Biology of B Virus in Macaque and Human Hosts: A Review

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B virus is a zoonotic alphaherpesvirus enzootic in Asian monkeys of the genus *Macaca*. At least 25 cases of human disease caused by B virus have occurred to date, leading to death in 16 instances. Advances in the technology available for the diagnosis of B virus infection and in the agents for its treatment are improving the prognosis for cases in human beings. Efforts are under way at several institutions in the United States to establish B virus–free colonies of rhesus macaques for use in biomedical research. Unfortunately, the epidemiology of B virus in group-housed macaques is poorly understood. The elucidation of factors important in the transmission of B virus between monkeys will greatly enhance efforts to eradicate this virus and may help to minimize further human exposure to the agent.

B virus is an alphaherpesvirus that is enzootic in Asian monkeys of the genus *Macaca* and that generally produces only mild localized lesions—or no overt lesions at all—in the natural host species. Human beings, certain New World primate species, and other mammals represent aberrant hosts in which B virus infection is usually fatal. The relatively few reported instances of human B virus disease have stimulated a volume of research and public policy emphasis that is disproportionate to the agent's global prominence relative to other alphaherpesviruses of primates [1, 2]. Furthermore, the enthusiasm for research on B virus has temporally paralleled human case reports, whose numbers peaked during the days when the battle against human poliovirus infection led to an unprecedented use of rhesus monkeys (*Macaca mulatta*) in biomedical laboratories [2]. A second relative increase in the frequency of human B virus disease has recently emerged, coincident with an increased demand by biomedical researchers for macaques for use in simian retrovirus studies.

Reports on B virus in the scientific literature primarily consist of anecdotal descriptions of cases in humans, serological surveys for antibodies to B virus in macaques, experimental studies in laboratory animals, and delineation of diagnostic methods to identify the agent. Advances in the technology available for the diagnosis of B virus infection have facilitated the rapid identification and treatment of human cases. As a result of public health considerations and requirements for simian retrovirus research, the National Institutes of Health are currently funding efforts to eradicate B virus and exogenous retroviruses through the formation of specific pathogen–free breeding colonies of rhesus monkeys [3]. Thus far, in all efforts to develop B virus–free groups, available serological tests have been used for the screening of preassembled groups of monkeys or of individual animals before group formation. However, the sensitivity and specificity of available tests when used for this purpose have yet to be defined. Also, the lack of sufficient information concerning the epidemiology and natural history of B virus in macaques has made it impossible to bolster these efforts through colony management schemes. A clearer understanding of these issues would promote more efficient elimination of B virus from infected groups and would help prevent its reintroduction into established specific pathogen–free colonies.

The Virus

Taxonomy and Host Diversity of the Primate Herpesviruses

Various names have been ascribed to B virus in the scientific literature, including herpes B virus, B virus, monkey B virus, *Herpes simiae*, *Herpesvirus simiae*, and Herpes B. Although many researchers appear to accept the Latinized form *Herpesvirus simiae*, this name has not gained official stature because approximately 35 other herpesviruses also occur naturally in simian species [4]. The Herpesvirus Study Group, appointed by the International Committee on Taxonomy of Viruses, offered the provisional designation "cercopithecine herpesvirus 1" (CHV-1) within the genus *Simplexvirus*, subfamily Alphaherpesvirinae, and family Herpesviridae [5]. This classification scheme was based primarily on the biological characteristics and serological relatedness of this virus to other members of its group. Genomic structure is also important for classification but has not yet been adequately defined for the virus. In the remainder of this review, CHV-1 will be referred to as B virus, the name familiar to most readers.

Of all the nonhuman primate herpesviruses, only B virus has been shown definitively to cause disease in human beings. However, the virulence of other primate herpesviruses in heterologous species has been well documented. Certain

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alphaherpesviruses and gammaherpesviruses may result in fatal neurological and systemic disease in primates other than the natural host species [6–9]. B virus itself has been shown to be fatal in at least two species of New World monkeys, cebus (capuchin) monkeys [10] and common marmosets (*Callithrix jacchus*) [11], with pathology similar to that observed in B virus–infected people. Similarly, human herpes simplex viruses (HSVs) of types 1 and 2 (HSV-1 and HSV-2) have caused pathology in a variety of New World monkey species [9] and also in chimpanzees [12], exemplifying the general lack of host restriction among certain members of this virus family.

Initial Isolation and Description of B Virus

B virus was the first simian host–associated virus recognized to exist. Gay and Holden [10] initially reported the existence of this agent following its occurrence in 1932 in a 29-year-old laboratory worker (identified with the initials Dr. W. B.) who was bitten on the hand by a normal-appearing rhesus monkey. Sabin and Wright [13] later provided a comprehensive review of this case. Three days after the bite, the patient's hand became inflamed, and regional lymphangitis and lymphadenitis developed subsequently. The patient was hospitalized 6 days after infection with a fever of 101.4°F and persistent pain and swelling at the site of the bite wound. Several small fluid-filled vesicles formed near the wound over the next 2 days. Bouts of generalized abdominal cramping ensued 10 days after infection and were followed by ascending flaccid paralysis and urinary retention. Analysis of the CSF indicated mononuclear pleocytosis (112 cells/mm^3) along with increased levels of albumen and globulins. Paresthesia of the arms was noted on day 16 after infection. The patient's condition deteriorated over the following day, as indicated by hypopnea, pulmonary edema, a convulsion accompanying a laryngospasm, and coma. Death from respiratory paralysis occurred after 5 hours of coma despite supportive measures that included mechanical ventilation and continued aspiration of pulmonary fluid.

The histologic diagnosis following autopsy was acute transverse myelitis with areas of focal necrosis and hemorrhage in the regional lymph nodes, spleen, and adrenal glands. Inflammatory exudates, composed primarily of microglial cells, were noted in the gray and white matter of the spinal cord. Additional lesions in the medulla, pons, and frontal lobe were composed of perivascular mononuclear cell infiltrates. Surprisingly few gross lesions (other than slight edema of the spinal cord) were apparent at autopsy. The cause of death was listed as encephalomyelitis [10, 13].

The persistence of herpetiform lesions near the bite wound of patient W.B. was, at the time, considered most predictive of an etiology for this case, although no virus was identified from vesicular fluid in the initial lesions. Suspensions composed primarily of the patient's spinal cord, when inoculated into rabbits via the intradermal route, produced clinical signs and pathology similar to those observed in the patient. No significant pathology resulted when attempts were made to transmit the virus back to rhesus monkeys, but two cebus monkeys died following intracerebral inoculation of the material. Through cross-neutralization tests, Gay and Holden [10] reported the agent to be antigenically related to well-described isolates of HSV and designated it "W virus," after the first initial of the patient. This report formed part of a series in which the authors attempted to establish that epidemic encephalitis of human beings was caused by an adapted neurotropic strain of HSV. They classified the isolate as a strain of HSV with exceptionally high virulence for rabbits.

A review of this case by Sabin and Wright [13] included additional experimental work in rabbits, rhesus monkeys, dogs, mice, and guinea pigs with tissue specimens collected from the patient at autopsy. These authors demonstrated that the isolate represented a unique entity biologically distinct from HSV in rabbits, although it had produced herpes-typical lesions in both rabbit and human tissues. Again, no specific disease was produced in rhesus monkeys or other species that were experimentally inoculated. Sabin and Wright designated the isolate "B virus," after the second initial of the patient, and included it as a provisional new member of the herpesviruses on the basis of its observed biological activity. Henceforth, the name B virus was widely adopted.

In further studies [14] Sabin was successful in maintaining rabbit-propagated B virus through four serial passages in rhesus monkeys and then back to rabbits, in which it produced paralytic disease and death. In rhesus monkeys neurological disease occurred only after intracerebral inoculation, whereas vesicopustular and visceral lesions without demonstrable CNS involvement were identified after transcutaneous and intraperitoneal inoculation. Thus the disease in rhesus monkeys clinically resembled typical HSV infection in human beings; other candidate viruses were excluded on the basis of this observation and histologic findings.

In the above studies tissue involvement was confirmed by the identification of typical Cowdry type A inclusion bodies and by the reproduction of lesions in rabbits and monkeys. Sera obtained from rhesus monkeys during the convalescent phase of experimental infection neutralized rabbit-passaged B virus. Furthermore, although neutralizing antibodies to B virus were detected in only one of 13 rhesus monkeys maintained by Sabin [14], four of seven normal-appearing rhesus monkeys tested in the laboratory of Gay and Holden [10] were inherently antibody-positive. This observation was used as a possible explanation for the failure of the latter authors to produce disease by experimental infection of rhesus monkeys and as an argument that B virus may be enzootic in that species [14].

Architecture and Environmental Stability of the Virion

B virus resembles the closely related HSVs in many respects, including pathogenesis, morphology, antigenic and

genomic structure, and apparent molecular mechanisms of host cell infection and replication. In this author's opinion, these similarities argue for functional employment of the body of knowledge about HSV where similar details regarding the biology of B virus in host cells have yet to be described. In fact, homology shown to exist between corresponding antigenic epitopes of these viruses (as discussed below) has complicated efforts to differentiate them by way of serological tests.

Morphological studies performed by Ludwig et al. [15] characterized B virus in some detail. The virus was found to be an enveloped particle that was ~160–180 nm in diameter and contained a toroidal core with rotational symmetry (measuring 40 nm) and double-stranded DNA in linear form. The molecular weight (MW) of B viral DNA was $\sim 107 \pm 8.1 \times 10^6$, corresponding to 162.1 ± 12.3 kilobase pairs. The authors reported that B virus had the highest guanine-plus-cytosine content among known herpesviruses, at 75 moles percent. Analyses of terminal portions of the B virus genome [15] suggested that distinct isomeric populations may naturally exist in patterns similar to those described for HSVs and bovine mammillitis viruses [16, 17].

Ludwig and co-workers [15] used a denaturing SDS-PAGE system to identify ~23 major polypeptides produced by purified B virus, with MWs of 18–220 kD. Immunoprecipitation data with convalescent-phase rhesus sera suggested that four to six of these products were glycoproteins. Hilliard et al. [18] radiolabeled polypeptides from B virus and identified ~50 different viral proteins (MW, 26–239 kD), at least nine of which were shown to be glycoproteins. These results compare with the ~33 viral polypeptides, including eight glycoproteins, that have been identified for HSV-1. Recent studies by Eberle et al. [19] showed antigenic relatedness between the glycoprotein designated gB for the HSVs and a similar entity identified on the surface of B virus and other alphaherpesviruses of nonhuman primates. Antibodies to these gB-equivalent epitopes failed to cross-neutralize the set of heterologous viruses in these experiments. However, homologues to another surface glycoprotein, corresponding to gD for the HSVs, were also shown to exist for B virus and to be involved in the generation of cross-neutralizing antibodies. Other workers [15, 20, 21] located two glycosylated protein epitopes on B virus corresponding to gA/B and gD of HSVs and associated both sites with the majority of cross-precipitating antibody activity observed to exist among HSV-1, HSV-2, B virus, and bovine mammillitis virus.

Storage of B virus in tissue culture fluid (pH 7.2, 4°C) was shown to produce only a slight loss in viability over a period of at least 8 weeks [22]. A single episode of freezing at either −20°C or −72°C resulted in an initial loss of 2 logs of infectivity of tissue culture fluid–stored specimens, but no further decreases were reported during the subsequent 8 weeks. Three repeated freeze-thaw cycles thereafter did not lower the titers of virus. However, all infectivity was lost at the end of 2 weeks of specimen storage at 40°C [22]. As in the case of other enveloped viruses, inactivation by lipid solvents, ultraviolet light, and heat may be expected. Thus cell-free virus is not likely to persist for long in the environment [23, 24].

Interaction of B Virus with Permissive Cells

Despite isolation of B virus from the initial human case in 1932 [10, 13], it was not until 20 years later that the agent was recovered from a natural infection in macaques [25]. In this instance B virus was found in a suspension of spinal cord tissue from a rhesus monkey that had been used for human poliovirus research. The virus was successfully propagated in cotton rats, mice, guinea pigs, rabbits, hamsters, chick embryos, and continuous cell cultures of monkey kidney epithelial and human HeLa cells. This study demonstrated not only a lack of host restriction for B virus similar to that which had been shown to exist for HSV-1, but also the disconcerting potential for inadvertent amplification of a new hazardous infectious agent. The potential public health implications of this observation were discussed in a study by Wood and Shimada [26], who recovered B virus from cell suspensions of rhesus and cynomolgus monkey kidney tissue used in the preparation of poliovirus vaccines for human beings. Fortunately, B virus was found to be readily inactivated by the treatment with formalin and heat that was routinely used at the time for the production of poliovirus vaccine [22, 26]. The Centers for Disease Control [27] recently published a set of recommendations intended to reduce the potential for human exposure to B virus in laboratories where cultured primary rhesus monkey kidney cells are utilized.

Morphological changes in rhesus kidney epithelial cells infected in vitro with B virus have been reported by Reissig and Melnick [28]. Following attachment to surface receptors on host cells, the virus penetrated through the plasma membrane via fusion with the viral envelope. The naked capsid was then transported to nuclear pores, where viral DNA was released into the nucleus. As with other DNA viruses, B virus progeny were produced in the cell nucleus [15, 28]. The growth curve for B virus was compared with that of HSV-1 and HSV-2, although the former was somewhat shorter in duration [15, 18, 28]. Infectious B virus was detectable as early as 6 hours after infection, and titers of virus stabilized by ~24–36 hours [18]. The appearance of Cowdry type A intranuclear inclusion bodies during the replicative cycle was typical of several other herpesviruses and coincided with host cell shutdown of macromolecular synthesis, margination of chromatin, cellular swelling, and ultimately cellular lysis. Polykaryocytes (syncytia) were sometimes evident during the course of B virus infection; studies reviewed by Roizman and Sears [29] involving HSV-1 and HSV-2 suggest that this observation is associated with structural and antigenic alterations of host cell membranes.

B Virus and Latency

B virus, similar to other alphaherpesviruses, is capable of establishing latent infections in the host that presumably per-

sist throughout life despite the presence of specific humoral antibody. Subsequent reactivation from latency causes renewed production and liberation of infectious virus (recurrences), with or without overt clinical symptomatology in the host [7, 30–33]. Just as neuronal tissue tropisms have been described for HSV-1 and HSV-2 [29, 34, 35], latent B virus has been detected in trigeminal [30–32] and lumbosacral sensory ganglia [32] of macaques—corresponding to initial sites of viral entry and replication in the oral-facial and genital epithelial mucosae, respectively. However, Wall et al. [36] failed to distinguish unique genital-associated strains of B virus from oral-associated strains on the basis of restriction endonuclease genomic analysis. These authors were able to differentiate B virus isolates of rhesus origin from those of cynomolgus origin irrespective of tissue site, however. Further studies are needed to determine whether distinct macaque species–associated strains of B virus exist naturally.

The presence of latent B virus in the trigeminal ganglia was suspected in a person who initially presented with herpes ophthalmic zoster and trigeminal neuralgia but reported no direct exposure to monkeys during the 10 years before illness [37]. Definitive etiologic diagnosis for this case was possible via combined serological and virological testing. Latent infections with B virus have been established in rabbits after experimental inoculation of the agent [38, 39].

Molecular mechanisms relevant to the establishment and maintenance of B virus latency have not been reported. It is likely that latency represents a complex molecular system interrelating several viral regulatory proteins with the viral genome and the host cell environment. The model of HSV latency proposed by Roizman and Sears [29, 40] involves an interaction between immediate-early (alpha) regulatory proteins and host cell type, with reactivation stemming from the cumulative effects of local and systemic stimuli causing the replication of viral DNA to exceed a certain limiting threshold.

Pathology and Pathogenesis

B Virus Disease in Macaques

Most reports of natural infections of macaques with B virus have involved rhesus and cynomolgus or long-tailed monkeys (*Macaca fascicularis*), but isolations from bonnet (*Macaca radiata*), Japanese (*Macaca fuscata*), Taiwan (*Macaca cyclopis*), and stump-tailed (*Macaca arctoides*) macaques have also been reported [7, 33, 41–43]. In addition, probable infections with B virus were found in three patas monkeys (*Erythrocebus patas*) and one black-and-white colobus monkey (*Colobus abyssinicus*) that were housed near a colony of rhesus monkeys in a zoo in the United States [44]. As has been mentioned, experimental infection with B virus has produced fatal neurological disease in at least two species of New World monkeys, cebus (capuchin) monkeys [10] and common marmosets (*C. jacchus*) [11]. No reports indicate the presence of B virus among African Old World monkeys, but the closely related alphaherpesvirus SA 8 occurs naturally in baboons (*Papio* species) with a pathogenesis apparently similar to that described for B virus [45–47]. This section is limited to the pathology associated with infections in macaques.

Clinical findings of B virus disease in rhesus monkeys have been reported by various authors [7, 48, 49]. Initial lesions consisted of fluid-filled vesicles on the dorsum of the tongue, at the mucocutaneous border of the lips, or anywhere else in the buccal cavity. These vesicles eventually ruptured, giving rise to fibronecrotic scabs that generally healed by granulation without scarring in 7–14 days. Secondary infections of these lesions by bacteria and fungi sometimes resulted, while B virus–associated conjunctivitis of varying severity was also observed occasionally. Scabs yielding infectious B virus were noted on the skin overlying different regions of the body, ranging from 0.5 cm to 2.0 cm in diameter and sometimes demonstrating Cowdry type A inclusions [32, 49]. Histologically, the oral lesions appeared similar to the changes produced in cell cultures infected in vitro [28], with ballooning cell degeneration and syncytial cell formation. Oral lesions were sometimes found concurrently with foci of inflammation and necrosis in the hepatic parenchyma and renal interstitium; except when inclusion bodies were present, however, the appearance of these changes was not specific to B virus [7, 49]. Lesions in the CNS were confined to mononuclear perivascular cuffing and gliosis without neuronal degeneration in the tracts and roots of the facial and trigeminal nerves and associated regions of the pons and medulla.

Systemic illness associated with B virus in macaques has been reported on only three occasions. According to one report [50], a rhesus monkey undergoing oral phenylbutazone therapy for 36 days developed disseminated herpesvirus infection, with ulcerative lesions in the mouth, esophagus, and stomach and diffuse necrosis of the liver, spleen, and adrenal glands. No effort was made in this instance to confirm the identity of the infectious agent, however. A second case of disseminated infection was reported by Daniel et al. [51], who isolated B virus from oral, rectal, lingual, and pulmonary specimens from a rhesus monkey that died of cerebral infarction. Seroconversion to B virus in this animal was confirmed during a 19-day course of disease. Finally, Espana [52] reported an apparent respiratory outbreak of B virus infection among group-housed bonnet monkeys, with the development of interstitial hemorrhagic pneumonia and focal hepatitis in 40 of 79 individuals in the population; 16 of those cases resulted in death. That overt disease due to B virus is typically uncommon among macaques is indicated by the results of Keeble [49], who observed only 332 cases of overt lesions of the tongue and lips among 14,400 group-housed rhesus monkeys examined over a 2-year period, for a rate of 2.3%.

The pathogenesis of B virus infection in macaques appears to closely parallel that described for the HSVs [34], with primary replication in epithelial tissues followed by uptake of virus by sensory and autonomic nerve endings and axonal transport to the nucleus of nerve cell bodies, where latent infections are established. B virus may be recovered from sites of replication in the epidermis during the first 1 or 2 weeks following infection [32], although overt lesions may or may not be apparent at the time [14, 48]. For HSVs the host responds to these initial events by nonspecific cell-mediated immune mechanisms principally involving natural killer cells and mononuclear phagocytes [23, 53, 54], thereby contributing to the destruction of infected cells. Thus immunopathogenic mechanisms are partly responsible for the formation of lesions.

Although neutralizing antibodies to B virus were shown to arise in an experimentally infected monkey by 17 days after infection [32], their presence does not appear to eliminate infection [7, 30–33, 39]. A partial explanation is the importance of direct cell-to-cell transmission of B virus, as demonstrated in vitro with monkey kidney epithelial cells [55]. Further detail is lacking regarding the specific immunologic regulation of natural B virus infections in macaques. Studies involving the related human HSVs [53, 54] suggest that both antibody-mediated and cell-mediated mechanisms help to control the extent of infection.

The suspicion that trigeminal ganglia of macaques harbor latent B virus was confirmed initially in 1975 by Boulter [30] and later that year by Vizoso [31] in experiments using explant cultivation of ganglia harvested from naturally infected rhesus monkeys. Earlier reports [24, 49] had implicated the trigeminal ganglia as a likely site for latency on the basis of clinical observations of natural infections in macaques and the resemblance of these infections to recurrent HSV-1-associated labial infections of human beings [56]. Zwartouw and Boulter [32] subsequently identified latent B virus in the lumbosacral sensory ganglia subserving the genital area of five of 11 cynomolgus monkeys tested; three of these five animals were also infected at the trigeminal site. In addition, six of 18 cynomolgus monkeys in one group asymptomatically shed B virus from genital and/or oral epithelium during an apparent outbreak of the disease; in their analysis, the authors concluded that B virus was transmitted venerally by a mechanism similar to that documented for HSV-2 (the cause of genital herpes) in human beings [32, 33]. According to Tribe [57], the late Dr. Alan Goffe of Wellcome Research Laboratories had postulated in 1966 that B virus was transmitted during sexual activity. However, unlike the situation reported in an outbreak involving the related herpesvirus SA 8 among group-housed animals of *Papio* species [47], no overt genital lesions associated with B virus in macaques were described [32]. The relative importance of either mode of natural transmission of B virus among macaques—genital or oral—remains to be determined.

B Virus Infection in Humans

Since the initial report recognizing B virus as a distinct biological entity [13], 25 well-documented cases of human infection have been described [2, 58–62]. Approximately two-thirds of these cases arose in the United States, with the remainder reported from Canada and Great Britain. Twenty-two cases (88%) progressed to various degrees of encephalomyelitis, which ultimately led to 16 deaths. Several additional case reports lacking complete documentation exist in the literature [2]. It is likely that the actual number of human cases has been significantly underestimated, particularly if hypothesized asymptomatic cases are considered [61–63].

A disturbing observation was the high frequency with which historical human survivors of disease caused by B virus were left with moderate to severe neurological impairment, sometimes requiring institutionalization [2]. Fortunately, the recent development of compounds efficacious in the treatment of B virus disease [11, 62, 64–66] may favorably alter the clinical prognosis for future cases. Rapid diagnosis and initiation of therapy are of paramount importance if the replication of B virus is to be arrested before its extension to the CNS [67]; standardized guidelines for prevention and diagnosis of B virus infection have been established, providing a ready reference for individuals working with macaques or their tissues [27, 68].

The mode of exposure to B virus has not been established for all human cases reported. Part of the difficulty in documenting this point has stemmed from the diversity of exposures possibly important among persons working directly with macaques, while incomplete knowledge of the pathogenesis of human B virus disease has added to the uncertainties. Frequently, it has been necessary to rely on secondary sources of information suggestive of specific direct exposure to monkeys, such as trauma logs or interviews with spouses and co-workers. In 20 of the 25 documented cases, there was an indication that the patient had somehow been directly inoculated with tissue or fluid from a monkey [2, 58–60, 62], i.e., via monkey bites, monkey scratches, or cage scratches (15 cases); direct contamination of a preexisting wound with monkey saliva (one case); cuts sustained from tissue culture bottles containing monkey kidney cells (two cases); or needle-stick injuries following use in macaques (two cases).

The remaining five documented cases involved the cleaning of a rhesus skull without the use of protective gloves but with no reported injury, apparent transmission from a B virus herpetiform lesion on a patient's forearm (bite wound) to an area of contact dermatitis on his wife's finger, possible respiratory exposure to B virus (two cases in patients presenting without ascending paralytic disease), and an unknown mode of exposure in an animal attendant who died 2 days after the onset of acute cranial neurological symptoms without substantial fever. (The animal attendant came into frequent contact with macaques during his work, and his wife re-

ported that he had often sustained scratches while handling animals; the last medically recorded bite wound occurred 4 months before his death, however. The authors of this report hypothesized a primary brain infection following entry of B virus through the nose, mouth, eye, or skin of the head, since the patient presented without ascending paralytic disease [69].) Although the single documented case of human-to-human transmission of B virus [59, 62] during a recent outbreak of the disease was distressing, no further transmission was noted among 159 contacts of those cases [62]; thus the risk of secondary transmission among humans is probably low. The apparent lack of confirmed human cases in Asian countries where B virus is enzootic among macaques is intriguing, especially in light of the high frequency of direct contact reported between monkeys and human beings [70]. It is likely that reduced access to medical care and limited availability of B virus diagnostic facilities have precluded the identification of sporadic human cases in that geographic region.

The pathogenesis of B virus infection in human beings appears to resemble that in macaques in that the neuronal route is primarily responsible for the dissemination of the virus within infected hosts [67, 71]. After infection, replication of the agent within the skin is usually associated with local and regional inflammatory changes. Regional lymph nodes draining the site of entry of the virus may be hemorrhagic and focally necrotic. Subsequent propagation of the virus within the peripheral nervous system rapidly progresses to central loci in the spinal cord and, eventually, to the brain. Inflammation and necrosis are typically evident in the pons and medulla, but intranuclear inclusion bodies are not a consistent finding [24]. Whitley [67] contrasted the generalized encephalitis frequently resulting from B virus infection of humans with the temporal lobe–restricted geography usually associated with HSV-1 encephalitis. Alternatively, the multiple-organ involvement observed with systemic human B virus disease [24, 67] resembled that reported to occur with HSV-associated visceral infections [72].

The clinical course of human B virus disease has typically followed—with varying severity—the description of the initial case summarized above. However, the small number of human cases reported has limited the ability to generalize in this regard; advances in diagnostic imagery and immunologic monitoring of infection [73] should help improve the clinical characterization of B virus disease. Available information suggests that the incubation period from exposure to symptomatic disease in human beings varies from <2 days [74] to >10 years (for the apparent reactivation of a latent infection already described) [37]. Death has ensued from as early as 2 days to ~6 months after infection [2, 62]. Improvements in intensive care medical technology will no doubt influence these data as additional cases develop [67].

Diagnosis of B Virus Infection

Virological Approaches

B virus has been propagated successfully in tissue culture and in laboratory animals, as indicated above. Most work has centered around the use of Vero cells or other established epithelial cell lines in vitro [18, 51, 75, 76] because of the added technical difficulties encountered in the use of laboratory animals; LLC-MK2 cells are reported to support the growth of B virus but not of the related HSVs [32]. Tissue swab specimens from oral, conjunctival, and genital epithelium—the documented portals of B virus shedding from macaques—are routinely submitted for B virus isolation. One report described the isolation of B virus from anal swab specimens obtained from a rhesus monkey that died following systemic B virus disease [51].

For cases of human B virus infection or suspected exposure, swab specimens, CSF, and punch-biopsied material from possible sites of viral inoculation (bite and scratch wounds) have been collected and tested for the agent [62]. B virus has also been identified in specimens of urine and stool from infected persons [62]. Direct examination of infected monolayers has indicated the presence of B virus by detection of characteristic cytopathic effects and inclusion bodies [28, 75], but neutralization studies with specific antisera are required [77]. A direct fluorescent antibody test for the in situ demonstration of B virus in tissues of experimentally infected mice has been reported [78].

Hilliard et al. [79] have described a technique for rapid and specific identification of B virus DNA (following Vero-cell propagation of virus from test specimens) by use of restriction endonuclease analysis. In another approach Eberle and Hilliard [80] used SDS-PAGE to identify the presence of radiolabeled B virus–infected cell polypeptides with high specificity. Together, these techniques have provided a standardized mechanism for rapid virological diagnosis of B virus disease in humans and nonhuman primates that is useful both in research and in clinical settings [62]. The obvious limitations of these methods are their reliance on the presence of sufficient titers of infectious virus in test specimens to allow for initial propagation and the permissiveness of established cell lines for field strains. This combination of difficulties has made the detection of asymptomatic shedding of B virus by macaques exceptionally difficult [32, 33, 39]. In addition, studies aimed at detecting latent B virus have depended on its initial reactivation through explant cultivation or cocultivation of neuronal ganglia, with a high probability of many false-negative results [32, 33]. Recently developed molecular methods for direct detection of viral nucleic acid, including in situ hybridization or amplification by the polymerase chain reaction, are not subject to these limitations and have proven valuable in studies of the related HSVs [81–84]. Application of these techniques to the study of B virus

would help tremendously in the rapid detection and characterization of B virus in macaque and human tissues.

Serological Approaches

Options for serological diagnosis of B virus infection have been hampered by extensive cross-reactivity between antibodies to HSV and those to B virus [85] and by necessarily rigorous biosafety requirements for laboratory research involving B virus [86]. Sera from B virus–infected macaques tend to contain high titers of neutralizing antibodies to HSV, but human sera containing antibodies to HSV can also neutralize B virus, albeit to a lesser extent [31, 87]. The high prevalence of HSV antibodies among human beings [34, 88] has therefore complicated efforts to confirm historical cases of human infection with B virus. Previously, human sera were evaluated for antibodies to B virus by serum neutralization or complement fixation [77, 87, 89], with B virus and HSV antigens used separately or in cross-adsorption tests. Hutt et al. [85] warned that if neutralization tests on macaque sera were based solely on HSV-1 antigen, a 50% error in the diagnosis of B virus infection could result. These authors also demonstrated enhanced serum neutralization of B virus when active guinea pig complement was incorporated. Unfortunately, the use of HSV-1 antigen alone has been advocated as a viable alternative for use in serum neutralization tests in macaques when either an appropriate biocontainment laboratory or B virus is lacking [31, 90, 91]. The consequence is a reduction in the test's sensitivity—but not of its specificity, since macaques appear not to serve as hosts for the HSVs [92]. Other approaches to the serological diagnosis of B virus infection have included a plaque-reduction assay and an indirect immunofluorescence test [77, 85].

There have recently been several notable advances in the technology available for the diagnosis of B virus infection. Katz et al. [93] have developed a competition enzyme immunoassay for differentiation of antibodies to B virus, SA 8 virus, and HSV-1 in human and simian sera. Incorporation of goat antibodies to human immunoglobulins for the enzyme conjugate in the assay helped improve the sensitivity of the test. A variant of this test involving the use of monoclonal antibody conjugates specific to various immunoglobulin isotypes has proven capable of quantifying IgM vs. various subclasses of IgG-specific responses to B virus infection. In this manner it has been possible to identify and characterize early antibody responses arising both in serum and in CSF of humans and macaques; these assays serve as useful tools for epidemiological investigations and for monitoring of patients' responses to therapy [73]. Concurrent evaluation of antibody reactivities to specific B virus polypeptides by way of immunoblot analysis [94] has added to the specificity of serological test results. Other investigators [76] have exploited the susceptibility of B virus to inactivation by contact with psoralen in conjunction with ultraviolet light, generating a dot-immunobinding assay for antibody detection that does not require high-level biocontainment facilities. Preadsorption of test sera with HSV antigen in that assay reportedly eliminated heterotypic binding without reducing test sensitivity.

The extensive sharing of immunologic epitopes by B virus, SA 8 virus, HSV-1, and HSV-2 should not be underemphasized. Antibodies to B virus are detectable in approximately half of the general human population irrespective of previous contact with monkeys [2, 25, 95, 96] and are considered to represent a heterotypic response attributable to HSV-directed immunoglobulins. In a survey of 113 laboratory workers, Cabasso et al. [96] found an increasing prevalence of antibodies to B virus with increasing age; the epidemiological pattern resembled that for antibodies to HSV-1 and HSV-2. Furthermore, pooled experimental lots of both simian and human γ-globulins were found to contain antibodies to B virus and those to HSV concurrently. Vizoso [75] discussed this phenomenon in the context of the diversity and inherent overlap in antigenic structure and biological behavior apparent among some herpesviruses. He considered the identification and classification of these agents to be biased by the context in which associated diseases and antibodies became manifest. In addition, he interpreted the pathogenesis of herpesviruses within hosts as a process subject to natural selection. Southern and Oldstone [97] presented a similar perspective for viruses in general and predicted a reevaluation of schemes of virus classification based on shared structure-function relationships underlying the molecular mechanisms of viral pathogenesis.

Epidemiology of B Virus in Macaques

On the basis of published data, B virus appears to be enzootic in all species of macaques. This characteristic is evident from serological surveys, rates of isolation, and/or observations of symptomatic disease in free-ranging rhesus [70, 98, 99], bonnet [100], cynomolgus [42, 90, 101], stumptailed [7, 33], Japanese [41], and Taiwan [41, 43] monkeys. A number of surveys have documented a high prevalence of infection among rhesus and cynomolgus monkeys maintained in domestic research colonies [33, 48, 49, 90, 91, 102–105]. Insufficient evidence exists to suggest that B virus is normally more prevalent in some macaque species or geographic regions than others. Nonetheless, a single unique population of cynomolgus monkeys introduced almost 400 years ago to the island of Mauritius does appear to be free of both B virus and type D simian retrovirus [106]. A research colony of rhesus monkeys established by Charles River Laboratories in 1974 on Key Lois, Florida, represents a second distinct population of B virus–free macaques [107]. Neither

of these groups arose through natural evolutionary processes, however.

A classification bias of unknown degree is apparent in the majority of published serological surveys of B virus; inadequacies in diagnostic methodology make precise interpretation or comparison of findings difficult. Most surveys have relied on serum neutralization and have lacked standardization with respect to the type of cell system employed, the concentration of B virus used, and the use of complement, all of which are known to affect sensitivity [2]. This problem has been further complicated by the frequent lack of proper methods of sampling, including a failure to consider sample size and a disregard for the attributes of the specific populations of monkeys studied. Most previous surveys must therefore be treated as convenience samples, nullifying direct comparison of the B virus prevalences reported. Finally, lack of information in published surveys regarding animals' specific origin, age, gender, housing, and time in captivity has eliminated the possibility of complete epidemiological interpretation of these data [2]. Thus many surveys have provided only general indications of trends or patterns that have yet to withstand critical scientific assessment.

Two observations from serological surveys have been consistent among studies: the proportion of macaques positive for antibody to B virus has increased after capture and caging of the animals in dense groups, and increasing age of the monkeys has been positively associated with the presence of antibodies to B virus. Sampling of wild-caught rhesus monkeys indicates B virus infection in 10%–35% of individuals overall, while up to 100% of group-housed captive rhesus monkeys have been seropositive in some surveys [2, 14, 33, 102–104, 108, 109]. These differences were interpreted by di Giacomo and Shah [102] to be a result of overcrowding in captivity. Keeble [49] suggested that among macaques B virus was transmitted primarily via salivary contamination of common food and water sources and secondarily via direct inoculation from bites and scratches. Separate studies confirmed that close contact was necessary for transmission of B virus by showing that individual caging eliminated the spread of infection [24, 57, 99, 102, 105, 110, 111]. Despite the reported respiratory-associated outbreak of B virus disease in a group of bonnet monkeys discussed above [52], the lack of transmission of the virus among adjacently caged macaques [33, 102] argues against the overall significance of the respiratory route. Aerosol transmission has been experimentally demonstrated in rabbits, rhesus monkeys, and guinea pigs [112]; however, a high inoculum ($\geq$13,000 $TCID_{50}$) was required before the titer of antibody to B virus rose significantly in rhesus monkeys.

The positive age association borne out by several surveys [33, 57, 90, 99, 101–105, 109] appears to resemble that observed in different human populations for the related HSVs [88, 113, 114]. The proportions of seropositive wild-caught macaques classified as juveniles, young adults, and adults have ranged from 0 to 28%, from 0 to 66%, and from 72% to 92%, respectively [33, 70, 90, 99, 102, 105]. This trend toward an older age group has also been apparent in a number of surveys of group-housed populations of captive macaques, although the proportion of monkeys seropositive has been moderately higher for all age strata [33, 102–105, 109]. In a recent serological survey conducted by this author and others [109], increasing age was highly predictive of B virus antibody positivity in rhesus monkeys (odds ratio = 11.34, $P < .001$). Overall, 97% of monkeys $\geq$2.5 years old were seropositive, whereas only 22% of younger monkeys were seropositive.

Van Steenis et al. [101] interpreted the distribution of antibody among wild-caught cynomolgus macaques as indicating a mechanism of slow horizontal transmission of virus. Zwartouw et al. [33] stated that most transmission of B virus was venereal; this assertion was based on the differential success of attempts to isolate B virus from oral vs. genital sites and from the associated sensory ganglia [32], the housing conditions under which B virus was and was not transmitted, and the age-stratified distribution of antibodies to B virus. These authors presumed that sexual instead of nonsexual modes of exposure predominated among postpubertal monkeys, but the agent was sometimes demonstrated concurrently in both oral and genital sites. In one outbreak of B virus infection involving 18 group-housed macaques, the single male present did not seroconvert until 2 weeks after six of the females [33].

Zwartouw et al. [33] also noted that the reactivation of B virus from latency was most facilitated by social stresses imposed by the assembly of adult monkeys into breeding groups, even if neutralizing antibody was initially either absent or present in low titer. Stress-associated reactivation was also reported by Keeble [49], who observed a seasonal rise in overt B virus disease among group-housed rhesus monkeys following the arrival of monsoons in his locality. At least three reports have described outbreaks of overt B virus disease developing in macaques concurrently with stresses imposed by inclement weather or transport from one location to another [42, 52, 115]. No trend in genital or oral shedding of B virus associated with the breeding season has been reported. Such an association would favor the importance of breeding activities (and concurrent social stressors) as they relate to the transmission of B virus among macaques.

It is interesting that no evidence has been presented for the vertical transmission of B virus between an infected female monkey and her offspring; such a pattern has been noted for HSV in human beings. Infant macaques born of seropositive dams apparently are themselves seropositive, but their titers decline after a few months and are presumed to represent antibodies of maternal origin [2, 33]. γ-Globulin is known to cross the placenta of primates [2]. Zwartouw et al. [33] documented shedding of B virus from the oral and genital mucosae of a cynomolgus monkey; although shedding continued

for 12 days, this animal's 10-month-old infant did not become infected. In a separate study, Zwartouw and Boulter [32] demonstrated a lack of reactivation and shedding of B virus among five pregnant female monkeys sampled for 3 months around parturition. Likewise, Weir et al. [111] failed to find evidence of shedding of B virus by 13 seropositive pregnant macaques tested weekly for 3 weeks after cesarean section or vaginal delivery.

Notwithstanding the progress to date, it is obvious that much remains to be learned regarding the epidemiology of B virus in macaques. The significance of venereal transmission of the agent among macaques must be substantiated in comparison with alternative mechanisms by which new hosts are exposed through a variety of portals over time. Serological and virological data have established that infections with B virus occur regularly in prepubertal monkeys unless they are caged separately. Prospective virological studies correlated with B virus serology, the need for which has been stated [67], would permit proper temporal assessment of exposure variables relative to infection and would ideally include efforts to distinguish sexual from nonsexual forms of contact among monkeys at risk. Aggressive behaviors are known to increase in groups of macaques during the breeding season [116], and this change might possibly confound the findings of Zwartouw et al. cited above [33]. The elucidation of factors important to the transmission of B virus between monkeys would greatly facilitate the agent's eradication from research colonies. Furthermore, human exposure to B virus could be minimized if the primary periods and routes of shedding of the virus by infected monkeys were defined.

Treatment and Prevention of Human Infection with B Virus

Antiserum and Vaccine Development

The observation that high-titered human antiserum to HSV neutralized B virus in vitro lent support to the idea that large doses of human γ-globulin might be useful in postexposure immunoprophylaxis for B virus infection [96]. Furthermore, this serological cross-reactivity was used as an explanation for the apparent rarity of cases of B virus infection in human beings despite frequent contact with infected monkeys [2, 15, 24, 108]. In one report [117] the concurrent inoculation of B virus and of antiserum to B virus was shown to protect rabbits from clinically overt disease. In that study it appeared that protection was due to the destruction of infected epithelial cells as opposed to the direct neutralization of the inoculum. Reliance on heterotypic protection by anti-HSV globulins was not advocated by the authors; rather, they suggested the use of simian antisera with confirmed activity against B virus. Unfortunately, the use of human γ-globulin for a limited number of cases of B virus infection has not been notably efficacious [68, 117], and prior exposure to HSV has not appeared to alter the clinical outcome of human B virus disease [62].

The recognition of neutralizing antibodies to B virus stimulated efforts to produce a B virus vaccine for use in humans and nonhuman primates [38, 98, 118]. A formalin-inactivated product that protected rabbits against challenge with a large inoculum of infectious B virus was developed [118]. Immunization of 253 people effectively reduced—but not to zero—the proportion of the population that was negative for antibody to B virus. Frequent booster injections were required, and the results indicated a heterologous HSV antibody response. A limited trial of this vaccine in wild-caught rhesus monkeys was inconclusive because of poor husbandry of the trapped animals. No B virus vaccine of proven efficacy is currently available [68].

The protection observed following vaccination of rabbits against B virus disease was suggested in a study by Hull and Nash [98] to result from a viremic phase of infection in that species—a process not yet shown to exist for humans [62]. In one study inoculation of rabbits with a suspension of HSV protected them against subsequent challenge with B virus via a heterotypic response, but B virus still was commonly found in the dorsal root ganglia in latent form [38]. In latently infected rabbits serum neutralizing antibodies were not present in high titers, and in several instances spontaneous reactivation of latent virus led to paralysis and death weeks to months after animals had survived the initial infection. That neutralizing antibodies may fail to eliminate B virus was shown in vitro by Black and Melnick [55], with cell-to-cell passage of virus predominant over cell-free dissemination in the supernatant. Cell-mediated immune mechanisms are known to be primarily responsible for limiting the severity of herpesvirus disease and for effecting clinical recovery [53, 54].

The development of vaccines to prevent or modulate HSV infections in human beings is currently an area of active research [119, 120]. The greatest benefit to date has been a reduction in the severity of primary or recurrent disease. The elimination of B virus from research colonies of macaques would require prevention of the initial establishment of latent infections that might subsequently reactivate. The current literature indicates that this goal has been achieved in only one circumstance involving analogous HSV-1 infections in a mouse model [121]. Furthermore, potential problems encountered in the serological verification of B virus–free status for macaque colonies would only be compounded by the availability of a vaccine strain of the agent.

Antiviral Drugs

Current approaches to the treatment of B virus disease in human beings have been directed by the availability of nucleoside analogues with established efficacy against related HSVs. Boulter and Grant [39] successfully prolonged the

survival of B virus–infected rabbits by parenteral administration of vidarabine (9-β-D-arabinofuranosyladenine). Later, Boulter et al. [66] investigated treatment with acyclovir (9-[(2-hydroxyethoxy)methyl]guanine) in B virus–infected Vero cells and experimentally inoculated rabbits; complete protection of rabbits from paralytic disease or death required an intravenous dosing schedule of at least 2 weeks' duration, with the initiation of treatment no more than 24 hours after inoculation of virus. No significant toxicity of this compound to mammalian cells in vitro has been documented. Other work [64] has demonstrated in vitro efficacy against B virus for the related nucleoside analogue ganciclovir (9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine, 9-[1,3-dihydroxy-2-propoxymethyl]guanine, or DHPG) in plaque-reduction assays using human fetal fibroblasts. Both products have been recommended for use against related human herpesvirus infections occurring alone or in combination with human immunodeficiency virus infection [122], but latent infections are not eliminated by such treatment.

Although the limited number of recent cases of human B virus infection has precluded appropriate quantitative evaluation of the antiviral efficacy of these products, a positive clinical outcome has been associated in at least four instances with the use of intravenous acyclovir (10–15 mg/kg every 8 hours) followed by oral therapy [59–62, 65, 67]. Zwartouw et al. [11] demonstrated greater efficacy of orally administered ganciclovir than of acyclovir in B virus–infected rabbits (even if treatment was delayed until 5 days after infection) and advocated the former as therapy for advanced B virus disease in humans. In three of four specific cases in which the protocol for the treatment of human B virus infection included ganciclovir (5 mg/kg intravenously every 12 hours for one case), therapy was unsuccessful, but the antiviral and pharmacokinetic properties of this agent support further consideration of its use. The patient's neurological condition at the initiation of therapy appears to be highly predictive of the outcome of treatment—an observation emphasizing the need for rapid virological and serological confirmation of infection [59, 62, 65]. Since patients confirmed to be shedding B virus may have established latent infections capable of subsequent reactivation, lifelong antiviral therapy is probably warranted. Moreover, the cessation of active shedding of virus should precede any alteration in the schedule of treatment.

Formation of B Virus–Free Macaque Populations

The establishment of B virus–free colonies of macaques has been proposed as a primary mechanism for the prevention of further human exposure to the agent [3, 33, 68, 123]. This approach is a logical extension of general guidelines established to reduce the likelihood of human contact with infected monkeys or their tissues [68, 108, 124]. Complete or partial success in this endeavor has been realized on several occasions [33, 57, 102, 107, 110, 125], but verification of the specific pathogen–free status of such groups has been limited by uncertainties about the accuracy (particularly the sensitivity) of available diagnostic tests. Some workers [102, 110] have indicated the successful exclusion of B virus via single housing of monkeys, but this practice has not been viewed as a favorable long-term solution since it promotes the development of socially maladjusted animals with poor breeding potential. Advances in laboratory technology for the diagnosis of B virus infection should be accompanied by efforts to evaluate the properties (particularly the sensitivity and specificity) of new tests within representative populations of macaques. The gold standard against which serological tests should be compared is difficult to envision since B virus is capable of remaining latent within the neurological tissues of the host. Some help in this regard may be found in the application of new molecular methods for the direct detection of viral nucleic acid, as discussed above. Finally, improved epidemiological understanding of B virus in macaques should complement the use of diagnostic screening tests for the eradication of B virus.

Summary

This review has highlighted developments in the biology of B virus in macaque and human hosts through an analysis of the major literature in the field. It has been a priority of this endeavor to present similarities between B virus and closely related alphaherpesviruses in their respective natural host species. Exciting developments in diagnostic technology have made possible tremendous advances in the study of B virus biology, but much obviously remains to be learned about the epidemiology and natural history of the disease. On a practical level the combined results of diagnostic and epidemiological research on B virus can be applied to the development of schemes to eradicate B virus from domestic macaque research colonies, thereby helping to safeguard human beings against infection in the future.

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Note Added in Proof

An additional fatal case of B virus disease occurred in a Texas Primate Center veterinarian during late October 1991 (Dan Dalgard, personal communication). This patient presented initially with nonspecific complaints, including myalgia, headache, nausea, and persistent fever. Neurological signs, including diplopia and dysphagia, did not become evident until the fifth day of disease. No self-report or written record of monkey bite or scratch wounds existed; the mode of B virus exposure for this

case was undetermined at the time this article went to press. This event emphasizes the need to consider the possibility of B virus infection in all individuals with medical complaints who have been in direct contact with macaques or their tissues, even when neurological involvement is not initially recognized. Continuing investigations may help to refine existing guidelines for the prevention of human B virus infection.

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