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Overviews

Monkey B Virus (*Cercopithecine herpesvirus 1*)

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Macaques are a particularly valuable nonhuman primate model for a wide variety of biomedical research endeavors. B virus (*Cercopithecine herpesvirus* 1; BV) is an α-herpesvirus that naturally infects conventional populations of macaques. Serious disease due to BV is rare in macaques, but when transmitted to humans, BV has a propensity to invade the central nervous system and has a fatality rate greater than 70% if not treated promptly. The severe consequences of human BV infections led to the inclusion of BV in the original NIH list of target viruses for elimination by development of specific pathogen-free rhesus colonies. In macaques and especially in humans, diagnosis of BV infection is not straightforward. Furthermore, development and maintenance of true BV specific pathogen-free macaque colonies has proven difficult. In this overview we review the natural history of BV in macaques, summarize what is known about the virus at the molecular level, and relate this information to problems associated with diagnosis of BV infections and development of BV-free macaque colonies.

Abbreviations: BSL, Biosafety Level; BV, B virus (*Cercopithecine herpesvirus 1*); ChHV, chimpanzee herpesvirus; HSV, herpes simplex virus; HVP2, *Herpesvirus papio 2* (*Cercopithecine herpesvirus 16*); HVS1, *Herpesvirus saimiri 1*; mAb, monoclonal antibody; ORF, open reading frame; RL, long repeat region; RS, short repeat region; SA8, simian agent 8 (*Cercopithecine herpesvirus 2*); SPF, specific pathogen free; CNS, central nervous system

Nonhuman primate research models are extremely valuable in biomedical testing. In the US particularly, macaques are historically ingrained in research protocols and provide a particular value over nonmacaque primates in certain types of research (for example, a nonhuman primate model for AIDS). Research macaques carry numerous viruses that can affect occupational health and safety as well as research results. Currently, conventional macaques are used in research widely due to a lack of availability of specific pathogen-free (SPF) macaques (that is, conventional animals being derived from colonies where specified infectious agents are enzootic, regardless of the test status of the individual macaque). Breeding of SPF research macaques is aimed primarily at the elimination of viruses that can confound or preclude certain research protocols (for example, simian retroviruses) as well as targeting agents that pose a considerable potential zoonotic threat. Each target agent poses a unique challenge in identifying infected animals and eliminating the viruses from the population.

B virus (*Cercopithecine herpesvirus* 1; BV) is a macaque α-herpesvirus that is similar to the herpes simplex viruses (HSV1 and HSV2) of humans. From an animal health standpoint, BV is not a serious problem in its natural macaque host. However, the fatal effect of zoonotic BV infection in humans has driven the effort to eliminate BV from research macaques. Historically, the case fatality rate in untreated human BV infection has been greater than 70%, a rate similar to untreated HSV encephalitis in humans.[16,66,84] Development and maintenance of true BV SPF macaque colonies has proven difficult for reasons that will be discussed in later sections of this manuscript. Several excellent reviews of BV in its macaque host and of human BV cases are available.[16,49,58,84,119,122] Here we briefly review the current state of knowledge regarding BV and refer the reader to these prior reviews for more details and references.

Although BV is immediately thought of whenever fatal neurologic herpesvirus infections and primates are involved, BV is not the only α-herpesvirus that occurs in monkeys. Related viruses have been isolated from chimpanzees,[65] baboons,[24,63,67] vervets,[68] langurs,[27] and several species of South American monkeys.[45,62,74] Serologic studies also suggest the existence of related α-herpesviruses in other primate species as well.[18,26,27,40,56] All monkey species are likely to have their own unique α-herpesviruses and those will probably all be related to some extent both genetically and antigenically. Primate α-herpesviruses other than BV can also cause fatal neurologic disease when they cross the species barrier. Fatal HSV infections have been reported in a number of nonhuman primate species,[39,47,64,70,91,101] and simian herpesviruses other than BV have been shown to cause fatal infections in primate species other than their natural host (Table 1). *Herpesvirus saimiri* 1 (HVS1), an α-herpesvirus of squirrel monkeys, produces severe and frequently fatal disease with disseminated multifocal necrosis of visceral organs and occasional involvement of the central nervous system (CNS) in both marmosets and owl monkeys,[29,45,52,62,74] and there is 1 unconfirmed report of a human case of encephalitis due to HVS1, where IgG titers to HVS1 increased but no virus was ever isolated.[102] Recently, a fatal infection in a black and white colobus monkey was described and shown to be due to infection with baboon *Herpesvirus papio* 2 (HVP2).[112]

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Table 1. HSV-like α-herpesviruses of nonhuman primates

Virus	Natural host	Fatal disease in other primate species	Genome sequenced?	Antigenic crossreactivity with HSV: ELISA / neutralization
ChHV	Chimpanzee	Not reported	Partial[65]	++++ / not reported[65]
BV	Macaque	Human;[49,84,119] DeBrazza, colobus, and patas monkeys[64,111,124]	Complete[89]	+++ / ++[21,42]
HVP2	Baboon	Colobus monkey[112]	Complete[114]	+++ / ++[21,42]
SA8	Vervet	Not reported	Complete[113]	+++ / ++[21,42]
HVS1	Squirrel monkey	Owl monkey;[29,52,62] marmoset;[45,74] human?[102]	Partial[20,83]	++ / –[21,42]
HVA1	Spider monkey	Not reported	Partial[20]	++ / –[21,42]

The references cited in the table are for general summary purposes and are not all-inclusive.

In mice, HVP2 also produces a rapidly fatal infection of the CNS very similar to human BV infections.[97] In addition, BV infections are not uniformly lethal in all nonmacaque species. Both humans and DeBrazza monkeys have survived BV infections,[2,8,15,46,84,104,111] and asymptomatic enzootic BV infection of brown capuchins has been reported.[12] Therefore, aside from its propensity to cause lethal infections when transmitted to humans, BV is very much like other closely related primate α-herpesviruses.

Molecular Biology of BV

Although known by several names (most commonly *Herpesvirus simiae*, monkey B virus, and herpes B) over the years since its isolation in 1932, BV has officially been designated *Cercopithecine herpesvirus 1* by the International Committee on the Taxonomy of Viruses.[31] BV has a virion structure typical of herpesviruses, with an icosahedral capsid embedded in an amorphous protein tegument and surrounded by a lipid membrane envelope.

Because of biosafety concerns and the relative rarity of human BV infections, little research has been conducted actually using BV; much of what is 'known' about BV is extrapolated from study of HSV1 and HSV2. Although all other primate α-herpesviruses are Risk Group 2 pathogens and can be studied under Biosafety Level (BSL) 2 containment, BV is classified as a Risk Group 4 pathogen that requires BSL3 or BSL4 facilities. The classification of BV as a 'select agent' by the Centers for Disease Control and Prevention together with the failure of the National Institutes of Health to include BV on their list of agents qualifying for bioterrorism research funding has further restricted and even discouraged research on this virus.

The genome sequence of the laboratory standard vaccine strain of BV (strain E2490)[50] has been reported by several laboratories,[78,79,89] and sequences for a number of individual genes of field isolates of BV have also been determined.[3,60,80,89,107,108] The DNA genomes of the simian α-herpesviruses have a very high G+C content, with BV having the highest of all α-herpesviruses, at 74.5%. Overall, the simian virus genomes are very similar to the genomes of HSV1 and HSV2 in their genetic organization: homologs of almost every HSV gene are present in the same order and orientation in the simian virus genomes. Consistent with the proposed coevolution of herpesviruses and their host species, phylogenetic analyses based on DNA sequence data indicate that BV is more closely related to the baboon and vervet viruses (*C. herpesvirus* 16 [HVP2] and *C. herpesvirus 2* [simian agent 8; SA8], respectively) than to the human HSV or chimpanzee (ChHV) viruses.[65,71] Where HSV1 and HSV2 differ from one another at the genetic level, BV and the other simian viruses more closely resemble HSV2 and ChHV than HSV1. For example, the *US4* gene, which encodes the gG glycoprotein, is considerably larger in HSV2 and ChHV than in HSV1, and the simian virus *US4* genes are similar in size to the HSV2 *US4* gene.[21,78,89,113,114]

Despite their organizational genetic similarity to human HSV, there are several differences in the simian virus genomes. Some of these differences are molecular details of genome organization. For example, many herpesvirus genes are arranged in cotranscriptional units, where multiple mRNAs use a single common polyA-mRNA termination site. As a result, mRNAs for the open reading frame (ORFs) located most 5′ in each set contain the coding sequence not only for the 5′ ORF but also 1 or 2 additional ORFs located 3′ to it before the polyA-mRNA termination site. The grouping of ORFs into transcriptional units varies somewhat between the human and simian viruses. For example, in HSV, the *US3* through *US7* genes are arranged in 2 transcriptional units, *US3+4* and *US5–7*, whereas in the simian viruses, the transcriptional units are *US3–5* and *US6+7*.[21,72,73] The functional significance of these differences is not known.

Several substantial differences also exist between the genomes of HSV and the simian viruses. One such difference is in the short repeat (RS) region of the genome. The region located between the 3′ end of the immediate early regulatory *ICP4* gene (*RS1*) and the end of the RS region is significantly larger in all of the monkey viruses than in HSV (1.8 kb versus 1.0 kb). Computer-based analyses suggest that a conserved microRNA may be encoded within this region of the BV, HVP2, and SA8 genomes.[19] A second example is in the long repeat (RL) region. In HSV1, HSV2, and ChHV, a gene (*RL1*) located in the RL region encodes the γ34.5 protein. Although the γ34.5 protein plays a central role in determining the neurovirulence of HSV in mice[123], there is no apparent homolog of the *RL1* gene in any of the simian viruses.[89,113,114] Even so, the sequence length between the flanking *RL2* gene and the end of the RL region is nearly the same in the human and simian viruses. Deletion of a portion of this *RL1* region in HVP2 only slightly decreased neurovirulence of HVP2 in mice, suggesting that this region does not play a central role in neurovirulence of the virus, as it does in HSV.[19] In HSV, ORFs on the opposite strand overlap the *RL1* ORF.[61,92] Again, computer analyses have failed to identify homologous ORFs in the simian viruses.[19,89,113,114] Therefore, the functional significance of this region of the BV genome (and those of HVP2 and SA8) is unknown at present.

From predictions based on DNA sequence data, variation in the amino acid (AA) sequence identity of homologous BV and HSV proteins ranges from 26.5% to 87.5%, with an average of 62.5% identity.[89] In contrast, AA sequence similarity values range from 70% to 99% (average, 95%) between rhesus and cynomolgous BV genotypes, 56% to 98% (average, 87%) between BV and HVP2, and 50% to 97% (average, 83%) between BV and SA8. This level

of AA sequence homology is consistent with previous studies that detected antigenic crossreactivity of almost all BV proteins with homologous HSV proteins.[22,42,88,115] Not surprisingly, many of the most conserved proteins are structural components of the virion capsid and enzymes. Although some glycoproteins are among the least conserved proteins, other glycoproteins show a strong degree of conservation. Again, this finding is consistent with the observation that many BV glycoproteins share antigenic determinants with homologous HSV glycoproteins, and explains the long-recognized crossreactivity of BV and HSV in neutralization assays.[22,36,51,115,116]

Despite the recognized crossreactivity of BV and HSV in virus neutralization assays and ELISAs, there is also a fair amount of virus specificity in that BV-immune sera react more strongly with BV antigen than HSV antigen. This difference can be explained by the fact that although most BV proteins do exhibit antigenic crossreactivity with their HSV homologs, many contain BV-specific epitopes in addition to crossreactive epitopes. In addition, several BV glycoproteins exhibit decreased sequence conservation with their HSV counterparts. Both the gG and gC glycoproteins have been found to be largely BV-specific antigens with respect to HSV, although they do crossreact to a greater degree with the homologous glycoproteins of HVP2 and SA8.[22,86,89,107]

Regarding the limited geographical occurrence of fatal BV infections in US and European research settings, rhesus monkeys may carry a strain of BV that is more lethal than are BV isolates from other macaque species. To address this possibility, a 1.2-kb region of the genome of several BV isolates obtained from various macaque species was amplified by PCR and sequenced.[108] This study revealed the existence of different genotypes of BV, each of which was particular to a specific macaque species. This initial observation has been confirmed by a number of subsequent studies.[80,111] However, testing of various BV isolates from different macaque species in mice has not supported the hypothesis that rhesus BV isolates are more lethal than nonrhesus isolates. Rhesus BV isolates exhibit a broad spectrum of neurovirulence in mice, ranging from producing no clinical signs of disease at one extreme to the rapid invasion of the CNS and death at the other.[94] Testing of different BV genotypes from nonrhesus macaques has revealed a similarly wide range of neurovirulence in mice.[93]

Little research on experimental pathogenesis has been done with BV. Historically, rabbits have been the animal model of choice, due to their extreme sensitivity to BV infection. Rabbits rapidly succumb to BV infection when inoculated by almost any route, including inhalation.[51] More recently, several investigators have used the mouse as a model system.[37,94] However, the age and particularly the strain of mice can have a dramatic effect on the ability of BV to establish a productive, lethal infection. Balb/c mice are susceptible to CNS disease by some BV strains, whereas C57 mice are resistant to all BV isolates tested.[94] The bias of the C57 immune response toward a Th1 cellular response rather than the Th2 antibody response bias exhibited by Balb/c mice likely is involved in the different susceptibility of these mouse strains. The very robust γ-IFN response in Balb/c mice suggests that immune-mediated tissue destruction may also play an important role in their susceptibility to BV.[19] In vitro studies have shown that the innate β-IFN response of mice to neurovirulent HVP2 strains is poor, and β-IFN does not limit viral replication, whereas apathogenic HVP2 strains induce a robust β-IFN response, and viral replication is effectively controlled.[96] Infection of mice lacking the β-IFN receptor (and thus unable to respond to β-IFN) with apathogenic strains of HVP2 results in invasion of the CNS and death with the same time course as neurovirulent strains.[95] These studies suggest the possibility that the ability of the host to mount an effective local β-IFN response at the site of infection could be a critical factor in determining the ability of BV to successfully infect nonmacaque species.

The sensitivity of BV to various antiherpetic drugs has been investigated, but most such studies predate many of the current antiviral drugs used to treat herpesvirus infections. BV is susceptible to acyclovir, but it is approximately 10-fold less sensitive than HSV.[7,128] Further, despite the similarity of the HSV and BV thymidine kinase enzymes (approximately 65% amino acid sequence similarity and equivalent K_m values for thymidine), the BV enzyme does not phosphorylate acyclovir or related antiviral compounds, which is a prerequisite for their antiviral effectiveness.[33] Testing of a number of current antiviral drugs against several BV isolates showed that almost all drugs were less inhibitory against BV than HSV.[33] Even so, several drugs are effective against BV in vitro, including penciclovir and ganciclovir. Notably, mutants resistant to high levels of these drugs occur spontaneously in tissue culture.[19]

Biology of BV in the Natural Host

Understanding the biology of BV in macaques is critical in devising attempts to identify and eliminate the virus from a macaque colony. BV is horizontally transmitted between macaques, and the prevalence of BV induced antibodies in a population is generally related to age, with the percentage of positives increasing progressively from infant to juvenile, adolescent, young adult, and mature adult status.[120,121,127,129] Transmission in macaques can occur through oral, ocular, or genital contact of mucous membranes or open skin lesions. Macaques younger than 1 y can be infected with BV associated with intimate contact with an infected mother. In baboons, horizontal oral transmission of HVP2 from mother to infant as well as between infants has been reported.[23] In both macaques and baboons, viral exposure and infection increases markedly as animals become socially and reproductively active in the prepubescent and pubertal period (2 to 4 y of age).[25,85,120,121,129] In wild populations of macaques and in conventional colonies where animals are not segregated, the prevalence of anti-BV antibodies in adults can range from 70% to nearly 100%.[54,82]

As for HSV, the majority of animals experiencing primary infection with BV typically do not exhibit any overt clinical signs of disease, although orofacial or genital lesions are seen occasionally.[1,58,59,119,122] Initial virus replication occurs locally at the mucosal site of infection and induces an immune response in the host, resulting in the appearance of both antiviral antibody and cell-mediated immune responses. As the virus replicates, it enters sensory neurons serving the site of infection and is transported intraaxonally to the neuron cell body. In the normal course of infection, the virus then becomes latent in neurons of sensory ganglia serving the site of infection. During latency, the virus exists in a nonreplicating state and is protected from the host's immune response. In some instances, primary infections may not terminate with the establishment of latency, but rather continue to progress as generalized infections that spread throughout the body and are frequently fatal.[1,9,14,105]

Once a latent infection has been established, the virus remains in sensory neurons for the life of the host. Periodically, the virus

can reactivate from the latent state. Virus is transported down the neuronal axon to the mucosal epithelium, where it undergoes productive replication. These recurrent infections result in shedding of infectious virus. Most recurrent infections are not accompanied by clinically apparent lesions; thus, healthy animals can shed infectious virus asymptomatically. The frequency of virus shedding appears to be quite low (2% to 3%) under typical husbandry conditions.[48,49,120] Stress related to social challenges, transportation, immunosuppression, or a new housing environment can trigger reactivation and shedding of BV in infected macaques.[10,75,127] Furthermore, in rhesus monkeys reactivation of BV and shedding appears to occur primarily during the breeding season.[48,75,120,127,129] Potential factors associated with reactivation due to stressors and breeding could relate to hormonal changes.

Human BV Infection

In 1932, a medical researcher was bitten on the finger by a macaque being used in poliovirus research.[36,98] The researcher developed typical herpetic lesions on the finger, but the infection then progressed to involve the CNS. The patient eventually died from an acute ascending myeloencephalitis. A herpesvirus was isolated from several tissues and, although initially identified as HSV, was subsequently shown to be distinct from HSV and was designated as 'the B virus'.[98]

Since this first case, a number of additional human infections have occurred sporadically over the years.[16,49,84,119,122] Although not numerous, BV infections in humans are notorious for their severity. Untreated, human BV infections have a fatality rate of 70% to 80%, with many survivors having marked neurologic deficits and others experiencing a progressive decline in neurologic functions. Almost all human cases have been associated with bites or scratches received from macaques. However, additional modes of transmission have been implicated in some cases, including splashing of macaque urine into the eye, needlestick injury, and contamination of cuts with material from primary macaque cells in the laboratory. Not surprisingly, most persons infected with BV have been animal care personnel, veterinarians, and laboratory researchers.

With the rapid prophylactic treatment of persons exposed to BV and the ready availability of more antiviral drugs, more people are surviving BV infection.[2,8,15,46,104] Because α-herpesviruses rapidly enter the sensory nervous system during the early stages of infection, the potential exists for survivors to harbor latent BV. The appearance of clinical signs without any known potential BV exposure immediately prior to the appearance of clinical signs or after initial resolution of BV infections both suggest that latency not only occurs but that reactivation of latent virus can be associated with clinical symptoms as well.[15,32,46,104] Survivors harboring latent BV raises the specter of human-to-human transmission of BV, but only 1 such case has ever been described.[46] In this case, the infected person had extensive close and direct contact with a BV patient (her spouse) during the acute disease phase of infection. Testing of more than 130 other persons, including health care personnel, who had contact with these 2 patients failed to detect any additional cases of BV. The authors concluded that the risk of human-to-human transmission in the absence of close, direct contact is low.

The clinical course of BV infections in humans can vary considerably.[122] Initial symptoms of infection usually develop 1 to 3 wk after exposure, although in a few cases, initial symptoms developed considerably later. In addition, initial symptoms can vary, including nonspecific flu-like illness, vesicular herpetic lesions at the injury site, and symptoms associated with infection of the peripheral or central nervous systems. The progression of clinical symptoms associated with advancing infection also varies among persons. The virus usually spreads along nerves to the spinal cord and brain. Brainstem encephalomyelitis develops in the terminal stages of the infection. Once BV reaches the brainstem, the outcome is almost always death. Many persons surviving BV infection have residual neurologic sequelae, and progressive neurologic deterioration also can occur. In one case, a person developed clinical symptoms of infection years after their last known exposure, raising the possibility that asymptomatic primary infections can occur, with fatal disease due to reactivation of latent BV.

One peculiar aspect of human BV infections is that they have been reported only in the US and Europe in persons working with macaques or macaque tissues in a husbandry or research environment. A serologic survey of persons working with macaques for many years revealed no evidence of asymptomatic BV infections.[35] In India and Southeast Asia, people often live and work in close proximity to wild macaques and experience repeated exposure events (bites and scratches), but there are no reports of fatal BV infections in these areas. Wild macaques are known to be seropositive for BV, and a recent study documented the frequent occurrence of bites and scratches among both monkey temple workers and tourists visiting these sites.[30,54] Although serologic analysis of monkey temple workers suggests that some persons may have experienced BV infection,[53] they showed no evidence of clinically apparent infections.

Several explanations could account for the lack of fatal infections in Asia. One possibility is that due to the rarity of clinical cases of human BV infections, fatal BV infections may not be diagnosed accurately. Further, captive macaques in the US may be maintained in a comparatively more stressful environment than that of wild macaques. Because various kinds of stress are known to result in reactivation and shedding of infectious BV, captive macaques may have a greater probability of shedding high levels of virus at any given time, increasing the likelihood of transmitting BV. However, several Asian nations do have large captive macaque facilities that are operated like US breeding colonies, but again no incidents of human BV infection have been reported in these facilities. Regardless of geographic setting, there may be an inherent inequality in comparing the risk of human exposure when handling captive macaques on study versus random encounters with wild macaques. Manipulation of macaques on study will increase the frequency of potential exposures. In addition, the research protocol may involve immunosuppressive test articles or physical or behavioral stress on the macaques, either of which could potentiate reactivation and shedding of infectious BV. In any case, the reason for the limited geographic occurrence of human BV infections remains a mystery.

Although SPF macaques are available for use in biomedical research, nonSPF macaques continue to be widely used. Because BV is so prevalent in macaques, human exposures continue to occur. Guidelines have been developed for first aid after an exposure incident, testing of persons potentially exposed to BV and the monkey involved, and recommendations for prophylactic antiviral treatment.[9] All relevant information regarding sample collection and specimen shipping are available via the Internet from the National B Virus Reference Laboratory.[41]

Approaches to BV SPF Colony Development

A goal of developing and providing BV-free macaques is not a recent concept. In 1972, DiGiacomo and Shah[17] noted a virtual absence of BV in laboratory reared infant rhesus and that BV spread within the colony could be limited by individually caging the animals. In 1976, after the capture of wild rhesus monkeys in India, Charles River Laboratories followed a plan of individual caging with repeated testing and special handling to preclude cross-contamination in order to derive a select founder population for a breeding colony of BV test-negative rhesus. These animals were segregated on an island in the Florida Keys for production.[82] Pursuing the development and maintenance of SPF macaques requires a commitment of financial resources to 1) perform diagnostic screening of founder animals, as well as continued surveillance testing of the colony; 2) provide proper housing and welfare of animals during screening or quarantine; and 3) absorb production losses due to culling of infected or suspect animals from the population.

Although the ideal goal is to eradicate BV from research macaques, the practical goal may be to reduce the risk of occupational exposure and infection. Reducing the risk of occupational exposure to BV should not focus solely on removing BV from the colony animals. Risk reduction also relies on appropriate technical training of the research staff, implementation of safety and postexposure protocols, correct use of personal protective equipment, study design to limit manipulation of the animal as much as possible, and acclimatization of the research macaques to their environment and the study procedures. Development of truly BV-free colonies of macaques has proven to be very difficult.[38,43,99,117,118,125,127] Although efforts to establish BV SPF colonies have made considerable progress, failure of available antibody testing to detect seronegative latent BV-infected animals remains an obstacle in eliminating all infected animals. In one retrospective survey, even with the institution of an aggressive surveillance program to establish a BV SPF rhesus colony, seropositive macaques were detected as late as 7 y into the program.[43] The practical goal of reducing occupational infection with BV requires full commitment to implementation of employee training and education regarding the importance of reporting, following up on each contamination exposure event, and the correct use of personal protective equipment. Regardless of the recorded SPF status of a macaque, every case of human infectious exposure should be pursued as though the animal were positive and shedding BV.[11]

Diagnosis of BV Infections

Diagnosis of BV infection in macaques usually is accomplished by serologic testing. Although use of homologous BV antigen is most desirable, working with large quantities of infectious virus to produce BV antigen preparations poses considerable biohazard concerns. Several serologic assays use HVP2 or SA8 antigens to take advantage of the close genetic and antigenic relationship between BV and other simian α-herpesviruses.[57,81,110,126] In some cases, the HVP2- and SA8-based assays are just as sensitive for detection of BV-positive macaques as are those that incorporate BV antigen. Although HSV1 is used as an alternative antigen, the signal:noise ratio is not as great as those for assays using monkey virus antigens, making HSV-based assays slightly less sensitive.[81] Notably, none of these assays can identify which virus is infecting a particular positive monkey; it is assumed that macaques are infected with macaque BV.

Diagnosis of human BV infections is a far more difficult problem for a number of reasons. Because of the severe nature of BV infections in people, rapid diagnosis is essential. Although serologic tests are a mainstay of diagnostics, they rely on detection of antiviral antibodies and thus are unable to detect infections until at least 7 to 10 d after infection, when an antiviral immune response has developed. As mentioned earlier, most BV proteins crossreact with analogous proteins of HSV.[22,42,88,115] Because most adult humans are infected with HSV1, HSV2, or both, anti-HSV antibodies in human sera will react with BV antigen, giving false-positive results. Furthermore, when BV infects an HSV-immune person, an anamnestic response to crossreactive antigens occurs similar to the "original antigenic sin" phenomenon described for influenza virus.[34] This stimulation of antibodies to crossreactive antigens makes detection of BV-specific antibodies all the more difficult.[20] Many patients are treated prophylactically with antiviral drugs after a suspected BV exposure incident. This practice can impede BV replication, thereby lessening the development of an immune response to BV. Therefore, serologic assays for detection of human BV infections must be both sensitive and virus-specific to detect antibodies that are specifically directed against BV.

Development of serologic assays that can reliably differentiate BV from HSV infections is also problematic. One approach used is to adsorb human sera with HSV antigen, thus removing serum antibodies that react with HSV antigen prior to testing for anti-BV antibodies.[55] Although this approach can reduce the sensitivity of assays, it has successfully been used to diagnose BV infections. Another approach has been to use recombinant DNA technology to express individual BV genes and use the recombinant proteins as antigens.[86,90,107,109] The high G+C content of BV genes makes efficient prokaryotic expression of BV genes difficult and inefficient, but expression using the insect baculovirus system has proven more successful.[86] Because most BV proteins possess crossreactive antigenic determinants that are present on analogous HSV proteins, expression of smaller portions of particular antigens has been used to produce antigens that are more BV-specific than are the intact proteins. Despite advances in the molecular biology of BV and the promise of recombinant antigens, no truly BV-specific serologic assays are available at present.

Monoclonal antibodies (mAbs) react to individual epitopes and have proven useful in diagnostic assays for many different viruses. In an attempt to capitalize on this promise, several laboratories have developed mAbs to BV.[5,6,13,76] Not surprisingly, the vast majority of anti-BV mAbs recognize epitopes common to BV and HSV. Although BV-specific mAbs have been isolated, the epitopes they are directed against have proven not to be consistently recognized by infected macaques, limiting the usefulness of these reagents in diagnostic assays. In one case, a BV-specific mAb to the gB glycoprotein (which is consistently recognized in infected animals) was isolated. However, the mAb was not diagnostically useful because its binding was inhibited by crossreactive antibodies in immune serum that presumably were directed against a nearby epitope, resulting in false-negative results.[6]

Test sensitivity is of vital importance for SPF colony development, because retaining unidentified infected animals (that is, infected but with negative test results) in the colony will lead to the eventual failure of the SPF status of the colony and the opportunity for BV transmission to additional animals.[38,125,127] Specificity of a test method may be somewhat less of a concern during initial screening of the colony. However as the prevalence of true

BV-infected animals decreases, the consequences of removing a false-positive animal can be considerable. A lot of time and money is invested in a well-established breeder animal, and the effect of disturbing the social order of an intact breeding colony by removal of an animal must also be considered. The use of BV recombinant glycoproteins reportedly provides high diagnostic potential for detection of antibodies to BV.[86] Results from this study indicate that an ELISA test using multiple recombinant glycoprotein antigens (gB, gC, gD, and membrane-associated gG) may provide the highest sensitivity and specificity for diagnostic testing. In addition, the gG antigen is useful in discriminating between antibodies elicited to BV rather than by other closely related α-herpesviruses. Recombinant BV antigens are safe to use in the clinical laboratory setting, are safe and economical to produce, and can easily be standardized. Such diagnostic tools may be very valuable assets in monitoring SPF colonies.

For rapid diagnosis, PCR assays have the advantage that they can rapidly and specifically detect minute quantities of virus rather than relying on development of a host immune response to the virus. PCR can be used to test swabs from the site of a bite or scratch for the presence of BV. In the case of bites or scratches resulting from a monkey, the animal itself can also be tested to determine whether it was actively shedding BV at the time of the incident, thereby providing some idea of the likelihood of transmitting the virus. However, PCR is not useful for identification of latently infected monkeys (or humans), due to the infrequent and intermittent nature of virus shedding.

Several laboratories have developed PCR assays for detection of BV, and some have even proven their use in diagnosing human infections due to BV versus HSV.[4,44,87,103,106] Although its extreme sensitivity makes PCR ideal for detecting small amounts of virus in diagnostic specimens, this sensitivity can be a limitation as well. BV isolates from different macaque species exhibit substantial variation in their DNA sequences. In addition, sequence variation occurs among different strains of BV from rhesus monkeys.[89,108] The availability of genome sequence data for a single attenuated BV strain makes it difficult to quantitatively assess the extent of sequence variation among BV isolates. Even so, for a PCR test to be a reliable diagnostic assay, it needs to be not only specific for BV (versus HSV) but also must be validated by using multiple BV strains and genotypes to ensure the ability of the assay to detect all BV strains.

Establishment and Maintenance of SPF Colonies

Initial development of an SPF colony is based on restricting physical contact of candidate macaques, repeated screening to identify infected animals, and immediate removal of positive animals from those that have tested negative for BV. Removal requires culling of known infected animals from the SPF program coupled with stringent management practices to prevent cross-contamination from conventional macaques. If selecting candidates from a conventional population, age should be considered. Macaques younger than 18 mo are less likely to be infected with BV than are older animals.[58,120,121] However, the time and cost associated with rearing younger animals to prime reproductive age will be higher, and the behavioral effect of initial single-cage housing of young monkeys must be addressed. In this respect, the animal's psychological welfare and future value also must be considered. The risk of developing self-injurious behavior appears to be increased, particularly in male rhesus monkeys, by adverse life experiences during the first 2 y of life and by subsequent stress.[77] In addressing BV SPF colony formation in rhesus monkeys, Schapiro and colleagues[100] noted the importance of behavioral management of young animals to mold future social and parenting success.

Monkeys selected as SPF candidates by negative test status must undergo continued BV surveillance by use of standardized test methods and schedules. Tailored protocols need to be devised for husbandry, care, and use of the selected animals to prevent cross-contamination. Frequent testing is advisable during the first several years an animal is in the SPF program. This process requires a period of continued individual housing or housing in small peer groups to minimize program losses due to infections, because all animals in a cohoused group where one animal tests positive must either be removed from the program or tested more frequently to ensure their continued negative status.

Multiple schemes have been used for BV SPF colony development, largely because different institutions have different management priorities and financial and facility constraints. Individual or small group (2 or 3 animals) caging is recommended for rhesus macaques for 6 to 18 mo during the initial screening period. Because of the variable patterns of seroconversion in some animals,[43,118] 6 mo should be the minimal initial testing interval. Experience indicates that the use of several antibody test methods as well as confirmation of equivocal test results by using different diagnostic laboratories helps to improve the overall accuracy and reliability of a surveillance program.

Identification of actual BV infection in macaques is complicated by the fact that after infection, most animals do not display obvious clinical signs such as conjunctivitis, orofacial or genital herpetic lesions. Such lesions may be rare in animals after initial infection or reactivation of the virus brought on by stress or immunosuppression.[49] Regardless, identification of herpetic lesions is sufficient grounds for culling animals from the SPF program.

As discussed earlier, diagnostic techniques to identify BV-infected macaques are complicated due to the nature of the virus and limitations of different test methods. Viral testing by PCR is more sensitive than viral culture methods.[117] However, testing by PCR or virus isolation can at best be only randomly successful in live monkeys, due to the fact that latent virus in sensory ganglia will not be detected; these tests will be positive only when the latent virus reactivates and infectious virus is shed, and this process occurs sporadically and infrequently.[120] Further, BV typically is shed in oral and genital secretions in the absence of any detectable lesions. To improve overall surveillance for BV, the trigeminal and sacral ganglia of animals culled from an SPF group can be harvested for PCR testing. This additional screening is especially important in well-established colonies, where the expected true prevalence of BV infection is relatively low. Such postmortem testing should definitely be instituted for culled animals that have shown nonnegative or ambiguous BV antibody test results after previous negative test results.[118] The danger inherent in collecting and handling tissues from potentially infected macaques should be appreciated.

Numerous challenges confront using antibody tests to identify infected monkeys. Even with stringent enforcement of testing and segregation of negative animals, BV-positive animals may surface after many years.[43] Although BV antibody test methods have been refined over years of work, no test is perfect. Some macaque serum samples exhibit high nonspecific background,

confusing test interpretation. Reliance on BV antibody testing is frustrated further by a variable course of antibody development in some macaques. Fluctuations in serum antibody titers over time have been noted. Limitations of test sensitivity and specificity also can be a problem, especially in well-established SPF programs. Colony management must include a plan to respond to specific nonnegative test results from individual animals over serial testing.

An IgG antibody response to primary BV infection in rhesus monkeys is generally detectable within 14 to 21 d. Over time, however, because BV establishes latency, antibody titers can decline when animals are not exposed repeatedly to the agent. Furthermore, not all macaques display a conventional serologic response to infection. Some macaques repeatedly tested in SPF colonies have shown a pattern of progressively increasing seroreactivity, with a few interspersed episodes of negative test results. Other macaques may show a cycling pattern of reactivity, with multiple episodes of negative test results.[117] In addition, a subpopulation of SPF rhesus macaques in the cited study exhibited changes in their levels of seroreactivity. Review of all BV tests for individual animals in an SPF colony revealed that 89% had consistently negative serologic tests by ELISA and Western blot; the remaining 11% showed a variable pattern of test results.[117] In this subgroup, a strong ELISA titer (greater than 1:500) or a positive result on a confirmatory test was considered the best indicator of seroconversion. Evaluating patterns of seroreactivity thus can be very useful in identifying BV-infected animals during SPF colony development and ongoing maintenance surveillance.

The positive predictive value of BV testing is a function of true prevalence of BV infection within the cohort being tested. As the true prevalence of infection falls, so too does the positive predictive value of the test. For this reason, wide ranging epidemiologic surveillance of a colony is necessary for evaluation of overall antibody prevalence and possible associations with changes in seroreactivity. For clarification, a few examples of how true prevalence of an agent affects the predictive value of a test are warranted. Consider a population of 10,000 macaques being screened with a test that has a diagnostic sensitivity of 98% and a diagnostic specificity of 95%. If the estimated prevalence of BV-infected animals is 30% of the population (that is, 3000 animals), the positive predictive value (to identify true positive animals among all positive test results) is 89.4% (350 false-positive tests), and the negative predictive value (to identify true negative animals among all negative test results) is 99.1% (60 false-negative tests). However, if the estimated true prevalence of BV infected animals drops to 1% of the population (that is, 100 animals), the positive predictive value is reduced to 16.5% (495 false-positive tests), whereas the negative predictive value approaches 100% (2 false-negative tests). This example demonstrates why the evaluation of test results in well-established SPF colonies is so challenging.[41]

To emphasize the difficulty in interpreting nonnegative BV test results from samples derived from macaque populations that historically appear to be free of BV, consider the unique example of cynomolgus monkeys from the island country of Mauritius. These monkeys were introduced as the sole nonhuman primate species on the island more than 400 y ago, presumably originating from monkeys kept as pets by sailors. In one study, 102 free-ranging cynomolgous monkeys on Mauritius were trapped and screened for antibodies to HSV1, and all were found to be negative.[69] Serologic screening for BV carried out at 2 different diagnostic laboratories on several thousand sera from Mauritius macaques drawn soon after importation to the United States in 1995 through 1998 confirmed that these monkeys were seronegative, with rare equivocal or positive test results.[28] However, the Mauritius cynomolgus monkeys, whether free-ranging or in captive colonies, have not participated in any testing program to cull or control BV, nor have any of the rare nonnegative animals been segregated from the rest of the population. If animals with equivocal test results are truly infected with BV, then in the absence of any actions to prevent virus transmission, a dramatic change over time in the seropositive status of adults from an initial 0% to the greater than 70% positivity typical of adult macaque populations would be expected. However, this expectation has not been realized. In fact, no animals from this population have ever been identified as being BV-infected, even with additional test methods. This result, together with the epidemiology and management of the colonies on Mauritius, suggests that the rare nonnegative test results are likely due to test limitations in sensitivity or specificity. Although the Mauritius population appears to be truly SPF for BV, this issue remains a topic of debate.

Several other possibilities may explain rare reactive or equivocal test results encountered in testing for BV in SPF colonies. One is that such results could represent an immune response to an as-yet unidentified herpesvirus that is somewhat antigenically related to BV. Another possibility is that these animals may be infected pre- or perinatally, but they never experience a recurrent infection of sufficient severity to induce a typical immune response to BV. Were this the case, some animals should reactivate latent BV during their life and shed virus, with a subsequent rise in antibody titers within the population; this pattern has not been noted in the Mauritius macaques. Further, the nonnegative test results may represent some sort of nonspecific immunoreactivity. In both macaque and baboon SPF colonies, animals that exhibit repeated equivocal test results have been identified, and sometimes this equivocal reactivity extends to multiple infectious agents. Future monitoring of SPF programs may reveal additional confounding or associated factors relative to variable test results. These factors may include age, length of time in the colony, administration of biologics, medicine or test articles, seasonality, and so forth. Given the questions surrounding the nature and meaning of equivocal test results, the safest action is to remove these animals from the SPF program. Interpretation of nonnegative test results from animal within an SPF program should be evaluated critically by the colony management in collaboration with personnel from the diagnostic laboratory to meet the program goals.

Summary

BV is a natural pathogen of macaque monkeys, and in any typical population over 75% of adult animals are infected. As for HSV, the primary mode of transmission of BV is by direct contact with an animal actively shedding infectious virus. Oral infections predominate in infant and juvenile macaques, whereas sexual transmission appears to be the primary mode of transmission in adult animals. BV establishes latent infections in sensory ganglia and can spontaneously reactivate, resulting in periodic shedding of infectious virus in the absence of visible lesions. Although BV infection is of little consequence in macaques, the virus can produce fatal infections involving the CNS in other primate species, including humans.

At the molecular level, BV is closely related to other α-herpes-

viruses of nonhuman primates and is most closely related to HVP2 of baboons and SA8 of vervets. Why BV is so lethal in humans, whereas human infections by the very similar viruses of baboons and vervets have never been reported, remains an open question. BV, HVP2, and SA8 share extensive DNA and amino acid sequence homology and exhibit extensive antigenic cross-reactivity due to shared epitopes. Although the level of antigenic crossreactivity is less than with other simian viruses, BV also shares many antigenic determinants with HSV. HSV is prevalent in the human population, therefore most adults have anti-HSV antibodies, thus complicating serologic diagnosis of human BV infections. Sequencing of the BV genome has led to the identification of BV genes encoding proteins that are likely to be more virus-specific in their antigenicity, opening new possibilities for development of more sensitive and specific diagnostics tests for BV through recombinant DNA technology.

The severity of BV infections in humans makes BV the primary zoonotic concern for persons working with and around macaque monkeys. To address this concern, much time, expense, and effort has gone into the development of SPF macaque breeding colonies. Although such colonies are currently producing animals for use in biomedical research, there continue to be breaks in the BV SPF status of these colonies, raising questions about the sensitivity and specificity of the assays used for screening purposes as well as the true meaning of equivocal test results. Use of SPF monkeys undoubtedly decreases the probability of occupational zoonotic BV infections, but as long as breaks continue to occur in SPF colonies, injuries sustained by personnel working with SPF macaques must be treated the same as though the animals harbor BV. Although much remains to be learned about BV, recent classification of BV as a select agent and the accompanying restrictions are likely to severely limit active research on this important pathogen in the United States.

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