cell line producing LCV_{Cja} was established from one of several lymphomatous marmosets at the WNPRC.¹⁰ Ten more cell lines producing LCV_{Cja} have since been established independently, only one of which originated from a non-WNPRC marmoset.⁵⁶ The establishment of cell lines producing LCV_{Cja} required cultivation of common marmoset lymphocytes *in vitro* for 6–8 weeks before transformation became apparent.⁵⁶ LCV_{Cja} is transforming for marmoset B-lymphocytes *in vitro*, but it does not transform rhesus or human B-lymphocytes.¹⁰

The size and composition of the LCV_{Cja} genome is generally similar to that of EBV and LCV_{Mmu} (GenBank Acc. No. NC_004367).¹⁰¹ The length of the genome is 161,345 bp (assuming the presence of 7.5 copies of IR1 and 35 copies of TR). Homologues of most EBV and LCV_{Mmu} genes are present in the LCV_{Cja} genome; however, homologues of 14 Old World LCV genes are absent. Most of these “absentees” are lytic genes which are not essential for LCV replication *in vitro*. The absence of some genes such as those encoding the viral interleukin 10 and CSF-1 receptor may be relevant to the lower transmissibility of LCV_{Mmu} as compared to Old World monkey LCVs and EBV. Other notable EBV genes for

which homologues are absent in LCV_{Cja} are those genes encoding EBER RNAs, BARF0, and a 150-kDa glycoprotein (product of BDLF3). Interestingly, EBV mutants with a BDLF3 gene deletion infect epithelial cells more efficiently than the wild-type virus.⁸ Whether or not LCV_{Cja} has enhanced capacity to infect epithelial cells is not known.

15.6. SUMMARY

The prototype lymphocryptovirus is human EBV. Natural infection with LCVs is prevalent in most, if not all, species of Old World monkeys and apes. Some New World monkey species are also naturally infected with LCVs.

The most notable biological characteristic of primate LCVs is the ability to transform or immortalize B-lymphocytes. Some simian LCVs can immortalize human B-cells in vitro, although with lower efficiency. Reciprocally, EBV can transform B-cells of some simian species, also with a lower efficiency than for human B-lymphocytes. Interestingly, the most widely used transforming strain of EBV (EBV-B95-8) is produced by cells of tamarin origin.

Simian LCVs and EBV share many properties. Their genomes are collinear, and although sequence similarity varies across the genome, in some regions it exceeds 90%. Viral antigens, particularly capsid antigens, are strongly cross-reactive. Similar to EBV, simian LCVs are excreted in the oral cavity and are readily transmitted by saliva. Most adult monkeys are infected with LCVs.

Simian LCV can cause B-cell lymphomas in macaques immunosuppressed by either SIV infection or pharmacologically. At the same time, two major malignancies associated with EBV, Burkitt's lymphoma and nasopharyngeal carcinoma, have not been observed in monkeys.

REFERENCES

- Agiba, V. Z., B. A. Lapin, V. V. Timanovskaya, M. C. Dzhachvliany, L. V. Kokosha, G. N. Chuvirov, and A. G. Djatchenko. 1980. Isolation of lymphotropic baboon herpesvirus (HVP) from oral swabs of hamadryas baboons of the Sukhumi monkey colony. *Exp. Pathol. (Jena)* 18(5):269–274.
- Agiba, V. Z., L. A. Yakovleva, B. A. Lapin, I. A. Sangulija, V. V. Timanovskaya, D. S. Markarjan, G. N. Chuvirov, and E. A. Salmanova. 1975. The establishment of continuous lymphoblastoid suspension cell cultures from hematopoietic organs of baboon (*Papio hamadryas*) with malignant lymphoma. *Exp. Pathol. (Jena)* 10(5–6):318–332.
- Baskin, G. B., L. N. Martin, S. R. Rangan, B. J. Gorimus, M. Murphey-Corb, R. H. Wolf, and K. F. Soike. 1986. Transmissible lymphoma and simian acquired immunodeficiency syndrome in rhesus monkeys. *J. Natl. Cancer Inst.* 77:127–139.
- Bird, A. G., S. M. McLachlan, and S. Britton. 1981. Cyclosporin A promotes spontaneous outgrowth in vitro of Epstein–Barr virus-induced B-cell lines. *Nature* 289(5795):300–301.
- Blake, N. W., A. Moghaddam, P. Rao, A. Kaur, R. Glickman, Y. G. Cho, A. Marchini, T. Haigh, R. P. Johnson, A. B. Rickinson, and F. Wang. 1999. Inhibition of antigen presentation by the glycine/alanine repeat domain is not conserved in simian homologues of Epstein–Barr virus nuclear antigen 1. *J. Virol.* 73(9):7381–7389.
- Blaschke, S., H. Hannig, C. Buske, F. J. Kaup, G. Hunsmann, and W. Bodemer. 2001. Expression of the simian Epstein–Barr virus-encoded latent membrane protein-1 in malignant lymphomas of SIV-infected rhesus macaques. *J. Med. Virol.* 65(1):114–120.
- Bocker, J. F., K.-H. Tiedemann, G. W. Bornkamm, and H. Zur Hausen. 1980. Characterization of an EBV-like virus in African green monkey lymphoblasts. *Virology* 101:291–295.
- Borza, C. M. and L. M. Hutt-Fletcher. 1998. Epstein–Barr virus recombinant lacking expression of glycoprotein gp150 infects B cells normally but is enhanced for infection of epithelial cells. *J. Virol.* 72(9):7577–7582.
- Chen, H. L., K. Hayashi, T. R. Koirala, H. Ino, K. Fujimoto, Y. Yoshikawa, C. R. Choudhury, and T. Akagi. 1997. Malignant lymphoma induction in rabbits by oral inoculation of crude virus fraction prepared from Ts-B6 cells (cynomolgus B-lymphoblastoid cells harboring Epstein–Barr virus-related simian herpesvirus). *Acta Med. Okayama* 51(3):141–147.
- Cho, Y., J. Ramer, P. Rivailler, C. Quink, R. L. Garber, D. R. Beier, and F. Wang. 2001. An Epstein–Barr-related herpesvirus from marmoset lymphomas. *Proc. Natl. Acad. Sci. U. S. A.* 98(3):1224–1229.
- Cho, Y. G., A. V. Gordadze, P. D. Ling, and F. Wang. 1999. Evolution of two types of rhesus lymphocryptovirus similar to type 1 and type 2 Epstein–Barr virus. *J. Virol.* 73(11):9206–9212.
- Chu, C. T., C. S. Yang, and A. Kawamura. 1971. Antibodies to Epstein–Barr virus in a Burkitt's lymphoma cell line in Taiwan monkey (*M. cyclopis*). *Appl. Environ. Microbiol.* 21:539–540.
- de Thoisy, B., J. F. Pouliquen, V. Lacoste, A. Gessain, and M. Kazanji. 2003. Novel gamma-1

- herpesviruses identified in free-ranging new world monkeys (golden-handed tamarin [*Saguinus midas*], squirrel monkey [*Saimiri sciureus*], and white-faced saki [*Pithecia pithecia*]) in French Guiana. *J. Virol.* 77(16):9099–9105.
14. Deinhardt, F., L. Falk, L. G. Wolfe, A. Schudel, M. Nonoyama, P. Lai, B. Lapin, and L. Yakovleva. 1978. Susceptibility of marmosets to Epstein–Barr virus-like baboon herpesviruses. *Primates Med.* 10:163–170.
15. Djatchenko, A. G., V. V. Kakubava, B. A. Lapin, V. Z. Agrba, L. A. Yakovleva, and E. I. Samilchuk. 1976. Continuous lymphoblastoid suspension cultures from cells of haematopoietic organs of baboons with malignant lymphoma—biological characterization and biological properties of the herpes virus associated with culture cells. *Exp. Pathol. (Jena)* 12(3–4):163–168.
16. Djatchenko, A. G., L. V. Kokosha, B. A. Lapin, L. A. Yakovleva, and V. Z. Agrba. 1980. Detection of baboon herpesvirus DNA in tissues of hemoblastosis diseases and healthy monkeys of the Sukhumi nursery. *Exp. Oncol.* 2(4):31–32.
17. Dunkel, V. C., T. W. Pry, and G. Henle. 1972. Immunofluorescence tests for antibodies to Epstein–Barr virus with sera of lower primates. *J. Natl. Cancer Inst.* 49:435–440.
18. Ehlers, B., K. Borchers, C. Grund, K. Frolich, H. Ludwig, and H. J. Buhk. 1999. Detection of new DNA polymerase genes of known and potentially novel herpesviruses by PCR with degenerate and deoxyinosine-substituted primers. *Virus Genes* 18(3):211–220.
19. Ehlers, B., A. Ochs, F. Leendertz, M. Goltz, C. Boesch, and K. Matz-Rensing. 2003. Novel simian homologues of Epstein–Barr virus. *J. Virol.* 77(19):10695–10699.
20. Falk, L., F. Deinhardt, M. Nonoyama, L. G. Wolfe, C. Bergholz, B. Lapin, L. Yakovleva, V. Agrba, G. Henle, and W. Henle. 1976. Properties of a baboon lymphotropic herpesvirus related to Epstein–Barr virus. *Int. J. Cancer* 18:798–807.
21. Falk, L. A., G. Henle, W. Henle, F. Deinhardt, and A. Schudel. 1977. Transformation of lymphocytes by Herpesvirus papio. *Int. J. Cancer* 20(2):219–226.
22. Faucher, S., K. Dimock, and K. E. Wright. 2002. Characterization of the Cyno-EBV LMP1 homologue and comparison with LMP1s of EBV and other EBV-like viruses. *Virus Res.* 90(1–2):63–75.
23. Feichtinger, H., E. Kaaya, P. Putkonen, S. L. Li, M. Ekman, R. Gendelman, G. Biberfeld, and P. Biberfeld. 1992. Malignant lymphoma associated with human AIDS and with SIV-induced immunodeficiency in macaques. *AIDS Res. Hum. Retroviruses* 8:339–348.
24. Ferrari, M. G., E. D. Rivadeneira, R. Jarrett, L. Stevceva, S. Takemoto, P. Markham, and G. Franchini. 2001. HV(MNE), a novel lymphocryptovirus related to Epstein–Barr virus, induces lymphoma in New Zealand White rabbits. *Blood* 98(7):2193–2199.
25. Ferrari, M. G., L. Stevceva, P. Markham, and G. Franchini. 2003. Species-specific transformation of T cells by HV(MNE). *Virology* 317(2):299–307.
26. Fogg, M. H., A. Carville, J. Cameron, C. Quink, and F. Wang. 2005. Reduced prevalence of Epstein–Barr virus-related lymphocryptovirus infection in sera from a new world primate. *J. Virol.* 79(15):10069–10072.
27. Fogg, M. H., D. Garry, A. Awad, F. Wang, and A. Kaur. 2006. The BZLF1 homolog of an Epstein–Barr-related gamma-herpesvirus is a frequent target of the CTL response in persistently infected rhesus macaques. *J. Immunol.* 176(6):3391–3401.
28. Fogg, M. H., A. Kaur, Y. G. Cho, and F. Wang. 2005. The CD8⁺ T-cell response to an Epstein–Barr virus-related gammaherpesvirus infecting rhesus macaques provides evidence for immune evasion by the EBNA-1 homologue. *J. Virol.* 79(20):12681–12691.
29. Frank, A., W. A. Andiman, and G. Miller. 1976. Epstein–Barr virus and nonhuman primates: natural and experimental infection. *Adv. Cancer Res.* 23:171–201.
30. Franken, M., B. Annis, A. N. Ali, and F. Wang. 1995. 5' Coding and regulatory region sequence divergence with conserved function of the Epstein–Barr virus LMP2A homolog in herpesvirus papio. *J. Virol.* 69(12):8011–8019.
31. Franken, M., O. Devergne, M. Rosenzweig, B. Annis, E. Kieff, and F. Wang. 1996. Comparative analysis identifies conserved tumor necrosis factor receptor-associated factor 3 binding sites in the human and simian Epstein–Barr virus oncogene LMP1. *J. Virol.* 70(11):7819–7826.
32. Fuentes-Panana, E. M., S. Swaminathan, and P. D. Ling. 1999. Transcriptional activation signals found in the Epstein–Barr virus (EBV) latency C promoter are conserved in the latency C promoter sequences from baboon and Rhesus monkey EBV-like lymphocryptoviruses (cercopithecine herpesviruses 12 and 15). *J. Virol.* 73(1):826–833.
33. Fujimoto, K. and S. Honjo. 1991. Presence of antibody to Cyno-EBV in domestically bred cynomolgus monkeys (*Macaca fascicularis*). *J. Med. Primatol.* 20:42–45.
34. Fujimoto, K., K. Terato, J. Miyamoto, H. Ishiko, M. Fujisaki, F. Cho, and S. Honjo. 1990. Establishment

- of a B-lymphoblastoid cell line infected with Epstein–Barr-related virus from a cynomolgus monkey (*Macaca fascicularis*). *J. Med. Primatol.* 19:21–30.
35. Gerber, P. and S. M. Birch. 1967. Complement-fixing antibodies in sera of human and non-human primates to viral antigen derived from Burkitt's lymphoma cells. *Proc. Natl. Acad. Sci. U. S. A.* 58:478–484.
 36. Gerber, P., S. S. Kalter, G. Schidlovsky, W. D. Peterson, and M. D. Daniel. 1977. Biological and antigenic characteristics of Epstein–Barr virus-related herpesviruses and chimpanzees and baboons. *Int. J. Cancer* 20:448–459.
 37. Gerber, P., G. R. Prichett, and E. D. Kieff. 1976. Antigens and DNA of a chimpanzee agent related to Epstein–Barr virus. *J. Virol.* 19:1090–1099.
 38. Gerber, P. and E. N. Rosenblum. 1968. The incidence of complement-fixing antibodies to herpes simplex and herpes-like viruses in man and rhesus monkey. *Proc. Soc. Exp. Biol. Med.* 128:541–546.
 39. Gerner, C. S., A. Dolan, and D. J. McGeoch. 2004. Phylogenetic relationships in the Lymphocryptovirus genus of the Gammaherpesvirinae. *Virus Res.* 99(2):187–192.
 40. Goldman, N., J. C. Landon, and J. Reischer. 1968. Fluorescent antibody and gel diffusion reactions of human and chimpanzee sera with cells cultured from Burkitt's tumours and normal chimpanzee blood. *Cancer Res.* 28:2489–2495.
 41. Habis, A., G. Baskin, L. Simpson, I. Fortgang, M. Murphrey-Corb, and L. S. Levy. 2000. Rhesus lymphocryptovirus infection during the progression of SAIDS and SAIDS-associated lymphoma in the rhesus macaque. *AIDS Res. Hum. Retroviruses* 16(2):163–171.
 42. Hayashi, K. and T. Akagi. 2000. An animal model for Epstein–Barr virus (EBV)-associated lymphomagenesis in the human: malignant lymphoma induction of rabbits by EBV-related herpesvirus from cynomolgus. *Pathol. Int.* 50(2):85–97.
 43. Hayashi, K., H. L. Chen, H. Yanai, T. R. Koirala, N. Ohara, N. Teramoto, T. Oka, T. Yoshino, K. Takahashi, K. Miyamoto, K. Fujimoto, Y. Yoshikawa, and T. Akagi. 1999. Cyno-EBV (EBV-related herpesvirus from cynomolgus macaques) induces rabbit malignant lymphomas and their tumor cell lines frequently show specific chromosomal abnormalities. *Lab. Invest.* 79(7):823–835.
 44. Hayashi, K., Z. Jin, S. Onoda, H. Joko, N. Teramoto, N. Ohara, W. Oda, T. Tanaka, Y. X. Liu, T. R. Koirala, T. Oka, E. Kondo, T. Yoshino, K. Takahashi, and T. Akagi. 2003. Rabbit model for human EBV-associated hemophagocytic syndrome (HPS): sequential autopsy analysis and characterization of IL-2-dependent cell lines established from herpesvirus papio-induced fatal rabbit lymphoproliferative diseases with HPS. *Am. J. Pathol.* 162(5):1721–1736.
 45. Hayashi, K., H. Joko, T. R. Koirala, S. Onoda, Z. S. Jin, M. Munemasa, N. Ohara, W. Oda, T. Tanaka, T. Oka, E. Kondo, T. Yoshino, K. Takahashi, M. Yamada, and T. Akagi. 2003. Therapeutic trials for a rabbit model of EBV-associated Hemophagocytic Syndrome (HPS): effects of vidarabine or CHOP, and development of Herpesvirus papio (HVP)-negative lymphomas surrounded by HVP-infected lymphoproliferative disease. *Histol. Histopathol.* 18(4):1155–1168.
 46. Hayashi, K., N. Ohara, N. Teramoto, S. Onoda, H. L. Chen, T. Oka, E. Kondo, T. Yoshino, K. Takahashi, J. Yates, and T. Akagi. 2001. An animal model for human EBV-associated hemophagocytic syndrome: herpesvirus papio frequently induces fatal lymphoproliferative disorders with hemophagocytic syndrome in rabbits. *Am. J. Pathol.* 158(4):1533–1542.
 47. Hayashi, K., N. Teramoto, and T. Akagi. 2002. Animal in vivo models of EBV-associated lymphoproliferative diseases: special references to rabbit models. *Histol. Histopathol.* 17(4):1293–1310.
 48. Heberling, R. L., C. P. Bieber, and S. S. Kalter. 1982. Establishment of a lymphoblastoid cell line from a lymphomatous cynomolgus monkey. In: Yohn, D. S. and J. R. Blakeslee (eds), *Advances in Comparative Leukemia Research 1981*. New York: Elsevier North Holland, Inc.
 49. Heller, M., P. Gerber, and E. Kieff. 1981. Herpesvirus papio DNA is similar in organization to Epstein–Barr virus DNA. *J. Virol.* 37(2):698–709.
 50. Heller, M., P. Gerber, and E. Kieff. 1982. DNA of herpesvirus pan, a third member of the Epstein–Barr virus–Herpesvirus papio group. *J. Virol.* 41:931–939.
 51. Heller, M. and E. Kieff. 1981. Colinearity between the DNAs of Epstein–Barr virus and herpesvirus papio. *J. Virol.* 37(2):821–826.
 52. Howe, J. G. and M. D. Shu. 1988. Isolation and characterization of the genes for two small RNAs of herpesvirus papio and their comparison with Epstein–Barr virus-encoded EBER RNAs. *J. Virol.* 62:2790–2798.
 53. Ino, H., K. Hayashi, H. Yanai, N. Teramoto, T. R. Koirala, H. L. Chen, T. Oka, T. Yoshino, K. Takahashi, and T. Akagi. 1997. Analysis of the genome of an Epstein–Barr-virus (EBV)-related herpesvirus in a cynomolgus monkey cell line (Si-IIA). *Acta Med. Okayama* 51(4):207–212.

54. Ishida, T. and K. Yamamoto. 1987. Survey of non-human primates for antibodies reactive with Epstein–Barr virus (EBV) antigens and susceptibility of their lymphocytes for immortalization with EBV. *J. Med. Primatol.* 16:359–371.
55. Jenson, H. B., Y. Ench, S. J. Gao, K. Rice, D. Carey, R. C. Kennedy, J. R. Arrand, and M. Mackett. 2000. Epidemiology of herpesvirus papio infection in a large captive baboon colony: similarities to Epstein–Barr virus infection in humans. *J. Infect. Dis.* 181(4):1462–1466.
56. Jenson, H. B., Y. Ench, Y. Zhang, S. J. Gao, J. R. Arrand, and M. Mackett. 2002. Characterization of an Epstein–Barr virus-related gammaherpesvirus from common marmoset (*Callithrix jacchus*). *J. Gen. Virol.* 83(Pt 7):1621–1633.
57. Jiang, H., Y. G. Cho, and F. Wang. 2000. Structural, functional, and genetic comparisons of Epstein–Barr virus nuclear antigen 3A, 3B, and 3C homologues encoded by the rhesus lymphocryptovirus. *J. Virol.* 74(13):5921–5932.
58. Kahnt, K., K. Matz-Rensing, P. Hofmann, C. Stahl-Hennig, and F. J. Kaup. 2002. SIV-associated lymphomas in rhesus monkeys (*Macaca mulatta*) in comparison with HIV-associated lymphomas. *Vet. Pathol.* 39(1):42–55.
59. Kalter, S. S., R. L. Heberling, and J. J. Ratner. 1972. EBV antibody in sera of non-human primates. *Nature* 238(5363):353–354.
60. Kokosha, L. V., V. Z. Agbba, B. A. Lapin, L. A. Yakovleva, N. N. Arshba, T. P. Markova, and V. V. Pimanovskaja. 1977. Continuous lymphoblastoid suspension cultures from cells of haematopoietic organs of baboons with malignant lymphoma. Report III: immunological studies. *Exp. Pathol. (Jena)* 13(4–5):247–254.
61. Kutok, J. L., S. Klumpp, M. Simon, J. J. MacKey, V. Nguyen, J. M. Middeldorp, J. C. Aster, and F. Wang. 2004. Molecular evidence for rhesus lymphocryptovirus infection of epithelial cells in immunosuppressed rhesus macaques. *J. Virol.* 78(7):3455–3461.
62. Landon, J. C., L. B. Ellis, and D. F. Fabricui. 1968. Leukocytes suspension culture from baboon. *Proc. Am. Assoc. Cancer Res.* 9:39.
63. Landon, J. C., L. B. Ellis, V. H. Leve, and D. P. Fabricio. 1968. Herpes-type virus in cultured leukocytes from chimpanzees. *J. Natl. Cancer Inst.* 40:181–192.
64. Landon, J. C. and L. Malan. 1971. Seroepidemiological studies of Epstein–Barr virus antibodies in monkeys. *J. Natl. Cancer Inst.* 46:881–884.
65. Lapin, B. A., V. V. Timanovskaya, and L. A. Yakovleva. 1985. Herpesvirus HVMA: a new representative in the group of the EBV-like B-lymphotropic her-
- pesviruses of primates. *Haematol. Blood Transfus.* 29:312–313.
66. Lee, Y. S., M. Nonoyama, and H. Rabin. 1981. Co-linear relationships of herpesvirus papio DNA to Epstein–Barr virus DNA. *Virology* 110(1):248–252.
67. Lee, Y. S., A. Tanaka, R. Y. Lau, M. Nonoyama, and H. Rabin. 1980. Comparative studies of herpesvirus papio (baboon herpesvirus) DNA and Epstein–Barr virus DNA. *J. Gen. Virol.* 51(Pt 2):245–253.
68. Lee, Y. S., A. Tanaka, R. Y. Lau, M. Nonoyama, and H. Rabin. 1981. Linkage map of the fragments of Herpesvirus papio DNA. *J. Virol.* 37:710–720.
69. Levitskaya, J., M. Coram, V. Levitsky, S. Imreh, P. M. Steigerwald-Mullen, G. Klein, M. G. Kurilla, and M. G. Masucci. 1995. Inhibition of antigen processing by the internal repeat region of the Epstein–Barr virus nuclear antigen-1. *Nature* 375(6533):685–688.
70. Li, S. L., P. Biberfeld, and I. Ernberg. 1994. DNA of lymphoma-associated herpesvirus (HVMF1) in SIV-infected monkeys (*Macaca fascicularis*) shows homologies to EBNA-1, -2 and -5 genes. *Int. J. Cancer* 59(2):287–295.
71. Li, S. L., H. Feichtinger, E. Kaaya, P. Migliorini, P. Putkonen, G. Biberfeld, J. M. Middeldorp, P. Biberfeld, and I. Ernberg. 1993. Expression of Epstein–Barr-virus-related nuclear antigens and B-cell markers in lymphomas of SIV-immunosuppressed monkeys. *Int. J. Cancer* 55(4):609–615.
72. Ling, P. D., J. J. Ryon, and S. D. Hayward. 1993. EBNA-2 of herpesvirus papio diverges significantly from the type A and type B EBNA-2 proteins of Epstein–Barr virus but retains an efficient transactivation domain with a conserved hydrophobic motif. *J. Virol.* 67:2990–3003.
73. Loeb, D. D., N. S. Sung, R. L. Pesano, C. J. Sexton, C. Hutchison III, and J. S. Pagano. 1990. Plasmid origin of replication of herpesvirus papio: DNA sequence and enhancer function. *J. Virol.* 64(6):2876–2883.
74. McCann, E. M., G. L. Kelly, A. B. Rickinson, and A. I. Bell. 2001. Genetic analysis of the Epstein–Barr virus-coded leader protein EBNA-LP as a co-activator of EBNA2 function. *J. Gen. Virol.* 82(Pt 12):3067–3079.
75. Meerbach, A., C. Friedrichs, R. Thust, and P. Wutzler. 2004. Transformation of rabbit lymphocytes by an Epstein–Barr virus-related herpesvirus from *Macaca arctoides*. *Arch. Virol.* 149(6):1083–1094.
76. Moghaddam, A., J. Koch, B. Annis, and F. Wang. 1998. Infection of human B lymphocytes with lymphocryptoviruses related to Epstein–Barr virus. *J. Virol.* 72(4):3205–3212.
77. Moghaddam, A., M. Rosenzweig, D. Lee-Parritz, B. Annis, R. P. Johnson, and F. Wang. 1997. An animal

- model for acute and persistent Epstein–Barr virus infection. *Science* 276(5321):2030–2033.
78. Neubauer, R. H., H. Rabin, and A. M. Brown. 1978. Selective stimulation and differentiation of early antigens in lymphoblastoid cell lines producing Epstein–Barr-like viruses. *J. Gen. Virol.* 41:295–301.
 79. Neubauer, R. H., H. Rabin, B. C. Strnad, B. A. Lapin, L. A. Yakovleva, and E. Indzie. 1979. Antibody responses to *Herpesvirus papio* antigens in baboons with lymphoma. *Int. J. Cancer* 23(2):186–192.
 80. Neubauer, R. H., H. Rabin, B. C. Strnad, M. Nonoyama, and W. A. Nelson-Rees. 1979. Establishment of a lymphoblastoid cell line and isolation of an Epstein–Barr-related virus of gorilla origin. *J. Virol.* 31:845–848.
 81. Ohno, S., J. Luka, L. Falk, and G. Klein. 1977. Detection of a nuclear, EBNA-type antigen in apparently EBNA-negative *Herpesvirus papio* (HPV)-transformed lymphoid lines by the acid-fixed nuclear binding technique. *Int. J. Cancer* 20:941–946.
 82. Ohno, S., J. Luka, L. A. Falk, and G. Klein. 1978. Serological reactivities of human and baboon sera against EBNA and *Herpesvirus papio*-determined nuclear antigen (HUPNA). *Eur. J. Cancer* 14:955–960.
 83. Ohno, S., J. Luka, and G. Klein. 1979. Evidence for antigenic distinctness of the Epstein–Barr virus-determined nuclear antigen and the *Herpesvirus papio*-determined nuclear antigen. *Cancer Lett.* 6(6):325–329.
 84. Omerovic, J. and R. Longnecker. 2007. Functional homology of gHs and gLs from EBV-related gamma-herpesviruses for EBV-induced membrane fusion. *Virology* 365(1):157–165.
 85. Payton, M. E., J. M. d'Offay, M. E. Prado, D. H. Black, B. Damania, G. L. White, and R. Eberle. 2004. Comparative transmission of multiple herpesviruses and simian virus 40 in a baboon breeding colony. *Comp. Med.* 54(6):695–704.
 86. Peng, R., A. V. Gordadze, E. M. Fuentes Panama, F. Wang, J. Zong, G. S. Hayward, J. Tan, and P. D. Ling. 2000. Sequence and functional analysis of EBNA-LP and EBNA2 proteins from nonhuman primate lymphocryptoviruses. *J. Virol.* 74(1):379–389.
 87. Pesano, R. L. and J. S. Pagano. 1986. *Herpesvirus papio* contains a plasmid origin of replication that acts in cis interspecies with an Epstein–Barr virus trans-acting function. *J. Virol.* 60:1159–1162.
 88. Phakdeewirot, P., S. Payungporn, S. Chutinimitkul, A. Theamboonlers, and Y. Poovorawan. 2006. Prevalence and molecular characterization of the polymerase gene of gibbon lymphocryptovirus. *J. Med. Primatol.* 35(3):136–143.
 89. Pingel, S., H. Hannig, K. Matz-Rensing, F. J. Kaup, G. Hunsmann, and W. Bodemer. 1997. Detection of Epstein–Barr virus small RNAs EBER1 and EBER2 in lymphomas of SIV-infected rhesus monkeys by *in situ* hybridization. *Int. J. Cancer* 72(1):160–165.
 90. Prepens, S., K. A. Kreuzer, F. Leendertz, A. Nitsche, and B. Ehlers. 2007. Discovery of herpesviruses in multi-infected primates using locked nucleic acids (LNA) and a bigenic PCR approach. *J. Virol.* 4:84.
 91. Rabin, H., R. H. Neubauer, R. F. Hopkins, E. K. Dzhikidze, Z. V. Shevtsova, and B. A. Lapin. 1977. Transforming activity and antigenicity of an Epstein–Barr-like virus from lymphoblastoid cell lines of baboon with lymphoid disease. *Intervirology* 8:240–249.
 92. Rabin, H., R. H. Neubauer, R. F. Hopkins, and M. Nonoyama. 1978. Further characterization of a herpesvirus-positive orang-utan cell line and comparative aspects of *in vitro* transformation with lymphotropic Old World primate herpesviruses. *Int. J. Cancer* 21:762–767.
 93. Rabin, H., B. C. Strnad, R. H. Neubauer, A. M. Brown, R. F. Hopkins, and R. A. Mazur. 1980. Comparisons of nuclear antigens of Epstein–Barr virus (EBV) and EBV-like simian viruses. *J. Virol.* 48:265–272.
 94. Ramer, J. C., R. L. Garber, K. E. Steele, J. F. Boyson, C. O'Rourke, and J. A. Thomson. 2000. Fatal lymphoproliferative disease associated with a novel gamma-herpesvirus in a captive population of common marmosets. *Comp. Med.* 50(1):59–68.
 95. Rangan, S. R., L. N. Martin, B. E. Bozelka, N. Wang, and B. J. Gormus. 1986. Epstein–Barr virus-related herpesvirus from a rhesus monkey (*Macaca mulatta*) with malignant lymphoma. *Int. J. Cancer* 38:425–432.
 96. Rao, P., H. Jiang, and F. Wang. 2000. Cloning of the rhesus lymphocryptovirus viral capsid antigen and Epstein–Barr virus-encoded small RNA homologues and use in diagnosis of acute and persistent infections. *J. Clin. Microbiol.* 38(9):3219–3225.
 97. Rasheed, S., R. W. Rongey, J. Bruszweski, W. A. Nelson-Rees, H. Rabin, R. H. Neubauer, G. Esra, and M. B. Gardner. 1977. Establishment of a cell line with associated Epstein–Barr-like virus from a leukemic orangutan. *Science* 198:407–409.
 98. Rezikyan, S., E. E. Kaaya, M. Ekman, A. F. Voevodin, H. Feichtinger, P. Putkonen, E. Castanos-Velez, G. Biberfeld, and P. Biberfeld. 1995. B-cell lymphomagenesis in SIV-immunosuppressed cynomolgus monkeys. *Int. J. Cancer* 61(4):574–579.
 99. Rivadeneira, E. D., M. G. Ferrari, R. F. Jarrett, A. A. Armstrong, P. Markham, T. Birkebak, S. Takemoto,

- C. Johnson-Delaney, J. Pecon-Slattery, E. A. Clark, and G. Franchini. 1999. A novel Epstein–Barr virus-like virus, HV(MNE), in a Macaca nemestrina with mycosis fungoides. *Blood* 94(6):2090–2101.
100. Rivailleur, P., A. Carville, A. Kaur, P. Rao, C. Quink, J. L. Kutok, S. Westmoreland, S. Klumpp, M. Simon, J. C. Aster, and F. Wang. 2004. Experimental rhesus lymphocryptovirus infection in immunosuppressed macaques: an animal model for Epstein–Barr virus pathogenesis in the immunosuppressed host. *Blood* 104(5):1482–1489.
101. Rivailleur, P., Y. G. Cho, and F. Wang. 2002. Complete genomic sequence of an Epstein–Barr virus-related herpesvirus naturally infecting a new world primate: a defining point in the evolution of oncogenic lymphocryptoviruses. *J. Virol.* 76(23):12055–12068.
102. Rivailleur, P., H. Jiang, Y. G. Cho, C. Quink, and F. Wang. 2002. Complete nucleotide sequence of the rhesus lymphocryptovirus: genetic validation for an Epstein–Barr virus animal model. *J. Virol.* 76(1):421–426.
103. Rivailleur, P., C. Quink, and F. Wang. 1999. Strong selective pressure for evolution of an Epstein–Barr virus LMP2B homologue in the rhesus lymphocryptovirus. *J. Virol.* 73(10):8867–8872.
104. Rose, T. M. 2005. CODEHOP-mediated PCR – a powerful technique for the identification and characterization of viral genomes. *J. Virol.* 2(March 15):20.
105. Ruf, I. K., A. Moghaddam, F. Wang, and J. Sample. 1999. Mechanisms that regulate Epstein–Barr virus EBNA-1 gene transcription during restricted latency are conserved among lymphocryptoviruses of Old World primates. *J. Virol.* 73(3):1980–1989.
106. Ryon, J. J., E. D. Fixman, C. Houchens, J. Zong, P. M. Lieberman, Y. N. Chang, G. S. Hayward, and S. D. Hayward. 1993. The lytic origin of herpesvirus papio is highly homologous to Epstein–Barr virus ori-Lyt: evolutionary conservation of transcriptional activation and replication signals. *J. Virol.* 67:4006–4016.
107. Schatzl, H., M. Tschikobava, D. Rose, A. Voevodin, H. Nitschko, E. Sieger, U. Busch, K. von der Helm, and B. Lapin. 1993. The Sukhumi primate monkey model for viral lymphomagenesis: high incidence of lymphomas with presence of STLV-I and EBV-like virus. *Leukemia* 7(Suppl 2):S86–S92.
108. Schmitz, H. 1981. Immunofluorescent staining of nuclear antigen in lymphoid cells transformed by *Herpesvirus papio* (HPV). *J. Immunol. Methods* 42:337–342.
109. Siler, C. A. and N. Raab-Traub. 2008. Rhesus lymphocryptovirus latent membrane protein 2A activates beta-catenin signaling and inhibits differentiation in epithelial cells. *Virology* 377(2):273–279.
110. Suzuki, J., S. Goto, A. Kato, C. Hashimoto, N. Miwa, S. Takao, T. Ishida, A. Fukuoka, H. Nakayama, K. Doi, and K. Isowa. 2005. Malignant NK/T-cell lymphoma associated with simian Epstein–Barr virus infection in a Japanese macaque (*Macaca fuscata*). *Exp. Anim.* 54(1):101–105.
111. Tellam, J., M. Rist, G. Connolly, N. Webb, C. Faizou, F. Wang, and R. Khanna. 2007. Translation efficiency of EBNA1 encoded by lymphocryptoviruses influences endogenous presentation of CD8⁺ T cell epitopes. *Eur. J. Immunol.* 37(2):328–337.
112. Timanovskaya, V. V., A. F. Voevodin, L. A. Iakovleva, D. S. Markarian, and M. T. Ivanov. 1988. Malignant lymphoma in rabbits induced by herpesvirus-containing materials of *Macaca arctoides* monkeys. *Eksp. Onkol.* 10(3):47–51.
113. VanDevanter, D. R., P. Warrener, L. Bennett, E. R. Schultz, S. Coulter, R. L. Garber, and T. M. Rose. 1996. Detection and analysis of diverse herpesviral species by consensus primer PCR. *J. Clin. Microbiol.* 34(7):1666–1671.
114. Voevodin, A. F. and I. Hirsch. 1985. Immunoprecipitation of Epstein–Barr virus (EBV)-specific proteins by prelymphomatous and normal baboon sera containing antibodies reactive with EBV early antigen. *Acta Virol. (Praha)* 29(3):242–246.
115. Voevodin, A. F., T. I. Ponomarjeva, and B. A. Lapin. 1985. Seroepizootiology of the herpesvirus Papio (HVP) infection in healthy baboons (*Papio hamadryas*) of high- and low-lymphoma risk populations. *Exp. Pathol.* 27(1):33–39.
116. Voevodin, A. F., L. A. Yakovleva, B. A. Lapin, and T. I. Ponomarjeva. 1983. Increased antibody responses to Herpes virus papio (HVP) antigens in pre-lymphomatous baboons (*Papio hamadryas*) of the Sukhumi high lymphoma stock. *Int. J. Cancer* 32(5):637–639.
117. von Knebel Doeberitz M., G. W. Bornkamm, and H. zur Hausen. 1983. Establishment of spontaneously outgrowing lymphoblastoid cell lines with Cyclosporin A. *Med. Microbiol. Immunol.* 172(2):87–99.
118. Voss, G., S. Nick, C. Stahl-Hennig, K. Ritter, and G. Hunsmann. 1992. Generation of macaque B lymphoblastoid cell lines with simian Epstein–Barr-like viruses: transformation procedure, characterization of the cell lines and occurrence of simian foamy virus. *J. Virol. Methods* 39:185–195.
119. Wang, F., P. Rivailleur, P. Rao, and Y. Cho. 2001. Simian homologues of Epstein–Barr virus. *Philos. Trans. R. Soc. Lond B Biol. Sci.* 356(1408):489–497.

120. Wu, L. and L. M. Hutt-Fletcher. 2007. Compatibility of the gH homologues of Epstein–Barr virus and related lymphocryptoviruses. *J. Gen. Virol.* 88(Pt 8):2129–2136.
121. Wutzler, P., A. Meerbach, I. Farber, H. Wolf, and K. Scheibner. 1995. Malignant lymphomas induced by an Epstein–Barr virus-related herpesvirus from *Macaca arctoides*—a rabbit model. *Arch. Virol.* 140(11):1979–1995.
122. Yakovleva, L. A., V. V. Timanovskaya, A. F. Vorodin, L. V. Indzhiia, B. A. Lapin, M. T. Ivanov, and D. S. Markaryan. 1987. Modelling of malignant lymphoma in rabbits, using oncogenic viruses of non-human primates. *Haematol. Blood Transfus.* 31:445–447.
123. Yates, J. L., S. M. Camiolo, S. Ali, and A. Ying. 1996. Comparison of the EBNA1 proteins of Epstein–Barr virus and herpesvirus papio in sequence and function. *Virology* 222(1):1–13.
124. Zhao, B., R. Dalbies-Tran, H. Jiang, I. K. Ruf, J. T. Sample, F. Wang, and C. E. Sample. 2003. Transcriptional regulatory properties of Epstein–Barr virus nuclear antigen 3C are conserved in simian lymphocryptoviruses. *J. Virol.* 77(10):5639–5648.

16

Rhadinoviruses

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16.1. INTRODUCTION

The prototype virus of the *Rhadinovirus* genus is the simian virus best known as herpesvirus saimiri (HVS). This virus was isolated from a healthy squirrel monkey (*Saimiri sciureus*) in 1968.⁷⁹ Later a similar virus named Herpesvirus ateles (HVA) was isolated from a spider monkey (*Ateles geoffroyii*).^{75,83} Both HVS and HVA are apathogenic in their natural hosts. At the same time, they are profoundly oncogenic in other species of New World monkeys: lymphoma develops in 100% of animals within 2 months after inoculation.^{77,78,80–82} Interestingly, HVS- and HVA-induced lymphomas are

of T-cell origin and in vitro both viruses transform T-lymphocytes of various simian species into immortal cell lines. Moreover, one of the HVS strains (C488) efficiently immortalizes not only simian but also human T-lymphocytes in vitro.¹⁰ HVS and HVA were extensively studied in the 1970s–early 1990s and the culmination of this research was the establishment in 1992 of a new herpesvirus genus, the *Rhadinovirus*, with HVS as the prototype.⁹⁰

In 1994, the first and the only known human rhadinovirus was discovered.²¹ This virus, named human herpesvirus 8 (HHV-8), turned to be the causal agent of Kaposi's sarcoma (KS), a malignancy which is one of the hallmarks of AIDS (reviewed in Ganem⁴⁵). At that time, the only known simian rhadinoviruses were of New World monkeys, and the discovery of HHV-8 initiated a search for similar viruses in Old World NHPs. The first findings of such viruses were reported in 1997.^{30,92} Old World simian rhadinoviruses have since been shown to be much more closely related to HHV-8 than HVS and HVA, and the focus of simian rhadinovirus research has shifted to these “HHV-8-like” agents (reviewed in Damanis and Desrosiers²³ and O'Connor and Kedes⁸⁶). These viruses are covered in Section 16.4. The “classical” simian rhadinoviruses HSV and HVA are covered in Section 16.5.

16.2. CLASSIFICATION AND NOMENCLATURE

The genus *Rhadinovirus* belongs to the *Gammaherpesvirinae* subfamily. The name rhadinovirus is derived from Greek *rhadinos* (slender, taper). This unusual name was prompted by the fragility of HVS genomic DNA (see Section 16.3). The closest relatives of rhadinoviruses are lymphocryptoviruses (the

Lymphocryptovirus genus), which also belong to the *Gammaherpesvirinae* subfamily; hence, the alternative names of lymphocryptoviruses and rhadinoviruses: γ 1- and γ 2-herpesviruses, respectively.

The latest published version of the International Committee on Taxonomy of Viruses (ICTV) classification (8th Report) includes three simian rhadinoviruses species: *Saimiriine herpesvirus* 2 (SaHV-2), *Ateline herpesvirus* 2 (AtHV-2), and *Cercopithecine herpesvirus* 17 (CeHV-17). In May 2008, this version of the ICTV classification was updated, but the amendment had not been published by the time of writing (summer 2008).

The introduced changes to the existing nomenclature are that CeHV-17 is renamed *Macacine herpesvirus* 5 (McHV-5), and the rank of *Ateline herpesvirus* 3 (AtHV-3) is elevated from strain to species. The old and new ICTV names of simian rhadinoviruses, as well as their synonymous names are listed in Table 16.1. It is worth mentioning that AtHV-3 was listed at the species level in the 6th ICTV report, was later demoted to the rank of isolate/strain within AtHV-2 species, and finally was reinstated as a separate species.

ICTV-names of simian rhadinoviruses are rarely used. The designations HVS, HVA, and RRV are much more common in the literature. However, the names HVS and HVA are also used for designation of simplexviruses iso-

lated from the squirrel and spider monkeys (see Chapter 12). This problem was remedied by assigning numbers to the virus names: HVS-1 and HVA-1 to the simplexviruses and HVS-2 and HVA-2 to rhadinoviruses. However, this informal nomenclature is also rarely used. Usually, HVS/HVA naming does not pose a significant problem; the affiliation of these viruses is usually clear from the context. For the sake of simplicity, the unofficial but best known names are used in this chapter for the infectious simian rhadinoviruses, that is, HVS for SaHV-2, HVA for AtHV-2 and AtHV-3, and RRV for McHV-5 (CeHV-17).

In addition to the simian rhadinoviruses included in the official ICTV classification, a number of frag-viruses related to HHV-8 and RRV have been detected in various Old World simian species.^{48–50, 63, 64, 91, 92, 96, 105} These viruses are designated as RV (rhadinoviruses) with a three-letter subscript indicating the genus or species of their natural hosts (Table 16.2). The only exception is the frag-virus associated with the retroperitoneal fibromatosis (RF) (Section 16.4.2). The name of this virus, RF-associated herpesvirus (RFHV), is derived from the name of the disease it is associated with.

The ICTV classification includes an “unassigned” gammaherpesvirus species *Callitrichine herpesvirus* 1 (CalHV-1) which was originally named *Herpesvirus*

Table 16.1. Simian Rhadinovirus Species

ICTV New Name	ICTV Old Name	Synonymous Names*	Host Species	Original Reference
<i>Macacine herpesvirus</i> 5 (McHV-5)	<i>Cercopithecine herpesvirus</i> 17 (CeHV-17)	Rhesus rhadinovirus (RRV) , Macaca mulatta rhadinovirus	<i>Macaca mulatta</i>	30
<i>Saimiriine herpesvirus</i> 2 (SaHV-2)	<i>Saimiriine herpesvirus</i> 2 (SaHV-2)	Herpesvirus saimiri (HVS) , Herpesvirus saimiri 2 (HVS-2)	<i>Saimiri sciureus</i>	79
<i>Ateline herpesvirus</i> 2 (AtHV-2)	<i>Ateline herpesvirus</i> 2 (AtHV-2)	Herpesvirus ateles (HVA) , Herpesvirus ateles 2 (HVA-2)	<i>Ateles geoffroyii</i>	83
<i>Ateline herpesvirus</i> 3 (AtHV-3) [†]	<i>Ateline herpesvirus</i> 3 (AtHV-3)	Herpesvirus ateles (HVA) , Herpesvirus ateles 3 (HVA-3)	<i>Ateles paniscus</i>	41
<i>Callitrichine herpesvirus</i> 1 [‡] (Ca1HV-1)	<i>Callitrichine herpesvirus</i> 1 (Ca1HV-1)	Herpesvirus sanguinus	<i>Saguinus</i> spp.	76

*Most commonly used names are in bold.

[†]Newly recognized species.

[‡]Unassigned gamma-herpesvirus species.

Table 16.2. Representative Simian Frag-Viruses Assignable to RV-1 and RV-2 Lineages

Generic Name*	Specific Name* (Original Name)	Host Species	GenBank Acc. No.
RV-1mac	RV-1 _{Mne} (RFHV _{Mn})	<i>Macaca nemestrina</i>	AF005478
	RV-1 _{Mmu} (RFHV _{Mm})	<i>M. mulatta</i>	AF005479
RV-1agm	RV-1 _{Cae} (ChRV1)	<i>Chlorocebus aethiops</i>	AJ251573
RV-1pan	RV-1 _{Ptr} (PanRHV1a)	<i>Pan troglodytes</i>	AF250879
	RV-1 _{Ptr} (PanRHV1b)		AF250880 AF250881 AF250882 AJ292986
RV-1gor	RV-1 _{Ggo} (GorRHV1)	<i>Gorilla gorilla</i>	AF250886 AY177144
RV-1mnd	RV-1 _{Msp} (MndRHV1)	<i>Mandrillus sphinx</i>	AF282943
RV-2agm	RV-2 _{Cae} (ChRV2)	<i>Chlorocebus aethiops</i>	AJ251574
RV-2pan	RV-2 _{Ptr} (PanRHV2)	<i>P. troglodytes</i>	AF346489 AF346490 EU085378
RV-2gib	RV-2 _{Hle} (HyloRHV2)	<i>Hylobates leucogenys</i>	AY465375
RV-2bab	RV-2 _{Pan} (PapRV2)	<i>Papio anubis</i>	AY270026
	RV-2 _{Pcy} (PcyRV2)	<i>P. cynocephalus</i>	AY270027
RV-2mnd	RV-2 _{Msp} (MndRHV2)	<i>Mandrillus leucophaeus</i>	AF282937 AF282938 AF282939 AF282940
RV-2mac	RV-2 _{Mne} (MneRV2)	<i>M. nemestrina</i>	AF204167
	RV-2 _{Mmu} (MGVMm)	<i>M. mulatta</i>	DQ792460
	RV-2 _{Mfa} (MGVMf)	<i>M. fascicularis</i>	AF159033 AF159032 AY138583

*Adapted from Damania and Desrosiers²³ with modification.

saguinus. This virus, first described in 1971,⁷⁶ still remains virtually uncharacterized. Possibly, CalHV-1 is a rhadinovirus. This supposition is prompted by the similarity of the terminase gene fragment of CalHV-1 (GenBank Acc. No. AF091070) to the homologous sequence of HVA and other several nonprimate rhadinovirus genes. However, the degree of homology (approximately 70%) and the length of the region (approximately 140 bp) are insufficient for unequivocal taxonomic identification of CalHV-1. The second known fragment of the CalHV-1 genome (polymerase gene,

GenBank Acc. No. CHU63458) has little similarity with the sequences of other known rhadinoviruses.

16.3. GENOMIC ORGANIZATION

The macrostructure of primate rhadinovirus genomes is similar and relatively simple as compared with other herpesviruses: a single long unique region (LUR) containing all protein-coding genes is framed by the terminal repeats (TRs). The G + C content in the unique and terminal regions is significantly different, being much

higher in the TR segments. During the original characterization of HVS genomic DNA, the LUR and TR genomic segments were separated based on their different buoyant density; hence, the designations DNA-L (light) and DNA-H (heavy) which appear in the literature for these segments of HVS and HVA genome. A tendency for genomic DNA to spontaneously fragment into DNA-L and DNA-H during isolation was the “fragility trait” that gave the *Rhadinovirus* genus its name.

All open reading frames (ORFs) are located within the LUR. There are about 80 ORFs in the HVS and HVA genomes, and 80–90 in the HHV-8 and RRV genomes (Tables 16.3 and 16.4). The LUR of HVS was the first among primate rhadinovirus genomes to be sequenced⁵ and ORFs identified in this genome were consecutively numbered from left to right, regardless of the coding strand (ORF-1, -2, -3, -4, etc.). This simple nomenclature has withstood the test of time: ORFs in the HVA, HHV-8, and RRV genomes are named identically to their HSV orthologs.^{1,6,94,97}

The genomes of HVS and HVA are almost completely collinear (Table 16.3). The HHV-8 and RRV genomes, as compared to the HVS/HVA genomes, have several insertions which are designated K1, K2, and so forth, in the HHV-8 genome (K stands for *Kaposi sarcoma*) and R1, R2, and so forth, in the RRV genome (R stands for *Rhesus monkey*) (Table 16.4). “K-” and “R-” inserts contain either a single ORF or several genes. Most of the K and R ORFs are homologous (e.g., K1/R1, K4/R4, K8/R8, K8.1/R8.1, and some others). The exceptions are ORFs K3, K4.1, K4.2, K6, K7, K12; homologs of these ORFs are absent in the RRV genome. The most notable difference is the absence of the K12 homolog in the RRV genome; this HHV-8 gene encodes kaposin, a presumptive oncogene.

16.4. OLD WORLD SIMIAN RHADINOVIRUSES

The Old World simian rhadinoviruses, including frag-viruses, segregate into two monophyletic lineages named RV-1 and RV-2. This dichotomy is well supported by phylogenetic analysis of genomic sequences, predominantly from the polymerase gene.^{17,18,36,48–50,63,64,96,101} The only human rhadinovirus is HHV-8 which clusters within the RV-1 lineage.

In terms of the ability to efficiently replicate in cell culture, the Old World simian rhadinoviruses can be divided into two groups: cultivable and noncultivable.

Interestingly, none of the simian RV-1 viruses detected so far are cultivable. Thus, none have been isolated as a bona fide virus; all known simian RV-1s are frag-viruses (Table 16.2). Whether this is a reflection of a genuine replication defectiveness of RV-1 viruses or if there is a technical reason for this (e.g., permissive cells exist but have not yet found) is not clear. Some simian species, such as pig-tailed macaques, African green monkeys, mandrills, and common chimpanzees, harbor both RV-1 and RV-2 viruses.

All cultivable simian rhadinoviruses belong to RV-2 lineage. These viruses have all been isolated from macaque species (*M. mulatta*, *M. nemestrina*, and *M. fascicularis*) (Table 16.5). Species-specific lineages of macaque RV-2s are clearly identifiable by phylogenetic analysis of gB gene sequences.⁷

The best studied among RV-2 viruses is rhesus rhadinovirus (RRV). Virtually all that is known about the RV-2 viruses has been derived from the study of two RRV isolates: RRV_{26–95}³⁰ and RRV₁₇₅₇₇.¹⁰⁶ RRV is the only Old World simian rhadinovirus currently recognized by the ICTV as a species (*Macacine herpesvirus 5* or *Cercopithecine herpesvirus 17*).

Thanks to their ability to replicate in vitro, RRV and related macaque viruses are readily amenable to molecular characterization and manipulation (Section 16.4.1). At the same time, knowledge regarding the *in vivo* pathogenesis of RRV infections has lagged behind. Although RRV is undoubtedly very useful as a surrogate model of HHV-8 infection, there is a fundamental difference in the biological properties of these two viruses. In tissue culture the HHV-8 lytic replication program is enacted only in a very small proportion of virus-positive cells; B-cells and fibroblasts are nonpermissive for HHV-8. In contrast, RRV behaves in fibroblast cell cultures as a typical lytic virus.

RFHV/RF association, as a model of HHV-8 pathogenesis, may be a more relevant than the RRV model.^{17,91,92} However, progress in this field is severely hampered by the lack of infectious RFHV (Section 16.4.2).

16.4.1. Rhesus Rhadinovirus

The first RRV (RRV_{26–95}) was isolated in 1997 by co-culturing rhesus monkey peripheral blood mononuclear cells (PBMCs) with a primary culture of rhesus monkey skin fibroblasts.³⁰ The second extensively studied strain of RRV (RRV₁₇₅₇₇) was isolated in 1999 from a simian

Table 16.3. Homology of HVS-C488 ORFs with Their Orthologs in HVS-A11, HVA, RRV, and HHV-8 Genomes*

ORF	% aa Identity HVS-A11	% aa Identity HVA	% aa Identity RRV	% aa Identity HHV-8	Predicted Viral Protein/Function
1	nh 23.2/StpA	32.8/Tio 40.0/Tio	nh 40.0/ORF73	nh nh	Tyrosine-kinase interacting protein, Tip Saimiri transformation-associated protein, Stp
2	80.5	nh	57.2	43.1	vDHFR
3	93.6	70.1	nh; 25.4/ORF75	nh; 26.9/ORF75	vFGARAT
4a	61.4	60.3	34.2	34.1	Complement control protein homolog, membrane form
4b	57.3	57.6	ns	ns	Complement control protein homolog, soluble form
5	51.3	nh	nh	nh	
6	97.5	86.5	54.3	55.0	Major single-stranded DNA-binding protein, mDNA-BP
7	96.6	83.5	47.7	44.8	Processing and transport protein
8	96.5	86.0	55.4	56.0	Glycoprotein B
9	97.8	87.7	62.6	62.3	DNA polymerase
10	98.8	82.6	25.6	25.4	Raji LF1 EBV
11	98.3	76.3	32.4	31.1	Raji LF2 EBV
12	90.5	nh	nh 26.6/K3; 22.6/K5		
13	98.0	nh	nh	nh	vIL-17
14	94.8	49.6	nh	nh	vSag
15	91.4	nh	nh	nh	vCD59
16	92.5	65.6	23.9	22.5	vBcl2
17	96.0	74.0	47.0	44.3	Protease/capsid protein minor capsid scaffold protein
18	97.3	80.5	49.6	48.8	
19	97.8	79.6	47.9	45.4	Virion tegument protein
20	96.0	68.0	39.1	43.8	Fusion protein
21	96.0	75.2	34.8	31.7	Thymidine kinase
22	88.4	83.8	32.9	35.7	Glycoprotein H
23	99.2	82.0	31.7	36.9	
24	98.1	81.5	46.8	46.9	
25	99.3	89.4	67.5	65.9	Major capsid protein
26	100.0	90.1	58.2	57.9	Capsid protein VP23
27	99.3	69.6	30.8	33.0	
28	82.4	73.2	22.4	32.4	
29	97.7	87.0	59.5	59.6	DNA-packaging protein, terminase
30	94.7	82.7	33.3	33.3	
31	99.0	90.2	42.5	44.8	
32	97.1	71.7	34.5	33.2	

Table 16.3. (Continued)

ORF	% aa Identity HVS-A11	% aa Identity HVA	% aa Identity RRV	% aa Identity HHV-8	Predicted Viral Protein/Function
33	98.5	80.3	39.1	37.5	
34	98.1	87.0	43.3	43.5	
35	98.0	77.6	40.4	32.9	
36	98.8	81.6	29.4	32.2	Phosphotransferase, possible tyrosine kinase
37	99.0	87.8	53.0	50.5	Alkaline exonuclease
38	95.5	71.2	33.3	41.4	N-myristoylated in HSV
39	98.4	84.7	57.8	51.8	Glycoprotein M, integral membrane protein
40	95.6	69.2	30.5	28.1	Helicase–primase complex
41	96.3	75.0	33.1	31.1	Helicase–primase complex
40/41	95.9	70.2	—	—	Spliced in EBV (BBLF2 + 3)
42	98.5	80.8	41.2	41.2	
43	99.6	89.2	57.3	60.7	Minor capsid protein, virion protein
44	99.5	89.8	62.8	61.5	Helicase–primase complex, helicase
45	95.0	61.8	35.7	31.9	
46	95.2	85.3	58.7	59.4	Uracil DNA glycosylase
47	69.3	73.2	29.6	29.8	Glycoprotein L (CMV), N-myristylation signal
48	63.8	51.9	31.9	28.8	Glu and Asp-rich, repetitive structure
49	94.7	73.7	25.6	22.3	
50a	70.6	ns	ns	ns	Rta homolog, exons 1+2
50b	70.4	71.2	28.9	27.0	Rta homolog, exon 2
51	39.0	38.2	ph	ph	Putative virus-specific glycoprotein
52	62.3	66.0	33.9	32.2	
53	63.3	60.7	35.6	35.6	Putative glycoprotein N
54	94.1	74.2	39.4	33.9	Deoxyuridine triphosphatase, dUTPase
55	99.5	84.0	48.7	47.5	
56	98.1	80.7	43.9	45.4	Helicase–primase complex, DNA-replication, primase
57	98.6	ns	ns	26.3	IE52, spliced form
57b	98.7	75.1	34.7	29.2	Exon2 of 1E52
58	98.3	83.0	30.6	28.5	
59	95.1	74.9	37.7	36.1	Processivity factor, subunit of DNA-polymerase
60	98.7	91.8	62.5	64.9	Ribonucleotide reductase, small subunit

Table 16.3. (Continued)

ORF	% aa Identity HVS-A11	% aa Identity HVA	% aa Identity RRV	% aa Identity HHV-8	Predicted Viral Protein/Function
61	97.4	89.8	54.0	53.4	Ribonucleotide reductase, large subunit
62	99.1	85.2	42.8	39.8	Probable capsid assembly and DNA maturation protein
63	97.8	75.9	35.4	32.0	Legumain protein
64	94.4	72.0	32.3	31.7	Large tegument protein
65	70.5	61.9	39.9	35.5	Capsid protein
66	97.9	80.8	35.4	37.4	
67	97.4	84.4	51.8	53.8	Tegument protein
67.5	98.8	85.0	50.0	41.6	
68	97.7	78.0	49.3	45.6	Probable major envelope glycoprotein
69	99.2	88.8	51.0	54.2	
70	98.0	85.5	65.6	66.0	Thymidylate synthase
71	94.0	44.8	14.5	20.0	vFL1P, FLICE interacting protein, inhibitor of apoptosis
72	98.4	74.0	31.3	34.8	vCyclin
73	76.1	44.2	21.3	30.7	LANA homolog, repetitive structure
74	96.0	70.1	33.6	35.9	vGPCR, IL-8 receptor
75	95.7	73.9	35.5	36.9	Virion protein, vFGARAT

* Adapted from Ensser *et al.*³⁷ with permission.

nh, no homology; ns, no splicing data available; ph, positional homology.

immunodeficiency virus (SIV)-infected rhesus monkey with a lymphoproliferative disorder.¹⁰⁶

Primary rhesus monkey fibroblast cultures turned out to be fully permissive for RRV.³⁰ Replication of RRV in these cells is accompanied by the development of cytopathic effect (CPE) which becomes visible 8–11 days after inoculation and by day 13–16 the virus completely destroys the cell culture. RRV-induced CPE is focal; that is, at low multiplicity of infection it manifests as discrete areas of disrupted monolayer encircled by the rounded cells and syncytia. This ability allows quantitation of RRV infectivity by a classical plaque assay.^{30,32} RRV can also be readily propagated in engineered permanent rhesus monkey cell lines.^{32,60} These cells are immortalized by genetic transfer of the catalytic subunit of telomerase. RRVs grown in primary fibroblasts and in telomerase-transformed permanent cell lines appear to be indistinguishable. Typically, the yield of infectious

RRV one week after the inoculation of permissive cells reaches 10⁶ pfu/mL.⁸⁴

Latent infection with RRV can be established in vitro by infecting human BJAB or 293-HEK cell lines.³¹ Reactivation of the virus in these cells can be achieved by the treatment with a phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) which is commonly used for reactivation of herpesviruses in latently infected cells.

Persistent RRV infection can be established in rhesus monkey B-lymphoblastoid cell lines transformed with rhesus lymphocryptovirus (RhLCV).¹² After infection with RRV, 5–40% of cells become latently infected with RRV. Spontaneous induction of lytic replication occurs in a very small proportion of cells. Whereas cells latently infected with RRV also harbor latent RhLCV, concomitant reactivation of RRV and RhLCV never takes place in the same cell.

Table 16.4. Characteristics of RRV ORFs

RRV ORFs	HHV-8 Ortholog	% aa Identity	% aa Identity	Predicted Viral Protein	Localization in Virion [†]	Expression Group [‡]
		RRV ₁₇₅₇₇ RRV _{26–95} *	RRV ₁₇₅₇₇ HHV-8*			
R1	K1	98.1	28.0	K ITAM signaling molecule (KIS) ^{22,25}		
2		99.9	45.5	Dihydrofolate reductase (DHFR)		
4	4	64.5	35.7	Complement-binding protein (CP) ⁷⁰	E	I
6	6	100	63.3	ssDNA-binding protein (ssDBP)	T	
7	7	99.6	52.2	Transport protein (TP)	T	
8	8	96.7	66.4	Glycoprotein B (gB) ⁷	E	e
9	9	100	67.4	DNA polymerase (POL)	T	I
10	10	100	35.3	dUTPase-related protein		
11	11	100	33.0	dUTPase-related protein	T	
R2	K2	100	22.6	Viral interleukin-6 (vIL-6) ⁵⁹		
	2	99.9	45.5	Dihydrofolate reductase (DHFR)		
	K3					
70		98.8	65.8	Thymidylate synthase (TS)		
R4	K4	94.8	32.6	Viral macrophage inflammatory protein II (vMIP-II; vCCL-2)		
	K4.1					
	K4.2					
	K5					
	K6					
	K7					
16	16	100	46.0	Viral B-cell-lymphoma 2 (vBcl-2)		
17	17	99.4	45.8	Capsid assembly protein, protease (PRO)/Scaffolding protein (SCAF) [§]	C	e
18	18	100	58.0	Packaging protein; capsid	C	e
19	19	99.3	52.9		T	
20	20	99.4	46.9		T	
21	21	98.9	46.4	Thymidine kinase (TK) ⁴⁷	T	
22	22	74.2	41.2	Glycoprotein H (gH) ¹⁴	E	
24	24	99.6	59.1		T	
25	25	99.6	72.3	Major capsid protein (MCP); capsid	C	I
26	26	98.4	64.3	Triplex-2 minor capsid protein (TRI-2)	C	
27	27	100	27.8		T	
28	28	100	26.5	Glycoprotein 150 (gp150)	E	I
29b	29b	99.4	66.4	DNA-packaging protein/terminase	T	I
30	30	98.7	38.2			
31	31	100	46.1			
32	32	100	42.9	Transport protein	T	I
33	33	100	42.7	Myristylated protein-binding protein (MyrPBP)	T	
29a	29a	100	61.2	DNA-packaging protein/terminase	T	
34	34	99.7	49.4			
35	35	100	37.2		T	e

Table 16.4. (Continued)

RRV ORFs	HHV-8 Ortholog	% aa Identity RRV ₁₇₅₇₇ RRV _{26–95} *	% aa Identity RRV ₁₇₅₇₇ HHV-8*	Predicted Viral Protein	Localization in Virion [†]	Expression Group [‡]
36	36	100	48.7	Virion serine protein kinase (vPK)	T	1
37	37	100	63.9	Alkaline DNA exonuclease shutoff and exonuclease (SOX)	T	
38	38	100	45.0	Myristylated protein (MyrP)	T	1
39	39	100	59.5	Glycoprotein M (gM)	E	
40	40	99.8	33.0	Helicase–primase subunit		
41	41	99.5	28.7	Helicase–primase subunit		
42	42	99.6	48.0		T	
43	43	100	62.0	Portal protein (PORT)	C	
44	44	100	66.0	Helicase–primase subunit		
45	45	99.4	37.0	vIRF-7 binding protein (vIRF-7BP)	T	
46	46	83.5	56.8	Uracil DNA glucosidase (UDG)		
47	47	56.4	31.2	Glycoprotein L (gL) ¹⁴	E	e
48	48	100	29.6		T	
49	49	99.7	53.8		T	
50	50	99.7	44.9	Replication and transcription activator (RTA) ^{24,65,67,100}		e
R8	K8			K-basic-leucin zipper replication-associated protein (KbZIP/RAP) ⁶⁷		e
R8.1	K8.1			Glycoprotein 8.1	E	
52	52	100	47.7		T	
53	53	100	49.0	Glycoprotein N (gN)	E	
54	54	100	43.1	dUTPase	T	
55	55	100	55.2	Palmitoylated protein (PalmP)	T	
56	56	100	52.7	Helicase–primase subunit		
57	57	99.8	47.1	mRNA transcript accumulation protein (MTA)	T	
R9.1	K9	98.3	28.7	Viral interferon regulatory factor 1 (vIRF-1)		
R9.2		99.5	23.2	Viral interferon regulatory factor 2 (vIRF-2)		
R9.3		100	24.3	Viral interferon regulatory factor 3 (vIRF-3)		1
R9.4		99.6	25.5	Viral interferon regulatory factor 4 (vIRF-4)		
R9.5		100	29.5	Viral interferon regulatory factor 5 (vIRF-5)		
R9.6		100	22.9	Viral interferon regulatory factor 6 (vIRF-6)		1
R9.7.		99.4	25.0	Viral interferon regulatory factor 7 (vIRF-7)		

Table 16.4. (Continued)

RRV ORFs	HHV-8 Ortholog	% aa Identity RRV ₁₇₅₇₇ RRV _{26–95} *	% aa Identity RRV ₁₇₅₇₇ HHV-8*	Predicted Viral Protein	Localization in Virion [†]	Expression Group [‡]
R9.8		100	26.8	Viral interferon regulatory factor 8 (vIRF-8)		
58	58	99.7	38.9	Epithelial ligand (EpiL)	E	
59	59	100	51.8	DNA polymerase processivity factor (PF-8)		
60	60	100	70.1	Ribonucleotide reductase small subunit (RNR-S)	T	
61	61	99.0	61.6	Ribonucleotide reductase large subunit (RNR-L)	T	e
62	62	99.4	57.6	Triplex-1 (TRI-1)	C	
63	63	99.8	43.5	Large tegument protein binding protein (LTPBP)	T	
64	64	99.6	41.3	Large tegument protein (LTP)	T	
65	65	100	45.5	Small capsomer interacting protein (SCIP)	C	l
66	66	99.8	47.3	Egress protein	T	e
67	67	87.8	67.1		T	l
67.5	67.5	98.8	62.5			l
68	68	100	49.1		T	e
69	69	100	65.6	Origin of replication lytic <i>oriL</i> located downstream to ORF69 ⁸⁷		l
R13 ⁷¹	K12 K13	99.4	33.1	Kaposin		
				Viral FLICE-inhibitory protein cellular homologous (vFLIP)	e	
72	72	99.6	39.4	Viral cyclin (v-cyc)		
73	73	99.8	18.3	Latency-associated nuclear antigen 1 (LANA-1)		
R14	K14	100	32.8	Viral OX-2 membrane glycoprotein (vOX2)		
74	74	100	42.0	Viral G protein coupled receptor (vGPCR)/vIL-8 receptor ^{38,89}		e
75	75	99.8	44.9	Viral phosphoribosylformylglycineamide amidotransferase (vFGARAT)	T	l
R15	K15			Functional homolog of human CD200 ⁸⁹		

^{*}Data from Alexander *et al.*⁶[†]Data from O'Connor and Kedes⁸⁵; E, envelope; C, capsid; T, tegument.[‡]Data from Dittmer *et al.*³⁴; e, early; l, late.[§]Encoded by ORF17.5 within ORF17.

Table 16.5. Infectious Old World Monkey Rhadinovirus Isolates (RRV Group)

Name	Isolates*	Host Species	Genomic Sequences†	References
Rhesus rhadinovirus (RRV)	RRV _{26–95} RRV _{492–98} RRV _{309–95} RRV ₁₇₅₇₇	<i>Macaca mulatta</i>	AF210726‡ AF083501‡	6, 7, 30 97, 106
Pig-tailed macaque rhadinovirus (PRV)	PRV ₁₉₅₄₅ PRV ₉₈₁₂₆	<i>M. nemestrina</i>	NA§	7, 69
Cynomolgus rhadinovirus (CRV)	CRV _{27–97} CRV _{472–98} CRV _{23–97}	<i>M. fascicularis</i>	NA§	7

*Isolates shown in bold have been used for in vivo inoculations.

†GenBank access numbers.

‡Complete sequence of long unique region.

NA, not available.

The availability of a high yield in vitro propagation system facilitates obtaining highly purified RRV preparations. As a result, the structure of RRV is better characterized than its human counterpart, HHV-8. More than 30 virion-associated proteins have been identified in highly purified RRV preparations⁸⁵ (Table 16.4). The three-dimensional structure of RRV capsid has been reconstructed at 15°A resolution which is significantly higher than the resolution achieved for HHV-8 (24°A). In general, the capsid structure of RRV and HHV-8 are very similar.¹⁰⁷ Three distinct capsid species are produced in cells lytically infected with RRV: empty capsids (A), capsids containing an inner ring-like structure (B), and filled capsids containing viral DNA (C). C capsids predominate in purified RRV preparations, whereas in similar HHV-8 preparations a mixture of A and B capsids predominates.⁸⁷ Presumably, C capsids are transient structural intermediates destined to become infectious virions.

16.4.1.1. GENES AND THEIR EXPRESSION

Complete LUR sequences are available for RRV_{26–95} (GenBank Acc. No. AF210726), RRV₁₇₅₇₇ (GenBank Acc. No. AF083501), as well as for a rhadinovirus isolate from a Japanese macaque (GenBank Acc. No. AY528864). However, data on the Japanese macaque virus, other than the LUR sequence, remain unpublished.

Little is known about characteristics of RRV TRs; only the small fragments of these TRs have been sequenced. It is presumed by analogy with HHV-8 that RRV TRs have a very high G + C content and a variable number of the repeated units that are different for each terminus of the genome. All ORFs encoding viral proteins are located within LUR. Those ORFs which are shared by RRV, HHV-8, and HVS/HVA are collinear except for ORF2. HHV8-ORF2 is located between K2 and K3, whereas RRV-ORF2 is the second ORF at the left end of LUR (after R1). Known characteristics of RRV ORFs and the proteins encoded by them are presented in Table 16.4. The vast majority of the predicted viral proteins are 100–98% homologous in both RRV strains. Notable exceptions are three envelope proteins: glycoprotein L (gL/ORF47), complement-binding protein (CP/ORF4), and glycoprotein H(gH/ORF22). The amino acid sequence identity values for these proteins in RRV_{26–95} and RRV₁₇₅₇₇ are 56.4%, 65.5%, and 74.2%, respectively. A fourth protein, which is significantly divergent in RRV strains (83.5%), is the uracil DNA glucosidase (UDG/ORF46). The degree of similarity between the RRV and HHV-8 proteins is low to moderate (range 18.3–72.3%); however, in most cases it is sufficient to establish homology between the RRV and HHV-8 proteins. The most conserved (72.3% aa identity) is the major capsid protein (MCP/ORF25) whereas the least conserved (18.3% identity) is ORF73 that encodes the latency-associated nuclear antigen 1

(LANA-1). For the majority of RRV and HHV-8 proteins, homology is in the range of 40–60% aa sequence identity.

The temporal pattern of RRV-LUR transcription has been determined using a battery of the RT-PCT assays optimized for quantitative detection of mRNAs encoded by all RRV ORFs.³⁴ Three groups of transcripts (immediate–early, early, and late) have been identified in lytically infected cells (Table 16.4).

The RRV genes adjacent to TRs (R1 and R15) encode membrane proteins that interact with cellular signal transduction pathways. The product of R1, a transmembrane glycoprotein, may initiate events ultimately leading to B-cell activation.^{22,25} In *in vitro* tests, this protein displays oncogenic properties: the expression of R1 in Rat-1 cells leads to morphological transformation of these cells and they become tumorigenic for *nude* mice.²⁵ Interestingly, the T-cell transforming activity of HVS is not affected by substitution of the *syp* oncogene (“native” for HVS) with the R1 gene from RRV.²⁵ The R15 gene encodes a transmembrane protein with the multiple (up to 12) transmembrane domains. The R15 protein has a number of activities characteristic of signaling molecules such as recruitment of Src kinases and tumor necrosis factor receptor-associated factors (TRAFs), activation of NF- κ B, and others.¹⁶ The significance of these *in vitro* characteristics of the R1 and R15 proteins for the *in vivo* properties of RRV is not clear.

The RRV genome, similar to HHV-8, contains multiple genes encoding homologs of cellular genes such as interleukin 6 (vIL-6/R2), macrophage inflammatory protein (vMIP/R4), interferon regulatory proteins (vIRFs/R9.1-R9.8), interleukin 8 receptor (vIL-8R or vGPCR/ORF74), oX-2 glycoprotein (vOX-2/R14), and Fas-associated with death domain-like interleukin 1b converting enzyme (FLICE) inhibitory protein (vFLIP/R13). It is presumed that these cellular genes became fixed in the genome of rhabdoviruses because they were in some way advantageous for viral survival. However, the exact roles of these gene products, particularly *in vivo*, are not known.

Viral microRNAs are expressed in RRV-infected cells. They are encoded at approximately the same genomic locations as HHV-8 microRNAs. However, the sequences of RRV and HHV-8 microRNAs are unrelated.⁹⁵ Although there are many speculations regarding their possible roles, their functions remain unknown.

16.4.1.2. MODE OF TRANSMISSION AND PREVALENCE OF INFECTION

RRV appears to be a contagious virus. Otherwise, it is difficult to explain how in some captive breeding colonies most adult rhesus monkeys become infected. However, how exactly RRV is transmitted is not known. Analogy with HHV-8 suggests that RRV is transmitted by saliva; however, direct proof of this is absent.

Nothing is known about the prevalence of RRV infection in the wild. Data on the prevalence of RRV infection in captive macaques are limited to a few samples. The prevalence of RRV infection in adult rhesus monkeys from the specific pathogen-free colony at the New England National Primate Research Center (NENPRC) was 93% as shown by RRV-specific antibody ELISA.³⁰ Slightly lower, but also high (75%) was the seroprevalence of RRV infection in the Oregon National Primate Research Center (ONPRC).¹⁰⁶ At the same time, the prevalence of RRV infection in the rhesus monkey colony at Tulane National Primate Research Center (TNPRC) as determined by polymerase chain reaction (PCR) was significantly lower (6%).⁹³ Whether this apparent discrepancy is due to differences in diagnostic methodologies or accurately reflects wide variability of RRV prevalence in the captive colonies is not clear.

16.4.1.3. IMMUNE RESPONSES

Infection with RRV, both natural and experimental, is accompanied by an antibody immune response. Anti-RRV antibodies are usually determined by indirect ELISA using lysates of purified virus as an antigen.^{13,30,39,69,106} This test reliably discriminates RRV-seropositive and -seronegative samples; equivocal “gray-zone” results are rare. Seroconversion occurs within 2 weeks after experimental inoculation of macaques with RRV.⁶⁹ Most, but not all, RRV-positive macaques have neutralizing anti-RRV antibodies.¹³ Such antibodies can be detected and titrated using a classical approach based on the abrogation of CPE-inducing activity of RRV as indicator of neutralization. However, more modern versions of RRV neutralization test based on the use of recombinant RRVs with “reporter cassettes” have also been described.¹³ In these tests, the expression of readily measurable markers [secreted-engineered alkaline phosphatase (SEAP) or green fluorescent protein (GFP)] is used for quantitation of viral neutralization. During primary infection the appearance of neutralizing antibodies lags behind the appearance of “binding” antibodies,

that is, antibodies measured by the whole virus lysate ELISA. The titers of neutralizing and antibodies measurable by ELISA do not correlate with one another.¹³ The kinetics of the antibody response against individual RRV proteins is not known.

There are no published data on T-cell and innate immune responses against RRV.

16.4.1.4. DIAGNOSIS OF INFECTION

RRV and its “cousins” (PRV, CRV) can readily be isolated and titrated by cocultivation of PBMCs with primary rhesus fibroblast cultures.^{7,30,106} The titration RRV in such cultures may be used for determining “infectious virus load.”⁶⁹ However, more often the RRV viral load is measured by quantitative real-time PCR for RRV DNA.^{13,18,33,39}

RRV and PRV in PBMCs, B-lymphocytes, and lymph nodes of infected macaques are readily detectable by qualitative PCR.^{8,69,93,106} Apparently, RRV is preferentially harbored by CD20⁺ B-cells.^{8,69} Data on the diagnostic sensitivity and specificity of RRV PCR tests as compared with anti-RRV antibodies tests have not been reported.

16.4.1.5. PATHOGENICITY

Natural infection with RRV in macaques is subclinical.³⁰ Data on experimental infection with RRV in macaques are limited to two studies performed in the late 1990s in the ORPRC and NENPRC.^{69,106} In both studies, the pathogenicity of RRV was tested in immunocompetent and SIV-immunosuppressed macaques.

The ORPRC group inoculated four RRV-seronegative rhesus monkeys with RRV₁₇₅₇₇; two of these monkeys were experimentally infected with SIV 2 months before RRV inoculation.¹⁰⁶

The NENPRC group used two macaque rhadinovirus isolates, rhesus monkey isolate RRV_{26–95} and pig-tailed macaque isolate PRV₁₉₅₄.⁶⁹ Ten SIV-positive rhesus monkeys and 11 SIV-negative macaques (6 rhesus monkeys and 5 pig-tailed macaques) were inoculated with either RRV_{26–95} or PRV₁₉₅₄. SIV-positive monkeys were either preinfected with SIV (superinfection group, $n = 6$) or simultaneously coinfecte

d with RRV and one with PRV. Thus, the design of this experiment was very complex, involving cross-species inoculations. The presence of numerous variables resulted in multiple splitting of the experimental groups.

Inoculation of immunocompetent SIV-negative macaques with RRV or PRV alone had no pathological consequences except the development of transient peripheral lymphadenopathy in 8 out of 11 animals inoculated with RRV_{26–95} or PRV₁₉₅₄.^{69,106} This lymphadenopathy completely resolved by week 12 after inoculation.⁶⁹

Experimental RRV and PRV infection in SIV-immunosuppressed rhesus monkeys was associated with pathology, but the spectra of pathological findings reported by each group differed. The ONPRC group described development of a hyperplastic B-lymphoproliferative disease in both rhesus monkeys experimentally coinfected with RRV₁₇₅₇₇ and SIV. Interestingly, the rhesus monkey from which RRV₁₇₅₇₇ was isolated had similar pathology.¹⁰⁶ The disorder was characterized by persistent angiofollicular lymphadenopathy, hepatomegaly, splenomegaly, and hypergammaglobulinemia. The observed disorder resembled the multicentric plasma cell variant of Castleman’s disease (MCD), a rare B-lymphoproliferative pathology developing almost exclusively in patients coinfecte

d with HIV-1 and HHV-8.

The NENPRC group did not observe an overt disease that could be specifically linked with macaque rhadinovirus infection.⁶⁹ Of note, 8 out of 10 SIV-positive monkeys infected with either RRV- or PRV-developed SAIDS. All 4 monkeys in the coinoculation group died of SAIDS. The survival in this group was significantly shorter than in the superinfection group, 155 days versus 560 days on average. However, clinical and pathological manifestations of SAIDS in the dually infected rhesus monkeys (disseminated mycobacterial infection, *Pneumocystis carinii* pneumonia, *Cryptosporidium* enteritis, wasting) could not be attributed to rhadinovirus infection. A B-lymphoproliferative disorder was not observed in any of the monkeys that died of SAIDS.

There are many possibilities as to why the results obtained by the ONPRC and NENPRC groups are not fully consistent. Indeed, too many important variables were different in these experiments. However, after 1999 no studies addressing RRV pathogenicity have been published. The only relevant publication reports the lack

of association between RRV and B-cell lymphomas in SIV-immunosuppressed rhesus monkeys.⁹³

16.4.1.6. ENGINEERED RHESUS RHADINOVIRUSES

Recombinant infectious RRV has been generated from cloned RRV genomic fragments by homologous recombination^{13,33} and as bacterial artificial chromosomes (BACs).³⁹ Such engineered viruses are amenable to targeted modifications to introduce traits useful for analysis of viral replication, latency, and pathogenesis. For example, recombinant RRV expressing GFP can be readily traced in infected cells and tissues. Biological properties of RRV-GFP are presumed to be the same as the wild-type RRV, although this is based solely on their in vitro one-step growth curves being identical.^{13,33} Another tag successfully used is SEAP.¹³ The RRV-GFP- and RRV-SEAP-based neutralization assays using engineered viruses are more rapid and less subjective than the classical neutralization test. Importantly, desired modifications can be introduced into genome of engineered RRV.

The most promising among the currently available methodologies for generation of infectious recombinant herpesviruses is, apparently, cloning of an entire viral genome as BAC. An infectious RRV-BAC, based on the genome of RRV₁₇₅₇₇, has been successfully produced and shown to be equivalent to the parental virus in in vitro tests.³⁹ Importantly, a significant increase in the number of CD20⁺ cells in the peripheral blood has also been observed in all rhesus macaques ($n = 4$) inoculated sequentially with SIV and the RRV-BAC. However, B-lymphoproliferative disease has not reported in these animals (although the length of the observation period could be too short).³⁹

The RRV-BAC approach has excellent potential for revealing roles of individual RRV proteins and other genomic determinants in vivo. This field is yet to be fully explored.

16.4.2. Retroperitoneal Fibromatosis-Associated Herpesvirus

An unusual pathology, the fibroproliferative lesions localized in the retroperitoneal space, was first reported in pig-tailed macaques (*M. nemestrina*) housed at the Washington National Primate Research Center (WaNPRC).^{46,103,104} The disease was named retroperitoneal fibromatosis (RF). Similar pathology was subsequently described in other macaque species.^{71,72,98}

Intriguingly, “spindleoid” cells present in RF lesions resemble the spindle cells seen in KS lesions, and these cells are believed to be a malignant component of KS.^{68,103,104} At the same time, KS and RF have differences: RF lacks increased vascularity and hemorrhages typical of KS lesions; cutaneous lesions, characteristic to KS, have rarely been observed in RF-affected macaques; and profound fibrosis characteristic of RF lesions is not observed in KS.

Most macaques affected by RF have been shown to be infected with one of the subtypes of simian betaretrovirus, simian D-type retrovirus 2 (SRV-2).^{15,19,71,72,88,98} SRV infection in macaques is profoundly immunosuppressive and the development of RF may be attributed to the immunosuppression caused by this virus. However, RF has been observed in SRV-2-negative macaques.^{9,17} Thus, SRV-2-induced immunosuppression may be a contributing factor but it is unlikely that this virus alone is a causal agent of RF. Also, there is no clue as to why SRV-2 infection may predispose animals to RF more efficiently than other immunosuppressive retroviral infections (SRVs other than SRV-2, SIV).

The paradigm shift regarding possible etiology of RF occurred after discovery HHV-8 as the causal agent of KS. RF tissues were tested for the presence of an HHV-8-related virus and such a virus, more specifically a frag-virus, was found.⁹² The frag-virus, named RF-associated herpesvirus (RFHV), has been detected in RF samples from both pig-tailed and rhesus macaques (RFHV_{Mne} and RFHV_{Mmu}, respectively). All attempts to isolate RFHV as an infectious agent have failed.¹⁷ Phylogenetic analysis clearly places RFHV in the RV-1 lineage.

Available data points at RFHV as the strongest candidate for being the causal agent of RF. First, all RF samples tested contain RFHV-specific DNA and the quantity of RFHV-DNA in the RF-lesions greatly exceeds that in other organs of RF-affected macaques. Second, immunostaining with monoclonal antibodies specific for a common epitope of the latency-associated nuclear antigen of RFHV and HHV-8 (LANA) reveals the presence of this antigen in the vast majority of spindleoid cells in RF lesions. RV-2-specific DNA has also been detected in RF-affected macaques.²⁰ However, the number of RV-2 DNA copies in the RF lesions is about 100-fold lower than that of RFHV and is not significantly different in normal and RF tissues in RF-affected animals.¹⁷

Why RFHV and related simian frag-viruses (RV-1 lineage) elude isolation as bona fide viruses is not clear.

Table 16.6. Lymphomagenicity Host Range of HVS Subgroups A, B, and C

HVS Subgroups*	Host Species	A	B	C
<i>Saimiri sciureus</i>		—	—	—
<i>Saguinus oedipus</i>		+	+	+
<i>Callitrix jacchus</i>		+	—	+
<i>Macaca mulatta</i>		—	—	+

*Representative strains: HVS-A (A11), HVS-B (S295C, SMHI), and HVS-C (C488, 484).

Lack of systems for testing biological properties of RFHV hampers progress in studying the role of RFHV in the development of RF.

16.5. NEW WORLD MONKEY RHADINOVIRUSES

New World monkey rhadinoviruses, as mentioned before, have profound acute T-lymphotropic oncogenicity in vivo and T-cell transforming activity in vitro (Table 16.6).

These viruses have been extensively used for investigating basic mechanisms underlying development of lymphoid malignancies as well as tools for investigating T-cell immunology (reviewed in Fickenscher and Fleckenstein⁴³).

16.5.1. Genomic Organization and Gene Products

The whole genome sequences are known for two HVS strains (A11, C488) and 1 HVA strain (AtHV-3).^{1,5,37} Seventy-seven ORFs have been identified in the LUR (DNA-L) of HVS and HVA. The protein-coding part of the HVA and HVS genomes are almost perfectly collinear. A notable exception is the absence (or inactivation) in HVA of homologs of dihydrofolate reductase (DHFR), viral IL-17, and CD59 genes found in the HVS genome.¹ Average sequence homology of viral proteins is 75% (range 30.4–92.5%). Major characteristics of HVS/HVA ORFs are presented in Table 16.3.

Contrary to LUR sequences, the TR (DNA-H) sequences of HSV and HAV have very little in common. The only shared motifs in the TRs are the cleavage-packaging site defining the junctions between TR and LUR as well as the genome cleavage motifs.¹

16.5.2. Herpesvirus Saimiri

The natural host of HVS is squirrel monkeys (*Saimiri sciureus*).⁷⁹ The virus spreads horizontally, presumably, through saliva. Most squirrel monkeys acquire HVS during the first two years of life. The infection is subclinical and persistent for life. HVS can be readily isolated from nucleated peripheral blood cells by cocultivation with owl monkey kidney cells (OMK) which are highly permissive for the virus.²⁷ HVS induces CPE in OMK cells and ultimately lyses infected cells. Usually, OMK cultures infected with HVS die within a week after the start of cocultivation; however, it may take as long as 2–3 weeks. At the peak of CPE, HVS titers in the culture medium reach 10⁶–10⁷ pfu/mL. Cell-free virus is relatively stable and can be stored at +4°C.⁴³

HVS is acutely oncogenic in nonnatural New World monkeys' hosts such as tamarins (*Saguinus* spp.), common marmosets (*Callithrix jacchus*), and owl monkeys (*Aotus trivirgatus*).^{53–55,77,78,80–82} Peripheral T-cell lymphomas in these species develop after intramuscular or intravenous inoculation of virus. When the regular dose of the virus (approximately 10⁶ pfu) is used, lymphomas develop in all inoculated animals within 2 months. Interestingly, T-cell lymphomas can also be induced by intramuscular inoculation with purified HVS genomic DNA.⁴⁴

HVS strains are classified into three subgroups: A, B, and C.^{29,73,74} Initially, segregation of HVS isolates into subgroups was based on differences in their pathogenicity in various nonnatural hosts (Table 16.6). Subsequently, this classification was reinforced by phylogenetic analysis of genomic sequences. Subgroup C strains generally have the strongest oncogenic potential; they are T-lymphotropic not only to New World monkey species but also to rhesus monkeys. Subgroup B strains are the weakest in terms of oncogenicity.

The leftmost region of the LUR is required for the T-cell transforming and lymphomagenic activity.^{11,28,51,52,57,58,66} HVS subgroup C strains, the most potent in this respect, contain two genes in this region: *stpC* (saimiri transformation-associated protein of the subgroup C) and *tip* (tyrosine kinase interaction protein). Subgroup A and B strains do not have the *tip* gene, and their counterparts of *stpC* are named *stpA* and *stpB*, respectively. The deletion of any of three genes (*stpC*, *tip*, *stpA*) abolishes the transforming activity in vitro. However, none of these transforming genes is required for replication.^{35,43}

The ability of HVS to infect human cells has been known since the mid-1970s.²⁶ Various human cell types (epithelial, mesenchymal, and hematopoietic) have been shown to be infectable by HVS. Infected human cells maintain the HVS genome as an episome.⁹⁹ However, in early studies the presence of the viral genome apparently did not alter growth characteristics of human nonlymphoid cells *in vitro*. It is now clear that the reason for this was the use of the subgroup A and B strains. Human T-cells can be immortalized by HVS-C488, a subgroup C strain.¹⁰ Transforming activity, although weaker than that of HVS-C488, is characteristic of all subgroup C HVS strains. HVS-transformed human T-cells proliferate indefinitely *in vitro* and produce no infectious virus even after treatment with the compounds known to reactivate latent herpesviruses such as TPA.⁴² HVS-transformed human T-cell lines can be established by infecting PBMCs, cord blood cells, thymocytes, and antigen-specific established cell lines. Importantly, human T-cells transformed by HVS have a normal karyotype and retain characteristics of the parental T-cell including antigen specificity, surface phenotype, and other major functional properties.^{62,102} This makes HVS-induced human T-cell transformation an extremely useful tool for studying human T-cells and T-cell-mediated immunity.

16.5.3. Herpesvirus Ateles

The first isolate of HVA (strain 810) was identified in a primary kidney cell culture from a healthy spider monkey (*Ateles geoffroyii*) imported from Guatemala.⁸³ Shortly thereafter, similar but not identical viruses (HVA strains 73, 87, 93, 94) were isolated from Columbian spider monkeys (*Ateles paniscus*).⁴¹ HVA-73 is considered as the prototype HVA, which is currently recognized as the AtHV-3 species.

HVA replicates well in OMK cells. However, in contrast to HVS, high virus titers in culture medium are not reached; the virus remains strongly cell-associated.²⁷ Subclinical infection with HVA is common in various species of spider monkeys.⁴³ HVA causes T-lymphomas in cotton-top tamarin (*Saguinus oedipus*) and owl monkeys (*Aotus trivirgatus*) which are very similar to HVS-induced T-cell lymphomas in these same species.⁵³ HVA also transforms T-lymphocytes of these and some other New World monkey species *in vitro*.^{40,56,61} However, none of the HVA strains is transforming for human T-lymphocytes.⁴³

The transformation-associated gene of HVA is homologous to both the *StpC* and *Tip* genes of HVS, hence its name *Tio* (“two in one”). The *Tio* protein is constitutively expressed in HVA-transformed cells, binds to Src family kinases (Lck and Src), and is phosphorylated by these kinases.²⁻⁴

16.6. SUMMARY

The *Rhadinovirus* genus belongs to the subfamily *Gammaherpesvirinae* within the *Herpesviridae* family. The alternative generic name for rhadinoviruses is $\gamma 2$ -herpesviruses; their closest relatives are $\gamma 1$ -herpesviruses (genus *Lymphocryptovirus*). Four simian rhadinovirus species are currently recognized by the ICTV: *Saimiriine herpesvirus 2* (SaHV-2); *Ateline herpesvirus 2* (AtHV-2); *Ateline herpesvirus 3* (AtHV-3); and *Macacine herpesvirus 5* (McHV-5) [old ICTV *Cercopithecine herpesvirus 17* (CeHV-17)]. Simian rhadinoviruses are better known under their common names: herpesvirus saimiri (HVS) for SaHV-2; herpesvirus atelles (HVA) for AtHV-2 and AtHV-3; and rhesus rhadinovirus (RRV) for McHV-7/CeHV-17. The human counterpart of the simian rhadinoviruses is KS-associated herpesvirus (KSHV), classified by the ICTV as *human herpesvirus 8* (HHV-8) species within the *Rhadinovirus* genus. HHV-8 is the causal agent of KS, a malignancy commonly observed in AIDS patients.

Macrostructure of the primate rhadinovirus genomes is similar: a single LUR containing all protein-coding genes is framed by the TRs. The G + C content in the TR regions is much higher than the LUR. Human and simian viruses share many genes. Old World simian rhadinoviruses are more closely related to HHV-8 than are HVS and HVA. Two lineages of Old World simian rhadinoviruses are known (RV-1 and RV-2). The RV-1 lineage is the closest to the human HHV-8 lineage. All known simian RV-1 viruses are frag-viruses; the most notable among them is RFHV. RFHV is a presumable causal agent of RF, a disease seen in immunosuppressed macaques somewhat resembling human KS. The RV-2 lineage includes RRV and related isolates as well as numerous frag-viruses detected in various Old World monkey and ape species. Some simian species harbor both RV-1 and RV-2 viruses.

RRV is the best-characterized Old World monkey rhadinovirus. In many respects this virus resembles HHV-8, but there are substantial differences between them. In contrast to the predominantly latent HHV-8,

the “default mode” for RRV is lytic infection. RRV has been reported to cause B-lymphoproliferative diseases in SIV-immunosuppressed rhesus monkeys, although this outcome has not been observed in other studies. No KS-like disease has been associated with RRV.

New World monkey rhadinoviruses (HVS and HVA) are apathogenic in their natural hosts (squirrel and spider monkeys, respectively). At the same time, they are strongly T-lymphomagenic for some other New World monkey species.

HVS strains are classified into three subgroups (A, B, and C) based on the difference in pathogenicity and genomic sequences. Both HVS and HVA can immortalize T-lymphocytes of many simian species in vitro. Moreover, subgroup C HVS strains efficiently immortalize human T-cells. HVS-transformed human T-cells retain a normal karyotype and major functional characteristics of the parental T-cell including antigen specificity and surface phenotype. The T-cell oncogenicity/transforming activity of HVS and HVA is not found in human rhadinoviruses, nor are homologues of the HVS and HVA oncogenes (*stp/tip* and *tio*, respectively).

REFERENCES

- Albrecht, J. C. 2000. Primary structure of the Herpesvirus ateles genome. *J. Virol.* 74(2):1033–1037.
- Albrecht, J. C., B. Biesinger, I. Muller-Fleckenstein, D. Lengenfelder, M. Schmidt, B. Fleckenstein, and A. Ensler. 2004. Herpesvirus ateles Tio can replace herpesvirus saimiri StpC and Tip oncoproteins in growth transformation of monkey and human T cells. *J. Virol.* 78(18):9814–9819.
- Albrecht, J. C., U. Friedrich, C. Kardinal, J. Koehn, B. Fleckenstein, S. M. Feller, and B. Biesinger. 1999. Herpesvirus ateles gene product Tio interacts with nonreceptor protein tyrosine kinases. *J. Virol.* 73(6):4631–4639.
- Albrecht, J. C., I. Muller-Fleckenstein, M. Schmidt, B. Fleckenstein, and B. Biesinger. 2005. Tyrosine phosphorylation of the Tio oncoprotein is essential for transformation of primary human T cells. *J. Virol.* 79(16):10507–10513.
- Albrecht, J. C., J. Nicholas, D. Biller, K. R. Cameron, B. Biesinger, C. Newman, S. Wittmann, M. A. Craxton, H. Coleman, B. Fleckenstein, and R. W. Honess. 1992. Primary structure of the herpesvirus saimiri genome. *J. Virol.* 66(8):5047–5058.
- Alexander, L., L. Denekamp, A. Knapp, M. R. Auerbach, B. Damania, and R. C. Desrosiers. 2000. The primary sequence of rhesus monkey rhadinovirus isolate 26-95: sequence similarities to Kaposi’s sarcoma-associated herpesvirus and rhesus monkey rhadinovirus isolate 17577. *J. Virol.* 74(7):3388–3398.
- Auerbach, M. R., S. C. Czajak, W. E. Johnson, R. C. Desrosiers, and L. Alexander. 2000. Species specificity of macaque rhadinovirus glycoprotein B sequences. *J. Virol.* 74(1):584–590.
- Bergquam, E. P., N. Avery, S. M. Shiigi, M. K. Axthelm, and S. W. Wong. 1999. Rhesus rhadinovirus establishes a latent infection in B lymphocytes in vivo. *J. Virol.* 73(9):7874–7876.
- Bielefeldt-Ohmann, H., D. H. Barouch, A. M. Bakke, A. G. Bruce, M. Durning, R. Grant, N. L. Letvin, J. T. Ryan, A. Schmidt, M. E. Thouless, and T. M. Rose. 2005. Intestinal stromal tumors in a simian immunodeficiency virus-infected, simian retrovirus-2 negative rhesus macaque (*Macaca mulatta*). *Vet. Pathol.* 42(3):391–396.
- Biesinger, B., I. Muller-Fleckenstein, B. Simmer, G. Lang, S. Wittmann, E. Platzer, R. C. Desrosiers, and B. Fleckenstein. 1992. Stable growth transformation of human T lymphocytes by herpesvirus saimiri. *Proc. Natl. Acad. Sci. U. S. A.* 89(7):3116–3119.
- Biesinger, B., J. J. Trimble, R. C. Desrosiers, and B. Fleckenstein. 1990. The divergence between two oncogenic herpesvirus saimiri strains in a genomic region related to the transforming phenotype. *Virology* 176(2):505–514.
- Bilello, J. P., S. M. Lang, F. Wang, J. C. Aster, and R. C. Desrosiers. 2006. Infection and persistence of rhesus monkey rhadinovirus in immortalized B-cell lines. *J. Virol.* 80(7):3644–3649.
- Bilello, J. P., J. S. Morgan, B. Damania, S. M. Lang, and R. C. Desrosiers. 2006. A genetic system for rhesus monkey rhadinovirus: use of recombinant virus to quantitate antibody-mediated neutralization. *J. Virol.* 80(3):1549–1562.
- Bilello, J. P., J. S. Morgan, and R. C. Desrosiers. 2008. Extreme dependence of gH and gL expression on ORF57 and association with highly unusual codon usage in rhesus monkey rhadinovirus. *J. Virol.* 82(14):7231–7237.
- Bosch, M. L., E. Harper, A. Schmidt, K. B. Strand, S. Thormahlen, M. E. Thouless, and Y. Wang. 1999. Activation in vivo of retroperitoneal fibromatosis-associated herpesvirus, a simian homologue of human herpesvirus-8. *J. Gen. Virol.* 80(Pt 2):467–475.
- Brinkmann, M. M. and T. F. Schulz. 2006. Regulation of intracellular signalling by the terminal membrane proteins of members of the Gammaherpesvirinae. *J. Gen. Virol.* 87(Pt 5):1047–1074.
- Bruce, A. G., A. M. Bakke, H. Bielefeldt-Ohmann, J. T. Ryan, M. E. Thouless, C. C. Tsai, and T.

- M. Rose. 2006. High levels of retroperitoneal fibromatosis (RF)-associated herpesvirus in RF lesions in macaques are associated with ORF73 LANA expression in spindleoid tumour cells. *J. Gen. Virol.* 87(Pt 12):3529–3538.
18. Bruce, A. G., A. M. Bakke, M. E. Thouless, and T. M. Rose. 2005. Development of a real-time QPCR assay for the detection of RV2 lineage-specific rhadinoviruses in macaques and baboons. *J. Virol.* 79(2):2.
19. Bryant, M. L., P. A. Marx, S. M. Shiigi, B. J. Wilson, W. P. McNulty, and M. B. Gardner. 1986. Distribution of type D retrovirus sequences in tissues of macaques with simian acquired immune deficiency and retroperitoneal fibromatosis. *Virology* 150(1):149–160.
20. Burnside, K. L., J. T. Ryan, H. Bielefeldt-Ohmann, A. G. Bruce, M. E. Thouless, C. C. Tsai, and T. M. Rose. 2006. RFHVMn ORF73 is structurally related to the KSHV ORF73 latency-associated nuclear antigen (LANA) and is expressed in retroperitoneal fibromatosis (RF) tumor cells. *Virology* 354(1):103–115.
21. Chang, Y., E. Cesarman, M. S. Pessin, F. Lee, J. Culpepper, D. M. Knowles, and P. S. Moore. 1994. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 266(5192):1865–1869.
22. Damania, B., M. DeMaria, J. U. Jung, and R. C. Desrosiers. 2000. Activation of lymphocyte signaling by the R1 protein of rhesus monkey rhadinovirus. *J. Virol.* 74(6):2721–2730.
23. Damania, B. and R. C. Desrosiers. 2001. Simian homologues of human herpesvirus 8. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 356(1408):535–543.
24. Damania, B., J. H. Jeong, B. S. Bowser, S. M. DeWire, M. R. Staudt, and D. P. Dittmer. 2004. Comparison of the Rta/Orf50 transactivator proteins of gamma-2-herpesviruses. *J. Virol.* 78(10):5491–5499.
25. Damania, B., M. Li, J. K. Choi, L. Alexander, J. U. Jung, and R. C. Desrosiers. 1999. Identification of the R1 oncogene and its protein product from the rhadinovirus of rhesus monkeys. *J. Virol.* 73(6):5123–5131.
26. Daniel, M. D., D. Silva, D. Jackman, P. Sehgal, R. B. Baggs, R. D. Hunt, N. W. King, and L. V. Mellendez. 1976. Reactivation of squirrel monkey heart isolate (herpesvirus saimiri strain) from latently infected human cell cultures and induction of malignant lymphoma in marmoset monkeys. *Bibl. Haematol.* 43:392–395.
27. Daniel, M. D., D. Silva, and N. Ma. 1976. Establishment of owl monkey kidney 210 cell line for virological studies. *In Vitro* 12:290.
28. Desrosiers, R. C., A. Bakker, J. Kamine, L. A. Falk, R. D. Hunt, and N. W. King. 1985. A region of the herpesvirus saimiri genome required for oncogenicity. *Science* 228(4696):184–187.
29. Desrosiers, R. C. and L. A. Falk. 1982. Herpesvirus saimiri strain variability. *J. Virol.* 43(1):352–356.
30. Desrosiers, R. C., V. G. Saserville, S. C. Czajak, X. Zhang, K. G. Mansfield, A. Kaur, R. P. Johnson, A. A. Lackner, and J. U. Jung. 1997. A herpesvirus of rhesus monkeys related to the human Kaposi's sarcoma-associated herpesvirus. *J. Virol.* 71(12):9764–9769.
31. DeWire, S. M. and B. Damania. 2005. The latency-associated nuclear antigen of rhesus monkey rhadinovirus inhibits viral replication through repression of Orf50/Rta transcriptional activation. *J. Virol.* 79(5):3127–3138.
32. DeWire, S. M., M. A. McVoy, and B. Damania. 2002. Kinetics of expression of rhesus monkey rhadinovirus (RRV) and identification and characterization of a polycistronic transcript encoding the RRV Orf50/Rta, RRV R8, and R8.1 genes. *J. Virol.* 76(19):9819–9831.
33. DeWire, S. M., E. S. Money, S. P. Krall, and B. Damania. 2003. Rhesus monkey rhadinovirus (RRV): construction of a RRV-GFP recombinant virus and development of assays to assess viral replication. *Virology* 312(1):122–134.
34. Dittmer, D. P., C. M. Gonzalez, W. Vahrson, S. M. DeWire, R. Hines-Boykin, and B. Damania. 2005. Whole-genome transcription profiling of rhesus monkey rhadinovirus. *J. Virol.* 79(13):8637–8650.
35. Duboise, S. M., J. Guo, S. Czajak, R. C. Desrosiers, and J. U. Jung. 1998. STP and Tip are essential for herpesvirus saimiri oncogenicity. *J. Virol.* 72(2):1308–1313.
36. Duprez, R., E. Boulanger, Y. Roman, and A. Gesain. 2004. Novel gamma-2-herpesvirus of the Rhadinovirus 2 lineage in gibbons. *Emerg. Infect. Dis.* 10(5):899–902.
37. Ensser, A., M. Thurau, S. Wittmann, and H. Fickenscher. 2003. The genome of herpesvirus saimiri C488 which is capable of transforming human T cells. *Virology* 314(2):471–487.
38. Estep, R. D., M. K. Axthelm, and S. W. Wong. 2003. A G protein-coupled receptor encoded by rhesus rhadinovirus is similar to ORF74 of Kaposi's sarcoma-associated herpesvirus. *J. Virol.* 77(3):1738–1746.
39. Estep, R. D., M. F. Powers, B. K. Yen, H. Li, and S. W. Wong. 2007. Construction of an infectious rhesus rhadinovirus bacterial artificial chromosome for the analysis of Kaposi's sarcoma-associated herpesvirus-related disease development. *J. Virol.* 81(6):2957–2969.

40. Falk, L., D. Johnson, and F. Deinhardt. 1978. Transformation of marmoset lymphocytes in vitro with Herpesvirus atelis. *Int. J. Cancer* 21(5):652–657.
41. Falk, L. A., S. M. Nigida, F. Deinhardt, L. G. Wolfe, R. W. Cooper, and J. I. Hernandez-Camacho. 1974. Herpesvirus atelis: properties of an oncogenic herpesvirus isolated from circulating lymphocytes of spider monkeys (*Ateles* sp.). *Int. J. Cancer* 14(4):473–482.
42. Fickenscher, H., B. Biesinger, A. Knappe, S. Wittmann, and B. Fleckenstein. 1996. Regulation of the herpesvirus saimiri oncogene stpC, similar to that of T-cell activation genes, in growth-transformed human T lymphocytes. *J. Virol.* 70(9):6012–6019.
43. Fickenscher, H. and B. Fleckenstein. 2001. Herpesvirus saimiri. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 356(1408):545–567.
44. Fleckenstein, B., M. D. Daniel, R. D. Hunt, J. Werner, L. A. Falk, and C. Mulder. 1978. Tumour induction with DNA of oncogenic primate herpesviruses. *Nature* 274(5666):57–59.
45. Ganem, D. 2007. Kaposi's sarcoma-associated herpesvirus. In: Knipe, D. M. and P. M. Howley (eds), *Fields Virology*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, Wolters Kluwer Business, pp. 2847–2888.
46. Giddens, W. E., Jr., C. C. Tsai, W. R. Morton, H. D. Ochs, G. H. Knitter, and G. A. Blakley. 1985. Retroperitoneal fibromatosis and acquired immunodeficiency syndrome in macaques. Pathologic observations and transmission studies. *Am. J. Pathol.* 119(2):253–263.
47. Gill, M. B., J. E. Murphy, and J. D. Fingeroth. 2005. Functional divergence of Kaposi's sarcoma-associated herpesvirus and related gamma-2 herpesvirus thymidine kinases: novel cytoplasmic phosphoproteins that alter cellular morphology and disrupt adhesion. *J. Virol.* 79(23):14647–14659.
48. Greensill, J. and T. F. Schulz. 2000. Rhadinoviruses (gamma2-herpesviruses) of Old World primates: models for KSHV/HHV8-associated disease? *AIDS* 14(Suppl 3):S11–S19.
49. Greensill, J., J. A. Sheldon, K. K. Murthy, J. S. Bessonette, B. E. Beer, and T. F. Schulz. 2000. A chimpanzee rhadinovirus sequence related to Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8: increased detection after HIV-1 infection in the absence of disease. *AIDS* 14(17):F129–F135.
50. Greensill, J., J. A. Sheldon, N. M. Renwick, B. E. Beer, S. Norley, J. Goudsmit, and T. F. Schulz. 2000. Two distinct gamma-2 herpesviruses in African green monkeys: a second gamma-2 herpesvirus lineage among old world primates? *J. Virol.* 74(3):1572–1577.
51. Heck, E., U. Friedrich, M. U. Gack, D. Lengenfelder, M. Schmidt, I. Muller-Fleckenstein, B. Fleckenstein, A. Ensser, and B. Biesinger. 2006. Growth transformation of human T cells by herpesvirus saimiri requires multiple Tip-Lck interaction motifs. *J. Virol.* 80(20):9934–9942.
52. Heck, E., D. Lengenfelder, M. Schmidt, I. Muller-Fleckenstein, B. Fleckenstein, B. Biesinger, and A. Ensser. 2005. T-cell growth transformation by herpesvirus saimiri is independent of STAT3 activation. *J. Virol.* 79(9):5713–5720.
53. Hunt, R. D., L. V. Melendez, F. G. Garcia, and B. F. Trum. 1972. Pathologic features of Herpesvirus atelis lymphoma in cotton-topped marmosets (*Saguinus oedipus*). *J. Natl. Cancer Inst.* 49(6):1631–1639.
54. Hunt, R. D., L. V. Melendez, N. W. King, and F. G. Garcia. 1972. Herpesvirus saimiri malignant lymphoma in spider monkeys. A new susceptible host. *J. Med. Primatol.* 1(2):114–128.
55. Hunt, R. D., L. V. Melendez, N. W. King, C. E. Gilmore, M. D. Daniel, M. E. Williamson, and T. C. Jones. 1970. Morphology of a disease with features of malignant lymphoma in marmosets and owl monkeys inoculated with Herpesvirus saimiri. *J. Natl. Cancer Inst.* 44(2):447–465.
56. Johnson, D. R. and M. Jondal. 1981. Herpesvirus atelis and herpesvirus saimiri transform marmoset T cells into continuously proliferating cell lines that can mediate natural killer cell-like cytotoxicity. *Proc. Natl. Acad. Sci. U. S. A.* 78(10):6391–6395.
57. Jung, J. U. and R. C. Desrosiers. 1992. Herpesvirus saimiri oncogene STP-C488 encodes a phosphoprotein. *J. Virol.* 66(3):1777–1780.
58. Jung, J. U., J. J. Trimble, N. W. King, B. Biesinger, B. W. Fleckenstein, and R. C. Desrosiers. 1991. Identification of transforming genes of subgroup A and C strains of Herpesvirus saimiri. *Proc. Natl. Acad. Sci. U. S. A.* 88(16):7051–7055.
59. Kaleeba, J. A., E. P. Bergquam, and S. W. Wong. 1999. A rhesus macaque rhadinovirus related to Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 encodes a functional homologue of interleukin-6. *J. Virol.* 73(7):6177–6181.
60. Kirchoff, V., S. Wong, J. S. St, and G. S. Pari. 2002. Generation of a life-expanded rhesus monkey fibroblast cell line for the growth of rhesus rhadinovirus (RRV). *Arch. Virol.* 147(2):321–333.
61. Kiyotaki, M., K. R. Solomon, and N. L. Letvin. 1988. Herpesvirus atelis immortalizes in vitro a CD3⁺CD4⁺CD8⁺ marmoset lymphocyte with NK function. *J. Immunol.* 140(3):730–736.

62. Kumar, A., H. L. Stipp, D. Sheffer, and O. Narayan. 1999. Use of herpesvirus saimiri-immortalized macaque CD4(+) T cell clones as stimulators and targets for assessment of CTL responses in macaque/AIDS models. *J. Immunol. Methods* 230(1–2):47–58.
63. Lacoste, V., P. Mauclere, G. Dubreuil, J. Lewis, M. C. Georges-Courbot, and A. Gessain. 2001. A novel gamma 2-herpesvirus of the Rhadinovirus 2 lineage in chimpanzees. *Genome Res.* 11(9):1511–1519.
64. Lacoste, V., P. Mauclere, G. Dubreuil, J. Lewis, M. C. Georges-Courbot, J. Rigoulet, T. Petit, and A. Gessain. 2000. Simian homologues of human gamma-2 and betaherpesviruses in mandrill and drill monkeys. *J. Virol.* 74(24):11993–11999.
65. Langlais, C. L., J. M. Jones, R. D. Estep, and S. W. Wong. 2006. Rhesus rhadinovirus R15 encodes a functional homologue of human CD200. *J. Virol.* 80(6):3098–3103.
66. Lee, H., J. J. Trimble, D. W. Yoon, D. Regier, R. C. Desrosiers, and J. U. Jung. 1997. Genetic variation of herpesvirus saimiri subgroup A transforming protein and its association with cellular src. *J. Virol.* 71(5):3817–3825.
67. Lin, S. F., D. R. Robinson, J. Oh, J. U. Jung, P. A. Luciw, and H. J. Kung. 2002. Identification of the bZIP and Rta homologues in the genome of rhesus monkey rhadinovirus. *Virology* 298(2):181–188.
68. London, W. T., J. L. Sever, D. L. Madden, R. V. Henrickson, M. Gravell, D. H. Maul, M. C. Dalakas, K. G. Osborn, S. A. Houff, and M. B. Gardner. 1983. Experimental transmission of simian acquired immunodeficiency syndrome (SAIDS) and Kaposi-like skin lesions. *Lancet* 2(8355):869–873.
69. Mansfield, K. G., S. V. Westmoreland, C. D. DeBakker, S. Czajak, A. A. Lackner, and R. C. Desrosiers. 1999. Experimental infection of rhesus and pig-tailed macaques with macaque rhadinoviruses. *J. Virol.* 73(12):10320–10328.
70. Mark, L., O. B. Spiller, M. Okroj, S. Chanas, J. A. Aitken, S. W. Wong, B. Damania, A. M. Blom, and D. J. Blackbourn. 2007. Molecular characterisation of the Rhesus Rhadinovirus (RRV) ORF4 gene and the RRV complement control protein (RCP) it encodes. *J. Virol.* 81(8): 4166–4176.
71. Marx, P. A., M. L. Bryant, K. G. Osborn, D. H. Maul, N. W. Lerche, L. J. Lowenstein, J. D. Kluge, C. P. Zaiss, R. V. Henrickson, S. M. Shiigi, B. J. Wilson, A. Malley, L. C. Olson, W. P. McNulty, L. O. Arthur, R. V. Gilden, C. S. Barker, E. Hunter, R. J. Munn, G. Heidecker, and M. B. Gardner. 1985. Isolation of a new serotype of simian acquired immune deficiency syndrome type D retrovirus from Celebes black macaques (*Macaca nigra*) with immune deficiency and retroperitoneal fibromatosis. *J. Virol.* 56(2):571–578.
72. Marx, P. A. and L. J. Lowenstein. 1987. Mesenchymal neoplasms associated with type D retroviruses in macaques. *Cancer Surv.* 6(1):101–115.
73. Medveczky, P., E. Szomolanyi, R. C. Desrosiers, and C. Mulder. 1984. Classification of herpesvirus saimiri into three groups based on extreme variation in a DNA region required for oncogenicity. *J. Virol.* 52(3):938–944.
74. Medveczky, M. M., E. Szomolanyi, R. Hesselton, D. DeGrand, P. Geck, and P. G. Medveczky. 1989. Herpesvirus saimiri strains from three DNA subgroups have different oncogenic potentials in New Zealand white rabbits. *J. Virol.* 63(9):3601–3611.
75. Melendez, L. V., H. Castellanos, H. H. Barahona, M. D. Daniel, R. D. Hunt, C. E. Fraser, F. G. Garcia, and N. W. King. 1972. Two new herpesviruses from spider monkeys (*Ateles geoffroyi*). *J. Natl. Cancer Inst.* 49(1):233–238.
76. Melendez, L. V., M. D. Daniel, H. H. Barahona, C. E. Fraser, R. D. Hunt, and F. G. Garcia. 1971. New herpesviruses from South American monkeys. Preliminary report. *Lab. Anim. Sci.* 21(6):1050–1054.
77. Melendez, L. V., M. D. Daniel, and R. D. Hunt. 1970. Herpesvirus saimiri induced malignant lymphoma: recovery of the viral agent from the fatally affected animals. *Bibl. Haematol.* 36:751–753.
78. Melendez, L. V., M. D. Daniel, R. D. Hunt, C. E. Fraser, F. G. Garcia, N. W. King, and M. E. Williamson. 1970. Herpesvirus saimiri. V. Further evidence to consider this virus as the etiological agent of a lethal disease in primates which resembles a malignant lymphoma. *J. Natl. Cancer Inst.* 44(5):1175–1181.
79. Melendez, L. V., M. D. Daniel, R. D. Hunt, and F. G. Garcia. 1968. An apparently new herpesvirus from primary kidney cultures of the squirrel monkey (*Saimiri sciureus*). *Lab. Anim. Care* 18(3):374–381.
80. Melendez, L. V., R. D. Hunt, M. D. Daniel, B. J. Blake, and F. G. Garcia. 1971. Acute lymphocytic leukemia in owl monkeys inoculated with herpesvirus saimiri. *Science* 171(976):1161–1163.
81. Melendez, L. V., R. D. Hunt, M. D. Daniel, C. E. Fraser, F. G. Garcia, and M. D. Williamson. 1970. Lethal reticuloproliferative disease induced in Cebus albifrons monkeys by herpesvirus saimiri. *Int. J. Cancer* 6(3):431–435.
82. Melendez, L. V., R. D. Hunt, M. D. Daniel, F. G. Garcia, and C. E. Fraser. 1969. Herpesvirus saimiri. II. Experimentally induced malignant lymphoma in primates. *Lab. Anim. Care* 19(3):378–386.

83. Melendez, L. V., R. D. Hunt, N. W. King, H. H. Barahona, M. D. Daniel, C. E. Fraser, and F. G. Garcia. 1972. Herpesvirus atèles, a new lymphoma virus of monkeys. *Nat. New Biol.* 235(58):182–184.
84. O'Connor, C. M., B. Damania, and D. H. Kedes. 2003. De novo infection with rhesus monkey rhadinovirus leads to the accumulation of multiple intranuclear capsid species during lytic replication but favors the release of genome-containing virions. *J. Virol.* 77(24):13439–13447.
85. O'Connor, C. M. and D. H. Kedes. 2006. Mass spectrometric analyses of purified rhesus monkey rhadinovirus reveal 33 virion-associated proteins. *J. Virol.* 80(3):1574–1583.
86. O'Connor, C. M. and D. H. Kedes. 2007. Rhesus monkey rhadinovirus: a model for the study of KSHV. *Curr. Top. Microbiol. Immunol.* 312:43–69.
87. Pari, G. S., D. AuCoin, K. Colletti, S. A. Cei, V. Kirchoff, and S. W. Wong. 2001. Identification of the rhesus macaque rhadinovirus lytic origin of DNA replication. *J. Virol.* 75(23):11401–11407.
88. Philipp-Staheli, J., T. Marquardt, M. E. Thouless, A. G. Bruce, R. F. Grant, C. C. Tsai, and T. M. Rose. 2006. Genetic variability of the envelope gene of Type D simian retrovirus-2 (SRV-2) subtypes associated with SAIDS-related retroperitoneal fibromatosis in different macaque species. *J. Virol.* 3:11.
89. Pratt, C. L., R. D. Estep, and S. W. Wong. 2005. Splicing of rhesus rhadinovirus R15 and ORF74 bicistronic transcripts during lytic infection and analysis of effects on production of vCD200 and vGPCR. *J. Virol.* 79(6):3878–3882.
90. Roizman, B., R. C. Desrosiers, B. Fleckenstein, C. Lopez, A. C. Minson, and M. J. Studdert, for The Herpesvirus Study Group of the International Committee on Taxonomy of Viruses. 1992. The family Herpesviridae: an update. *Arch. Virol.* 123(3–4):425–449.
91. Rose, T. M., J. T. Ryan, E. R. Schultz, B. W. Raden, and C. C. Tsai. 2003. Analysis of 4.3 kilobases of divergent locus B of macaque retroperitoneal fibromatosis-associated herpesvirus reveals a close similarity in gene sequence and genome organization to Kaposi's sarcoma-associated herpesvirus. *J. Virol.* 77(9):5084–5097.
92. Rose, T. M., K. B. Strand, E. R. Schultz, G. Schaefer, G. W. Rankin Jr., M. E. Thouless, C. C. Tsai, and M. L. Bosch. 1997. Identification of two homologs of the Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in retroperitoneal fibromatosis of different macaque species. *J. Virol.* 71(5):4138–4144.
93. Ruff, K., G. B. Baskin, L. Simpson, M. Murphy-Corb, and L. S. Levy. 2003. Rhesus rhadinovirus infection in healthy and SIV-infected macaques at Tulane National Primate Research Center. *J. Med. Primatol.* 32(1):1–6.
94. Russo, J. J., R. A. Bohenzky, M. C. Chien, J. Chen, M. Yan, D. Maddalena, J. P. Parry, D. Peruzzi, I. S. Edelman, Y. Chang, and P. S. Moore. 1996. Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proc. Natl. Acad. Sci. U. S. A.* 93(25):14862–14867.
95. Schafer, A., X. Cai, J. P. Bilello, R. C. Desrosiers, and B. R. Cullen. 2007. Cloning and analysis of microRNAs encoded by the primate gamma-herpesvirus rhesus monkey rhadinovirus. *Virology* 364(1):21–27.
96. Schultz, E. R., G. W. Rankin Jr., M. P. Blanc, B. W. Raden, C. C. Tsai, and T. M. Rose. 2000. Characterization of two divergent lineages of macaque rhadinoviruses related to Kaposi's sarcoma-associated herpesvirus. *J. Virol.* 74(10):4919–4928.
97. Searles, R. P., E. P. Bergquam, M. K. Axthelm, and S. W. Wong. 1999. Sequence and genomic analysis of a Rhesus macaque rhadinovirus with similarity to Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8. *J. Virol.* 73(4):3040–3053.
98. Shiigi, S. M., B. J. Wilson, A. Malley, R. A. Chandler, C. F. Howard Jr., L. C. Olson, J. L. Palotay, W. P. McNulty, and P. A. Marx. 1986. Association of SAIDS/RF-related signs with current or past SAIDS type 2 retrovirus infection in a colony of Celebes black macaques. *Lab. Anim. Sci.* 36(1):20–23.
99. Simmer, B., M. Alt, I. Buckreus, S. Berthold, B. Fleckenstein, E. Platzer, and R. Grassmann. 1991. Persistence of selectable herpesvirus saimiri in various human haematopoietic and epithelial cell lines. *J. Gen. Virol.* 72(Pt 8):1953–1958.
100. Staudt, M. R. and D. P. Dittmer. 2007. The Rta/Orf50 transactivator proteins of the gamma-herpesviridae. *Curr. Top. Microbiol. Immunol.* 312:71–100.
101. Strand, K., E. Harper, S. Thormahlen, M. E. Thouless, C. Tsai, T. Rose, and M. L. Bosch. 2000. Two distinct lineages of macaque gamma herpesviruses related to the Kaposi's sarcoma associated herpesvirus. *J. Clin. Virol.* 16(3):253–269.
102. Troidl, B., B. Simmer, H. Fickenscher, I. Muller-Fleckenstein, F. Emmrich, B. Fleckenstein, and E. Gebhart. 1994. Karyotypic characterization of human T-cell lines immortalized by Herpesvirus saimiri. *Int. J. Cancer* 56(3):433–438.
103. Tsai, C. C., W. E. Giddens Jr., W. R. Morton, S. L. Rosenkranz, H. D. Ochs, and R. E. Benveniste. 1985. Retroperitoneal fibromatosis and acquired immunodeficiency syndrome in macaques: epidemiologic studies. *Lab. Anim. Sci.* 35(5):460–464.

104. Tsai, C. C., T. F. Warner, H. Uno, W. E. Giddens Jr., and H. D. Ochs. 1985. Subcutaneous fibromatosis associated with an acquired immune deficiency syndrome in pig-tailed macaques. *Am. J. Pathol.* 120(1):30–37.
105. Whitby, D., A. Stossel, C. Gamache, J. Papin, M. Bosch, A. Smith, D. H. Kedes, G. White, R. Kennedy, and D. P. Dittmer. 2003. Novel Kaposi's sarcoma-associated herpesvirus homolog in baboons. *J. Virol.* 77(14):8159–8165.
106. Wong, S. W., E. P. Bergquam, R. M. Swanson, F. W. Lee, S. M. Shiigi, N. A. Avery, J. W. Fanton, and M. K. Axthelm. 1999. Induction of B cell hyperplasia in simian immunodeficiency virus-infected rhesus macaques with the simian homologue of Kaposi's sarcoma-associated herpesvirus. *J. Exp. Med.* 190(6):827–840.
107. Yu, X. K., C. M. O'Connor, I. Atanasov, B. Damania, D. H. Kedes, and Z. H. Zhou. 2003. Three-dimensional structures of the A, B, and C capsids of rhesus monkey rhadinovirus: insights into gammaherpesvirus capsid assembly, maturation, and DNA packaging. *J. Virol.* 77(24):13182–13193.

Section 2.2:

Other Simian DNA Viruses

17

Parvoviruses

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- 17.2 Classification and nomenclature
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17.1. INTRODUCTION

Parvoviruses are nonenveloped DNA viruses that, as their name implies (*parvus* = small in Latin), are small both in size (18–26 nm) and in the coding capacity of their genome (reviewed in reference 1). The hallmark of parvoviruses is an unusual single-stranded DNA genome. Parvoviruses can be divided into two groups: autonomous and nonautonomous. The best known autonomous parvovirus is human B19. Primary infection with this virus is manifested as the childhood disease *erythema infectiosum*, also known as the 5th disease. A characteristic sign of this mild and self-limiting illness is facial erythema—hence its colloquial name “slapped-cheek” disease. B19 is tropic to the erythroid precursor and because of this, it is most dangerous to patients with compromised erythropoiesis. In patients with sickle cell anemia, thalassemia, and similar conditions, parvovirus B19 causes the transient arrest of erythropoiesis, the aplastic crisis. Congenital infection with B19 is suspected to be the cause of nonimmune *hydrops fetalis*. Claims have been made that B19 infection is associated

with chronic joint diseases; however, the supporting evidence is not convincing.

Simian counterparts of parvovirus B19 have been isolated from macaques.^{7,11} Similarly to their human relative, they are erythrotropic and may cause anemia, particularly in immunosuppressed monkeys.^{4,12} Several outbreaks of parvoviral anemia have been reported in captive macaque colonies.

Nonautonomous parvoviruses can replicate only with the help of adenoviruses or, less frequently, herpes viruses—hence their name: adeno-associated viruses (AAVs). Most of the interest in AAVs is due to their potential as gene therapy vectors.⁵ AAVs have been isolated from simian cells; however, whether or not these viruses can be considered simian is not clear.

17.2. CLASSIFICATION AND NOMENCLATURE

Parvoviruses comprise the family *Parvoviridae*. All human and simian parvoviruses belong to the subfamily *Parvovirinae*. B19 virus and its simian counterparts belong to the *Erythrovirus* genus. AAVs belong to the *Dependovirus* genus. Phylogenetic analysis clearly places AAVs isolated from human and simian cells in the same group with the avian parvoviruses.⁹ Interestingly, these AAV-related avian parvoviruses are replication competent.

Three closely related species of the simian parvoviruses are currently recognized by the International Committee on Taxonomy of Viruses (ICTV) within the *Erythrovirus* genus: (1) simian parvovirus (SPV) or cynomolgus macaque parvovirus; (2) rhesus macaque parvovirus (RmPV); and (3) pig-tailed macaque parvovirus (PMPV). Information on the origin and the

Table 17.1. Simian Parvoviruses (Erythroviruses)

ICTV-Recognized Species	Synonymous Abbreviated Names	Host Species	Original Reference	Genomic DNA Sequence*
Simian parvovirus (SPV) [†]	SPV _{Mfa}	<i>Macaca fascicularis</i>	11	U26342
Rhesus macaque parvovirus (RmPV)	RhPV, SPV _{Mmu}	<i>M. mulatta</i>	7	AF221122
Pig-tailed macaque parvovirus (PMPV)	PtPV, SPV _{Mne}	<i>M. nemestrina</i>	7	AF221123

* GenBank Access numbers.

† Prototype simian parvovirus (erythrovirus).

synonymous names of these viruses are presented in Table 17.1.

The abbreviation SPV is used as the designation for cynomolgus macaque parvovirus and as a generic name for all known simian erythroviruses (usually named parvoviruses). To avoid ambiguity in this chapter, the abbreviation SPV is used only as the generic designation; the abbreviated names of individual simian parvoviruses include a subscript indicating the species from which they originate. For example, the prototype simian erythrovirus from cynomolgus macaques (*M. fascicularis*) is designated as SPV_{Mfa}.

The virions of simian erythroviruses are icosahedral and 24 nm in diameter. The capsid consists of 60 capsomers. Morphologically all parvoviruses are indistinguishable, although some fine differences in the surface topology can be revealed by crystallographic analysis. However, no crystallographic data are available for the simian erythroviruses.

Phylogenetic analysis of erythroviral genomes reliably clusters the simian and human viruses. Within this primate erythrovirus clade, the human and simian lineages are clearly separated.⁹ Interestingly, the closest “relative” of the primate erythroviruses is chipmunk parvoviruses¹⁷ (Figure 17.1).

17.3. ERYTHROVIRUSES

17.3.1. Genomic Organization and Gene Products

Simian erythroviruses have a single-stranded DNA genome approximately 5,000 nt in length. The almost complete genome sequences are known for all SPV species.^{2,7} The exact terminal sequences in these genomes are yet to be determined. The erythroviral genome contains only three protein-encoding genes that are flanked by noncoding palindromic sequences (Figure 17.2). These terminal sequences form complex secondary structures serving as a primer for the

synthesis of the complementary strand. Each virion contains one genomic DNA molecule of either positive-sense or negative-sense polarity. Only the positive-sense molecules encode the proteins.

5'-non-coding region (about 300 nt in length) contains a single promoter sequence TATAAA. This regulatory region is followed by the NS-1 gene, which spans about 2,350 nt. This gene encodes a nonstructural protein. The region between the NS-1 gene and the 3'-non-coding region is occupied by two overlapping structural genes, VP1 and VP2. There is a short overlap (8 nt in SPV_{Mfa} genome) between the start of the VP1 gene and the end of the NS-1 genes. The VP1 gene spans about 2,450 nt. The product of this gene, VP1 protein, has an enzymatic domain, a calcium-dependent phospholipase A₂ activity (PLA₂). At the N-terminus of VP1 there are sequence

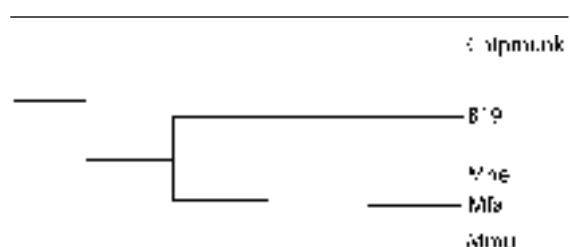


Figure 17.1. Phylogeny of primate erythroviruses. Phenogram shows kinship between simian (Mmu, Mfa, Mne) and human (B19) erythroviruses and their closest relative among nonprimate parvoviruses (Chipmunk). Length of branches is not proportional to the genetic distances. Mmu, rhesus monkey parvovirus; Mfa, cynomolgus macaque parvovirus; Mne, pig-tailed macaque parvovirus; B19, human parvovirus B19; Chipmunk, chipmunk parvovirus.

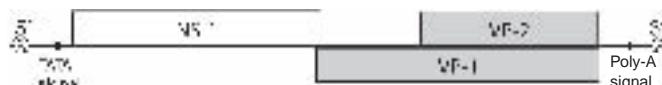


Figure 17.2. “Macro” map of a primate erythroivirus genome. Coding part of the genome contains three genes, encoding nonstructural (NS-1) and structural (VP-1, VP-2) proteins. Coding part is flanked by the nontranslated regions that contain palindromic sequences forming complex secondary structures. Translation initiation signal (TATA) and poly-A signal are located in 5'- and 3'-terminal nontranslated regions, respectively.

motifs required for nuclear transport and the assembly of the virions. The VP2 gene spans 1,676 nt and is coterminous with the VP1 gene at the 3'-end. The product of this gene, VP2 protein, lacks enzymatic activity.

Sequence similarity of SPV genomes is, on average, about 70%. In the most conserved regions, the similarity reaches 95–96%. The least conserved are three genomic fragments located in the regions corresponding to the nucleotides 833–950, 1,793–1,923, and 2,429–2,682 in the SPV genome. The similarity of SPV and B19 is about 55%.

The predicted lengths and molecular masses of simian erythroviral proteins are presented in Table 17.2.

17.3.2. Overview of Replication Cycle

SPVs apparently use the same receptor as parvovirus B19, the globoside.³ The virus enters a host cell through clathrin-mediated endocytosis. Inside the endosome, the virus remains intact for a relatively long time (a few hours). Virus release from the endosome into the cytosol requires PLA₂ activity. Release occurs in the vicinity of the nuclear membrane and the virions are transported into the nucleus. The mechanism of nuclear transport is poorly understood. The release of the genomic DNA occurs in the nucleus. Apparently, a short piece of genomic

DNA (20–30 nt at the 5'-end) becomes exposed at the capsid surface. Cellular DNA polymerase initiates DNA synthesis at this extra-capsid genomic segment. It appears that the polymerase “pulls-out” genomic DNA from the capsid before disintegration of the capsid starts. However, the details of this step are not known. The mechanism of genome replication is quite complex; it includes formation of double-stranded and multi-stranded replication intermediates. The hallmark of this process is self-priming by the terminal “hairpin” secondary structures. Viral replication occurs in actively dividing cells and is not accompanied by inhibition of cellular synthesis because it depends on the cellular DNA replication machinery. In contrast to double-stranded DNA viral genomes, the replication of single-stranded DNA is an error-prone process—hence, the profound variability of erythroviral genomes.

Protein-coding RNAs are transcribed from the negative-sense DNA and undergo complex splicing.^{8,16} Viral mRNAs are delivered to the cytoplasm and translated. The capsid proteins are transported to the nucleus and assemble there into empty capsids. Viral genomic DNAs, both positive- and negative-sense molecules, are incorporated in these capsids randomly, one molecule per capsid. After assembly, the virions are retained in the nucleus where they form paracrystalline arrays.

Table 17.2. Size and Amino Acid Identity of Primate Erythrovirus Proteins*

Protein Virus	NS-1	VP-1 Unique Part	VP-1 Complete	VP-2 Protein
Simian parvovirus	687aa	262aa	818aa	556aa
Rhesus macaque parvovirus	682aa/76 [†]	264aa/70	820aa/83	556aa/89
Pig-tailed macaque parvovirus	672aa/66	229aa/54	785aa/76	556aa/86
Human parvovirus B19	672aa/41	227aa/45	781aa/59	554aa/86

* As compared to the prototype simian parvovirus.

[†] Percentage of identical amino acids.

Eventually the virus is released, first from the nucleus and then from the cell. The mechanism of these processes is poorly understood.

SPV is tropic to erythroid cells. Two human erythroid cell lines, KU812Ep6 and UT7/Epo, are infectable by SPV.³ Both cell lines are also permissive for parvovirus B19; however, it is not clear if the replication of SPV in these cell lines can be maintained long term. SPV replication in short-term cultures of macaque erythroid progenitor cells leads to the inhibition of cell growth.³ Primary and permanent fibroblastoid and epithelioid cell cultures are nonpermissive for SPV.

17.3.3. Natural Host and Prevalence of Infection

The natural hosts of known SPVs are presumed to be macaques. However, data on the prevalence of this infection in wild macaques are absent. SPV infection is prevalent in captive macaque colonies. Antibodies against SPV antigens are detectable in about 50% of captive cynomolgus macaques and 35% of rhesus monkeys.⁴ Data on the presence of SPV-related viruses in simian species, other than macaques, are absent.

17.3.4. Mode of Transmission

Direct contact between animals is not necessary for transmission of SPV in captivity; that is, the virus can be transmitted among macaques kept in individual cages that prevent physical contacts between the animals. Thus, the transmission of SPV in captivity, likely, is airborne.⁴ The virus may also be transmitted through procedures associated with routine animal care.

SPV can infect human cells *in vitro*,³ which indirectly indicates that simian-to-human transmission of the virus, in principle, is possible. The available data are inconclusive. On the one hand, the prevalence of antibodies reacting with SPV antigens is higher in animal handlers exposed to the SPV-positive monkeys, compared to unexposed individuals (17% versus 6%). On the other hand, there are no data directly proving the presence of SPV in humans, and no illness attributable to SPV was observed in animal handlers exposed to the virus during an outbreak of SPV anemia.³

17.3.5. Pathogenicity

SPV infection in healthy macaques is inapparent, or mild and self-limited.¹² However, in immunosuppressed animals the virus causes anemia, which can be severe and even life-threatening.^{4,6,13,15} The disease in

macaques mimics many features of parvovirus B19-induced pathology in humans.

Macaques with SPV disease look ill, lose weight, and are anorexic. Diarrhea and dehydration are common. Moderate generalized lymphadenopathy and splenomegaly are observed. The characteristic hematological signs of the disease are normocytic/normochromic anemia and reticulocytopenia. Marked loss of mature erythroid and myeloid cells, increased number of megakaryocytes, and the presence of many undifferentiated cells are observed in bone marrow preparations. Giant pronormoblasts with intranuclear inclusion bodies are also observed. The inclusion bodies consist of tightly packed parvovirus particles forming the paracrystalline array.⁴

17.3.6. Immune Responses

SPV infection is accompanied by a readily detectable antibody response against structural proteins VP1 and VP2. The kinetics of the antibody response is not known. However, IgG antibodies against SPV persist after the clearance of virus as indicated by the presence of anti-SPV in about 50% of healthy macaques.^{3,13} The anti-SPV antibody response in cynomolgus macaques is not significantly inhibited by treatment with antithymocyte globulin or monoclonal anti-CD40L antibodies.¹⁵ Nothing is known about cellular and innate immunity against SPVs.

17.3.7. Diagnosis of Infection

The most reliable method for diagnosing SPV infection in macaques is the detection of viral DNA. In early studies this was achieved by dot-blot molecular hybridization assay, using a cloned 723-bp SPV genomic fragment as a probe.^{4,11} Several qualitative PCR tests for SPV have been described; however, data on their diagnostic performance have not been reported.^{3,7,11} More recently, real-time PCR for SPV has been used for the determination of viral load, but no details of this test have been reported.¹⁵ Isolation of SPV is not a diagnostic option owing to the absence of permissive cell lines. Detection of antibodies against SPV, unless seroconversion is demonstrated, indicates that a seropositive animal has had exposure to SPV. Seropositivity by itself is not sufficient to diagnose ongoing SPV infection.

Several tests for anti-SPV antibodies have been described.^{3,11} The most specific among these tests is a Western blot assay based on the recombinant SPV

VP2 protein.³ SPV ELISA based on SPV virus-like particles, consisting of baculovirus-expressed VP2 protein, has been described; however, their diagnostic performance has not been evaluated.^{3,4}

17.3.8. Prevention and Treatment

Control of simian parvovirus infection in primate centers is not practiced routinely. However, it is desirable to use SPV-negative monkeys in experiments involving various kinds of immunosuppression. Specific anti-SPV drugs are not available. However, treatment with steroids has been shown to be effective therapy for acute SPV infection.¹⁵

17.4. SIMIAN AAVS

AAVs have been isolated from the stocks of some simian adenoviruses. These viruses are closely related to AAV-1 and AAV-6 isolated from human adenovirus stocks.¹⁴ Viruses related to AAV-4 and AAV-8 have been found in the uncultured materials from cynomolgus macaques.¹⁰ However, this finding has not been independently confirmed.

As mentioned, despite the association of AAVs with human and simian adenoviruses, they are related phylogenetically to avian viruses.⁹ It is not known how the “symbiosis” of AAVs and primate adenoviruses was established.

AAV vectors for gene therapy are based on the AAV-2 serotype isolated from human cells. None of the AAVs isolated from simian cells is currently used as a gene therapy vector.⁵

17.5. SUMMARY

Parvoviruses (the family *Parvoviridae*) are small nonenveloped viruses with an “unusual” single-stranded DNA genome. There are two groups of parvoviruses: autonomous (subfamily *Parvovirinae*) and nonautonomous (*Dependovirinae*). The first can replicate independently, but only in actively dividing cells; the second are replication-incompetent and produce progeny only if the host cell is coinfecte^d with “helper” viruses, usually adenoviruses. All known autonomous simian parvoviruses belong to the *Erythrovirus* genus. Nonautonomous parvoviruses, the adeno-associated viruses (AAVs), which were isolated from nonhuman primate cells, belong to the *Dependovirus* genus. All parvoviruses are indistinguishable morphologically.

Parvovirus virions are the smallest among animal DNA viruses. Three closely related species of the simian erythrovirus species are currently recognized by the ICTV: (1) simian parvovirus (SPV), a cynomolgus macaque virus; (2) rhesus macaque parvovirus (RmPV); (3) pig-tailed macaque parvovirus (PMPV). These simian viruses are closely related to human parvovirus B19. In a similar way to B19 virus, SPV is tropic to erythroid cells and can induce anemia in immunocompromised macaques. SPV infection is inapparent in immunocompetent macaques.

Control of simian parvovirus infection in primate centers is not practiced routinely. However, it is desirable to use SPV-negative monkeys in experiments involving various kinds of immunosuppression. Specific anti-SPV drugs are not available. However, steroids have been shown to be effective for the therapy of acute SPV infection.

AAVs isolated from primate cells, despite their association with human and simian adenoviruses, cannot be considered as private viruses. Phylogenetic analysis of their genomes indicates that these AAVs are likely avian viruses. AAVs are not associated with any pathology. AAVs may be of potential use as vectors for gene therapy, and most of the interest these viruses attract is due to this application.

REFERENCES

- Berns, K. and C. R. Parrish. 2007. Parvoviridae. In: Knipe, D. M. and P. M. Howley (eds), *Fields Virology*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, Wolters Kluwer Business, pp. 2437–2477.
- Brown, K. E., S. W. Green, M. G. O’Sullivan, and N. S. Young. 1995. Cloning and sequencing of the simian parvovirus genome. *Virology* 210(2):314–322.
- Brown, K. E., Z. Liu, G. Gallinella, S. Wong, I. P. Mills, and M. G. O’Sullivan. 2004. Simian parvovirus infection: a potential zoonosis. *J. Infect. Dis.* 190(11):1900–1907.
- Brown, K. E. and Young, N. S. 1997. The simian parvoviruses. *Rev. Med. Virol.* 7(4):211–218.
- Coura, R. S. and N. B. Nardi. 2007. The state of the art of adeno-associated virus-based vectors in gene therapy. *J. Virol.* 4:99.
- Foresman, L., O. Narayan, and D. Pinson. 1999. Progressive anemia in a pig-tail macaque with AIDS. *Contemp. Top. Lab. Anim. Sci.* 38(4):20–22.
- Green, S. W., I. Malkovska, M. G. O’Sullivan, and K. E. Brown. 2000. Rhesus and pig-tailed macaque

- parvoviruses: identification of two new members of the erythrovirus genus in monkeys. *Virology* 269(1):105–112.
8. Liu, Z., J. Qiu, F. Cheng, Y. Chu, Y. Yoto, M. G. O'Sullivan, K. E. Brown, and D. J. Pintel. 2004. Comparison of the transcription profile of simian parvovirus with that of the human erythrovirus B19 reveals a number of unique features. *J. Virol.* 78(23):12929–12939.
 9. Lukashov, V. V. and J. Goudsmit. 2001. Evolutionary relationships among parvoviruses: virus-host co-evolution among autonomous primate parvoviruses and links between adeno-associated and avian parvoviruses. *J. Virol.* 75(6):2729–2740.
 10. Mori, S., L. Wang, T. Takeuchi, and T. Kanda. 2004. Two novel adeno-associated viruses from cynomolgus monkey: pseudotyping characterization of capsid protein. *Virology* 330(2):375–383.
 11. O'Sullivan, M. G., D. C. Anderson, J. D. Fikes, F. T. Bain, C. S. Carlson, S. W. Green, N. S. Young, and K. E. Brown. 1994. Identification of a novel simian parvovirus in cynomolgus monkeys with severe anemia: a paradigm of human B19 parvovirus infection. *J. Clin. Invest.* 93(4):1571–1576.
 12. O'Sullivan, M. G., D. K. Anderson, J. A. Goodrich, H. Tulli, S. W. Green, N. S. Young, and K. E. Brown. 1997. Experimental infection of cynomolgus monkeys with simian parvovirus. *J. Virol.* 71(6):4517–4521.
 13. O'Sullivan, M. G., D. K. Anderson, J. E. Lund, W. P. Brown, S. W. Green, N. S. Young, and K. E. Brown. 1996. Clinical and epidemiological features of simian parvovirus infection in cynomolgus macaques with severe anemia. *Lab. Anim. Sci.* 46(3):291–297.
 14. Schmidt, M., E. Grot, P. Cervenka, S. Wainer, C. Buck, and J. A. Chiorini. 2006. Identification and characterization of novel adeno-associated virus isolates in ATCC virus stocks. *J. Virol.* 80(10):5082–5085.
 15. Schroder, C., S. Pfeiffer, G. Wu, A. M. Azimzadeh, A. Aber, R. N. Pierson III, and M. G. O'Sullivan. 2006. Simian parvovirus infection in cynomolgus monkey heart transplant recipients causes death related to severe anemia. *Transplantation* 81(8):1165–1170.
 16. Vashisht, K., K. S. Faaberg, A. L. Aber, K. E. Brown, and M. G. O'Sullivan. 2004. Splice junction map of simian parvovirus transcripts. *J. Virol.* 78(20):10911–10919.
 17. Yoo, B. C., D. H. Lee, S. M. Park, J. W. Park, C. Y. Kim, H. S. Lee, J. S. Seo, K. J. Park, and W. S. Ryu. 1999. A novel parvovirus isolated from Manchurian chipmunks. *Virology* 253(2):250–258.

18

Polyomaviruses

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18.1. INTRODUCTION

The prototype polyomavirus is a murine virus that causes multiple tumor types in newborn mice—hence the name “polyoma” (meaning many tumors). The first simian polyomavirus was discovered in 1960 in primary cell cultures from rhesus macaques used for the production of polio vaccine.⁵² The virus, named simian vacuolating agent or simian virus 40 (SV-40) has been shown to be oncogenic in hamsters *in vivo* and transforming to hamster and human cells *in vitro*.^{28,49,50}

After the discovery of SV-40, it was shown that numerous lots of polio vaccine produced between 1955 and 1961 were contaminated with live SV-40. This problem was corrected by switching vaccine production from rhesus to African green monkey kidney cell cultures; the latter are free of SV-40. Nevertheless, millions of humans immunized with polio vaccine were exposed

to SV-40. Whether or not SV-40 exposure resulted in any pathological consequences remains unclear (Section 18.4.1).

SV-40 is a “virological celebrity” in the positive as well as the negative sense. This virus has been extensively and successfully used as a model system for gaining insights into basic biological processes, such as transcription, DNA replication, RNA processing, and oncogenic transformation. The SV-40 genome was the first viral genome to be cloned.²² The genomic DNA of SV-40 is the most intensively manipulated DNA molecule (per base pair) in the history of molecular biology.

In the 1970s, SV-40-related viruses were isolated from humans^{15,34} and monkeys.^{57,67} Two well-characterized human polyomaviruses, JC and BK, are medically important (reviewed in Imperiale and Major²¹). Human infection with the JC and BK viruses is very common. Usually, these infections are asymptomatic. However, both viruses may be pathogenic, particularly in immunocompromised hosts. JC virus may cause a fatal disease, progressive multifocal leukoencephalopathy, whereas BK virus is involved in kidney pathology in immunocompromised patients, particularly after kidney transplantation. The significant increase in the number of posttransplant patients and the advent of AIDS greatly increased the medical significance of the JC and BK viruses. Two additional human polyomaviruses (KI and WU) were described in 2007^{2,17} and their pathogenic potential is yet to be assessed.

In addition to SV-40, several other simian polyomaviruses have been described. Two of these viruses, isolated from baboons and African green monkeys, are well characterized, whereas data on the others are

Table 18.1. Simian Polyomaviruses

Virus Name*	Synonymous Names	Original References	Genomic Sequences†
<i>Simian virus 40 (SV-40)</i>	Vacuolating virus	50	NC_001699‡
<i>Baboon polyomavirus 1 (BaPyV-1)</i>	Simian agent (SA-12), Simian virus 12 (SV-12), Polyomavirus papionis 1	28	NC_007611‡
<i>African green monkey polyomavirus (AgmPyV)</i>	African green monkey lymphotropic papovavirus, Lymphotropic papovavirus (LPV), Lymphotropic polyomavirus, Polyomavirus cercopitheci, B-lymphotropic papovavirus K38	65	K02562‡ M30540‡
<i>Baboon polyomavirus 2 (BaPyV-2)</i>	Polyomavirus papionis 2	14	Not available
<i>Cynomolgus polyomavirus (CynoPy)</i>	Crab-eating macaque polyomavirus, Polyomavirus fascicularis	57	Partial large T-antigen sequence§
<i>Chimpanzee polyomavirus (ChPyV)</i>		21	AY691168
<i>Squirrel monkey polyomavirus (SquiPyV)</i>		59	AM748741‡

*ICTV species in italic.

†GenBank access numbers.

‡Complete genome sequences.

§Not deposited in the GenBank; published in Valis *et al.*⁵⁷

fragmentary. A second variant of baboon polyomavirus has been isolated in cell culture but no sequence data are available. In contrast, the polyomaviruses from the chimpanzee and squirrel monkey are frag-viruses, that is, known only from genomic sequences. The evidence for the existence of a cynomolgus macaque polyomavirus is mainly based on morphological data. Many more simian polyomaviruses likely exist, but this field has not been extensively explored.

18.2. CLASSIFICATION AND NOMENCLATURE

In earlier versions of the International Committee on Taxonomy of Viruses (ICTV) classification, polyomaviruses and papillomaviruses were combined in the now defunct family *Papovaviridae* ("papova" was an acronym for *papillomas + polyoma + "vacuolating agent,"* that is, SV-40) which included the genera *Polyomavirus* and *Papillomavirus*. The name polyomavirus

was usually used for the murine polyomaviruses, whereas the other members of the *Polyomavirus* genus were commonly named papovaviruses. In 2000, the taxonomic status of both *Polyomavirus* and *Papillomavirus* genera was raised to the family level. Since then, all members of the *Polyomaviridae* family have been named polyomaviruses and the designation "papovavirus" has been gradually disappearing.

Three species of simian polyomaviruses are currently recognized by the ICTV: *simian virus 40 (SV-40)*, *African green monkey polyomavirus (AgmPyP)*, and *baboon polyomavirus (BaPyV)* (Table 18.1).

The situation with BaPyV is perplexing. Two different polyomaviruses from baboons have been reported. The first virus, BaPyV-1 or simply BaPyV, has been known for years as SA-12.^{30,57} This virus is well characterized; its complete genome sequence is known.⁹ The data on the second baboon virus (BaPyV-2) are confined to a single report published in 1989.¹⁶ There is no genomic

sequence for this virus, not even partial sequence data. Nevertheless, BaPyV-2 is included in the latest ICTV classification, whereas BaPyV-1 is not.

In addition to the officially recognized simian polyomaviruses there are three “candidate” polyomaviruses from the cynomolgus macaque,⁵⁹ chimpanzee,²³ and squirrel monkey.⁶⁰ There have been several reports of the isolation of a “stump-tailed macaque polyomavirus” and closely related viruses from rhesus and cynomolgus macaques.^{19,39,46,66} However, all these viruses were shown to be of bovine origin^{35,40} and were most likely contaminants from fetal bovine serum.

18.2.1. Phylogeny

The most comprehensive polyomavirus phylogeny is based on the analysis of more than 100 complete genome sequences, including 17 isolates of SV-40, 2 isolates of AgmPyV, and 1 isolate of BaPyV-1.³⁸ A simian polyomavirus-focused version of this phylogeny is presented in Figure 18.1.

One of the clades in this phylogeny includes human (JC, BK) and simian (BaPyV-1 and SV-40) viruses. Interestingly, the baboon virus BaPyV-1 is closer to BK virus than BK is to the JC virus. However, the JC/BK/BaPyV-1/SV-40 clade does not include African green monkey polyomavirus (AgmPyV) and squirrel monkey polyomavirus (SquiPyV).

The closest relatives of AgmPyV are murine and hamster polyomaviruses. This phylogenetic position of AgmPyV is consistent with the hypothesis that this virus originates in a rodent species. If true, the hypothetical ancestral rodent virus could have infected African green monkeys as a result of interspecies transmission in nature or captivity. Alternatively, the progenitor of AgmPyV could have infected African green monkey cells in vitro as a laboratory contaminant. It is also possible that a second primate polyomavirus lineage exists. An intriguing similarity between the genomic sequences of AgmPyV and polyomavirus MCPyV recently shown to be associated with Merkel cell carcinoma in humans¹² is consistent with such possibility. In addition, there are data, although preliminary, indicating that AgmPyV and chimpanzee polyomavirus (ChPyV) are closely related; they are clustered together in a single gene (VP1) phylogeny.⁶⁰ However, this has yet to be verified by analysis of the complete genome sequences.

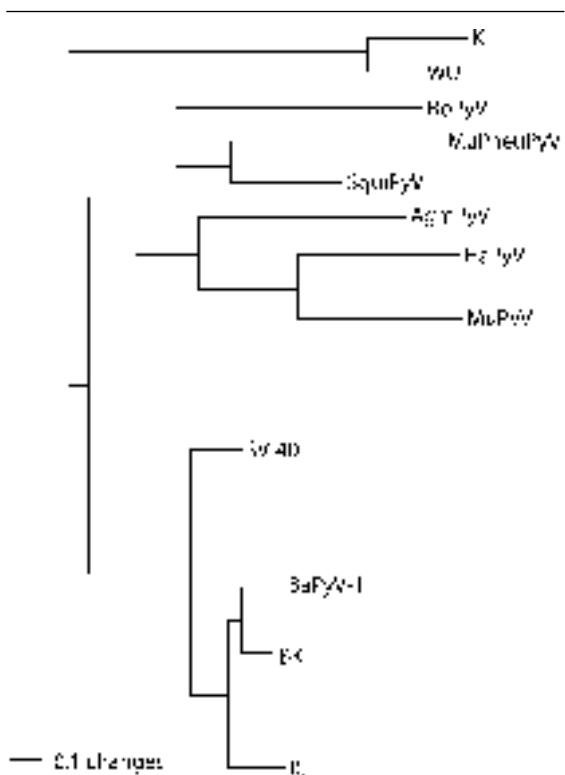


Figure 18.1. Phylogenetic tree of human, simian, and representative mammalian polyomaviruses. KI, WU, BK, JC, human polyomaviruses; BoPyV, bovine polyomavirus; MuPneuPyV, murine pneumotropic polyomavirus; SquiPyV, squirrel monkey polyomavirus; AgmPyV, African green monkey polyomavirus; HaPy, hamster polyomavirus; MuPyV, murine polyomavirus; BaPyV-1, baboon polyomavirus 1. Consensus tree; maximum likelihood analysis with the bootstrapping. Bootstrap support for all, but one, nodes of the tree is 100%. Bootstrap support for the BaPyV/BK clade node is 98%. (Phylogenetic tree was kindly provided by Dr. Marcos Pérez-Losada and Dr. Keith A. Crandall.)

The closest relative of SquiPyV is murine pneumotropic polyomavirus. These viruses, together with bovine polyomavirus, belong to a monophyletic clade that is clearly separated from the main primate clade

(BK/BaPyV/JC/SV-40) and the presumptive primate clade (AgmPyV/ChPyV) in polyomavirus phylogeny. Thus, the origin of SquiPyV is not completely clear.

18.3. GENERAL FEATURES

18.3.1. Genome Composition and Gene Products

The molecular and structural details of SV-40 are known, perhaps, better than those of any animal virus. Other simian polyomaviruses, and even human polyomaviruses, are clearly far behind SV-40 in this respect. However, many properties of polyomaviruses are generic and an overview of common properties is presented in this section. The details that are specific for the individual simian polyomaviruses are described in Sections 18.4–18.9.

The virions of polyomaviruses are nonenveloped, relatively small (40–45 nm) particles. The icosahedral capsid is composed of 72 capsomers. The polyomavirus genome is covalently closed circular dsDNA. Cellular histones H2A, H2B, H3, and H4 are bound to the genomic DNA and together they form a viral minichromosome which structurally and functionally resembles cellular chromatin. The minichromosome inside the capsid is tightly packed and is described by the term “supercoiled” DNA.

The size of the polyomavirus genome is small, about 5,000 bp. Just six proteins are encoded: VP1, VP2, VP3, large T antigen, small T antigen, and the so-called “agnoprotein” (Figure 18.2; Table 18.2).

Only three of these proteins (VP1, VP2, VP3) are structural. Each capsomer is composed of five molecules of VP1 and one molecule of either VP2 or VP3. Unusually, for capsids with icosahedral symmetry, the pentamers and hexamers are morphologically indistinguishable. The capsomers are linked to each other through contacts between extended C-terminuses of VP1 molecules. The contacts are stabilized by bound calcium ions. The N-terminus of the VP2 molecules in the capsomers is myristylated. The outer surface of the virions is composed of extended regions of VP1 molecules. VP2 and VP3 are not exposed at the virion surface.

The T antigens, large and small, are nonstructural proteins. They were originally detected using sera from rodents bearing polyomavirus-induced tumors—hence the T-antigen designation—T being an abbreviation for tumor. T antigens are encoded by the “minus” (complementary) strand of the genomic DNA.

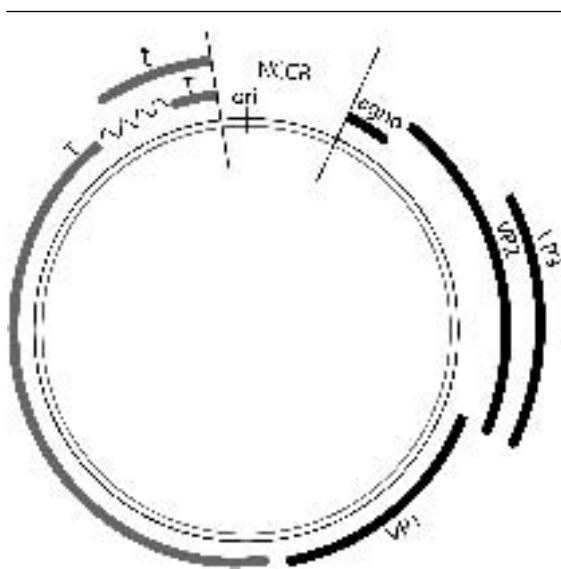


Figure 18.2. Polyomavirus genome map. Genomic double-stranded DNA is circular. Structural (VP1, VP2, VP3, and agnoprotein) and major nonstructural (large T-antigen and small t-antigen) proteins are encoded by different strands of genomic DNA (shown in gray and black). There are two exons in the large T-antigen gene. Noncoding control region (NCCR) contains origin of replication (*ori*) site.

The agnoprotein is a small, highly basic protein which facilitates the assembly of virions and their cell-to-cell spread. It may have other functions which are being uncovered.²⁶ All simian polyomaviruses, with the possible exception of AgmPyV, have agnoprotein. Agnoprotein is not essential for replication *in vitro*, but it is believed to be important for infection *in vivo*.

18.3.2. Overview of Replication Cycle

Polyomavirus replication cycles start from the attachment of the VP1 “protruding” moiety to the cell surface receptor. The identity of the receptor is known only for SV-40. In the early 1990s, it was reported that SV-40 used Class I major histocompatibility complex as a receptor.⁸ This result has been frequently cited; however, more recent studies have challenged this finding and identified ganglioside GM as the SV-40 receptor.⁵⁶

Table 18.2. Sizes of the Genome and Proteins of Primate Polyomaviruses

Virus	Genome Size (bp)	T-Ag	t-Ag	VP1	VP2	VP3
Simian virus 40	5,243	695*	172	362	351	232
Baboon polyomavirus	5,230	699	172	364	352	234
African green monkey polyomavirus	5,270	697	189	368	356	237
Squirrel monkey polyomavirus	5,075	655	163	357	332	207
Chimpanzee polyomavirus	?	?	?	395	?	?
Human BK virus	5,153	695	172	362	351	232
Human JC virus	5,121†	688	172	354	344	225

? Not known.

*Number of amino acids.

†The most common variant; genome size varies from 5,111 to 5,134.

After binding to the receptor, polyomavirus is internalized into small vesicles and delivered to the nuclear membrane. The exact mechanism of transport from the cell to the nuclear membrane is not known even for SV-40. Apparently, the transport occurs in two “runs,” first from the cell membrane to the endoplasmic reticulum (ER) and second from the ER to the nuclear membrane. The transport of virus-containing vesicles is mediated by microtubules.

The exact timing and location of uncoating is not known. Likely, the uncoating occurs shortly before fusion of the vesicle and nuclear membranes.

The viral genome in the nucleus structurally and functionally resembles cellular chromatin. Viral DNA is transcribed by cellular RNA polymerase II in a manner that is similar to the transcription of the cellular genes. In fact, the basic features of eukaryotic transcription machinery have been discovered using SV-40 replication as a model of the process.

The nonstructural, “early” genes are transcribed first; mRNAs are delivered to the cytoplasm and translated there. The early proteins, the most important of which is the large T antigen, are transported to the nucleus where they initiate transition of the host cell into the S phase of the cell cycle. The activated cellular DNA replication machinery produces replicas of both cellular and viral DNA.

The transcription of the late genes begins almost simultaneously with the onset of viral genome replication. The structural proteins synthesized in the cytoplasm are transported to the nucleus where virions are assembled. The key role in the assembly process belongs to VP1. This protein alone can self-assemble into capsomers and

virus-like particles (VLPs) when expressed in prokaryotic or eukaryotic cells.

18.3.3. Cell Transformation and Oncogenicity

The replication of polyomaviruses in vitro in permissive cells is lytic; that is, the virus-infected cells are lysed. However, in nonpermissive cells, the polyomavirus infection results in cell transformation. The transformed cells have altered more aggressive growth characteristics: immortalization (the ability to grow indefinitely in vitro), anchorage independence (the ability to grow in semisolid medium), loss of contact inhibition (the ability to form foci of multilayered growth), and reduced serum requirement. Polyomavirus-transformed cells are usually, but not always, tumorigenic; that is, they grow in vivo as malignant tumors in histocompatible or immunocompromised hosts.

Cell transformation may be considered as the in vitro analog of cell malignization in vivo, although in vivo oncogenesis is a more complex phenomenon. SV-40-induced transformation has been instrumental in unraveling important basic mechanisms in oncogenesis.

In vivo, SV-40 induces malignant tumors in newborn rodents. Cells transformed by SV-40 in vitro and SV-40-induced tumor cells in vivo both contain the viral genome, but do not produce complete virus. In these cells, only the early viral proteins, large and small T antigens, are expressed. T antigens are responsible for the tumor phenotype of the transformed/malignant cells. Cell transformation in vitro can be induced by the transfection of expression vectors containing T-antigen genes. Mice transgenic for the SV-40 T antigens develop a

malignant tumor, which can be targeted to specific organs by the inclusion of tissue-specific promoters in the T-antigen transgene.

T antigens cause malignant transformation by inactivating the functions of the tumor suppressor proteins (p53 and Rb) and the proteins involved in apoptosis. In a nonpermissive intracellular environment, the expression of the viral genome is deregulated toward the overexpression of T antigens, which block cellular mechanisms normally controlling cell proliferation.

The oncogenicity of SV-40 and other simian polyomaviruses is a purely artificial phenomenon.

None of the simian polyomaviruses is oncogenic in their natural hosts or transforming for the homologous cells in vitro.

18.4. SIMIAN VIRUS-40

The major properties of SV-40 are described in the previous sections. This section contains information on SV-40 infection in monkeys and its clinical manifestation; in addition, studies on the association of SV-40 with human malignancies are briefly reviewed.

The natural host of SV-40 is presumed to be the rhesus monkey. However, surprisingly little is known about SV-40 infection in the wild, how prevalent it is and whether or not it is harbored exclusively by rhesus monkeys. Moreover, available data indicate that SV-40 infection may not be common in wild macaques, including rhesus monkeys.²⁰ In the most recent serological survey, a low prevalence of SV-40 has been found in recently caught Barbary macaques (*M. sylvanus*) from Gibraltar (1.4%, $n = 130$) and Mauritian cynomolgus macaques (*M. fascicularis*) (8.8%, $n = 136$).⁶¹ It is quite possible that these populations are free from SV-40 infection in their natural habitat, and those few animals which tested positive contracted the infection in captivity. It has to be mentioned, however, that both Gibraltarian and Mauritian macaque populations originated from a few founders and may not be fully representative of the species they belong to.

Extremely high prevalence of SV-40 infection has been reported in a “semi-free” macaque population, the Nepalese temple rhesus monkeys (90%, $n = 39$).²⁴ However, the sample investigated is too small; also, this population cannot be considered as a good “proxy” of the truly wild rhesus monkey populations.

In captive macaques, the SV-40 infection is very common (40–75%) in all species tested (*M. mulatta*, *M.*

fascicularis, *M. sylvanus*, *M. tonkeana*, *M. fuscata*, *M. nigra*, *M. arctoides*, *M. thibetana*, *M. nemestrina*).^{20,61}

The prevalence of SV-40 infection in animals of the same species imported from different countries varies. SV-40 prevalence in cynomolgus macaques originating from China (92%, $n = 101$) is significantly higher than that in Vietnamese cynomolgus macaques (43%, $n = 348$). Rhesus macaques imported from China have the highest seroprevalence of SV-40 (95%, $n = 161$) compared to rhesus monkeys of other origins.⁶¹

Very little is known about the SV-40 mode of transmission. A relatively small percentage (about 10%) of captive rhesus monkeys is positive for SV-40 during the first year of life.^{32,61} Thus, vertical and perinatal transmissions play little role, if any, in the spread of SV-40 in captivity. Prevalence of infection increases dramatically, to about 55–60%, during the second year of life and does not increase significantly after reaching sexual maturity.⁶¹ Therefore, sexual transmission can be excluded, at least as a predominant mode of transmission. It is not clear if direct contact between monkeys is required, or if the virus can be contracted from a contaminated environment. The latter cannot be excluded because SV-40 has been isolated from samples taken from cage surfaces or various objects “used” by the monkeys.⁴

As a rule, SV-40 infection in macaques is subclinical. Pathological findings attributable to this virus are very rare. A case of SV-40-positive interstitial pneumonia and renal tubular necrosis in a young adult rhesus monkey has been described.⁴⁸ Several cases of SV-40 diseases, tubulointerstitial nephritis and progressive multifocal leukoencephalopathy, have been described in rhesus monkeys with simian immunodeficiency virus (SIV)-induced AIDS.^{18,27}

Whether or not SV-40 is harbored by the simian species other than macaques is not clear. Surprisingly, antibodies against SV-40 T antigen, as well as neutralizing antibodies, have been found in about 50% of baboons of different origins, including wild animals.⁶⁵ In 23% of sampled captive baboons ($n = 46$) the presence of SV-40 has been confirmed by the virus-specific polymerase chain reaction (PCR). The most intriguing finding, the serological evidence of SV-40 infection in wild baboons, has not yet been confirmed by PCR or virus isolation.

Different SV-40 isolates are indistinguishable in neutralization assays and ELISAs. Antigenic cross-reactivity between SV-40 and human poliomaviruses is observed in various serological tests, including the most

commonly used VLP-based ELISAs.^{63,64} This cross-reactivity is stronger with the BK virus than with the JC virus; however, even in the former case it is quite weak. Nevertheless, the specificity of the results of the SV-40 VLP ELISA has to be confirmed by competitive inhibition using homologous and heterologous VLPs, particularly when human sera are tested.^{11,63} SV-40 can also be reliably distinguished from the BK and JC viruses by PCR, including real-time quantitative tests.^{31,51}

Phylogenetic analysis of the completely sequenced SV-40 genomes distinguishes three major clades or genotypes. The highest proportion of variable sites is characteristic for the sequence encoding the C-terminus of T antigen.^{13,37} Phylogenetic analysis using only this fragment of the SV-40 genome produces the same results as analysis of the complete genome.

No correlation between the biological properties of SV-40 isolates and their genotypes has been observed.¹³ The origin of the virus, either from monkey or human cells, also does not correlate with the genotype. It should be mentioned that one report has described a stronger oncogenic activity of an SV-40 isolate from human cells as compared to isolates from monkey cells.⁶² However, in this study the genotypes had not been determined.

18.4.1. Simian-to-Human Transmissions

About 98 million Americans were exposed to live SV-40 through vaccination with inactivated polio vaccine from 1955 to 1963.⁴² During this period, an even higher number of individuals were potentially exposed to SV-40 in those countries that undertook universal vaccination of children with live oral polio vaccine. The ultimate outcome of this exposure is still not known. The real degree of human exposure to SV-40 was probably much smaller, although reliable estimates are not available. What proportion of the vaccine batches was contaminated with live SV-40? How many individuals and how many times were they injected with live SV-40 or received it orally? What was the dose distribution of live SV-40 in contaminated vaccine lots? What was the efficiency of virus “seeding” in groups exposed to different doses of SV-40? The estimates of these key parameters, even approximate, are missing.

Epidemiological studies addressing the possibility of pathological consequences of human exposure to SV-40 found no evidence of excess morbidity and mortality in potentially exposed populations.⁴⁴ However, these data are insufficient to exclude the risk completely.

The second wave of the “SV-40 scare” was provoked in the early 2000s by publications reporting the presence of SV-40 in some human tumors (mesotheliomas, brain tumors, osteosarcomas, and non-Hodgkin’s lymphomas) (reviewed in Shah⁴⁴). Contradictory results have been reported and an unequivocal interpretation of these data is not possible. However, the predominant view is that no credible evidence exists that SV-40 infection has become established in human populations and that it plays a role in the development of human malignancies.⁴⁴

At the same time, the possibility of transient “dead-end” human infections with SV-40 cannot be excluded. Neutralizing antibodies against SV-40 have been detected in humans.⁴³ The most recent study in this field employed a highly sensitive and specific method for the detection of antibodies against SV-40, a competitive ELISA using SV-40 VLPs as the antigen.¹¹ Using this method antibodies against SV-40 VLPs have been detected in zoo workers; those individuals who have direct contact with nonhuman primates are more likely to be seropositive than general zoo workers (10% versus 3%).

18.5. BABOON POLYOMAVIRUS 1

The baboon polyomavirus 1 (BaPyV-1) was first isolated from an African green monkey (*Cercopithecus pygerythrus*) kidney culture and named simian agent 12 (SA-12).³⁰ However, subsequent studies have shown that African green monkeys are not a natural host of SA-12 and that infection with this virus is prevalent in baboons.^{7,57} Apparently, baboons are the natural host of SA-12, although the presence of this virus in other sympatric African monkey species cannot be excluded.

The properties of BaPyV-1 are similar to those of SV-40 and human polyomaviruses.^{3,10,29,33,45,47} Most notably, BaPyV-1 transforms hamster kidney cells in vitro and is oncogenic for newborn hamsters.^{57,58}

BaPyV-1 infection in baboons is subclinical. The pathogenicity of BaPyV-1 in immunosuppressed animals cannot be excluded; however, no such cases have been described.

The diagnosis of BaPyV-1 infection can be achieved by virus isolation and detection of antibodies and viral DNA. Anti-BaPyV-1 antibodies are still detected mainly by the “old-fashioned” hemagglutination inhibition test.¹⁶ Neutralization assays are also used. No ELISA specific for anti-BaPyV-1 is available. A BaPyV-1-specific quantitative PCR has been described,⁹ but its

utility for the detection of the virus in “real life” samples has not been tested.

Almost all that is known about BaPyV-1 was published in the late 1970s. The only recent advance in this field is the sequencing of the complete genome of the virus.^{9,38} Two independently determined sequences of the BaPyV-1 genome have been reported (GenBank Acc. Nos. AY614708 and DQ435829). These sequences are 99% similar; the difference is most likely due to errors in the DQ435829 sequence. The size of the BaPyV-1 genome, based on the more reliable sequence, is 5,230 bp. The genome composition is typical for polyomaviruses; no exceptional features have been reported.⁹ The closest relative of BaPyV-1 among primate polyomaviruses is the BK virus. Both BaPyV-1 and BK viruses belong to the same lineage as the SV-40 and JC viruses.

18.6. BABOON POLYOMAVIRUS 2

The virus designated baboon polyomavirus 2 (BaPyV-2) was isolated in 1989 from baboon kidney culture.¹⁶ Very little is known about this virus and all that is known is restricted to the original report. The identification of BaPyV-2 is based on its typical polyomavirus morphology. Genomic sequences of the virus are not available. No cross-reactivity has been found between antigens of BaPyV-2 and BaPyV-1, SV-40, JC and BK viruses, as determined by immunofluorescence, immune electron microscopy and hemagglutination inhibition tests. No genomic sequences of BaPyV-2 are available, not even a partial sequence. In contrast to BaPyV-1, the BaPyV-2 virus does not agglutinate baboon erythrocytes, a finding that allows discrimination between anti-BaPyV-2 and anti-BaPyV-1 antibodies in the same serum.

Thirteen percent ($n = 118$) of wild-caught olive baboons (*Papio anubis*) have been shown to be seropositive for BaPyV-2, as determined by the BaPyV-2-specific hemagglutination inhibition test. In the same group, 21% of baboons were seropositive for BaPyV-1, but only two animals were seropositive for both viruses.¹⁶ These data suggest that baboons are a natural host of BaPyV-2. However, the information available is clearly insufficient for an unequivocal conclusion.

18.7. AFRICAN GREEN MONKEY POLYOMAVIRUS

AgmPyV was first isolated from an African green monkey B-lymphoblastoid cell line in 1979.⁶⁷ The unusual

feature of this polyomavirus is its B-cell tropism. That is why AgmPyV is better known as the lymphotropic or B-lymphotropic polyomavirus (papovavirus).^{41,53,55}

The complete genome sequence of AgmPyV was reported in the mid-1980s.^{14,36} The composition of the AgmPyV genome and its expression pattern are typical of polyomaviruses.^{1,6,25,41,53,55} AgmPyV transforms hamster embryonic cells in vitro; the transformed cells are tumorigenic in newborn and 10-day-old hamsters.⁵⁴

The natural host of AgmPyV is presumed to be African green monkey, but supporting data are controversial. In the original study, antibodies against AgmPyV were not found in African green monkeys.⁶⁷ Later, however, antibodies against AgmPyV were reported in various simian species and even in humans.^{5,41,53,55} Isolation of AgmPyV from wild monkeys has not been reported. As has been mentioned earlier, the genomic sequences of AgmPyV are more similar to the sequences of rodent polyomaviruses than to the sequences of SV-40 and BaPyV-1.³⁸ This result does not prove that AgmPyV is not a simian virus; however, this possibility cannot be ruled out.

Since the mid-1980s, AgmPyV remained in oblivion; however, recently this virus has been “resurrected” by finding which have determined it to be the closest relative of the human polyomavirus associated with the Merkel cell carcinoma.¹²

18.8. CYNOMOLGUS MACAQUE POLYOMAVIRUS

This virus was found in cynomolgus macaques after allogenic kidney transplantation. Viral particles having typical polyomavirus morphology, as well as characteristic intranuclear inclusions, were detected in 21% of kidney grafts ($n = 57$).⁵⁹ The virus was detectable during the period when the animals were severely immunosuppressed (3–11 weeks after transplantation). However, this polyomavirus (CynoPyV) has never been isolated. The only known characteristic of CynoPyV is the sequence of a short fragment (128 bp) of its large T-antigen gene. Within this fragment, the homology of CynoPyV and SV-40 is 90 and 83% at the nucleotide and amino acid levels, respectively.

CynoPyV was associated with kidney pathology reminiscent of the BK virus nephropathy observed in posttransplant patients. Tubulointerstitial nephritis was diagnosed in 50% of CynoPyV-positive animals. Characteristic nuclear inclusions were also detected in

the epithelium of a grafted ureter in the animals with nephritis.

Since 1999, no additional studies on CynoPyV have been published.

18.9. CHIMPANZEE POLYOMAVIRUS

ChPyV is a frag-virus; a fragment of its genome was amplified by PCR from feces of a common chimpanzee (*Pan troglodytes*) with severe diarrhea. The nested “broad-spectrum PCR”, that is, the PCR presumed to be generic for polyomaviruses, was used.²³

Attempts to isolate infectious virus from the same sample in cell cultures of human (HeLa, 293T) and monkey (MA104, Vero, MARC-145) origin have failed. ChPyV DNA was not detected in fecal samples from two chimpanzees housed together with the index case.

The genomic sequence of ChPyV covers the entire VP1-encoding gene (GenBank Acc. No. AY691168; 1,204 bp). The predicted size of the ChPyV VP1 is 395 amino acids; the largest among known polyomaviruses, mainly due to a 10 amino-acid insertion in one of the outer loops (loop EF). Other than this, the protein is similar to its homologs in other polyomaviruses. Based on the VP1 sequence, the closest relative of ChPyV is AgmPyV.^{23,60}

18.10. SQUIRREL MONKEY POLYOMAVIRUS

Squirrel monkey polyomavirus is the latest addition to the list of simian polyomaviruses.⁶⁰ This virus is known only from its genomic sequence. However, at the genomic level, SquiPyV is well characterized, its complete genome sequence is determined, and all genes and known regulatory sequence elements have been identified.

The sample from which genomic SquiPyV DNA has been amplified originated from a Bolivian squirrel monkey (*Saimiri biuviensis*). Initially, viral DNA was detected in this sample using nested generic VP1 PCR designed for the detection of ChPyV.²³ This sample has been the only positive among 44 DNA samples from New World monkeys of 18 species representing all genera of the Platyrrini. After amplification and sequencing of the VP1 gene fragment, the remaining part of the circular genomic DNA was amplified by nested long PCR using primers facing outwards.

The complete genome is 5,075 bp in length, the smallest among known primate polyomavirus genomes. All predicted SquiPyV proteins, except agnoprotein, are

also smaller than their homologs in other primate polyomaviruses (Table 18.2). Other than this, the genome composition is typical of primate polyomaviruses.

As expected, the most divergent is the noncoding regulatory region. However, all major functional elements, such as the origin of replication (*ori*), T-antigen-binding motifs, TATA-boxes, binding sites for the transcription factors Sp1, NF-1, and AP-1, are conserved.

The putative SquiPyV agnoprotein is significantly more divergent than its homologs in other primate polyomaviruses. In contrast to the homologs which differ from each other mainly in the C-terminal part, the amino acid substitutions in SquiPyV agnoprotein are evenly distributed over the whole length of the protein, as compared with the consensus sequence.

Phylogenetic analysis of the SquiPyV VP1 gene sequence places SquiPyV in the same clade with the murine pneumotropic and bovine polyomaviruses. This result is consistent with a nonsimian origin of SquiPyV. However, there is strong evidence against this possibility: SquiPyV-specific DNA has been detected by PCR in 50% (5 out of 10) of DNA samples extracted from uncultured tissues (spleens) of monkeys of different *Saimiri* species housed in different facilities.⁶⁰ Thus, SquiPyV is likely to be an authentic monkey virus. However, the alternative explanation that squirrel monkeys are highly susceptible to the polyomavirus excreted by some rodents with which they have contact, cannot be excluded.

18.11. SUMMARY

Polyomaviruses are small, nonenveloped DNA viruses with a circular dsDNA genome of about 5,000 bp. The family *Polyomaviridae* includes three simian polyomavirus species: *simian virus 40* (SV-40), *African green monkey polyomavirus* (AgmPyV), and *baboon polyomavirus 1* (BaPyV-1).

Two other simian polyomaviruses, from a chimpanzee (ChPyV) and from a squirrel monkey (SquiPyV), are known only from genomic sequence data. Polyomavirus associated with nephritis in immunosuppressed cynomolgus macaques with transplanted kidneys has also been described, but it was not isolated and only a very short fragment of its genomic sequence is known. Very little is also known about polyomavirus isolated from baboons (BaPyV-2), other than it is distinguishable from BaPuV-1.

The simian origin of SV-40 and BaPyV-1 is not disputed, whereas the evidence that AgmPyV is an

authentic simian virus remains inconclusive. The possibility exists that this virus originates from rodents.

Genome composition, replication, and expression patterns are similar for all simian polyomaviruses. The early (large and small T antigens) and late (VP1, VP2, and VP3) genes are encoded by different strands of genomic DNA. The coding regions of the genome are separated by the noncoding regulatory region containing the origin of replication and multiple sequence motifs regulating and coordinating the replication of genomic DNA and the expression of viral genes.

An interesting property of polyomaviruses is their ability to transform cells in vitro and cause multiple tumor types in vivo. However, the oncogenicity of polyomaviruses is a laboratory phenomenon. In natural conditions, polyomavirus infections are subclinical. However, in immunocompromised hosts polyomaviruses can be pathogenic. The pathogenic potential of primate polyomaviruses has been mainly explored with human polyomaviruses (JC and BK). Very little is known about the pathogenicity of simian polyomaviruses in the context of immunosuppression, although pathology attributable to SV-40 has been described in rhesus monkeys with AIDS.

REFERENCES

- Abraham, G. and H. Manor. 1987. Transcription map of the African green monkey lymphotropic papovavirus. *Virology* 157(1):89–98.
- Allander, T., K. Andreasson, S. Gupta, A. Bjerkner, G. Bogdanovic, M. A. Persson, T. Dalianis, T. Ramqvist, and B. Andersson. 2007. Identification of a third human polyomavirus. *J. Virol.* 81(8):4130–4136.
- Benton, C. V., R. V. Gilden, and K. V. Shah. 1982. Species- and genus-specific antigenic determinants of simian agent 12 VP1 as defined with monoclonal antibodies. *Virology* 117(2):490–495.
- Bofill-Mas, S., N. Albinana-Gimenez, P. A. Pipkin, P. D. Minor, and R. Girones. 2004. Isolation of SV40 from the environment of a colony of cynomolgus monkeys naturally infected with the virus. *Virology* 330(1):1–7.
- Brade, L., N. Muller-Lantzsch, and H. zur Hausen. 1981. B-lymphotropic papovavirus and possibility of infections in humans. *J. Med. Virol.* 6(4):301–308.
- Brade, L., N. Mueller-Lantzsch, S. Kaiser, and M. Scharrer. 1983. Biochemical studies on structural and nonstructural proteins of the African green monkey B-lymphotropic papovavirus (LPV). *Virology* 127(2):469–474.
- Braun, L., S. S. Kalter, L. A. Yakovleva, V. R. Kaschula, and K. V. Shah. 1980. Neutralizing antibodies to simian papovavirus SA12 in Old World primates in laboratory colonies: high prevalence in baboons. *J. Med. Primatol.* 9(4):240–246.
- Breau, W. C., W. J. Atwood, and L. C. Norkin. 1992. Class I major histocompatibility proteins are an essential component of the simian virus 40 receptor. *J. Virol.* 66(4):2037–2045.
- Cantalupo, P., A. Doering, C. S. Sullivan, A. Pal, K. W. Peden, A. M. Lewis, and J. M. Pipas. 2005. Complete nucleotide sequence of polyomavirus SA12. *J. Virol.* 79(20):13094–13104.
- Cunningham, T. P. and J. M. Pipas. 1985. Simian agent 12 is a BK virus-like papovavirus which replicates in monkey cells. *J. Virol.* 54(2):483–492.
- Engels, E. A., W. M. Switzer, W. Heneine, and R. P. Viscidi. 2004. Serologic evidence for exposure to simian virus 40 in North American zoo workers. *J. Infect. Dis.* 190(12):2065–2069.
- Feng, H., M. Shuda, Y. Chang, and P. S. Moore. 2008. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 319(5866):1096–1100.
- Forsman, Z. H., J. A. Lednicky, G. E. Fox, R. C. Willson, Z. S. White, S. J. Halvorson, C. Wong, A. M. Lewis Jr., and J. S. Butel. 2004. Phylogenetic analysis of polyomavirus simian virus 40 from monkeys and humans reveals genetic variation. *J. Virol.* 78(17):9306–9316.
- Furuno, A., T. Kanda, and K. Yoshiike. 1986. Monkey B-lymphotropic papovavirus genome: the entire DNA sequence and variable regions. *Jpn. J. Med. Sci. Biol.* 39(4):151–161.
- Gardner, S. D., A. M. Field, D. V. Coleman, and B. Hulme. 1971. New human papovavirus (BK) isolated from urine after renal transplantation. *Lancet* 1(7712):1253–1257.
- Gardner, S. D., W. A. Knowles, J. F. Hand, and A. A. Porter. 1989. Characterization of a new polyomavirus (Polyomavirus papionis-2) isolated from baboon kidney cell cultures. *Arch. Virol.* 105(3–4):223–233.
- Gaynor, A. M., M. D. Nissen, D. M. Whiley, I. M. Mackay, S. B. Lambert, G. Wu, D. C. Brennan, G. A. Storch, T. P. Sloots, and D. Wang. 2007. Identification of a novel polyomavirus from patients with acute respiratory tract infections. *PLoS Pathog.* 3(5):e64.
- Horvath, C. J., M. A. Simon, D. J. Bergsagel, D. R. Pauley, N. W. King, R. L. Garcea, and D. J. Ringler. 1992. Simian virus 40-induced disease in rhesus monkeys with simian acquired immunodeficiency syndrome. *Am. J. Pathol.* 140(6):1431–1440.
- Howley, P. M., N. Newell, K. V. Shah, M. F. Law, P. Gruss, G. Sauer, and T. J. Kelly Jr. 1979. Identification

- of the primate papovavirus HD as the stump-tailed macaque virus. *J. Virol.* 30(1):400–403.
20. Ichikawa, T., N. Minamoto, T. Kinjo, N. Matsubayashi, K. Matsubayashi, and I. Narama. 1987. A serological survey of simian virus 40 in monkeys. *Microbiol. Immunol.* 31(10):1001–1008.
 21. Imperiale, M. J. and E. O. Major. 2007. Polyomaviruses. In: Knipe, D. M. and P. M. Howley (eds), *Fields Virology*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, Wolters Kluwer Business, pp. 2263–2298.
 22. Jackson, D. A., R. H. Symons, and P. Berg. 1972. Biochemical method for inserting new genetic information into DNA of Simian Virus 40: circular SV40 DNA molecules containing lambda phage genes and the galactose operon of *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 69(10):2904–2909.
 23. Johne, R., D. Enderlein, H. Nieper, and H. Muller. 2005. Novel polyomavirus detected in the feces of a chimpanzee by nested broad-spectrum PCR. *J. Virol.* 79(6):3883–3887.
 24. Jones-Engel, L., G. A. Engel, J. Heidrich, M. Chalise, N. Poudel, R. Viscidi, P. A. Barry, J. S. Allan, R. Grant, and R. Kyes. 2006. Temple monkeys and health implications of commensalism, Kathmandu, Nepal. *Emerg. Infect. Dis.* 12(6):900–906.
 25. Kanda, T., K. Yoshiike, and K. K. Takemoto. 1983. Alignment of the genome of monkey B-lymphotropic papovavirus to the genomes of simian virus 40 and BK virus. *J. Virol.* 46(1):333–336.
 26. Khalili, K., M. K. White, H. Sawa, K. Nagashima, and M. Safak. 2005. The agnogene of polyomaviruses: a multifunctional auxiliary protein. *J. Cell Physiol.* 204(1):1–7.
 27. King, N. W., R. D. Hunt, and N. L. Letvin. 1983. Histopathologic changes in macaques with an acquired immunodeficiency syndrome (AIDS). *Am. J. Pathol.* 113(3):382–388.
 28. Kirschstein, R. L. and P. Gerber. 1962. Ependymomas produced after intracerebral inoculation of SV40 into new-born hamsters. *Nature* 195:299–300.
 29. Lecatsas, G., H. H. Malherbe, and M. Strickland-Cholmley. 1977. Electronmicroscopical characterisation of simian papovavirus SA12. *J. Med. Microbiol.* 10(4):477–478.
 30. Malherbe, H. H. and R. Harwin. 1963. The cytopathic effects of vervet monkey viruses. *S. Afr. Med. J.* 37:407–411.
 31. McNees, A. L., Z. S. White, P. Zanwar, R. A. Vilchez, and J. S. Butel. 2005. Specific and quantitative detection of human polyomaviruses BKV, JCV, and SV40 by real time PCR. *J. Clin. Virol.* 34(1):52–62.
 32. Minor, P., P. A. Pipkin, K. Cutler, and G. Dunn. 2003. Natural infection and transmission of SV40. *Virology* 314(1):403–409.
 33. Newell, N., K. V. Shah, and T. J. Kelly Jr. 1979. Evolutionary relationships of the primate papomaviruses: base sequence homology among the genomes of simian virus 40, stump-tailed macaque virus, and SA12 virus. *J. Virol.* 30(2):624–636.
 34. Padgett, B. L., D. L. Walker, G. M. ZuRhein, R. J. Eckroade, and B. H. Dessel. 1971. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet* 1(7712):1257–1260.
 35. Parry, J. V., M. H. Lucas, J. E. Richmond, and S. D. Gardner. 1983. Evidence for a bovine origin of the polyomavirus detected in foetal rhesus monkey kidney cells, FRHK-4 and -6. *Arch. Virol.* 78(4):151–165.
 36. Pawlita, M., A. Clad, and H. zur Hausen. 1985. Complete DNA sequence of lymphotropic papovavirus: prototype of a new species of the polyomavirus genus. *Virology* 143(1):196–211.
 37. Peden, K., L. Sheng, R. Omeir, M. Yacobucci, M. Klutch, M. Laassri, K. Chumakov, A. Pal, H. Murata, and A. M. Lewis Jr. 2008. Recovery of strains of the polyomavirus SV40 from rhesus monkey kidney cells dating from the 1950s to the early 1960s. *Virology* 370(1):63–76.
 38. Perez-Losada, M., R. G. Christensen, D. A. McClellan, B. J. Adams, R. P. Viscidi, J. C. Demma, and K. A. Crandall. 2006. Comparing phylogenetic codivergence between polyomaviruses and their hosts. *J. Virol.* 80(12):5663–5669.
 39. Reissig, M., T. J. Kelly Jr., R. W. Daniel, S. R. Rangan, and K. V. Shah. 1976. Identification of the stumptailed macaque virus as a new papovavirus. *Infect. Immun.* 14(1):225–231.
 40. Richmond, J. E., J. V. Parry, and S. D. Gardner. 1984. Characterisation of a polyomavirus in two foetal rhesus monkey kidney cell lines used for the growth of hepatitis A virus. *Arch. Virol.* 80(2–3):131–146.
 41. Segawa, K. and K. K. Takemoto. 1983. Identification of B-lymphotropic papovavirus-coded proteins. *J. Virol.* 45(2):872–875.
 42. Shah, K. and N. Nathanson. 1976. Human exposure to SV40: review and comment. *Am. J. Epidemiol.* 103(1):1–12.
 43. Shah, K. V. 1966. Neutralizing antibodies to simian virus 40 (SV40) in human sera from India. *Proc. Soc. Exp. Biol. Med.* 121(1):303–307.
 44. Shah, K. V. 2007. SV40 and human cancer: a review of recent data. *Int. J. Cancer* 120(2):215–223.
 45. Shah, K. V., R. W. Daniel, and T. J. Kelly Jr. 1977. Immunological relatedness of papovaviruses of the simian

- virus 40-polyoma subgroup. *Infect. Immun.* 18(2):558–560.
46. Shah, K. V., S. R. Rangan, M. Reissig, R. W. Daniel, and F. Z. Bellhan. 1977. Congenital transmission of a papovavirus of the stump-tailed macaque. *Science* 195(4276):404–406.
47. Shah, K. V., J. D. Valis, and R. W. Daniel. 1978. Hemagglutination with simian papovavirus SA12. *J. Clin. Microbiol.* 7(4):396–398.
48. Sheffield, W. D., J. D. Strandberg, L. Braun, K. Shah, and S. S. Kalter. 1980. Simian virus 40-associated fatal interstitial pneumonia and renal tubular necrosis in a rhesus monkey. *J. Infect. Dis.* 142(4):618–622.
49. Shein, H. M. and J. F. Enders. 1962. Transformation induced by simian virus 40 in human renal cell cultures. I. Morphology and growth characteristics. *Proc. Natl. Acad. Sci. U. S. A.* 48:1164–1172.
50. Shein, H. M., J. F. Enders, J. D. Levinthal, and A. E. Burkett. 1963. Transformation induced by simian virus 40 in newborn Syrian hamster renal cell cultures. *Proc. Natl. Acad. Sci. U. S. A.* 49:28–34.
51. Shi, L., J. Ho, L. A. Norling, M. Roy, and Y. Xu. 1999. A real time quantitative PCR-based method for the detection and quantification of simian virus 40. *Biologicals* 27(3):241–252.
52. Sweet, B. and M. Hilleman. 1960. The vacuolating virus, S.V. 40. *Proc. Soc. Exp. Biol. Med.* 105:420–427.
53. Takemoto, K. K., A. Furuno, K. Kato, and K. Yoshiike. 1982. Biological and biochemical studies of African green monkey lymphotropic papovavirus. *J. Virol.* 42(2):502–509.
54. Takemoto, K. K. and T. Kanda. 1984. Lymphotropic papovavirus transformation of hamster embryo cells. *J. Virol.* 50(1):100–105.
55. Takemoto, K. K. and K. Segawa. 1983. A new monkey lymphotropic papovavirus: characterization of the virus and evidence of a related virus in humans. *Prog. Clin. Biol. Res.* 105:87–96.
56. Tsai, B., J. M. Gilbert, T. Stehle, W. Lencer, T. L. Benjamin, and T. A. Rapoport. 2003. Gangliosides are receptors for murine polyoma virus and SV40. *EMBO J.* 22(17):4346–4355.
57. Valis, J. D., N. Newell, M. Reissig, H. Malherbe, V. R. Kaschula, and K. V. Shah. 1977. Characterization of SA12 as a simian virus 40-related papovavirus of chacma baboons. *Infect. Immun.* 18(1):247–252.
58. Valis, J. D., J. D. Strandberg, and K. V. Shah. 1979. Transformation of hamster kidney cells by simian papovavirus SA12. *Proc. Soc. Exp. Biol. Med.* 160(2):208–212.
59. van Gorder, M. A., P. P. Della, J. W. Henson, D. H. Sachs, A. B. Cosimi, and R. B. Colvin. 1999. Cynomolgus polyoma virus infection: a new member of the polyoma virus family causes interstitial nephritis, ureteritis, and enteritis in immunosuppressed cynomolgus monkeys. *Am. J. Pathol.* 154(4):1273–1284.
60. Verschoor, E. J., M. J. Groenewoud, Z. Fagrouch, A. Kewalapati, S. van Gessel, M. J. Kik, and J. L. Heeney. 2008. Molecular characterization of the first polyomavirus from a New World primate: squirrel monkey polyomavirus. *J. Gen. Virol.* 89(Pt 1):130–137.
61. Verschoor, E. J., H. Niphuis, Z. Fagrouch, P. Christian, K. Sasnauskas, M. C. Pizarro, and J. L. Heeney. 2008. Seroprevalence of SV40-like polyomavirus infections in captive and free-ranging macaque species. *J. Med. Primatol.* 37(4):196–201.
62. Vilchez, R. A., C. F. Brayton, C. Wong, P. Zanwar, D. E. Killen, J. L. Jorgensen, and J. S. Butel. 2004. Differential ability of two simian virus 40 strains to induce malignancies in weanling hamsters. *Virology* 330(1):168–177.
63. Viscidi, R. P. and B. Clayman. 2006. Serological cross reactivity between polyomavirus capsids. *Adv. Exp. Med. Biol.* 577:73–84.
64. Viscidi, R. P., D. E. Rollison, E. Viscidi, B. Clayman, E. Rubalcaba, R. Daniel, E. O. Major, and K. V. Shah. 2003. Serological cross-reactivities between antibodies to simian virus 40, BK virus, and JC virus assessed by virus-like-particle-based enzyme immunoassays. *Clin. Diagn. Lab. Immunol.* 10(2):278–285.
65. Westfall, L. W., M. H. Shearer, C. A. Jumper, G. L. White, J. F. Papin, R. Eberle, J. S. Butel, R. K. Bright, and R. C. Kennedy. 2008. Evidence of simian virus 40 exposure in a colony of captive baboons. *Virology* 377(1):54–62.
66. Wognum, A. W., C. J. Sol, J. van der Noordaa, G. van Steenis, and A. D. Osterhaus. 1984. Isolation and characterization of a papovavirus from cynomolgus macaque kidney cells. *Virology* 134(1):254–257.
67. zur Hausen, H. and L. Gissmann. 1979. Lymphotropic papovaviruses isolated from African green monkey and human cells. *Med. Microbiol. Immunol.* 167(3):137–153.

19

Papillomaviruses

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19.1. INTRODUCTION

Papillomaviruses, as their name suggests, have been discovered as the causal agents of mucocutaneous papillomas, colloquially known as warts. Human papillomaviruses (HPVs) are among a few known “centenarian” viruses. Indeed, the transmissibility of human warts by cell-free extracts was demonstrated for the first time in 1907. However, despite such an early start the papillomavirus field remained little explored for many decades. The main reason for this was the lack of an *in vitro* system for culturing papillomaviruses.

The situation changed dramatically in the late 1970s–early 1980s when the advent of molecular cloning and other “molecular” techniques made it possible to characterize the papillomaviruses quite comprehensively, without culturing them *in vitro*. In a relatively short time, more than 100 types of HPVs were identified and some of them have been shown to be responsible for the development of multiple epithelial malignancies, most notably cervical cancer. It is estimated that about 10% of human cancer cases are caused by papillomaviruses. Importantly, the majority of cervical cancer cases are

caused by just a few HPV types (HPV-16 and HPV-18 are the most common). These cancers are now preventable by highly effective papillomavirus vaccines. The development of papillomavirus vaccine is, perhaps, the most spectacular “success story” in human virology during the last 10 years.

Several simian counterparts of HPVs are known; however, this field is relatively unexplored. To a large extent this is explained by the fact that the explosive growth of HPV research was not dependent on nonhuman primate (NHP) models. However, the recently described model, based on the experimental infection of macaques with simian papillomaviruses,²⁸ may be useful for the development of therapeutic papillomavirus vaccines and other treatment modalities.

19.2. CLASSIFICATION AND NOMENCLATURE

From the early 1960s until 2000, papillomaviruses were grouped together with the polyomaviruses in the family *Papovaviridae* (“pa-po-va” stands for papilloma + polyoma + vacuolizing agent, i.e., SV-40). In 2000, these two groups, the *Papillomavirus* and *Polyomavirus* genera, were officially “divorced.” The taxonomic status of each genus was raised to the family level. Thus, in the current International Committee on Taxonomy of Viruses (ICTV) classification there is the *Papillomaviridae* family. The family *Papovaviridae* is now defunct.

Within the *Papillomaviridae*, there are 16 genera that contain papillomaviruses from many mammalian and avian species. Morphologically, papillomaviruses are small (52–55 nm) spherical particles having icosahedral symmetry (Figure 19.1).

The papillomavirus capsid is composed of 72 pentameric capsomers. The capsomers have two different

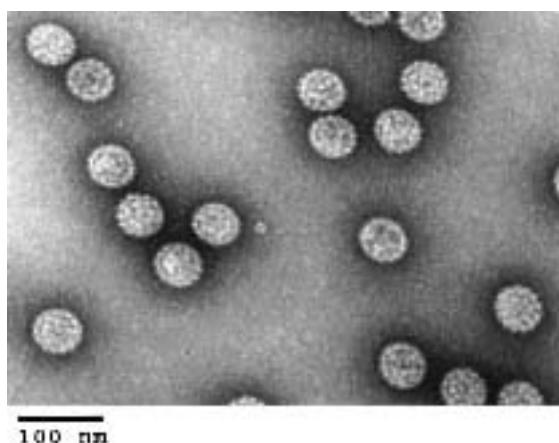


Figure 19.1. Electron micrograph of negatively stained rhesus monkey papillomavirus (RhPV). RhPV virions (isolate RhPV1) were purified by cesium chloride gradient ultracentrifugation from culture medium of HEK 293T cell line transfected with an expression vector for RhPV1 L1 and L2 capsid proteins and circular RhPV1 genome¹⁹ (Ozbun *et al.*, unpublished) and negatively stained with 2% uranyl acetate. Virions are of uniform size (55 nm) and capsomers are clearly visible. (Image was kindly provided by Dr. Michelle A. Ozbun.)

conformations depending on the number of neighboring capsids, five at the vertices and six elsewhere. Each capsomer is composed of two proteins, L1 and L2. The L1 protein is the major structural protein comprising approximately 80% of the virion weight. The genomic DNA encapsulated inside the capsid is associated with cellular histones.

Modern papillomavirus taxonomy is based on the degree of genomic sequence homology. Clustering of papillomaviruses into genera and lower taxa, based on the sequence of the complete genome, is the same as that based on the sequence within the major capsid protein gene (L1). For this reason, L1 sequence homology is used as the classification criterion. The viruses are included in the same genus if the homology between their L1 genes exceeds 60%. Within each genus, papillomaviruses are classified as species, types, and subtypes also according to the degree of the L1 sequence homology: 61–70% for species, 71–90% for types, 91–98%

for subtypes. Papillomaviruses with 99% L1 sequence homology are named variants.

Papillomavirus genera tend to include viruses from the same species. Three genera, the *Alfa-*, *Beta-*, and *Gamma-papillomavirus*, include almost exclusively HPVs. The only exception in the latest version of the ICTV classification (8th Report) is the presence of simian papillomaviruses in the *Alfapapillomavirus* genus. These are rhesus monkey papillomavirus (RhPV), pygmy chimpanzee papillomavirus (PCPV), and common chimpanzee papillomavirus (PCPV-C). It has to be mentioned that in most publications the common chimpanzee papillomavirus name is abbreviated as CCPV, whereas in the ICTV classification this virus is confusingly designated as “pygmy chimpanzee papillomavirus C.” RhPV is the only simian papillomavirus currently recognized as a species. CCPV and CCPV-C are classified as the isolates/strains belonging to the *human papillomavirus* 6 species.

The previous version of the ICTV classification contained two papillomaviruses from colobus monkeys (*Colobus guereza*)—colobus papillomaviruses 1 and 2 (CgPV-1 and CgPV-2). Currently these viruses are not included in the ICTV classification, possibly because only a partial sequence of their genomes is known. Interestingly, based on the available genomic sequence, CoPV-2 should be included in the *Betapapillomavirus* genus.

The complete genome of the MfPV-1 strain of cynomolgus macaque papillomavirus has been reported recently (GenBank Acc. No. EF028290). The closest matches for this sequence are HPVs belonging to the *Betapapillomavirus* genus. MfPV-1 will probably be included in the next version of the ICTV classification.

Papillomaviruses belonging to the same genus tend to have similar biological properties. Alfapapillomaviruses are collectively referred to as “genital-mucosal” papillomaviruses because they usually infect genital and other mucosal surfaces. Betapapillomaviruses usually infect skin and are referred to as “cutaneous” papillomaviruses.

The major species and types of alfa-, beta-, and gamma-papillomaviruses are presented in Table 19.1.

The nomenclature of HPV species and types/subtypes is somewhat confusing. Traditionally in medical literature HPV are identified by their types. Just a few HPV species include only one type (e.g., the HPV-50 species includes only the HPV-50 type), whereas the majority of HPV species include many types (e.g., the HPV-6

Table 19.1. Major Species and Types of Alfa-, Beta-, and Gamma-Papillomaviruses

Genus	Type Species	Species	Simian Viruses
<i>Alfapapillomavirus</i>	HPV-32	HPV-2, -6, -7, -10, -16, -18, -26, -32, -34, -53, -54, -61, -71, -cand90	Rhesus monkey papillomavirus (RhPV), Pigmy chimpanzee papillomavirus (PCPV), Common chimpanzee papillomavirus (CCPV) or PCPV-C, Colobus papillomavirus 1 (CoPV-1)
<i>Betapapillomavirus</i>	HPV-5	HPV-5, -9, -49, -92, -96	Cynomolgus macaque papillomavirus (MfPV), Colobus papillomavirus 2 (CoPV-2)
<i>Gammapapillomavirus</i>	HPV-4	HPV-4, -48, -50, -60, -88	None

species includes the types HPV-6, -11, -13, -44, -55, -74). Thus, the same designation (e.g., HPV-6) may be the name of the species or the type.

In addition to the “bona fide” simian papillomaviruses, a number of simian frag-papillomaviruses have been described.^{1,4,28} Particularly rich “harvest” of such viruses was collected from the healthy skin of chimpanzees, gorillas, cynomolgus macaques, and spider monkeys.¹ This study reported profound diversity of frag-papillomaviruses in these species: 13, 4, 26, and 1 genomic sequence variants in 9 chimpanzees, 3 gorillas, 14 cynomolgus macaques, and 2 spider monkeys. However, these data have not been independently confirmed and cannot be considered as unequivocally convincing. Nevertheless, it well may be that the diversity of simian papillomaviruses is much greater than is currently known.

19.3. GENOMIC ORGANIZATION AND GENE PRODUCTS

The genomes of human and simian papillomaviruses are similar, although there are some differences in the repertoire of early genes of monkey and human/ape viruses. Double-stranded genomic DNA is a circular molecule about 8,000 bp in length. All papillomavirus genes are encoded by the same strand of DNA. Two of the genes, L1 and L2 (L stands for “late”) are structural. They encode capsid proteins. The “L genes” are expressed only in productively infected cells. The remaining genes, E1, E2, E4, E5, E6, and E7 (E stands for “early”), are nonstructural. They encode proteins required for viral replication. Importantly, two of the “E genes,” E6 and E7, are the oncogenes for the high-risk HPVs.

The L1 and L2 genes occupy adjacent positions spanning approximately 40% of the genome. The E genes cover roughly half of the genome. There are many gene overlaps in the “E” region of the genome. Between the L1 and E6 genes there is a noncoding region containing the origin of replication and transcription control elements. This region of the genome is designated as the long control region (LCR) or, less frequently, as the upstream regulatory region (URR) (Figure 19.2). Major characteristics of the simian papillomavirus genes and their predicted products are presented in Table 19.2.

The papillomavirus genome expression pattern is quite complex. More than a dozen alternatively spliced HPV RNA species have been described (reviewed in Howley and Lowy⁹).

19.4. OVERVIEW OF REPLICATION CYCLE

The hallmark of papillomavirus replication is the link between productive virus replication and the differentiation state of the epithelial cell. Figuratively speaking, the exclusive right for the production of complete papillomavirus virions belongs to differentiated epithelial cells. In multilayer squamous skin epithelium the assembly of papillomavirus virions starts in the upper spinous layer and is completed in the granular layer.

For this reason, human and simian papillomaviruses cannot be propagated *in vitro* in monolayer or suspension cell lines, which puts a rigid constraint on unraveling the mechanism of papillomavirus replication. However, production of infectious HPV can be achieved by transfection of HEK 293T cells with an expression vector for L1 and L2 capsid proteins and circular HPV genome.¹⁹

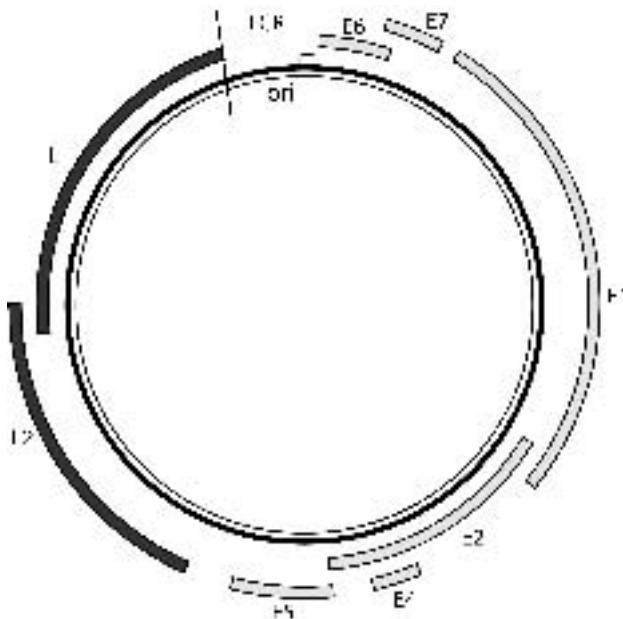


Figure 19.2. Schematic map of simian papilloma virus genome. Genomic DNA is circular, about 8,000 bp in length. One strand, shown in bold, encodes all viral proteins. Late (L) and early (E) genes (shown in dark-gray and light-gray, respectively) do not overlap. Noncoding LCR contains the origin of replication (ori) and transcription control elements.

Nothing concrete is known about the replication of simian papillomaviruses and it is presumed that the replication mechanisms are the same for HPV and its simian counterparts. It is worth mentioning, however, that the mechanisms of HPV replication are also largely deduced from the data obtained using bovine papillomas virus which can be cultured *in vitro*.

The primary targets for papillomaviruses are basal keratinocytes. The identity of papillomavirus receptor(s) has not been established with any certainty. Papillomavirus entry into the cell likely occurs through clathrin-dependent receptor-mediated endocytosis.⁹ The uncoating occurs in the endosome. Viral genomic DNA is released in the cytoplasm and transported to the

nucleus. Viral capsid protein L2 plays an important role in the uncoating and nuclear transport of the genomic DNA. The transcription of viral DNA is a complex process due to the existence of multiple promoters and complex splicing. The transcription patterns are also different in various types of host cells. The nonstructural "E" genes are always transcribed and translated first. Generally, the more differentiated an epithelial host cell is, the more complete is the set of transcribed and translated E genes. The replication of viral DNA is mediated by cellular DNA polymerase. Active viral DNA replication occurs only in the differentiated epithelial cells located in the upper spinous layer. The synthesis of the structural capsid proteins L1 and L2 is also restricted to the

Table 19.2. Predicted Proteins of Simian Papillomaviruses

Protein Virus	L1	L2	E1	E2	E4	E5	E6	E7
Common chimpanzee papillomavirus	502*	463	649	377	118	95	153	100
Pigmy chimpanzee papillomavirus	503	464	649	378	109	95	151	99
Rhesus monkey papillomavirus	502	467	626	367	?†	43, 45‡	192	114
Cynomolgus macaque papillomavirus	513	524	604	504	?	?	139	99

*Number of amino acids.

†ORF is not identified.

‡Stop codon in the middle of E5-homologous segment (ORF 5a and 5b).

? No data.

differentiated cells of the same layer. The mature virions are accumulated in the dying and dead epithelial cells in the granular layer and desquamating *stratum corneum*. The recombinant L1 protein expressed in mammalian and nonmammalian cells, either alone or in combination with L2, self-assembles in virus-like particles (VLPs). VLPs are morphologically indistinguishable from intact virions; they are also strongly immunogenic. Papillomavirus vaccines are composed of VLPs.

19.5. MECHANISMS OF ONCOGENESIS

Some HPVs are etiologically linked with several types of cancer, most importantly with cervical carcinoma. Other HPV-associated cancers are skin cancers, particularly in *epidermodysplasia verruciformes* patients, and some head and neck cancers.

In terms of oncogenicity, the most dangerous HPV types are HPV-16 and HPV-18. However, many other types are also high-risk (HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, -82).⁹

Only early genes are expressed in papillomavirus-transformed/malignant cells, and E7 and E6 are the major players in the mechanisms underlying the oncogenicity of papillomaviruses. An important component of these mechanisms is functional inactivation of the tumor suppressor proteins Rb and p53, by the E7 and E6 proteins, respectively. Many details of multiple interactions between the proteins involved in the regulation of epithelial cell growth, which are triggered by the overexpression of E7 and E6, are described in the HPV and bovine papillomavirus systems.⁹ Many of them are shared, presumably, with simian papillomaviruses. However, very little is known definitely about the degree of similarity in this respect.

Typically, but not always, the papillomavirus genome is integrated into the cellular genome in cancer cells. Integration is usually associated with the deletion of large segments of the genome. However, the E7/E6 genes always remain intact and expressed.

E7 and E6 proteins of oncogenic papillomaviruses also induce chromosomal instability in nonproductively infected host cells by affecting centrosome duplication and activating telomerase.

19.6. PAPILLOMAVIRUS IN THE SIMIAN SPECIES

Lesions similar to those caused by the HPVs have been observed in several simian species.^{2,3,12,18,20} Papillo-

maviruses were detected in these lesions using electron microscopy, immunological and molecular methods. The degree to which these “isolates” are characterized varies. In the most advanced cases, the complete genome has been sequenced. Concrete data relevant to the individual simian papillomaviruses is presented in Sections 19.6.1–19.6.3.

19.6.1. Rhesus Monkey Papillomavirus

The first and the only simian papillomavirus recognized by the ICTV as a species, rhesus monkey papillomavirus (RhPV), was described in 1988.¹⁰ This virus was shown to be sexually transmitted in a rhesus monkey colony.¹⁶ Papillomavirus-specific sequences were detected in the lymph node metastasis of a penile squamous cell carcinoma from a rhesus monkey using HPV probes. The RhPV genome was integrated in this tumor; the integration breakpoint was located in the L1 gene.¹⁵ The complete genome of RhPV was sequenced and shown to be highly homologous to the HPV-16 genome (maximum nucleotide sequence homology 91%).^{4,5,8,15} Cotransfection of the cloned RhPV genome and activated *ras* oncogene into primary epithelial rat cells conferred the tumor phenotype on them: the ability for anchorage-independent growth and tumorigenicity in *nude* mice.²³ In this respect, RhPV is similar to HPV-16, a human oncogenic virus. The transforming activity of RhPV, being glucocorticoid hormone (dexamethasone)-independent, is even more potent than that of HPV-16.²³

Recombinant RhPV proteins expressed in bacterial cells were used as the antigens for detection of anti-RhPV antibodies in several captive rhesus monkey colonies. Reactivity with at least one RhPV protein was detected in 38–62% of animals.¹⁴ Virus-specific DNA was detected in a fraction of seropositive animals, 10–50% depending on the method used for the detection of viral DNA. The frequency of viral DNA-positive–seronegative cases in the paired samples panel ($n = 24$) was 8%.

The apparent diversity of papillomaviruses infecting rhesus macaques is profound. Thirteen RhPV sequence variants (RhPV-a through RhPV-m, plus prototype RhPV-1) are distinguishable within just the L1 gene sequence.⁴

19.6.2. Cynomolgus Macaque Papillomavirus

The first data indicating the existence of cynomolgus macaque papillomavirus were reported in 1997.⁵ A

frag-virus, named MfPV-a, was amplified by polymerase chain reaction (PCR) from one out of seven of the genital specimens tested using consensus MY09-MY11 primers targeting a fragment of the L1 gene.

A few years later, papillomavirus particles were detected in penile papillomas (known in human pathology as *Condyloma acuminatum*) which were observed in a wild-caught *Macaca fascicularis*. The attempts to identify this virus using generic HPV PCRs (MY09-MY11, GP5-GP6) have failed.⁶

Systematic histological examination of cervical and vaginal specimens obtained from 385 female cynomolgus macaques revealed intraepithelial neoplasms in 20 animals.²⁷ In most cases, the lesions were benign vaginal papillomas or mild to severe intraepithelial dysplasias. Invasive cervical carcinomas were diagnosed in two animals. Immunostaining using three papillomavirus-specific antibodies (against bovine papillomavirus 1, HPV-16/18, and HPV-1/6/11/16/18/31) was positive with at least one antibody in all 20 cases. Staining was positive most frequently when hyperimmune anti-bovine papillomavirus 1 serum was used.

Cynomolgus macaques are infected, apparently, with multiple variants of papillomaviruses.²⁸ Based on the sequence of a relatively short L1 gene fragment, seven papillomavirus variants have been identified in this species. Interestingly, the most prevalent variant, named RhPV-d, is shared by rhesus and cynomolgus monkeys. This observation is unexpected because papillomaviruses are usually strictly species-specific.

Transmission experiments showed that RhPV-d virus is infectious in cynomolgus monkeys. Depending on the papillomavirus markers used, the success rate of transmission was 30–40% and the incubation period ranged from 6–24 weeks.²⁸ This model appears to be promising, although its utility for studying prophylactic and therapeutic approaches has yet to be proved.

19.6.3. Chimpanzee Papillomaviruses

Papillomaviruses are found in both pygmy and common chimpanzees (*Pan paniscus* and *Pan troglodytes*, respectively).^{24–26} The complete genomes of both viruses, named PCPV (pygmy chimpanzee PV) and CCPV (common chimpanzee PV), have been sequenced (GenBank Acc. Nos. X62844 and NC_001838). As expected, these two papillomaviruses are the closest relatives among all papillomaviruses (89% nucleotide chimpanzee homology). Phylogenetic analysis places them

in the same lineage as the HPV-6 species.^{8,25} However, the closest relatives of PCPV and CCPV, among HPVs belonging to this lineage, are HPV-13 (85% nucleotide homology) and HPV-44, -74, -55 (80% homology); the prototype of the HPV-6 lineage is more distant. The genomes of PCPV/CCPV and related HPVs are strictly collinear.

PCPV originated from oral epithelial hyperplasia. An outbreak of this disease was observed in a colony of pygmy chimpanzee.²⁶ The origin of CCPV is not clear.

19.6.4. Colobus Papillomaviruses

A number of reports described papillomaviruses in colobus monkeys (*Colobus guereza*). The first finding was reported in 1980.²⁶ Viral particles with papillomavirus morphology were detected in the papillomatous lesions on the hands and feet of four colobus monkeys. No molecular or immunological characterization of this virus has been reported.

Several years later, papillomavirus-like particles were detected by electron microscopy in the nuclei of penile papillomas in a colobus monkey.¹³ This virus was shown to be related to HPV-11, as indicated by molecular cross-hybridization at low stringency conditions.

Shortly thereafter HPV-related virus was found in the cutaneous papilloma of another colobus monkey.¹⁰ To distinguish colobus papillomaviruses originating from penile and cutaneous papillomas, they were named Colobus papilloma virus 1 (CoPV-1) and 2 (CoPV-2), respectively.

Subsequent studies showed that partial genomic sequences of CoPV-1 and CoPV-2 (GenBank Acc. Nos. U72629 and U72630) are less related to each other than they are to the genital and cutaneous HPVs.^{4,21} Phylogenetic analysis of partial L1 gene sequences places CoPV-1 and CoPV-2 in different papillomavirus genera, *Alfapapillomavirus* and *Betapapillomavirus*, respectively.^{5,7} Unfortunately, complete genome sequences of CoPV-1 and CoPV-2 are not known and it is not clear if the materials in which these viruses were originally detected are still available.

19.7. IMMUNOGENICITY OF HPV VACCINES IN NHPs

NHPs, including chimpanzees, are not susceptible to HPV and therefore cannot be used for testing the protective efficacy of HPV vaccine. However, several studies have been published where the immunogenicity

of various HPV vaccine preparations was tested in NHPs.^{11,17,22}

In one of these studies it was shown that African green monkeys immunized with HPV-11 VLPs produce specific neutralizing antibodies not only in serum, but also in cervicovaginal secretions.¹¹ These data provided suggestive evidence that systemic immunization with HPV VLPs induces mucosal immunity potentially protective to sexually transmitted HPV.

Researchers at Merck and Co. a producer of the FDA-approved HPV vaccine, use chimpanzees and rhesus monkeys for preclinical immunogenicity studies of the quadrivalent (HPV-6, -11, -16, -18) vaccine and the effectiveness of the proprietary aluminum-based adjuvant.^{17,22} It was shown that the vaccine induces neutralizing antibodies and cytokine, both Th1 and Th2 responses, but rarely a cytotoxic T-lymphocyte response. The immunogenicity of the vaccine was better when it was administered in the adjuvant. The by-product of these studies was development of the method for the simultaneous detection of antibodies belonging to different types and subtypes of rhesus monkey immunoglobulins (IgG total, IgG1, IgG2, IgG4, IgA, and IgM).²² This method can be adapted for the detection of antibodies against other simian viruses.

19.8. SUMMARY

Papillomaviruses are small, nonenveloped DNA viruses with circular dsDNA and a characteristic capsid consisting of 72 capsomers. Papillomaviruses comprise the family *Papillomaviridae* that includes more than 100 HPVs and several simian papillomaviruses which are closely related to some HPVs. An unusual and characteristic biological property of papillomaviruses is a tight link between their replication and the differentiation state of host epithelial cells. As a result, papillomaviruses cannot be propagated in vitro using established cell lines or primary monolayer cultures.

Papillomaviruses are mainly known as the causal agents of various mucocutaneous tumors, most notably cervical cancer. It is estimated that about 10% of all human cancers are caused by HPVs. An effective vaccine against the most dangerous sexually transmitted HPV types (HPV-16 and HPV-18) has been recently introduced.

The knowledge of simian papillomaviruses is fragmentary. The complete genome sequences of four simian papillomaviruses are known: pygmy and com-

mon chimpanzee papillomaviruses (PCPV and CCPV); rhesus and cynomolgus macaque papillomaviruses (RhPV and MfPV). Papillomaviruses are also described in colobus monkeys. However, the diversity of simian papillomaviruses is probably much greater than is currently known.

Simian papillomavirus can cause genital neoplasia in NHPs. Experimental transmission of macaque papillomaviruses is a promising model of human genital cancer, although it is not yet fully developed.

REFERENCES

1. Antonsson, A. and B. G. Hansson. 2002. Healthy skin of many animal species harbors papillomaviruses which are closely related to their human counterparts. *J. Virol.* 76(24):12537–12542.
2. Boever, W. J. and T. Kern. 1976. Papillomas in black and white colobus monkeys (*Colobus polykomus*). *J. Wildl. Dis.* 12(2):180–181.
3. Brown, R. J., W. E. Britz, J. L. Kupper, and W. P. Trevethan. 1972. Cutaneous horn in a rhesus monkey. *Lab. Anim. Sci.* 22(1):112–113.
4. Chan, S. Y., H. U. Bernard, M. Ratterree, T. A. Birkebak, A. J. Faras, and R. S. Ostrow. 1997. Genomic diversity and evolution of papillomaviruses in rhesus monkeys. *J. Virol.* 71(7):4938–4943.
5. Chan, S. Y., R. S. Ostrow, A. J. Faras, and H. U. Bernard. 1997. Genital papillomaviruses (PVs) and epidermodysplasia verruciformis PVs occur in the same monkey species: implications for PV evolution. *Virology* 228(2):213–217.
6. Gordon, H. P., D. A. Reim, and S. A. McClain. 2000. Condyloma acuminatum in a cynomolgus monkey (*Macaca fascicularis*). *Contemp. Top. Lab. Anim. Sci.* 39(2):30–33.
7. Gottschling, M., A. Kohler, E. Stockfleth, and I. Nindl. 2007. Phylogenetic analysis of beta-papillomaviruses as inferred from nucleotide and amino acid sequence data. *Mol. Phylogenet. Evol.* 42(1):213–222.
8. Gottschling, M., A. Stamatakis, I. Nindl, E. Stockfleth, A. Alonso, and I. G. Bravo. 2007. Multiple evolutionary mechanisms drive papillomavirus diversification. *Mol. Biol. Evol.* 24(5):1242–1258.
9. Howley, P. M. and D. R. Lowy. 2007. Papillomaviruses. In: Knipe, D. M. and P. M. Howley (eds), *Fields Virology*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, Wolters Kluwer Business, pp. 2299–2354.
10. Kloster, B. E., D. A. Manias, R. S. Ostrow, M. K. Shaver, S. W. McPherson, S. R. Rangen, H. Uno, and A. J. Faras. 1988. Molecular cloning and characterization of the DNA of two papillomaviruses from monkeys. *Virology* 166(1):30–40.

11. Lowe, R. S., D. R. Brown, J. T. Bryan, J. C. Cook, H. A. George, K. J. Hofmann, W. M. Hurni, J. G. Joyce, E. D. Lehman, H. Z. Markus, M. P. Neepur, L. D. Schultz, A. R. Shaw, and K. U. Jansen. 1997. Human papillomavirus type 11 (HPV-11) neutralizing antibodies in the serum and genital mucosal secretions of African green monkeys immunized with HPV-11 virus-like particles expressed in yeast. *J. Infect. Dis.* 176(5):1141–1145.
12. Lucke, B., H. Ratcliffe, and C. Breedis. 1950. Transmissible papilloma in monkeys. *Am. Soc. Exp. Pathol.* 9:337.
13. O'Banion, M. K., J. P. Sundberg, A. L. Shima, and M. E. Reichmann. 1987. Venereal papilloma and papillomavirus in a colobus monkey (*Colobus guereza*). *Intervirology* 28(4):232–237.
14. Ostrow, R. S., S. M. Coughlin, R. C. McGlennen, A. N. Johnson, M. S. Ratterree, J. Scheffler, N. Yaegashi, D. A. Galloway, and A. J. Faras. 1995. Serological and molecular evidence of rhesus papillomavirus type 1 infections in tissues from geographically distinct institutions. *J. Gen. Virol.* 76(Pt 2):293–299.
15. Ostrow, R. S., K. V. LaBresh, and A. J. Faras. 1991. Characterization of the complete RhPV 1 genomic sequence and an integration locus from a metastatic tumor. *Virology* 181(1):424–429.
16. Ostrow, R. S., R. C. McGlennen, M. K. Shaver, B. E. Kloster, D. Houser, and A. J. Faras. 1990. A rhesus monkey model for sexual transmission of a papillomavirus isolated from a squamous cell carcinoma. *Proc. Natl. Acad. Sci. U. S. A.* 87(20):8170–8174.
17. Palker, T. J., J. M. Monteiro, M. M. Martin, C. Kakareka, J. F. Smith, J. C. Cook, J. G. Joyce, and K. U. Jansen. 2001. Antibody, cytokine and cytotoxic T lymphocyte responses in chimpanzees immunized with human papillomavirus virus-like particles. *Vaccine* 19(27):3733–3743.
18. Patterson, M. M., A. B. Rogers, K. G. Mansfield, and M. D. Schrenzel. 2005. Oral papillomas and papilliform lesions in rhesus macaques (*Macaca mulatta*). *Comp. Med.* 55(1):75–79.
19. Pyeon, D., P. F. Lambert, and P. Ahlquist. 2005. Production of infectious human papillomavirus independently of viral replication and epithelial cell differentiation. *Proc. Natl. Acad. Sci. U. S. A.* 102(26):9311–9316.
20. Rangan, S. R., A. Gutter, G. B. Baskin, and D. Anderson. 1980. Virus associated papillomas in colobus monkeys (*Colobus guereza*). *Lab. Anim. Sci.* 30(5):885–889.
21. Reszka, A. A., J. P. Sundberg, and M. E. Reichmann. 1991. In vitro transformation and molecular characterization of Colobus monkey venereal papillomavirus DNA. *Virology* 181(2):787–792.
22. Ruiz, W., W. L. McClements, K. U. Jansen, and M. T. Esser. 2005. Kinetics and isotype profile of antibody responses in rhesus macaques induced following vaccination with HPV 6, 11, 16 and 18 L1-virus-like particles formulated with or without Merck aluminum adjuvant. *J. Immune Based Ther. Vaccines* 3(1):2.
23. Schneider, J. F., R. C. McGlennen, K. V. LaBresh, R. S. Ostrow, and A. J. Faras. 1991. Rhesus papillomavirus type 1 cooperates with activated ras in transforming primary epithelial rat cells independent of dexamethasone. *J. Virol.* 65(6):3354–3358.
24. Sundberg, J. P., A. L. Shima, and D. L. Adkison. 1992. Oral papillomavirus infection in a pygmy chimpanzee (*Pan paniscus*). *J. Vet. Diagn. Invest.* 4(1):70–74.
25. Van Ranst, M., A. Fuse, P. Fitien, E. Beuken, H. Pfister, R. D. Burk, and G. Opdenakker. 1992. Human papillomavirus type 13 and pygmy chimpanzee papillomavirus type 1: comparison of the genome organizations. *Virology* 190(2):587–596.
26. Van Ranst, M., A. Fuse, H. Sobis, W. De Meirichy, S. M. Syrjanen, A. Billiau, and G. Opdenakker. 1991. A papillomavirus related to HPV type 13 in oral focal epithelial hyperplasia in the pygmy chimpanzee. *J. Oral Pathol. Med.* 20(7):325–331.
27. Wood, C. E., H. Borgerink, T. C. Register, L. Scott, and J. M. Cline. 2004. Cervical and vaginal epithelial neoplasms in cynomolgus monkeys. *Vet. Pathol.* 41(2):108–115.
28. Wood, C. E., Z. Chen, J. M. Cline, B. E. Miller, and R. D. Burk. 2007. Characterization and experimental transmission of an oncogenic papillomavirus in female macaques. *J. Virol.* 81(12):6339–6345.

20

Hepadnaviruses

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- 20.2 Classification, nomenclature, and morphology
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20.1. INTRODUCTION

The prototype hepadnavirus, human hepatitis B virus (HBV), as its name suggests is a causal agent of hepatitis B. Hepatitis B, although being preventable now, still has a profound health impact. It is estimated that there are 400 million HBV carriers in the world and about a quarter of them are expected to die from late complications of hepatitis B, such as liver cirrhosis and hepatocellular cancer.

HBV research has a remarkable history. The discovery of HBV was honored by award of the Nobel Prize in Medicine to Baruch Blumberg in 1976. The full history of this discovery has been described.² HBV was the first virus to be virtually eliminated from the blood supply as a result of the introduction of a highly effective test for the detection of the virus. The HBV vaccine is also one of the most successful human vaccines and was the first recombinant human vaccine to be widely used in clinical practice. Importantly, immunization with HBV vaccine prevents not only hepatitis B but also substantially reduces the incidence of liver cirrhosis and hepatocellular carcinoma.

Hepadnaviruses are a very interesting virus group from a basic research aspect. The genome of these viruses exists in DNA and RNA forms at the different stages of the replication cycle, and a reverse transcription step is essential for their replication. Although there are some similarities in the mechanisms of replication of hepadnaviruses and spumaviruses (see Chapter 9), hepadnaviruses are clearly distinct from the retroviruses.

Hepadnavirus infection in nonhuman primates (NHPs) was described soon after discovery of the human HBV.⁹ Over the following 20 years a number of reports on HBV infection in NHPs, primarily apes, were published (reviewed in Robertson and Margolis²³). However, for a long time simian and human HBVs could not be reliably distinguished and the dominant view was that they were variants of the same virus. The change in this paradigm occurred in the late 1980s and early 1990s when the complete genomes of several simian HBVs were sequenced and were shown to be clearly distinct from all genotypes of human HBV.

Perhaps the key question in the long-term perspective of hepadnavirus research is whether or not simian and human reservoirs are epidemiologically linked. If the answer to this question is positive, then the prospect of HBV eradication from the human population by universal vaccination may be doubtful.

20.2. CLASSIFICATION, NOMENCLATURE, AND MORPHOLOGY

The name “hepatna” is derived from the Latin word *hepar* (liver) and DNA. This name implies that hepadnaviruses are DNA viruses. However, hepadnaviruses cannot be considered as “ordinary” DNA viruses. Together with the retroviruses they are combined into the group referred to as “RNA and DNA reverse transcribing

viruses." The formal taxon for hepadnaviruses in the International Committee on Taxonomy of Viruses (ICTV) classification is the *Hepadnaviridae* family. There are two genera in this family: *Orthohepadnavirus* and *Avihepadnavirus*. The latter includes avian hepadnaviruses. All primate hepadnaviruses belong to the *Orthohepadnavirus* genus.

The chimpanzee (ChHBV), gibbon (GiHBV), and orangutan (OrHBV) hepadnaviruses are clearly distinguishable from human and other orthohepadnaviruses by phylogenetic analysis. Nevertheless, these viruses are not recognized as viral species in the latest ICTV classification. The only simian hepadnavirus which has been "awarded" a species rank is the woolly monkey hepatitis B virus (WmHBV).

Virions of all hepadnavirus have two structural elements, the core and the envelope. The size of the complete virions, also referred to as Dane particles, is about 42 nm. The nucleocapsid or "core" is icosahedral and about 28 nm in diameter. It consists of 120 capsomers, each composed of two molecules of the core proteins. The envelope is made up of three different forms of the surface antigen (HBsAg) and cellular lipids. In addition to Dane particles, hepadnaviral preparations contain an excess of spherical (22 nm in diameter) and tubular particles consisting of HBsAg. These particles are not infectious.

20.3. GENOME COMPOSITION AND GENE PRODUCTS

The genome of hepadnaviruses is remarkable in several respects. It is the only partially double-stranded DNA viral genome, one of the smallest (approximately 3,200 bp), and perhaps the most "economically packed" genome among animal viruses. All hepadnavirus proteins are encoded by the minus-strand and every nucleotide in this strand participates in protein encoding.

The composition of human and simian HBV genomes is identical. HBV virions contain the relaxed circular (RC) genomic DNA which is composed of two strands, the "minus" and "plus." The minus-strand spans the entire genome whereas the plus-strand spans only about two-thirds of the genome. The length of the plus-strand varies in different virions, but all plus-strands are 5'-coterminal; that is, they all have the same 5'-end.

There are four genes (S, C, P, and X) encoding the surface, core, polymerase, and pX proteins, respectively. The largest gene overlaps the three other genes; the S

gene is located completely within the P gene, but in a different reading frame. The reading frame of the C gene is also different, whereas the S and X genes are in the same reading frame (Figure 20.1).

The S and C genes each encode more than one protein (Figure 20.2). There are three "sub-genes" within the S gene (pre-S1, pre-S2, and S proper) which encode the large (L), medium (M), and small (S) proteins forming HBV surface antigen (HBsAg). The C gene consists of the pre-C and C proper sub-genes that

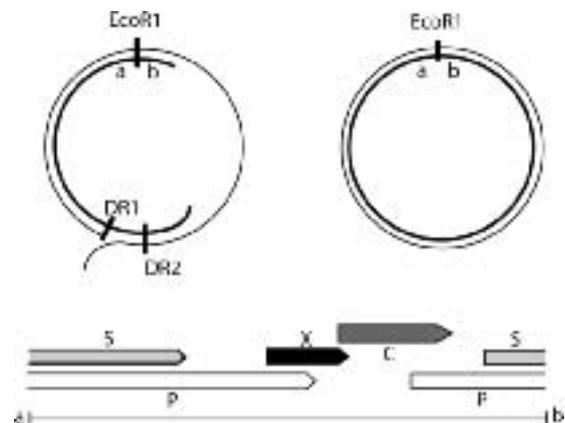


Figure 20.1. Hepadnavirus schematic genome map. (Top left) Virion genomic DNA consisting of two strands, the "minus" and "plus." The minus-strand spans the entire genome (outer circle) whereas the plus-strand spans only about two-thirds of the genome (inner circle). Sequences of plus and minus strands are not complementary in the region between DR1 and DR2 (direct repeats). (Top right) Viral DNA after the completion of synthesis of the plus-strand (inner circle). All viral proteins are encoded by the plus-strand. EcoR1, a single cleavage site for EcoR-1 restriction enzyme defines the first (a) and the last (b) positions in the genome. (Bottom) Plus-strand of the genome "opened" for clarity only. Positions of the genes (open reading frames—ORFs) and the direction of their transcription/translation are shown by the arrows. S, C, P, and X, ORFs encoding the surface, core, polymerase, and pX proteins, respectively.

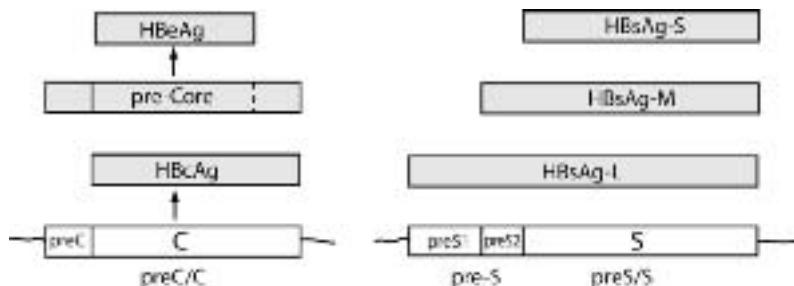


Figure 20.2. Processing of hepadnavirus structural proteins. Two viral proteins are encoded by C (core) genes. The major core antigen (HBcAg) is encoded by C ORF. The second C protein (pre-Core protein) is translated from entire pre-C/C ORF and undergoes truncation at both ends. The truncated pre-Core protein is named E antigen (HBeAg). HBcAg and HBeAg make up the capsid (core) of the virion. Three forms of the surface antigen (HBsAg), the large (L), medium (M), and small (S) are translated from pre-S1 + pre-S2 + S, pre-S2 + S and S ORFs, respectively. HBsAg-S is the most abundant in virions and subviral particles. HBsAg-L is enriched in the virion envelope (approximately 15% of envelope protein) but only traces of this protein are present in the subviral particles. HBsAg-M constitutes 10–15% of viral envelope proteins both in the virions and subviral particles.

encode two proteins: the core antigen (HBcAg) and a truncated form of HBcAg designated the “e antigen” (HBeAg). The HBcAg is encoded by the C proper sub-gene whereas HBeAg is encoded by both the pre-C and C proper sub-genes. The HBeAg precursor undergoes posttranslational processing which cleaves off a 15-kDa polypeptide from the C-terminus. As a result, HBeAg is smaller than HBcAg. The HBeAg polypeptide chain is also folded differently to HBcAg—hence the different antigenicity of these two antigens. The virion capsid (core) is composed of HBcAg and HBeAg.

The capsid is enclosed in an envelope that consists of HBsAg and cell-derived lipids. Molecules of HBsAg can also self-assemble into 15–25 nm spherical particles and tubules 20 nm in diameter. Neither type of HBsAg particles contain genomic DNA or any other viral proteins, and are thus noninfectious.

The polymerase (P) is the largest viral protein; it consists of four domains: terminal protein (TP), spacer (SP), reverse transcriptase (RT), and RNaseH (RH). The RT domain is further subdivided into seven subdomains designated A to G. The catalytic site of RT, the highly conserved YMDD motif, is located in the C subdomain.

The smallest viral protein, pX, is the least conserved among hepadnaviral proteins. Its function(s) is substantially less clear than are the functions of other viral pro-

teins. However, it is known that this protein is required for efficient infection *in vivo*.

Properties of the human and simian HBV proteins are very similar. Some amino acid substitutions in simian HBV proteins are characteristic of the simian viruses.²³

20.4. OVERVIEW OF REPLICATION CYCLE

HBV and related simian viruses are strictly hepatotropic. The main and possibly the only cell target permissive for HBV replication *in vivo* is the hepatocyte.

The virus binds to a cellular receptor via the pre-S1 domain of L-HBsAg. The cell surface receptor for HBVs has proven elusive. Although a number of host proteins have been claimed to contribute to HBV entry in various model systems, none of them has been convincingly identified as the HBV receptor.

The mechanisms of entry of the virion into the cell, and the uncoating and release of genomic DNA into the nucleus, are virtually unknown. It is clear, however, that the virions strip off the envelope in the cytoplasm; the genomic DNA is released from the nucleocapsids, possibly within a nuclear pore, and is delivered into the nucleus.

After delivery of the genomic DNA to the nucleus, the plus-strand is extended the full length of the

minus-strand by the cellular DNA replication machinery. As a result, the relaxed circular DNA (rc-DNA) is converted into covalently closed circular DNA (cccDNA). The minus-strand of cccDNA is then transcribed by cellular RNA polymerase II. Four mRNA species (3.5, 2.4, 2.1, and 0.8 kb) are produced. The largest mRNA plays a dual role. On the one hand, it serves as pregenomic RNA (pgRNA) which is used as a template by the viral RT for the synthesis of genomic DNA molecules. On the other hand, the viral core proteins are translated from this RNA. The surface proteins pre-S1 (HBsAg-L), S (HBsAg-S), and pX are translated from 2.4 kb, 2.1 kb, and 0.8 kb mRNAs, respectively.

Importantly, assembly of virions and encapsulation of pgRNA precede the start of reverse transcription. Thus, conversion of the pgRNA genome into a DNA genome occurs inside hepadnavirus virions. The RNA-to-DNA conversion may take two alternative routes. In the majority of virions (80–95%) the pgRNA genome is partially converted into a double-stranded DNA genome. These virions are fully replication-competent. In a small fraction (5–20%) the end product of the reverse transcription is a double-stranded linear DNA. These virions are defective; they can initiate infection but cannot produce infectious progeny.

The release of complete virions (Dane particles) from the infected cell is not accompanied by rupture of the cell membrane. The exact mechanism of the release is unknown.

20.5. SIMIAN HBV-RELATED VIRUSES

HBV-related hepadnaviruses have been found in common chimpanzees (*Pan troglodytes* subspecies), gibbons (*Hylobates* and *Nomascus* species), gorillas (*Gorilla gorilla*), orangutans (*Pongo pygmaeus*), and woolly monkeys (*Lagothrix lagotricha*).

Although findings of serological HBV markers of NHP species, other than those listed above, were reported in the early years of hepadnavirus research^{3,22} none of them was subsequently confirmed by the recovery and unequivocal identification of genomic DNA. The latest well-controlled survey failed to detect hepadnavirus infection either serologically or by polymerase chain reaction (PCR) in African monkeys (*Chlorocebus aethiops*, *Cercopithecus torquatus*, *C. mona*, *C. nictitans*, *C. erythrrotis*, *Cebus albifrons*, *Mandrillus sphinx*, *M. leucophaeus*), or in New World monkeys (*Saimiri sciureus*, *Callithrix jacchus*, *Saguinus oedipus*).²⁶

Thus, although the available data on the distribution of hepadnaviruses among simian species are still fragmentary, it is unlikely that they are harbored by many monkey species. Apparently, simian hepadnavirus infections are primarily restricted to apes.

20.5.1. Detection of Simian HBVs

Permanent cell lines and primary cultures routinely used for virus isolation are not permissive for orthohepadnaviruses.

Active HBV infection in simian hosts can be detected by serological tests designed for HBsAg and/or by PCR. The detection of HBsAg is a reliable marker of HBV infection in NHPs. Even the most divergent simian HBV, the WmHBV, can be detected by the human HBsAg test. However, detection of HBsAg alone cannot be considered conclusive proof of HBV infection in NHPs. Even when high-quality HBsAg tests are used, false-positive results may occur in rare cases. In addition, the tests for human HBsAg do not discriminate between human and simian viruses.

Currently, PCR is the method of choice for detecting simian HBV carriers. A number of such tests are used by different research groups.^{13–15,24,26,29} More than 40 complete genome sequences of simian HBVs are currently available, providing the ability to design PCR tests that are either generic, or specific for certain groups of simian HBVs.

20.5.2. Chimpanzee Hepadnavirus

Carriers of HBV-like virus have been consistently detected among wild, wild-caught, and captive common chimpanzees.^{3,5,6,9,10,13,16,26–28,35} The cumulative prevalence of ChHBV carriers among almost 600 animals tested is about 10%.

The first complete genomic sequence of ChHBV was reported in 1988.³⁰ However, the “separateness” of ChHBV from all HBV genotypes was only conclusively established a few years later.^{19,21}

At the time of writing (Spring 2008), 13 ChHBV genomes have been completely sequenced.³⁴ Four ChHBV clades (ChHBV-1 to -4) can be distinguished by phylogenetic analysis (Figure 20.3).

Known ChHBV variants originate from *P. t. troglodytes*, *P. t. schweinfurthii*, *P. t. vellerosus*, and *P. t. verus*. However, only one isolate (Ch-RC170-1Gab) has been recovered from a wild animal, a common

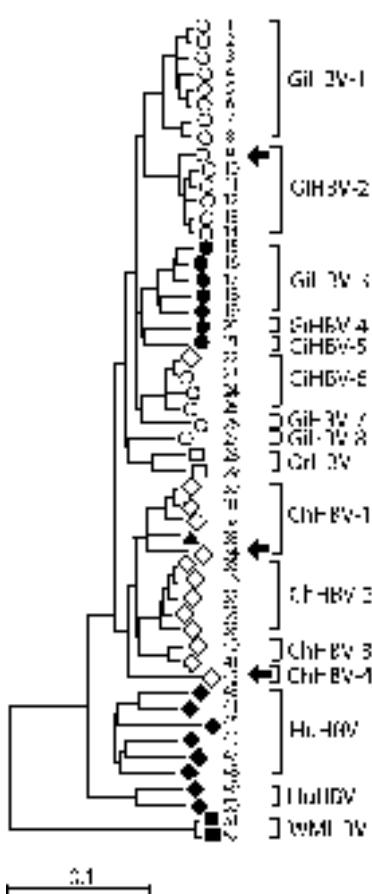


Figure 20.3. Phylogeny of primate hepadnaviruses. Phylogenetic tree (neighbor-joining analysis) of complete genome sequences. (Adapted from Yang *et al.*³⁴ with permission.) **1 through 42 and 51, 52—simian HBVs:** GiHBV-1 through 8, gibbon HBV genotypes; PrHBV, orangutan HBV genotype; ChHBV-1 through 4, common chimpanzee genotypes; WMHBV, woolly monkey HBV genotype. Recombinant genomes are indicated by arrow. Host species: ○, *Hylobates* spp.; ●, *Nomascus* spp.; □, *Pongo pygmaeus*; ▲, *Gorilla gorilla*; ◇, *Pan triglodytes*; ♦, *Homo sapiens*; ■, *Lagothrix lagotricha*. GenBank Accession Numbers for simian HBVs (**recombinants** in bold): 1, AY781180; 2, AY781181; 3, AY781177; 4, AY781179; 5, AY781182; 6, AY781178; 7, AY781183; 8, AY781187; 9, **AJ131572**; 10,

chimpanzee (*P. t. troglodytes*), living in the Lopé reserve in Gabon.^{12,13}

The composition of the ChHBV genome is the same as that of human HBV, although there are several amino acid substitutions in the predicted proteins.

ChHBV is presumed to be transmitted in natural conditions both from mother-to-infant and sexually. The virus is also shed in the feces, and this has been successfully used for noninvasive sampling of wild chimpanzees and gorillas.¹³ However, ChHBV prevalence determined by the analysis of fecal samples may underestimate the true prevalence of this infection in wild chimpanzee populations, as it is not known if fecal shedding occurs in all virus carriers.

No transmission of virus from ChHBV-positive chimpanzees to animal caretakers has been documented.²² In contrast, a few cases of accidental human-to-chimpanzee transmission of HBV are known. Three of 16 HBV-positive chimpanzees in the London Zoo were infected with human HBV, whereas the other 13 animals carried ChHBV.^{6,27} How these chimpanzees acquired the human virus is not clear.

Figure 20.3. (Continued) AB037927; 11, AB037928; 12, AY781184; 13, AY781185; 14, AY781186; 15, AJ131568; 16, AY077736; 17, AY330914; 18, AJ131574; 19, AY077735; 20, AJ131573; 21, AY330915; 22, AJ131575; 23, AY330913; 24, U46935; 25, AJ131569; 26, AJ131571; 27, AY330917; 28, AF193863; 29, AF193864; 30, AF242585; 31, AY330911; 32, AF222322; 33, AJ131567; 34, **AF498266**; 35, D00220; 36, AB032433; 37, AB032432; 38, AF222323; 39, AF242568; 40, AF305327; 41, AY330912; 42, **AB046525**. Note two “anomalous” clustering (sequences 33 and 22). The first case (clustering gorilla and chimpanzee HBVs) may be explained by the interspecies transmission in nature, whereas the second case (clustering of gibbon and chimpanzee HBVs) remains unexplained. **43 through 50—representative human HBVs genotypes:** 43, 44, 45, 46, 47, 48, 49, and 50, HuHBV genotypes D, E, G, A, B, C, F, and H, respectively.

20.5.3. Gibbon Hepadnavirus

The first serological data suggesting that gibbons are infected with HBV were reported in 1969.⁹ However, only in 1991 it was shown that gibbon hepadnavirus (GiHBV) is distinct from all human HBVs.¹⁷ A few years later the uniqueness of GiHBV was conclusively confirmed by phylogenetic analysis using the complete genome sequence.²⁰

GiHBV infection is prevalent in gibbons of different *Hylobates* and *Nomascus* species and subspecies.^{1,4,8,18,24,26} Fifty-five out of 213 mostly captive gibbons (25.8%) tested by different investigators had active GiHBV infections.²⁶ GiHBV infection has also been detected in wild gibbons: 4 out of 9 (44%) pileated gibbons (*Hylobates pileatus*) were GiHBV-positive.²⁴ However, the sample tested is too small to make a reliable prevalence estimate.

GiHBV is perhaps the best-studied simian hepadnavirus, at least in terms of sequence information available. Complete genome sequences of 26 GiHBV isolates are known. The genomic diversity of GiHBV is the greatest among all nonhuman HBVs (Figure 20.3). Currently, eight clades (GiHBV-1 to GiHBV-8) have been identified by phylogenetic analyses of complete genome sequences. Three clades (GiHBV-3, -4, -5) are closely related and include GiHBVs from the *Nomascus* species. GiHBV isolates from the *Hylobates* species belong to widely separated clades GiHBV-1, -2 and GiHV-6, -7, -8.³⁴ One recombinant GiHBV isolate (GenBank Acc. No. AJ131572) has also been reported.³⁴ Most of this genome sequence groups in clade GiHBV-2, whereas a 498-bp fragment located in the pX region clearly belongs to clade GiHBV-3.

Geographical distribution of the GiHBV clades is not random, apparently. Although an exact geographical origin is known for only a few GiHBV isolates, it appears likely that phylogenetic clustering is congruent with geography.^{24,26}

Whether or not GiHBV is pathogenic for the natural host is not known, although the elevation of alanine transaminase (ALT) in the liver of chronic carriers has been described.¹⁸

Little is known about the mode of transmission of GiHBV. It is presumed that the virus can be transmitted both horizontally and vertically. However, supporting data are sparse. Only a single case of mother-to-infant transmission of GiHBV has been documented.¹⁸

Chimpanzees and orangutans are susceptible to infection with GiHBV.^{17,26} No evidence of gibbon-to-human transmission of GiHBV has been found.¹⁸

20.5.4. Orangutan Hepadnavirus

Serological surveys of captive orangutans using tests for diagnosis of human HBV infection have revealed high prevalence of infection with a related hepadnavirus (OrHBV).^{32,33} Out of 195 animals tested, 83 were positive for either HBsAg or anti-HBs antibodies, and 15 of these were found to be chronic carriers (defined as HBsAg-positive for more than a year). Importantly, 2 out of 3 wild orangutans tested shortly after capture were HBsAg-positive.

All but 1 of 18 OrHBV-positive animals investigated longitudinally were clinically healthy and had normal liver enzyme levels. One animal had a moderately elevated level of ALT, although histopathological examination did not reveal any abnormalities. Thus, OrHBV infection in orangutans is apparently subclinical, although the number of observations is too small to make an unequivocal conclusion.

Sequence analysis of genomic fragments amplified from the blood of seven chronic OrHBV carriers confirmed that the viruses infecting these animals were closely related to, but clearly distinct from, human HBV. Two variants of OrHBV have been identified.^{31,32} The first variant (prototype OrHBV-Somad, GenBank Acc. No. AF193863) was present in 12 orangutans originating from East Kalimantan in Borneo and in 7 out of 13 animals from other regions of Borneo. The second variant (OrHBV-Papa, GenBank Acc. No. AF193864) was present in the other 6 non-East Kalimantan orangutans. Sequence identity of OrHBV-Somad and OrHBV-Papa genomes is 95%.³¹ This level of sequence homology is insufficient to designate these two variants as separate HBV genotypes. As may be expected based on the overlapping habitats and geographical clustering of GiHBV isolates, the closest relative of OrHBV is GiHBV.²⁶ While it is possible that some HBV isolates identified in orangutans could have originated from gibbons,²⁶ most OrHBV are distinct from GiHBV.³⁴

20.5.5. Woolly Monkey Hepadnavirus

This virus, or more precisely its genomic DNA, has been identified in a captive woolly monkey (*Lagothrix*

lagotricha).⁷ Retrospective analysis of sera from six woolly monkeys from the same breeding group showed that four animals were positive for serological markers of HBV infection.

The genomic sequence of WmHBV is clearly different from all human and ape HBV sequences; it is the most divergent sequence among the primate HBVs.⁷ Interestingly, the closest relative of WmHBV is not a simian virus, but one of the human HBV genotypes (genotype F) present almost exclusively in the indigenous South American population.

WmHBV can be transmitted experimentally to chimpanzees and spider monkeys; however, the virus is apparently apathogenic in these species.

20.5.6. Recombinant Viruses

Perhaps the most interesting recent finding in the simian HBV field is the identification of human–simian HBV recombinants.^{11,25,28,34} Currently, three human–simian recombinant genomes are known. Two of these are recombinants of chimpanzee and human HBV (GenBank Acc. Nos. AF498266 and AB046525) and the third is a recombinant of gibbon and human HBV (GenBank Acc. No. AB048704).

20.6. SUMMARY

The prototype hepadnavirus is human HBV. Simian counterparts of human HBV are found almost exclusively in ape species (chimpanzees, gibbons, orangutans, and gorillas). The only exception is woolly monkey HBV. All primate hepadnaviruses are included in the genus *Orthohepadnavirus* that belongs to the family *Hepadnaviridae*, and are closely related.

Hepadnavirus virions are small spherical particles consisting of an icosahedral core and an envelope that contains viral proteins and cellular lipids. Completely double-stranded genomic DNA encapsidated in the core of infectious virions is unusual. The minus-strand of the HBV genome is circular (about 3,200 nt in length), whereas the complementary plus-strand is shorter. Thus, the genome of infectious hepadna virions is only partially double-stranded. There are only two structural genes, S and C, encoding surface and core antigens, HBsAg and HBcAg, respectively. These antigens consist of several proteins, encoded by overlapping “subgenes”: pre-S1, pre-S2, and S proper and pre-C and

C proper. The third hepadnavirus antigen, HBeAg is a product of posttranslational processing of pre-C and C polypeptides. The viral core is composed of HBcAg and HBeAg, whereas the main component of the envelope is HBsAg.

The major nonstructural protein, polymerase, is encoded by the P gene. This protein combines the reverse transcriptase (RT) and RNAase H (RH) activities. The function of the smallest and the least conserved protein pX, the product of P gene, is not clear. However, pX is required for efficient replication. Genome replication is a complex, multistage process that includes formation of whole-genome length pgRNA through transcription of the minus-strand of the virion DNA by cellular RNA polymerase II. It is pgRNA which is initially encapsulated into newly assembled capsids. Conversion of the pgRNA into a DNA genome occurs inside the virions and takes two alternative routes. In the majority of virions a partially double-stranded DNA is synthesized and these virions are fully replication-competent. In a small fraction, the end product of reverse transcription is a double-stranded linear DNA, and these virions are defective.

By analogy with human HBV, simian HBVs are presumed to be transmitted both vertically and sexually. Blood-borne transmission is also a possibility under natural conditions although this has not been documented. The most numerous groups of simian HBVs are isolates from gibbons (GiHBV) and common chimpanzees (ChHBV). The only known isolate from a gorilla apparently originated from the common chimpanzee. HBV isolates from orangutans (OrHBV) are apparently indigenous to this species; however, relatively recent interspecies transmission from sympatric gibbons cannot be excluded. All simian HBVs appear to be weakly pathogenic in their natural hosts.

An intriguing question is whether or not simian and human HBV reservoirs are connected. If they are significantly connected then the prospect of HBV eradication from the human population by universal vaccination is doubtful. Recombinants between simian and human HBVs have been identified. Thus, coinfections do appear to occur in natural conditions, either in simian or human hosts. The significance of these findings for public health is not clear. The fact that no cases of human infection with simian HBV have been documented is encouraging.

REFERENCES

- Aiba, N., H. Nishimura, Y. Arakawa, and K. Abe. 2003. Complete nucleotide sequence and phylogenetic analyses of hepatitis B virus isolated from two pileated gibbons. *Virus Genes* 27(3):219–226.
- Blumberg, B. S. 1977. Australia antigen and the biology of hepatitis B. *Science* 197(4298):17–25.
- Eichberg, J. W. and S. S. Kalter. 1980. Hepatitis A and B: serologic survey of human and nonhuman primate sera. *Lab. Anim. Sci.* 30(3):541–543.
- Grethe, S., J. O. Heckel, W. Rietschel, and F. T. Hufert. 2000. Molecular epidemiology of hepatitis B virus variants in nonhuman primates. *J. Virol.* 74(11):5377–5381.
- Hu, X., A. Javadian, P. Gagneux, and B. H. Robertson. 2001. Paired chimpanzee hepatitis B virus (ChHBV) and mtDNA sequences suggest different ChHBV genetic variants are found in geographically distinct chimpanzee subspecies. *Virus Res.* 79(1–2):103–108.
- Hu, X., H. S. Margolis, R. H. Purcell, J. Ebert, and B. H. Robertson. 2000. Identification of hepatitis B virus indigenous to chimpanzees. *Proc. Natl. Acad. Sci. U. S. A.* 97(4):1661–1664.
- Lanford, R. E., D. Chavez, K. M. Brasky, R. B. Burns III, and R. Rico-Hesse. 1998. Isolation of a hepadnavirus from the woolly monkey, a New World primate. *Proc. Natl. Acad. Sci. U. S. A.* 95(10):5757–5761.
- Lanford, R. E., D. Chavez, R. Rico-Hesse, and A. Mootnick. 2000. Hepadnavirus infection in captive gibbons. *J. Virol.* 74(6):2955–2959.
- Lichter, E. A. 1969. Chimpanzee antibodies to Australia antigen. *Nature* 224(5221):810–811.
- MacDonald, D. M., E. C. Holmes, J. C. Lewis, and P. Simmonds. 2000. Detection of hepatitis B virus infection in wild-born chimpanzees (*Pan troglodytes verus*): phylogenetic relationships with human and other primate genotypes. *J. Virol.* 74(9):4253–4257.
- Magiorkinis, E. N., G. N. Magiorkinis, D. N. Paraskevis, and A. E. Hatzakis. 2005. Re-analysis of a human hepatitis B virus (HBV) isolate from an East African wild born *Pan troglodytes schweinfurthii*: evidence for interspecies recombination between HBV infecting chimpanzee and human. *Gene* 349:165–171.
- Makuwa, M., S. Souquiere, O. Bourry, P. Rouquet, P. Telfer, P. Maucleire, M. Kazanji, P. Roques, and F. Simon. 2007. Complete-genome analysis of hepatitis B virus from wild-born chimpanzees in central Africa demonstrates a strain-specific geographical cluster. *J. Gen. Virol.* 88(Pt 10):2679–2685.
- Makuwa, M., S. Souquiere, S. L. Clifford, A. Mouinga-Ondeme, M. Bawe-Johnson, E. J. Wickings, S. Latour, F. Simon, and P. Roques. 2005. Identification of hepatitis B virus genome in faecal sample from wild living chimpanzee (*Pan troglodytes troglodytes*) in Gabon. *J. Clin. Virol.* 34(Suppl 1):S83–S88.
- Makuwa, M., S. Souquiere, P. Telfer, C. Apetrei, M. Vray, I. Bedjabaga, A. Mouinga-Ondeme, R. Onanga, P. A. Marx, M. Kazanji, P. Roques, and F. Simon. 2006. Identification of hepatitis B virus subgenotype A3 in rural Gabon. *J. Med. Virol.* 78(9):1175–1184.
- Makuwa, M., S. Souquiere, P. Telfer, O. Bourry, P. Rouquet, M. Kazanji, P. Roques, and F. Simon. 2006. Hepatitis viruses in non-human primates. *J. Med. Primatol.* 35(6):384–387.
- Makuwa, M., S. Souquiere, P. Telfer, E. Leroy, O. Bourry, P. Rouquet, S. Clifford, E. J. Wickings, P. Roques, and F. Simon. 2003. Occurrence of hepatitis viruses in wild-born non-human primates: a 3 year (1998–2001) epidemiological survey in Gabon. *J. Med. Primatol.* 32(6):307–314.
- Mimms, L. T., L. R. Solomon, J. W. Ebert, and H. Fields. 1993. Unique preS sequence in a gibbon-derived hepatitis B virus variant. *Biochem. Biophys. Res. Commun.* 195(1):186–191.
- Noppornpanth, S., B. L. Haagmans, P. Bhattacharayya, P. Ratanakorn, H. G. Nieters, A. D. Osterhaus, and Y. Poovorawan. 2003. Molecular epidemiology of gibbon hepatitis B virus transmission. *J. Gen. Virol.* 84(Pt 1):147–155.
- Norder, H., A. M. Courouce, and L. O. Magnus. 1994. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology* 198(2):489–503.
- Norder, H., J. W. Ebert, H. A. Fields, I. K. Mushahwar, and L. O. Magnus. 1996. Complete sequencing of a gibbon hepatitis B virus genome reveals a unique genotype distantly related to the chimpanzee hepatitis B virus. *Virology* 218(1):214–223.
- Norder, H., B. Hammas, S. D. Lee, K. Bile, A. M. Courouce, I. K. Mushahwar, and L. O. Magnus. 1993. Genetic relatedness of hepatitis B viral strains of diverse geographical origin and natural variations in the primary structure of the surface antigen. *J. Gen. Virol.* 74(Pt 7):1341–1348.
- Robertson, B. H. 2001. Viral hepatitis and primates: historical and molecular analysis of human and nonhuman primate hepatitis A, B, and the GB-related viruses. *J. Viral Hepat.* 8(4):233–242.
- Robertson, B. H. and H. S. Margolis. 2002. Primate hepatitis B virus—genetic diversity, geography and evolution. *Rev. Med. Virol.* 12(3):133–141.
- Sall, A. A., S. Starkman, J. M. Reynes, S. Lay, T. Nhim, M. Hunt, N. Marx, and P. Simmonds. 2005. Frequent infection of *Hylobates pileatus* (pileated gibbon) with

- species-associated variants of hepatitis B virus in Cambodia. *J. Gen. Virol.* 86(Pt 2):333–337.
25. Simmonds, P. and S. Midgley. 2005. Recombination in the genesis and evolution of hepatitis B virus genotypes. *J. Virol.* 79(24):15467–15476.
26. Starkman, S. E., D. M. MacDonald, J. C. Lewis, E. C. Holmes, and P. Simmonds. 2003. Geographic and species association of hepatitis B virus genotypes in non-human primates. *Virology* 314(1):381–393.
27. Takahashi, K., B. Brotman, S. Usuda, S. Mishiro, and A. M. Prince. 2000. Full-genome sequence analyses of hepatitis B virus (HBV) strains recovered from chimpanzees infected in the wild: implications for an origin of HBV. *Virology* 267(1):58–64.
28. Takahashi, K., S. Mishiro, and A. M. Prince. 2001. Novel hepatitis B virus strain from a chimpanzee of Central Africa (*Pan troglodytes troglodytes*) with an unusual antigenicity of the core protein. *Intervirology* 44(5):321–326.
29. Vartanian, J. P., P. Pineau, M. Henry, W. D. Hamilton, M. N. Muller, R. W. Wrangham, and S. Wain-Hobson. 2002. Identification of a hepatitis B virus genome in wild chimpanzees (*Pan troglodytes schweinfurthi*) from East Africa indicates a wide geographical dispersion among equatorial African primates. *J. Virol.* 76(21):11155–11158.
30. Vaudin, M., A. J. Wolstenholme, K. N. Tsiquaye, A. J. Zuckerman, and T. J. Harrison. 1988. The complete nucleotide sequence of the genome of a hepatitis B virus isolated from a naturally infected chimpanzee. *J. Gen. Virol.* 69(Pt 6):1383–1389.
31. Verschoor, E. J., K. S. Warren, S. Langenhuizen, Heriyanto, R. A. Swan, and J. L. Heeney. 2001. Analysis of two genomic variants of orang-utan hepadnavirus and their relationship to other primate hepatitis B-like viruses. *J. Gen. Virol.* 82(Pt 4):893–897.
32. Warren, K. S., J. L. Heeney, R. A. Swan, Heriyanto, and E. J. Verschoor. 1999. A new group of hepadnaviruses naturally infecting orangutans (*Pongo pygmaeus*). *J. Virol.* 73(9):7860–7865.
33. Warren, K. S., H. Niphuis, Heriyanto, E. J. Verschoor, R. A. Swan, and J. L. Heeney. 1998. Seroprevalence of specific viral infections in confiscated orangutans (*Pongo pygmaeus*). *J. Med. Primatol.* 27(1):33–37.
34. Yang, J., Q. Xi, R. Deng, J. Wang, J. Hou, and X. Wang. 2007. Identification of interspecies recombination among hepadnaviruses infecting cross-species hosts. *J. Med. Virol.* 79(11):1741–1750.
35. Zuckerman, A. J., A. Thornton, C. R. Howard, K. N. Tsiquaye, D. M. Jones, and M. R. Brambell. 1978. Hepatitis B outbreak among chimpanzees at the London Zoo. *Lancet* 2(8091):652–654.

21

Adenoviruses

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21.1. INTRODUCTION

Adenoviruses are medium-sized DNA viruses with the compelling characteristic of being perhaps the most “photogenic” of all primate viruses (Figure 21.1). They are important human pathogens causing predominantly respiratory tract illnesses, as well as other diseases such as acute hemorrhagic cystitis, epidemic keratoconjunctivitis, gastroenteritis, myocarditis, hepatitis, and meningoencephalitis. More than 50 types of human adenoviruses are known.

Human adenoviruses have been widely used as models for unraveling the structural principles of “viral architecture” and mechanisms of the fundamental biological processes. The most important in this context was the discovery of RNA splicing made in 1977 as a “by-product” of studying adenoviral RNAs.⁶ The discovery of adenovirus oncogenicity for rodents in 1962 initiated an avalanche of studies exploring different aspects of viral oncogenesis.⁴⁰ However, the interest in this field waned when it became clear that adenoviruses are not oncogenic *in vivo* in humans.

The first simian adenovirus, currently designated as simian adenovirus 21, was isolated in 1956 by

W. P. Rowe, who was one of the discoverers of human adenoviruses.³⁵ Virtually all known simian adenoviruses were isolated in the late 1950s–1960s in the laboratories of the pioneers of simian virology R. N. Hull, H. Malherbe, and S. Kalter.^{5,13,17,19,21,22,24,28,34} These viruses have not been “popular” objects for research during the last 35 years and their pathogenic potential in nonhuman primates (NHPs) is virtually unexplored. Some interest in the simian adenoviruses is reemerging mostly in the context of their potential usefulness as gene delivery and vaccine vectors.

21.2. CLASSIFICATION AND NOMENCLATURE

Adenoviruses (family *Adenoviridae*) are harbored by many vertebrates, from fish to humans. All known adenoviruses found in mammalian species, and hence all primate adenoviruses, are included in the *Mastadenovirus* genus.

Human and simian adenoviruses (the term *mastadenovirus* is used only in the classification) traditionally were classified into “types.”^{15,33,36} The isolates belonging to the same type can be cross-neutralized by the antisera raised against individual isolates, hence the terms *neutralization serotypes* or simply *serotype*, which were widely used in the past. The modern classification of human adenoviruses is based on the phylogenetic analysis of their genomic sequences.²⁷ Closely related types/serotypes of human adenoviruses are clustered in the groups formally classified as species within the *Mastadenovirus* genus. Six such species are recognized in the latest International Committee on Taxonomy of Viruses (ICTV) classification (8th Report), namely, *human adenovirus A, B, C, D, E, and F*. Recently, an

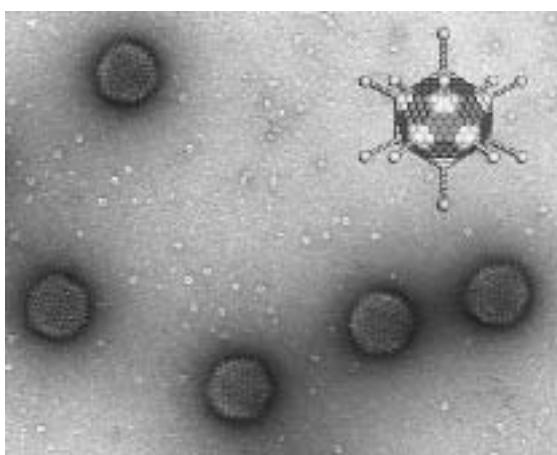


Figure 21.1. Adenovirus morphology.
Negatively stained human adenovirus virions. Icosahedral capsids are formed by tightly packed structural units (capsomers). Numerous fibers and capsomers detached from the virions during purification are clearly visible. (Electron micrograph is kindly provided by Mrs. Margaret E. Bisher.) Inset (upper right corner)—adenovirus capsid model. Fibers extend from pentamers (white vortex capsids). Nonvortex capsomers are hexamers. (Image is created by Dr. Richard Feldmann; the image can be obtained from public audiovisual library of National Cancer Institute, USA.)

additional species, *human adenovirus G*, has been suggested (prototype—*human adenovirus type 52*).²⁰

Most of the known simian adenoviruses are either clearly distinct from human adenoviruses in terms of genomic sequence similarity or uncharacterized in this respect (Table 21.1). These viruses are currently classified as “tentative species” within the *Mastadenovirus* genus. At the same time, six simian adenoviruses (Table 21.2) are so closely related to some human adenoviruses that they can be considered as members (isolates/strains) of the human adenovirus species. In other words, based on the genomic sequence they cannot be reliably distinguished from human adenoviruses and their “simian affiliation” is based on the species from which they were isolated. All known chimpanzee adenoviruses are classified as members of *human adenovirus B* and *E* species.^{23,27} Among adenoviruses isolated from mon-

keys only one (SAdV-19) is sufficiently similar to human adenoviruses to be included in the *human adenovirus F* species, which contains human adenoviruses type 40 and 41. Two more monkey adenoviruses (SAdV-1 and SAdV-7) are closely related to human adenovirus type 52 (suggested *human adenovirus G* species), which is also an enteric adenovirus. No simian “representatives” are known in the *human adenovirus A, C, and D* species.

Phylogeny of simian adenoviruses is still incomplete. Several genomic regions (VA-RNA, TP, pol, IIIa, IV, and E1A genes) have been analyzed. However, only a few isolates were included in the analysis and phylogenetic position of most tentative species (SAdV-2, -4, -5, -6, -8, -18, and -20) is unknown.^{1,2,23,25,26,38}

21.3. MORPHOLOGY AND STRUCTURAL PROTEINS

Adenovirus virions are nonenveloped icosahedral particles with a diameter of about 90 nm. The capsid of adenoviruses is composed of 252 capsomers. There are two types of capsomers, the pentons ($n = 240$) and hexons ($n = 12$), each surrounded by five and six neighboring capsomers, respectively. The pentons are located at the vertices of the icosahedron; the hexons cover the faces and edges. A distinctive morphological feature of adenoviruses is the long (about 40 nm) fibers projecting from the vertices of the icosahedron (Figure 21.1).

There are 11 structural proteins that make up the virion (Table 21.3).

The hexons are composed of protein II. Minor capsid proteins IIIa, VIII, and IX interconnect the hexons. The pentons are composed of protein III (penton) and IV (fiber). Protein VI underlies the penton (penton base). Some adenoviruses, for example, group F, have two fiber proteins. The fiber protein is folded in a way that its distal end is globular—hence the designation of the terminal fiber structure—the knob. Inside the capsid, there is a core consisting of five proteins: V, VII, μ , TP, and p23. Protein V is the major core building block. Genomic DNA is encapsulated inside the core. Protein μ , or strictly speaking peptide μ , of just 19 amino acids, is a minor core component. Protein VII binds to protein V and penton base protein VI thus linking the capsid and core. The so-called terminal protein (TP) is attached to the 5'-ends of the genomic DNA. There are also about 10 molecules of viral cysteine protease (p23) inside the core.

Table 21.1. Simian Adenoviruses (SAdV) Currently Classified as Tentative Species

ICTV Designation	Original Designation	Species of Origin	Genomic Sequences	References
SAdV-1*	SV-1	<i>Macaca fascicularis</i>	NC_006879 [†]	19
SAdV-2	SV-11	<i>M. mulatta</i>	NA [‡]	19
SAdV-3*	SV-15	<i>M. mulatta</i>	AY598782 [†]	19
SAdV-4	SV-17	<i>M. mulatta</i>	NA	19
SAdV-5	SV-20	<i>M. mulatta</i>		19
SAdV-6	SV-23	<i>M. mulatta</i>		19
SAdV-7*	SV-25	<i>M. mulatta</i>	DQ792570 [†]	19
SAdV-8	SV-30	<i>M. fascicularis</i>	NA	19
SAdV-9	SV-31	<i>Macaca</i> spp.	NA	19
SAdV-10	SV-32	<i>Macaca</i> spp.	NA	19
SAdV-11	SV-33	<i>M. mulatta</i>	NA	19
SAdV-12	SV-34	<i>M. mulatta</i>	NA	19
SAdV-13	SV-36	<i>Macaca</i> spp.	NA	19
SAdV-14	SV-37	<i>M. mulatta</i>	NA	19
SAdV-15	SV-38	<i>M. mulatta</i>	NA	17
SAdV-16	SA-7	<i>Chlorocebus aethiops</i>	NA	28
SAdV-17	SA-17	<i>C. aethiops</i>	NA	21
SAdV-18	SA-18	<i>C. aethiops</i>	NA	21
SAdV-20	V340	<i>C. aethiops</i>	NA	24

*Complete genome sequence is available.

[†]GenBank access number.

[‡]Genomic sequences are not available.

21.4. GENOME COMPOSITION AND GENE EXPRESSION

Complete genomes of eight simian adenoviruses have been sequenced, namely, the genomes of five chimpanzee viruses (SAdV-21 through SAdV-25) as well as three monkey viruses (SAdV-1, SAdV-3, and

SAdV-7)^{25,26,37} (Tables 21.1 and 21.2). The E1A gene region of several other adenoviruses has also been sequenced.^{1,2}

All simian adenoviral genomes are collinear. Their structure is very similar to that of human adenoviral genomes (Figure 21.2).

Table 21.2. Simian Adenovirus (SAdV) Serotypes Belonging to the Human Adenovirus (HAdV) Species

ICTV Designation	Original Designation	Human Adenovirus Species	Host Species	Complete Genome	References
SAdV-19	AA153	HAdV-F	<i>Papio cynocephalus</i>	NA	13, 22
SAdV-21	C-1	HAdV-B	<i>Pan troglodytes</i>	AC_000010*	35
SAdV-22	Pan5	HAdV-E	<i>P. troglodytes</i>	AY530876*	34
SAdV-23	Pan6	HAdV-E	<i>P. troglodytes</i>	AY530877*	34
SAdV-24	Pan7	HAdV-E	<i>P. troglodytes</i>	AY530878*	34
SAdV-25	Pan9, C68 [†]	HAdV-E	<i>P. troglodytes</i>	AF394196*	5

*GenBank access number.

[†]Commonly used designation.

Table 21.3. Adenoviral Structural Proteins

Abbreviated Name	Alternative Name	Location in Virion	Monomer or Polymer in Virion	Estimated Number of Molecules per Virion
II	Hexon	Capsid, facing outwards	Trimer	720
III	Penton base	Capsid, facing outwards	Pentamer	56
IIIa		Capsid, facing outwards	Monomer	68
IV	Fiber	Fiber	Trimer	35
V		Core, linking core and capsid	Monomer	157
TP	Terminal	Core, bound to DNA ends	Monomer	2
VI	Minor capsid	Capsid	Hexamer	342
VII		Core	Monomer	833
VIII		Capsid, stabilizing links between hexons	Monomer	127
IX	Capsid, stabilizing links between hexons		Trimer	247
M		Core	Monomer	100
P23	Protease	Core	Monomer	10

The genome length varies from 31 kb (rhesus adenovirus) to 36 kb (chimpanzee adenoviruses). Each end of the double-stranded linear genomic DNA has inverted repeat sequence (IRS) containing origin of replication (*ori*) sequence. Both strands of genomic DNA are coding but not continuously. Different blocks of genes are encoded by different strands. Early genes are clustered in three major blocks, named E1A/E1B, E3, and E4. E1A/E1B and E4 gene blocks are located at the opposite termini of the genome next to the IRS and are encoded by different strands. Most of the genes encoding structural proteins are located between E1A/E1B and E3 early gene blocks. The fiber gene, which is duplicated in the enteric adenoviruses, is located between E3 and E4 early gene blocks. A characteristic feature of

mammalian adenoviruses is a presence of the genes encoding small virus-associated RNA (VA RNA). There is a single VA gene in the genomes of monkey adenoviruses and two adjacent VA genes in the genomes of chimpanzee adenoviruses.

21.5. OVERVIEW OF THE REPLICATION CYCLE

The replication cycle of human adenoviruses has been extensively studied and is well understood. At the same time, very little is known about the replication of simian adenoviruses. However, human and simian adenoviruses in general share many properties and it is reasonable to suggest that the mechanism of their replication is also similar.



Figure 21.2. "Macro" map of adenovirus genome. TP, terminal protein; E, early genes (gray boxes); L, late genes (white boxes). Direction of transcription is indicated by arrow-side. VA, genes encoding small virus-associated RNAs.

The replication cycle starts by the attachment of the virus to the cellular receptor via the receptor-binding site located in the fiber. The receptors for simian adenoviruses are not known. An analogy with human adenoviruses is of no help because human adenoviruses use different cell surface molecules as the receptors. The virus enters the cell through clathrin-mediated endocytosis. In addition to the fiber protein (IV), the penton base protein (III) is also involved in the entry process. The uncoating starts before completion of the entry; fibers are shed off and “fiberless” nucleocapsids “entrapped” in the endosomes are transported to the nuclear membrane. In the vicinity of the nuclear membrane, the capsid disintegrates and the nucleoprotein complex consisting of the genomic DNA and proteins VII and TP is delivered to the nucleus. The transcription of adenoviral genome is staged. The immediate early gene (E1A) is transcribed first by the host RNA polymerase II. The primary transcription RNA products undergo multiple splicing and the resulting mRNAs are exported to the cytoplasm. The cellular protein synthesis machinery translates these mRNA into immediate early proteins. These proteins are posttranslationally modified (phosphorylated) and transported to the nucleus. The E1A proteins switch on the transcription of the RNAs encoding early proteins. These RNAs are also transcribed by the cellular RNA polymerase II. The early primary transcripts, similar to the immediate–early transcripts, are processed by the cellular splicing machinery and the early mRNAs are exported to the cytoplasm. The early proteins translated from these mRNAs are imported to the nucleus. The set of early proteins includes products required for the replication of viral DNA and the products triggering the third and final stage of genome expression, the transcription of the late RNAs. In contrast to the immediate–early and early RNAs, the late RNAs are transcribed from multiple viral genomic DNA molecules accumulating in the nucleus as result of ongoing DNA replication. Late mRNAs encoding the structural proteins are exported to the cytoplasm for translation. The late protein IVa2 and one of the early proteins (E4-orf6) play an active role at this stage of the viral cycle. The translation and posttranslational processing of the structural proteins require, in addition to the host cellular machinery, participation of special viral RNAs, named VA RNAs and the late L4 protein. The VA RNAs are transcribed during the early phase of replication by the cellular RNA polymerase III but become engaged only at the late phase of the viral cycle. The L4 protein serves as a chaperone ensuring the

proper aggregation of the hexon proteins in the trimers. The structural proteins are transported to the nucleus where the nucleocapsids are assembled. The mechanism of assembly is not fully understood. However, it is known that a special DNA sequence motif, called the packaging signal, is required for assembly and two viral proteins (IVa2 and L4) play an important role in this process. The newly assembled virions are “immature” in that they are composed of precursor polyproteins and are not infectious. A viral protease that is included in the virions during assembly cleaves the precursor proteins into final products. The resulting mature virions are infectious. The release of the virions is associated with the rupture of the cell. Whether the release of virions is due to a purely mechanical cell rupture or if additional mechanisms of virion egress are involved is not clear. In any case, the end result of the lytic adenovirus replication cycle is host cell death and the release of cell-free infectious progeny virus.

Lytic infection is not the only possible outcome of adenoviral infection. All human adenoviruses are capable of inducing growth transformation of rodent cells in vitro; however, only some of them are oncogenic for rats and hamsters in vivo. Highly oncogenic human adenoviruses belong to the *human adenovirus A* species which does not include simian adenovirus isolates. Nevertheless, some simian adenoviruses have been shown to be oncogenic for rodents.¹⁸ However, models of viral oncogenesis based on the use of simian adenoviruses have not been further explored. By analogy with human adenoviruses, it is expected that E1A and E1B genes of simian adenoviruses are potential oncogenes. However, their oncogenic potential cannot be realized in primate cells. Why adenoviruses perform so differently in primate and rodent host cells is not completely clear, although it may be partially due to the differences in the expression of telomerase.

21.6. ADENOVIRAL INFECTIONS IN NHPs

Adenovirus infection is presumed to be common in simian species. However, no sufficiently representative survey for this infection in NHPs either captive or wild has been reported.

Infection with simian adenoviruses in NHPs is predominantly subclinical. However, rare cases of severe diseases in captive monkeys and apes associated with adenoviruses have been described.^{3,4,7,8,10,11,14,16,24,29,30} The most significant among these

diseases was fatal adenoviral pneumonia in newborn monkeys. Twenty-six such cases have been described.⁷ The association of the pneumonia with adenovirus was suggested based on the presence of basophilic intranuclear inclusions and intranuclear paracrystalline arrays of adenovirus particles. Adenoviral pancreatitis has been described in captive rhesus monkeys.^{8,30} However, this disease is very rare (3 cases out of 1,000 necropsies). Two cases of adenoviral “pneumoenteritis” have been described in African green monkeys (*Chlorocebus aethiops*)²⁴ and two cases of necrotizing adenoviral hepatitis have been reported in the common chimpanzee (*Pan troglodytes*).^{10,14,16} Adenoviral pathology has also been described in simian immunodeficiency virus (SIV)-immunosuppressed rhesus monkeys.^{3,4,32,44} However, adenoviral diseases cannot be considered a typical manifestation of simian AIDS.

There are no published data on experimental induction of a disease by inoculation of NHPs with well-defined simian adenovirus preparations.

21.6.1. Diagnosis

Simian adenoviruses are largely neglected agents. As a result, the published data on the detection of these viruses in NHP reflects the state of the art in the 1960s–1970s. Adenoviral disease is usually suspected by a pathologist when basophilic intranuclear inclusions are observed in the affected tissues. If electron microscopy of thin sections reveals intranuclear crystalline arrays of adenovirus-like particles, the diagnosis of the adenoviral disease is considered as confirmed (Figures 21.3 and 21.4).

The presence of adenovirus in the affected tissues can also be confirmed by the detection of viral antigens or viral DNA. However, the potential of modern methodologies to diagnose simian adenovirus infections has not yet been realized. Monoclonal antibodies for detection of simian adenovirus antigens are not available. Generic polymerase chain reaction (PCR) tests for the detection of simian adenoviruses are definitely feasible; however, no such tests have been described. The same is true regarding the development of quantitative real-time PCR for simian adenoviruses. It is also worth mentioning that mere presence of the virus in the lesions is insufficient for establishing causal relationship between the virus and disease.

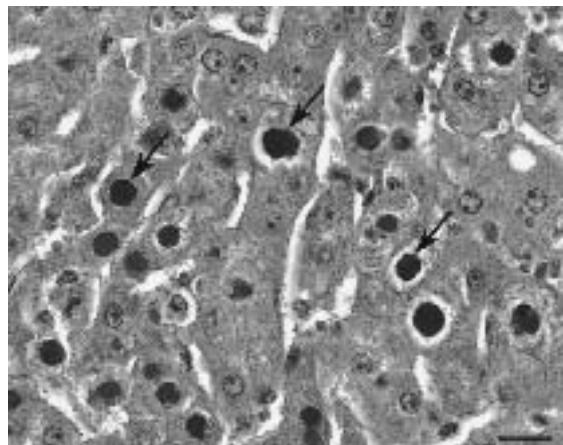


Figure 21.3. Adenoviral intranuclear inclusions. Homogeneous basophilic intranuclear inclusions (arrows) completely fill the enlarged nuclei. Scalebar = 2 μm. (Adapted from Zöller *et al.*⁴⁴; with permission.) See color version page 12.

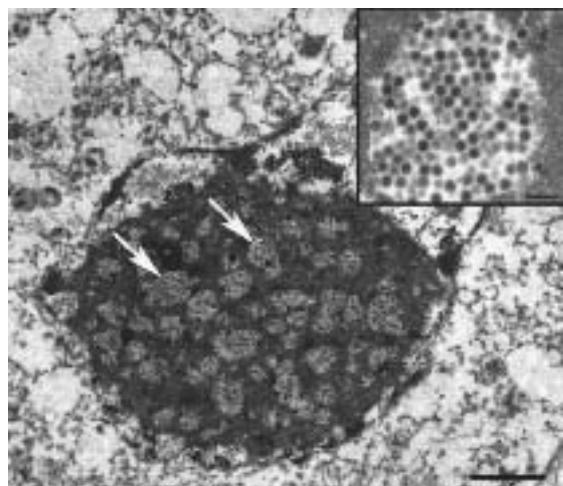


Figure 21.4. Adenovirus virions in nucleus. Intranuclear crystalline array of adenovirus particles (white arrows) in a necrotic hepatocyte from rhesus monkey with hepatitis. Scalebar = 2 μm. Inset—adenovirus particles at higher magnification. Scalebar = 200 nm. (Adapted from Zöller *et al.*⁴⁴; with permission.)

Although the advent of diagnostic PCR tests has decreased the significance of virus isolation, this method continues to be of major importance in diagnosing adenoviral infections. Simian adenoviruses are similar to their human counterparts in that they replicate well in the human epithelial cell lines. Human lung adenocarcinoma cells A549 are considered to be particularly permissive for primate adenoviruses. However, some other human epithelioid cell lines, such as HeLa and Hep2, are also permissive for most primate adenoviruses. Adenoviral infection of these cell lines is accompanied by characteristic cytopathic effect (CPE) that is manifested as rounding and swelling of the cells followed by their detachment in the form of “grape-like” clusters. The kinetics of the CPE development (range 1–28 days) depends on the dose of the virus. Isolation of simian adenoviruses can be sped up by centrifugation of the specimen directly onto permissive indicator cells, the so-called “shell vial” technique. In contrast to respiratory adenoviruses, the enteric adenoviruses are fastidious. Successful isolation and propagation of human enteric adenoviruses in the human cell line 293, also called Graham 293 or HEK 293, suggests that this cell line could be also useful for the isolation of simian enteric adenoviruses.

The classical serological test for adenoviruses is the neutralization assay. This test is very reliable; however, since it detects only type-specific antibodies its usefulness for diagnosing adenoviral infections is limited. The hemagglutination inhibition test for identification of adenoviruses and detection of type-specific antibodies is no longer used. ELISAs based on recombinant proteins of simian adenoviruses or peptides would be optimal for the detection of group- or type-specific antibodies against these viruses in NHP hosts; however, such tests are yet to be developed.

21.7. SIMIAN ADENOVIRUSES AS VECTORS FOR GENE THERAPY AND LIVE RECOMBINANT VACCINES

Human adenoviruses are widely used as vectors for gene therapy or as live recombinant vaccines. In contrast to retroviral vectors, adenoviruses do not integrate their DNA into the host genome and they are genetically stable. Adenoviral vectors are also easy to propagate. Importantly, they can infect nondividing cells. Adenoviral vectors can also be delivered as aerosols. Obviously, adenoviral vectors also have disadvantages. One

of the most restricting among them is the high prevalence of antibodies against human adenoviruses in humans. These preexisting antibodies can neutralize the adenovirus vector and limit its effectiveness. The use of chimpanzee adenoviruses as vectors in humans has been suggested for overcoming the preexisting antibody problem.^{9,12,31,38,39,41,43} The rational behind this is the assumption that humans do not have antibodies against simian adenoviruses. However, this assumption may not be true because antibodies against chimpanzee adenoviruses have been reported in humans, particularly in Africa.⁴²

21.8. SUMMARY

Adenoviruses are medium-sized DNA viruses with a characteristic morphology: icosahedral capsid composed of 252 capsomers and long fibers projecting from each of 12 vertices of the icosahedron. Adenoviruses have a double-stranded linear DNA genome about 32,000 bp in length. Adenoviruses are important human pathogens predominantly causing respiratory tract illnesses. Human and simian adenoviruses belong to the genus *Mastadenovirus*, family *Adenoviridae*. Human adenoviruses are classified into groups A, B, C, D, and E. All known simian adenoviruses can be divided into two groups. The first group includes all known chimpanzee adenoviruses (SAdV-21, -22, -23, -24, and -25) and one simian adenovirus isolated from monkey species (SAdV-19). These simian viruses are classified as isolates/species belonging to human adenovirus B and E species based on the close similarity of their genomic sequences with those of human adenoviruses. No simian counterpart of human adenoviruses belonging to species A, C, and D are known. The second group includes simian viruses classified as tentative species within the genus *Mastadenovirus*. These viruses are sufficiently distant from human adenoviruses although kinship of most tentative simian adenovirus species with other primate adenoviruses is yet to be established.

Infection with simian adenoviruses in NHPs is predominantly subclinical. However, rare cases of adenovirus-associated diseases (pneumonia, pancreatitis, hepatitis) in captive monkeys and apes have been described. Adenovirus-associated pathology is more frequent in SIV-immunosuppressed macaques; however, adenoviral diseases are not considered as a typical manifestation of simian AIDS.

REFERENCES

- Avvakumov, N., A. E. Kajon, R. C. Hoeben, and J. S. Mymryk. 2004. Comprehensive sequence analysis of the E1A proteins of human and simian adenoviruses. *Virology* 329(2):477–492.
- Avvakumov, N., R. Wheeler, J. C. D'Halluin, and J. S. Mymryk. 2002. Comparative sequence analysis of the largest E1A proteins of human and simian adenoviruses. *J. Virol.* 76(16):7968–7975.
- Baskin, G. B., M. Murphrey-Corb, E. A. Watson, and L. N. Martin. 1988. Necropsy findings in rhesus monkeys experimentally infected with cultured simian immunodeficiency virus (SIV)/delta. *Vet. Pathol.* 25(6):456–467.
- Baskin, G. B. and K. F. Soike. 1989. Adenovirus enteritis in SIV-infected rhesus monkeys. *J. Infect. Dis.* 160(5):905–907.
- Baschnig, M., Jr, N. G. Rogers, C. J. Gibbs Jr., and D. C. Gajdusek. 1971. Characterization of four new adenovirus serotypes isolated from chimpanzee tissue explants. *Am. J. Epidemiol.* 94(2):166–171.
- Berget, S. M., C. Moore, and P. A. Sharp. 1977. Spliced segments at the 5' terminus of adenovirus 2 late mRNA. *Proc. Natl. Acad. Sci. U. S. A.* 74(8):3171–3175.
- Boyce, J. T., W. E. Giddens Jr., and M. Valerio. 1978. Simian adenoviral pneumonia. *Am. J. Pathol.* 91(2):259–276.
- Chandler, F. W. and H. M. McClure. 1982. Adenoviral pancreatitis in rhesus monkeys: current knowledge. *Vet. Pathol. Suppl.* 7:171–180.
- Cohen, C. J., Z. Q. Xiang, G. P. Gao, H. C. Ertl, J. M. Wilson, and J. M. Bergelson. 2002. Chimpanzee adenovirus CV-68 adapted as a gene delivery vector interacts with the coxsackievirus and adenovirus receptor. *J. Gen. Virol.* 83(Pt 1):151–155.
- Davis, K. J., G. B. Hubbard, K. F. Soike, T. M. Butler, and T. P. Lipscomb. 1992. Fatal necrotizing adenoviral hepatitis in a chimpanzee (*Pan troglodytes*) with disseminated cytomegalovirus infection. *Vet. Pathol.* 29(6):547–549.
- Espana, C. 1974. Viral epizootics in captive nonhuman primates. *Lab. Anim. Sci.* 24(1):167–176.
- Farina, S. F., G. P. Gao, Z. Q. Xiang, J. J. Rux, R. M. Burnett, M. R. Alvira, J. Marsh, H. C. Ertl, and J. M. Wilson. 2001. Replication-defective vector based on a chimpanzee adenovirus. *J. Virol.* 75(23):11603–11613.
- Fuentes-Marins, R., A. R. Rodriguez, S. S. Kalter, A. Hellman, and R. A. Crandell. 1963. Isolation of enteroviruses from the "normal" baboon (*Papio doguera*). *J. Bacteriol.* 85:1045–1050.
- Hillis, W. D., A. C. Garner, and A. I. Hillis. 1969. A new simian adenovirus serologically related to human adenovirus type 2 and a chimpanzee with "viral hepatitis". *Am. J. Epidemiol.* 90(4):344–353.
- Hillis, W. D. and R. Goodman. 1969. Serologic classification of chimpanzee adenoviruses by hemagglutination and hemagglutination inhibition. *J. Immunol.* 103(5):1089–1095.
- Hillis, W. D., A. W. Holmes, and V. Davison. 1968. Serologic characterization of adenoviruses isolated from chimpanzees associated with viral hepatitis. *Proc. Soc. Exp. Biol. Med.* 129(2):366–369.
- Hull, R. N. 1968. The simian viruses. *Virol. Monogr.* 2:1–16.
- Hull, R. N., I. S. Johnson, C. G. Culbertson, C. B. Reimer, and H. F. Wright. 1965. Oncogenicity of the simian adenoviruses. *Science* 150(699):1044–1046.
- Hull, R. N., J. R. Minner, and C. C. Mascoli. 1958. New viral agents recovered from tissue cultures of monkey kidney cells. III. Recovery of additional agents both from cultures of monkey tissues and directly from tissues and excreta. *Am. J. Hyg.* 68(1):31–44.
- Jones, M. S., B. Harrach, R. D. Ganac, M. M. Gozum, W. P. Dela Cruz, B. Riedel, C. Pan, E. L. Delwart, and D. P. Schnurr. 2007. New adenovirus species found in a patient presenting with gastroenteritis. *J. Virol.* 81(11):5978–5984.
- Kalter, S. S., D. Ablashi, C. Espana, R. L. Heberling, R. N. Hull, E. H. Lennette, H. H. Malherbe, S. McConnell, and D. S. Yohn. 1980. Simian virus nomenclature, 1980. *Intervirology* 13(6):317–330.
- Kalter, S. S., C. S. Kim, and E. A. Sueltenfuss. 1967. Further characterization of agents isolated from normal baboon (*Papio* sp.). *J. Infect. Dis.* 117(4):301–306.
- Kidd, A. H., D. Garwicz, and M. Oberg. 1995. Human and simian adenoviruses: phylogenetic inferences from analysis of VA RNA genes. *Virology* 207(1):32–45.
- Kim, C. S., E. S. Sueltenfuss, and S. S. Kalter. 1967. Isolation and characterization of simian adenoviruses isolated in association with an outbreak of pneumoenteritis in vervet monkeys (*Cercopithecus aethiops*). *J. Infect. Dis.* 117(4):292–300.
- Kovacs, G. M., A. J. Davison, A. N. Zakhartchouk, and B. Harrach. 2004. Analysis of the first complete genome sequence of an Old World monkey adenovirus reveals a lineage distinct from the six human adenovirus species. *J. Gen. Virol.* 85(Pt 10):2799–2807.
- Kovacs, G. M., B. Harrach, A. N. Zakhartchouk, and A. J. Davison. 2005. Complete genome sequence of simian adenovirus 1: an Old World monkey adenovirus with two fiber genes. *J. Gen. Virol.* 86(Pt 6):1681–1686.
- Madisch, I., G. Harste, H. Pommer, and A. Heim. 2005. Phylogenetic analysis of the main neutralization

- and hemagglutination determinants of all human adenovirus prototypes as a basis for molecular classification and taxonomy. *J. Virol.* 79(24):15265–15276.
- 28. Malherbe, H. and R. Harwin. 1963. The cytopathic effects of vervet monkey viruses. *S. Afr. Med. J.* 37:407–411.
 - 29. Martin, B. J., R. C. Dysko, and C. E. Chriss. 1991. Pancreatitis associated with simian adenovirus 23 in a rhesus monkey. *Lab. Anim. Sci.* 41(4):382–384.
 - 30. McClure, H. M., F. W. Chandler, and J. C. Hierholzer. 1978. Necrotizing pancreatitis due to simian adenovirus type 31 in a rhesus monkey. *Arch. Pathol. Lab. Med.* 102(3):150–153.
 - 31. McCoy, K., N. Tatsis, B. Korioth-Schmitz, M. O. Lasaro, S. E. Hensley, S. W. Lin, Y. Li, W. Giles-Davis, A. Cun, D. Zhou, Z. Xiang, N. L. Letvin, and H. C. Ertl. 2007. Effect of preexisting immunity to adenovirus human serotype 5 antigens on the immune responses of nonhuman primates to vaccine regimens based on human- or chimpanzee-derived adenovirus vectors. *J. Virol.* 81(12):6594–6604.
 - 32. Ochs, H. D., W. R. Morton, C. C. Tsai, M. E. Thouless, Q. Zhu, L. D. Kuller, Y. P. Wu, and R. E. Benveniste. 1991. Maternal–fetal transmission of SIV in macaques: disseminated adenovirus infection in an offspring with congenital SIV infection. *J. Med. Primatol.* 20(4):193–200.
 - 33. Rapoza, N. P. 1967. A classification of simian adenoviruses based on hemagglutination. *Am. J. Epidemiol.* 86(3):736–745.
 - 34. Rogers, N. G., M. Basnight, C. J. Gibbs, and D. C. Gajdusek. 1967. Latent viruses in chimpanzees with experimental kuru. *Nature* 216(5114):446–449.
 - 35. Rowe, W. P., J. M. Hartley, and R. J. Huebner. 1956. Additional serotypes of the APC virus group. *Proc. Soc. Exp. Biol. Med.* 97(2):465–470.
 - 36. Rowe, W. P., J. M. Hartley, and R. J. Huebner. 1958. Serotype composition of the adenovirus group. *Proc. Soc. Exp. Biol. Med.* 97(2):465–470.
 - 37. Roy, S., G. Gao, D. S. Clawson, L. H. Vandenberghe, S. F. Farina, and J. M. Wilson. 2004. Complete nucleotide sequences and genome organization of four chimpanzee adenoviruses. *Virology* 324(2):361–372.
 - 38. Roy, S., Y. Zhi, G. P. Kobinger, J. Figueiredo, R. Calcedo, J. R. Miller, H. Feldmann, and J. M. Wilson. 2006. Generation of an adenoviral vaccine vector based on simian adenovirus 21. *J. Gen. Virol.* 87(Pt 9):2477–2485.
 - 39. Tatsis, N., L. Tesema, E. R. Robinson, W. Giles-Davis, K. McCoy, G. P. Gao, J. M. Wilson, and H. C. Ertl. 2006. Chimpanzee-origin adenovirus vectors as vaccine carriers. *Gene Ther.* 13(5):421–429.
 - 40. Trentin, J. J., Y. Yabe, and G. Taylor. 1962. The quest for human cancer viruses. *Science* 137:835–841.
 - 41. Wilson, J. M., G. Gao, Q. Wang, Z. Dai, R. Calcedo, X. Sun, and G. Li. 2008. Adenovirus-based vaccine generate cytotoxic T lymphocytes to epitopes of NS1 from Dengue virus that are present in all major serotypes. *Hum. Gene Ther.* 19(9):927–936.
 - 42. Xiang, Z., Y. Li, A. Cun, W. Yang, S. Ellenberg, W. M. Switzer, M. L. Kalish, and H. C. Ertl. 2006. Chimpanzee adenovirus antibodies in humans, sub-Saharan Africa. *Emerg. Infect. Dis.* 12(10):1596–1599.
 - 43. Zhi, Y., J. Figueiredo, G. P. Kobinger, H. Hagan, R. Calcedo, J. R. Miller, G. Gao, and J. M. Wilson. 2006. Efficacy of severe acute respiratory syndrome vaccine based on a nonhuman primate adenovirus in the presence of immunity against human adenovirus. *Hum. Gene Ther.* 17(5):500–506.
 - 44. Zöller, M., K. Mätz-Rensing, and F.-J. Kaup. 2008. Adenoviral hepatitis in a SIV-infected rhesus monkey (*Macaca mulatta*). *J. Med. Primatol.* 37(4):184–187.

Section 3:

Miscellaneous RNA and DNA Viruses

22

Miscellaneous Viruses

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22.1. INTRODUCTION

The placement of several simian viruses in this chapter under a miscellaneous umbrella by no means conveys an inferior status compared to other viruses in the preceding chapters. The knowledge of these viruses is still too fragmentary to present them in the same format as in Chapters 3 through 21. At the same time, it is possible that future interest in these neglected miscellaneous agents will increase significantly, as has happened more than once in the history of virology. On the other hand, some of the current “darlings” of virologists could equally well slide into oblivion.

22.2. HEPACI AND RELATED VIRUSES

Hepaciviruses (genus *Hepacivirus*) belong to the family *Flaviviridae*. The prototype hepatitis C virus (HCV), the causative agent of human hepatitis C. A simian counterpart of HCV is not known. However, there is one simian virus, GB virus B (GBV-B) that is classified as a tentative hepatitis C virus species. GBV-B

is the closest known relative of HCV. Two other simian viruses, GB virus A (GBV-A) and chimpanzee GB virus C (GBV-C_{Pr}), are also related to HCV, although more distantly than GBV-B. These viruses are currently classified as “unassigned” flavivirus species.

The GBV story began in the mid-1960s when Friedrich Deinhardt and co-workers induced hepatitis in tamarins (*Saguinus* spp.) by inoculation with serum from a surgeon (with the initials G.B.) who occupationally contracted non-A–non-B hepatitis.²⁵ The disease was serially transmitted in tamarins.^{26,66,79,80} The presumptive etiological agent was named GB virus (GBV) and was suspected to be a cause of non-A–non-B hepatitis. However, all attempts to reproduce the transmission of non-A–non-B hepatitis to tamarins and other New World monkeys failed. At the same time, it was established that non-A–non-B hepatitis is readily transmissible to common chimpanzees (*Pan troglodytes*).^{101,102} As a result, interest in GBV waned. The virus, contained in an 11th passage tamarin serum pool, was put in cold storage.

Several years after the discovery of HCV (1989) it became apparent that a small but significant proportion of the parenterally transmitted hepatitis (about 10%) was negative for both HBV and HCV markers. By that time (mid-1990s) new molecular tools for virus discovery had become available. Using one such tool, representational difference analysis,⁵⁹ two viruses were identified in the 11th GBV passage tamarin serum pool.^{67,92} These viruses were named GBV-A and GBV-B, and they both turned out to be of simian origin. Shortly thereafter a third virus, GBV-C, was identified in the same material using another virus discovery tool [polymerase chain reaction (PCR) amplification with degenerate primers generic for HCV, GBV-A, and GBV-B].⁹¹ GBV-C was clearly

a human virus. Initially, it was suspected to be an etiological agent of “hepatitis G” (presumptive “non-A, -B, -C, -D, -E” human hepatitis virus)—hence its second name—HGV.⁵⁸ However, subsequent studies found no evidence for pathogenicity of GBV-CHGV^{3,4} and the term *hepatitis G* is no longer used. A variant of GBV-C, GBV-C_{Ptr}, has been found in common chimpanzees.^{2,11}

GBVs have single-stranded RNA genome of positive polarity. GBV-A and GBV-C genomes are collinear with the HCV genome with one exception: they lack the gene encoding the RNA-binding structural protein (C protein). GBV-B and HCV have the same set of genes and their genomes are fully collinear. There are three major elements in the GBV genomes: a 5'-untranslated region (UTR), a single large open reading frame (ORF) spanning about 9,000 nt, and a 3'-UTR. The genomic RNA does not have a cap structure. The initiation of protein translation, as in picornaviruses, is mediated by an internal ribosomal binding site (IRES) formed by a string of about 340 nt located in the 5'-UTR. The ORF includes, in 5' -to- 3'-order, two or three nonstructural genes (C, E1, and E2), one presumably structural gene (p7), and six nonstructural genes (NS2, NS3, NS4a, NS4b, NS5a, and NS5b). The primary product of translation from this ORF is a single polyprotein which is post-translationally processed into 9 or 10 mature proteins. By analogy with HCV, it is presumed that the polyprotein is cleaved first between the NS2 and NS3 genes. This cleavage is mediated by the proteinase activity of the NS3 multifunctional enzyme. The cleavages within the carboxy-terminal half of the polyprotein are mediated by the serine protease activity of the NS3 enzyme with participation of the NS4a protein as a cofactor. The cleavages within amino-terminal half of the polyprotein are carried out by the cellular proteases, so-called signal peptidases. Extensive secondary structures are characteristic for the 3'-UTR spanning about 200 nt. 3'-UTR is composed of several distinct regions: highly variable, UC-rich, and 3'-terminal.

22.2.1. GB Virus B

GBV-B is the closest relative of HCV among the GB viruses.^{41,67,74} The origin of GBV-B remains a mystery. On the one hand, natural GBV-B infection in New World monkeys has not been found.⁹ On the other hand, it is unlikely that GBV-B is a human virus. No evidence of GBV-B infection in humans has been reported.⁹² GBV-B RNA was not detected in the pa-

tient GB serum used as the inoculum.¹⁵ GBV-B is not infectious for chimpanzees,¹⁵ whereas it readily infects tamarins (*Saguinus* spp.)^{16,89} and common marmosets (*Callithrix jacchus*).^{12,53}

GBV-B, in contrast to its “cousins” (GBV-A and GBV-C), causes hepatitis in experimentally infected tamarins, marmosets, and owl monkeys.^{12,16,53,67} The most developed of these is the tamarin model.¹⁷ GBV-B-induced hepatitis in tamarins while in marmosets it produces a mild disease diagnosed by elevation of liver enzyme levels and pathomorphological findings in liver biopsies. The disease resolves spontaneously in both tamarins and marmosets.^{8,12,15,16,51,53,63,69,87,89} Thus, the frequent transition to chronicity which is a major feature of human hepatitis C is not reproduced in the GBV-B models.

The resolution of acute hepatitis induced by GBV-B in tamarins is likely mediated by cellular immunity.¹¹⁰ A systemic T-cell-mediated immune response is directed against nonstructural proteins NS3, NS4A, and NS5B, and is temporally associated with the clearance of virus from the blood. A local T-cell response in the liver is also detectable during the resolution of the infection. Neutralizing antibodies are not detected in monkeys that have cleared GBV-B infection, but even so these animals proved to be immune to subsequent virus challenge.¹⁷ Moreover, no anti-GBV-B antibodies are detectable in tamarins experimentally infected with GBV-B.¹⁷ It remains to be determined whether or not anti-GBV-B antibody responses develop in tamarins or if the tests used (peptide-based ELISA) are adequate for their detection. In any case, there is currently no antibody test for diagnosing GBV-B infection. The virus can only be detected by molecular techniques, including quantitative real-time PCR^{17,69} or a biological assay, that is, inoculation of the tamarins.

GBV-B models may potentially be useful for pre-clinical testing of new anti-HCV drugs. The rational behind this idea is the similarity of some essential steps in the replication of HCV and GBV-B. The knowledge of functional and structural similarities and differences between HCV and GBV-B proteins is however still fragmentary.^{19,41,86,88,103} Importantly, the NS3 proteases of both viruses cleave the heterogenous virus precursor proteins, although less efficiently than the homologous proteins.^{19,88} Protease inhibitors are one of the most promising classes of new anti-HCV drugs. Another potential drug target, the IRES, has a similar structure in HCV and GBV-B.⁴¹ However, drugs

targeting IRES remain a purely theoretical option at this time.

GBV-B replicates efficiently in tamarin primary hepatocyte cultures.⁸ Such cultures may be maintained for up to 1.5 months, which is long enough to allow in vitro screening of various compounds for potential anti-HCV activity and investigating their mode of action at the cellular level.⁵²

A promising approach is an “upgrade” of tamarin and marmoset GBV-B models by using engineered hybrid viruses containing various regions of GBV-B genome substituted with homologous HCV genomic fragments.^{42,82} The potential of this model is yet to be explored.

22.2.2. GB Virus A

GBV-A was first identified in a *Saguinus labiatus* tamarin as a frag-virus.⁹² However, it was subsequently shown that this virus most probably originated from another tamarin species, *S. nigricollis*.¹⁴ Using molecular techniques, complete genome sequences of GBV-A have been determined for viruses originating from *S. labiatus*, *Callithrix jacchus* × *penicillata* hybrid, and *Aotus trivirgatus*.^{21,34,54,55} Using GBV-A-specific PCR, the virus was also detected in *C. jacchus*, *C. mystax*, and *C. oedipus*.

GBV-A infection in New World monkeys is persistent but subclinical. GBV-A genomic sequences detected in animals belonging to the same species cluster together and these species-specific genotypes of GBV-A are well supported by phylogenetic analysis.²¹ Thus, GBV-A viruses appear to have coevolved with their New World simian hosts. The closest relatives of GBV-A are the GBV-C viruses. In contrast to HCV, the pace of evolution for both viruses is relatively slow.^{2,21,68}

22.2.3. Chimpanzee GB Virus C

The only true simian GBV-C currently known is the virus from chimpanzees. This virus, GBV-C_{Ptr}, was described simultaneously and independently by two research groups in 1998.^{2,11} The first group² detected GBV-C_{Ptr}, or more precisely some fragments of its genome, in 3 common chimpanzees out of 39 tested. Two viral RNA-positive animals (*P. troglodytes troglodytes*) originated from Cameroon and Nigeria (animal numbers ch23 and ch33, respectively) and the third one, *P. troglodytes verus* (animal no. ch30), originated from an unspecified location in West Africa. All three

of these animals were wild-caught. Blood samples used for these analyses were drawn shortly after their capture. Thus, it is likely that the chimpanzees were infected with GBV-C_{Ptr} before they were brought into captivity. For animal ch30, two samples were obtained about 2 years apart. Two genomic fragments were amplified from each of the three chimpanzees, a 176-bp 5'-NTR fragment and a 176-bp NS5 gene fragment. The amplification of a larger 426 bp NS3 gene fragment was successful in only one case (animal no. ch33). Phylogenetic analysis of all amplified fragment sequences showed that the GBV-C_{Ptr} lineage is clearly separate from the human HBV-CHGVs lineages. Interestingly, the genomic diversity of GBV-C_{Ptr} proved to be greater than that of human GBV-C/HGVs.²

The second research group¹¹ recovered GBV-C_{Ptr} genomic sequences from captive chimpanzees inoculated with various materials from hepatitis patients using representational difference analysis.⁵⁹ An almost complete genome sequence of GBV-C_{Ptr} (original name GBV-C_{tro}) has been determined (GenBank Acc. No. AF070476; 9,250 bp). The overall sequence similarity of GBV-C_{Ptr} and human GBV-C/HGB sequences is approximately 71%, and the genomic structure of GBV-CHGB and GBV-C_{Ptr} is strictly collinear. The closest relative of GBV-C_{tro} in terms of sequence similarity is the GBV-C_{Ptr} frag-virus detected in *P. troglodytes verus* (animal no. ch30). GBV-C_{tro} has not been found in human samples ($n = 276$). Among simian species only macaques ($n = 16$) have been tested for the presence of GBV-C_{tro} or closely related sequences, and all were negative.

The biological properties of GBV-C_{Ptr} are not known. However, it is unlikely that the virus is pathogenic. GBV-C/HGV, the human relative of GBV-C_{Ptr}, can be readily transmitted to chimpanzees, but the infection is self-limiting and subclinical.¹⁸ No new data relevant to GBV-C_{Ptr} has been reported since 1998.

22.3. REOVIRUSES

The hallmarks of reoviruses (family *Reoviridae*) are their double-stranded and segmented RNA genome. The name of this family is an acronym derived from their original description as *respiratory*, *enteric*, *orphan* viral agents (“orphan” meaning the absence of any known association with a disease). The prototype genus for the *Reoviridae* family is the *Orthoreovirus* genus. Viruses belonging to this genus are usually referred to as

reoviruses, though strictly speaking this name is family-specific. Despite orthoreoviruses being known for about 50 years, they still remain “orphans,” because no unequivocal association with a human disease has been established. In contrast, a pathogenic simian orthoreovirus is known (see Section 22.3.1).

One of the genera within the *Reoviridae* family, the *Rotavirus* genus, includes very important human pathogens. Rotaviruses are estimated to cause approximately 450,000–700,000 deaths annually from acute gastroenteritis in infants and young children, mostly in the developing world. Rotaviruses isolated from simian species are described in Section 22.3.2.

22.3.1. Orthoreoviruses

Serological data suggesting orthoreovirus infection in nonhuman primates (NHPs) were reported in the early 1970s; however, infections were not confirmed by virus isolation.⁴⁸ In 1995, orthoreovirus was isolated from a diseased baboon, one of eight juveniles affected during an outbreak of acute progressive meningoencephalitis in a baboon colony at the Southwest National Primate Research Center, San Antonio, USA.^{33,57} This virus is named baboon orthoreovirus (BRV), although it is not known whether or not baboons are the natural host of BRV. BRV is currently included in the International Committee on Taxonomy of Viruses (ICTV) classification as a sole member of subgroup III within the *Orthoreovirus* genus. No other outbreak caused by BRV in baboon colonies has been reported. Experimental transmission of BRV to two juvenile baboons resulted in the development of meningoencephalitis in both inoculated animals.

BRV belongs to the so-called fusogenic (syncytium-inducing) reoviruses and it has been extensively used for the in vitro analysis of molecular mechanisms underlying this activity which is unusual for nonenveloped viruses.^{22–24,32,33,85}

22.3.2. Rotaviruses

The first rotavirus discovered was simian rotavirus (SA11) isolated from a rectal swab of African green monkey (*Chlorocebus aethiops*) in 1958 in the laboratory of one of the patriarchs of simian virology, H. Malherbe.^{61,62} This virus attracted little interest until the mid-1970s when rotaviruses were found to be the major cause of gastroenteritis in human infants. In contrast to many human rotaviruses, SA11 grows efficiently

in vitro and in the late 1970s and 1980s it was distributed to laboratories all over the world.^{27,49,50,60,81,98} Perhaps most of the knowledge regarding rotavirus replication, genome composition, and virion structure was obtained using SA11 or its derivatives. The SA11-derived stains currently available are not “pure” simian viruses; they are reassortment recombinants between simian (SA11 progenitor) and bovine (“O” agent) rotaviruses.⁹³ Apparently, the “O” agent isolated in Malherbe’s laboratory in 1965⁶² contaminated the SA11 progenitor-producing cell cultures before distribution of the virus. This explains discrepancies in the properties of different SA11-derived strains and emphasizes the need for precise knowledge of genome composition for accurate interpretation of the results of experiments utilizing SA11.⁹³

It is not clear whether rotaviruses circulate in natural NHP populations. Data unequivocally showing that wild monkeys or apes harbor rotaviruses are absent. On the other hand, there are data suggesting a possibility of natural rotavirus infections in monkeys. Antibodies reacting with human rotavirus antigens in ELISA have been found in wild-caught cynomolgus macaques,⁶ but whether the infection was acquired in the wild or captivity was not clear. Natural reassortment between bovine and simian rotaviruses in cattle in India has been described.³⁶ Emergence of such reassortant viruses would require coinfection of a host with two different rotaviruses, one of which is presumed to be transmitted from monkeys.

A significant proportion of captive monkeys and apes are seropositive for rotaviruses.^{6,46,50,78} Several rotavirus strains have been isolated from captive rhesus monkeys and pig-tailed macaques.^{44,64,90,100,107–109} One of these strains (PTRV) is apparently of bovine origin.⁴⁴ The origin of other rotaviruses isolated from macaques is unclear, but they are presumed to be simian. These viruses can be readily transmitted to seronegative macaques by intragastric inoculation. However, gastroenteritis does not develop in the experimentally infected monkeys.^{64,90,109,112}

22.4. CALICIVIRUSES

The family *Caliciviridae* currently includes four genera, *Norovirus*, *Sapovirus*, *Vesivirus*, and *Lagovirus*. Noroviruses and sapoviruses, especially the former, are important human pathogens. The noroviruses are the major cause of nonbacterial epidemic gastroenteritis,

and are notorious for causing gastroenteritis outbreaks on cruise ships.

The first isolation of a calicivirus from NHPs was reported in 1983.⁹⁶ The virus was isolated from a pygmy chimpanzee (*Pan paniscus*) housed in the San Diego Zoo and was named primate calicivirus 1, isolate Pan1 (PCV-1-Pan1). What was apparently the identical virus was subsequently isolated from other NHPs housed in the same zoo [leaf langur (*Presbytis cristata*), lowland gorilla (*Gorilla gorilla*), spider monkey (*Ateles fusciceps*), and douc langur (*Pygathrix nemaeus*)].^{94,95} Such a diverse range of simian species infected with what is presumably the same virus indicates that there was a common source of infection, possibly a nonprimate species. Since 1985, no findings of PCV-1 in NHPs have been reported. The complete genomic sequence of PCV-1-Pan1, also designated as Pan1, has been reported.⁸³ Based on its genomic structure Pan1 can be placed in the *Vesivirus* genus; its closest relatives among known vesiviruses are the feline caliciviruses. Thus, it is unlikely that PCV-1-Pan1 is a genuine simian calicivirus.

More recently caliciviruses have been found in captive macaques using electron microscopy (EM)¹⁰⁶ or a combination of generic calicivirus-specific PCR and virus isolation.³⁵ In the latter case, the isolate from a rhesus monkey named the Tulane virus (TV), was successfully propagated in vitro and its complete genome sequence was determined.³⁵ The virus undoubtedly belongs to the *Caliciviridae* family, its closest relatives being noroviruses. However, TV cannot be placed in any of the currently recognized calicivirus genera. It has been proposed that TV should be classified as a prototype for a new calicivirus genus *Recovirus* (rhesus enteric calicivirus).³⁵ However, the proposed nomenclature has yet to be approved by the ICTV.

22.5. CORONAVIRUSES

Viruses included in the family *Coronaviridae*, like the arteriviruses (see Chapter 11), belong to the *Nidovirales* order. Within the *Coronaviridae* there are two genera, *Coronavirus* and *Torovirus*. Formally a common name, coronavirus can be used for all members of the *Coronaviridae* family. However, in practice it is used virtually exclusively only for viruses included in the *Coronavirus* genus. Coronaviruses have been recognized as a separate entity based on their characteristic crown-like morphology when observed by EM of negatively stained preparations. Human coronaviruses are responsible for

a substantial proportion of upper respiratory tract viral illnesses. The causative agent of severe acquired respiratory syndrome (SARS) is also a coronavirus.

EM was and still is a major tool for the detection of coronaviruses, although molecular methods are gradually overtaking EM. Coronavirus-like particles have been described in fecal samples obtained from rhesus monkeys, cynomolgus and pig-tailed macaques, baboons, chimpanzees, and marmosets.^{37–39,97,106} Apart from the EM findings, very little is known about simian coronaviruses. In the late 1980s, two strains of coronavirus were isolated from a rhesus monkey (CVRM 281) and a hamadryas baboon (CVP 2500) at the Sukhumi Primate Center. Inoculation of rhesus monkeys with the CVRM 281 strain resulted in the development of enterocolitis and pneumonia.^{37–39}

22.6. PARAMYXOVIRUSES

Paramyxoviruses comprise the *Paramyxovirinae* subfamily within the *Paramyxoviridae* family. Several important human pathogens, most notably measles, mumps, respiratory syncytial and parainfluenza viruses are all paramyxoviruses.

ICTV classification includes three simian paramyxoviruses: *simian virus 10* (SV-10), *simian virus 5* (SV-5), and *simian virus 41* (SV-41). SV-10 is included in the genus *Respirovirus* that includes human parainfluenza virus 1 and 3; SV-5 and SV-41 are included in the genus *Rubulavirus* that includes mumps virus and human parainfluenza virus 2 and 4. Despite the presence of the attribute “simian” in their name, it remains unproven that the natural hosts of these viruses are actually simian species. In fact, it is probable that these viruses originated in nonsimian species. The reason why they are named “simian” is historical; SV-5, SV-10, and SV-41 were isolated as common contaminants of primary monkey kidney cultures.^{47,61,111} SV-5 may be a canine virus²⁰ but this is not unequivocally proven. SV-41 and SV-10 are probably variants of human parainfluenza viruses.^{45,72,104} SV-5 has been extensively used as a model for investigation of the mechanisms of paramyxovirus replication. SV-41 and SV-10 are virtually forgotten viruses.

22.7. POXVIRUSES

Poxviruses (family *Poxviridae*) are best known for two viruses of paramount importance—*variola virus* and

vaccinia virus. The first is the causative agent of smallpox, one of the deadliest diseases in the history of mankind. The other virus, *vaccinia*, is “the terminator” of smallpox: it is used as a vaccine that was introduced by Sir Edward Jenner in 1796 and ultimately eliminated variola virus from natural circulation.

Both *variola* and *vaccinia* viruses belong to the family *Orthopoxvirus*, subfamily *Chordopoxvirinae*, which includes poxviruses from vertebrate species. *Monkeypox virus* is a member of the same family. However, the name is a misnomer. Although monkeys are infectable with the monkeypox virus under natural conditions, simian species are not the major natural host of this virus. The main reservoir of monkeypox virus is an African squirrel species (*Funisciurus* spp.). Monkeypox virus infection in NHPs is covered in Chapter 24.

Among eight genera within the *Chordopoxvirinae* subfamily only one, the *Yatapoxvirus* genus, includes a simian poxvirus: Yaba monkey tumor virus (YMTV). Another yatapoxvirus, a Yaba-like disease virus (YLDV), is possibly also of simian origin.

YMTV was described in 1958 as the causative agent of subcutaneous tumors in captive rhesus monkeys housed at primate facilities in Yaba, Nigeria.¹⁰ The virus was identified as a poxvirus.^{71,73} Subcutaneous inoculation of rhesus monkeys with YMTV resulted in rapid development of subcutaneous tumors, presumably of histiocytic origin.^{5,73,99} These tumors were not invasive and regressed spontaneously 2–3 months postinoculation.⁹⁹ Intravenous inoculation of rhesus monkeys resulted in the developments of multiple tumors in the heart, lung, muscles, and subcutaneous tissues which were fatal.⁹⁹ Interestingly, subcutaneous inoculation of human subjects with YMTV resulted in the developments of histiocytic subcutaneous tumors similar to those observed in monkeys. Human YMTV-induced growth is also noninvasive and regresses spontaneously.^{40,65}

From the late 1960s to 2003, YMTV remained in oblivion. In 2003, the complete genomic sequence (genome size 134,721 bp) of YMTV was reported.¹³ The most recent study of YMTV identified a functional inhibitor of interleukin-18 that is encoded in the viral genome.⁷⁰

Yaba-like disease in monkeys is manifested as a mild fever and epidermal lesions indistinguishable from those caused by human Tanapox virus (TPV).^{28,30,31} YLDV and TPV are closely related,^{56,113} apparently representing different strains of the same virus. Al-

though seropositivity for YLDV has been described in wild-caught NHPs,²⁹ the question of whether or not this virus is harbored by simian species under natural conditions has to be readdressed using modern methodologies.

22.8. ANELLOVIRUSES (TT VIRUSES)

Anelloviruses (genus *Anellovirus*, after Latin word *anello* = ring) are small, nonenveloped viruses with a circular, negative-sense, single-stranded DNA genome (3,000–4,000 nt). In the latest ICTV classification (8th Report) the genus *Anellovirus* remains unassigned to any viral family or order. The prototype anellovirus is *Torque teno virus* (TTV). The name is a derivative of Latin words *torques* (necklace) and *tenuis* (thin); it replaced the original name “TT virus” which are the initials of the patient from whom TTV was recovered in 1997. Both old and new designations are abbreviated identically (TTV). Another anellovirus is *Torque teno mini virus* (TTMV) which is currently classified as a tentative species.

The TTV was discovered as frag-virus in serum of a patient with non-A, -B, -C, -E, -D hepatitis using representational difference analysis (reviewed in Hino and Miyata⁴³). Initially, this virus was suspected to be the etiological agent of hepatitis and some other human diseases. However, no convincing evidence of its pathogenicity has been found (reviewed in Hino and Miyata⁴³). TTV infection has also been shown to be very common in humans and some animals, including NHPs. TTV-specific DNA sequences have been detected by PCR in apes and various species of Old and New World monkeys kept in captivity.^{1,75–77,84,105} Whether TTVs detected in captive NHPs are acquired from humans or nonsimian species is not clear. The genomic diversity of TTVs is profound but host- to species-specific TTV lineages are not apparent. The diversity of the best-studied “human TTVs” (i.e., TTVs detected in human materials) is so broad that none of the “simian TTVs” can be reliably distinguished, except by origin of the material from which TTV frag-viruses were recovered.⁴³ The presence of TTV frag-viruses in wild NHPs has been reported only in the Eastern common chimpanzee (*Pan troglodytes schweinfurthii*).⁷

Due to lack of disease association, the interest in TTVs is waning and no reports in the TTV in NHPs have been published since 2004.

REFERENCES

1. Abe, K., T. Inami, K. Ishikawa, S. Nakamura, and S. Goto. 2000. TT virus infection in nonhuman primates and characterization of the viral genome: identification of simian TT virus isolates. *J. Virol.* 74(3):1549–1553.
2. Adams, N. J., L. E. Prescott, L. M. Jarvis, J. C. Lewis, M. O. McClure, D. B. Smith, and P. Simmonds. 1998. Detection in chimpanzees of a novel flavivirus related to GB virus-C/hepatitis G virus. *J. Gen. Virol.* 79(Pt 8):1871–1877.
3. Alter, H. J., Y. Nakatsuji, J. Melpolder, J. Wages, R. Wesley, J. W. Shih, and J. P. Kim. 1997. The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. *N. Engl. J. Med.* 336(11):747–754.
4. Alter, M. J., M. Gallagher, T. T. Morris, L. A. Moyer, E. L. Meeks, K. Krawczynski, J. P. Kim, and H. S. Margolis, for Sentinel Counties Viral Hepatitis Study Team. 1997. Acute non-A-E hepatitis in the United States and the role of hepatitis G virus infection. *N. Engl. J. Med.* 336(11):741–746.
5. Ambrus, J. L., E. T. Feltz, J. T. Grace Jr., and G. Owens. 1963. A virus-induced tumor in primates. *Natl. Cancer Inst. Monogr.* 10:447–458.
6. Awang, A. and K. Yap. 1990. Group A rotavirus infection in animals from an animal house and in wild-caught monkeys. *J. Diarrhoeal Dis. Res.* 8(3):82–86.
7. Barnett, O. E., M. Worobey, E. C. Holmes, and A. Cooper. 2004. Detection of TT virus among chimpanzees in the wild using a noninvasive technique. *J. Wildl. Dis.* 40(2):230–237.
8. Beames, B., D. Chavez, B. Guerra, L. Notvall, K. M. Brasky, and R. E. Lanford. 2000. Development of a primary tamarin hepatocyte culture system for GB virus-B: a surrogate model for hepatitis C virus. *J. Virol.* 74(24):11764–11772.
9. Beames, B., D. Chavez, and R. E. Lanford. 2001. GB virus B as a model for hepatitis C virus. *ILAR J.* 42(2):152–160.
10. Bearcroft, W. G. and M. F. Jamieson. 1958. An outbreak of subcutaneous tumours in rhesus monkeys. *Nature* 182(4629):195–196.
11. Birkenmeyer, L. G., S. M. Desai, A. S. Muerhoff, T. P. Leary, J. N. Simons, C. C. Montes, and I. K. Mushahwar. 1998. Isolation of a GB virus-related genome from a chimpanzee. *J. Med. Virol.* 56(1):44–51.
12. Bright, H., A. R. Carroll, P. A. Watts, and R. J. Fenton. 2004. Development of a GB virus B marmoset model and its validation with a novel series of hepatitis C virus NS3 protease inhibitors. *J. Virol.* 78(4):2062–2071.
13. Brunetti, C. R., H. Amano, Y. Ueda, J. Qin, T. Miyamura, T. Suzuki, X. Li, J. W. Barrett, and G. McFadden. 2003. Complete genomic sequence and comparative analysis of the tumorigenic poxvirus Yaba monkey tumor virus. *J. Virol.* 77(24):13335–13347.
14. Bukh, J. and C. L. Apgar. 1997. Five new or recently discovered (GBV-A) virus species are indigenous to New World monkeys and may constitute a separate genus of the Flaviviridae. *Virology* 229(2):429–436.
15. Bukh, J., C. L. Apgar, S. Govindarajan, and R. H. Purcell. 2001. Host range studies of GB virus-B hepatitis agent, the closest relative of hepatitis C virus, in New World monkeys and chimpanzees. *J. Med. Virol.* 65(4):694–697.
16. Bukh, J., C. L. Apgar, and M. Yanagi. 1999. Toward a surrogate model for hepatitis C virus: an infectious molecular clone of the GB virus-B hepatitis agent. *Virology* 262(2):470–478.
17. Bukh, J., R. E. Engle, S. Govindarajan, and R. H. Purcell. 2008. Immunity against the GBV-B hepatitis virus in tamarins can prevent productive infection following rechallenge and is long-lived. *J. Med. Virol.* 80(1):87–94.
18. Bukh, J., J. P. Kim, S. Govindarajan, C. L. Apgar, S. K. Foung, J. Wages Jr., A. J. Yun, M. Shapiro, S. U. Emerson, and R. H. Purcell. 1998. Experimental infection of chimpanzees with hepatitis G virus and genetic analysis of the virus. *J. Infect. Dis.* 177(4):855–862.
19. Butkiewicz, N., N. Yao, W. Zhong, J. Wright-Minogue, P. Ingravallo, R. Zhang, J. Durkin, D. N. Standring, B. M. Baroudy, D. V. Sangar, S. M. Lemon, J. Y. Lau, and Z. Hong. 2000. Virus-specific cofactor requirement and chimeric hepatitis C virus/GB virus B nonstructural protein 3. *J. Virol.* 74(9):4291–4301.
20. Chanock, R., K. Johnson, and M. Cook. 1961. The hemadsorption technique with special reference to the problem of naturally occurring simian parainfluenza viruses. *Am. Rev. Respir. Dis.* 83:125–129.
21. Charrel, R. N., P. De Micco, and X. de Lamballerie. 1999. Phylogenetic analysis of GB viruses A and C: evidence for cospeciation between virus isolates and their primate hosts. *J. Gen. Virol.* 80(Pt 9):2329–2335.
22. Dawe, S., J. Boutilier, and R. Duncan. 2002. Identification and characterization of a baboon reovirus-specific nonstructural protein encoded by the bicistronic s4 genome segment. *Virology* 304(1):44–52.
23. Dawe, S., J. A. Corcoran, E. K. Clancy, J. Salsman, and R. Duncan. 2005. Unusual topological arrangement of structural motifs in the baboon reovirus fusion-associated small transmembrane protein. *J. Virol.* 79(10):6216–6226.

24. Dawe, S. and R. Duncan. 2002. The S4 genome segment of baboon reovirus is bicistronic and encodes a novel fusion-associated small transmembrane protein. *J. Virol.* 76(5):2131–2140.
25. Deinhardt, F., A. W. Holmes, R. B. Capps, and H. Popper. 1967. Studies on the transmission of human viral hepatitis to marmoset monkeys. I. Transmission of disease, serial passages, and description of liver lesions. *J. Exp. Med.* 125(4):673–688.
26. Deinhardt, F., D. Peterson, G. Cross, L. Wolfe, and A. W. Holmes. 1975. Hepatitis in marmosets. *Am. J. Med. Sci.* 270(1):73–80.
27. Dimitrov, D. H., D. Y. Graham, and M. K. Estes. 1985. Detection of rotaviruses by nucleic acid hybridization with cloned DNA of simian rotavirus SA11 genes. *J. Infect. Dis.* 152(2):293–300.
28. Downie, A. W. 1972. The epidemiology of tanapox and yaba virus infections. *J. Med. Microbiol.* 5(4):xiv.
29. Downie, A. W. 1974. Serological evidence of infection with Tana and Yaba pox viruses among several species of monkey. *J. Hyg. (Lond)* 72(2):245–250.
30. Downie, A. W. and C. Espana. 1972. Comparison of Tanapox virus and Yaba-like viruses causing epidemic disease in monkeys. *J. Hyg. (Lond)* 70(1):23–32.
31. Downie, A. W. and C. Espana. 1973. A comparative study of Tanapox and Yaba viruses. *J. Gen. Virol.* 19(1):37–49.
32. Duncan, R. 1999. Extensive sequence divergence and phylogenetic relationships between the fusogenic and nonfusogenic orthoreoviruses: a species proposal. *Virology* 260(2):316–328.
33. Duncan, R., F. A. Murphy, and R. R. Mirkovic. 1995. Characterization of a novel syncytium-inducing baboon reovirus. *Virology* 212(2):752–756.
34. Erker, J. C., S. M. Desai, T. P. Leary, M. L. Chalmers, C. C. Montes, and I. K. Mushahwar. 1998. Genomic analysis of two GB virus A variants isolated from captive monkeys. *J. Gen. Virol.* 79(Pt 1):41–45.
35. Farkas, T., K. Sestak, C. Wei, and X. Jiang. 2008. Characterization of a rhesus monkey calicivirus representing a new genus of Caliciviridae. *J. Virol.* 82(11):5408–5416.
36. Ghosh, S., V. Varghese, S. Samajdar, M. Sinha, N. Kobayashi, and T. N. Naik. 2007. Molecular characterization of bovine group A rotavirus G3P[3] strains. *Arch. Virol.* 152(10):1935–1940.
37. Goncharuk, E. I., Z. V. Shevtsova, R. I. Krylova, N. B. Rumel', and V. I. Stetsenko. 1994. [The experimental coronavirus infection of monkeys]. *Mikrobiol. Z.* 56(3):65–71.
38. Goncharuk, E. I., Z. V. Shevtsova, N. B. Rumel', and V. V. Fedorinov. 1993. [The properties of simian coronavirus]. *Vopr. Virusol.* 38(3):126–129.
39. Goncharuk, E. I., Z. V. Shevtsova, N. B. Rumel', and R. I. Krylova. 1994. [Spontaneous coronavirus infection in monkeys]. *Zh. Mikrobiol. Epidemiol. Immunobiol.* August–September (Suppl 1):109–114.
40. Grace, J. T., Jr. and E. A. Mirand. 1963. Human susceptibility to a simian tumor virus. *Ann. N. Y. Acad. Sci.* 108:1123–1128.
41. Grace, K., M. Gartland, P. Karayannidis, M. J. McGarvey, and B. Clarke. 1999. The 5' untranslated region of GB virus B shows functional similarity to the internal ribosome entry site of hepatitis C virus. *J. Gen. Virol.* 80(Pt 9):2337–2341.
42. Haqshenas, G., X. Dong, H. Netter, J. Torresi, and E. J. Gowans. 2007. A chimeric GB virus B encoding the hepatitis C virus hypervariable region 1 is infectious in vivo. *J. Gen. Virol.* 88(Pt 3):895–902.
43. Hino, S. and H. Miyata. 2007. Torque teno virus (TTV): current status. *Rev. Med. Virol.* 17(1):45–57.
44. Hoshino, Y., S. Honma, R. W. Jones, N. Santos, O. Nakagomi, T. Nakagomi, A. Z. Kapikian, and M. E. Thouless. 2006. A rotavirus strain isolated from pigtailed macaque (*Macaca nemestrina*) with diarrhea bears a P6[1]:G8 specificity. *Virology* 345(1):1–12.
45. Hull, R. N. 1968. The simian viruses. *Virol. Monogr.* 2:1–16.
46. Jiang, B., H. M. McClure, R. L. Fankhauser, S. S. Monroe, and R. I. Glass. 2004. Prevalence of rotavirus and norovirus antibodies in non-human primates. *J. Med. Primatol.* 33(1):30–33.
47. Kalter, S. S., D. Ablashi, C. Espana, R. L. Heberling, R. N. Hull, E. H. Lennette, H. H. Malherbe, S. McConnell, and D. S. Yohn. 1980. Simian virus nomenclature, 1980. *Intervirology* 13(6):317–330.
48. Kalter, S. S. and R. L. Heberling. 1971. Reovirus antibody in primates. *Am. J. Epidemiol.* 93(5):403–412.
49. Kalter, S. S., R. L. Heberling, A. R. Rodriguez, and T. L. Lester. 1983. Infection of baboons ("*Papio cynocephalus*") with rotavirus (SA11). *Dev. Biol. Stand.* 53:257–261.
50. Kalter, S. S., A. R. Rodriguez, and R. L. Heberling. 1982. Rotavirus (SA11) antibody in nonhuman primates. *Lab. Anim. Sci.* 32(3):291–293.
51. Kyuregyan, K. K., V. F. Poleschuk, N. A. Zamyatina, O. V. Isaeva, M. I. Michailov, S. Ross, J. Bukh, M. Roggendorf, and S. Viazov. 2005. Acute GB virus B infection of marmosets is accompanied by mutations in the NS5A protein. *Virus Res.* 114(1–2):154–157.
52. Lanford, R. E., D. Chavez, B. Guerra, J. Y. Lau, Z. Hong, K. M. Brasky, and B. Beames. 2001.

- Ribavirin induces error-prone replication of GB virus B in primary tamarin hepatocytes. *J. Virol.* 75(17):8074–8081.
53. Lanford, R. E., D. Chavez, L. Notvall, and K. M. Brasky. 2003. Comparison of tamarins and marmosets as hosts for GBV-B infections and the effect of immunosuppression on duration of viremia. *Virology* 311(1):72–80.
54. Leary, T. P., S. M. Desai, J. C. Erker, and I. K. Mushahwar. 1997. The sequence and genomic organization of a GB virus A variant isolated from captive tamarins. *J. Gen. Virol.* 78(Pt 9):2307–2313.
55. Leary, T. P., S. M. Desai, J. Yamaguchi, M. L. Chalmers, G. G. Schlauder, G. J. Dawson, and I. K. Mushahwar. 1996. Species-specific variants of GB virus A in captive monkeys. *J. Virol.* 70(12):9028–9030.
56. Lee, H. J., K. Essani, and G. L. Smith. 2001. The genome sequence of Yaba-like disease virus, a yatapoxvirus. *Virology* 281(2):170–192.
57. Leland, M. M., G. B. Hubbard, H. T. Sentmore III, K. F. Soike, and J. K. Hilliard. 2000. Outbreak of Orthoreovirus-induced meningoencephalomyelitis in baboons. *Comp. Med.* 50(2):199–205.
58. Linnen, J., J. Wages Jr, Z. Y. Zhang-Keck, K. E. Fry, K. Z. Krawczynski, H. Alter, E. Koonin, M. Gallagher, M. Alter, S. Hadziyannis, P. Karayannidis, K. Fung, Y. Nakatsuji, J. W. Shih, L. Young, M. Piatak Jr., C. Hoover, J. Fernandez, S. Chen, J. C. Zou, T. Morris, K. C. Hyams, S. Ismay, J. D. Lifson, G. Hess, S. K. Foung, H. Thomas, D. Bradley, H. Margolis, and J. P. Kim. 1996. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science* 271(5248):505–508.
59. Lisitsyn, N., N. Lisitsyn, and M. Wigler. 1993. Cloning the differences between two complex genomes. *Science* 259(5097):946–951.
60. Lopez, S. and C. F. Arias. 1992. Simian rotavirus SA11 strains. *J. Virol.* 66(3):1832.
61. Malherbe, H. and R. Harwin. 1963. The cytopathic effects of vervet monkey viruses. *S. Afr. Med. J.* 37:407–411.
62. Malherbe, H. H. and M. Strickland-Cholmley. 1967. Simian virus SA11 and the related O agent. *Arch. Gesamte Virusforsch.* 22(1):235–245.
63. Martin, A., F. Bodola, D. V. Sangar, K. Goettge, V. Popov, R. Rijnbrand, R. E. Lanford, and S. M. Lemon. 2003. Chronic hepatitis associated with GB virus B persistence in a tamarin after intrahepatic inoculation of synthetic viral RNA. *Proc. Natl. Acad. Sci. U. S. A.* 100(17):9962–9967.
64. McNeal, M. M., K. Sestak, A. H. Choi, M. Basu, M. J. Cole, P. P. Aye, R. P. Bohm, and R. L. Ward. 2005. Development of a rotavirus-shedding model in rhesus macaques, using a homologous wild-type rotavirus of a new P genotype. *J. Virol.* 79(2):944–954.
65. McNulty, W. P., Jr., W. C. Lobitz Jr., F. Hu, C. A. Maruffo, and A. S. Hall. 1968. A pox disease in monkeys transmitted to man. Clinical and histological features. *Arch. Dermatol.* 97(3):286–293.
66. Melnick, J. L. and W. P. Parks. 1970. Hepatitis in marmosets. *J. Infect. Dis.* 121(3):353–354.
67. Muerhoff, A. S., T. P. Leary, J. N. Simons, T. J. Pilot-Matias, G. J. Dawson, J. C. Erker, M. L. Chalmers, G. G. Schlauder, S. M. Desai, and I. K. Mushahwar. 1995. Genomic organization of GB viruses A and B: two new members of the Flaviviridae associated with GB agent hepatitis. *J. Virol.* 69(9):5621–5630.
68. Nakao, H., H. Okamoto, M. Fukuda, F. Tsuda, T. Mitsui, K. Masuko, H. Iizuka, Y. Miyakawa, and M. Mayumi. 1997. Mutation rate of GB virus Hepatitis G virus over the entire genome and in subgenomic regions. *Virology* 233(1):43–50.
69. Nam, J. H., K. Faulk, R. E. Engle, S. Govindarajan, M. St Claire, and J. Bukh. 2004. In vivo analysis of the 3' untranslated region of GB virus B after in vitro mutagenesis of an infectious cDNA clone: persistent infection in a transfected tamarin. *J. Virol.* 78(17):9389–9399.
70. Nazarian, S. H., M. M. Rahman, S. J. Werden, D. Vileneuve, X. Meng, C. Brunetti, C. Valeriano, C. Wong, R. Singh, J. W. Barrett, Y. Xiang, and G. McFadden. 2008. Yaba monkey tumor virus encodes a functional inhibitor of interleukin-18. *J. Virol.* 82(1):522–528.
71. Nicholas, A. H. and W. P. McNulty. 1968. In vitro characteristics of a poxvirus isolated from rhesus monkeys. *Nature* 217(5130):745–746.
72. Nishio, M., M. Tsurudome, H. Bando, and Y. Ito. 1990. Immunological relationships of simian virus 41 (SV41) to other paramyxoviruses and serological evidence of SV41 infection in human populations. *J. Gen. Virol.* 71(Pt 9):2093–2097.
73. Niven, J. S., J. A. Armstrong, C. H. Andrews, H. G. Pereira, and R. C. Valentine. 1961. Subcutaneous “growths” in monkeys produced by a poxvirus. *J. Pathol. Bacteriol.* 81:1–14.
74. Ohba, K., M. Mizokami, J. Y. Lau, E. Orito, K. Ikeo, and T. Gojobori. 1996. Evolutionary relationship of hepatitis C, pesti-, flavi-, plantviruses, and newly discovered GB hepatitis agents. *FEBS Lett.* 378(3):232–234.
75. Okamoto, H., M. Fukuda, A. Tawara, T. Nishizawa, Y. Itoh, I. Hayasaka, F. Tsuda, T. Tanaka, Y. Miyakawa, and M. Mayumi. 2000. Species-specific TT viruses and cross-species infection in nonhuman primates. *J. Virol.* 74(3):1132–1139.

76. Okamoto, H., T. Nishizawa, M. Takahashi, A. Tawara, Y. Peng, J. Kishimoto, and Y. Wang. 2001. Genomic and evolutionary characterization of TT virus (TTV) in tupaia and comparison with species-specific TTVs in humans and non-human primates. *J. Gen. Virol.* 82(Pt 9):2041–2050.
77. Okamoto, H., T. Nishizawa, A. Tawara, Y. Peng, M. Takahashi, J. Kishimoto, T. Tanaka, Y. Miyakawa, and M. Mayumi. 2000. Species-specific TT viruses in humans and nonhuman primates and their phylogenetic relatedness. *Virology* 277(2):368–378.
78. Otsyula, M., J. Yee, M. Suleman, R. Tarara, J. Martins, P. Woods, R. Glass, and M. Jennings. 1996. Rotavirus infection in African, non-human primates. *Ann. Trop. Med. Parasitol.* 90(6):659–661.
79. Parks, W. P. and J. L. Melnick. 1969. Attempted isolation of hepatitis viruses in marmosets. *J. Infect. Dis.* 120(5):539–547.
80. Parks, W. P., J. L. Melnick, W. R. Voss, D. B. Singer, H. S. Rosenberg, J. Alcott, and A. M. Casazza. 1969. Characterization of marmoset hepatitis virus. *J. Infect. Dis.* 120(5):548–559.
81. Petschow, B. W., R. E. Litov, L. J. Young, and T. P. McGraw. 1992. Response of colostrum-deprived cynomolgus monkeys to intragastric challenge exposure with simian rotavirus strain SA11. *Am. J. Vet. Res.* 53(5):674–678.
82. Rijnbrand, R., Y. Yang, L. Beales, F. Bodola, K. Goettge, L. Cohen, R. E. Lanford, S. M. Lemon, and A. Martin. 2005. A chimeric GB virus B with 5' nontranslated RNA sequence from hepatitis C virus causes hepatitis in tamarins. *Hepatology* 41(5):986–994.
83. Rinehart-Kim, J. E., W. M. Zhong, X. Jiang, A. W. Smith, and D. O. Matson. 1999. Complete nucleotide sequence and genomic organization of a primate calicivirus, Pan-1. *Arch. Virol.* 144(1):199–208.
84. Romeo, R., P. Hegerich, S. U. Emerson, M. Colombo, R. H. Purcell, and J. Bukh. 2000. High prevalence of TT virus (TTV) in naive chimpanzees and in hepatitis C virus-infected humans: frequent mixed infections and identification of new TTV genotypes in chimpanzees. *J. Gen. Virol.* 81(Pt 4):1001–1007.
85. Salsman, J., D. Top, J. Boutilier, and R. Duncan. 2005. Extensive syncytium formation mediated by the reovirus FAST proteins triggers apoptosis-induced membrane instability. *J. Virol.* 79(13):8090–8100.
86. Sbardellati, A., E. Scarselli, V. Amati, S. Falcinelli, A. S. Kekule, and C. Traboni. 2000. Processing of GB virus B non-structural proteins in cultured cells requires both NS3 protease and NS4A cofactor. *J. Gen. Virol.* 81(Pt 9):2183–2188.
87. Sbardellati, A., E. Scarselli, E. Verschoor, A. De Tomasi, D. Lazzaro, and C. Traboni. 2001. Generation of infectious and transmissible virions from a GB virus B full-length consensus clone in tamarins. *J. Gen. Virol.* 82(Pt 10):2437–2448.
88. Scarselli, E., A. Urbani, A. Sbardellati, L. Tomei, R. De Francesco, and C. Traboni. 1997. GB virus B and hepatitis C virus NS3 serine proteases share substrate specificity. *J. Virol.* 71(7):4985–4989.
89. Schaluder, G. G., G. J. Dawson, J. N. Simons, T. J. Pilot-Matias, R. A. Gutierrez, C. A. Heynen, M. F. Knigge, G. S. Kurpiewski, S. L. Buijk, T. P. Leary, A. S. Muerhoff, S. M. Desai, and I. K. Mushahwar. 1995. Molecular and serologic analysis in the transmission of the GB hepatitis agents. *J. Med. Virol.* 46(1):81–90.
90. Sestak, K., M. M. McNeal, A. Choi, M. J. Cole, G. Ramesh, X. Alvarez, P. P. Aye, R. P. Bohm, M. Mohamadzadeh, and R. L. Ward. 2004. Defining T-cell-mediated immune responses in rotavirus-infected juvenile rhesus macaques. *J. Virol.* 78(19):10258–10264.
91. Simons, J. N., T. P. Leary, G. J. Dawson, T. J. Pilot-Matias, A. S. Muerhoff, G. G. Schlauder, S. M. Desai, and I. K. Mushahwar. 1995. Isolation of novel virus-like sequences associated with human hepatitis. *Nat. Med.* 1(6):564–569.
92. Simons, J. N., T. J. Pilot-Matias, T. P. Leary, G. J. Dawson, S. M. Desai, G. G. Schlauder, A. S. Muerhoff, J. C. Erker, S. L. Buijk, M. L. Chalmers, C. L. Van Sant, and I. K. Mushahwar. 1995. Identification of two flavivirus-like genomes in the GB hepatitis agent. *Proc. Natl. Acad. Sci. U. S. A.* 92(8):3401–3405.
93. Small, C., M. Barro, T. L. Brown, and J. T. Patton. 2007. Genome heterogeneity of SA11 rotavirus due to reassortment with "O" agent. *Virology* 359(2):415–424.
94. Smith, A. W., D. E. Skilling, M. P. Anderson, and K. Benirschke. 1985. Isolation of primate calicivirus Pan paniscus type 1 from a douc langur (*Pygathrix nemaeus* l.). *J. Wildl. Dis.* 21(4):426–428.
95. Smith, A. W., D. E. Skilling, and K. Benirschke. 1985. Calicivirus isolation from three species of primates: an incidental finding. *Am. J. Vet. Res.* 46(10):2197–2199.
96. Smith, A. W., D. E. Skilling, P. K. Ensley, K. Benirschke, and T. L. Lester. 1983. Calicivirus isolation and persistence in a pygmy chimpanzee (*Pan paniscus*). *Science* 221(4605):79–81.
97. Smith, G. C., T. L. Lester, R. L. Heberling, and S. S. Kalter. 1982. Coronavirus-like particles in nonhuman primate feces. *Arch. Virol.* 72(1–2):105–111.

98. Soike, K. F., G. W. Gary, and S. Gibson. 1980. Susceptibility of nonhuman primate species to infection by simian rotavirus SA-11. *Am. J. Vet. Res.* 41(7):1098–1103.
99. Sproul, E. E., R. S. Metzgar, and J. T. Grace Jr. 1963. The pathogenesis of Yaba virus-induced histiocytomas in primates. *Cancer Res.* 23:671–675.
100. Stuker, G., L. S. Oshiro, and N. J. Schmidt. 1980. Antigenic comparisons of two new rotaviruses from rhesus monkeys. *J. Clin. Microbiol.* 11(2):202–203.
101. Tabor, E., D. A. Peterson, M. April, L. B. Seeff, and R. J. Gerety. 1980. Transmission of human non-A, non-B hepatitis to chimpanzees following failure to transmit GB agent hepatitis. *J. Med. Virol.* 5(2):103–108.
102. Tabor, E., L. B. Seeff, and R. J. Gerety. 1979. Lack of susceptibility of marmosets to human non-A, non-B hepatitis. *J. Infect. Dis.* 140(5):794–797.
103. Takikawa, S., R. E. Engle, S. U. Emerson, R. H. Purcell, M. St Claire, and J. Bukh. 2006. Functional analyses of GB virus B p13 protein: development of a recombinant GB virus B hepatitis virus with a p7 protein. *Proc. Natl. Acad. Sci. U. S. A.* 103(9):3345–3350.
104. Tsurudome, M., H. Bando, M. Nishio, Y. Iwamoto, M. Kawano, K. Kondo, H. Komada, and Y. Ito. 1990. Antigenic and structural properties of a paramyxovirus simian virus 41 (SV41) reveal a close relationship with human parainfluenza type 2 virus. *Virology* 179(2):738–748.
105. Verschoor, E. J., S. Langenhuijzen, and J. L. Heeney. 1999. TT viruses (TTV) of non-human primates and their relationship to the human TTV genotypes. *J. Gen. Virol.* 80(Pt 9):2491–2499.
106. Wang, Y., X. Tu, C. Humphrey, H. McClure, X. Jiang, C. Qin, R. I. Glass, and B. Jiang. 2007. Detection of viral agents in fecal specimens of monkeys with diarrhea. *J. Med. Primatol.* 36(2):101–107.
107. Westerman, L. E., B. Jiang, H. M. McClure, L. J. Snipes-Magaldi, D. D. Griffin, G. Shin, J. R. Gentsch, and R. I. Glass. 2006. Isolation and characterization of a new simian rotavirus, YK-1. *J. Virol.* 3:40.
108. Westerman, L. E., H. M. McClure, B. Jiang, J. W. Almond, and R. I. Glass. 2005. Serum IgG mediates mucosal immunity against rotavirus infection. *Proc. Natl. Acad. Sci. U. S. A.* 102(20):7268–7273.
109. Westerman, L. E., J. Xu, B. Jiang, H. M. McClure, and R. I. Glass. 2005. Experimental infection of pigtailed macaques with a simian rotavirus, YK-1. *J. Med. Virol.* 75(4):616–625.
110. Woppard, D. J., G. Haqshenas, X. Dong, B. F. Pratt, S. J. Kent, and E. J. Gowans. 2008. Virus-specific T-cell immunity correlates with control of GB virus B infection in marmosets. *J. Virol.* 82(6):3054–3060.
111. Yoshida, E., H. Yamamoto, and H. Shimojo. 1965. Contamination of cynomolgus monkey kidney cell cultures by hemagglutinating simian virus (SV 5). *Jpn. J. Med. Sci. Biol.* 18(3):151–156.
112. Zhao, W., M. Xia, T. Bridges-Malveo, M. Cantu, M. M. McNeal, A. H. Choi, R. L. Ward, and K. Sestak. 2005. Evaluation of rotavirus dsRNA load in specimens and body fluids from experimentally infected juvenile macaques by real-time PCR. *Virology* 341(2):248–256.
113. Zimmermann, P., I. Thordesen, D. Frangoulidis, and H. Meyer. 2005. Real-time PCR assay for the detection of tanapox virus and yaba-like disease virus. *J. Virol. Methods* 130(1–2):149–153.

Section 4:

Experimental and Natural

Infection of Nonhuman Primates with

Nonsimian Viruses

23

Experimental Infection of Nonhuman Primates with Viruses of Medical Importance

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23.1. INTRODUCTION

Modeling human viral disease by inoculation of non-human primates (NHPs) with the causative virus, either

known or suspected, is undoubtedly one of the main themes of simian virology. This field was pioneered by Carl Landsteiner and Erwin Popper who proved the viral etiology of human poliomyelitis in 1908 by experimentally inducing the disease in monkeys by inoculation of clinical specimens from patients.¹⁹³

The explosive growth of basic and medical virology from the 1950s to the 1970s was accompanied by a dramatic increase in the use of NHPs for virological research. During this period, monkeys and apes were experimentally inoculated with virtually all medically significant viruses as well as with clinical specimens from diseases suspected to be of viral origin. Many of these attempted NHP models were poorly developed or not further explored at all.

With the advent of AIDS in 1981, a new era of simian virology began that was highly focused on HIV/AIDS-related research. Among the most important was the simian immunodeficiency virus (SIV) macaque model of AIDS (which is covered in Chapter 4). In the 1980s, limited importation and the higher cost of breeding monkeys and apes significantly increased the cost of NHP research. In addition, many alternative “high-tech” animal models such as “nude,” transgenic, and knock-out mice became available. The overall effect of these developments was a significant decrease in use of NHPs for modeling viral diseases other than AIDS in the 1980s–1990s. However, the situation is ever changing and the trend during the 2000s has been toward a greater and more diversified use of NHP models of human viral diseases.

Although models using small laboratory animals, primarily mice, are an extremely useful and versatile research tool; they rarely match NHP models in mimicking the pathogenesis and immune responses seen in humans affected with a viral disease. Of course this does not mean that NHP models are required for each human viral disease. Moreover, even the theoretically closest NHP model may not mimic some important features of a human disease. Nevertheless, the experimental infection of NHPs with many medically important viruses is paramount for preclinical testing of new vaccination and treatment modalities as well as for gaining insights into pathogenic mechanisms that are difficult or impossible to assess in human patients.

Space limitations and an extremely diverse range of topics dictate a concise and eclectic form for this chapter as well as preferential citation of original papers and reviews from the past 5 years.

23.2. VIRAL HEPATITIS

Although NHP models have been used for studying viruses causing hepatitis A, B, C, D, and E, particularly important has been and continues to be the common chimpanzee model of hepatitis C—hence, the nonalphabetical order of presentation in this section.

23.2.1. Hepatitis C

Modeling hepatitis C virus (HCV) infection and hepatitis C in the common chimpanzee (*Pan troglodytes*) began in 1978 when non-A-non-B hepatitis was first transmitted to chimpanzees.^{9,151} The exploration of this model provided strong evidence for the existence of the parenterally transmitted hepatitis virus other than the known hepatitis A and B viruses.^{42,101,102,285,291} Plasma collected from a chimpanzee during one of these experiments which had very high titers of the virus⁴² was essential for the discovery of HCV in 1989.⁵⁵ Chimpanzees remain the only animal species infectable with HCV. Although successful transmission of HCV to rhesus monkeys has been described, these results have not been independently confirmed.⁴⁵

After discovery of HCV, the focus of the HCV/chimpanzee model shifted from identification and characterization of the causal agent toward vaccine development.^{44,54,96,219} Unfortunately, this task has proven to be formidably complex. HCV, like HIV, has proven to be a “fast moving target.” In other words, the virus is prone to rapid genetic and antigenic changes and is capable of evading immune responses.^{37,97,98,100,105,209} The immune response against HCV that is readily detectable in HCV-infected persons appears to be impotent as far as preventing progression of the acute infection into chronic hepatitis with all its fatal consequences including liver cirrhosis and hepatocellular cancer. The existence of multiple HCV genotypes further complicates the situation. Superinfection of chimpanzees with another HCV genotype was readily achieved,⁹⁶ and inter-genotype recombination has been shown to occur in persistently infected chimpanzees.¹¹⁵

The natural course of HCV infection in chimpanzees is substantially different from that in humans.^{98,194} HCV-induced hepatitis in chimpanzees is milder than human hepatitis C, although cases of severe HCV-induced hepatitis in chimpanzees have been reported.⁹⁹ The progression to chronic hepatitis is markedly less common in chimpanzees than in humans and in

contrast to humans liver cirrhosis is extremely rare in chimpanzees.^{194,218} Although the chimpanzee model is far from ideal in mimicking pathogenesis of human hepatitis C, currently there is no viable alternative, particularly for testing protective efficacy of candidate HCV vaccines.^{43,156,194}

Transmission of HCV by contaminated factor VIII concentrate has been modeled in chimpanzees.²⁶² Despite the proven presence of multiple HCV genotypes in the inoculum, only a single genotype has been transmitted.

Theoretically, the chimpanzee model is the best option for preclinical testing of new anti-HCV drugs. However, it is rarely used for this purpose due to the limited availability of chimpanzees and other constraints on the experiments involving these endangered animals.⁵²

A number of experimental HCV vaccines and vaccination protocols aimed at protection against infection have been tested with variable success. Some groups have reported protection,^{30,54,195,220,361} whereas others did not achieve protection.^{96,110,292,294,295} Recent studies of candidate HCV vaccines using the chimpanzee model have focused on induction of protective T-cell responses.^{91,109,221,245,265,294,295,315} Cell-mediated immune protection of vaccinated chimpanzees against challenge with heterologous HCV strains have been achieved.^{91,109} However, escape mutants (resulting from mutations in the NS3 and NS5A genes) were detected in chimpanzees that had initially mounted a strong anti-HCV cellular immune response.²⁹⁵ Thus, although the developers of HCV vaccines are cautiously optimistic, an effective HCV vaccine usable in humans still appears to be a quite distant option.

23.2.2. Hepatitis B

Soon after the discovery of hepatitis B virus (HBV) it was shown that common chimpanzees are readily infectable with this virus.^{27,145,228} Transmission of the virus was achieved by inoculation with HBV surface antigen (HBsAg)-positive human blood, serum, saliva, and semen.^{8,28,35,189} Chimpanzees can also be infected by administration of molecularly cloned genomic HBV DNA produced in vitro by transfection of human or rat cells.^{2,324,341} Other apes are probably also susceptible to human HBV infection. However, direct proof of this is available only for gibbons.^{23,322} Old and New World monkeys are known to be resistant to human

HBV infection. One possible exception is chacma baboons (*Papio ursinus*).^{25,176} However, these results have not been independently confirmed, and other baboon species have been reported to be resistant to human HBV infection.²⁴¹ Barbary macaques (*Macaca sylvanus*) can be infected by intrahepatic inoculation of cloned HBV DNA, but they are resistant to infection by the virus.¹²⁴ Thus, among NHPs only chimpanzees can be used for modeling human HBV infection and hepatitis B.

The chimpanzee model was extensively explored in the early years of HBV research in the 1970s and 1980s. HBV infection in chimpanzees in general is similar to the infection in humans, but tends to be milder.^{89,189,326} After primary infection, the immune response either clears the virus or a chronic infection is established. Chronic HBV infection in chimpanzees is associated with hepatitis, but liver cirrhosis has not been reported. However, two cases of hepatocellular carcinoma have been described in HBV-positive chimpanzees.²⁸⁶ The major use of the chimpanzee model has been in the framework of HBV vaccine development. Both HBV vaccines currently approved for human use (plasma-derived and recombinant yeast-derived) were tested in chimpanzees in preclinical trials and shown to be protective.^{36,48,269,297} A number of experimental approaches to vaccination against HBV have been tested in chimpanzees and shown to be protective against challenge with infectious HBV. These include immunization with pre-S peptide vaccines,^{114,158,266} live recombinant poxvirus vaccine,²⁵⁶ prime-boost vaccination,²⁷² DNA vaccines,^{64,273} anti-idiotypic vaccine,¹⁷⁷ and oral live recombinant adenovirus.²¹⁰

After successful introduction of HBV vaccines into clinical practice, the use of the chimpanzee model has waned significantly. However, some investigational anti-HBV products are still tested in chimpanzees.^{153,185,323,384} Using the chimpanzee model, it was shown that intravenous administration of humanized monoclonal antibodies against pre-S1 protein that are directed against an epitope mapping to amino acid residues 37–45 protects against virus challenge.¹⁵³ Another monoclonal antibody directed against an epitope mapping to amino acid residues 17–21 in the pre-S protein has also been shown to be protective.³⁸⁴ However, this epitope is present in only five genotypes of human HBV (A, B, C, F, and H). Therefore, this monoclonal antibody does not neutralize viruses belonging to other HBV genotypes (D, E, and G).

Using the chimpanzee model, it has been shown that the prime-boost strategy of therapeutic immunization with a DNA vaccine as the prime, and canarypox recombinant HBV vaccine as the booster, is ineffective.³²³

23.2.3. Hepatitis A

Human hepatitis A had been successfully transmitted to chimpanzees and marmosets long before hepatitis A virus (HAV) was conclusively identified.^{74,75} NHP models of human hepatitis A were quite intensively explored in the 1970s.^{81,152,222,227}

New World monkeys, particularly tamarins (*Saguinus mystax*, *S. labiatus*) and owl monkeys (*Aotus trivirgatus*), are highly susceptible to HAV-induced hepatitis. In contrast, HAV is usually nonpathogenic for commonly used Old World monkey species (*Macaca mulatta*, *M. fascicularis*).^{11,379} Stump-tailed macaques (*M. arctoides*) are a possible exception in this respect, although data on the susceptibility of this species to HAV-induced hepatitis are not entirely consistent.^{11,296} Common chimpanzees are susceptible to HAV-induced hepatitis, but this model is rarely used due to the availability of the cheaper and more assessable tamarin model. HAV-induced hepatitis A is usually milder in monkeys and apes than in humans. However, all of the major features of human hepatitis A are reproduced.

23.2.4. Hepatitis E

Hepatitis E virus (HEV) is the sole member of the genus *Hepevirus* and the family *Hepeviridae*. This virus shares many properties with HAV. Both viruses are transmitted mainly by the fecal–oral route. Hepatitis E, like hepatitis A, is an acute disease which does not become chronic. In general, hepatitis E is more severe than hepatitis A (mortality 1–2% versus 0.5%); however, in pregnant women the mortality from hepatitis E can be much higher (20% and more) (reviewed in Mushahwar²⁵⁹).

Inoculation of NHP with materials from patients with water-borne non-A hepatitis (now known as hepatitis E) played an important role in the identification of HEV.^{22,41,172} A number of simian species (from chimpanzee to marmosets) are susceptible to experimental infection with HEV; however, cynomolgus macaques and rhesus monkeys are most commonly used for modeling HEV infection and hepatitis E.^{12,61,231,347,350,352,353,373,382} Depending on the strain and dosage of the virus, experimental infection with HEV may be subclinical or manifested as hepatitis.^{3,350}

In contrast to humans, pregnancy does not aggravate the course of experimental HEV infections in cynomolgus macaques.³⁵² It is worth mentioning that HEV seropositivity has been reported in macaques [cynomolgus, rhesus, and Japanese (*M. fuscata*)] which were not inoculated with HEV.^{144,188} However, direct proof that macaques can be naturally infected with HEV (or related virus) is absent.

Macaque models have been extensively used for pre-clinical testing of various experimental vaccines against HEV and hepatitis E.^{170,171,200,201,298,351,383,386} One of these vaccines, consisting of a recombinant capsid protein expressed in insect cells,²⁹⁸ has been successfully tested in a phase 2 field trial.³²⁷

23.3. HIGHLY PATHOGENIC RNA VIRUSES CAUSING HEMORRHAGIC FEVERS

Highly pathogenic RNA virus infections is an umbrella term for viruses that are transmitted to humans from animal reservoirs and cause acute, severe, frequently fatal hemorrhagic fevers, and encephalitis in humans.

The most notable signs of viral hemorrhagic fevers are a highly elevated body temperature, multiple hemorrhages, and bleeding in the most severe cases—hence the name of these viruses and the diseases they cause. Taxonomically, hemorrhagic fever viruses belong to various families: *Filoviridae*, *Arenaviridae*, *Bunyaviridae*, and *Flaviviridae*. However, the pathogenesis of hemorrhagic fevers caused by these viruses has many common features. The development of the disease is largely attributed to an overzealous proinflammatory host response triggered by the viral infection. The main pathogenic mechanism leading to a fatal outcome is impairment of the integrity of the vascular system resulting in the loss of plasma. The cause of death in fatal cases is hypovolemic shock. This type of response is observed in accidental nonnatural hosts like humans and NHPs. In nature, all hemorrhagic fever viruses except Dengue virus are maintained in wild or domesticated animal reservoirs. In the reservoir species these viruses cause inapparent or mild infections. Hemorrhagic fever viruses are transmitted to humans by arthropod vectors (mosquitoes or ticks for flaviviruses), through exposure to the rodent's excreta (arenaviruses and some bunyaviruses) or through direct contact with diseased animals (filoviruses).

Animal models are of critical importance for the development of vaccines against these diseases, their

treatment, and prophylaxis.¹³¹ NHP models of hemorrhagic fevers undoubtedly mimic the pathogenesis of human hemorrhagic fevers and immune responses against the infection far better than small animal models. Information on these models is summarized in Sections 23.3.1–23.3.4.

23.3.1. Filoviral Hemorrhagic Fevers (Ebola and Marburg)

Ebola (EBOV) and Marburg (MARV) viruses are the only known filoviruses (the family *Filoviridae*). Four species of EBOV [*Zaire ebolavirus* (EBOV-Z), *Sudan ebolavirus* (EBOV-S), *Reston ebolavirus* (EBOV-R), and *Ivory Coast ebolavirus* (EBOV-IC)] and one species of MARV [*Lake Victoria marburgvirus* (LV-MARV)] are currently recognized by the International Committee on Taxonomy of Viruses (ICTV). The lineages to which EBOVs and MARV belong are sufficiently separated to be ranked taxonomically as two genera, *Ebolavirus* and the *Marburgvirus*, respectively. The name of the family was suggested by the unusual filamentous shape of virions. The names of the viruses and genera have geographical origins. Marburg is a city in Germany where the disease caused by MARV [Marburg hemorrhagic fever (MHF)] was first reported. Ebola is a river in the Democratic Republic of Congo (formerly Zaire) in the vicinity where the first known outbreak of Ebola hemorrhagic fever (EHF) occurred. Both diseases are clinically similar, highly contagious and usually fatal. The mortality ranges from 40 to 100%, being 80–90% for the majority of the human outbreaks (reviewed in Hensley *et al.*¹⁴²).

EBOV can cause devastating outbreaks of EHF in wild gorillas and chimpanzees. Moreover, the origin of some human outbreaks has been traced to contact with the carcasses of great apes affected with EHF (see Chapter 24). It is also worth mentioning that the first outbreak of MHF was the result of occupational exposure of laboratory workers to African monkeys infected with MARV.

Cynomolgus macaques, rhesus monkeys, baboons, and African green monkeys can be experimentally infected with EBOV and MARV.^{29,40,211,127,242,270,319} Macaques are susceptible to all EBOV and MARV strains. African green monkeys are susceptible to EBOV and MARV with the exception of EBOV-R and to some extent EBOV-S. Baboons, in general, are less susceptible to EBOV and MARV than macaques as the induced

disease is less severe. Interestingly, EBOV-R, which is nonpathogenic for humans, is pathogenic for macaques (see Chapter 24). Most studies involving experimental infection of NHPs with filoviruses have been performed using EBOV and macaques.

The clinical presentation of EHF in macaques and humans is similar, but the course of the disease in monkeys is more rapid and invariably fatal. Macaques experimentally infected with EBOV die within 6–9 days after inoculation; this time frame is too short for the development of an adaptive antiviral immune response. The macaque model of EHF differs in this respect from human EHF, where not all cases are fatal. Presumably, this difference is due to a dose disparity; “natural” doses are probably lower than those used for experimental infections. However, direct estimates of doses in naturally occurring infections are absent.

The primary target cells for EBOV are macrophages and dendritic cells (DC).^{120,123} Late in the course of infection endothelial cells are also infected. EBOV infection of these cells induces the release of proinflammatory cytokines and the so-called tissue factor (TF) that disrupts the DC-mediated orchestration of immune responses. This leads to severe impairment of immunity, particularly innate immunity, and vascular dysfunction. EBOV infection inhibits production of interferons (IFN) and IFN signaling pathways. The blockade of IFN signaling cannot be overcome by treatment with high dose IFN.¹⁶¹ EBOV-Z infection in cynomolgus macaques leads to a dramatic decrease in the number of the natural killer (NK) cells, which are almost completely depleted by day 4 after inoculation.³⁰⁴ Profound depletion of CD4⁺ and CD8⁺ lymphocytes also develops. Interestingly, EBOV does not replicate in NK or lymphocytes and the decline of these subpopulations is due to the activation of so-called “bystander apoptosis.”¹¹⁸ Importantly, this phenomenon is observed only in human and simian EHF and is not reproduced in the rodent models of EBOV infection.

Although EBOV and MARV infect endothelial cells, vascular dysfunction and loss of the endothelial barrier are mostly attributable to soluble damaging factors such as elevated levels of tumor necrosis factor α (TNF- α) and nitric oxide (NO). NO is a potent vasodilator and contributes to the development of vasodilatory shock. TNF- α induces expression of TF that leads to the development of disseminated intravascular coagulation (DIC), a characteristic feature of EHF.¹²³ The formation of microvascular thrombi in combination with increased

permeability of the endothelium results in bleeding and necrosis. Experimental treatment aiming at inhibition of DIC development has been tested with some success in the macaque/EBOV model.¹¹⁹ Administration of the recombinant nematode-derived anticoagulant protein c2 (rNARC2) within the first 24-h postinoculation protected about one third of EBOV-infected macaques and prolonged survival of the remaining animals. In the absence of treatment, EHF is invariably fatal in this model.

Currently, the major use of EBOV/macaque models is for testing various experimental vaccines against EHF and MHF.^{31,308,375,376} Traditional whole inactivated virus vaccines against EHF and MHF are ineffective.²⁴² A number of modern vaccine strategies which appeared promising when tested in the rodent models, turned out to be ineffective when tested in NHPs.¹²² However, during the past 5 years several new experimental vaccines/immunization protocols have been reported to be protective against EBOV or MARV in the macaque model.^{46,47,62,167,337–339,360,376} The major types of the vaccines/vaccination strategies which have been actively developed are DNA prime/recombinant adenovirus boost immunization^{337–339} and recombinant vaccines-based human parainfluenza virus type 3,^{46,47,376} vesicular stomatitis virus,^{117,167} virus-like particles (VLPs),^{343,360,375} and adenoviruses.³⁴² All these approaches appear promising, although side-by-side comparisons have not been reported.

Postexposure vaccination, if effective, would be extremely useful in controlling Ebola and Marburg outbreaks. In the macaque model protection by postexposure vaccination is achievable.^{63,103,117} Another postexposure option, passive immunoprophylaxis/immunotherapy with various antibody preparations, has also been tested in NHP models. The administration of horse anti-EBOV hyperimmune serum was shown to be protection in the baboon (*Papio hamadryas*) model.³⁹ However, passive immunotherapy was ineffective when tested using the more adequate macaque model.^{159,160}

23.3.2. Flaviviral Hemorrhagic Fevers

23.3.2.1. YELLOW FEVER

Yellow fever virus (YFV) occupies a special place in the history of virology. Walter Reed made history when he proved in 1901 that yellow fever is caused by a filterable agent, i.e., a virus, and that it is transmitted by mosquitoes. What is less known is that the viral etiology

of yellow fever was not considered proven until 1928 when Adrian Stokes and co-workers described the successful transmission of yellow fever to rhesus monkeys and presented convincing proof that the disease was caused by the virus. This benchmark paper³³⁵ was reprinted in 2001 with an accompanying biography of Adrian Stokes who died of yellow fever which he contracted while carrying out the experiments reported by his team in 1928.

Yellow fever is distinguished from other viral hemorrhagic fevers by more severe liver injury manifested as jaundice—hence the name of the disease. Rhesus monkeys and cynomolgus macaques are readily infected with YFV and develop a disease that is virtually indistinguishable from severe human yellow fever.^{247,332,335} Diseased monkeys usually die within a week of inoculation.

Thanks to mosquito control and the availability of an effective vaccine, yellow fever is a much smaller public health problem than it was in the nineteenth and the first part of the twentieth centuries. Nevertheless, sporadic outbreaks of yellow fever continue to occur, particularly in Africa, with a global annual mortality estimated at 30,000.¹³⁰

The live vaccine against yellow fever is based on the attenuated YFV strain 17D. A single immunization with this vaccine induces protective immunity in humans; the 17D-based vaccine has an excellent safety record over the more than 60 years of its use. The macaque model was instrumental in the development of this vaccine. Current quality control procedures for vaccine production still include the monkey neurovirulence test.^{223,225}

23.3.2.2. KYASANUR FOREST DISEASE

Bonnet macaques (*Macaca radiata*) are infectable with Kyasanur forest disease virus (KFDV). Experimental infection with KFDV in this species is manifested as necrotic changes in the intestine, spleen, and lymph nodes.¹⁷⁹

23.3.2.3. DENGUE HEMORRHAGIC FEVER

Dengue virus (DENV) infection is a major health problem in the geographical regions where the virus is endemic. Clinically it can present as a mild form (dengue fever) or as a more severe form (dengue hemorrhagic fever) which is frequently accompanied by a fatal dengue shock syndrome. It is estimated that there are approximately 100 million cases of dengue fever and

about 500,000 cases of dengue hemorrhagic fever per year. Dengue fever, a nonlethal disease, has been known for more than 200 years, while dengue hemorrhagic fever, much more severe disease, was only recognized in the 1950s. The development of dengue hemorrhagic fever is attributed to an aberrant immune response, the immune enhancement effect. This develops in individuals immune to one DENV type after superinfection with another DENV type (four types of DENV are known). The mechanism of this phenomenon is not fully understood.

Although macaques can be infected with DENV, they do not develop disease.¹³¹ Nevertheless macaques are used for studying the immune responses to experimental DENV infection³²⁰ and testing experimental treatments⁴ and candidate DENV vaccines.^{186,187,240} An effective tetravalent DENV vaccine is urgently needed and the NHP primate model clearly has to be integrated in the vaccine development process.^{186,187,368}

23.3.3. Arenaviral Hemorrhagic Fevers

Arenaviruses (genus *Arenavirus*, family *Arenaviridae*) that cause hemorrhagic fever in humans can be divided into two groups: the Old World group represented by Lassa virus (LASV) and the New World group that includes several viruses endemic in different parts of Latin America.

23.3.3.1. LASSA FEVER

Lassa fever is a disease endemic in West Africa and is responsible for approximately 5,000 deaths/year in this region. The case fatality rate for Lassa fever is about 2–5%. Lassa fever is the only viral hemorrhagic fever for which there is an approved efficient treatment protocol (intravenous Ribavirin).

Rhesus monkeys can be infected with LASV. The virus induces severe multi-organ pathology: hepatocellular necrosis, interstitial pneumonia, pulmonary arteritis, adrenal gland necrosis, encephalitis, and uveitis.^{49,358} LASV-induced disease in macaques does not exactly mimic human Lassa fever; however, the major pathogenetic features are apparently held in common.^{108,163} Importantly, the macaque model was predictive regarding the efficacy of treatment of Lassa fever with ribavirin.^{163,165,331} It has also been shown using the macaque model that ribavirin in combination with passive immunotherapy is more efficacious than ribavirin alone.¹⁶⁵ Successful vaccination

against LASV-induced disease in macaques has also been reported.^{106,107,121} The replication-competent vaccine that is based on attenuated recombinant vesicular stomatitis virus expressing LASV glycoprotein holds promise. This vaccine was shown to induce protection after a single intramuscular injection.¹²¹

In addition to the macaque model, LASV can be transmitted experimentally to marmosets.⁵⁰ LASV-infected marmosets develop disease that closely resembles Lassa fever.

Another alternative NHP model of Lassa fever is based on the use of the murine lymphocytic choriomeningitis virus (LCMV), a mouse virus that is closely related to LASV.^{83,212,213,314} The disease in macaques induced by LCMV is similar to LASV-induced disease. The use of this model still carries risk because LCMV is also pathogenic for humans.

23.3.3.2. SOUTH AMERICAN HEMORRHAGIC FEVERS

South American hemorrhagic fevers have a restricted geographical distribution—hence their names: Argentinean hemorrhagic fever, Bolivian hemorrhagic fever, and Venezuelan hemorrhagic fever. The causative agents of these diseases are named Junin virus (JUNV), Machupo virus (MACV), and Guanarito virus (GTOV), respectively.

Argentinean hemorrhagic fever has modeled in rhesus monkeys^{132,178,235–237} and common marmosets.^{17,129,363–366} The disease in macaques can be induced by intravenous inoculation with JUNV¹³² and by exposure to virus-containing aerosol.¹⁷⁸ Some JUNV strains are predominantly viscerotropic, whereas others are neurotropic.^{132,235} This is similar to what is observed in human patients with Argentinean hemorrhagic fever. The macaque/JUNV model was used for testing efficiency of chemoprophylaxis by ribavirin.²³⁴ The drug was found to be protective only if administered simultaneously with inoculation of JUNV. In the early 1990s a live-attenuated JUNV vaccine (named Candid # 1) was evaluated in rhesus monkeys and found to be safe, immunogenic, and protective.^{236,237} However, this vaccine has apparently not been further developed. The marmoset model of Argentinean hemorrhagic fever was actively explored in the late 1979s–1980s,^{17,129,363–366} but more recent publications in this field are absent.

Bolivian hemorrhagic fever has also been modeled in macaques^{128,174,346} as well as African green monkeys

(*Chlorocebus aethiops*),^{238,357} mainly in the 1970s. These models have not been further explored.

23.3.4. Bunyaviral Hemorrhagic Fevers

Bunyaviruses (family *Bunyaviridae*) that cause hemorrhagic fevers belong to three genera, *Phlebovirus* [Rift Valley fever virus (RVFV)], *Nairovirus* [Crimean-Congo hemorrhagic fever virus (CCHFV)], and *Hantavirus* [Hantaan, Puumala (PUUV), Sin Nombre, and others]. NHP models have been described for RVFV and PUUV.

23.3.4.1. RIFT VALLEY FEVER

Rift Valley fever, the disease caused by RVFV, is a relatively mild illness endemic in sub-Saharan Africa. However, in some cases the disease is complicated by a hemorrhagic syndrome and, less frequently, by encephalitis which is usually fatal.

The rhesus monkey model of Rift Valley fever mimics features of the human disease, and fatal hemorrhagic complications also develop in approximately 20% of inoculated monkeys.^{57,254,277} The rhesus/RVFV model was used for preclinical evaluation of prophylactic use of interferon, and some beneficial effect was observed.^{252,253}

A formalin-killed vaccine against RVFV approved for use in humans is available. The drawback of this vaccine is the requirement for annual booster immunizations. The development of live vaccines against Rift Valley fever has not been persistently pursued. However, one experimental live vaccine against RVFV was tested in the rhesus monkey model and was shown to be adequately attenuated.²⁵⁵

23.3.4.2. HANTAVIRAL HEMORRHAGIC FEVER

The prototype hantavirus, Hantaan virus, was named after the Hantaan river in Korea. The first recognized cases of the disease caused by this virus were identified near this river in the early 1950s. Other hantaviruses also received “geographical” names (Seul, Dobrava, Puumala, Sin Nombre). Most hantaviruses cause hemorrhagic manifestations accompanied with renal damage, the so-called hemorrhagic fever with renal syndrome. The Sin Nombre virus causes a severe pulmonary disease named hantavirus pulmonary syndrome.

The only hantaviral disease modeled in NHPs is nephropathia epidemica, a relatively mild kidney illness endemic in Scandinavia. The disease is caused by the

Puumala virus (PUUV). This virus can be experimentally transmitted to cynomolgus macaques.^{135,180–182} PUUV-infected macaques develop lethargy, anorexia, mild proteinuria, and microhematuria. Kidney damage is largely confined to medullar tubular cells. In general, the disease induced in monkeys is similar to the nephropathia epidemica.¹⁸¹ Using this model it has been demonstrated that classical serotherapy with convalescent phase sera from experimentally infected monkeys can protect cynomolgus macaques challenged with virulent PUUV.^{180,182}

23.4. HIGHLY PATHOGENIC RNA VIRUSES CAUSING ENCEPHALITIS

Disease caused by highly pathogenic RNA encephalitis viruses usually presents as meningoencephalitis with limited involvement of the limbs. A key pathogenic feature of these viruses is tropism for the central nervous system (CNS) which implies a capacity to cross blood-brain barrier. Taxonomically, the highly pathogenic RNA encephalitis viruses belong to the following genera: *Flavivirus* (the family *Flaviviridae*), *Alphavirus* (the family *Togaviridae*), and *Henipavirus* (the family *Paramyxoviridae*).

Humans and NHPs are accidental hosts for the encephalitis viruses. The viruses are transmitted from their natural reservoir species by ticks and mosquitoes or through close contact with infected animals. Importantly, only a relatively small proportion of transmissions to accidental hosts results in development of disease.

Animal models of disease/infection are of paramount importance for the development of antiviral therapies and vaccines (reviewed in Holbrook and Gowen¹⁵⁰). Information on the experimental infection of NHPs with encephalitis viruses is summarized in Sections 23.4.1–23.4.3.

23.4.1. Flaviviral Encephalitis

23.4.1.1. JAPANESE ENCEPHALITIS

Japanese encephalitis virus (JEV) is the prototype of a group of related viruses comprising the so-called Japanese encephalitis serocomplex. This virus group includes JEV, West Nile encephalitis virus (WNV), and St. Louis encephalitis virus (SLEV). JEV causes a polio-like disease with peripheral neuropathies that is fatal in 25% of cases. Japanese encephalitis continues to be a significant public health problem in some regions of

Asia, with approximately 50,000 cases reported annually.

JEV is readily transmitted to rhesus monkeys and cynomolgus macaques. The pathological consequences of experimental infection with JEV depend on the route of inoculation. Intranasal inoculation results in lethal disease, whereas i.v. inoculation results in subclinical infection.^{260,301}

Vaccines against Japanese encephalitis, both killed and live-attenuated, have been used for decades. Nevertheless, the development of new vaccines continues. The cynomolgus/JEV model is an essential part of this process.^{73,246,302,345} Among the experimental vaccines being developed, a chimeric yellow fever/Japanese encephalitis virus vaccine (ChimeriVax-JE) is at the most advanced stage.^{73,246} However, this vaccine has not yet reached the stage of human trials.

23.4.1.2. WEST NILE ENCEPHALITIS

West Nile encephalitis virus (WNV) has been known since 1937, but interest in this virus increased greatly after initial outbreaks in North America in 1999. Since then, cases of WNV encephalitis, predominantly in the elderly, are registered in the United States and Canada every summer. Fortunately, the number of fatal cases is relatively small. Rhesus monkeys can be infected by WNV by intracerebral inoculation. Virulence of various WNV isolates in this model differs significantly and the establishment of persistent infections is common.²⁸⁰ Baboons are also infectable with WNV, although the infection is subclinical and is cleared within 2 months postinoculation.³⁶⁹ The macaque model was used for preclinical testing of the candidate WNV vaccine ChimeriVax-WN02 (an attenuated recombinant virus in which the premembrane and envelope genes of YFV vaccine strain 17D are replaced by the corresponding genes of WNV). The vaccine has been shown to be immunogenic and safe.^{14,250} A similar vaccine based on the dengue type 4 virus has also been tested in macaques and found to be promising.²⁷⁹

23.4.1.3. TICK-BORNE ENCEPHALITIS

Flaviviruses causing tick-borne encephalitis (TBEV) are traditionally divided into two subtypes: Western (Central European encephalitis viruses) and Far-eastern (Russian spring-summer encephalitis viruses). All TBEVs are lethal to mice and have been extensively studied in this model. However, NHP models

have also been explored.^{179,281,389} TBEVs can be transmitted experimentally to both African (patas monkeys and vervets) and Asian (rhesus monkeys and bonnet macaques) NHPs. The frequency and severity of clinical manifestations varies significantly among different TBEVs. Far-eastern TBEVs tend to be more virulent. TBEV infection in NHPs commonly becomes persistent.²⁸¹ The rhesus monkey model was used for testing the candidate live recombinant vaccines against tick-borne encephalitis (attenuated chimeric DENV/TBEV viruses). The vaccines were shown to be highly immunogenic and lacking neurovirulence.^{278,318} However, recently reported data suggest that the degree of attenuation of these vaccines may be insufficient.²²⁶

23.4.1.4. ST. LOUIS ENCEPHALITIS

St. Louis encephalitis virus (SLEV) is found only in South and North America where it causes epidemics with intervals of 5–15 years. SLEV was first isolated by inoculation of rhesus monkeys and mice with material from patients affected during the epidemic in St. Louis in 1933—hence the name of the virus. SLEV is transmissible to rhesus macaques by intracerebral inoculation. The virulence of various isolates in macaques and mice differs significantly. The results obtained using rodent and macaque models are highly correlated.²⁴⁹ Results showed promise in the macaque model for a candidate vaccine against St. Louis encephalitis that is based on the attenuated chimeric DENV/SLEV.³⁸

23.4.1.5. POWASSAN ENCEPHALITIS

Powassan virus (POWV) is the only tick-borne encephalitis flavivirus found in North America. Rhesus monkeys are infectable with this virus through intracerebral inoculation; experimentally infected monkeys develop encephalitis.^{112,113}

23.4.2. Alphaviral Encephalitis

Encephalitis alphaviruses (genus *Alphavirus*, family *Togaviridae*) are mosquito-borne viruses that cause severe encephalitis primarily in horses—hence their names: Venezuelan equine encephalitis (VEEV), Western equine encephalitis (WEEV), and Eastern equine encephalitis (EEEV). Occasionally, these viruses cause encephalitis in humans.

23.4.2.1. VENEZUELAN EQUINE ENCEPHALITIS

VEEV can be transmitted experimentally to rhesus monkeys and cynomolgus macaques through aerosol and by injection.^{125,164,248,288} In contrast to the disease in humans, the disease in macaques is characterized by involvement of lymphoid tissue rather than the CNS. The cynomolgus/VEEV model has been actively used for the development of a new vaccine against Venezuelan equine encephalitis based on the attenuated strain V3526 produced by genetic modification.²⁸⁷ This candidate vaccine has been successfully tested in the cynomolgus model and found to be safer and more effective than the vaccine TC-83 currently used in humans.^{303,307}

23.4.2.2. WESTERN EQUINE ENCEPHALITIS

Western equine encephalitis virus (WEEV) readily infects cynomolgus macaques through aerosol exposure.³⁰⁶ The monkeys infected by this route develop severe encephalitis within 4–6 days postexposure. In the late 1970s and early 1980s, it was shown that experimental intrauterine infection of rhesus monkey fetuses with attenuated WEEV may be teratogenic.^{207,251} Such a possibility must be taken into account in determining the safety of experimental vaccines against WEEV.

23.4.2.3. EASTERN EQUINE ENCEPHALITIS

Susceptibility of rhesus monkeys to EEEV was first described in 1939.³⁷² Intracerebral inoculation of rhesus monkeys with the virus results in the development of fatal encephalitis; however, after i.v. inoculation the infection is subclinical. The most recent development in modeling EEE in monkeys is experimental infection of cynomolgus macaques through exposure to aerosolized EEEV.³⁰⁵ The lethal encephalitis that develops after such exposure is similar to the disease in humans caused by EEEV.

23.4.3. Bunyaviral Encephalitis

La Crosse virus (LACV) belongs to the genus *Orthobunyavirus* (family *Bunyaviridae*). This virus is a major cause of pediatric encephalitis in North America. Rhesus monkeys are readily infectable with LACV; however, the infection is subclinical.³³

23.5. SMALLPOX AND MONKEYPOX

Smallpox is a highly lethal and contagious disease caused by the variola virus (VARV) which belongs to

the family *Orthopoxvirus* within the subfamily *Chordopoxvirinae* of the family *Poxviridae*. For centuries, smallpox was one of the most feared diseases. At the same time, the struggle with smallpox produced one of the most significant breakthroughs in the history of medicine: the concept of vaccination conceived by Edward Jenner who developed a vaccine against smallpox in 1797. Almost 200 years later, in 1977, the WHO declared smallpox eradicated. This outstanding success was achieved by mass immunization with the live vaccine of Jenner which is based on the vaccinia virus.

Unfortunately, the smallpox story does not end with this success. VARV is a prime candidate for development into a biological weapon. Although an international convention signed in 1972 banned the development of biological weapons, there is a risk of VARV being used for bioterrorism. Live VARV is kept cryopreserved in two special-containment laboratories, one in the United States and the other in Russia. Fortunately, VARV has never escaped from these laboratories. Nevertheless, extreme vigilance and preparedness is needed to deal with this potential threat. NHP models are essential for the development of effective means to deal with the accidental or intentional spread of VARV.

Experiments with live VARV are extremely costly and difficult to carry out due to the stringent bio-containment requirements.¹⁶² A viable alternative is to use monkeypox virus (MPV). This virus was first isolated in 1959 from captive cynomolgus macaques with a smallpox-like disease.³⁵⁶ MPV and the monkeypox disease share many features with VARV and smallpox (reviewed in Di Giulio and Eckburg⁸⁰). Importantly, the vaccinia virus-based vaccine against smallpox also protects against monkeypox. Humans are susceptible to monkeypox, but fortunately the disease is much less contagious than smallpox. Several relatively small outbreaks of monkeypox have been reported in countries of Central and Western Africa. In 2003, an outbreak of monkeypox occurred in the United States.⁸⁰ The source of this outbreak was MPV-infected wild-caught rodents imported to the United States from Africa, possibly Gambian giant rats that were sold as pets.

MPV has the potential to be weaponized, although such a possibility is remote. More importantly, MPV provides an excellent surrogate model which mimics major features of smallpox.³⁸¹ The monkeypox models in cynomolgus and rhesus macaques have been actively used mainly for development of optimized pre- and postexposure prophylaxis protocols.^{85,143,224,267,333,334}

Testing new vaccines in these models is usually done in comparison with Dryvax, the only vaccine against smallpox licensed in the United States, which is currently considered as the gold standard in this field.^{84,267} It has been shown that immunization with modified vaccinia virus Ankara (MVA) confers protection against monkeypox that is almost as strong as that induced by Dryvax. MVA is a highly attenuated replication-deficient strain of vaccinia virus which is presumed to be safer than classical vaccinia virus, particularly in immunocompromised hosts.

Several DNA vaccines against smallpox have also been tested using monkey models. These vaccines induce protective immunity against monkeypox, although less strong than the protection provided by Dryvax.^{143,154} Efforts have also been made to improve the standardization of Dryvax; a vaccine was prepared from a biologically cloned vaccinia virus strain used for production of Dryvax. This “refined” Dryvax vaccine was equivalent to conventional Dryvax in terms of its protective activity against monkeypox.²²⁴ The protection against smallpox in the macaques immunized with the vaccinia virus is mediated predominantly by virus-specific antibodies; apparently cell-mediated immunity plays a lesser role.⁸⁶ These data are encouraging because methods for the induction of virus-specific antibodies are much better developed than those aimed at inducing virus-specific cellular immunity.

The SIV/rhesus monkey model has been used for testing protective efficiency of immunization against smallpox and monkeypox in the context of immunosuppression mimicking HIV-induced immunosuppression. Immunization of SIV-positive rhesus macaques with the Dryvax vaccine resulted in development of skin lesions. This adverse effect can be prevented by preimmunization with the genetically engineered, replication-deficient poxvirus NYVAC.⁸⁷ However, even this improved immunization protocol did not protect SIV-immunosuppressed macaques from the development of smallpox after challenge.⁸⁵

The comparative efficiency of postexposure chemo- and immunoprophylaxis has been tested in the MPV/cynomolgus macaque model.³³³ Treatment with cidofovir and a related nucleoside analogue administered 24 h after challenge with a lethal dose of MPV were more effective in preventing death from monkeypox than was vaccination with a vaccinia-based vaccine (Elstree-RIVM; licensed in the Netherlands) administered at the same time.

23.6. RABIES

Rabies, the disease notorious for its torturous course and inevitably fatal outcome, is very rarely observed in developed countries these days. However, in the developing world rabies is still a significant public health problem. The relatively safe rabies vaccine derived from infected diploid tissue culture cells is expensive, so developing countries continue to use cheaper, old-fashioned “pasteurian” vaccines produced in sheep, goat, and suckling mouse brains. These vaccines cause more severe adverse reactions than any other antiviral vaccines used in humans.

Monkeys and apes, like humans, are susceptible to rabies.^{20,21,348,362} However, NHP models of rabies have rarely been used because much cheaper and well developed small animal models are available. Perhaps, the most interesting rabies-related data obtained using NHPs are the results of testing a rabies DNA vaccine.^{202–204} This vaccine expresses the major envelope protein of the rabies virus and showed excellent protective efficacy in cynomolgus monkeys challenged with a lethal dose of rabies virus. Moreover, even a single vaccination was sufficient for protection.²⁰² However, postexposure vaccination with the DNA vaccine was less successful. In this case the level of protection achieved in the vaccinated group was lower than in the “gold standard” group immunized with the diploid tissue culture cell-derived rabies vaccine: 50% and 75%, respectively.²⁰³ If further development of the DNA rabies vaccine is successful, the stability and low cost of this vaccine would be ideal for use in poor-resource settings.

23.7. RESPIRATORY TRACT INFECTIONS

Human respiratory infections are mostly modeled in small laboratory animals. However, since the 1940s NHPs have been occasionally used for medically important respiratory viruses, especially the influenza viruses. During last 5 years, some interest in NHP models of human viral respiratory tract infections has reemerged, primarily in the context of testing vaccines and new treatments against the SARS coronavirus and potentially pandemic influenza viruses.

23.7.1. Influenza

Many simian species, including New and Old World monkeys as well as apes, are infectable with human and avian influenza viruses.^{34,134,168,169,257,258,330} However,

these models have not been consistently utilized. The emergence of an avian influenza virus that is pathogenic for humans greatly stimulated interest in the development of new modalities for prevention and treatment of potentially pandemic influenza viruses. In this context, several research themes have been explored, particularly (1) development and characterization of macaque H5N1 influenza models^{18,60,191,312}; (2) testing immunogenicity and protective efficacy of experimental vaccines^{82,95,157,317}; and (3) “proof of principle” studies of treatments modulating immune responses, such as interleukin-7-induced enhancement of immune response to influenza vaccination in the elderly.¹⁵ “Resurrection” of the 1918 Spanish influenza virus, the virus that caused the most lethal pandemic in modern times, has opened new possibilities for gaining a better understanding what made this particular virus so lethal. The macaque model of “Spanish flu” appears to be very useful.¹⁸³ Using this model it was shown that aberrant innate immune responses triggered by this virus are mainly responsible for the extremely high virulence of the 1918 influenza virus. Apparently, this feature is shared by the H5N1 virus pathogenic for humans.³¹²

23.7.2. SARS

The epidemic of severe acquired respiratory syndrome (SARS) in 2002–2003, although relatively small, caused significant damage to the global economy particularly in China, Southeast Asia, and Canada. At its peak, SARS even briefly overshadowed the AIDS. Fortunately, the SARS epidemic was short-lived and, predictably, after it ended the attention given to the disease and its causal agent [SARS coronavirus (SARS-CoV)] has largely subsided. However, a possibility that SARS may reemerge does exist and the development of a SARS vaccine and effective treatment modalities is clearly warranted.

Experimental induction of SARS-like disease in cynomolgus macaques by inoculation with SARS-CoV was first reported in 2003 shortly after the isolation of this virus.^{111,190} However, these results have not been reproduced by other research groups^{197,230,316} and the situation with modeling SARS in NHP remains controversial.¹⁴⁹ There is no doubt that cynomolgus macaques, rhesus monkeys, African green monkeys, and common marmosets are readily infectable with SARS-CoV.^{65,133,137,230,299,316} However, the pathological consequences of infection reported by various groups vary

from a mild transient upper-respiratory illness or no disease at all^{230,316} to severe pneumonia and respiratory distress.^{111,137,190}

Several experimental SARS vaccines have been tested in cynomolgus macaques and rhesus monkeys including whole virus inactivated vaccines,^{300,385} adenoviral recombinant vaccines,^{116,184} and a recombinant modified vaccinia virus Ankara.⁵³ Using the cynomolgus macaque model, it has also been shown that prophylactic treatment with pegylated interferon-alpha significantly reduces viral replication and excretion as well as damage of type 1 pneumocytes, the primary target cells for the virus. Postexposure treatment with pegylated IFN-alpha was less effective, although some positive effect was observed.¹³⁶

23.7.3. Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) is the major cause of lower respiratory tract illness in infants. RSV is also increasingly recognized as a common cause of severe respiratory infection in the elderly. It has been known since the mid-1970s that a wide range of NHP species (squirrel monkeys, *Cebus* spp., rhesus monkeys, bonnet macaques, and chimpanzees) are infectable with RSV.^{19,32,229,310,311,329} Experimentally infected macaques develop pathology resembling RSV-induced lung disease in humans. NHP models of RSV infection are of critical importance primarily for RSV vaccine development. However, until recently, NHP models of RSV infection/disease were little explored and the whole field of RSV vaccine development remained at an impasse due to the unexpected failure of an inactivated RSV vaccine (instead of protection it caused immune-mediated enhancement).¹⁷³ During the last several years, efforts to develop an effective RSV vaccine have been renewed using modern methodologies for vaccine development.^{71,72,355,359,378} Importantly, the phenomenon of immune-mediated pathogenicity enhancement by the whole virus inactivated RSV vaccine is reproducible in bonnet (*M. radiata*) and cynomolgus macaque models.^{67,284} These models thus provide an important tool for assessment of candidate RSV vaccines for potential immune-mediated adverse reactions.

23.7.4. Human Metapneumovirus

Human metapneumovirus (hMPV) is a relatively new addition to the list of viral pathogens causing acute respiratory-tract illnesses in humans. This virus, first

described in 2001, belongs to the genus *Metapneumovirus* within the *Paramyxoviridae* family. hMPV is readily transmissible to cynomolgus macaques by experimental inoculation of wild-type and molecularly cloned isolates.^{192,354} Viral replication in experimentally infected monkeys is restricted to the respiratory tract and is associated with mild inflammatory changes in the airways. The main target cells for hMPV are ciliated respiratory epithelial cells. Shedding of virus peaks at day 4 postinoculation and completely subsides by day 10. Experimentally infected cynomolgus macaques develop protective immunity which lasts for 6–12 weeks after the primary infection.³⁵⁴ Importantly, it has been shown that as for RSV, vaccination of cynomolgus macaques with hMPV whole virus formalin-inactivated vaccines is associated with the development of the immune-mediated eosinophilic bronchitis and bronchiolitis.⁷⁰

23.8. HERPESVIRAL INFECTIONS

23.8.1. Generalized Herpes Simplex

NHPs are rarely used as animal models for experimental HSV infections due to the availability of more convenient and less expensive small animal models. Judging by the documented cases of accidental human-to-simian transmissions, many simian species appear to be susceptible to HSVs. However, controlled inoculation studies have only been reported for owl and cebus monkeys.^{26,104,175,239,261} In owl monkeys even a small dose of wild-type HSV (100 pfu) administered intravenously is fatal. Experimental genital infection of cebus monkeys with HSV-2 resulted in pathology that resembled genital herpes in humans.^{104,261} However, this model has not been further explored.

23.8.2. Varicella

Mild varicella can be induced in common chimpanzees by inoculation with VZV,⁵⁶ but this research model is not practical since these apes are an endangered species. VZV can also be experimentally transmitted to cotton-top marmosets (*Callithrix jacchus*).²⁹³ In VZV-infected marmosets the virus replicates preferentially in the lung tissue and pneumonia develops rather than a varicella-like disease.

23.8.3. Epstein–Barr Virus

New World monkeys such as cotton-top and white-lipped tamarins (*S. oedipus* and *S. fuscicollis*), com-

mon marmosets, owl monkeys, and squirrel monkeys are readily infectable with EBV. It has also been reported that rhesus and cynomolgus macaques can be infected with EBV.¹⁹⁹ However, unambiguous interpretation of these data is not possible because no proof has been provided that the virus detected in inoculated animals is in fact EBV.

Malignant lymphomas can be experimentally induced by EBV in New World monkeys.^{1,93,94,166,243,325,340,367} Cotton-top tamarins (wrongly referred to as marmosets in many early reports) appear to be particularly susceptible.¹⁶⁶ This model of EBV-induced oncogenesis has not been explored further. However, it is worth mentioning that the most widely used strain of EBV (EBV-B95-8) is a by-product of EBV-research in New World monkeys performed in the 1970s.²⁴⁴

Common marmosets experimentally infected with EBV develop persistent infection with oral shedding of the virus.⁵⁸ This model has been used for testing experimental EBV vaccines.^{59,215} The protective effect of immunization with EBV gp340 preparations was found to be very limited and use of this model has been discontinued.

23.8.4. Human Herpesvirus 6

The progression toward SAIDS in SIV-infected pigtailed macaques (*M. nemestrina*) is markedly accelerated by coinfection with subtype A of human herpesvirus 6 (HHV-6A). HHV-6A infection in these dually inoculated macaques has been documented by the seroconversion and detection of HHV-6A DNA in plasma of inoculated animals.²¹⁴ These data have been interpreted as evidence that HHV-6A may promote progression to AIDS. However, neither independent confirmation nor additional studies along these lines has been reported.

23.8.5. Human Herpesvirus 8

Results of inoculation of SIV-positive and SIV-negative rhesus monkeys with human herpesvirus 8 (HHV-8) are inconclusive. None of the animals inoculated with HHV-8 derived from PEL cells developed an antibody response against HHV-8. Moreover, HHV-8-specific RNA was not detected in uncultured tissue of the inoculated monkeys. At the same time, HHV-8 DNA was detected by polymerase chain reaction (PCR) in cultivated peripheral blood mononuclear cells of all inoculated animals. No clinical manifestation attributable to HHV-8 was observed.³⁰⁹

23.9. VIRAL GASTROENTERITIS

23.9.1. Rotaviruses

Soon after the discovery of human rotaviruses in 1974, it was shown that these viruses could be transmitted to newborn rhesus monkeys³⁷¹ and cynomolgus macaques.²¹⁶ Whereas diarrhea was observed in monkeys inoculated directly with the human rotavirus, generally the pathogenicity was lost during serial passages in monkeys. In addition, only newborns or infants were readily infectable.¹⁹⁸ For this reason, experimental infection of macaques with human rotaviruses has not been further explored as a model of rotavirus gastroenteritis. More recently, however, it has been shown that five of five infant African green monkeys and one of two anubis baboons were infectable with human rotavirus by oral inoculation of noncultured human rotavirus A (G8P[6] genotype). However, the experimentally induced infection was subclinical.⁵¹

23.9.2. Noroviruses

A human norovirus (the Norwalk agent) has been transmitted to common chimpanzees by the intragastric inoculation. However, no clinical manifestations attributable to the inoculated virus were observed.³⁷⁰ Human noroviruses has also been transmitted experimentally (by intragastric inoculation) to common marmosets, cotton-top tamarins, rhesus monkeys, and pig-tailed macaques, but not cynomolgus macaques.^{313,336} However, only in pig-tailed macaques was the infection with human norovirus (Toronto strain of Norwalk-like virus) clinically manifested (diarrhea and vomiting sufficiently severe to cause dehydration).³³⁶ Susceptibility of pig-tailed macaques to norovirus gastroenteritis has not been independently confirmed.

23.10. HUMAN T-LYMPHOMA/LEUKEMIA VIRUS 1

Cynomolgus macaques are infectable with HTLV-1,³⁷⁴ but the infection is subclinical. Immunization of cynomolgus and pig-tailed macaques with various crude HTLV-1-derived preparations induces immune responses that are protective against challenge with live HTLV-1 and STLV-1.^{77–79,263,264}

Rhesus monkeys are also infectable with HTLV-1.³⁴⁹ Coinfection of rhesus monkeys with HTLV-1 and SIV results in activation of HTLV-1 infection as shown by the marked increase in the number of lymphocytes with

“bizarre” nuclei (so-called “flower cells”) which are commonly observed in patients with adult T-cell lymphoma/leukemia. However, the development of HTLV-1-positive T-cell lymphoma in the HTLV-1/SIV coinfecting rhesus monkeys has not been observed.³⁴⁹

23.11. MEASLES

Once considered as almost inevitable in childhood, measles is a rarity now in developed countries. This outstanding achievement is virtually entirely due to universal vaccination with a highly efficacious live measles vaccine [the tri-valent measles, mumps, rubella (MMR) vaccine]. However, in the developing world, measles continues to be the leading cause of childhood death from a vaccine-preventable disease.

Experiments on NHPs have played and continue to play an important role in the fight against measles. The first transmission of the measles virus (MV) to monkeys was described by J. Goldberger and J. F. Anderson in 1911.^{10,126} Experimental infection of rhesus monkeys was used during the development of the live measles vaccine to demonstrate apathogenicity of the attenuated vaccine strain of MV.⁹² The rhesus macaque model of measles is considered to be the most adequate animal model of measles available,^{7,16,90,232,233,268,388} although cynomolgus macaques are also highly susceptible to measles.^{90,321} In terms of the dynamics of virological and immunological parameters, experimental measles in cynomolgus macaques is similar to that in rhesus monkeys; however, MV-induced skin rash and conjunctivitis are less manifested in the cynomolgus model.⁹⁰ MV is also highly pathogenic for the marmosets and tamarins,^{5,6,208} but these models are less adequate in terms of mimicking human measles than the macaque models.

The rhesus monkey model of measles has been actively used for testing new candidate measles vaccines and for investigation of mechanisms of MV-induced immunosuppression and immunity. MV is a strongly immunosuppressive agent. Fortunately, in immunocompetent hosts MV-induced immunosuppression is transient. The mechanism of MV-induced immunosuppression is multifactorial; the virus affects humoral, cellular, and innate immunity.^{69,147,232,282} Despite the induction of transient immunosuppression, the immune response to MV is sufficiently effective to clear the virus and establish long-lasting protective immunity. In rhesus macaques with depleted B-cell or T-cell (CD8⁺)

populations, T-cell-mediated immunity has been shown to be primarily responsible for protection.^{274,275}

Although a highly efficacious measles vaccine is available, the development of new measles vaccines is continuing and macaque models are an integral part of this process.^{24,66,68,76,148,283,289,290} The main incentive behind this continued vaccine development is the WHO plan for global measles eradication which is to be implemented after completion of the global polio eradication program. Massive immunization against measles in developing countries will require a vaccine(s) that is inexpensive, stable in hot climates, and can be delivered by noninjectable means. Currently, none of the approved measles vaccines meets these conditions. Taking into account the high prevalence of HIV infection in some developing countries, the vaccine/immunization protocols also need to be optimized for use in HIV-infected individuals. The rhesus macaque model of coinfection with SIV and measles is useful in this context.²⁷⁶ Another unresolved problem related to mass immunization against measles of infants in the developing world is the presence of maternal antibodies that inhibit induction of an immune response against the conventional live vaccine. NHP models that mimic this situation by simultaneous immunization of newborn rhesus macaques with the measles vaccine and administration of anti-MV antibodies have been described.^{289,387} It has also been shown that coimmunization with a measles DNA vaccine expressing the MV hemagglutinin, fusion, and nucleoprotein genes together with a DNA vaccine expressing interleukin 2 (IL-2) protects immunized infants against development of high-level viremia despite the presence of passively administered antimeasles antibodies.^{289,290}

23.12. HUMAN POLYOMAVIRUSES

Some species of New World monkeys can be infected with the human JC and BK polyomaviruses. Inoculation with JC virus induced brain tumors, astrocytomas, and glioblastomas in owl and squirrel monkeys after an incubation period of 1–3 years.^{155,205,206,217} Similar doses of BK virus and SV-40 were not oncogenic. The JC virus-induced tumors contain the entire viral genome and express T-antigen, but do not produce complete virus. The utility of this model is limited due to the long incubation period. Also, this model does not mimic features of human pathology associated with the JC virus.

Pharmacologically immunosuppressed (with cyclophosphamide) squirrel monkeys are highly suscep-

tible to BK virus. Four weeks after intravenous inoculation, high virus loads are detected in blood and virtually all organs. Interstitial nephritis was observed in most (three out of four) animals inoculated with BK virus. Interestingly, under identical conditions squirrel monkeys are much less susceptible to SV-40.³⁸⁰

23.13. HUMAN ENTEROVIRUSES

Poiovirus induces a CNS disease in rhesus monkeys which is remarkably similar to human poliomyelitis.³⁴⁴ This model was instrumental in the development of vaccines against poliomyelitis (reviewed in Eggers⁸⁸). The essential component of oral polio vaccine quality control is testing each vaccine lot for residual neuropathogenicity in rhesus monkeys.

Among nonpolio enteroviral models, the most explored is experimental infection of cynomolgus macaques with human enterovirus 71 (EV71).^{138–141} EV71 is primarily known as an etiologic agent of herpangina (HA), the so-called hand, foot, and mouth disease (HFMD). It may also be associated with a poliomyelitis-like illness in infants and young children.²⁷¹ The virus caused HFMD/HA outbreaks involving thousands of cases in Taiwan in 1991 and in China in 2008. Fortunately, the mortality from HFMD/HA is low (about 0.3%).¹⁴⁶ EV71 induces paralytic diseases in cynomolgus macaques when administered subcutaneously, intravenously, or orally.^{138,140} The virus targets the CNS and can be detected in motor neurons but is absent in surrounding cells.¹⁴¹ A temperature-sensitive mutant of EV71 (strain BrCr) was shown to be nonpathogenic in cynomolgus macaques.¹³⁹ However, the most advanced EV71 vaccine candidate, the attenuated strain EV71 (S1-3'), induces mild neurological signs in cynomolgus monkeys.¹³ Immunization with the EV71 (S1-3')-based live vaccine protects cynomolgus macaques from a lethal challenge with EV71 (BrCr) and induces neutralizing antibodies against all EV71 genotypes. However, the neutralizing activity of immunized monkey sera is strongest against the homologous genotype (genotype A) and declines against other genotypes in the following order: B1 > C4 > B4 > C2.¹³

Coxsackie A4 (CVA4) and B4 (CVB4) viruses have also been experimentally transmitted to monkeys.^{328,377} After oral administration of CVA4 to rhesus monkeys, the virus replicates initially in the lower gastrointestinal tract and then enters the bloodstream and spreads

to other organs.³²⁸ This model has not been explored further.

An intriguing possibility is the association of coxsackie B viruses with insulin-dependent diabetes.²⁷¹ However, there is only one published report exploring the diabetogenic potential of CVB4 in monkeys.³⁷⁷ Although claims that CVB4 induces “transient diabetes in nonhuman primates” have been made, the data presented in this paper did not demonstrate changes in glucose tolerance or insulin secretion abnormalities in cynomolgus monkeys, rhesus macaques, or capuchins inoculated with CVB4.

An acute fatal disease, “superacute liver necrosis,” has been induced in African green and rhesus monkeys by inoculation with human enteroviruses ECHO-11 (E11) and ECHO-19 (E19).¹⁹⁶ The acute hepatic failure accompanied by pathology in multiple other organs develops within days after inoculation with E11 and E19. The viruses used in these experiments were isolated in the former USSR from children with acute enteroviral uveitis or an unspecified “severe enterovirus infection.” Interestingly, cases similar to the disease induced in monkeys have also been observed in humans in association with E11 and E19. However, this model has not been pursued further.

REFERENCES

1. Ablashi, D. V., G. S. Aulakh, J. Luetzeler, K. S. Sundar, G. R. Armstrong, and A. Faggioni. 1983. Fatal lymphoproliferative disease in a common marmoset (*Callithrix jacchus*) following inoculation of Ag876 strain of Epstein-Barr virus and a tumor-promoting agent: preliminary report. *Comp. Immunol. Microbiol. Infect. Dis.* 6(2):151–160.
2. Acs, G., M. A. Sells, R. H. Purcell, P. Price, R. Engle, M. Shapiro, and H. Popper. 1987. Hepatitis B virus produced by transfected Hep G2 cells causes hepatitis in chimpanzees. *Proc. Natl. Acad. Sci. U. S. A.* 84(13):4641–4644.
3. Aggarwal, R., S. Kamili, J. Spelbring, and K. Krawczynski. 2001. Experimental studies on sub-clinical hepatitis E virus infection in cynomolgus macaques. *J. Infect. Dis.* 184(11):1380–1385.
4. Ajariyakhajorn, C., M. P. Mammen Jr., T. P. Endy, M. Gettayacamin, A. Nisalak, S. Nimmanitya, and D. H. Libraty. 2005. Randomized, placebo-controlled trial of nonpegylated and pegylated forms of recombinant human alpha interferon 2a for suppression of dengue virus viremia in rhesus monkeys. *Antimicrob. Agents Chemother.* 49(11):4508–4514.
5. Albrecht, P., D. Lorenz, and M. J. Klutch. 1981. Encephalitogenicity of measles virus in marmosets. *Infect. Immun.* 34(2):581–587.
6. Albrecht, P., D. Lorenz, M. J. Klutch, J. H. Vickers, and F. A. Ennis. 1980. Fatal measles infection in marmosets pathogenesis and prophylaxis. *Infect. Immun.* 27(3):969–978.
7. Albrecht, P., A. L. Shabo, G. R. Burns, and N. M. Tauraso. 1972. Experimental measles encephalitis in normal and cyclophosphamide-treated rhesus monkeys. *J. Infect. Dis.* 126(2):154–161.
8. Alter, H. J., R. H. Purcell, J. L. Gerin, W. T. London, P. M. Kaplan, V. J. McAuliffe, J. Wagner, and P. V. Holland. 1977. Transmission of hepatitis B to chimpanzees by hepatitis B surface antigen-positive saliva and semen. *Infect. Immun.* 16(3):928–933.
9. Alter, H. J., R. H. Purcell, P. V. Holland, and H. Popper. 1978. Transmissible agent in non-A, non-B hepatitis. *Lancet* 1(8062):459–463.
10. Anderson, J. F. and J. Goldberger. 1911. The period of infectivity of the blood in measles. *Am. Assoc. LVII:113–114.*
11. Andzhaparidze, A. G., Z. V. Shevtsov, L. I. Korzaia, IuV Karetnyi, and M. S. Balaian. 1987. [Signs of natural infection with hepatitis A in brown macaques (*Macaca arctoides*)]. *Vopr. Virusol.* 32(5):541–544.
12. Arankalle, V. A., L. P. Chobe, and M. S. Chadha. 2006. Type-IV Indian swine HEV infects rhesus monkeys. *J. Viral Hepat.* 13(11):742–745.
13. Arita, M., N. Nagata, N. Iwata, Y. Ami, Y. Suzuki, K. Mizuta, T. Iwasaki, T. Sata, T. Wakita, and H. Shimizu. 2007. An attenuated strain of enterovirus 71 belonging to genotype a showed a broad spectrum of antigenicity with attenuated neurovirulence in cynomolgus monkeys. *J. Virol.* 81(17):9386–9395.
14. Arroyo, J., C. Miller, J. Catalan, G. A. Myers, M. S. Ratterree, D. W. Trent, and T. P. Monath. 2004. ChimeriVax-West Nile virus live-attenuated vaccine: preclinical evaluation of safety, immunogenicity, and efficacy. *J. Virol.* 78(22):12497–12507.
15. Aspinall, R., J. Pido-Lopez, N. Imami, S. M. Henson, P. T. Ngom, M. Morre, H. Niphuis, E. Remarque, B. Rosenwirth, and J. L. Heeney. 2007. Old rhesus macaques treated with interleukin-7 show increased TREC levels and respond well to influenza vaccination. *Rejuvenation. Res.* 10(1):5–17.
16. Auwaerter, P. G., P. A. Rota, W. R. Elkins, R. J. Adams, T. DeLozier, Y. Shi, W. J. Bellini, B. R. Murphy, and D. E. Griffin. 1999. Measles virus infection in rhesus macaques: altered immune responses and comparison of the virulence of six different virus strains. *J. Infect. Dis.* 180(4):950–958.

17. Avila, M. M., S. R. Samoilovich, R. P. Laguens, M. S. Merani, and M. C. Weissenbacher. 1987. Protection of Junin virus-infected marmosets by passive administration of immune serum: association with late neurologic signs. *J. Med. Virol.* 21(1):67–74.
18. Baas, T., C. R. Baskin, D. L. Diamond, A. Garcia-Sastre, H. Bielefeldt-Ohmann, T. M. Tumpey, M. J. Thomas, V. S. Carter, T. H. Teal, N. Van Hoeven, S. Proll, J. M. Jacobs, Z. R. Caldwell, M. A. Gritsenko, R. R. Hukkanen, D. G. Camp, R. D. Smith, and M. G. Katze. 2006. Integrated molecular signature of disease: analysis of influenza virus-infected macaques through functional genomics and proteomics. *J. Virol.* 80(21):10813–10828.
19. Babu, P. G., A. Selvan, S. Christuraj, J. David, T. J. John, and E. A. Simoes. 1998. A primate model of respiratory syncytial virus infection. *Indian J. Exp. Biol.* 36(8):758–762.
20. Baer, G. M., S. A. Moore, J. H. Shaddock, and H. B. Levy. 1979. An effective rabies treatment in exposed monkeys: a single dose of interferon inducer and vaccine. *Bull. World Health Organ.* 57(5):807–813.
21. Baer, G. M., J. H. Shaddock, S. A. Moore, P. A. Yager, S. S. Baron, and H. B. Levy. 1977. Successful prophylaxis against rabies in mice and Rhesus monkeys: the interferon system and vaccine. *J. Infect. Dis.* 136(2):286–291.
22. Balayan, M. S., A. G. Andjaparidze, S. S. Savinskaya, E. S. Ketiladze, D. M. Braginsky, A. P. Savinov, and V. F. Poleschuk. 1983. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal–oral route. *Intervirology* 20(1):23–31.
23. Bancroft, W. H., R. Snithbhan, R. M. Scott, M. Tingpalapong, W. T. Watson, P. Tanticharoenyos, J. J. Karwacki, and S. Srimarut. 1977. Transmission of hepatitis B virus to gibbons by exposure to human saliva containing hepatitis B surface antigen. *J. Infect. Dis.* 135(1):79–85.
24. Bankamp, B., G. Hodge, M. B. McChesney, W. J. Bellini, and P. A. Rota. 2008. Genetic changes that affect the virulence of measles virus in a rhesus macaque model. *Virology* 373(1):39–50.
25. Baptista, M., A. Kramvis, S. Jammeh, J. Naicker, J. S. Galpin, and M. C. Kew. 2003. Follow up of infection of chacma baboons with inoculum containing A and non-A genotypes of hepatitis B virus. *World J. Gastroenterol.* 9(4):731–735.
26. Barahona, H., L. V. Melendez, R. D. Hunt, and M. D. Daniel. 1976. The owl monkey (*Aotus trivirgatus*) as an animal model for viral diseases and oncologic studies. *Lab. Anim. Sci.* 26(6 Pt 2):1104–1112.
27. Barker, L. F., F. V. Chisari, P. P. McGrath, D. W. Dalgard, R. L. Kirschstein, J. D. Almeida, T. S. Edington, D. G. Sharp, and M. R. Peterson. 1973. Transmission of type B viral hepatitis to chimpanzees. *J. Infect. Dis.* 127(6):648–662.
28. Barker, L. F., J. E. Maynard, R. H. Purcell, J. H. Hoofnagle, K. R. Berquist, W. T. London, R. J. Gerety, and D. H. Krushak. 1975. Hepatitis B virus infection in chimpanzees: titration of subtypes. *J. Infect. Dis.* 132(4):451–458.
29. Baskerville, A., E. T. Bowen, G. S. Platt, L. B. McArdell, and D. I. Simpson. 1978. The pathology of experimental Ebola virus infection in monkeys. *J. Pathol.* 125(3):131–138.
30. Bassett, S. E., B. Guerra, K. Brasky, E. Miskovsky, M. Houghton, G. R. Klimpel, and R. E. Lanford. 2001. Protective immune response to hepatitis C virus in chimpanzees rechallenged following clearance of primary infection. *Hepatology* 33(6):1479–1487.
31. Bausch, D. G. and T. W. Geisbert. 2007. Development of vaccines for Marburg hemorrhagic fever. *Expert Rev. Vaccines* 6(1):57–74.
32. Belshe, R. B., L. S. Richardson, W. T. London, D. L. Sly, J. H. Lorfeld, E. Camargo, D. A. Prevar, and R. M. Chanock. 1977. Experimental respiratory syncytial virus infection of four species of primates. *J. Med. Virol.* 1(3):157–162.
33. Bennett, R. S., C. M. Cress, J. M. Ward, C. Y. Firestone, B. R. Murphy, and S. S. Whitehead. 2008. La Crosse virus infectivity, pathogenesis, and immunogenicity in mice and monkeys. *Virology* 373(1):5–25.
34. Berendt, R. F. and W. C. Hall. 1977. Reaction of squirrel monkeys to intratracheal inoculation with influenza/A/New Jersey/76 (swine) virus. *Infect. Immun.* 16(2):476–479.
35. Berquist, K. R., J. M. Peterson, B. L. Murphy, J. W. Ebert, J. E. Maynard, and R. H. Purcell. 1975. Hepatitis B antigens in serum and liver of chimpanzees acutely infected with hepatitis B virus. *Infect. Immun.* 12(3):602–605.
36. Bitter, G. A., K. M. Egan, W. N. Burnette, B. Samal, J. C. Fieschko, D. L. Peterson, M. R. Downing, J. Wypych, and K. E. Langley. 1988. Hepatitis B vaccine produced in yeast. *J. Med. Virol.* 25(2):123–140.
37. Blackard, J. T. and K. E. Sherman. 2007. Hepatitis C virus coinfection and superinfection. *J. Infect. Dis.* 195(4):519–524.
38. Blaney, J. E., Jr., J. Speicher, C. T. Hanson, N. S. Sathe, S. S. Whitehead, B. R. Murphy, and A. G. Pletnev. 2008. Evaluation of St. Louis encephalitis virus/dengue virus type 4 antigenic chimeric viruses in mice and rhesus monkeys. *Vaccine* 26(33):4150–4159.
39. Borisevich, I. V., V. V. Mikhailov, V. P. Krasnianskii, V. N. Gradoboev, E. V. Lebedinskaia, N. V.

- Potryvaeva, and G. D. Timan'kova. 1995. [Development and study of the properties of immunoglobulin against Ebola fever]. *Vopr. Virusol.* 40(6):270–273.
40. Bowen, E. T., G. S. Platt, D. I. Simpson, L. B. McArdell, and R. T. Raymond. 1978. Ebola haemorrhagic fever: experimental infection of monkeys. *Trans. R. Soc. Trop. Med. Hyg.* 72(2):188–191.
 41. Bradley, D. W., K. Krawczynski, E. H. Cook Jr., K. A. McCaustland, C. D. Humphrey, J. E. Spelbring, H. Myint, and J. E. Maynard. 1987. Enterically transmitted non-A, non-B hepatitis: serial passage of disease in cynomolgus macaques and tamarins and recovery of disease-associated 27- to 34-nm viruslike particles. *Proc. Natl. Acad. Sci. U. S. A.* 84(17):6277–6281.
 42. Bradley, D. W., K. A. McCaustland, E. H. Cook, C. A. Schable, J. W. Ebert, and J. E. Maynard. 1985. Post-transfusion non-A, non-B hepatitis in chimpanzees. Physicochemical evidence that the tubule-forming agent is a small, enveloped virus. *Gastroenterology* 88(3):773–779.
 43. Bukh, J. 2004. A critical role for the chimpanzee model in the study of hepatitis C. *Hepatology* 39(6):1469–1475.
 44. Bukh, J., C. L. Apgar, R. Engle, S. Govindarajan, P. A. Hegerich, R. Tellier, D. C. Wong, R. Elkins, and M. C. Kew. 1998. Experimental infection of chimpanzees with hepatitis C virus of genotype 5a: genetic analysis of the virus and generation of a standardized challenge pool. *J. Infect. Dis.* 178(4):1193–1197.
 45. Bukh, J., C. L. Apgar, S. Govindarajan, S. U. Emerson, and R. H. Purcell. 2001. Failure to infect rhesus monkeys with hepatitis C virus strains of genotypes 1a, 2a or 3a. *J. Viral Hepat.* 8(3):228–231.
 46. Bukreyev, A., P. E. Rollin, M. K. Tate, L. Yang, S. R. Zaki, W. J. Shieh, B. R. Murphy, P. L. Collins, and A. Sanchez. 2007. Successful topical respiratory tract immunization of primates against Ebola virus. *J. Virol.* 81(12):6379–6388.
 47. Bukreyev, A., L. Yang, S. R. Zaki, W. J. Shieh, P. E. Rollin, B. R. Murphy, P. L. Collins, and A. Sanchez. 2006. A single intranasal inoculation with a paramyxovirus-vectored vaccine protects guinea pigs against a lethal-dose Ebola virus challenge. *J. Virol.* 80(5):2267–2279.
 48. Buynak, E. B., R. R. Roehm, A. A. Tytell, A. U. Bertland, G. P. Lampson, and M. R. Hilleman. 1976. Development and chimpanzee testing of a vaccine against human hepatitis B. *Proc. Soc. Exp. Biol. Med.* 151(4):694–700.
 49. Callis, R. T., P. B. Jahrling, and A. DePaoli. 1982. Pathology of Lassa virus infection in the rhesus monkey. *Am. J. Trop. Med. Hyg.* 31(5):1038–1045.
 50. Carrion, R., Jr., K. Brasky, K. Mansfield, C. Johnson, M. Gonzales, A. Ticer, I. Lukashevich, S. Tardif, and J. Patterson. 2007. Lassa virus infection in experimentally infected marmosets: liver pathology and immunophenotypic alterations in target tissues. *J. Virol.* 81(12):6482–6490.
 51. Chege, G. K., A. D. Steele, C. A. Hart, D. R. Snodgrass, E. O. Omolo, and J. M. Mwenda. 2005. Experimental infection of non-human primates with a human rotavirus isolate. *Vaccine* 23(12):1522–1528.
 52. Chen, C. M., Y. He, L. Lu, H. B. Lim, R. L. Tripathi, T. Middleton, L. E. Hernandez, D. W. Beno, M. A. Long, W. M. Kati, T. D. Bosse, D. P. Larson, R. Wagner, R. E. Lanford, W. E. Kohlbrenner, D. J. Kempf, T. J. Pilot-Matias, and A. Molla. 2007. Activity of a potent hepatitis C virus polymerase inhibitor in the chimpanzee model. *Antimicrob. Agents Chemother.* 51(12):4290–4296.
 53. Chen, Z., L. Zhang, C. Qin, L. Ba, C. E. Yi, F. Zhang, Q. Wei, T. He, W. Yu, J. Yu, H. Gao, X. Tu, A. Gettie, M. Farzan, K. Y. Yuen, and D. D. Ho. 2005. Recombinant modified vaccinia virus Ankara expressing the spike glycoprotein of severe acute respiratory syndrome coronavirus induces protective neutralizing antibodies primarily targeting the receptor binding region. *J. Virol.* 79(5):2678–2688.
 54. Choo, Q. L., G. Kuo, R. Ralston, A. Weiner, D. Chien, G. Van Nest, J. Han, K. Berger, K. Thudium, C. Kuo, J. Kansopon, J. McFarland, A. Tabrizi, K. Ching, B. Moss, L. B. Cumings, M. Houghton, and E. Muchmore. 1994. Vaccination of chimpanzees against infection by the hepatitis C virus. *Proc. Natl. Acad. Sci. U. S. A.* 91(4):1294–1298.
 55. Choo, Q. L., G. Kuo, A. J. Weiner, L. R. Overby, D. W. Bradley, and M. Houghton. 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 244(4902):359–362.
 56. Cohen, J. I., T. Moskal, M. Shapiro, and R. H. Purcell. 1996. Varicella in Chimpanzees. *J. Med. Virol.* 50(4):289–292.
 57. Cosgriff, T. M., J. C. Morrill, G. B. Jennings, L. A. Hodgson, M. V. Slayter, P. H. Gibbs, and C. J. Peters. 1989. Hemostatic derangement produced by Rift Valley fever virus in rhesus monkeys. *Rev. Infect. Dis.* 11(Suppl 4):S807–S814.
 58. Cox, C., S. Chang, L. Karran, B. Griffin, and N. Wedderburn. 1996. Persistent Epstein–Barr virus infection in the common marmoset (*Callithrix jacchus*). *J. Gen. Virol.* 77(Pt 6):1173–1180.
 59. Cox, C., B. A. Naylor, M. Mackett, J. R. Arrand, B. E. Griffin, and N. Wedderburn. 1998. Immunization of common marmosets with Epstein–Barr virus (EBV) envelope glycoprotein gp340: effect on viral shedding

- following EBV challenge. *J. Med. Virol.* 55(4):255–261.
60. Crowe, J. E., Jr., E. C. Sannella, S. Pfeiffer, G. L. Zorn, A. Azimzadeh, R. Newman, G. G. Miller, and R. N. Pierson. 2003. CD154 regulates primate humoral immunity to influenza. *Am. J. Transplant.* 3(6):680–688.
 61. Cuyck-Gandre, H., R. Cockman-Thomas, J. D. Caudill, L. S. Asher, K. L. Armstrong, B. Hauroeder, N. J. Clements, L. N. Binn, and C. F. Longer. 1998. Experimental African HEV infection in cynomolgus macaques (*Macaca fascicularis*). *J. Med. Virol.* 55(3):197–202.
 62. Daddario-Dicaprio, K. M., T. W. Geisbert, J. B. Geisbert, U. Stroher, L. E. Hensley, A. Grolla, E. A. Fritz, F. Feldmann, H. Feldmann, and S. M. Jones. 2006. Cross-protection against Marburg virus strains by using a live, attenuated recombinant vaccine. *J. Virol.* 80(19):9659–9666.
 63. Daddario-Dicaprio, K. M., T. W. Geisbert, U. Stroher, J. B. Geisbert, A. Grolla, E. A. Fritz, L. Fernando, E. Kagan, P. B. Jahrling, L. E. Hensley, S. M. Jones, and H. Feldmann. 2006. Postexposure protection against Marburg haemorrhagic fever with recombinant vesicular stomatitis virus vectors in non-human primates: an efficacy assessment. *Lancet* 367(9520):1399–1404.
 64. Davis, H. L., M. J. McCluskie, J. L. Gerin, and R. H. Purcell. 1996. DNA vaccine for hepatitis B: evidence for immunogenicity in chimpanzees and comparison with other vaccines. *Proc. Natl. Acad. Sci. U. S. A.* 93(14):7213–7218.
 65. de Lang, A., T. Baas, T. Teal, L. M. Leijten, B. Rain, A. D. Osterhaus, B. L. Haagmans, and M. G. Katze. 2007. Functional genomics highlights differential induction of antiviral pathways in the lungs of SARS-CoV-infected macaques. *PLoS Pathog.* 3(8):e112.
 66. de Swart, R. L., T. Kuiken, J. Fernandez-de Castro, M. J. Papania, J. V. Bennett, J. L. Valdespino, P. Minor, C. L. Witham, S. Yuksel, H. Vos, G. van Amerongen, and A. D. Osterhaus. 2006. Aerosol measles vaccination in macaques: preclinical studies of immune responses and safety. *Vaccine* 24(40–41):6424–6436.
 67. de Swart, R. L., T. Kuiken, H. H. Timmerman, G. van Amerongen, B. G. van den Hoogen, H. W. Vos, H. J. Neijens, A. C. Andeweg, and A. D. Osterhaus. 2002. Immunization of macaques with formalin-inactivated respiratory syncytial virus (RSV) induces interleukin-13-associated hypersensitivity to subsequent RSV infection. *J. Virol.* 76(22):11561–11569.
 68. de Swart, R. L., C. LiCalsi, A. V. Quirk, G. van Amerongen, V. Nodelman, R. Alcock, S. Yuksel, G. H. Ward, J. G. Hardy, H. Vos, C. L. Witham, C. I. Grainger, T. Kuiken, B. J. Greenspan, T. G. Gard, and A. D. Osterhaus. 2007. Measles vaccination of macaques by dry powder inhalation. *Vaccine* 25(7):1183–1190.
 69. de Swart, R. L., M. Ludlow, L. de Witte, Y. Yanagi, G. van Amerongen, S. McQuaid, S. Yuksel, T. B. Geijtenbeek, W. P. Duprex, and A. D. Osterhaus. 2007. Predominant infection of CD150+ lymphocytes and dendritic cells during measles virus infection of macaques. *PLoS Pathog.* 3(11):e178.
 70. de Swart, R. L., B. G. van den Hoogen, T. Kuiken, S. Herfst, G. van Amerongen, S. Yuksel, L. Sprong, and A. D. Osterhaus. 2007. Immunization of macaques with formalin-inactivated human metapneumovirus induces hypersensitivity to hMPV infection. *Vaccine* 25(51):8518–8528.
 71. de Waal, L., U. F. Power, S. Yuksel, G. van Amerongen, T. N. Nguyen, H. G. Niesters, R. L. de Swart, and A. D. Osterhaus. 2004. Evaluation of BBG2Na in infant macaques: specific immune responses after vaccination and RSV challenge. *Vaccine* 22(8):915–922.
 72. de Waal, L., L. S. Wyatt, S. Yuksel, G. van Amerongen, B. Moss, H. G. Niesters, A. D. Osterhaus, and R. L. de Swart. 2004. Vaccination of infant macaques with a recombinant modified vaccinia virus Ankara expressing the respiratory syncytial virus F and G genes does not predispose for immunopathology. *Vaccine* 22(8):923–926.
 73. Dean, C. H., J. B. Alarcon, A. M. Waterston, K. Draper, R. Early, F. Guirakhoo, T. P. Monath, and J. A. Mikszta. 2005. Cutaneous delivery of a live, attenuated chimeric flavivirus vaccine against Japanese encephalitis (ChimeriVax)-JE) in non-human primates. *Hum. Vaccin.* 1(3):106–111.
 74. Deinhardt, F., G. Courtois, P. Dherte, P. Osterrieth, G. Ninane, G. Henle, and W. Henle. 1962. Studies of liver function tests in chimpanzees after inoculation with human infectious hepatitis virus. *Am. J. Hyg.* 75:311–321.
 75. Deinhardt, F., A. W. Holmes, R. B. Capps, and H. Popper. 1967. Studies on the transmission of human viral hepatitis to marmoset monkeys. I. Transmission of disease, serial passages, and description of liver lesions. *J. Exp. Med.* 125(4):673–688.
 76. Devaux, P., G. Hodge, M. B. McChesney, and R. Cattaneo. 2008. Attenuation of V- or C-defective measles viruses: infection control by the inflammatory and interferon responses of rhesus monkeys. *J. Virol.* 82(11):5359–5367.
 77. Dezzutti, C. S., D. E. Frazier, L. Y. Huff, P. C. Stromberg, and R. G. Olsen. 1990. Subunit vaccine protects *Macaca nemestrina* (pig-tailed macaque)

- against simian T-cell lymphotropic virus type I challenge. *Cancer Res.* 50:5687S–5691S.
78. Dezzutti, C. S., D. E. Frazier, L. J. Lafrado, and R. G. Olsen. 1990. Evaluation of a HTLV-1 subunit vaccine in prevention of experimental STLV-I infection in Macaca nemestrina. *J. Med. Primatol.* 19:305–316.
 79. Dezzutti, C. S., D. E. Frazier, and R. G. Olsen. 1990. Efficacy of an HTLV-1 subunit vaccine in prevention of a STLV-1 infection in pig-tailed macaques. *Dev. Biol. Stand.* 72:287–296.
 80. Di Giulio, D. B. and P. B. Eckburg. 2004. Human monkeypox: an emerging zoonosis. *Lancet Infect. Dis.* 4(1):15–25.
 81. Dienstag, J. L., S. M. Feinstone, R. H. Purcell, J. H. Hoofnagle, L. F. Barker, W. T. London, H. Popper, J. M. Peterson, and A. Z. Kapikian. 1975. Experimental infection of chimpanzees with hepatitis A virus. *J. Infect. Dis.* 132(5):532–545.
 82. DiNapoli, J. M., L. Yang, A. Suguitan Jr., S. Elankumaran, D. W. Dorward, B. R. Murphy, S. K. Samal, P. L. Collins, and A. Bukreyev. 2007. Immunization of primates with a Newcastle disease virus vectored vaccine via the respiratory tract induces a high titer of serum neutralizing antibodies against highly pathogenic avian influenza virus. *J. Virol.* 81(21):11560–11568.
 83. Djavani, M. M., O. R. Crasta, J. C. Zapata, Z. Fei, O. Folkerts, B. Sobral, M. Swindells, J. Bryant, H. Davis, C. D. Pauza, I. S. Lukashevich, R. Hammamieh, M. Jett, and M. S. Salvato. 2007. Early blood profiles of virus infection in a monkey model for Lassa fever. *J. Virol.* 81(15):7960–7973.
 84. Earl, P. L., J. L. Americo, L. S. Wyatt, L. A. Eller, J. C. Whitbeck, G. H. Cohen, R. J. Eisenberg, C. J. Hartmann, D. L. Jackson, D. A. Kulesh, M. J. Martinez, D. M. Miller, E. M. Mucker, J. D. Shamblin, S. H. Zwiers, J. W. Huggins, P. B. Jahrling, and B. Moss. 2004. Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox. *Nature* 428(6979):182–185.
 85. Edghill-Smith, Y., M. Bray, C. A. Whitehouse, D. Miller, E. Mucker, J. Manischewitz, L. R. King, M. Robert-Guroff, A. Hryniwicz, D. Venzon, C. Meseda, J. Weir, A. Nalca, V. Livingston, J. Wells, M. G. Lewis, J. Huggins, S. H. Zwiers, H. Golding, and G. Franchini. 2005. Smallpox vaccine does not protect macaques with AIDS from a lethal monkeypox virus challenge. *J. Infect. Dis.* 191(3):372–381.
 86. Edghill-Smith, Y., H. Golding, J. Manischewitz, L. R. King, D. Scott, M. Bray, A. Nalca, J. W. Hooper, C. A. Whitehouse, J. E. Schmitz, K. A. Reimann, and G. Franchini. 2005. Smallpox vaccine-induced antibodies are necessary and sufficient for protection against monkeypox virus. *Nat. Med.* 11(7):740–747.
 87. Edghill-Smith, Y., D. Venzon, T. Karpova, J. McNally, J. Nacsá, W. P. Tsai, E. Tryniszewska, M. Moniuszko, J. Manischewitz, L. R. King, S. J. Snodgrass, J. Parrish, P. Markham, M. Sowers, D. Martin, M. G. Lewis, J. A. Berzofsky, I. M. Belyakov, B. Moss, J. Tartaglia, M. Bray, V. Hirsch, H. Golding, and G. Franchini. 2003. Modeling a safer smallpox vaccination regimen, for human immunodeficiency virus type 1-infected patients, in immunocompromised macaques. *J. Infect. Dis.* 188(8):1181–1191.
 88. Eggers, H. J. 1999. Milestones in early poliomyelitis research (1840 to 1949). *J. Virol.* 73(6):4533–4535.
 89. Eichberg, J. W., L. B. Seeff, D. L. Lawlor, Z. Buskell-Bales, K. Ishak, J. H. Hoofnagle, A. L. Goldstein, and J. M. Langloss. 1987. Effect of thymosin immunostimulation with and without corticosteroid immunosuppression on chimpanzee hepatitis B carriers. *J. Med. Virol.* 21(1):25–37.
 90. El Mubarak, H. S., S. Yuksel, G. van Amerongen, P. G. Mulder, M. M. Mukhtar, A. D. Osterhaus, and R. L. de Swart. 2007. Infection of cynomolgus macaques (Macaca fascicularis) and rhesus macaques (Macaca mulatta) with different wild-type measles viruses. *J. Gen. Virol.* 88(Pt 7):2028–2034.
 91. Elmowalid, G. A., M. Qiao, S. H. Jeong, B. B. Borg, T. F. Baumert, R. K. Sapp, Z. Hu, K. Murthy, and T. J. Liang. 2007. Immunization with hepatitis C virus-like particles results in control of hepatitis C virus infection in chimpanzees. *Proc. Natl. Acad. Sci. U. S. A.* 104(20):8427–8432.
 92. Enders, J. F., S. L. Katz, M. V. Milovanovic, and A. Holloway. 1960. Studies on an attenuated measles-virus vaccine. I. Development and preparations of the vaccine: techniques for assay of effects of vaccination. *N. Engl. J. Med.* 263:153–159.
 93. Epstein, M. A., R. D. Hunt, and H. Rabin. 1973. Pilot experiments with EB virus in owl monkeys (*Aotus trivirgatus*). I Reticuloproliferative disease in an inoculated animal. *Int. J. Cancer* 12(2):309–318.
 94. Falk, L., F. Deinhardt, L. Wolfe, D. Johnson, J. Hilgers, and G. de The. 1976. Epstein–Barr virus: experimental infection of *Callithrix jacchus* marmosets. *Int. J. Cancer* 17(6):785–788.
 95. Fan, J., X. Liang, M. S. Horton, H. C. Perry, M. P. Citron, G. J. Heidecker, T. M. Fu, J. Joyce, C. T. Przysiecki, P. M. Keller, V. M. Garsky, R. Ionescu, Y. Rippeon, L. Shi, M. A. Chastain, J. H. Condra, M. E. Davies, J. Liao, E. A. Emini, and J. W. Shiver. 2004. Preclinical study of influenza virus A M2 peptide conjugate vaccines in mice, ferrets, and rhesus monkeys. *Vaccine* 22(23–24):2993–3003.

96. Farci, P., H. J. Alter, S. Govindarajan, D. C. Wong, R. Engle, R. R. Lesniewski, I. K. Mushahwar, S. M. Desai, R. H. Miller, N. Ogata, and R. H. Purcell. 1992. Lack of protective immunity against reinfection with hepatitis C virus. *Science* 258(5079):135–140.
97. Farci, P., H. J. Alter, D. C. Wong, R. H. Miller, S. Govindarajan, R. Engle, M. Shapiro, and R. H. Purcell. 1994. Prevention of hepatitis C virus infection in chimpanzees after antibody-mediated in vitro neutralization. *Proc. Natl. Acad. Sci. U. S. A.* 91(16):7792–7796.
98. Farci, P., W. T. London, D. C. Wong, G. J. Dawson, D. S. Vallari, R. Engle, and R. H. Purcell. 1992. The natural history of infection with hepatitis C virus (HCV) in chimpanzees: comparison of serologic responses measured with first- and second-generation assays and relationship to HCV viremia. *J. Infect. Dis.* 165(6):1006–1011.
99. Farci, P., S. J. Munoz, A. Shimoda, S. Govindarajan, D. C. Wong, A. Coiana, G. Peddis, R. Rubin, and R. H. Purcell. 1999. Experimental transmission of hepatitis C virus-associated fulminant hepatitis to a chimpanzee. *J. Infect. Dis.* 179(4):1007–1011.
100. Farci, P., A. Shimoda, D. Wong, T. Cabezon, D. De Gioannis, A. Strazzera, Y. Shimizu, M. Shapiro, H. J. Alter, and R. H. Purcell. 1996. Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. *Proc. Natl. Acad. Sci. U. S. A.* 93(26):15394–15399.
101. Feinstone, S. M., H. J. Alter, H. P. Dienes, Y. Shimizu, H. Popper, D. Blackmore, D. Sly, W. T. London, and R. H. Purcell. 1981. Non-A, non-B hepatitis in chimpanzees and marmosets. *J. Infect. Dis.* 144(6):588–598.
102. Feinstone, S. M., K. B. Mihalik, T. Kamimura, H. J. Alter, W. T. London, and R. H. Purcell. 1983. Inactivation of hepatitis B virus and non-A, non-B hepatitis by chloroform. *Infect. Immun.* 41(2):816–821.
103. Feldmann, H., S. M. Jones, K. M. Daddario-Dicaprio, J. B. Geisbert, U. Stroher, A. Grolla, M. Bray, E. A. Fritz, L. Fernando, F. Feldmann, L. E. Hensley, and T. W. Geisbert. 2007. Effective post-exposure treatment of Ebola infection. *PLoS Pathog.* 3(1):e2.
104. Felsburg, P. J., R. L. Heberling, and S. S. Kalter. 1972. Experimental genital infection of cebus monkeys with oral and genital isolates of Herpesvirus hominis types 1 and 2. *Arch. Gesamte Virusforsch.* 39(1):223–227.
105. Fernandez, J., D. Taylor, D. R. Morhardt, K. Mihalik, M. Puig, C. M. Rice, S. M. Feinstone, and M. E. Major. 2004. Long-term persistence of infection in chimpanzees inoculated with an infectious hepatitis C virus clone is associated with a decrease in the viral amino acid substitution rate and low levels of heterogeneity. *J. Virol.* 78(18):9782–9789.
106. Fisher-Hoch, S. P., L. Hutwagner, B. Brown, and J. B. McCormick. 2000. Effective vaccine for Lassa fever. *J. Virol.* 74(15):6777–6783.
107. Fisher-Hoch, S. P., J. B. McCormick, D. Auperin, B. G. Brown, M. Castor, G. Perez, S. Ruo, A. Conaty, L. Brammer, and S. Bauer. 1989. Protection of rhesus monkeys from fatal Lassa fever by vaccination with a recombinant vaccinia virus containing the Lassa virus glycoprotein gene. *Proc. Natl. Acad. Sci. U. S. A.* 86(1):317–321.
108. Fisher-Hoch, S. P., S. W. Mitchell, D. R. Sasso, J. V. Lange, R. Ramsey, and J. B. McCormick. 1987. Physiological and immunologic disturbances associated with shock in a primate model of Lassa fever. *J. Infect. Dis.* 155(3):465–474.
109. Folgori, A., S. Capone, L. Ruggeri, A. Meola, E. Sporeno, B. B. Ercole, M. Pezzanera, R. Tafi, M. Arcuri, E. Fattori, A. Lahm, A. Luzzago, A. Vitelli, S. Colloca, R. Cortese, and A. Nicosia. 2006. A T-cell HCV vaccine eliciting effective immunity against heterologous virus challenge in chimpanzees. *Nat. Med.* 12(2):190–197.
110. Forns, X., P. J. Payette, X. Ma, W. Satterfield, G. Eder, I. K. Mushahwar, S. Govindarajan, H. L. Davis, S. U. Emerson, R. H. Purcell, and J. Bukh. 2000. Vaccination of chimpanzees with plasmid DNA encoding the hepatitis C virus (HCV) envelope E2 protein modified the infection after challenge with homologous monoclonal HCV. *Hepatology* 32(3):618–625.
111. Fouchier, R. A., T. Kuiken, M. Schutten, G. van Amerongen, G. J. van Doornum, B. G. van den Hoogen, M. Peiris, W. Lim, K. Stohr, and A. D. Osterhaus. 2003. Aetiology: Koch's postulates fulfilled for SARS virus. *Nature* 423(6937):240.
112. Frolova, M. P., L. M. Isachkova, N. M. Shestopalova, and V. V. Pogodina. 1981. [Experimental monkey encephalitis caused by Powassan virus]. *Zh. Nevropatol. Psichiatr. Im S. S. Korsakova* 81(2):25–33.
113. Frolova, M. P., L. M. Isachkova, N. M. Shestopalova, and V. V. Pogodina. 1985. Experimental encephalitis in monkeys caused by the Powassan virus. *Neurosci. Behav. Physiol.* 15(1):62–69.
114. Fujisawa, Y., S. Kuroda, P. M. Van Eerd, H. Schellekens, and A. Kakinuma. 1990. Protective efficacy of a novel hepatitis B vaccine consisting of M (pre-S2 + S) protein particles (a third generation vaccine). *Vaccine* 8(3):192–198.
115. Gao, F., O. V. Nainan, Y. Khudyakov, J. Li, Y. Hong, A. C. Gonzales, J. Spelbring, and H. S. Margolis. 2007. Recombinant hepatitis C virus in

- experimentally infected chimpanzees. *J. Gen. Virol.* 88(Pt 1):143–147.
116. Gao, W., A. Tamin, A. Soloff, L. D'Aiuto, E. Nwanegbo, P. D. Robbins, W. J. Bellini, S. Barratt-Boyes, and A. Gamotto. 2003. Effects of a SARS-associated coronavirus vaccine in monkeys. *Lancet* 362(9399):1895–1896.
117. Geisbert, T. W., K. M. Daddario-Dicaprio, K. Williams, J. B. Geisbert, A. Leung, F. Feldmann, L. E. Hensley, H. Feldmann, and S. M. Jones. 2008. Recombinant vesicular stomatitis virus vector mediates postexposure protection against Sudan Ebola hemorrhagic fever in nonhuman primates. *J. Virol.* 82(11):5564–5568.
118. Geisbert, T. W., L. E. Hensley, T. R. Gibb, K. E. Steele, N. K. Jaax, and P. B. Jahrling. 2000. Apoptosis induced in vitro and in vivo during infection by Ebola and Marburg viruses. *Lab. Invest.* 80(2):171–186.
119. Geisbert, T. W., L. E. Hensley, P. B. Jahrling, T. Larsen, J. B. Geisbert, J. Paragas, H. A. Young, T. M. Fredeking, W. E. Rote, and G. P. Vlasuk. 2003. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet* 362(9400):1953–1958.
120. Geisbert, T. W., L. E. Hensley, T. Larsen, H. A. Young, D. S. Reed, J. B. Geisbert, D. P. Scott, E. Kagan, P. B. Jahrling, and K. J. Davis. 2003. Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection. *Am. J. Pathol.* 163(6):2347–2370.
121. Geisbert, T. W., S. Jones, E. A. Fritz, A. C. Shurtleff, J. B. Geisbert, R. Liebscher, A. Grolla, U. Stroher, L. Fernando, K. M. Daddario, M. C. Guttieri, B. R. Mothe, T. Larsen, L. E. Hensley, P. B. Jahrling, and H. Feldmann. 2005. Development of a new vaccine for the prevention of Lassa fever. *PLoS Med.* 2(6):e183.
122. Geisbert, T. W., P. Pushko, K. Anderson, J. Smith, K. J. Davis, and P. B. Jahrling. 2002. Evaluation in nonhuman primates of vaccines against Ebola virus. *Emerg. Infect. Dis.* 8(5):503–507.
123. Geisbert, T. W., H. A. Young, P. B. Jahrling, K. J. Davis, E. Kagan, and L. E. Hensley. 2003. Mechanisms underlying coagulation abnormalities in ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. *J. Infect. Dis.* 188(11):1618–1629.
124. Gheit, T., S. Sekkat, L. Cova, M. Chevallier, M. A. Petit, O. Hantz, M. Lesenechal, A. Benslimane, C. Trepo, and I. Chemin. 2002. Experimental transfection of Macaca sylvanus with cloned human hepatitis B virus. *J. Gen. Virol.* 83(Pt 7):1645–1649.
125. Gleiser, C. A., W. S. Gochenour Jr., T. O. Berge, and W. D. Tigertt. 1962. The comparative pathology of experimental Venezuelan equine encephalomyelitis infection in different animal hosts. *J. Infect. Dis.* 110:80–97.
126. Goldberger, J. and J. F. Anderson. 1911. An experimental demonstration of the presence of the virus of measles in the mixed buccal and nasal secretions. *JAMA* 57:496–578.
127. Gonchar, N. I., V. A. Pshenichnov, V. A. Pokhodjaev, K. L. Lopatov, and I. V. Firsova. 1991. [The sensitivity of different experimental animals to the Marburg virus]. *Vopr. Virusol.* 36(5):435–437.
128. Gonder, E. and G. Eddy. 1986. Indirect immunofluorescence, serum neutralization, and viremia responses of rhesus monkeys (*Macaca mulatta*) to Machupo virus. *J. Med. Virol.* 19(2):187–192.
129. Gonzalez, P. H., R. P. Laguens, M. J. Frigerio, M. A. Calello, and M. C. Weissenbacher. 1983. Junin virus infection of *Callithrix jacchus*: pathologic features. *Am. J. Trop. Med. Hyg.* 32(2):417–423.
130. Gould, E. A. and T. Solomon. 2008. Pathogenic flaviviruses. *Lancet* 371(9611):500–509.
131. Gowen, B. B. and M. R. Holbrook. 2008. Animal models of highly pathogenic RNA viral infections: hemorrhagic fever viruses. *Antiviral Res.* 78(1):79–90.
132. Green, D. E., B. G. Mahlandt, and K. T. McKee Jr. 1987. Experimental Argentine hemorrhagic fever in rhesus macaques: virus-specific variations in pathology. *J. Med. Virol.* 22(2):113–133.
133. Greenough, T. C., A. Carville, J. Coderre, M. Somasundaran, J. L. Sullivan, K. Luzuriaga, and K. Mansfield. 2005. Pneumonitis and multi-organ system disease in common marmosets (*Callithrix jacchus*) infected with the severe acute respiratory syndrome-associated coronavirus. *Am. J. Pathol.* 167(2):455–463.
134. Grizzard, M. B., W. T. London, D. L. Sly, B. R. Murphy, W. D. James, W. P. Parnell, and R. M. Chanock. 1978. Experimental production of respiratory tract disease in cebus monkeys after intratracheal or intranasal infection with influenza A/Victoria/3/75 or influenza A/New Jersey/76 virus. *Infect. Immun.* 21(1):201–205.
135. Groen, J., M. Gerding, J. P. Koeman, P. J. Roholl, G. van Amerongen, H. G. Jordans, H. G. Niesters, and A. D. Osterhaus. 1995. A macaque model for hantavirus infection. *J. Infect. Dis.* 172(1):38–44.
136. Haagmans, B. L., T. Kuiken, B. E. Martina, R. A. Fouchier, G. F. Rimmelzwaan, G. van Amerongen, D. van Riel, T. de Jong, S. Itamura, K. H. Chan, M. Tashiro, and A. D. Osterhaus. 2004. Pegylated

- interferon-alpha protects type 1 pneumocytes against SARS coronavirus infection in macaques. *Nat. Med.* 10(3):290–293.
137. Haagmans, B. L. and A. D. Osterhaus. 2006. Nonhuman primate models for SARS. *PLoS Med.* 3(5):e194.
138. Hashimoto, I. and A. Hagiwara. 1982. Pathogenicity of a poliomylitis-like disease in monkeys infected orally with enterovirus 71: a model for human infection. *Neuropathol. Appl. Neurobiol.* 8(2):149–156.
139. Hashimoto, I. and A. Hagiwara. 1983. Comparative studies on the neurovirulence of temperature-sensitive and temperature-resistant viruses of enterovirus 71 in monkeys. *Acta Neuropathol.* 60(3–4):266–270.
140. Hashimoto, I., A. Hagiwara, and H. Kodama. 1978. Neurovirulence in cynomolgus monkeys of enterovirus 71 isolated from a patient with hand, foot and mouth disease. *Arch. Virol.* 56(3):257–261.
141. Hashimoto, I., A. Hagiwara, and I. Uchino. 1985. Ultrastructural changes of motoneurons in monkeys infected with enterovirus 71. Brief report. *Arch. Virol.* 86(1–2):137–142.
142. Hensley, L. E., S. M. Jones, H. Feldmann, P. B. Jahrling, and T. W. Geisbert. 2005. Ebola and Marburg viruses: pathogenesis and development of countermeasures. *Curr. Mol. Med.* 5(8):761–772.
143. Heraud, J. M., Y. Edghill-Smith, V. Ayala, I. Kalisz, J. Parrino, V. S. Kalyanaraman, J. Manischewitz, L. R. King, A. Hryniwicz, C. J. Trindade, M. Hassett, W. P. Tsai, D. Venzon, A. Nalca, M. Vaccari, P. Silvera, M. Bray, B. S. Graham, H. Golding, J. W. Hooper, and G. Franchini. 2006. Subunit recombinant vaccine protects against monkeypox. *J. Immunol.* 177(4):2552–2564.
144. Hirano, M., X. Ding, H. T. Tran, T. C. Li, N. Takeda, T. Sata, S. Nakamura, and K. Abe. 2003. Prevalence of antibody against hepatitis E virus in various species of non-human primates: evidence of widespread infection in Japanese monkeys (*Macaca fuscata*). *Jpn. J. Infect. Dis.* 56(1):8–11.
145. Hirschman, R. J., N. R. Shulman, L. F. Barker, and K. O. Smith. 1969. Virus-like particles in sera of patients with infectious and serum hepatitis. *JAMA* 208(9):1667–1670.
146. Ho, M., E. R. Chen, K. H. Hsu, S. J. Twu, K. T. Chen, S. F. Tsai, J. R. Wang, and S. R. Shih. 1999. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. *N. Engl. J. Med.* 341(13):929–935.
147. Hoffman, S. J., F. P. Polack, D. A. Hauer, and D. E. Griffin. 2003. Measles virus infection of rhesus macaques affects neutrophil expression of IL-12 and IL-10. *Viral Immunol.* 16(3):369–379.
148. Hoffman, S. J., F. P. Polack, D. A. Hauer, M. Singh, M. A. Billeter, R. J. Adams, and D. E. Griffin. 2003. Vaccination of rhesus macaques with a recombinant measles virus expressing interleukin-12 alters humoral and cellular immune responses. *J. Infect. Dis.* 188(10):1553–1561.
149. Hogan, R. J. 2006. Are nonhuman primates good models for SARS? *PLoS Med.* 3(9):e411.
150. Holbrook, M. R. and B. B. Gowen. 2008. Animal models of highly pathogenic RNA viral infections: encephalitis viruses. *Antiviral Res.* 78(1):69–78.
151. Hollinger, F. B., G. L. Gitnick, R. D. Aach, W. Szmuness, J. W. Mosley, C. E. Stevens, R. L. Peters, J. M. Weiner, J. B. Werch, and J. J. Lander. 1978. Non-A, non-B hepatitis transmission in chimpanzees: a project of the transfusion-transmitted viruses study group. *Intervirology* 10(1):60–68.
152. Holmes, A. W., L. Wolfe, H. Rosenblatt, and F. Deinhardt. 1969. Hepatitis in marmosets: induction of disease with coded specimens from a human volunteer study. *Science* 165(895):816–817.
153. Hong, H. J., C. J. Ryu, H. Hur, S. Kim, H. K. Oh, M. S. Oh, and S. Y. Park. 2004. In vivo neutralization of hepatitis B virus infection by an anti-preS1 humanized antibody in chimpanzees. *Virology* 318(1):134–141.
154. Hooper, J. W., E. Thompson, C. Wilhelmsen, M. Zimmerman, M. A. Ichou, S. E. Steffen, C. S. Schmaljohn, A. L. Schmaljohn, and P. B. Jahrling. 2004. Smallpox DNA vaccine protects nonhuman primates against lethal monkeypox. *J. Virol.* 78(9):4433–4443.
155. Houff, S. A., W. T. London, G. DiChiro, B. L. Padgett, D. L. Walker, G. M. Zu Rhein, and J. L. Sever. 1983. Neuroradiological studies of JCV-induced astrocytomas in nonhuman primates. *Prog. Clin. Biol. Res.* 105:253–259.
156. Houghton, M. and S. Abrignani. 2005. Prospects for a vaccine against the hepatitis C virus. *Nature* 436(7053):961–966.
157. Itoh, Y., H. Ozaki, H. Tsuchiya, K. Okamoto, R. Torii, Y. Sakoda, Y. Kawaoka, K. Ogasawara, and H. Kida. 2008. A vaccine prepared from a non-pathogenic H5N1 avian influenza virus strain confers protective immunity against highly pathogenic avian influenza virus infection in cynomolgus macaques. *Vaccine* 26(4):562–572.
158. Itoh, Y., E. Takai, H. Ohnuma, K. Kitajima, F. Tsuda, A. Machida, S. Mishiro, T. Nakamura, Y. Miyakawa, and M. Mayumi. 1986. A synthetic peptide vaccine involving the product of the pre-S(2) region of hepatitis B virus DNA: protective efficacy in chimpanzees. *Proc. Natl. Acad. Sci. U. S. A.* 83(23):9174–9178.
159. Jahrling, P. B., J. Geisbert, J. R. Swearengen, G. P. Jaax, T. Lewis, J. W. Huggins, J. J. Schmidt,

- J. W. LeDuc, and C. J. Peters. 1996. Passive immunization of Ebola virus-infected cynomolgus monkeys with immunoglobulin from hyperimmune horses. *Arch. Virol. Suppl.* 11:135–140.
160. Jahrling, P. B., J. B. Geisbert, J. R. Swearengen, T. Larsen, and T. W. Geisbert. 2007. Ebola hemorrhagic fever: evaluation of passive immunotherapy in nonhuman primates. *J. Infect. Dis.* 196(Suppl 2):S400–S403.
161. Jahrling, P. B., T. W. Geisbert, J. B. Geisbert, J. R. Swearengen, M. Bray, N. K. Jaax, J. W. Huggins, J. W. LeDuc, and C. J. Peters. 1999. Evaluation of immune globulin and recombinant interferon-alpha2b for treatment of experimental Ebola virus infections. *J. Infect. Dis.* 179(Suppl 1):S224–S234.
162. Jahrling, P. B., L. E. Hensley, M. J. Martinez, J. W. LeDuc, K. H. Rubins, D. A. Relman, and J. W. Huggins. 2004. Exploring the potential of variola virus infection of cynomolgus macaques as a model for human smallpox. *Proc. Natl. Acad. Sci. U. S. A.* 101(42):15196–15200.
163. Jahrling, P. B., R. A. Hesse, G. A. Eddy, K. M. Johnson, R. T. Callis, and E. L. Stephen. 1980. Lassa virus infection of rhesus monkeys: pathogenesis and treatment with ribavirin. *J. Infect. Dis.* 141(5):580–589.
164. Jahrling, P. B., D. E. Hilmas, and C. D. Heard. 1977. Vascular clearance of Venezuelan equine encephalomyelitis viruses as a correlate to virulence for rhesus monkeys. *Arch. Virol.* 55(1–2):161–164.
165. Jahrling, P. B., C. J. Peters, and E. L. Stephen. 1984. Enhanced treatment of Lassa fever by immune plasma combined with ribavirin in cynomolgus monkeys. *J. Infect. Dis.* 149(3):420–427.
166. Johnson, D. R., L. G. Wolfe, G. Levan, G. Klein, I. Ernberg, and P. Aman. 1983. Epstein-Barr virus (EBV)-induced lymphoproliferative disease in cotton-topped marmosets. *Int. J. Cancer* 31(1):91–97.
167. Jones, S. M., H. Feldmann, U. Stroher, J. B. Geisbert, L. Fernando, A. Grolla, H. D. Klenk, N. J. Sullivan, V. E. Volchkov, E. A. Fritz, K. M. Daddario, L. E. Hensley, P. B. Jahrling, and T. W. Geisbert. 2005. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nat. Med.* 11(7):786–790.
168. Kalter, S. S. and R. L. Heberling. 1978. Serologic response of primates to influenza viruses. *Proc. Soc. Exp. Biol. Med.* 159(3):414–417.
169. Kalter, S. S., R. L. Heberling, T. E. Vice, F. S. Lief, and A. R. Rodriguez. 1969. Influenza (A2-Hong Kong-68) in the baboon (*Papio* sp.). *Proc. Soc. Exp. Biol. Med.* 132(1):357–361.
170. Kamili, S., J. Spelbring, D. Carson, and K. Krawczynski. 2004. Protective efficacy of hepatitis E virus DNA vaccine administered by gene gun in the cynomolgus macaque model of infection. *J. Infect. Dis.* 189(2):258–264.
171. Kamili, S., J. Spelbring, and K. Krawczynski. 2002. DNA vaccination against hepatitis E virus infection in cynomolgus macaques. *J. Gastroenterol. Hepatol.* 17(Suppl 3):S365–S369.
172. Kane, M. A., D. W. Bradley, S. M. Shrestha, J. E. Maynard, E. H. Cook, R. P. Mishra, and D. D. Joshi. 1984. Epidemic non-A, non-B hepatitis in Nepal. Recovery of a possible etiologic agent and transmission studies in marmosets. *JAMA* 252(22):3140–3145.
173. Kapikian, A. Z., R. H. Mitchell, R. M. Chanock, R. A. Shvedoff, and C. E. Stewart. 1969. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. *Am. J. Epidemiol.* 89(4):405–421.
174. Kastello, M. D., G. A. Eddy, and R. W. Kuehne. 1976. A rhesus monkey model for the study of Bolivian hemorrhagic fever. *J. Infect. Dis.* 133(1):57–62.
175. Katzin, D. S., J. D. Connor, L. A. Wilson, and R. S. Sexton. 1967. Experimental herpes simplex infection in the owl monkey. *Proc. Soc. Exp. Biol. Med.* 125(2):391–398.
176. Kedda, M. A., A. Kramvis, M. C. Kew, G. Lecatsas, A. C. Paterson, S. Aspinall, J. H. Stark, W. A. De Klerk, and B. Gridelli. 2000. Susceptibility of chacma baboons (*Papio ursinus orientalis*) to infection by hepatitis B virus. *Transplantation* 69(7):1429–1434.
177. Kennedy, R. C., J. W. Eichberg, R. E. Lanford, and G. R. Dreesman. 1986. Anti-idiotypic antibody vaccine for type B viral hepatitis in chimpanzees. *Science* 232(4747):220–223.
178. Kenyon, R. H., K. T. McKee Jr., P. M. Zack, M. K. Rippy, A. P. Vogel, C. York, J. Meegan, C. Crabbs, and C. J. Peters. 1992. Aerosol infection of rhesus macaques with Junin virus. *Intervirology* 33(1):23–31.
179. Kenyon, R. H., M. K. Rippy, K. T. McKee Jr., P. M. Zack, and C. J. Peters. 1992. Infection of *Macaca radiata* with viruses of the tick-borne encephalitis group. *Microb. Pathog.* 13(5):399–409.
180. Klingstrom, J., K. I. Falk, and A. Lundkvist. 2005. Delayed viremia and antibody responses in Puumala hantavirus challenged passively immunized cynomolgus macaques. *Arch. Virol.* 150(1):79–92.
181. Klingstrom, J., A. Plyusnin, A. Vaheri, and A. Lundkvist. 2002. Wild-type Puumala hantavirus infection induces cytokines, C-reactive protein, creatinine, and nitric oxide in cynomolgus macaques. *J. Virol.* 76(1):444–449.

182. Klingstrom, J., M. Stoltz, J. Hardestam, C. Ahlm, and A. Lundkvist. 2008. Passive immunization protects cynomolgus macaques against Puumala hantavirus challenge. *Antivir. Ther.* 13(1):125–133.
183. Kobasa, D., S. M. Jones, K. Shinya, J. C. Kash, J. Copps, H. Ebihara, Y. Hatta, J. H. Kim, P. Halfmann, M. Hatta, F. Feldmann, J. B. Alimonti, L. Fernando, Y. Li, M. G. Katze, H. Feldmann, and Y. Kawaoka. 2007. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. *Nature* 445(7125):319–323.
184. Kobinger, G. P., J. M. Figueiredo, T. Rowe, Y. Zhi, G. Gao, J. C. Sanmiguel, P. Bell, N. A. Wivel, L. A. Zitzow, D. B. Flieder, R. J. Hogan, and J. M. Wilson. 2007. Adenovirus-based vaccine prevents pneumonia in ferrets challenged with the SARS coronavirus and stimulates robust immune responses in macaques. *Vaccine* 25(28):5220–5231.
185. Komiya, Y., K. Katayama, H. Yugi, M. Mizui, H. Matsukura, T. Tomoguri, Y. Miyakawa, A. Tabuchi, J. Tanaka, and H. Yoshizawa. 2008. Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viremia between genotype A and genotype C. *Transfusion* 48(2):286–294.
186. Koraka, P., S. Benton, G. van Amerongen, K. J. Stittelaar, and A. D. Osterhaus. 2007. Characterization of humoral and cellular immune responses in cynomolgus macaques upon primary and subsequent heterologous infections with dengue viruses. *Microbes. Infect.* 9(8):940–946.
187. Koraka, P., S. Benton, G. van Amerongen, K. J. Stittelaar, and A. D. Osterhaus. 2007. Efficacy of a live attenuated tetravalent candidate dengue vaccine in naive and previously infected cynomolgus macaques. *Vaccine* 25(29):5409–5416.
188. Korzaia, L. I., B. A. Lapin, V. V. Keburia, and I. I. Lazareva. 2007. [Hepatitis E virus antibodies in the macaques and in the personnel serving the macaques of the Adler apery]. *Vopr. Virusol.* 52(1):36–40.
189. Krawczynski, K., A. M. Prince, and A. Nowoslawski. 1979. Immunopathologic aspects of the HBsAg carrier state in chimpanzees. *J. Med. Primatol.* 8(4):222–232.
190. Kuiken, T., R. A. Fouchier, M. Schutten, G. F. Rimmelzwaan, G. van Amerongen, D. van Riel, J. D. Laman, T. de Jong, G. van Doornum, W. Lim, A. E. Ling, P. K. Chan, J. S. Tam, M. C. Zambon, R. Gopal, C. Drosten, S. van der Werf, N. Escriou, J. C. Manuguerra, K. Stohr, J. S. Peiris, and A. D. Osterhaus. 2003. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet* 362(9380):263–270.
191. Kuiken, T., G. F. Rimmelzwaan, G. van Amerongen, and A. D. Osterhaus. 2003. Pathology of human influenza A (H5N1) virus infection in cynomolgus macaques (*Macaca fascicularis*). *Vet. Pathol.* 40(3):304–310.
192. Kuiken, T., B. G. van den Hoogen, D. A. van Riel, J. D. Laman, G. van Amerongen, L. Sprong, R. A. Fouchier, and A. D. Osterhaus. 2004. Experimental human metapneumovirus infection of cynomolgus macaques (*Macaca fascicularis*) results in virus replication in ciliated epithelial cells and pneumocytes with associated lesions throughout the respiratory tract. *Am. J. Pathol.* 164(6):1893–1900.
193. Landsteiner, K. and E. Popper. 1909. Uebertragung der Poliomyelitis acuta auf Affen. *Z. Immunitätsforsch* 2:377–390.
194. Lanford, R. E., C. Bigger, S. Bassett, and G. Klimpel. 2001. The chimpanzee model of hepatitis C virus infections. *ILAR J.* 42(2):117–126.
195. Lanford, R. E., B. Guerra, D. Chavez, C. Bigger, K. M. Brasky, X. H. Wang, S. C. Ray, and D. L. Thomas. 2004. Cross-genotype immunity to hepatitis C virus. *J. Virol.* 78(3):1575–1581.
196. Lashkevich, V. A., G. A. Koroleva, N. V. Tereshkina, A. N. Lukashev, L. V. Grigor'eva, and I. P. Titova. 1996. [Superacute lethal liver necrosis in monkeys infected with highly pathogenic variants of enteroviruses (ECHO 11 and ECHO 19 viruses)]. *Vopr. Virusol.* 41(5):198–206.
197. Lawler, J. V., T. P. Endy, L. E. Hensley, A. Garrison, E. A. Fritz, M. Lesar, R. S. Baric, D. A. Kulesh, D. A. Norwood, L. P. Wasieleski, M. P. Ulrich, T. R. Slezak, E. Vitalis, J. W. Huggins, P. B. Jahrling, and J. Paragas. 2006. Cynomolgus macaque as an animal model for severe acute respiratory syndrome. *PLoS Med.* 3(5):e149.
198. Leong, Y. K. and A. Awang. 1990. Experimental group A rotaviral infection in cynomolgus monkeys raised on formula diet. *Microbiol. Immunol.* 34(2):153–162.
199. Levine, P. H., S. A. Leiseca, J. F. Hewetson, K. A. Traul, A. P. Andrese, D. J. Granlund, P. Fabrizio, and D. A. Stevens. 1980. Infection of rhesus monkeys and chimpanzees with Epstein-Barr virus. *Arch. Virol.* 66(4):341–351.
200. Li, S. W., J. Zhang, Y. M. Li, S. H. Ou, G. Y. Huang, Z. Q. He, S. X. Ge, Y. L. Xian, S. Q. Pang, M. H. Ng, and N. S. Xia. 2005. A bacterially expressed particulate hepatitis E vaccine: antigenicity, immunogenicity and protectivity on primates. *Vaccine* 23(22):2893–2901.
201. Li, T. C., Y. Suzuki, Y. Ami, T. N. Dhole, T. Miyamura, and N. Takeda. 2004. Protection of

- cynomolgus monkeys against HEV infection by oral administration of recombinant hepatitis E virus-like particles. *Vaccine* 22(3–4):370–377.
202. Lodmell, D. L., M. J. Parnell, J. R. Bailey, L. C. Ewalt, and C. A. Hanlon. 2001. One-time gene gun or intramuscular rabies DNA vaccination of non-human primates: comparison of neutralizing antibody responses and protection against rabies virus 1 year after vaccination. *Vaccine* 20(5–6):838–844.
 203. Lodmell, D. L., M. J. Parnell, J. R. Bailey, L. C. Ewalt, and C. A. Hanlon. 2002. Rabies DNA vaccination of non-human primates: post-exposure studies using gene gun methodology that accelerates induction of neutralizing antibody and enhances neutralizing antibody titers. *Vaccine* 20(17–18):2221–2228.
 204. Lodmell, D. L., N. B. Ray, M. J. Parnell, L. C. Ewalt, C. A. Hanlon, J. H. Shaddock, D. S. Sanderlin, and C. E. Rupprecht. 1998. DNA immunization protects nonhuman primates against rabies virus. *Nat. Med.* 4(8):949–952.
 205. London, W. T., S. A. Houff, D. L. Madden, D. A. Fuccillo, M. Gravell, W. C. Wallen, A. E. Palmer, J. L. Sever, B. L. Padgett, D. L. Walker, G. M. ZuRhein, and T. Ohashi. 1978. Brain tumors in owl monkeys inoculated with a human polyomavirus (JC virus). *Science* 201(4362):1246–1249.
 206. London, W. T., S. A. Houff, P. E. McKeever, W. C. Wallen, J. L. Sever, B. L. Padgett, and D. L. Walker. 1983. Viral-induced astrocytomas in squirrel monkeys. *Prog. Clin. Biol. Res.* 105:227–237.
 207. London, W. T., N. H. Levitt, G. Altshuler, B. L. Curfman, S. G. Kent, A. E. Palmer, J. L. Sever, and S. A. Houff. 1982. Teratological effects of western equine encephalitis virus on the fetal nervous system of Macaca mulatta. *Teratology* 25(1):71–79.
 208. Lorenz, D. and P. Albrecht. 1980. Susceptibility of tamarins (*Saguinus*) to measles virus. *Lab. Anim. Sci.* 30(4 Pt 1):661–665.
 209. Lu, L., N. Tatsunori, C. Li, S. Waheed, F. Gao, and B. H. Robertson. 2008. HCV selection and HVR1 evolution in a chimpanzee chronically infected with HCV-1 over 12 years. *Hepatol. Res.* 38(7):704–716.
 210. Lubeck, M. D., A. R. Davis, M. Chengalvala, R. J. Natuk, J. E. Morin, K. Molnar-Kimber, B. B. Mason, B. M. Bhat, S. Mizutani, P. P. Hung, and R. H. Purcell. 1989. Immunogenicity and efficacy testing in chimpanzees of an oral hepatitis B vaccine based on live recombinant adenovirus. *Proc. Natl. Acad. Sci. U. S. A.* 86(17):6763–6767.
 211. Luchko, S. V., A. A. Dadaeva, E. N. Ustinova, L. P. Sizikova, E. I. Riabchikova, and L. S. Sandakhchiev. 1995. [Experimental study of Ebola hemorrhagic fever in baboon models]. *Biull. Eksp. Biol. Med.* 120(9):302–304.
 212. Lukashevich, I. S., J. D. Rodas, I. I. Tikhonov, J. C. Zapata, Y. Yang, M. Djavani, and M. S. Salvato. 2004. LCMV-mediated hepatitis in rhesus macaques: WE but not ARM strain activates hepatocytes and induces liver regeneration. *Arch. Virol.* 149(12):2319–2336.
 213. Lukashevich, I. S., I. Tikhonov, J. D. Rodas, J. C. Zapata, Y. Yang, M. Djavani, and M. S. Salvato. 2003. Arenavirus-mediated liver pathology: acute lymphocytic choriomeningitis virus infection of rhesus macaques is characterized by high-level interleukin-6 expression and hepatocyte proliferation. *J. Virol.* 77(3):1727–1737.
 214. Lusso, P., R. W. Crowley, M. S. Malnati, C. Di Serio, M. Ponzoni, A. Biancotto, P. D. Markham, and R. C. Gallo. 2007. Human herpesvirus 6A accelerates AIDS progression in macaques. *Proc. Natl. Acad. Sci. U. S. A.* 104(12):5067–5072.
 215. Mackett, M., C. Cox, S. D. Pepper, J. F. Lees, B. A. Naylor, N. Wedderburn, and J. R. Arrand. 1996. Immunisation of common marmosets with vaccinia virus expressing Epstein–Barr virus (EBV) gp340 and challenge with EBV. *J. Med. Virol.* 50(3):263–271.
 216. Majer, M., F. Behrens, E. Weinmann, R. Mauler, G. Maass, H. G. Baumeister, and T. Luthardt. 1978. Diarrhea in newborn cynomolgus monkeys infected with human rotavirus. *Infection* 6(2):71–72.
 217. Major, E. O., P. Mourrain, and C. Cummins. 1984. JC virus-induced owl monkey glioblastoma cells in culture: biological properties associated with the viral early gene product. *Virology* 136(2):359–367.
 218. Major, M. E., H. Dahari, K. Mihalik, M. Puig, C. M. Rice, A. U. Neumann, and S. M. Feinstone. 2004. Hepatitis C virus kinetics and host responses associated with disease and outcome of infection in chimpanzees. *Hepatology* 39(6):1709–1720.
 219. Major, M. E., K. Mihalik, J. Fernandez, J. Seidman, D. Kleiner, A. A. Kolykhalov, C. M. Rice, and S. M. Feinstone. 1999. Long-term follow-up of chimpanzees inoculated with the first infectious clone for hepatitis C virus. *J. Virol.* 73(4):3317–3325.
 220. Major, M. E., K. Mihalik, M. Puig, B. Rehermann, M. Nascimbeni, C. M. Rice, and S. M. Feinstone. 2002. Previously infected and recovered chimpanzees exhibit rapid responses that control hepatitis C virus replication upon rechallenge. *J. Virol.* 76(13):6586–6595.
 221. Manigold, T., E. C. Shin, E. Mizukoshi, K. Mihalik, K. K. Murthy, C. M. Rice, C. A. Piccirillo, and B. Rehermann. 2006. Foxp3+CD4+CD25+ T cells control virus-specific memory T cells in chimpanzees that recovered from hepatitis C. *Blood* 107(11):4424–4432.

222. Mao, J. S., Y. Y. Go, H. Y. Huang, P. H. Yu, B. Z. Huang, Z. S. Ding, N. L. Chen, J. H. Yu, and R. Y. Xie. 1981. Susceptibility of monkeys to human hepatitis A virus. *J. Infect. Dis.* 144(1):55–60.
223. Marchevsky, R. S., M. S. Freire, E. S. Coutinho, and R. Galler. 2003. Neurovirulence of yellow fever 17DD vaccine virus to rhesus monkeys. *Virology* 316(1):55–63.
224. Marriott, K. A., C. V. Parkinson, S. I. Morefield, R. Davenport, R. Nichols, and T. P. Monath. 2008. Clonal vaccinia virus grown in cell culture fully protects monkeys from lethal monkeypox challenge. *Vaccine* 26(4):581–588.
225. Mason, R. A., N. M. Tauraso, R. O. Spertzel, and R. K. Ginn. 1973. Yellow fever vaccine: direct challenge of monkeys given graded doses of 17D vaccine. *Appl. Microbiol.* 25(4):539–544.
226. Maximova, O. A., J. M. Ward, D. M. Asher, M. St Claire, B. W. Finneyfrock, J. M. Speicher, B. R. Murphy, and A. G. Pletnev. 2008. Comparative neuropathogenesis and neurovirulence of attenuated flaviviruses in nonhuman primates. *J. Virol.* 82(11):5255–5268.
227. Maynard, J. E., D. W. Bradley, C. R. Gravelle, J. W. Ebert, and D. H. Krushak. 1975. Preliminary studies of hepatitis A in chimpanzees. *J. Infect. Dis.* 131(2):194–197.
228. Maynard, J. E., W. V. Hartwell, and K. R. Berquist. 1971. Hepatitis-associated antigen in chimpanzees. *J. Infect. Dis.* 123(6):660–664.
229. McArthur-Vaughan, K. and L. J. Gershwin. 2002. A rhesus monkey model of respiratory syncytial virus infection. *J. Med. Primatol.* 31(2):61–73.
230. McAuliffe, J., L. Vogel, A. Roberts, G. Fahle, S. Fischer, W. J. Shieh, E. Butler, S. Zaki, M. St Claire, B. Murphy, and K. Subbarao. 2004. Replication of SARS coronavirus administered into the respiratory tract of African Green, rhesus and cynomolgus monkeys. *Virology* 330(1):8–15.
231. McCaustland, K. A., K. Krawczynski, J. W. Ebert, M. S. Balayan, A. G. Andjaparidze, J. E. Spelbring, E. H. Cook, C. Humphrey, P. O. Yarbough, M. O. Favorov, D. Carson, D. W. Bradley, and B. H. Robertson. 2000. Hepatitis E virus infection in chimpanzees: a retrospective analysis. *Arch. Virol.* 145(9):1909–1918.
232. McChesney, M. B., R. S. Fujinami, N. W. Lerche, P. A. Marx, and M. B. Oldstone. 1989. Virus-induced immunosuppression: infection of peripheral blood mononuclear cells and suppression of immunoglobulin synthesis during natural measles virus infection of rhesus monkeys. *J. Infect. Dis.* 159(4):757–760.
233. McChesney, M. B., C. J. Miller, P. A. Rota, Y. D. Zhu, L. Antipa, N. W. Lerche, R. Ahmed, and W. J. Bellini. 1997. Experimental measles. I. Pathogenesis in the normal and the immunized host. *Virology* 233(1):74–84.
234. McKee, K. T., Jr., J. W. Huggins, C. J. Trahan, and B. G. Mahlandt. 1988. Ribavirin prophylaxis and therapy for experimental argentine hemorrhagic fever. *Antimicrob. Agents Chemother.* 32(9):1304–1309.
235. McKee, K. T., Jr., B. G. Mahlandt, J. I. Maiategui, D. E. Green, and C. J. Peters. 1987. Virus-specific factors in experimental Argentine hemorrhagic fever in rhesus macaques. *J. Med. Virol.* 22(2):99–111.
236. McKee, K. T., Jr., J. G. Oro, A. I. Kuehne, J. A. Spisso, and B. G. Mahlandt. 1992. Candid No. 1 Argentine hemorrhagic fever vaccine protects against lethal Junin virus challenge in rhesus macaques. *Intervirology* 34(3):154–163.
237. McKee, K. T., Jr., J. G. Oro, A. I. Kuehne, J. A. Spisso, and B. G. Mahlandt. 1993. Safety and immunogenicity of a live-attenuated Junin (Argentine hemorrhagic fever) vaccine in rhesus macaques. *Am. J. Trop. Med. Hyg.* 48(3):403–411.
238. McLeod, C. G., Jr., J. L. Stookey, J. D. White, G. A. Eddy, and G. A. Fry. 1978. Pathology of Bolivian Hemorrhagic fever in the African green monkey. *Am. J. Trop. Med. Hyg.* 27(4):822–826.
239. Meignier, B., B. Martin, R. J. Whitley, and B. Roizman. 1990. In vivo behavior of genetically engineered herpes simplex viruses R7017 and R7020. II. Studies in immunocompetent and immunosuppressed owl monkeys (*Aotus trivirgatus*). *J. Infect. Dis.* 162(2):313–321.
240. Men, R., L. Wyatt, I. Tokimatsu, S. Arakaki, G. Shameem, R. Elkins, R. Chanock, B. Moss, and C. J. Lai. 2000. Immunization of rhesus monkeys with a recombinant of modified vaccinia virus Ankara expressing a truncated envelope glycoprotein of dengue type 2 virus induced resistance to dengue type 2 virus challenge. *Vaccine* 18(27):3113–3122.
241. Michaels, M. G., R. Lanford, A. J. Demetris, D. Chavez, K. Brasky, J. Fung, and T. E. Starzl. 1996. Lack of susceptibility of baboons to infection with hepatitis B virus. *Transplantation* 61(3):350–351.
242. Mikhailov, V. V., I. V. Borisevich, N. K. Chernikova, N. V. Potryvaeva, and V. P. Krasnianskii. 1994. [The evaluation in hamadryas baboons of the possibility for the specific prevention of Ebola fever]. *Vopr. Virusol.* 39(2):82–84.
243. Miller, G., T. Shope, D. Coope, L. Waters, J. Pagano, G. Bornkamm, and W. Henle. 1977. Lymphoma in cotton-top marmosets after inoculation with Epstein-Barr virus: tumor incidence, histologic spectrum antibody responses, demonstration of viral DNA, and characterization of viruses. *J. Exp. Med.* 145(4):948–967.

244. Miller, G., T. Shope, H. Lisco, D. Stitt, and M. Lipman. 1972. Epstein-Barr virus: transformation, cytopathic changes, and viral antigens in squirrel monkey and marmoset leukocytes. *Proc. Natl. Acad. Sci. U. S. A.* 69(2):383–387.
245. Mizukoshi, E., M. Nascimbeni, J. B. Blaustein, K. Mihalik, C. M. Rice, T. J. Liang, S. M. Feinstone, and B. Rehermann. 2002. Molecular and immunological significance of chimpanzee major histocompatibility complex haplotypes for hepatitis C virus immune response and vaccination studies. *J. Virol.* 76(12):6093–6103.
246. Monath, T. P., J. Arroyo, I. Levenbook, Z. X. Zhang, J. Catalan, K. Draper, and F. Guirakhoo. 2002. Single mutation in the flavivirus envelope protein hinge region increases neurovirulence for mice and monkeys but decreases viscerotropism for monkeys: relevance to development and safety testing of live, attenuated vaccines. *J. Virol.* 76(4):1932–1943.
247. Monath, T. P., K. R. Brinker, F. W. Chandler, G. E. Kemp, and C. B. Cropp. 1981. Pathophysiologic correlations in a rhesus monkey model of yellow fever with special observations on the acute necrosis of B cell areas of lymphoid tissues. *Am. J. Trop. Med. Hyg.* 30(2):431–443.
248. Monath, T. P., C. H. Calisher, M. Davis, G. S. Bowen, and J. White. 1974. Experimental studies of rhesus monkeys infected with epizootic and enzootic subtypes of Venezuelan equine encephalitis virus. *J. Infect. Dis.* 129(2):194–200.
249. Monath, T. P., C. B. Cropp, G. S. Bowen, G. E. Kemp, C. J. Mitchell, and J. J. Gardner. 1980. Variation in virulence for mice and rhesus monkeys among St. Louis encephalitis virus strains of different origin. *Am. J. Trop. Med. Hyg.* 29(5):948–962.
250. Monath, T. P., J. Liu, N. Kanessa-Thasan, G. A. Myers, R. Nichols, A. Deary, K. McCarthy, C. Johnson, T. Ermak, S. Shin, J. Arroyo, F. Guirakhoo, J. S. Kennedy, F. A. Ennis, S. Green, and P. Bedford. 2006. A live, attenuated recombinant West Nile virus vaccine. *Proc. Natl. Acad. Sci. U. S. A.* 103(17):6694–6699.
251. Moreland, A. F., R. D. Schimpff, and J. M. Gaskin. 1979. Fetal mortality and malformations associated with experimental infections of western equine encephalomyelitis vaccine virus in rhesus monkeys (*Macaca mulatta*). *Teratology* 20(1):65–74.
252. Morrill, J. C., C. W. Czarniecki, and C. J. Peters. 1991. Recombinant human interferon-gamma modulates Rift Valley fever virus infection in the rhesus monkey. *J. Interferon Res.* 11(5):297–304.
253. Morrill, J. C., G. B. Jennings, T. M. Cosgriff, P. H. Gibbs, and C. J. Peters. 1989. Prevention of Rift Valley fever in rhesus monkeys with interferon-alpha. *Rev. Infect. Dis.* 11(Suppl 4):S815–S825.
254. Morrill, J. C., G. B. Jennings, A. J. Johnson, T. M. Cosgriff, P. H. Gibbs, and C. J. Peters. 1990. Pathogenesis of Rift Valley fever in rhesus monkeys: role of interferon response. *Arch. Virol.* 110(3–4):195–212.
255. Morrill, J. C. and C. J. Peters. 2003. Pathogenicity and neurovirulence of a mutagen-attenuated Rift Valley fever vaccine in rhesus monkeys. *Vaccine* 21(21–22):2994–3002.
256. Moss, B., G. L. Smith, J. L. Gerin, and R. H. Purcell. 1984. Live recombinant vaccinia virus protects chimpanzees against hepatitis B. *Nature* 311(5981):67–69.
257. Murphy, B. R., V. S. Hinshaw, D. L. Sly, W. T. London, N. T. Hosier, F. T. Wood, R. G. Webster, and R. M. Chanock. 1982. Virulence of avian influenza A viruses for squirrel monkeys. *Infect. Immun.* 37(3):1119–1126.
258. Murphy, B. R., D. L. Sly, N. T. Hosier, W. T. London, and R. M. Chanock. 1980. Evaluation of three strains of influenza A virus in humans and in owl, cebus, and squirrel monkeys. *Infect. Immun.* 28(3):688–691.
259. Mushahwar, I. K. 2008. Hepatitis E virus: molecular virology, clinical features, diagnosis, transmission, epidemiology, and prevention. *J. Med. Virol.* 80(4):646–658.
260. Myint, K. S., B. Raengsakulrach, G. D. Young, M. Gettayacamin, L. M. Ferguson, B. L. Innis, C. H. Hoke Jr., and D. W. Vaughn. 1999. Production of lethal infection that resembles fatal human disease by intranasal inoculation of macaques with Japanese encephalitis virus. *Am. J. Trop. Med. Hyg.* 60(3):338–342.
261. Nahmias, A. J., W. T. London, L. W. Catalano, D. A. Fuccillo, J. L. Sever, and C. Graham. 1971. Genital herpesvirus hominis type 2 infection: an experimental model in cebus monkeys. *Science* 171(968):297–298.
262. Nainan, O. V., L. Lu, F. X. Gao, E. Meeks, B. H. Robertson, and H. S. Margolis. 2006. Selective transmission of hepatitis C virus genotypes and quasispecies in humans and experimentally infected chimpanzees. *J. Gen. Virol.* 87(Pt 1):83–91.
263. Nakamura, H., M. Hayami, Y. Ohta, K. Ishikawa, H. Tsujimoto, T. Kiyokawa, M. Yoshida, A. Sasagawa, and S. Honjo. 1987. Protection of cynomolgus monkeys against infection by human T-cell leukemia virus type-I by immunization with viral env gene products produced in *Escherichia coli*. *Int. J. Cancer* 40(3):403–407.
264. Nakamura, H., Y. Tanaka, A. Komuro-Tsujimoto, K. Ishikawa, K. Takadaya, H. Tozawa, H. Tsujimoto, S. Honjo, and M. Hayami. 1986. Experimental inoculation of monkeys with autologous lymphoid cell

- lines immortalized by and producing human T-cell leukemia virus type-I. *Int. J. Cancer* 38(6):867–875.
265. Nascimbeni, M., E. Mizukoshi, M. Bosmann, M. E. Major, K. Mihalik, C. M. Rice, S. M. Feinstone, and B. Rehermann. 2003. Kinetics of CD4⁺ and CD8⁺ memory T-cell responses during hepatitis C virus rechallenge of previously recovered chimpanzees. *J. Virol.* 77(8):4781–4793.
266. Neurath, A. R., B. Seto, and N. Strick. 1989. Antibodies to synthetic peptides from the preS1 region of the hepatitis B virus (HBV) envelope (env) protein are virus-neutralizing and protective. *Vaccine* 7(3):234–236.
267. Nigam, P., P. L. Earl, J. L. Americo, S. Sharma, L. S. Wyatt, Y. Edghill-Spano, L. S. Chennareddi, P. Silvera, B. Moss, H. L. Robinson, and R. R. Amara. 2007. DNA/MVA HIV-1/AIDS vaccine elicits long-lived vaccinia virus-specific immunity and confers protection against a lethal monkeypox challenge. *Virology* 366(1):73–83.
268. O'Brien, T. C., P. Albrecht, N. M. Tauraso, and G. R. Burns. 1972. Properties of a measles virus neuropathic for rhesus monkeys. *Arch. Gesamte Virusforsch.* 39(1):228–239.
269. Ogata, N., P. J. Cote, A. R. Zanetti, R. H. Miller, M. Shapiro, J. Gerin, and R. H. Purcell. 1999. Licensed recombinant hepatitis B vaccines protect chimpanzees against infection with the prototype surface gene mutant of hepatitis B virus. *Hepatology* 30(3):779–786.
270. P'iankov, O. V., A. N. Sergeev, O. G. P'iankova, and A. A. Chepurnov. 1995. [Experimental Ebola fever in Macaca mulatta]. *Vopr. Virusol.* 40(3):113–115.
271. Pallansch, M. A. and R. Roos. 2007. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe, D. M. and P. M. Howley (eds), *Fields Virology*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, Wolters Kluwer Business, pp. 839–893.
272. Pancholi, P., D. H. Lee, Q. Liu, C. Tackney, P. Taylor, M. Perkus, L. Andrus, B. Brotman, and A. M. Prince. 2001. DNA prime/canarypox boost-based immunotherapy of chronic hepatitis B virus infection in a chimpanzee. *Hepatology* 33(2):448–454.
273. Payette, P. J., X. Ma, R. D. Weeratna, M. J. McCluskie, M. Shapiro, R. E. Engle, H. L. Davis, and R. H. Purcell. 2006. Testing of CpG-optimized protein and DNA vaccines against the hepatitis B virus in chimpanzees for immunogenicity and protection from challenge. *Intervirology* 49(3):144–151.
274. Permar, S. R., S. A. Klumpp, K. G. Mansfield, A. A. Carville, D. A. Gorgone, M. A. Lifton, J. E. Schmitz, K. A. Reimann, F. P. Polack, D. E. Griffin, and N. L. Letvin. 2004. Limited contribution of humoral immunity to the clearance of measles viremia in rhesus monkeys. *J. Infect. Dis.* 190(5):998–1005.
275. Permar, S. R., S. A. Klumpp, K. G. Mansfield, W. K. Kim, D. A. Gorgone, M. A. Lifton, K. C. Williams, J. E. Schmitz, K. A. Reimann, M. K. Axthelm, F. P. Polack, D. E. Griffin, and N. L. Letvin. 2003. Role of CD8(+) lymphocytes in control and clearance of measles virus infection of rhesus monkeys. *J. Virol.* 77(7):4396–4400.
276. Permar, S. R., S. S. Rao, Y. Sun, S. Bao, A. P. Buzby, H. H. Kang, and N. L. Letvin. 2007. Clinical measles after measles virus challenge in simian immunodeficiency virus-infected measles virus-vaccinated rhesus monkeys. *J. Infect. Dis.* 196(12):1784–1793.
277. Peters, C. J., D. Jones, R. Trotter, J. Donaldson, J. White, E. Stephen, and T. W. Slone Jr. 1988. Experimental Rift Valley fever in rhesus macaques. *Arch. Virol.* 99(1–2):31–44.
278. Pletnev, A. G., M. Bray, K. A. Hanley, J. Speicher, and R. Elkins. 2001. Tick-borne Langat/mosquito-borne dengue flavivirus chimera, a candidate live attenuated vaccine for protection against disease caused by members of the tick-borne encephalitis virus complex: evaluation in rhesus monkeys and in mosquitoes. *J. Virol.* 75(17):8259–8267.
279. Pletnev, A. G., D. E. Swayne, J. Speicher, A. A. Rumyantsev, and B. R. Murphy. 2006. Chimeric West Nile/dengue virus vaccine candidate: preclinical evaluation in mice, geese and monkeys for safety and immunogenicity. *Vaccine* 24(40–41):6392–6404.
280. Pogodina, V. V., M. P. Frolova, G. V. Malenko, G. I. Fokina, G. V. Koreshkova, L. L. Kiseleva, N. G. Bochkova, and N. M. Ralph. 1983. Study on West Nile virus persistence in monkeys. *Arch. Virol.* 75(1–2):71–86.
281. Pogodina, V. V., M. P. Frolova, G. V. Malenko, G. I. Fokina, L. S. Levina, L. L. Mamonenko, G. V. Koreshkova, and N. M. Ralf. 1981. Persistence of tick-borne encephalitis virus in monkeys. I. Features of experimental infection. *Acta Virol.* 25(6):337–343.
282. Polack, F. P., S. J. Hoffman, W. J. Moss, and D. E. Griffin. 2002. Altered synthesis of interleukin-12 and type 1 and type 2 cytokines in rhesus macaques during measles and atypical measles. *J. Infect. Dis.* 185(1):13–19.
283. Polack, F. P., S. J. Hoffman, W. J. Moss, and D. E. Griffin. 2003. Differential effects of priming with DNA vaccines encoding the hemagglutinin and/or fusion proteins on cytokine responses after measles virus challenge. *J. Infect. Dis.* 187(11):1794–1800.
284. Ponnuraj, E. M., A. R. Hayward, A. Raj, H. Wilson, and E. A. Simoes. 2001. Increased replication of

- respiratory syncytial virus (RSV) in pulmonary infiltrates is associated with enhanced histopathological disease in bonnet monkeys (*Macaca radiata*) pre-immunized with a formalin-inactivated RSV vaccine. *J. Gen. Virol.* 82(Pt 11):2663–2674.
285. Popper, H., J. L. Dienstag, S. M. Feinstone, H. J. Alter, and R. H. Purcell. 1980. The pathology of viral hepatitis in chimpanzees. *Virchows Arch. A Pathol. Anat. Histol.* 387(1):91–106.
286. Porter, B. F., S. D. Goens, K. M. Brasky, and G. B. Hubbard. 2004. A case report of hepatocellular carcinoma and focal nodular hyperplasia with a myelolipoma in two chimpanzees and a review of spontaneous hepatobiliary tumors in non-human primates. *J. Med. Primatol.* 33(1):38–47.
287. Pratt, W. D., N. L. Davis, R. E. Johnston, and J. F. Smith. 2003. Genetically engineered, live attenuated vaccines for Venezuelan equine encephalitis: testing in animal models. *Vaccine* 21(25–26):3854–3862.
288. Pratt, W. D., P. Gibbs, M. L. Pitt, and A. L. Schmaljohn. 1998. Use of telemetry to assess vaccine-induced protection against parenteral and aerosol infections of Venezuelan equine encephalitis virus in non-human primates. *Vaccine* 16(9–10):1056–1064.
289. Premenko-Lanier, M., P. A. Rota, G. Rhodes, D. Verhoeven, D. H. Barouch, N. W. Lerche, N. L. Letvin, W. J. Bellini, and M. B. McChesney. 2003. DNA vaccination of infants in the presence of maternal antibody: a measles model in the primate. *Virology* 307(1):67–75.
290. Premenko-Lanier, M., P. A. Rota, G. H. Rhodes, W. J. Bellini, and M. B. McChesney. 2004. Protection against challenge with measles virus (MV) in infant macaques by an MV DNA vaccine administered in the presence of neutralizing antibody. *J. Infect. Dis.* 189(11):2064–2071.
291. Prince, A. M. 1985. Reliability of chimpanzee model for non-A, non-B hepatitis. *Lancet* 2(8464):1134.
292. Prince, A. M., B. Brotman, D. H. Lee, W. Pfahler, N. Tricoche, L. Andrus, and M. T. Shata. 2005. Protection against chronic hepatitis C virus infection after rechallenge with homologous, but not heterologous, genotypes in a chimpanzee model. *J. Infect. Dis.* 192(10):1701–1709.
293. Provost, P. J., P. M. Keller, F. S. Bunker, B. J. Keech, H. J. Klein, R. S. Lowe, D. H. Morton, A. H. Phelps, W. J. McAleer, and R. W. Ellis. 1987. Successful infection of the common marmoset (*Callithrix jacchus*) with human varicella-zoster virus. *J. Virol.* 61(10):2951–2955.
294. Puig, M., M. E. Major, K. Mihalik, and S. M. Feinstone. 2004. Immunization of chimpanzees with an envelope protein-based vaccine enhances specific humoral and cellular immune responses that delay hepatitis C virus infection. *Vaccine* 22(8):991–1000.
295. Puig, M., K. Mihalik, J. C. Tilton, O. Williams, M. Merchlinsky, M. Connors, S. M. Feinstone, and M. E. Major. 2006. CD4⁺ immune escape and subsequent T-cell failure following chimpanzee immunization against hepatitis C virus. *Hepatology* 44(3):736–745.
296. Purcell, R. H. and S. U. Emerson. 2001. Animal models of hepatitis A and E. *ILAR J.* 42(2):161–177.
297. Purcell, R. H. and J. L. Gerin. 1975. Hepatitis B subunit vaccine: a preliminary report of safety and efficacy tests in chimpanzees. *Am. J. Med. Sci.* 270(2):395–399.
298. Purcell, R. H., H. Nguyen, M. Shapiro, R. E. Engle, S. Govindarajan, W. C. Blackwelder, D. C. Wong, J. P. Prieels, and S. U. Emerson. 2003. Pre-clinical immunogenicity and efficacy trial of a recombinant hepatitis E vaccine. *Vaccine* 21(19–20):2607–2615.
299. Qin, C., J. Wang, Q. Wei, M. She, W. A. Marasco, H. Jiang, X. Tu, H. Zhu, L. Ren, H. Gao, L. Guo, L. Huang, R. Yang, Z. Cong, L. Guo, Y. Wang, Y. Liu, Y. Sun, S. Duan, J. Qu, L. Chen, W. Tong, L. Ruan, P. Liu, H. Zhang, J. Zhang, H. Zhang, D. Liu, Q. Liu, T. Hong, and W. He. 2005. An animal model of SARS produced by infection of *Macaca mulatta* with SARS coronavirus. *J. Pathol.* 206(3):251–259.
300. Qin, E., H. Shi, L. Tang, C. Wang, G. Chang, Z. Ding, K. Zhao, J. Wang, Z. Chen, M. Yu, B. Si, J. Liu, D. Wu, X. Cheng, B. Yang, W. Peng, Q. Meng, B. Liu, W. Han, X. Yin, H. Duan, D. Zhan, L. Tian, S. Li, J. Wu, G. Tan, Y. Li, Y. Liu, H. Liu, F. Lv, Y. Zhang, X. Kong, B. Fan, T. Jiang, S. Xu, X. Wang, C. Li, X. Wu, Y. Deng, M. Zhao, and Q. Zhu. 2006. Immunogenicity and protective efficacy in monkeys of purified inactivated Vero-cell SARS vaccine. *Vaccine* 24(7):1028–1034.
301. Raengsakulrach, B., A. Nisalak, M. Gettayacamin, V. Thirawuth, G. D. Young, K. S. Myint, L. M. Ferguson, C. H. Hoke Jr., B. L. Innis, and D. W. Vaughn. 1999. An intranasal challenge model for testing Japanese encephalitis vaccines in rhesus monkeys. *Am. J. Trop. Med. Hyg.* 60(3):329–337.
302. Raengsakulrach, B., A. Nisalak, M. Gettayacamin, V. Thirawuth, G. D. Young, K. S. Myint, L. M. Ferguson, C. H. Hoke Jr., B. L. Innis, and D. W. Vaughn. 1999. Safety, immunogenicity, and protective efficacy of NYVAC-JEV and ALVAC-JEV recombinant Japanese encephalitis vaccines in rhesus monkeys. *Am. J. Trop. Med. Hyg.* 60(3):343–349.
303. Rao, V., M. E. Hinz, B. A. Roberts, and D. Fine. 2006. Toxicity assessment of Venezuelan Equine Encephalitis virus vaccine candidate strain V3526. *Vaccine* 24(10):1710–1715.

304. Reed, D. S., L. E. Hensley, J. B. Geisbert, P. B. Jahrling, and T. W. Geisbert. 2004. Depletion of peripheral blood T lymphocytes and NK cells during the course of ebola hemorrhagic Fever in cynomolgus macaques. *Viral Immunol.* 17(3):390–400.
305. Reed, D. S., M. G. Lackemeyer, N. L. Garza, S. Norris, S. Gamble, L. J. Sullivan, C. M. Lind, and J. L. Raymond. 2007. Severe encephalitis in cynomolgus macaques exposed to aerosolized Eastern equine encephalitis virus. *J. Infect. Dis.* 196(3):441–450.
306. Reed, D. S., T. Larsen, L. J. Sullivan, C. M. Lind, M. G. Lackemeyer, W. D. Pratt, and M. D. Parker. 2005. Aerosol exposure to western equine encephalitis virus causes fever and encephalitis in cynomolgus macaques. *J. Infect. Dis.* 192(7):1173–1182.
307. Reed, D. S., C. M. Lind, M. G. Lackemeyer, L. J. Sullivan, W. D. Pratt, and M. D. Parker. 2005. Genetically engineered, live, attenuated vaccines protect nonhuman primates against aerosol challenge with a virulent IE strain of Venezuelan equine encephalitis virus. *Vaccine* 23(24):3139–3147.
308. Reed, D. S. and M. Mohamadzadeh. 2007. Status and challenges of filovirus vaccines. *Vaccine* 25(11):1923–1934.
309. Renne, R., D. Dittmer, D. Kedes, K. Schmidt, R. C. Desrosiers, P. A. Luciw, and D. Ganem. 2004. Experimental transmission of Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) to SIV-positive and SIV-negative rhesus macaques. *J. Med. Primatol.* 33(1):1–9.
310. Richardson, L. S., R. B. Belshe, W. T. London, D. L. Sly, D. A. Prevar, E. Camargo, and R. M. Chanock. 1978. Evaluation of five temperature-sensitive mutants of respiratory syncytial virus in primates: I. Viral shedding, immunologic response, and associated illness. *J. Med. Virol.* 3(2):91–100.
311. Richardson, L. S., R. B. Belshe, D. L. Sly, W. T. London, D. A. Prevar, E. Camargo, and R. M. Chanock. 1978. Experimental respiratory syncytial virus pneumonia in cebus monkeys. *J. Med. Virol.* 2(1):45–59.
312. Rimmelzwaan, G. F., T. Kuiken, G. van Amerongen, T. M. Bestebroer, R. A. Fouchier, and A. D. Osterhaus. 2001. Pathogenesis of influenza A (H5N1) virus infection in a primate model. *J. Virol.* 75(14):6687–6691.
313. Rockx, B. H., W. M. Bogers, J. L. Heeney, G. van Amerongen, and M. P. Koopmans. 2005. Experimental norovirus infections in non-human primates. *J. Med. Virol.* 75(2):313–320.
314. Rodas, J. D., I. S. Lukashevich, J. C. Zapata, C. Cairo, I. Tikhonov, M. Djavani, C. D. Pauza, and M. S. Salvato. 2004. Mucosal arenavirus infection of primates can protect them from lethal hemorrhagic fever. *J. Med. Virol.* 72(3):424–435.
315. Rollier, C., E. Depla, J. A. Drexhage, E. J. Verschoor, B. E. Verstrepen, A. Fatmi, C. Brinster, A. Fournillier, J. A. Whelan, M. Whelan, D. Jacobs, G. Maertens, G. Inchauspe, and J. L. Heeney. 2004. Control of heterologous hepatitis C virus infection in chimpanzees is associated with the quality of vaccine-induced peripheral T-helper immune response. *J. Virol.* 78(1):187–196.
316. Rowe, T., G. Gao, R. J. Hogan, R. G. Crystal, T. G. Voss, R. L. Grant, P. Bell, G. P. Kobinger, N. A. Wivel, and J. M. Wilson. 2004. Macaque model for severe acute respiratory syndrome. *J. Virol.* 78(20):11401–11404.
317. Ruat, C., C. Caillet, A. Bidaut, J. Simon, and A. D. Osterhaus. 2008. Vaccination of macaques with adjuvanted formalin-inactivated influenza A (H5N1) vaccines: protection against H5N1 challenge without disease enhancement. *J. Virol.* 82(5):2565–2569.
318. Rumyantsev, A. A., R. M. Chanock, B. R. Murphy, and A. G. Pletnev. 2006. Comparison of live and inactivated tick-borne encephalitis virus vaccines for safety, immunogenicity and efficacy in rhesus monkeys. *Vaccine* 24(2):133–143.
319. Ryabchikova, E. I., L. V. Kolesnikova, and S. V. Luchko. 1999. An analysis of features of pathogenesis in two animal models of Ebola virus infection. *J. Infect. Dis.* 179(Suppl 1):S199–S202.
320. Sariol, C. A., J. L. Munoz-Jordan, K. Abel, L. C. Rosado, P. Pantoja, L. Giavedoni, I. V. Rodriguez, L. J. White, M. Martinez, T. Arana, and E. N. Kraiselburd. 2007. Transcriptional activation of interferon-stimulated genes but not of cytokine genes after primary infection of rhesus macaques with dengue virus type 1. *Clin. Vaccine Immunol.* 14(6):756–766.
321. Sato, H., F. Kobune, Y. Ami, M. Yoneda, and C. Kai. 2008. Immune responses against measles virus in cynomolgus monkeys. *Comp. Immunol. Microbiol. Infect. Dis.* 31(1):25–35.
322. Scott, R. M., R. Snithian, W. H. Bancroft, H. J. Alter, and M. Tingpalapong. 1980. Experimental transmission of hepatitis B virus by semen and saliva. *J. Infect. Dis.* 142(1):67–71.
323. Shata, M. T., W. Pfahler, B. Brotman, D. H. Lee, N. Tricoche, K. Murthy, and A. M. Prince. 2006. Attempted therapeutic immunization in a chimpanzee chronic HBV carrier with a high viral load. *J. Med. Primatol.* 35(3):165–171.
324. Shih, C., M. Y. Yu, L. S. Li, and J. W. Shih. 1990. Hepatitis B virus propagated in a rat hepatoma cell line is infectious in a primate model. *Virology* 179(2):871–873.

325. Shope, T., D. Dechairo, and G. Miller. 1973. Malignant lymphoma in cottontop marmosets after inoculation with Epstein-Barr virus. *Proc. Natl. Acad. Sci. U. S. A.* 70(9):2487–2491.
326. Shouval, D., P. R. Chakraborty, N. Ruiz-Opazo, S. Baum, I. Spigland, E. Muchmore, M. A. Gerber, S. N. Thung, H. Popper, and D. A. Shafritz. 1980. Chronic hepatitis in chimpanzee carriers of hepatitis B virus: morphologic, immunologic, and viral DNA studies. *Proc. Natl. Acad. Sci. U. S. A.* 77(10):6147–6151.
327. Shrestha, M. P., R. M. Scott, D. M. Joshi, M. P. Mammen Jr., G. B. Thapa, N. Thapa, K. S. Myint, M. Fourneau, R. A. Kuschner, S. K. Shrestha, M. P. David, J. Seriwatana, D. W. Vaughn, A. Safary, T. P. Endy, and B. L. Innis. 2007. Safety and efficacy of a recombinant hepatitis E vaccine. *N. Engl. J. Med.* 356(9):895–903.
328. Simkova, A. and A. Petrovicova. 1972. Experimental infection of rhesus monkeys with Coxsackie A 4 virus. *Acta Virol.* 16(3):250–257.
329. Simoes, E. A., A. R. Hayward, E. M. Ponnuraj, J. P. Straumanis, K. R. Stenmark, H. L. Wilson, and P. G. Babu. 1999. Respiratory syncytial virus infects the Bonnet monkey, *Macaca radiata*. *Pediatr. Dev. Pathol.* 2(4):316–326.
330. Snyder, M. H., M. L. Clements, D. Herrington, W. T. London, E. L. Tierney, and B. R. Murphy. 1986. Comparison by studies in squirrel monkeys, chimpanzees, and adult humans of avian-human influenza A virus reassortants derived from different avian influenza virus donors. *J. Clin. Microbiol.* 24(3):467–469.
331. Stephen, E. L. and P. B. Jahrling. 1979. Experimental Lassa fever virus infection successfully treated with ribavirin. *Lancet* 1(8110):268–269.
332. Stephen, E. L., M. L. Sammons, W. L. Pannier, S. Baron, R. O. Spertzel, and H. B. Levy. 1977. Effect of a nuclease-resistant derivative of polyriboinosinic-polyribocytidyl acid complex on yellow fever in rhesus monkeys (*Macaca mulatta*). *J. Infect. Dis.* 136(1):122–126.
333. Stittelaar, K. J., J. Neyts, L. Naesens, G. van Amerongen, R. F. van Lavieren, A. Holy, E. De Clercq, H. G. Niesters, E. Fries, C. Maas, P. G. Mulder, B. A. van der Zeijst, and A. D. Osterhaus. 2006. Antiviral treatment is more effective than smallpox vaccination upon lethal monkeypox virus infection. *Nature* 439(7077):745–748.
334. Stittelaar, K. J., G. van Amerongen, I. Kondova, T. Kuiken, R. F. van Lavieren, F. H. Pistoor, H. G. Niesters, G. van Doornum, B. A. van der Zeijst, L. Mateo, P. J. Chaplin, and A. D. Osterhaus. 2005. Modified vaccinia virus Ankara protects macaques against respiratory challenge with monkeypox virus. *J. Virol.* 79(12):7845–7851.
335. Stokes, A., J. H. Bauer, and N. P. Hudson. 2001. The transmission of yellow fever to *Macacus rhesus*. 1928. *Rev. Med. Virol.* 11(3):141–148.
336. Subekti, D. S., P. Tjaniadi, M. Lesmana, J. McArdle, D. Iskandriati, I. N. Budiarsa, P. Walujo, I. H. Suparto, I. Winoto, J. R. Campbell, K. R. Porter, D. Sajuthi, A. A. Ansari, and B. A. Oyofo. 2002. Experimental infection of *Macaca nemestrina* with a Toronto Norwalk-like virus of epidemic viral gastroenteritis. *J. Med. Virol.* 66(3):400–406.
337. Sullivan, N. J., T. W. Geisbert, J. B. Geisbert, D. J. Shedlock, L. Xu, L. Lamoreaux, J. H. Custers, P. M. Popernack, Z. Y. Yang, M. G. Pau, M. Roederer, R. A. Koup, J. Goudsmit, P. B. Jahrling, and G. J. Nabel. 2006. Immune protection of nonhuman primates against Ebola virus with single low-dose adenovirus vectors encoding modified GPs. *PLoS Med.* 3(6):e177.
338. Sullivan, N. J., T. W. Geisbert, J. B. Geisbert, L. Xu, Z. Y. Yang, M. Roederer, R. A. Koup, P. B. Jahrling, and G. J. Nabel. 2003. Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. *Nature* 424(6949):681–684.
339. Sullivan, N. J., A. Sanchez, P. E. Rollin, Z. Y. Yang, and G. J. Nabel. 2000. Development of a preventive vaccine for Ebola virus infection in primates. *Nature* 408(6812):605–609.
340. Sundar, S. K., P. H. Levine, D. V. Ablashi, S. A. Leiseca, G. R. Armstrong, J. L. Cicmanec, G. A. Parker, and M. Nonoyama. 1981. Epstein-Barr virus-induced malignant lymphoma in a white-lipped marmoset. *Int. J. Cancer* 27(1):107–111.
341. Sureau, C., J. W. Eichberg, G. B. Hubbard, J. L. Romet-Lemonne, and M. Essex. 1988. A molecularly cloned hepatitis B virus produced in vitro is infectious in a chimpanzee. *J. Virol.* 62(8):3064–3067.
342. Swenson, D. L., D. Wang, M. Luo, K. L. Warfield, J. Woraratanadharma, D. H. Holman, J. Y. Dong, and W. D. Pratt. 2008. Vaccine to confer to nonhuman primates complete protection against multistrain Ebola and Marburg virus infections. *Clin. Vaccine Immunol.* 15(3):460–467.
343. Swenson, D. L., K. L. Warfield, T. Larsen, D. A. Alves, S. S. Coberley, and S. Bavari. 2008. Monovalent virus-like particle vaccine protects guinea pigs and nonhuman primates against infection with multiple Marburg viruses. *Expert Rev. Vaccines* 7(4):417–429.
344. Takahashi, Y., S. Misumi, A. Muneoka, M. Masuyama, H. Tokado, K. Fukuzaki, N. Takamune, and S. Shoji. 2008. Nonhuman primate intestinal villous

- M-like cells: an effective poliovirus entry site. *Biochem. Biophys. Res. Commun.* 368(3):501–507.
345. Tanabayashi, K., R. Mukai, A. Yamada, T. Takasaki, I. Kurane, M. Yamaoka, A. Terazawa, and E. Konishi. 2003. Immunogenicity of a Japanese encephalitis DNA vaccine candidate in cynomolgus monkeys. *Vaccine* 21(19–20):2338–2345.
346. Terrell, T. G., J. L. Stookey, G. A. Eddy, and M. D. Kastello. 1973. Pathology of Bolivian hemorrhagic fever in the rhesus monkey. *Am. J. Pathol.* 73(2):477–494.
347. Ticehurst, J., L. L. Rhodes Jr., K. Krawczynski, L. V. Asher, W. F. Engler, T. L. Mensing, J. D. Caudill, M. H. Sjogren, C. H. Hoke Jr., J. W. LeDuc, D. W. Bradley, and L. N. Binn. 1992. Infection of owl monkeys (*Aotus trivirgatus*) and cynomolgus monkeys (*Macaca fascicularis*) with hepatitis E virus from Mexico. *J. Infect. Dis.* 165(5):835–845.
348. Tollis, M., B. Dietzschold, C. B. Volia, and H. Koprowski. 1991. Immunization of monkeys with rabies ribonucleoprotein (RNP) confers protective immunity against rabies. *Vaccine* 9(2):134–136.
349. Traina-Dorge, V. L., L. N. Martin, R. Lorino, E. L. Winsor, and M. A. Beilke. 2007. Human T cell leukemia virus type 1 up-regulation after simian immunodeficiency virus-1 coinfection in the nonhuman primate. *J. Infect. Dis.* 195(4):562–571.
350. Tsarev, S. A., S. U. Emerson, T. S. Tsareva, P. O. Yarbough, M. Lewis, S. Govindarajan, G. R. Reyes, M. Shapiro, and R. H. Purcell. 1993. Variation in course of hepatitis E in experimentally infected cynomolgus monkeys. *J. Infect. Dis.* 167(6):1302–1306.
351. Tsarev, S. A., T. S. Tsareva, S. U. Emerson, S. Govindarajan, M. Shapiro, J. L. Gerin, and R. H. Purcell. 1994. Successful passive and active immunization of cynomolgus monkeys against hepatitis E. *Proc. Natl. Acad. Sci. U. S. A.* 91(21):10198–10202.
352. Tsarev, S. A., T. S. Tsareva, S. U. Emerson, M. K. Rippy, P. Zack, M. Shapiro, and R. H. Purcell. 1995. Experimental hepatitis E in pregnant rhesus monkeys: failure to transmit hepatitis E virus (HEV) to offspring and evidence of naturally acquired antibodies to HEV. *J. Infect. Dis.* 172(1):31–37.
353. Tsarev, S. A., T. S. Tsareva, S. U. Emerson, P. O. Yarbough, L. J. Legters, T. Moskal, and R. H. Purcell. 1994. Infectivity titration of a prototype strain of hepatitis E virus in cynomolgus monkeys. *J. Med. Virol.* 43(2):135–142.
354. van den Hoogen, B. G., S. Herfst, M. de Graaf, L. Sprong, R. van Lavieren, G. van Amerongen, S. Yuksel, R. A. Fouchier, A. D. Osterhaus, and R. L. de Swart. 2007. Experimental infection of macaques with human metapneumovirus induces transient protective immunity. *J. Gen. Virol.* 88(Pt 4):1251–1259.
355. Vaughan, K., G. H. Rhodes, and L. J. Gershwin. 2005. DNA immunization against respiratory syncytial virus (RSV) in infant rhesus monkeys. *Vaccine* 23(22):2928–2942.
356. von Magnus, P., E. K. Anderson, K. B. Petersen, and A. Birch-Anderson. 1959. A pox-like disease in cynomolgus monkeys. *Acta Pathol. Microbiol. Scand.* 46:156–176.
357. Wagner, F. S., G. A. Eddy, and O. M. Brand. 1977. The African green monkey as an alternate primate host for studying Machupo virus infection. *Am. J. Trop. Med. Hyg.* 26(1):159–162.
358. Walker, D. H., K. M. Johnson, J. V. Lange, J. J. Gardner, M. P. Kiley, and J. B. McCormick. 1982. Experimental infection of rhesus monkeys with Lassa virus and a closely related arenavirus, Mozambique virus. *J. Infect. Dis.* 146(3):360–368.
359. Ward, T. M., V. Traina-Dorge, K. A. Davis, and W. L. Gray. 2008. Recombinant simian varicella viruses expressing respiratory syncytial virus antigens are immunogenic. *J. Gen. Virol.* 89(Pt 3):741–750.
360. Warfield, K. L., D. L. Swenson, G. G. Olinger, W. V. Kalina, M. J. Aman, and S. Bavari. 2007. Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge. *J. Infect. Dis.* 196(Suppl 2):S430–S437.
361. Weiner, A. J., X. Paliard, M. J. Selby, A. Medina-Selby, D. Coit, S. Nguyen, J. Kansopon, C. L. Arian, P. Ng, J. Tucker, C. T. Lee, N. K. Polakos, J. Han, S. Wong, H. H. Lu, S. Rosenberg, K. M. Brasky, D. Chien, G. Kuo, and M. Houghton. 2001. Intrahepatic genetic inoculation of hepatitis C virus RNA confers cross-protective immunity. *J. Virol.* 75(15):7142–7148.
362. Weinmann, E., M. Majer, and J. Hilfenhaus. 1979. Intramuscular and/or intralumbar postexposure treatment of rabies virus-infected cynomolgus monkeys with human interferon. *Infect. Immun.* 24(1):24–31.
363. Weissenbacher, M. C., M. M. Avila, M. A. Calello, M. S. Merani, J. B. McCormick, and M. Rodriguez. 1986. Effect of ribavirin and immune serum on Junin virus-infected primates. *Med. Microbiol. Immunol.* 175(2–3):183–186.
364. Weissenbacher, M. C., M. A. Calello, O. J. Colillas, S. N. Rondinone, and M. J. Frigerio. 1979. Argentine hemorrhagic fever: a primate model. *Intervirology* 11(6):363–365.
365. Weissenbacher, M. C., M. A. Calello, M. S. Merani, J. B. McCormick, and M. Rodriguez. 1986. Therapeutic effect of the antiviral agent ribavirin in Junin

- virus infection of primates. *J. Med. Virol.* 20(3):261–267.
366. Weissenbacher, M. C., C. E. Coto, M. A. Calello, S. N. Rondinone, E. B. Damonte, and M. J. Frigerio. 1982. Cross-protection in nonhuman primates against Argentine hemorrhagic fever. *Infect. Immun.* 35(2):425–430.
367. Werner, J., H. Wolf, J. Apodaca, and H. zur Hausen. 1975. Lymphoproliferative disease in a cotton-top marmoset after inoculation with infectious mononucleosis-derived Epstein-Barr virus. *Int. J. Cancer* 15(6):1000–1008.
368. Whitehead, S. S., J. E. Blaney, A. P. Durbin, and B. R. Murphy. 2007. Prospects for a dengue virus vaccine. *Nat. Rev. Microbiol.* 5(7):518–528.
369. Wolf, R. F., J. F. Papin, R. Hines-Boykin, M. Chavez-Suarez, G. L. White, M. Sakalian, and D. P. Dittmer. 2006. Baboon model for West Nile virus infection and vaccine evaluation. *Virology* 355(1):44–51.
370. Wyatt, R. G., H. B. Greenberg, D. W. Dalgard, W. P. Allen, D. L. Sly, T. S. Thornhill, R. M. Chanock, and A. Z. Kapikian. 1978. Experimental infection of chimpanzees with the Norwalk agent of epidemic viral gastroenteritis. *J. Med. Virol.* 2(2):89–96.
371. Wyatt, R. G., D. L. Sly, W. T. London, A. E. Palmer, A. R. Kalica, D. H. Van Kirk, R. M. Chanock, and A. Z. Kapikian. 1976. Induction of diarrhea in colostrum-deprived newborn rhesus monkeys with the human reovirus-like agent of infantile gastroenteritis. *Arch. Virol.* 50(1–2):17–27.
372. Wyckoff, R. W. 1939. Encephalomyelitis in monkeys. *Science* 89(2319):542–543.
373. Xia, N. S., J. Zhang, Y. J. Zheng, S. X. Ge, X. Z. Ye, and S. H. Ou. 2004. Transfusion of plasma from a blood donor induced hepatitis E in Rhesus monkey. *Vox Sang.* 86(1):45–47.
374. Yamamoto, N., M. Hayami, A. Komuro, J. Schneider, G. Hunsmann, M. Okada, and Y. Hinuma. 1984. Experimental infection of cynomolgus monkeys with a human retrovirus, adult T-cell leukemia virus. *Med. Microbiol. Immunol. (Berl)* 173:57–64.
375. Yang, C., L. Ye, and R. W. Compans. 2008. Protection against filovirus infection: virus-like particle vaccines. *Expert Rev. Vaccines* 7(3):333–344.
376. Yang, L., A. Sanchez, J. M. Ward, B. R. Murphy, P. L. Collins, and A. Bukreyev. 2008. A paramyxovirus-vectorized intranasal vaccine against Ebola virus is immunogenic in vector-immune animals. *Virology* 377(2):255–264.
377. Yoon, J. W., W. T. London, B. L. Curfman, R. L. Brown, and A. L. Notkins. 1986. Coxsackie virus B4 produces transient diabetes in nonhuman primates. *Diabetes* 35(6):712–716.
378. Yusibov, V., V. Mett, V. Mett, C. Davidson, K. Musiychuk, S. Gilliam, A. Farese, T. Macvittie, and D. Mann. 2005. Peptide-based candidate vaccine against respiratory syncytial virus. *Vaccine* 23(7–18):2261–2265.
379. Zamiatina, N. A., A. G. Andzhaparidze, M. S. Balaian, A. V. Sobol', and I. P. Titova. 1990. [Susceptibility of Macaca rhesus to infection with hepatitis A virus strains isolated from man and monkeys]. *Vopr. Virusol.* 35(1):30–33.
380. Zaragoza, C., R. M. Li, G. A. Fahle, S. H. Fischer, M. Raffeld, A. M. Lewis Jr., and J. B. Kopp. 2005. Squirrel monkeys support replication of BK virus more efficiently than simian virus 40: an animal model for human BK virus infection. *J. Virol.* 79(2):1320–1326.
381. Zaucha, G. M., P. B. Jahrling, T. W. Geisbert, J. R. Swearengen, and L. Hensley. 2001. The pathology of experimental aerosolized monkeypox virus infection in cynomolgus monkeys (*Macaca fascicularis*). *Lab. Invest.* 81(12):1581–1600.
382. Zhang, J., S. X. Ge, G. Y. Huang, S. W. Li, Z. Q. He, Y. B. Wang, Y. J. Zheng, Y. Gu, M. H. Ng, and N. S. Xia. 2003. Evaluation of antibody-based and nucleic acid-based assays for diagnosis of hepatitis E virus infection in a rhesus monkey model. *J. Med. Virol.* 71(4):518–526.
383. Zhang, M., S. U. Emerson, H. Nguyen, R. Engle, S. Govindarajan, W. C. Blackwelder, J. Gerin, and R. H. Purcell. 2002. Recombinant vaccine against hepatitis E: duration of protective immunity in rhesus macaques. *Vaccine* 20(27–28):3285–3291.
384. Zhang, P., M. Y. Yu, R. Venable, H. J. Alter, and J. W. Shih. 2006. Neutralization epitope responsible for the hepatitis B virus subtype-specific protection in chimpanzees. *Proc. Natl. Acad. Sci. U. S. A.* 103(24):9214–9219.
385. Zhou, J., W. Wang, Q. Zhong, W. Hou, Z. Yang, S. Y. Xiao, R. Zhu, Z. Tang, Y. Wang, Q. Xian, H. Tang, and L. Wen. 2005. Immunogenicity, safety, and protective efficacy of an inactivated SARS-associated coronavirus vaccine in rhesus monkeys. *Vaccine* 23(24):3202–3209.
386. Zhou, Y. H., R. H. Purcell, and S. U. Emerson. 2005. A truncated ORF2 protein contains the most immunogenic site on ORF2: antibody responses to non-vaccine sequences following challenge of vaccinated and non-vaccinated macaques with hepatitis E virus. *Vaccine* 23(24):3157–3165.
387. Zhu, Y., P. Rota, L. Wyatt, A. Tamin, S. Rozenblatt, N. Lerche, B. Moss, W. Bellini, and M. McChesney. 2000. Evaluation of recombinant vaccinia virus-measles vaccines in infant rhesus macaques with

- preeexisting measles antibody. *Virology* 276(1):202–213.
388. Zhu, Y. D., J. Heath, J. Collins, T. Greene, L. Antipa, P. Rota, W. Bellini, and M. McChesney. 1997. Experimental measles. II. Infection and immunity in the rhesus macaque. *Virology* 233(1):85–92.
389. Zlontnik, I., D. P. Grant, and G. B. Carter. 1976. Experimental infection of monkeys with viruses of the tick-borne encephalitis complex: degenerative cerebellar lesions following inapparent forms of the disease or recovery from clinical encephalitis. *Br. J. Exp. Pathol.* 57(2):200–210.

24

Natural Infection of Nonhuman Primates with Nonsimian Viruses

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24.1. INTRODUCTION

In their natural habitats monkeys and apes have contact with thousands of nonprimate animal species and they are exposed to the multiple viruses harbored by the “neighbors.” Most such exposures have no pathological consequences. Simian species have a well-developed armamentarium for neutralizing viruses that are present in their natural range for thousands or even millions of years. However, extensive urbanization during the last 50–60 years changed, sometimes dramatically, the traditional habitats of simian species and brought many of them to the verge of extinction. Exposure of wild nonhuman primates (NHPs) to nonsimian viruses that are new to them represents a potential threat to their survival. The

largest documented outbreaks of fatal viral diseases in wild NHPs that were likely the result of habitat changes are covered in Section 24.2.

The potential for exposure to viruses and other microbes that are “foreign” to NHPs greatly increases in captivity. The major contributing factors are crowding conditions that are not uncommon in the primate facilities as well as contacts with the species which are impossible or extremely rare in nature. Obviously, the exposure of NHPs to human pathogens, including viruses, greatly increases in captivity. To some extent this is also true for the free-living habituated NHPs, primarily apes. “Natural,” or more accurately, nonexperimental transmissions of nonsimian animal and human and viruses to captive NHPs are concisely covered in Sections 24.3 and 24.4.

24.2. OUTBREAKS CAUSED BY NONPRIMATE VIRUSES IN WILD NHPs

24.2.1. Ebola Disease

The natural reservoir of Ebola virus (EBOV) is unknown. The prime suspects are fructiferous bats.²⁵ Monkeys and apes contract EBOV from this natural host(s). NHP outbreaks of Ebola hemorrhagic fever (EHF) likely happen much more frequently than human epidemics.²⁷ African great apes serve as an interim amplifying host for EBOV. Humans contract the virus from diseased or dead gorillas and chimpanzees.^{26,46} EBOV isolates specific for human epidemiological chains (identified by the unique combination of SNPs) have been traced back to EBOV-positive ape carcass with which an index case had contact.²⁶ Also, the outbreaks of EHF in apes were

accompanied by the similar outbreaks in the duiker antelopes (*Cephalophus* spp.), an animal that scavenges on ape carcasses.²⁶

Wild African great apes, particularly gorillas, are highly susceptible to EBOV.^{1,2,26,50} The outbreaks typically occur at the beginning of the dry season.²⁶ The mortality rate for EHF caused by EBOV-Z in the gorillas in the Lossi Sanctuary, Republic of Congo, was 90–95%.¹ The estimate of the EHF fatality rate in the common chimpanzees is also close to 90%.^{1,26} Outbreaks of EHF caused a dramatic decline in the wild population of gorillas and chimpanzees. It has been estimated that the outbreaks of EHF among eastern gorillas (*Gorilla gorilla beringei*) in the Lossi Sanctuary killed about half of the animals of this species.¹ The mortality due to EHF in western gorilla (*Gorilla gorilla gorilla*) from Loloué clearing Odzala-Kokoua National Park, Republic of Congo, was 97 and 77% for males from the groups and the solitary males, respectively.²

The vaccination of wild apes against EHF theoretically is the only way to stop the devastating effects of EBOV on the gorilla and chimpanzee wild populations. Delivering the vaccine presents a challenge. The existing vaccines can be delivered by darts, but high vaccination coverage cannot be achieved by this method and is hardly realistic. Oral vaccines delivered by baiting are a much better option, but development of such vaccines and proper bait is still in the early stages of development.

Outbreaks of EHF or MHF have not been reported in wild monkeys. The reason for that, particularly in the light of the susceptibility of African green monkeys and baboons to the experimental infection with EBOV and MARV remains unknown.

24.2.2. Kyasanur Forest Disease

Kyasanur forest disease virus (KFDV) was first isolated from wild monkeys affected with fatal hemorrhagic disease.^{11,12} The disease was named after the locale in Karnataka, India, where the epizootic was first recognized in 1957. The epizootics of Kyasanur forest disease affected wild langurs (*Presbytis entellus*) and bonnet macaques (*Macaca radiata*). During 1964–1973, more than 1,000 deaths of monkeys of these species was recorded.⁴⁹ The reason why Kyasanur forest disease emerged in the wild monkey populations in the 1950s is not clear. It has been suggested that the main factor was the increased exposure of monkeys to ticks

(*Haemaphysalis* spp.) infected with KFDV at the forest floor. A behavioral change occurred due to urbanization of forests in the natural habitat of these monkey species. The shrinking forest caused these species to descend to the forest floor where they became infected by tick vectors.¹¹ No large outbreaks of Kyasanur forest disease in wild monkeys in India have been reported since the mid-1970s.

24.3. OUTBREAKS CAUSED BY NONPRIMATE VIRUSES IN CAPTIVE NHPs

24.3.1. Enteroviral Encephalomyocarditis

Encephalomyocarditis virus (EMCV) belongs to the *Cardiovirus* genus, one of the genera within the *Picornaviridae* family. Natural hosts of this virus are mice and rats. The EMCV easily crosses species barriers and infects animals belonging to many vertebrate species, including NHPs as well as humans. A number of outbreaks of EMCV-caused fatal disease in captive NHPs, mostly baboons, are described.^{5,16,22,43} Fatal cases of EMCV infection in a free-ranging habituated troop of hamadryas baboons have also been reported.⁵ The disease caused by the EMCV in NHPs is characterized by a rapid course; the most common presentation is sudden death. The most significant pathological finding is nonsuppurative necrotizing myocarditis. In the experimental setup, the disease in baboons can be prevented by the administration of interferon within 24 h of inoculation with a lethal dose of EMCV.²⁸ However, this prophylactic measure is not practical. Several vaccines against EMCV were tested in NHPs.^{6,17,41} One of the vaccines, genetically engineered Mengo virus with deletions in the 5'-noncoding poly-C tracts, was shown to be protective in baboons and macaques challenged with lethal dose of EMCV.⁴¹ Although potentially useful, the vaccination against EMCV is not used in practice. The main and way to prevent EMCV-caused outbreaks in primate facilities is effective rodent control.

24.3.2. Cowpox

An outbreak of poxvirus disease in common marmosets (*Callithrix jacchus*) was reported in 1982.¹⁰ Possibly, this outbreak was caused by cowpox virus, but the causal agent was not precisely identified.

Two other outbreaks caused by cowpox virus have been recently reported. The first outbreak occurred in a sanctuary for exotic animals where monkeys of different species were housed together. Serological evidence of

infection with cowpox virus was found in 9 of 16 barbary macaques (*M. sylvanus*), 2 of 2 pig-tailed macaques (*M. nemestrina*), 3 of 6 cynomolgus macaques (*M. fascicularis*), and 1 of 4 rhesus monkeys. In 3 barbary macaques the infection was clinically manifested as multiple oral lesions. Apparently, cowpox virus was transmitted to the monkeys from wild brown rats (*Rattus norvegicus*).³²

The second outbreak, presumably caused by the cowpox virus, occurred in a colony of New World monkeys (marmosets and tamarins of different species).³³ The disease at the early stages was manifested as erosive-ulcerative oral lesions; at the advanced stages multiple hemorrhagic skin lesions were present predominantly on the face, scrotal region, soles and palms. The fatality rate was about 35%. The virus was provisionally identified as a cowpox virus. The source of this outbreak is unknown.

24.3.3. Ebola Disease

In 1989, an outbreak of fatal hemorrhagic fever has occurred during shipment and quarantine of cynomolgus macaque imported from the Philippines to a primate facility in Reston, Virginia, USA. The "Reston virus" isolated from these macaques was identified as a variant of EBOV and was designated EBOV-R.^{4,8,9,13,18,23} Fortunately, EBOV-R appears to be nonpathogenic to the humans.^{13,38}

Four EBOV-R strains are currently known, namely, Reston, Texas, Sienna, and Philippines.⁴⁵ The strains were named according to the locales where the outbreaks occurred in macaques. All groups of these macaques have been traced to a single primate facility in the Philippines. EBOV-R infection is not endemic in the cynomolgus macaques³⁸ and it remains a mystery how monkeys kept and bred in this facility contracted the virus. No outbreaks caused by EBOV-R or any other EBOV in captive NHPs have been reported after 1996.

24.3.4. Fatal Paramyxovirus Infection

An outbreak of acute severe respiratory illness in marmosets (*Callithrix jacchus*) occurred in 1999 in a marmoset colony housed in the animal facilities in Tianjin, China. The morbidity and mortality were 100 and 33%, respectively. The disease was caused by a paramyxovirus (Tianjin strain) closely related to Sendai virus, but distinguishable from all previously known genotypes.²⁹ The disease has been reproduced in marmosets by the inoculation of the isolated virus. Experimental mice and

rats housed in the same facility were not affected. Mice were also resistant to experimental infection with the Tianjin strain. The source of the outbreak is unknown.

24.4. TRANSMISSION OF HUMAN VIRUSES TO CAPTIVE AND HABITUATED NHPs

24.4.1. Measles Virus

Measles virus is readily transmissible from humans to captive NHPs. Outbreaks of the measles with significant mortality has been reported in macaques (*M. mulatta*, *M. fascicularis*, *M. fuscata*)^{3,30,35,44,52} and silvered leaf-monkeys (*Presbytis cristatus*).³⁹ Surprisingly, measles in apes has not been reported. However, potentially, the danger of contracting measles exists for captive monkeys of any species. Naturally acquired (nonexperimental) measles in NHPs is very similar to the human disease and the experimental measles. Human-to-simian transmission of measles virus to NHPs may result in a subclinical infection.^{19,30} However, the frequency of such outcomes and the duration of virus shedding in subclinically infected monkeys are unknown. Fortunately, human measles is a rarity in the developed countries now and measles outbreaks in primate facilities are also rare. Nevertheless, vigilance regarding possibility of measles outbreak has to be maintained. If such outbreak occurs, it should be controlled by quarantine measures assuming that the virus is highly contagious and the vaccination of contacts with live measles vaccine. Measles virus seronegative animal caretakers must also be vaccinated.

24.4.2. Varicella-Zoster Virus

Varicella-like disease has been described in several great ape species (common chimpanzee, gorilla, and orangutan) in the 1960s–1970s.^{15,36,51} It is likely that these cases were caused by the varicella-zoster virus (VZV), but this was not proven. Two proven cases of varicella in a captive gorilla (*Gorilla gorilla*) caused by VZV have been reported.^{31,40} Natural transmissions of VZV to monkeys have not been described.

24.4.3. Herpes Simplex Viruses

HSV-1 and HSV-2 infections in captive gibbons,^{7,42,48} chimpanzees,³⁷ and gorillas¹⁴ resulting in fatal disease were described in the late 1960s–early 1980s. However, data reported in these early publications are not sufficient to unequivocally conclude that in these cases the viruses were transmitted from humans to apes.

The more recently reported human-to-simian transmission of HSV-1 to captive marmosets (*Callitrix jacchus*),³⁴ white-faced saki monkey (*Pithecia pithecia*),⁴⁷ white-handed gibbon (*Hylobates lar*),²⁴ and orangutan (*Pongo pygmaeus pygmaeus*)²¹ are conclusively confirmed by sequencing viral fragments amplified from affected organs by polymerase chain reaction. In the case of the white-handed gibbon, HSV-1 caused meningoencephalitis; the animal was seropositive for at least several years. Thus, the disease apparently developed due to reactivation of a latent HSV-1 infection.²⁴ In the case of the juvenile orangutan, HSV-1 caused a generalized disease; skin and internal organs, particularly liver, were affected. It is not clear whether the disease was due to primarily HSV-1 infection or reactivation of the virus.²¹ The outbreak of vesiculoulcerative stomatitis in a family group of marmosets was likely due to primary HSV-1 infection.³⁴ The outbreak of fatal HSV-1 disease in another New World monkey species, white-faced saki monkeys (*Pithecia pithecia*) kept in the Center for Reproduction of Endangered Species (San Diego, USA), was also the result of primary infection with HSV-1.⁴⁷

24.4.4. Human Metapneumovirus

Outbreaks of a respiratory illness with high fatality rate occurred in the habituated group of wild common chimpanzees at the Mahale Mountains National Park, Tanzania in 2003, 2005, and 2006.²⁰ Measles and influenza viruses were excluded as causes of the illness. The prime suspect currently is human metapneumovirus but this is yet to be proved.

REFERENCES

- Bermejo, M., J. D. Rodriguez-Treijeiro, G. Illera, A. Barroso, C. Vila, and P. D. Walsh. 2006. Ebola outbreak killed 5000 gorillas. *Science* 314(5805):1564.
- Caillaud, D., F. Levrero, R. Cristescu, S. Gatti, M. Dewas, M. Douadi, A. Gautier-Hion, M. Raymond, and N. Menard. 2006. Gorilla susceptibility to Ebola virus: the cost of sociality. *Curr. Biol.* 16(13):R489–R491.
- Choi, Y. K., M. A. Simon, D. Y. Kim, B. I. Yoon, S. W. Kwon, K. W. Lee, I. B. Seo, and D. Y. Kim. 1999. Fatal measles virus infection in Japanese macaques (*Macaca fuscata*). *Vet. Pathol.* 36(6):594–600.
- Dalgard, D. W., R. J. Hardy, S. L. Pearson, G. J. Pucak, R. V. Quander, P. M. Zack, C. J. Peters, and P. B. Jahrling. 1992. Combined simian hemorrhagic fever and Ebola virus infection in cynomolgus monkeys. *Lab. Anim. Sci.* 42(2):152–157.
- Dzhikidze, E. K., R. I. Krylova, E. I. Balaeva, and V. G. Chalian. 1982. [Encephalomyocarditis in hamadryas baboons]. *Vopr. Virusol.* 27(4):418–422.
- Emerson, C. L. and J. L. Wagner. 1996. Antibody responses to two encephalomyocarditis virus vaccines in rhesus macaques (*Macaca mulatta*). *J. Med. Primatol.* 25(1):42–45.
- Emmons, R. W. and E. H. Lennette. 1970. Natural herpesvirus hominis infection of a gibbon (*Hylobates lar*). *Arch. Gesamte Virusforsch.* 31(3):215–218.
- Geisbert, T. W. and P. B. Jahrling. 1990. Use of immunoelectron microscopy to show Ebola virus during the 1989 United States epizootic. *J. Clin. Pathol.* 43(10):813–816.
- Geisbert, T. W., P. B. Jahrling, M. A. Hanes, and P. M. Zack. 1992. Association of Ebola-related Reston virus particles and antigen with tissue lesions of monkeys imported to the United States. *J. Comp. Pathol.* 106(2):137–152.
- Gough, A. W., N. J. Barsoum, S. I. Gracon, L. Mitchell, and J. M. Sturgess. 1982. Poxvirus infection in a colony of common marmosets (*Callithrix jacchus*). *Lab. Anim. Sci.* 32(1):87–90.
- Gould, E. A. and T. Solomon. 2008. Pathogenic flaviviruses. *Lancet* 371(9611):500–509.
- Goverdhan, M. K., P. K. Rajagopalan, D. P. Narasimha Murthy, S. Upadhyaya, M. Boshell, H. Trapido, and T. Ramachandra Rao. 1974. Epizootiology of Kyasanur forest disease in wild monkeys of Shimoga district, Mysore State (1957–1964). *Indian J. Med. Res.* 62(4):497–510.
- Hayes, C. G., J. P. Burans, T. G. Ksiazek, R. A. Del Rosario, M. E. Miranda, C. R. Manaloto, A. B. Barrientos, C. G. Robles, M. M. Dayrit, and C. J. Peters. 1992. Outbreak of fatal illness among captive macaques in the Philippines caused by an Ebola-related filovirus. *Am. J. Trop. Med. Hyg.* 46(6):664–671.
- Heldstab, A., D. Ruedi, W. Sonnabend, and F. Deinhardt. 1981. Spontaneous generalized Herpesvirus hominis infection of a lowland gorilla (*Gorilla gorilla gorilla*). *J. Med. Primatol.* 10(2–3):129–135.
- Heuschele, W. P. 1960. Varicella (chicken pox) in three young anthropoid apes. *J. Am. Vet. Med. Assoc.* 136:256–257.
- Hubbard, G. B., K. F. Soike, T. M. Butler, K. D. Carey, H. Davis, W. I. Butcher, and C. J. Gauntt. 1992. An encephalomyocarditis virus epizootic in a baboon colony. *Lab. Anim. Sci.* 42(3):233–239.
- Huneke, R. B., M. G. Michaels, C. L. Kaufman, and S. T. Ildstad. 1998. Antibody response in baboons (*Papio cynocephalus anubis*) to a commercially

- available encephalomyocarditis virus vaccine. *Lab. Anim. Sci.* 48(5):526–528.
- 18. Jahrling, P. B., T. W. Geisbert, D. W. Dalgard, E. D. Johnson, T. G. Ksiazek, W. C. Hall, and C. J. Peters. 1990. Preliminary report: isolation of Ebola virus from monkeys imported to USA. *Lancet* 335(8688):502–505.
 - 19. Jones-Engel, L., G. A. Engel, M. A. Schillaci, B. Lee, J. Heidrich, M. Chalise, and R. C. Kyes. 2006. Considering human-primate transmission of measles virus through the prism of risk analysis. *Am. J. Primatol.* 68(9):868–879.
 - 20. Kaur, T., J. Singh, S. Tong, C. Humphrey, D. Clevenger, W. Tan, B. Szekely, Y. Wang, Y. Li, Muse E. Alex, M. Kiyono, S. Hanamura, E. Inoue, M. Nakamura, M. A. Huffman, B. Jiang, and T. Nishida. 2008. Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees (*Pan troglodytes*) at Mahale Mountains National Park, Western Tanzania. *Am. J. Primatol.* 70(8):755–765.
 - 21. Kik, M. J., J. H. Bos, J. Groen, and G. M. Dorresteijn. 2005. Herpes simplex infection in a juvenile orangutan (*Pongo pygmaeus pygmaeus*). *J. Zoo Wildl. Med.* 36(1):131–134.
 - 22. Krylova, R. I. and E. K. Dzhikidze. 2005. Encephalomyocarditis in monkeys. *Bull. Exp. Biol. Med.* 139(3):355–359.
 - 23. Ksiazek, T. G., P. E. Rollin, P. B. Jahrling, E. Johnson, D. W. Dalgard, and C. J. Peters. 1992. Enzyme immunoassay for Ebola virus antigens in tissues of infected primates. *J. Clin. Microbiol.* 30(4):947–950.
 - 24. Landolfi, J. A., J. F. Wellehan, A. J. Johnson, and M. J. Kinsel. 2005. Fatal human herpesvirus type 1 infection in a white-handed gibbon (*Hyalobates lar*). *J. Vet. Diagn. Invest.* 17(4):369–371.
 - 25. Leroy, E. M., B. Kumulungui, X. Pourrut, P. Rouquet, A. Hassanin, P. Yaba, A. Delicat, J. T. Paweska, J. P. Gonzalez, and R. Swanepoel. 2005. Fruit bats as reservoirs of Ebola virus. *Nature* 438(7068):575–576.
 - 26. Leroy, E. M., P. Rouquet, P. Formenty, S. Souquiere, A. Kilbourne, J. M. Froment, M. Bermejo, S. Smit, W. Karesh, R. Swanepoel, S. R. Zaki, and P. E. Rollin. 2004. Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science* 303(5656):387–390.
 - 27. Leroy, E. M., P. Telfer, B. Kumulungui, P. Yaba, P. Rouquet, P. Roques, J. P. Gonzalez, T. G. Ksiazek, P. E. Rollin, and E. Nerrienet. 2004. A serological survey of Ebola virus infection in central African non-human primates. *J. Infect. Dis.* 190(11):1895–1899.
 - 28. Levin, R., A. Yaari, J. Gitelman, T. Bino, H. Rosenberg, and A. Kohn. 1986. EMC virus infection in baboons as a model for studies on antiviral substances. *Antiviral Res.* 6(5):277–283.
 - 29. Li, M., L. Y. Shi, L. J. Yuan, X. M. Li, Q. Wang, and W. X. Wang. 2008. [Complete genome sequence analysis of a newly isolated paramyxovirus Tianjin strain]. *Bing. Du Xue. Bao.* 24(1):1–6.
 - 30. MacArthur, J. A., P. G. Mann, V. Oreffo, and G. B. Scott. 1979. Measles in monkeys: an epidemiological study. *J. Hyg. (Lond.)* 83(2):207–212.
 - 31. Marenikova, S. S., N. N Maltseva, E. M. Shekukhina, L. S. Shenkman, and V. I. Korneeva. 1973. A generalized herpetic infection simulating smallpox in a gorilla. *Intervirology* 2:280–286.
 - 32. Martina, B. E., G. van Doornum, G. M. Dorresteijn, H. G. Niesters, K. J. Stittelaar, M. A. Wolters, H. G. van Bolhuis, and A. D. Osterhaus. 2006. Cowpox virus transmission from rats to monkeys, the Netherlands. *Emerg. Infect. Dis.* 12(6):1005–1007.
 - 33. Matz-Rensing, K., H. Ellerbrok, B. Ehlers, G. Pauli, A. Floto, M. Alex, C. P. Czerny, and F. J. Kaup. 2006. Fatal poxvirus outbreak in a colony of New World monkeys. *Vet. Pathol.* 43(2):212–218.
 - 34. Matz-Rensing, K., K. D. Jentsch, S. Rensing, S. Langhuyzen, E. Verschoor, H. Niphuis, and F. J. Kaup. 2003. Fatal Herpes simplex infection in a group of common marmosets (*Callithrix jacchus*). *Vet. Pathol.* 40(4):405–411.
 - 35. McChesney, M. B., R. S. Fujinami, N. W. Lerche, P. A. Marx, and M. B. Oldstone. 1989. Virus-induced immunosuppression: infection of peripheral blood mononuclear cells and suppression of immunoglobulin synthesis during natural measles virus infection of rhesus monkeys. *J. Infect. Dis.* 159(4):757–760.
 - 36. McClure, H. M. and M. E. Keeling. 1971. Viral diseases noted in the Yerkes Primate Center colony. *Lab. Anim. Sci.* 21(6):1002–1010.
 - 37. McClure, H. M., R. B. Swenson, S. S. Kalter, and T. L. Lester. 1980. Natural genital herpesvirus hominis infection in chimpanzees (*Pan troglodytes* and *Pan paniscus*). *Lab. Anim. Sci.* 30(5):895–901.
 - 38. Miranda, M. E., M. E. White, M. M. Dayrit, C. G. Hayes, T. G. Ksiazek, and J. P. Burans. 1991. Seroepidemiological study of filovirus related to Ebola in the Philippines. *Lancet* 337(8738):425–426.
 - 39. Montrey, R. D., D. L. Huxsoll, P. K. Hildebrandt, B. W. Booth, and S. Arimbalam. 1980. An epizootic of measles in captive silvered leaf-monkeys (*Presbytis cristatus*) in Malaysia. *Lab. Anim. Sci.* 30(4 Pt 1):694–697.
 - 40. Myers, M. G., L. W. Kramer, and L. R. Stanberry. 1987. Varicella in a gorilla. *J. Med. Virol.* 23(4):317–322.

41. Osorio, J. E., G. B. Hubbard, K. F. Soike, M. Girard, S. van der Werf, J. C. Moulin, and A. C. Palmenberg. 1996. Protection of non-murine mammals against encephalomyocarditis virus using a genetically engineered Mengo virus. *Vaccine* 14(2):155–161.
42. Ramsay, E., E. L. Stair, A. E. Castro, and M. I. Marks. 1982. Fatal Herpesvirus hominis encephalitis in a white-handed gibbon. *J. Am. Vet. Med. Assoc.* 181(11):1429–1430.
43. Reddacliff, L. A., P. D. Kirkland, W. J. Hartley, and R. L. Reece. 1997. Encephalomyocarditis virus infections in an Australian zoo. *J. Zoo Wildl. Med.* 28(2):153–157.
44. Remfry, J. 1976. A measles epizootic with 5 deaths in newly-imported rhesus monkeys (*Macaca mulatta*). *Lab. Anim.* 10(1):49–57.
45. Rollin, P. E., R. J. Williams, D. S. Bressler, S. Pearson, M. Cottingham, G. Pucak, A. Sanchez, S. G. Trappier, R. L. Peters, P. W. Greer, S. Zaki, T. Demarcus, K. Hendricks, M. Kelley, D. Simpson, T. W. Geisbert, P. B. Jahrling, C. J. Peters, and T. G. Ksiazek. 1999. Ebola (subtype Reston) virus among quarantined non-human primates recently imported from the Philippines to the United States. *J. Infect. Dis.* 179(Suppl 1):S108–S114.
46. Rouquet, P., J. M. Froment, M. Bermejo, P. Yaba, A. Delicat, P. E. Rollin, and E. M. Leroy. 2005. Wild animal mortality monitoring and human Ebola outbreaks, Gabon and Republic of Congo, 2001–2003. *Emerg. Infect. Dis.* 11(2):283–290.
47. Schrenzel, M. D., K. G. Osborn, A. Shima, R. B. Klieforth, and G. A. Maalouf. 2003. Naturally occurring fatal herpes simplex virus 1 infection in a family of white-faced saki monkeys (*Pithecia pithecia*). *J. Med. Primatol.* 32(1):7–14.
48. Smith, P. C., T. M. Yuill, R. D. Buchanan, J. S. Stanton, and V. Chaicumpa. 1969. The gibbon (*Hylobates lar*); a new primate host for Herpesvirus hominia. I. A natural epizootic in a laboratory colony. *J. Infect. Dis.* 120(3):292–297.
49. Sreenivasan, M. A., H. R. Bhat, and P. K. Rajagopalan. 1986. The epizootics of Kyasanur forest disease in wild monkeys during 1964 to 1973. *Trans. R. Soc. Trop. Med. Hyg.* 80(5):810–814.
50. Walsh, P. D., K. A. Abernethy, M. Bermejo, R. Beyers, P. De Wachter, M. E. Akou, B. Huijbregts, D. I. Mamboonga, A. K. Toham, A. M. Kilbourn, S. A. Lahm, S. Latour, F. Maisels, C. Mbina, Y. Mihindou, S. N. Obiang, E. N. Effa, M. P. Starkey, P. Telfer, M. Thibault, C. E. Tutin, L. J. White, and D. S. Wilkie. 2003. Catastrophic ape decline in western equatorial Africa. *Nature* 422(6932):611–614.
51. White, R. J., L. Simmons, and R. B. Wilson. 1972. Chickenpox in young anthropoid apes: clinical and laboratory findings. *J. Am. Vet. Med. Assoc.* 161(6):690–692.
52. Willy, M. E., R. A. Woodward, V. B. Thornton, A. V. Wolff, B. M. Flynn, J. L. Heath, Y. S. Villamarzo, S. Smith, W. J. Bellini, and P. A. Rota. 1999. Management of a measles outbreak among Old World nonhuman primates. *Lab. Anim. Sci.* 49(1):42–48.

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Figure 1.5. Vervet monkeys (*Chlorocebus pygerythrus*, possibly *C. p. callidus*), the Lake Nakuru region, Kenya. (Image is kindly provided by Dr. Jean P. Boubli.)

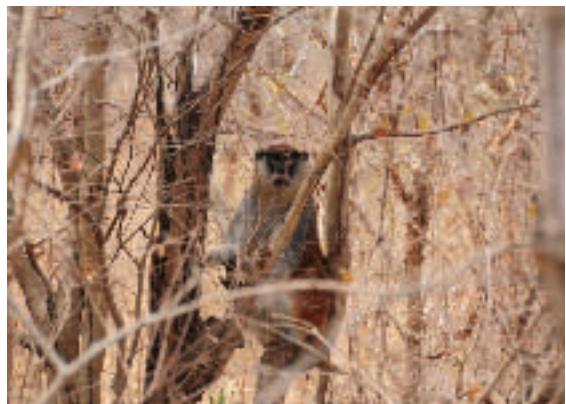


Figure 1.7. Male patas monkey (*Erythrocebus patas*), Nigeria. (Image is kindly provided by Dr. Janette Wallis.)



Figure 1.6. Tantalus monkey (*Chlorocebus tantalus*), Nigeria. (Image is kindly provided by Dr. Janette Wallis.)



Figure 1.8. Juvenile red-eared guenon (*Cercopithecus erythrotis*), Limbe Zoo, Limbe, Cameroon. (Photo by Preston Marx.)



Figure 1.9. Juvenile greater spot-nosed monkey (*Cercopithecus nictitans*), Medical Research Station, Kumba, Cameroon. (Photo by Preston Marx.)

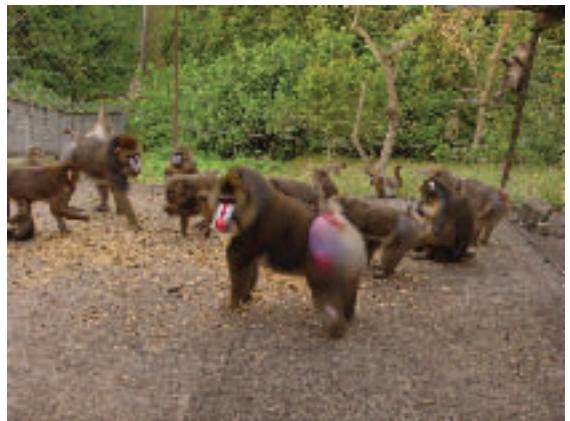


Figure 1.11. Adult male mandrill (*Mandrillus sphinx*) (center), International Center for Medical Research, Franceville, Gabon. (Photo by Preston Marx.)



Figure 1.10. Olive baboons (*Papio anubis*), Yankari Game Reserve, Nigeria. (Image is kindly provided by Dr. Janette Wallis.)



Figure 1.12. Adult male drill (*Mandrillus leucophaeus*), Limbe Zoo, Limbe, Cameroon. (Photo by Preston Marx.)



Figure 1.13. Adult sooty mangabey (*Cercocebus atys*), Tulane National Primate Research Center, USA. (Image is kindly provided by Mrs. Robin Rodrigues.)



Figure 1.15. Adult female and infant rhesus monkeys (*Macaca mulatta*), Swoyambhu Temple, Kathmandu, Nepal. (Image is kindly provided by Prof. Randall C. Kyes.)



Figure 1.14. Adult red-capped mangabey (*Cercocebus torquatus*), Yaoundé, Cameroon. (Photo by Preston Marx.)



Figure 1.16. Adult male cynomolgus macaque (*Macaca fascicularis*), Tinjil Island, Indonesia. (Image is kindly provided by Prof. Randall C. Kyes.)



Figure 1.17. Juvenile male Celebes black macaque (*Macaca nigra*), Tangkoko Nature Reserve, North Sulawesi, Indonesia. (Image is kindly provided by Prof. Randall C. Kyes.)



Figure 1.19. Adult female and infant Javan silvery gibbon (*Hylobates moloch*), Primate Research Center at Bogor Agricultural University, Bogor, Indonesia. (Image is kindly provided by Prof. Randall C. Kyes.)



Figure 1.18. Adult female Northern Plains gray langur (*Semnopithecus entellus*), Jodhpur, India. (Image is kindly provided by Prof. Randall C. Kyes.)

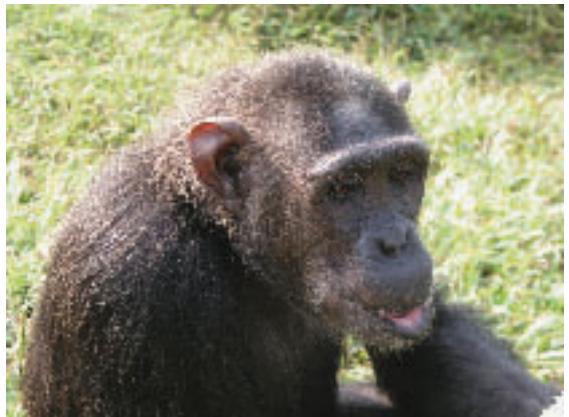


Figure 1.20. Adult common chimpanzee (*Pan troglodytes*), Bakumba, Gabon. (Photo by Preston Marx.)



Figure 1.21. Adult male lowland gorilla, International Center for Medical Research, Franceville, Gabon. (Photo by Preston Marx.)



Figure 1.23. Common marmosets (*Callithrix jacchus*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.)



Figure 1.22. Adult male Bornean orangutan (*Pongo pygmaeus*), Woodland Park Zoo, Seattle, Washington, USA. (Image is kindly provided by Prof. Randall C. Kyes.)



Figure 1.24. Golden-headed lion tamarin (*Leontopithecus chrysomelas*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.)



Figure 1.25. White-fronted capuchin (*Cebus albifrons*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.)



Figure 1.26. Common squirrel monkey (*Saimiri sciureus*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.)



Figure 1.27. Black-headed owl monkey, also called night monkey (*Aotus nigriiceps*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.)



Figure 1.28. Brown howler monkey (*Alouatta guariba*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.)



Figure 1.29. Black howler (*Alouatta pigra*), Balancan, Tabasco, Mexico. (Image is kindly provided by Dr. Juan Carlos Serio Silva.)



Figure 1.31. Gray woolly monkey (*Lagothrix cana*). (Image is kindly provided by Prof. Júlio César Bicca-Marques.)



Figure 1.30. Spider monkeys (*Ateles geoffroyi vellerosus*), Balancan, Tabasco, Mexico. (Image is kindly provided by Dr. Juan Carlos Serio Silva.)



Figure 1.32. Adult male Northern muriqui (*Brachyteles hypoxanthus*), Caratinga Biological Station, Minas Gerais, Brazil. (Image is kindly provided by Dr. Jean P. Boubli.)



Figure 1.33. Collared titi (*Callicebus torquatus*), Sustainable Development Reserve Amanã, Lake Amanã, Amazonas, Brazil. (Image is kindly provided by Marcela Alvares Oliviera.)



Figure 1.35. Neblina black-headed uakari (*Cacajao melanocephalus*), the Pico da Neblina National Park, Brazil; classified also as a separate species (*C. hosomi*). (Image is kindly provided by Dr. Jean P. Boubli.)



Figure 1.34. Rio Tapajos saki (*Pithecia irrorata*), Belo Horizonte Zoo, Minas Gerais, Brazil. (Image is kindly provided by Eduardo Franco.)

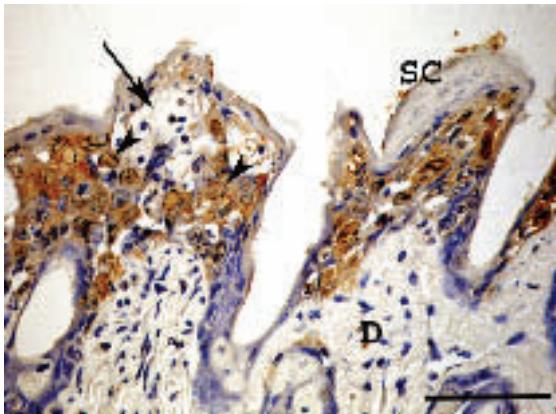


Figure 2.10. In situ detection of viral antigens by immunohistochemistry (IHC). Skin of a mouse inoculated with baboon simplexvirus (*Herpesvirus papio* 2). Epithelial cells beneath the stratum corneum (SC) are positive for viral antigens (red-brown staining). Positive cells exhibit degeneration, necrosis, and margination of chromatin (arrowheads). Virus-induced damage results in vesicle formation (arrow) and mild infiltrates of inflammatory cells in the dermis (D). Bar = 75 μ m. (Image is kindly provided by Dr. Jerry W. Ritchey.)

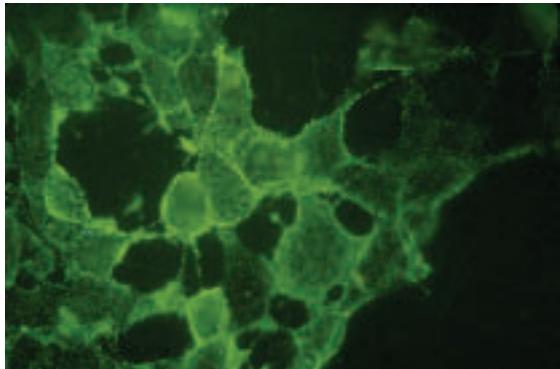


Figure 2.17. Indirect immunofluorescence assay. Acetone-fixed herpes simplex virus type 1 (HSV-1)-infected Vero cells were used as targets for detection of antibodies against the virus. Anti-HSV antibodies bound to the viral antigens are visualized by the secondary anti-IgG antibodies labeled with fluorescent dye (FITC—fluorescein isothiocyanate). Bright green fluorescence indicates positive reaction. (Image is kindly provided by Dr. Richard Eberle.)

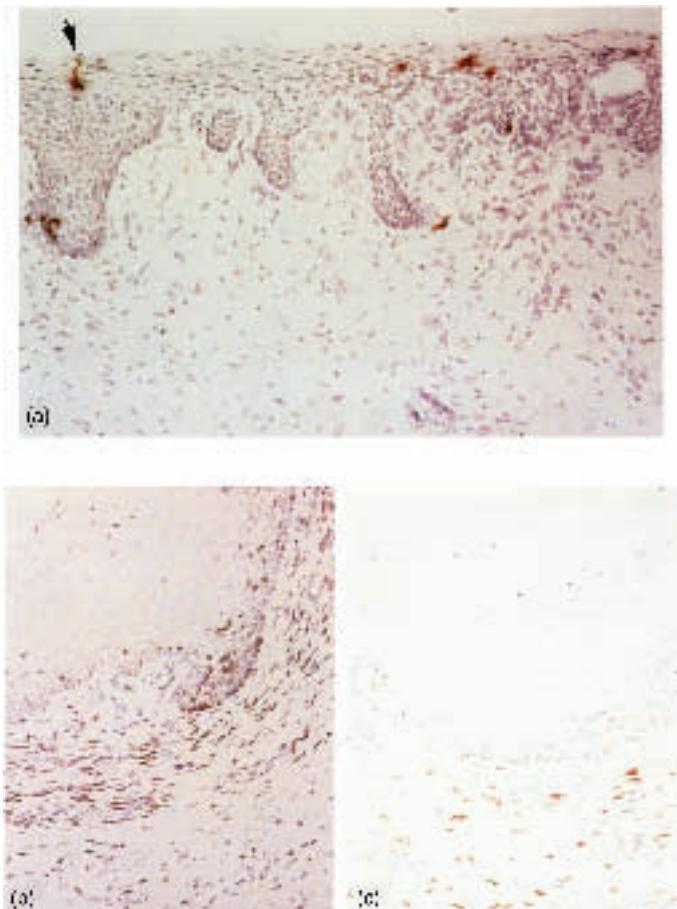


Figure 4.5. Immunohistochemistry staining of Langerhan cells, T cell, and macrophages in the vaginal mucosa. Simian immunodeficiency virus susceptible cells in the normal vaginal mucosa: (a) Langerhan cells (LCs); (b) T cells and C macrophages. Note in panel (a) (arrow), an LC at the vaginal surface with dendritic process sampling the lumen of the vagina. (Adapted from Miller *et al.*¹⁹⁶.)



Figure 5.3. A household pet sooty mangabey, *Cecocebus atys* in West Africa found to be naturally infected with SIVsm. The virus, SIVsmSL92b, is described in reference 2 and is closely related to HIV-2 group F. (Photo by Paul Telfer.)



Figure 5.4. Bush meat for sale at a road stand. Arm of a mandrill next to vegetables. Mandrills are naturally infected with one of two simian immunodeficiency viruses (SIVs), SIVmnd-1 and SIVmnd-2. (Photo by Preston A. Marx.)



Figure 6.5. Classic experiment in epidemiology to examine natural SRV-1 transmission in outdoor groups of rhesus monkeys at the California National Primate Research Center.⁴⁹ This outdoor enclosure was divided into three zones, left zone animals naturally affected with simian AIDS, center 10-foot open air barrier, and, right empty, no animals. Juvenile rhesus macaques (shown in figure) were introduced in the left and right zones. Nineteen of 23 rhesus juveniles in the left enclosure developed simian AIDS in 1 year or less. In contrast, all 21 juveniles rhesus in the right zone were not affected after 5 years of observation. This experiment excluded vectors (mosquitoes, rodents) and fomites, such as rainwater flowing between the cages, from a role in AIDS transmission. Physical contact was required. This experiment occurred before the cause of known of AIDS was known. Many theories were in circulation, including environmental factors. This experiment showed the cause was an infectious agent requiring close physical contact, a result that still stands. SRV-1 was isolated from an animal in the left enclosure, cloned and sequenced, and shown to be the etiologic agent of naturally occurring simian AIDS in Asian macaques.



Figure 13.2. Cutaneous manifestation of simian varicella. Rash on the torso of an African green monkey 10 days PI with SVV; maculopapular and vesicular eruptions are present simultaneously. (Image is kindly provided by Dr. Vicki L. Traina-Dorge.)

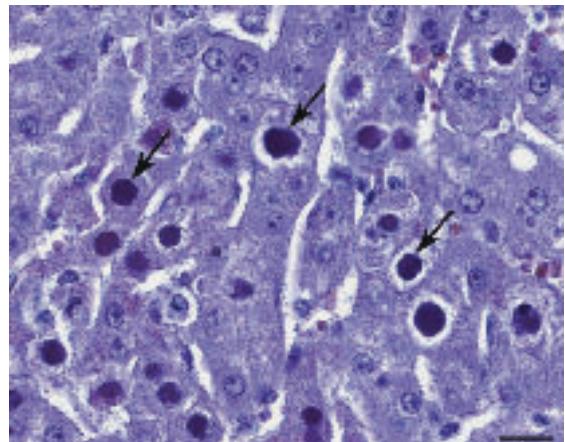


Figure 21.3. Adenoviral intranuclear inclusions. Homogeneous basophilic intranuclear inclusions (arrows) completely fill the enlarged nuclei. Scalebar = 2 μ m. (Adapted from Zöller *et al.*⁴⁴; with permission.)