atitis B; no immunization by vaccine is available. Especially in combination with ribavirin (Table 7.**5**), therapeutic use of interferon can lead to elimination of the virus in persistent infections and thus to prevention of cirrhosis of the liver and HCC.

Coronaviruses

■ Infections with coronaviruses are widespread in humans and animals. Human pathogens include causative agents of rhinitislike infections and the virus of the "severe acute respiratory syndrome" (SARS), which first erupted in China in 2002.

Diagnosis: serology or electron microscopy for common cold strains; PCR or isolation for SARS. ■

Pathogen. The *Coronaviridae* family includes several viral species that can infect vertebrates such as dogs, cats, cattle, pigs, rodents, and poultry. The name (corona, as in wreath or crown) refers to the appearance of the viruses (Fig. 8.**13**). One coronavirus species (human coronavirus, HuCV) is known since some time to be a human pathogen. It has at least two serotypes and probably a number of serological variants. In November 2002, a new coronavirus emerged in China and, after originally being mistaken as a new influenza recombinant, was identified as the causative agent of severe acute respiratory syndrome, or SARS, in spring 2003. Its origin, possibly from animals, is not known to date.

Coronavirus

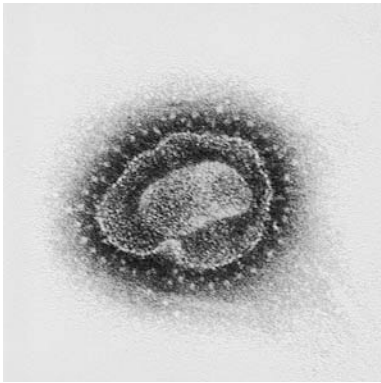

Fig. 8.**13** "Spikes" with club or drumstick-like swellings are located at regular, relatively generous intervals on the pleomorphic envelope, which measures 80–220 nm in diameter.

Coronavirus Replication and Viral Maturation

The coronavirus genome consists of the longest known, sense RNA strand exceeding 30 kb, which is integrated in the envelope in the form of a helical ribonucleoprotein. A hallmark of coronaviral RNA replication is the production of seven subgenomic mRNAs, each of which codes for one viral structural protein. The synthesis of progeny viral RNA takes place in association with specialized membrane structures, characterized as double-membrane vesicles. Viral maturation takes place in the rough endoplasmic reticulum after replacement of cellular proteins by viral proteins in the membranes. The viruses are then transported to the Golgi apparatus. The ensuing virus release mechanism is unknown. Recently, the receptor involved in the entry of the SARS virus into the cell was reported to be the angiotensin-converting enzyme 2 (ACE2).

Pathogenesis and clinical picture. *Common cold*-coronaviruses cause an everyday variety of respiratory infections, which are restricted to the ciliated epithelia of the nose and trachea. They are responsible for about 30% of common cold infections.

The immunity conferred by infection, apparently IgA-dependent, is short-lived. Reinfections are therefore frequent, whereby the antigenic variability of the virus may be a contributing factor. Various enteral coronaviruses with morphologies similar to the respiratory types have also been described in humans. Their pathogenicity, and hence their contribution to diarrhea, has not been clarified.

The *SARS virus* is transmitted aerogenically with an incubation time of two to 10 days. Clinically, fever and a marked shortness of breath is noted, developing into a severe atypical pneumonia with new pulmonary infiltrates on chest radiography. Shedding of virus is by respiratory discharges. Whether the virus present in other body fluids and excreta plays a decisive role for virus transmission is not yet clear.

Diagnosis.The *common-cold coronavirus* can be grown in organ cultures of human tracheal tissue or in human diploid cells. Isolating the viruses for diagnostic purposes is not routine. Serodiagnosis (complement-binding reaction, immunofluorescence or enzyme immunoassay) and electron microscopy are feasible methods.

The SARS virus can be identified by PCR or isolated in the Vero cell line.

Epidemiology and prevention. In November 2002, an outbreak of atypical pneumonia, later termed SARS, occurred in the southern Chinese city of Guangzhou (Guangdong Province). Only in February of 2003, the world was alerted about the lung disease, shortly before it escaped China, when a Guangdong resident in a Hong Kong hotel transmitted it to other guests who spread it to Toronto, Hanoi, Singapore, and elsewhere. Transmission of the virus is by droplets, but close contact ("household transmission")

with possibly other routes of transmission seems important. The only preventive measure to date is exposure prevention. Under therapy with ribavirin and intensive care, mortality of SARS is around 10%.

Retroviruses

■ Retroviruses possess an enzyme, reverse transcriptase, that can transcribe ssRNA into double-stranded DNA. This activity is reflected in the designation "retroviruses." Integration of the DNA thus derived from the viral genome in the host-cell genome is a precondition for viral replication. Certain retroviruses are also capable of **onco**genic cell transformation. Due to this potential and their **RNA** genome, these viruses are also called **oncorna**viruses (see Chapter 7).

Human pathogen retroviruses known to date include the types HTLV (human T-cell leukemia virus) I and II and HIV (human immunodeficiency virus) 1 and 2. The former are T-cell malignancy-causing pathogens, the latter cause acquired immune deficiency syndrome—AIDS.

AIDS manifests as a reduction of T helper cells after an average incubation time of 10 years. The collapse of cellular immunity results in occurrence of typical opportunistic infections (*Pneumocystis carinii* pneumonia, fungal and mycobacterial infections, CMV, and other viral infections) as well as lymphomas and Kaposi sarcoma. Transmission is by sexual intercourse, blood and blood products, as well as prenatal and perinatal infections.

8

Diagnosis: HIV infections are routinely detected by serology (antibodies or viral antigen). The circulating virus count (viral load) is determined by means of quantitative RT-PCR. The AIDS diagnosis is a clinical procedure that presupposes positive confirmation of HIV infection.

Therapy: inhibitors of reverse transcriptase and protease.

Prevention: exposure prophylaxis when contact with blood is involved (drug addicts, healthcare staff) and sexual intercourse. Postexposure prophylaxis and prophylaxis in pregnancy with chemotherapeutics. ■

Pathogen. The *Retroviridae* family is the classification group for all RNA viruses with reverse transcription of RNA to DNA in their reproductive cycles (RNA-dependent DNA synthesis) (p. 385). Only zoopathic retroviruses were known for many years. These viruses cause various kinds of tumors in animals. In 1980, retroviruses were also discovered in humans. This virus family includes seven genera, three of which play significant roles in human medicine:

- **HTLV-BLV retroviruses**, including HTL viruses types I and II and the bovine leukemia virus.
- **Spumaviruses**, which only occur in animals, two of which are (probably) from humans.
- **Lentiviruses**, with the human pathogens HIV 1 and 2, maedivirus (pneumonia), and visnavirus (encephalomyelitis) in sheep, viruses affecting goats and horses, and animal immune deficiency viruses.

A human pathogen retrovirus was isolated for the first time in 1980 from adults suffering from T-cell leukemias. It was designated as HTLV I (human T-cell leukemia virus). A short time later, a virus was isolated from hairy cell leukemia patients and named HTLV II.

HTLV I is found in adults with T-cell malignancy as well as in patients with neurological diseases (myelopathies). HTLV II appears to be associated with T-cell malignancy and other lymphoproliferative diseases. Its own etiological role is still under discussion.

Here is a summary of the human pathogenic aspects of HIV including its relation to acquired immune deficiency syndrome (AIDS).

The viral RNA genome, which is integrated in the genome of the host cell, contains the following genes and regulatory sequences (see. Fig. 8.**14**):

Genes essential to viral replication:

- ***tat*** gene: "transactive transcription," enhances the transcription and thus the expression of viral proteins by binding to the TAR (transactivation responsive region) in the LTR.
- ***rev*** gene: posttranscriptional activator for splicing and transport of viral mRNA (production of structural proteins).
- ***LTR*** sequence: promoter and enhancer elements.

Structural genes:

- ***gag*** gene: group-specific antigen.
- ***pol*** gene: codes for the reverse transcriptase, a protease and the integrase.
- ***env*** gene: envelope glycoprotein (gp).

Genes not essential to viral replication:

- **Virus infection factor (*vif*)**: makes the virus more infectious.
- **"Negative" factor (*nef*)**: inhibits or activates viral transcription as required, influences T-cell activation, reduces CD4 expression.
- ***vpr***: controls rate of replication.
- ***vpx***: only in HIV 2, controls rate of replication.
- ***vpu***: only in HIV 1, contributes to viral release, increases CD4 turnover.

Structure and Genomic Organization of HIV

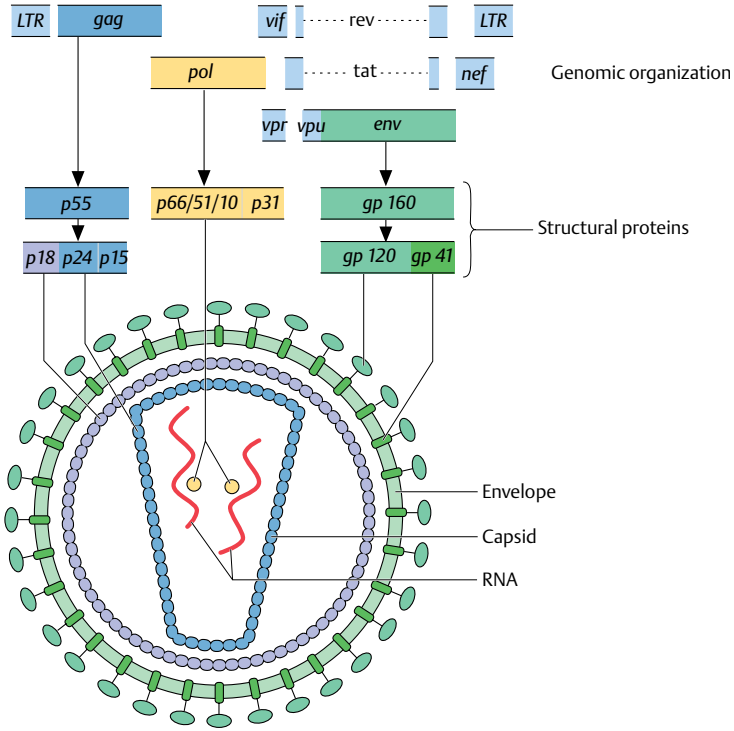

Fig. 8.**14**

Gene	Gene product	Function
Structural genes:		
gag	p55	p55 Nucleocapsid, precursor of p18, p24, p15
	p18	Matrix protein
	p24	Capsid protein
pol		Polymerase region
	p66/51/10	Reverse transcriptase/RNase/protease
	p31	Integrase
env	gp160	Glycoprotein, precursor of gp120, gp41
	gp120	Surface protein (binds to CD4 molecule of host cell)
	gp41	Transmembrane protein

Regulatory genes, see text.

Human Immune Deficiency Virus (HIV)

HIV replication

HIV can infect T4 lymphocytes and other cells bearing the CD4 marker on their surface. The CD4 molecule is the main receptor for HIV, or more precisely for its gp120 (Fig. 8.**14**). In addition, either the chemokine receptor CCR5 (macrophage-tropic R5 HIV strains) or CXCR4 (T cell-tropic X4 strains) is used as a coreceptor. Persons with (homozygotic) missing CCR5 are highly resistant to HIV infection. A number of other coreceptors are also active depending on the viral strain involved. HIV is then taken in by the cell. After uncoating, reverse transcription takes place in the cytoplasm. The rest of the viral replication process basically corresponds to the description of retroviral replication on p. 385. The interaction of the many different contributing control genes is responsible for the long latency period and subsequent viral replication (see also Fig. 8.**14**).

Replication of HIV takes the form of a lytic cycle, i.e., it results in destruction of the host cell, making it an exception among retroviruses. It must also be noted that the cell destruction mechanism has not been completely explained. Cell fusions are induced by X4 strains (syncytial formation). These processes occur late in the infection cycle and are associated with progression to AIDS. R5 strains do not induce syncytia, are present early in the course of the infection, and are mainly responsible for transmission of HIV. Besides virus-induced cell destruction (p. 392), apoptosis also appears to play an important role in the elimination of $CD4^+$ cells.

Pathogenesis and clinical picture. AIDS was described as a discrete pathology of the immune system in 1981. The pathogenicity of the disease is based on suppression of cellular immunity as a result of the loss of the $CD4^+$ T helper cells.

The primary infection either remains inapparent or manifests as "acute retroviral syndrome" with conjunctivitis, pharyngitis, exanthem, and lymphadenopathy, as well as a transitory meningoencephalitis in some cases. p24 antigen (Fig. 8.**14**) is detectable in serum after about 14 days, i.e., before the antibodies. This stage is followed by a long period of clinical latency (the incubation period is described as 10 years), during which the carrier is clinically normal but may be infectious. The HI virus can persist in a latent state in $CD4^+$ T lymphocytes, macrophages, and the Langerhans cells in the skin. Apparently, viral replication continues throughout this period, especially in lymphoid organs.

The drop in $CD4^+$ lymphocytes and the rise in the virus count (viral load, see below) in peripheral blood is followed by the lymphadenopathic stage. Opportunistic infections then set in, frequently combined with lymphomas, the otherwise rare Kaposi sarcoma, or so-called AIDS encephalopathy (subacute AIDS encephalitis, AIDS dementia complex). Similar neurological symptoms may also be induced because of HIV-induced immunosuppression,

Table 8.4 Diagnostic Definitions for AIDS in Adults

$CD4^+$ T cells/µl	Clinical categories		
	A	B	C
>500	A1	B1	**C1**
200–499	A2	B2	**C2**
<200	**A3**	**B3**	**C3**

A3, B3, and C1–3 confirm AIDS diagnosis

Clinical categories

A: Asymptomatic or acute (primary) HIV infection; persistent generalized lymphadenopathy (LAS)

B: Symptoms indicative of weakened cellular immune defenses, but no AIDS-defining diseases.

C: AIDS-defining diseases:

Viruses
HSV: chronic ulcer, esophagitis, bronchitis, pneumonia
VZV: generalized zoster
CMV: retinitis, encephalitis, pneumonia, colitis
JC virus: progressive multifocal leukoencephalopathy
HIV: encephalitis
HIV: wasting syndrome

Bacteria
Recurrent salmonellar septicemia
Recurrent pneumonia
Mycobacterial tuberculosis, pulmonary and extrapulmonary forms
Opportunistic mycobacteria (*M. avium*, etc.), disseminated or extrapulmonary

Protozoans
Cryptosporidium: chronic diarrhea
Isospora belli: chronic diarrhea
Toxoplasma gondii: encephalitis

Fungi
Candida: esophagitis, pneumonia, bronchitis
Histoplasma, *Cryptococcus neoformans*, coccidiosis: extrapulmonary, disseminated
Pneumocystis carinii: pneumonia

Malignomas
Kaposi sarcoma
Invasive cervical carcinoma
B-cell lymphoma EBV-positive

Toxoplasma or papovaviruses (PML, see p. 415), or lymphomas. Table 8.**4** presents the CDC (Centers for Disease Control) classification of the stages in the course of HIV infection. The number of $CD4^+$ T cells and the occurrence of so-called AIDS-defining diseases determine whether an HIV-positive patient is categorized as a case of AIDS (Table 8.**4**). The probability that an AIDS-defining disease will occur rises precipitously at $CD4^+$ cell counts below 200.

Laboratory diagnosis. The following diagnostic tools are currently available for confirmation of an HIV infection (not the same as manifest AIDS, see above):

- **HIV antibody detection.** EIA screening tests are now available using genetically engineered or synthesized viral antigens (first to third generation of screening tests). Every positive result requires confirmation by an alternative test (Western blot, see p. 123 and Fig. 2.**24**, p. 125, p24 antigen detection). The fourth-generation screening tests simultaneously detect antibodies to HIV 1 and 2 and p24 antigen (combination test) and are thus capable of detecting primary infections that are still antibody-negative.

- **HIV antigen detection.** In this test, a viral protein is detected in serum, usually capsid protein p24. The p24 antigen is detectable in serum as early as two weeks after infection and disappears again after eight to 12 weeks. Following a clinically stable latency period, HIV antigen can become detectable months or years later (transitory or persistent). This renewed appearance of HIV antigen is usually followed by manifest AIDS and is therefore a negative prognostic sign.

- **Rapid HIV test.** Antibody-based tests are available for rapid diagnosis in medical practices, hospitals, and health centers. Their specifications are equivalent to the third-generation screening tests.

- **PCR.** The most important application of the polymerase chain reaction (PCR, see p. 409) today is to determine the so-called viral load, whereby a commercially available quantitative RT-PCR (reverse transcriptase PCR) is used to determine the number of viral RNA molecules per ml of blood, taking into account the added standard amounts of HIV RNA (quantification standard). This test provides a prognostic estimate of how great the risk of progression to AIDS is (manifestation of an AIDS-defining disease). It can also be used to monitor the success of therapy with RT and protease inhibitors.

The following HIV diagnostic procedure is now recommended: an HIV antibody screening test should first be performed to diagnose an HIV infection. If the test result is positive, a second serum specimen should be tested to confirm the result and exclude confusion of sera. If the initial screening test is negative, but a (primary) HIV infection is justifiably suspected, HIV antigen can be tested, for instance using the combination test.

Epidemiology and prevention. HIV is transmitted by blood, blood products, and sexual intercourse. The virus can also be transmitted from mother to child in intrauterine infection, perinatal transmission, or the mother's milk. Infection via saliva or insect bite has not been confirmed. Accordingly, three rules of behavior are now propagated to prevent the spread of HIV: use a good-quality condom for each act of sexual intercourse. For i.v. drug consumption use only sterile syringes and needles; never share or pass on these injection utensils. Couples one of whom is HIV-positive should avoid an unplanned pregnancy.

Intensive efforts are being made to develop a vaccine (active immunization) and several vaccines will soon be ready for field trials. The types under consideration include split vaccines (p. 403), genome-free particles, attenuated viruses, naked DNA, and inactivated virions. It is not practicable to cover this field of research in detail here due to the fast-moving, and the necessarily tenuous, nature of the ongoing work.

Therapy. The recommended therapeutic procedure is also subject to rapid changes, whereby the common goal is to reduce the number of viruses as far as possible (<50 RNA copies per ml) and as soon as possible. Doing so can delay the occurrence of clinical symptoms, eliminate existing symptoms and slow or stop the development of resistance in the HI viruses.

In general, therapy is considered in reaction to the initial retroviral syndrome. Therapy is recommended in the first, asymptomatic stage at $CD4^+$ cell counts below 350, and if the count is higher than 350 only if the viral load is raised (consider therapy at 5000, therapy recommended at >30 000 RNA copies/ml). Pregnancy in an HIV-positive woman is a further therapeutic indication.

Three classes of substances are available for HIV therapy (see also p. 404f.):

- **Nucleosidic (or nucleotidic) reverse transcriptase inhibitors** (NRTI) (for example: azidothymidine, AZT; lamivudine, 3TC; didanosine, ddI, etc.). These are nucleoside analogs that bind to the active center of the enzyme are integrated in the DNA strands, resulting in "chain termination."
- **Nonnucleosidic reverse transcriptase inhibitors** (NNRTI) (for example: efavirenz, EFV; nevirapine, NVP, etc.). This class of substances also inhibits the production of viral cDNA by reverse transcriptase, but does not prevent viral production by infected cells.
- **Protease inhibitors** (PI) (for example: indinavir, IDV; ritonavir, RTV; saquinavir, SQV, etc.): PIs inhibit viral protease and thus viral maturation.

Combination Treatments of HIV Infections:

To avoid development of resistant HIV variants, a combination of at least three drugs from at least two substance classes is usually administered. The following combinations are currently established practice:

a) One PI and two NRTIs

b) One NNRTI and two NRTIs

c) Two PIs and one or two NRTIs

d) One PI and one NNRTI, alternatively with one or two NRTIs as well;

e) Three NRTIs

a) and b) appear to produce the best long-term results.

Standard vaccines can be used to prevent other infections, for example opportunistic infections in HIV-positive persons, especially children showing no symptoms. The dead vaccine type is recommended for polio. Live vaccine materials should generally not be used in persons showing AIDS symptoms.

Precautions for Healthcare Staff

All personnel in medical professions should know that HIV is not highly contagious and that precautions, as they apply to hepatitis B, are considered sufficient: wear protective gloves in all situations involving possible contact with blood. If blood droplets could be spattered or sprayed, masks and goggles should also be worn.

If exposure has occurred despite precautions (accidental injection, stab wound, contamination of a wound or mucosa with material containing HIV), immediate commencement of a combination therapy with one PI and two NRTIs for two to four weeks is indicated in addition to a thorough wound toilet and disinfection.

Viruses with Double-Stranded RNA Genomes

Reoviruses

Reoviruses possess a segmented, double-stranded RNA genome. Among the reoviruses, the rotaviruses are the most significant human pathogens. They cause diarrhea in small children and the elderly and can also produce severe sequelae in immunosuppressed patients.

Diagnosis: reovirus—isolation; rotavirus—antigen detection or electron microscopy. Isolation of this viral type in cell cultures is not a routine method.

Pathogen. The name ***reo****virus* is derived from the abbreviation for respiratory enteric orphan virus, recalling that no diseases were associated with the virus upon its discovery (hence "orphan virus"). The family *Reoviridae* includes, in addition to phytopathogenic and zoopathogenic strains, three genera in which human pathogens are classified:

- **Coltiviruses** include a large number of pathogens significant in veterinary medicine as well as the human pathogen virus that causes Colorado tick fever.
- **Reoviruses** in the narrower sense, with three serogroups.
- **Rotaviruses**, groups A to F, further subdivided into subgroups, serotypes, and electropherotypes (see below). The rotaviral genome consists of eleven segments of double-stranded RNA. Each segment codes for one viral protein. Some segments in other reoviruses code for two or three proteins.

Pathogenesis and clinical picture.

- **Coltiviruses.** Colorado tick fever usually runs a mild course with fever, myalgias, nausea, and vomiting, rarely encephalitis.
- **Reoviruses.** Implication of these viruses in diseases is still uncertain. It appears they are capable of infecting the respiratory and intestinal tracts of children. The fact that they are also found very frequently in asymptomatic persons makes it difficult to correlate them with specific clinical pictures.
- **Rotaviruses**. In the mid-seventies these viruses were recognized as diarrhea-causing viruses in infants and small children (Fig. 8.**15**). They are the most frequent cause of diarrhea in children aged six months to two years. It was recently discovered that they also play a role in infections of the elderly, and above all in immunosuppressed patients (e.g., bone marrow transplant patients), and can cause severe clinical pictures in these groups. Rotaviruses enter the body per os or by droplet infection, replicate in the villi of the small intestine and cause diarrhea, potentially resulting in exsiccosis.

Diagnosis. Colorado tick fever can be diagnosed serologically. Reovirus infections can be diagnosed by isolating the pathogens in cell cultures. Rotaviruses do not readily grow in cell cultures for diagnostic purposes. They can be detected more readily under an electron microscope or in antigen assays using commercially available solid phase tests (EIA) or passive agglutination. An elegant typing method for the different rotavirus strains involves analysis of the electrophoretic mobility of the 11 dsRNA strands of the viral genome.

Epidemiology. Humans are the sole natural reservoir of the infant pathogen rotaviruses. Generalized contamination is practically 100% when children reach school age, but carriers and reinfections are still possible despite immunity. Diarrheal infections are among the most important causes of death in

Rotaviruses

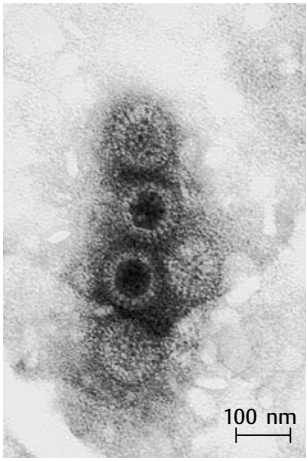

Fig. 8.**15** TEM image of rotaviruses in stool from an infant suffering from diarrhea. All of the viruses in the family Reoviridae possess a double icosahedral capsid. The outer capsid has a diameter of approximately 70 nm, the inner capsid approximately 40 nm. It contains the segmented RNA genome, comprising from 10 to 12 double-stranded subunits depending on the species.

small children in developing countries; 20% of these infections are due to rotaviruses. In the temperate zone, rotaviruses are implicated in fewer individual infections; here they more frequently cause winter outbreaks in hospitals and homes for small children. Rotaviruses remain viable for long periods on objects and skin (hands!) and are therefore spread rapidly by infected persons and healthy carriers. The most effective prophylactic approach is to practice stringent hygiene.

Viruses with Single-Stranded RNA Genomes, Antisense-Strand Orientation

Six viral families have an antisense RNA genome: the *Orthomyxoviridae*, the *Bunyaviridae*, the *Arenaviridae*, the *Paramyxoviridae*, the *Rhabdoviridae*, and the *Filoviridae*. Just like all other RNA viruses, they require a RNA-independent RNA polymerase, which enters the cell within the viral particle in the infective process.

Orthomyxoviruses

The representatives of this family are the different influenza A viruses. The A type is the most important of the three. It is the pathogen responsible for epidemics and pandemics, since its antigenicity structure changes within a narrower range due to point mutations (more frequent) and within a broader range due to recombination (less frequent). Type B tends to be endemic and type C is very rare. Influenza viruses are the classic flu pathogens, whereby the clinical picture is often characterized by bacterial superinfections as well.

Diagnosis: isolation in cell cultures, serology later in the course of the infection.

Prevention: dead vaccine for high-risk persons, e.g., with circulatory diseases.

Pathogen. This family has one genus, *Influenza virus*, with the three types influenza A, B, and C. Influenza A is by far the most important and most frequently observed influenza virus. It repeatedly causes epidemics and even pandemics at greater intervals, in contrast to influenza B, which tends to persist in endemic form and causes few outbreaks. Influenza C is rarely isolated, most frequently in youths. It plays on a minor role as an infective pathogen.

Structure and Replication

All influenza viruses show the same structure (see Fig. 7.**3**, p. 380) and a pronounced pleomorphism. In human tissues and fresh isolates, some filamentous forms several micrometers long are found, with increasingly round forms dominating after several laboratory passages. The genome of the influenza viruses is segmented and comprises eight separate antisense RNA strands, each of which codes for one specific protein. Together with the nucleoprotein, they form the helical nucleocapsid. Closely association with this structure is the RNA polymerase complex, which consists of three high-molecular-weight proteins with different functions. The nucleocapsid itself is embedded in a protein (so-called membrane or matrix protein). The virus is enclosed by an envelope made of cell membrane lipids with viral protein inclusions (hemagglutinin and neuraminidase, responsible for infectivity and viral progeny release). Both proteins are seen under the electron microscope as protrusions ("spikes") on the virus surface.

Replication of the influenza viruses proceeds as described on p. 385 for the antisense-strand viruses, whereby the **cap** of the viral mRNA is acquired by way of a unique mechanism. First, a protein of the polymerase complex separates the cap, together with 10–13 nucleotides, from the cellular RNA molecules by cleavage. This short, cap-bearing sequence serves as the primer in the synthesis of viral mRNA, which therefore begins with a cellular cap and a small piece of cellular RNA.

Continued: **Structure and Replication**

The close association of cellular and viral transcription is also reflected in the fact that RNA synthesis in the myxoviruses takes place in the nucleus of the host cell and not, as in other RNA viruses, in the cytoplasm.

Pathogenesis and clinical picture. The aerogenically transmitted influenza viruses normally replicate in the mucosa of the nasopharynx, resulting in a pharyngitis or at most a tracheobronchitis, after an incubation period of 24–72 hours. Pulmonary dissemination of the infection can result from an upper respiratory infection or manifest without one, whereby the prognosis in the latter case is less favorable. Pneumonia caused solely by the influenza virus is rare. As a rule, bacterial superinfections with staphylococci, streptococci, pneumococci, or *Haemophilus* bacteria are responsible. These infections, which used to be the normal cause of influenza deaths (*Haemophilus influenzae* in the "Spanish flu" of 1918), can be controlled with antibiotics.

Diagnosis. Influenza viruses can be grown and isolated in cell cultures if the diagnostic specimen is obtained very early, i.e., in the first one or two days of the infection. Throat lavages and swabs provide suitable material. The latter must be placed in a suitable transport medium without delay to prevent them from drying out. Identification of the cultured viruses is achieved based on the hemagglutinating properties of the myxoviruses in the hemagglutination inhibition test or by means of immunofluorescence.

If the specimen was obtained too late for virus isolation, a diagnosis can be arrived at by serological means, whereby a rise in the antibody titer of patient serum proves infection.

Table 8.**5** Classification and Antigen Structure of Influenza A Viruses

Viral prototype	Predominance	Antigen formula	
		Hemagglutinin (H)	Neuraminidase (N)
A/WS/33 A/PR8/34	1932–1946	HO	N1
A/Cambridge/46 A/F/M1/47	1946–1957	H1	N1
A/Singapore/57	1957–1968	H2	N2
A/Hong Kong/68	1968	H3	N2
A/USSR/77	1977	H1	N1

Epidemiology. Influenza A viruses are genetically variable. Slight antigenic changes are the general rule (antigenic drift, quasispecies, p. 391) and are explained by selection of point mutants in the hemagglutinin under immunological pressure. More profound changes (antigenic shifts) explain the periodic occurrence of influenza A epidemics and pandemics (Table 8.**5**).

Antigenic Shift

It is assumed that an antigenic shift occurs when gene segments are exchanged between different influenza strains as follows: there are two major reservoirs of influenza A viruses, humans and certain (aquatic) bird species whereby, in the latter, influenza viruses occur with 13 hemagglutinin types and nine neuraminidase types in nearly all possible combinations. Mixed infections with avian and human virus strains are observed in pigs, made possible by certain farming practices, e.g., in Asia where duck and/or pig husbandry are practiced together with fish breeding. This makes it possible for different viral strains to infect the same host and for two strains to infect the same host cell, which can result in a recombination of gene elements from different influenza A strains. Table 8.**5** shows antigenic changes in hemagglutinin and neuraminidase observed in the human influenza A virus since the 1930s.

Prevention and therapy. An inactivated adsorbate vaccine and some split vaccines (new: intranasal application) are available for influenza prophylaxis. The vaccine is recommended especially for persons whose occupation exposes them to such infections as well as persons with cardiovascular problems in their medical histories.

The therapeutic options include amantadine, which inhibits the viral uncoating process, and more recently neuraminidase inhibitors. These substances shorten the duration of illness by blocking the release of the viruses from the host cells and their further dissemination in the body.

Bunyaviruses

■ The **bunyavirus and phlebovirus species** are transmitted by arthropods. They cause benign, febrile infections, more rarely infections of the CNS and hemorrhagic fever. All bunyaviruses feature a single-stranded antisense RNA genome with three segments. Certain types of hantaviruses are the pathogens responsible for "hemorrhagic fever with renal syndrome" (HFRS), other types cause the "hantavirus pulmonary syndrome" (HPS). The hantavirus species are transmitted from mouse species to humans aerogenically.

Diagnosis: serological.

Prevention: exposure prophylaxis. ■

Pathogen. The family *Bunyaviridae* comprises over 200 viral species, among them four human pathogen genera: *Bunyavirus*, *Nairovirus*, *Phlebovirus*, and *Hantavirus*. The bunyaviruses are spherical, 80–110 nm in size, and posses envelopes with spikes formed on membranes of the smooth endoplasmic reticulum. The genome consists of three antisense-strand RNA segments, whereby each segment produces a separate ribonucleoprotein complex, resulting in a unique feature of the virion: it contains three helical nucleocapsids.

Pathogenesis and Clinical Picture.

- **Genus *Bunyavirus*:** these viruses are transmitted by arthropods. They cause benign forms of encephalitis such as California encephalitis and La-Crosse virus infections, both endemic to the USA, and the Oropouche virus in Brazil.

- **Genus *Nairovirus*:** the main human pathogen in this group is the Crimean-Congo hemorrhagic fever virus, with a lethality rate as high as 50%. The virus is endemic to southeastern Europe, Central Asia, China, Saudi Arabia, and Africa and is transmitted by ticks as well as by direct contact with infected animals or patients.

- **Genus *Phlebovirus*:** this group includes the pathogens that cause the benign Pappataci or phlebotomus fever ("sandfly fever"), which occurs in Europe (Italy, Yugoslavia), North Africa, Asia, and South America and is transmitted by the phlebotomus sandfly.

Rift Valley fever (RVF), an acute, febrile disease, rarely also involving hemorrhagic fever, is transmitted by mosquitoes and is endemic to Africa, usually following epizooties in livestock, in which case aerosol infection occurs (slaughtering). Epidemics have been reported with over 200 000 cases in Egypt and 25 000 cases in Senegal. Further epidemics have occurred in Somalia, Kenya, and Sudan.

- **Genus *Hantavirus*:** this genus includes several viral species (or serotypes) (Table 8.**6**) that can be classified in two groups according to the clinical symptoms they cause:

- the pathogens of **nephropathica epidemica** (NE) and **hemorrhagic fever with renal syndrome** (HFRS),

- and the **hantavirus pulmonary syndrome** (HPS).

The sources of infection are rodents (mice and rats). The infection is acquired by inhaling aerosols of urine, feces, and animal saliva. In NE and HFRS a renal dysfunction follows the influenzalike symptoms. HPS infection results in a rapidly progressive, acute dyspnea with pulmonary edema and is lethal in 60% of cases.

Table 8.6 Serotypes of Hantaviruses

Serotype	Syndrome	Geographic dissemination
Hantaan	HFRS, severe form	Asia, southeastern Europe
Belgrade	HFRS, severe form	Southeastern Europe
Puumala	NE	Central and northern Europe
Seoul	HFRS, mild form	Worldwide
Sin Nombre, etc.	HPS	US, Canada

Diagnosis. It is possible to isolate the virus from blood, but the procedure is too drawn-out and costly for routine diagnostics. Serology (IgM detection) is the method of choice, although the results can be difficult to interpret with bunyaviruses due to the rapidly changing antigenic variants produced in many of the viral species.

Epidemiology and prevention. The bunyaviruses and phleboviruses are transmitted by bloodsucking arthropods, whereby the cycle involves either human and vector only or, as with the togaviruses and flaviviruses, a mammal-arthropod-mammal cycle actually independent of humans, and in which human victims represent a dead end for the infectious agent. Hantaviruses are transmitted aerogenically to humans from rodents, in which the viruses persist apathogenically for the lifespan of the animal. The most recent isolates of the HPS pathogens have also apparently persisted in the reservoir animals for a long time, with occasional human infections as shown by retrospective analysis of blood and tissue specimens. Viral outbreaks are explained by sudden plagues of mice. Preventive measures include exposure prophylaxis (avoidance of insect bites and contact with rodents). An active vaccination is available for protection against Rift Valley fever.

Arenaviruses

The arenaviruses are "ambisense" viruses, meaning they possess genomic elements with minus (antisense) as well as plus (sense) polarity. It is quite possible for both coding orientations to occur on one and the same segment (of the segmented genome). Rodents are the natural reservoir of these viruses, from which they can infect humans. An infection with the LCM (lymphocytic choriomeningitis) virus is normally harmless. By contrast, infections

by the African Lassa and the South American Junin and Machupo viruses show high lethality rates (hemorrhagic fevers).

Diagnosis: Lassa: virus isolation in special laboratories (biosafety level 4). LCM: serology.

Pathogen. Most members of the *Arenaviridae* family were first identified in the 1960s. The protoype arenavirus, the pathogen that causes lymphocytic choriomeningitis (LCM), was identified 30 years earlier. Studies involving this virus have contributed a great deal to our understanding of cellular immunity in general and infection-related immunopathology in particular.

The human pathogens among the arenaviruses are the LCM virus (Europe, America), the Lassa virus (Africa), and the Junin and Machupo viruses (South America). All arenaviruses are spherical to pleomorphic and 50–300 nm in size (on average 110–130 nm). They consist of a "spiked" envelope derived from the plasma membrane, with an inner structure that appears to be granulated when viewed in ultrathin sections. It is to these granula the viral family owes its name (*arenosus* = sandy). They are considered to be host-cell ribosomes. The virion contains at least three strands of host RNA in addition to two viral RNA segments.

Ambisense Genome

The genome of the arenaviruses contains genomic components with sense (plus) polarity and others with antisense (minus) polarity (ambisense viruses, see p. 387) and is structured as follows: the smaller S part (S = small) codes in the 3′ part as an antisense-strand RNA for the nucleocapsid protein (NP) and in the 5′ part as sense-strand RNA for a viral glycoprotein. Each protein is translated separately from the subgenomic RNA; the NP, coded with the antisense orientation, is first transcribed into a sense-strand RNA. The L part (l = large) codes at the 3′ end in antisense-strand orientation for the viral polymerase and at the 5′ end in sense-strand orientation for a regulatory RNA-binding protein.

Pathogenesis and clinical picture. The source of nearly all human arenavirus infections is to be found in rodents. The virus enters the body per os, aerogenically or possibly also by skin contact. A pronounced viremia develops at first, followed by organ manifestations. In the case of LCM these are normally harmless and flulike, although they can also develop into meningitis or encephalitis, in rare cases with a lethal outcome. The Lassa virus is pantropic. It causes a hemorrhagic fever affecting nearly all inner organs and has a high rate of lethality. Death results from shock and anoxia. The clinical picture resulting from Junin and Machupo virus infections is similar. Compared to Lassa infections, CNS involvement is more frequent and the lethality rate is somewhat lower with these two viruses.

Diagnosis. In the acute stage, arenaviruses can be isolated from the patient's blood. Postmortem isolation is best done from liver tissue. In the hemorrhagic fevers, especially Lassa fever, the blood is highly infectious and handling it requires proper precautions and utmost care (aerosol formation!). Isolation of the virus is relatively easy in cell cultures. For reasons of safety, only special high-security laboratories are qualified to handle these organisms (e.g., at the Centers for Disease Control and Prevention in Atlanta, GA, USA).

Serodiagnosis is also feasible using standard serological techniques.

Epidemiology and prevention. All arenaviruses are endemic to rodents and are transmitted to humans by these animals.

No specific immunoprophylactic tools have been developed for any of these viruses. As far as exposure prophylaxis is concerned, it must be remembered that the LCM, Junin, and Machupo viruses are not transmitted among humans, but that the Lassa virus *is* transmitted by this route. The most stringent precautions are therefore called for when treating Lassa patients. Healthcare staff must wear special clothing and facemasks and special reduced-pressure plastic tents are recommended as patient cubicles. The therapeutic tools available for treatment are ribavirin and human immunoglobulin.

Paramyxoviruses

- This family includes the genera:
- ***Parmyxovirus*** with the parainfluenza viruses.
- ***Rubulavirus*** with the mumps virus.
- ***Morbillivirus*** with the measles virus.
- ***Pneumovirus*** with the respiratory syncytial virus (RS).
- **Nonclassified paramyxoviruses** (Hendra, Nipah).

The clinical manifestations include respiratory infections (parainfluenza, pseudocroup, mumps, RS, Nipah, Hendra), parotitis, and infections of other glandular organs (mumps), exanthem (measles), and CNS infections (mumps, measles, Nipah, Hendra).

Prevention: live vaccines are used to protect against measles and mumps; no immunoprophylactic tools are available against the other paramyxoviruses.

Pathogen. The family *Paramyxoviridae* is a heterogeneous one, both in its biology and pathogenic properties. It is divided into two subfamilies:

- ***Paramyxovirinae*** with the genera:
  - *Paramyxovirus* with the human pathogen species parainfluenza virus types 1 and 3.
  - *Rubulavirus* with the mumps virus and parainfluenza virus types 2 and 4.
  - *Morbillivirus* with the human pathogen measles virus and several zoopathic species that cause severe respiratory infections in various animal species (dogs [canine distemper], cats, cattle, seals, dolphins, turtles).
  - The nonclassified, closely related zoopathic and human pathogen *Hendra* and *Nipah* viruses.

- ***Pneumovirinae***, genus *Pneumovirus*, probably with several types of RS virus (respiratory syncytial virus).

Structure of the Paramyxoviruses

All paramyxoviruses have a similar structure (Fig. 8.**16**). They are pleomorphic. The smallest forms are 120–150 nm in size (with the exception of the somewhat smaller RS virus). They also possess an envelope that encloses the nucleocapsid. The genome consists of a continuous, single-stranded antisense RNA. The envelope is derived from the cell membrane. Various viral proteins are integrated in the envelope, visible in the form of spikes. The generic taxons are based on these spikes: parainfluenza and mumps viruses have two types of spikes, one containing the hemagglutinin (i.e., possessing hemagglutination activity) coupled with neuraminidase (HN protein), and the other the so-called fusion (F) protein, responsible for fusion of the envelope with the cell membrane. Measles viruses contain no neuraminidase and pneumoviruses possess only the F protein.

Parainfluenza Virus

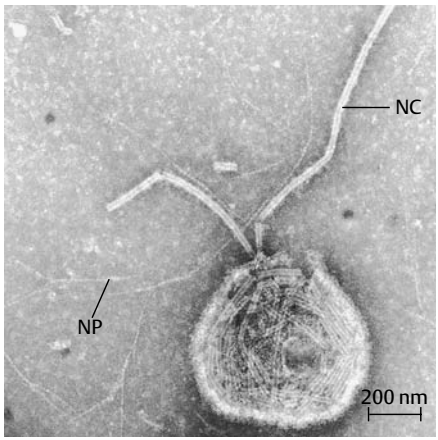

Fig. 8.**16** The envelope here is torn open, allowing the helical nucleocapsid (NC) to escape. NP: primary nucleoprotein helix (TEM).

Pathogenesis and clinical picture.

The **parainfluenza viruses** cause flulike infections, mainly in small children, which occasionally progress to bronchitis or even pneumonia. Occasionally, a dangerous croup syndrome develops. Bacterial superinfections are frequent, as are the usually harmless reinfections.

In **mumps virus** infections the virus first replicates in the respiratory tract, then causes a viremia, after which a parotitis is the main development as well as, fairly frequently, mumps meningitis. Complications include infection of various glandular organs. Orchitis can occur in postpuberty boys who contract mumps.

Measles. The pathogenesis of measles has not been fully explained. It is assumed that the virus, following primary replication in lymphoid tissues, is distributed hematogenously in two episodes. Thereafter the oral mucosa displays an enanthem and the tiny white "Koplik's spots." Then the fever once again rises and the typical measles exanthem manifests (Fig. 8.**17**). Possible complications include otitis in the form of a bacterial superinfection as well as pneumonia and encephalitis. A rare late sequel of measles (one case per million inhabitants) is subacute sclerosing panencephalitis (SSPE) in which nucleocapsids accummulate in brain cells, whereby few or no viral progeny are produced for lack of matrix protein. This disease occurs between the ages of one and 20, involves loss of memory and personality changes, and usually results in death within six to 12 months.

Nipah and Hendra virus infections are zoonoses endemic to Southeast Asia (Nipah) or Australia (Hendra). Both infections result in encephalitis with relatively high lethality rates (up to 40%) and in some cases severe interstitial pneumonias.

Measles Exanthem

Fig. 8.**17** The typical exanthem manifests during what is presumably the second hematogenous disseminative episode of the morbilliviruses.

RS viruses cause bronchiolitis or pneumonia, mainly in children up to six months of age, or rarely up to two years. Immune status appears to play an important role in the course of the infection. It has been determined that the course of the disease is more severe in children who have received dead vaccine material (similarly to measles). This is presumably due to antibodies, in the case of small children the mother's antibodies acquired by diaplacental transport. Immunosuppressed patients, for instance, bone marrow recipients, are also at risk for RSV.

Diagnosis. In addition to serodiagnostic methods, direct detection tests based on immunofluorescence or enzyme immunoassay are available for paramyxoviruses, some of them quite sensitive. Paramyxoviruses replicate readily in cell cultures from human tissues.

Epidemiology. Paramyxoviruses are transmitted by droplet infection. Generalized contamination levels in the population (except for Nipah and Hendra) are already very high in childhood (90% in 10-year-old children for parainfluenza virus types 1–3).

Nipah and Hendra viruses are zoonoses that are transmitted to humans from animals (Nipah: pigs, Hendra: horses). Various different animals can be infected by these pathogens, but bats (*Pteropus*) appear to be the natural reservoir for both viruses.

Prevention. Attenuated live vaccines are available for measles and mumps. The **dead** vaccine should not be used due to the aggravating effect mentioned above. No vaccines have as yet been developed for the other parainfluenza viruses.

Rhabdoviruses

Among the rhabdoviruses, the lyssaviruses, genotypes 1–7, are human pathogens. They are transmitted by the bite of an infected animal in its saliva and infections, once fully manifest, are always lethal (rabies, hydrophobia). The reservoir for type 1 is provided by wild animals in general (foxes, etc.), bats (sylvatic rabies), and, in Asia, dogs (urban rabies). Types 2–7 are restricted to Europe, Asia, Africa, and Australia with their main reservoir in bats.

Diagnosis: direct detection with IF in cornea cells and skin biopsies, postmortem isolation from brain tissues.

Prevention: due to the week-long and even month-long incubation period (except in types 2–4), postexposure prophylactic vaccination with combined active (dead vaccine) and passive (human immunoglobulin) vaccines is possible. Pre-exposure prophylaxis in the form of dead vaccine is administered to persons at high risk.

Pathogen. The rhabdoviruses of significance in human medicine are classified in seven genotypes. Type 1 is the classic, worldwide type that occurs in two forms: the "street virus" isolated from humans and animals and the "virus fixe" according to Pasteur. In 1882, Pasteur had transmitted the virus intracerebrally to rabbits. Following repeated passages of the virus in the rabbits, he had developed a dead vaccine type. Due to the brain-to-brain passages in the laboratory animals, the "virus fixe" became so highly adapted to brain tissue that it was unable to replicate in extraneural tissues. Types 2–4 were isolated from African bats, types 5 and 6 from European bats, and type 7 from Australian bats.

Rhabdoviruses are rodlike, 60 × 180 nm in size, with one end flat and one end rounded ("bulletshaped") and a spiked envelope surrounding a nucleocapsid similar to that of the myxoviruses. The genome consists of antisense-strand RNA.

Pathogenesis and clinical picture. Rabies viruses are almost always transmitted by the bite, sometimes also the scratch, of a rabid animal (exceptions, see below). The virus at first replicates at the portal of entry in muscle and connective tissue, then wanders along the nerve cells into the CNS, where more viral replication takes place. Using the same route, the virus then disseminates from the CNS into peripheral organs, above all the salivary glands, cornea, and kidneys. The primary clinical picture is an encephalitis with lethal outcome for humans and animals once it has broken out.

Clinical Course of Rabies

The disease goes through three stages. The initial, or prodromal, stage involves itching and burning at the portal of entry (bite wound), nausea, vomiting, and possibly a melancholy mood. In the second or excitative stage, cramps and spasms of the pharynx and larynx are the main symptoms, rendering swallowing very painful. The spasms can be induced by the mere sight of water ("hydrophobia"). Other mild acoustic and visual stimuli may elicit exaggerated reactions including attacks of cramps and violent anger, hitting, biting, and screaming. Death occurs within three to four days at the earliest. The third, paralytic, stage may develop instead of early death, with ascending paralysis and asphyxia, leading to exitus. Therapy is exclusively symptomatic. Since the patient experiences the disease in a fully conscious state, most of the medication serves to alleviate the pain and anxiety states. The disease runs essentially the same course in humans and animals, whereby the behavior of animals is often radically altered: wild animals lose their fear of humans and tame pets become aggressive. Rabies with the excitative stage is known as "furious rabies," without it as "dumb rabies."

Diagnosis. An intra-vitam laboratory diagnosis is established by examining an impression preparation from the cornea or skin biopsies with immunofluorescence. Postmortem, rabies viruses can be found in the brain tissue

of humans and animals by inoculating newborn mice or cell cultures with brain tissue or saliva.

Because antibody production begins so late, serodiagnosis is not practicable. Serological analysis is used to check for vaccine protection. Useful technical tools include an EIA or neutralization test (RFFIT, **r**apid **f**luorescent **f**ocus **i**nhibition **t**est in cell cultures). Special laboratories are used for the diagnostic testing.

Epidemiology. Lyssavirus type 1 is endemic to North America and Europe in wild animals (sylvatic rabies) and in certain tropical areas in domestic pets as well, in particular dogs (urban rabies). The reservoir for the remaining lyssavirus types are bloodsucking (hemovorous) as well as fructivorous and insectivorous bats.

The virus is excreted with the saliva of the diseased or terminal incubator animal and enters other animals or humans through scratch or bite wounds. The virus is highly labile, so transmission on contaminated objects is very rare. Human-to-human transmission has not been confirmed with the exception of cases in which rabies in corneal donors had gone unnoticed.

Prevention. The long incubation period of the rabies virus—in humans several weeks to several months, depending on the localization and severity of the bite wound—makes postexposure protective vaccination feasible. Development of the vaccine originated with Pasteur, who used a dead vaccine from the neural tissues of infected animals. Use of this original rabies vaccine often resulted in severe side effects with allergic encephalomyelitis. The vaccine types in use today are produced in diploid human embryonal cells (HDCV = **h**uman **d**iploid **c**ell **v**accine), hen fibroblasts or duck embryos. No further adverse reactions have been described with these vaccines, so that earlier apprehensions about the rabies vaccine are no longer justified.

The postexposure procedure depends on the type of contact, the species and condition of the biting animal and the epidemiological situation (Table 8.**7**). Exposure is constituted by a bite, wound contamination with saliva or licking of the mucosa, but not by simple petting. In endemic regions, any animal that bites unprovoked must be suspected of being rabid.

Postexposure prophylaxis begins with a rigorous wound toilet, the most important part of which is thorough washing out of the wound with soap, water, and a disinfectant agent. Passive immunization with 20 IU/kg human rabies immunoglobulin (RIG) is then begun, whereby half of the dose is instilled around the wound and the other half is injected i.m. Concurrently, active immunization is started with six doses of HDVC injected i.m. on days 0, 3, 7, 14, 30, and 90. The current therapeutic measures are summarized in Table 8.**7**.

Important: postexposure vaccination is apparently ineffective against the African viral strains (types 2–4).

Table 8.7 Rabies: Postexposure Prophylaxis (according to WHO recommendations issued in Geneva, 1992)

Animal species, epidemiological situation	**Condition of animals**	**Treatment of exposed person**[1]
Domestic pet		
Endemic area	–	HDCV and RIG[2]
Not from endemic area:		
Dog, cat	Healthy, can be observed for 10 days	None; if animal develops rabies within 10 days, begin immediately with HDCV/RIG
	Suspected rabies or rabid, unknown, escaped	HDCV and RIG
Other pets	–	Depends on epidemiological situation
Wild animal		
Wild carnivore, bats	Always consider rabid pending negative lab results	HDCV and RIG
Other wild animals:		
From endemic area	–	HDCV and RIG
Not from endemic area	–	Depends on epidemiological situation

HDCV: human diploid cell vaccine (active vaccination); RIG: rabies immunoglobulin from human source (passive vaccination).
[1] Treatment comprises administration of RIG and HDCV (see text). WHO recommendations also allow use of HDCV alone in cases of minor exposure (licking of skin).
[2] Discontinue treatment if animal under observation remains healthy for 10 days.

Persons exposed to an increased risk of contracting rabies can also be given pre-exposure protection with three doses of HDCV. Postexposure treatment is then limited to the wound toilet and HDCV injections.

Postexposure prophylaxis is impracticable in animals. Dogs and cats in particular must be vaccinated with living vaccine grown in duck embryos. In wild animals (foxes), oral bait vaccination programs have been successful. If the bait contains the attenuated rabies virus, exposure to it must be considered rabies exposure and the postexposure prophylactic procedure must be carried out. This does not apply to use of the recombinant vaccinia virus. However, see p. 428 on the pathogenicity of the vaccinia virus.

Filoviruses (Marburg and Ebola Viruses)

■ Two related African viruses are subsumed under the name filoviruses, Marburg and Ebola. These pathogens cause hemorrhagic fevers with high lethality rates. The few described Marburg virus outbreaks apparently involve monkey populations. Ebola outbreaks are apparently becoming more frequent. The natural reservoir of the filoviruses is unknown.

Diagnosis: by antigen assay, EM, and isolation. ■

Pathogen. The Marburg virus was isolated for the first time in 1967 as a result of three simultaneous outbreaks among laboratory staff in Marburg, Frankfurt, and Belgrade. The infection victims had been processing the organs of *Cercopithecus* (African green monkeys) from Uganda. Both the Marburg and Ebola viruses are threadlike, 14 µm-long viral particles, in some cases branched and 80 nm thick in diameter. Their surface consists of an envelope of host-cell membrane with viral spikes. The genome consists of antisense-strand RNA in a helical nucleocapsid 50 nm in diameter.

Pathogenesis and clinical picture. The Marburg and Ebola viruses cause so-called hemorrhagic fevers. The clinical picture first manifests with fever, headache, and neck pain, conjunctivitis and diarrhea, followed by hepatic, renal, and CNS involvement and finally, as a result of consumption coagulopathy, leads to extensive hemorrhaging and terminal shock. In terms of the anatomical pathology, nearly all organs show hemorrhages and fibrin deposits.

Diagnosis. Only designated laboratories with special safety facilities can undertake isolation work on these viruses. Detection is either in blood with an electron microscope or using immunofluorescence on tissue specimens. The pathogens can be grown in cell cultures. Serodiagnosis is also possible.

Epidemiology and prevention. The reservoir of the Marburg and Ebola viruses is unknown. Subsequent to the Marburg outbreak in 1967 among lab personnel in Europe, Marburg viruses have only been found in Africa. The Ebola virus, named after a river in Zaire, has caused several outbreaks in Africa since 1976 in which lethality rates of 50–90% were observed. Imported Ebola infections have also been seen in monkey colonies in the USA and Italy.

Protective suits and vacuum-protected plastic tents are no longer recommended for healthcare workers in contact with Marburg and Ebola patients (as with Lassa fever), since interhuman transmission is by excretions (smear infection) and in blood, but not aerogenic. Despite this fact, the high level of infectivity of any aerosols from patient material must be kept in mind during laboratory work and autopsies.

Subviral Pathogens: Viroids and Prions

Viroids are phytopathologically significant, noncoding RNA molecules that interfere with cellular regulation as antisense RNA. The hepatitis D virus has some structural similarity to viroids.

Prions consist of a cell-coded protein (PrP: prion protein) altered in its conformation and by point mutations. They are infectious and can cause normal cellular PrP to assume the pathological configuration. They cause the spongiform encephalopathies (Creutzfeldt-Jakob disease, CJD) in the classic and new variants (nvCJD), the Gerstmann-Sträussler-Scheinker (GSS) syndrome, and animal diseases (scrapie, BSE) characterized by neuronal vacuolization and loss and so-called amyloid plaques.

Prevention: exposure prophylaxis (iatrogenic and alimentary transmission).

Viroids

Viroids were discovered at the end of the sixties during investigations of plant diseases. They consist of infectious, naked ssRNA in closed circular form with extensive base-pairing to form a rod-shaped strand 50 nm long. This RNA is 10 times smaller than the smallest viral nucleic acid and comprises, depending on the type, only 250–350 nucleotides. It does not function as mRNA and does not code for proteins. Its mode of replication is unknown, but certainly involves cellular enzymes.

Viroids cause a number of plant diseases with considerable economic impact. The following hypothesis is currently under discussion to explain their pathogenicity mechanism: viroids possess complementary sequences to cellular 7S RNA, comprising, together with six proteins, the "signal recognition particle." This particle controls the posttranslational membrane insertion of proteins. Viroids can thus interfere as "antisense (or interfering) RNA" with the function of 7S RNA and thus with membrane formation.

The only significant human pathogen structurally related to the viroids is the hepatitis D pathogen (HDV, delta agent, see p. 429). HDV consists of a viroidlike, also circular, RNA into which an antisense RNA coding for the delta agent is inserted.

Prions

Pathogen. Attention was first drawn to certain encephalopathic agents whose physical properties differed greatly from those of viruses. For instance, they showed very high levels of resistance to sterilization and irradiation procedures. It was later determined that these pathogens—in complete contrast to viruses and viroids—require only protein, and no nucleic acid, as the basis of their infectivity and pathogenicity. This gave rise to the term "prion" for "proteinaceous infectious particle." An intensive search for nucleic acid in the "particles" was fruitless.

Prions are misfolded forms of a cellular protein. They consist of only a single protein (PrP, prion protein), which naturally occurs, for example, on the surface of neurons. The region coding for this protein of approx. 35 kDa is located in a single exon and is derived from a cellular gene expressed in both healthy and diseased brains. Disease-associated PrP (the best-known prion is the scrapie pathogen, the protein of which is called PrP^{sc} [sc for scrapie]), is a mutant, slightly shortened (27–30 kDa) form of the normal PrP^{c} (c for cell). It differs from normal PrP^{c} in its altered configuration, its nearly complete resistance to proteases and in the fact that it tends to accumulate inside the cell.

Pathogenesis. Infectious PrP^{sc} can transform naturally occurring PrP^{c} into PrP^{sc}, resulting in an autocatalytic chain reaction in which mainly the pathological protein is produced. This is why mice lacking the gene for PrP (genetically engineered "knockout mice") cannot be infected with the pathological PrP^{sc} prion. Deposits of large amounts of the pathological protein in the form of so-called amyloid plaques are visible under a microscope in brain tissue from infected humans and animals.

Clinical picture. The following encephalopathies are considered to be caused by prion infections:

In humans:

- Creutzfeldt-Jakob disease (CJD)
- New variant CJD (nvCJD or vCJD)
- Gerstmann-Sträussler-Scheinker (GSS) syndrome
- Kuru

In animals:

- Scrapie (sheep, goats)
- Transmissible mink encephalopathy (TME)
- Wasting disease (deer)
- Bovine spongiform encephalopathy (BSE, "mad cow disease")

All of these diseases are designated as transmissible encephalopathies characterized by incubation periods of a number of years, long durations of disease (one to several years in humans) and lethal courses with motor disturbances (animals) and progressive dementia (humans). Histologically, the brain shows no inflammation, but rather vacuolization of neurons, loss of neurons, proliferation of glial cells, and amyloid plaques (see above).

Diagnosis. The diagnostic procedure is histological. Since there is no immune response to the pathological PrP, serodiagnostic methods are useless. The pathological protein can, however, be detected in lymphoid tissue biopsies using monoclonal antibodies.

Epidemiology. CJD, which occurs sporadically (one case per million inhabitants per year) is produced anew in every case by mutations in PrP^{c}. The disease can be transmitted iatrogenically (brain electrodes, corneal transplants). The pathogenicity of the GSS prion (PrP^{gss}) is based on a single amino acid change. Genetic factors also appear to have a predisposing effect in view of the existence of familial forms of GSS. Kuru is a disease that was spread in New Guinea by cannibalistic rites, probably originating with a case of CJD. Kuru no longer occurs today.

Alimentary transmission is possible in animals. PrP^{sc} was transmitted to cattle by feeding them animal meal made from scrapie-infected sheep remains, resulting in BSE. Despite the fact that transmission of prions from one species to another is not a simple process in principle, BSE prions were transmitted to humans by the alimentary route, resulting in nvCJD. This route of transmission was confirmed by structural analyze of the BSE and nvCJD prions.

From 1995, when the first nvCJD patient died, to the end of 2000, 51 cases of CJD with lethal outcome have been described. In contrast to classic CJD, these infections occurred in young people, whereby the incidence of nvCJD in older persons can be masked by dementias from other causes. The expected outbreak of nvCJD because of the BSE epidemic is currently a topic of extensive discussion.

As a result of this new disease threat, some countries have now prohibited the feeding of animal meal to certain kinds of livestock (in particular ruminants).