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CHAPTER 34

Virology Research

Peter Barry[1,2], Marta Marthas[1], Nicholas Lerche[1], Michael B. McChesney[1] and Christopher J. Miller[1,2]

[1]California National Primate Research Center and [2]Center for Comparative Medicine, University of California, Davis, California 95616, USA

Introduction and scope

Viral infections are responsible for significant morbidity and mortality in humans. In 1918, the influenza A pandemic was responsible for over 40 million deaths worldwide. As of December 2002, 60 million people had been infected with HIV and one-third of those infected were dead. Further, emerging infectious diseases often have a viral etiology as evidenced by the recent outbreaks of a newly discovered coronavirus that produced Severe Acute Respiratory Syndrome (SARS) epidemics in Asia and North America and of monkeypox in the U.S. Chronic infections with hepatitis B and C viruses and herpes viruses are also significant public health threats and will remain so for the forseeable future. Clearly viral infections remain a significant threat to human health. Thus, considerable efforts are expended to develop vaccines and therapeutics to control both acute and persistent viral infections.

The outcome of human viral infections depends on a complex interaction between the virus and the host. Genetic polymorphisms in the human population and the dose, strain and route of virus inoculation all contribute to the wide range of clinical outcomes in viral infections. The study of viral pathogenesis, and the preclinical development of viral vaccines and therapeutics, requires the use of animal models. The exquisite specificity of viruses for their cellular receptors and the absolute requirement of viruses for appropriate intracellular machinery defines the host cell tropism of viruses and explains why viruses tend be adapted for infection of particular host species. The relative genetic homology of humans and nonhuman primates (NHPs) means that there is considerable conservation of the critical molecules used by viruses in their life cycle.

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Thus NHPs often are the only species that can be infected with human pathogens. Alternatively, a number of viruses endemic to NHPs are distinct but closely related to human pathogens and are good models for the related human infection. Further, the development, genetics, function and anatomy of the immune system of nonhuman primates and humans are very similar which makes nonhuman primates very useful for understanding human immunity.

Excellent reviews document the historical role of NHPs in viral research (Renegar, 1992; Soike *et al.,* 1984), including the critical role that monkeys played in elucidating the pathogenesis of poliovirus and in the development of the poliovirus vaccine (Sabin, 1965). This review will focus on contemporary studies employing NHPs to model viral diseases currently of greatest public health concern.

Acute viral diseases

Systemic infections

Hantavirus

Hantavirus (genus *Hantavirus*, family *Bunyaviridae*) infection in humans is associated with two severe and potentially fatal diseases. Hemorrhagic fever with renal syndrome (HFRS) is associated primarily with hantaviruses found in Europe and Asia, and is characterized by fever, thrombocytopenia, renal failure and, in severe cases, hemorrhage due to increased capillary permeability (Lee *et al.,* 1982). Hantavirus pulmonary syndrome (HPS) is associated with infection by hantaviruses indigenous to the Americas (e.g. Andes, Muerto Canyon, and Sin Nombre viruses) and is characterized by fever, myalgia, and rapid onset of severe respiratory distress (Nichol *et al.,* 1993). The mortality associated with HPS is 40–50% (Lednicky, 2003). The reservoirs of hantaviruses are specific rodent hosts, in which persistent, nonpathogenic infections are common (Lednicky, 2003; Schmaljohn and Hjelle, 1997). Transmission to humans is by inhalation of aerosolized rodent urine or feces (McElroy *et al.,* 2002). Human to human transmission of hantavirus has been documented only in cases of HPS caused by Andes virus (Padula *et al.,* 1998). No effective hantavirus vaccines have been developed and, while the viruses causing HFRS respond to antiviral therapy with ribivarin, there is currently no effective antiviral drug therapy for HPS (Chapman *et al.,* 1999).

Several species of NHPs are susceptible to experimental infection with hantaviruses (Groen *et al.,* 1995; Yanagihara *et al.,* 1988), and NHP models are emerging as valuable tools for investigating hantavirus pathogenesis and for evaluating antiviral therapies and candidate vaccines. Experimental infection of cynomolgus macaques with Puumala virus produced typical signs of HFRS, including lethargy, anorexia, proteinuria, and/or hematuria, along with cytokine, creatinine, nitric oxide and C-reactive protein responses similar to those of human HFRS (Klingstrom *et al.,* 2002). NHP models have also been used to evaluate candidate hantavirus vaccines. Prototype DNA vaccines, against hantaviruses causing HFRS, have elicited high titer neutralizing antibody in rhesus macaques (Hooper *et al.,* 2001).

In a first attempt to develop a model of HPS, cynomolgus macaques were experimentally infected with Andes virus by the intravenous or aerosol route. Viremia was detected by RT-PCR in 4 of 6 exposed animals, but none developed clinical signs of HPS. One animal developed a 3-fold decrease in baseline platelet count coincident with high viremia and consistent with thrombocytopenia seen in human HFRS. All animals, however, showed significantly reduced lymphocyte counts, and all developed IgM and IgG antibodies against viral nucleocapsid protein and neutralizing antibody titers (McElroy *et al.,* 2002). Future refinements of primate models should greatly facilitate progress in the treatment and prevention of hantavirus-related diseases.

Ebola virus

Ebola virus (family *Filoviridae*) has been responsible for outbreaks of hemorrhagic fever with high mortality in both humans and NHPs (Evans *et al.,* 2003; Walsh *et al.,* 2003). Many species of NHPs are susceptible to experimental infection with Ebola virus, the outcome of which is almost uniformly fatal. The lethality of Ebola virus infection in primates has been used as support for the conclusion that NHPs are not the natural reservoir host of Ebola in nature. The course of Ebola virus infection in NHPs closely follows that observed in infected humans (Baskerville *et al.,* 1978; Johnson *et al.,* 1995; Jaax *et al.,* 1996). As a result, nonhuman primates have been used extensively in studies of Ebola virus pathogenesis (Baskerville, 1978; Johnson *et al.,* 1995; Jaax *et al.,* 1996; Riyabchikova *et al.,* 1999; Ignatiev *et al.,* 2000). Studies in rhesus and cynomolgus macaques have demonstrated that lethal Ebola virus infections can occur by oral, conjunctival or aerosol

routes of exposure (Jaax *et al.,* 1996; Jahrling *et al.,* 1996; Johnson, E. *et al.,* 1995). Experimental infections, in African green monkeys and baboons, have confirmed that macrophages and monocytes are the first cells to be infected and play a major role in subsequent systemic distribution of virus (Riyabchikova *et al.,* 1999). Experimental studies have also documented the absence of an inflammatory response in Ebola virus-infected NHPs (Baskerville *et al.,* 1978; Jaax *et al.,* 1996), suggesting virus-induced impairment of immune function. Studies in baboons have shown that the severity of Ebola virus-induced coagulopathies is correlated with increases in serum levels of interferon and tumor necrosis factor-α (Ignatiev *et al.,* 2000). Interestingly, differences have been observed in Ebola virus pathogenesis in different species of nonhuman primates. Following infection with Ebola-Zaire, baboons developed signs of hemorrhagic disease, while African green monkeys, given the same dose, developed generalized fibrin thrombosis in the absence of overt hemorrhage (Riyabchikova *et al.,* 1999). These findings indicate that species-specific differences need to be considered when selecting a NHP model of Ebola virus infection, and that use of different model systems may be necessary to address the entire spectrum of human disease associated with Ebola virus infection.

NHP models have also figured prominently in efforts to develop an Ebola virus vaccine (Sullivan *et al.,* 2000; Sullivan *et al.,* 2003). Passive immunization of cynomolgus macaques with hyperimmune globulin alone, or in combination with recombinant interferon-α, was not protective (Geisbert *et al.,* 2002; Jaax *et al.,* 1996; Jahrling *et al.,* 1996; Jahrling *et al.,* 1999). Similarly, cynomolgus macaques, immunized with liposome-encapsulated irradiated Ebola virus, produced virus-neutralizing antibodies but were not protected against lethal challenge, suggesting that both humoral and cell-mediated immunity are required for protection (Rao *et al.,* 2002). Newer approaches to vaccine development have focused on the induction of both cellular and humoral immune responses, incorporating the use of DNA vaccines. A "prime-boost" protocol combining DNA immunization, followed by a boost with a recombinant adenoviral vector expressing Ebola viral proteins, induced both cellular and humoral immune responses in cynomolgus macaques. Immunized animals were completely protected from lethal challenge with Ebola-Zaire, indicating that a preventive vaccine against Ebola virus is feasible (Sullivan *et al.,* 2000). This prime-boost protocol, while effective as a preventive vaccine regimen, required more than 6 months to complete, precluding its use in controlling an acute epidemic (Sullivan *et al.,* 2003). Recent studies have demonstrated that immunization of cynomolgus macaques, with recombinant adenovirus vectors encoding Ebola virus glycoprotein and nucleoprotein, in the absence of DNA priming, resulted in earlier antibody production and induction of Ebola virus-specific CD8+ T-cell responses. Monkeys immunized with only a single dose of this construct and challenged 28 days later were completely protected against both high and low-dose viral challenge (Sullivan *et al.,* 2003).

Respiratory virus infections

The disease potential and pathology of respiratory pathogens differs in primates and rodent animal models for two major reasons. First, the anatomy and development of the respiratory system in rodents is significantly different from that in the human or nonhuman primate (Plopper *et al.,* 1992). Second, many viruses that are pathogenic in humans are also pathogenic in another primate species, but not in rodents, mainly due to the need for specific host cell factors for viral replication. Respiratory virus infections of humans have been modeled in a wide range of primates including chimpanzees (Belshe *et al.,* 1977; Hancock *et al.,* 2000; Teng *et al.,* 2000), macaques (Blake and Trask, 1921; Bukreyev *et al.,* 2002; Fouchier *et al.,* 2003; Ponnuraj *et al.,* 2001; Rimmelzwaan *et al.,* 2001; van den Hoogen *et al.,* 2001; Zaucha *et al.,* 2001) and African green monkeys (Durbin *et al.,* 2000; Kakuk *et al.,* 1993). Recently two new viral pathogens, a pneumovirus and a coronavirus, responsible for Severe Acute Respiratory Syndrome, were successfully transmitted to cynomolgus monkeys and this helped to establish the etiology of these newly discovered respiratory tract infections (Fouchier *et al.,* 2003; van den Hoogen *et al.,* 2001).

Primate models have been used for many years to study respiratory tract infection and immunity to measles virus (Figure 34.1) (Blake and Trask, 1921b; Putz *et al.,* 2003), influenza (Renegar, 1992; Rimmelzwaan *et al.,* 2001; Soike *et al.,* 1984), parainfluenza (Durbin *et al.,* 2000; Hall *et al.,* 1993; Schmidt *et al.,* 2002), respiratory syncytial virus (Belshe *et al.,* 1977; Cheng *et al.,* 2001; Hancock *et al.,* 2000; Kakuk *et al.,* 1993; Leaman *et al.,* 2002; Teng *et al.,* 2000) and, more recently, monkeypox (Zaucha *et al.,* 2001). The majority of published work is focused on vaccine development. Possibly the best studied example of differing host species pathogenicity, and hence the use of an animal model, is the case of measles virus. Both rodent and primate animal models have been used for measles vaccine research, but wildtype strains of measles virus

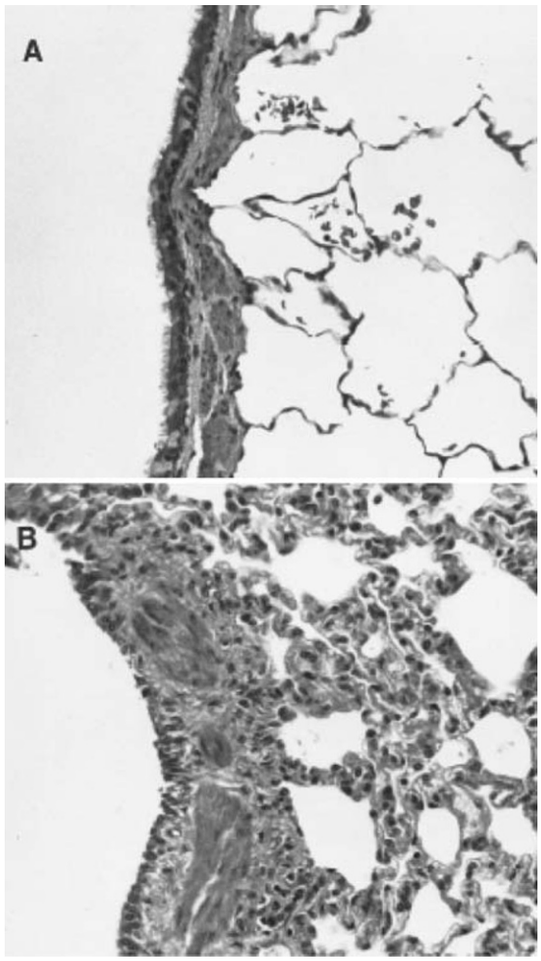

Figure 34.1 Histologic sections of lung tissue from rhesus monkeys 7 days after measles virus challenge. (A) Lung of a monkey that had received the live, attenuated measles vaccine 1 year prior to challenge. This is essentially normal lung. (B) Lung of an unvaccinated monkey. The wall of the respiratory bronchiole (left) is thickened with mononuclear cell infiltration and the alveolar septae are also thickened with inflammation and fibrin. These are features of viral bronchiolitis and interstitial pneumonia (Reprinted from McChesney, M.B. *et al.,* 1997, *Virology* 233, pp 74–84, with permission from Elsevier).

do not replicate in rodent species, with the exception of cotton rats, so that vaccine-induced protective immunity can be tested only in humans or susceptible nonhuman primates (Putz *et al.,* 2003). Similar restrictions are a concern for animal models of influenza virus (Renegar, 1992; Soike *et al.,* 1984).

Primate models have played a critical role in understanding the problem of enhanced or atypical lung disease that occurred in children vaccinated with whole-inactivated viral vaccines against measles or respiratory syncytial virus, when these children were exposed to wildtype virus. This vaccine-induced immunopathology has been extensively modeled in mice and a Type 2 CD4+ T cell has been found to play a pivotal role (Graham *et al.,* 2002). However, the laboratory mouse is not permissive for measles or respiratory syncytial virus replication and the question of protective versus immunopathologic vaccine immunity can only be addressed in a primate model. Using nonhuman primate models, it has now been shown that hypersensitivity reactions are largely responsible for the enhanced pathology associated with respiratory syncytial virus infection after immunization with formalin-inactivated RSV vaccines (De Swart *et al.,* 2002; Kakuk *et al.,* 1993; Ponnuraj *et al.,* 2001). Studies in macaques have demonstrated a role for immune complexes and eosinophils in the pathogenesis of atypical measles (Polack *et al.,* 1999).

Chronic viral diseases

Chronic viral infections are characterized by the ability of the pathogen to evade host antiviral immunity over the course of years. This reflects a highly evolved relationship between host and virus that involves the interactions of numerous viral proteins and the host immune system. This highly evolved relationship also means that human chronic viral pathogens often do not infect NHPs and thus, homologous viral pathogens of primates are used as models of human viral diseases. Further, for models of chronic viral diseases, the animal species must be long-lived and possess an immune system that is highly homologous to humans.

Primate T-lymphotropic viruses

Primate T-lymphotropic viruses (PTLV) comprise a closely related group of retroviruses in the Oncovirus subfamily of *Retroviridae* that includes viruses of humans and NHPs. The first member of this group to be identified was human T-lymphotropic virus type-I (HTLV-I), isolated from the blood of patients with Adult T-cell leukemia/lymphoma (ATLL) (Poiesz *et al.,* 1980). HTLV-I is the only human retrovirus known to be oncogenic and the etiologic link of this virus to ATLL is now well established (Johnson, J.M. *et al.,* 2001). HTLV-1 infection is also etiologically associated with a chronic, progressive myelopathy known as tropical spastic paraparesis/HTLV-1 associated

myelopathy (TSP/HAM) (Levin and Jacobson, 1997). The specific mechanisms underlying HTLV-1 pathogenesis and host immune response are not completely understood and there is currently no vaccine for prevention of HTLV-1 infection, or effective treatment for HTLV-1-related malignancies (Dekaban *et al.,* 1995; Franchini, 1995; Franchini and Streicher, 1995).

Other members of the PTLV group include HTLV-2 (Kalyanaraman *et al.,* 1982), a human virus not conclusively linked to cancer, and the cognate simian T-lymphotropic viruses types 1, 2, and 3 (STLV-1, -2, -3) (Kalyanaraman *et al.,* 1982; Meertens and Gessain, 2003; Miyoshi *et al.,* 1982). In NHPs with naturally acquired infections, STLV-I induces leukemia or lymphoma, predominantly in African species, with many clinical and pathological similarities to ATLL (Tsujimoto *et al.,* 1987). Both human and simian lymphomas occur in only a small proportion of infected individuals, usually after a prolonged period of latency (Blattner, 1989; Tsujimoto *et al.,* 1987). To date, no counterpart to TSP/HAM has been recognized in STLV-1-infected NHPs.

Two approaches have been used to develop and utilize NHP models of human HTLV-I infection and disease. One approach takes advantage of the fact that several species of NHPs are susceptible to experimental infection with HTLV-I, including various species of macaque (Beilke *et al.,* 1996; Ibuki *et al.,* 1997; Murata *et al.,* 1996), the squirrel monkey (Kazanji, 2000; Kazanji *et al.,* 2000) and the common marmoset (Yamanouchi *et al.,* 1985). Experimentally infected Japanese macaques (*M. fuscata*) have been used to demonstrate the efficacy of passive immunization with hypreimmune serum in preventing HTLV-I infection (Murata *et al.,* 1996). A cynomolgus macaque model was used to demonstrate the long-term persistence of protective immunity following immunization with recombinant vaccinia virus expressing the HTLV-I envelope gene (Ibuki *et al.,* 1997). Although NHPs infected with HTLV-I do not develop tumors, other HTLV-I-related pathological conditions, including polymyositis, uveitis and arthritis, were observed in a rhesus macaque following experimental infection with HTLV-I (Beilke *et al.,* 1996). The squirrel monkey model has been used to investigate the distribution of virus to various tissues, in early HTLV-I infection, and the relationship between viral gene expression and the host humoral and cellular immune response to infection (Kazanji, 2000; Kazanji *et al.,* 2000). Infection of common marmosets with HTLV-I by the oral route has provided support for the concept of milk-borne infection from HTLV-I-infected mothers to nursing infants (Yamanouchi *et al.,* 1985).

Another approach to HTLV-1 primate model development has exploited the naturally occurring infections with STLV-1 in many species of Old World NHPs. HTLV-1 and STLV-1 share a high degree of genetic relatedness (Dekaban *et al.,* 1995). The routes of transmission are the same (Lazo *et al.,* 1994), and the course of infection of STLV-1 in primates parallels that of HTLV-1 in humans (Tsujimoto *et al.,* 1987). The respective host-virus systems give rise to similar proportions of malignancies, and tumors induced by STLV-1 are indistinguishable from human ATLL (McCarthy *et al.,* 1990; Tsujimoto *et al.,* 1985). The method of replication of both STLV-1 and HTLV-1, namely clonal expansion of virus infected T-cells, is virtually identical (Gabet *et al.,* 2003; Morteaux *et al.,* 2003). The exact mechanisms of PTLV oncogenesis have not been fully elucidated. Results of recent studies suggest that the malignant transformation in ATLL is a "multi-hit" phenomenon, marked by discreet genetic events (Arima and Tei, 2001). The Tax gene, present in both STLV-1 and HTLV-1, is thought to play a major role in leukemogenesis, due to its pleiotropic actions (Dekaban *et al.,* 1995; Franchini, 1995; Franchini and Streicher, 1995). These include transcriptional suppression of DNA polymerase β (Jeang *et al.,* 1990) and the functional suppression of p16 and p53, genes that are important regulators of the cell cycle (Matsuoka, 2003). STLV-1 infection of nonhuman primates provides an excellent model system to investigate mechanisms of PTLV oncogenesis *in vivo*.

HIV infection and AIDS

For two decades, NHP models for AIDS, especially Simian immunodeficiency virus (SIV) infection of macaques, have provided important insights into the transmission and pathogenesis of AIDS as well as virus-specific immune responses to anti-SIV vaccine regimens. Although an effective vaccine against human AIDS is not yet available, significant progress is being made towards this goal (reviewed in Robinson, 2002).

Studies of primate lentivirus transmission, in NHP models, has permitted dissection of the earliest events that occur in an infected host, after exposure to virus under defined conditions. These include the effects of a viral inoculum dose, viral virulence and the route of exposure (reviewed in Pope and Haase, 2003). Experiments in NHP have also determined the temporal relationship between lentiviral replication, after known virus exposure/infection, systemic infection, the development of antiviral immune responses and disease progression. Perhaps most important for vaccine development,

NHP studies have compared lentivirus-specific immune responses early after virus exposure, in both vaccinated and unvaccinated animals, to assess potential correlates of vaccine-mediated protection (reviewed in McMichael and Hanke, 2003). The results of these experiments have begun to identify key strategies that could be used for reducing or eliminating HIV transmission from one infected person to another. Understanding each of these areas is essential to developing a vaccine to prevent human HIV infection or AIDS and these studies are neither feasible nor ethical in humans.

Primate species/virus systems

Table 34.1 compares the key similarities and differences between the major NHP models of HIV/AIDS and human HIV-infection. The chimpanzee/HIV-1 model uses HIV-1 but HIV infection in chimps is generally non-pathogenic and thus does not model AIDS in humans. This limitation, coupled with the very small number of chimps, and their endangered species status, make the chimp/HIV model for AIDS largely impractical for HIV vaccine research. A variety of NHP species can be infected with isolates of HIV-2, which is genetically similar to SIV.

Many African NHP species are naturally infected with species-specific SIV variants, but these endemic SIV infections apparently do not result in AIDS. Asian macaques are not naturally infected with SIV but experimental inoculation of macaques, with SIV variants isolated from African monkeys, results in AIDS (reviewed in Gardner, 2003). A number of macaque and baboon species have been used for transmission, pathogenesis and vaccine studies with HIV-2 (Locher *et al.,* 2001). Infection of macaques with SIV or hybrid SIV/HIV viruses, containing the HIV-1 envelope (SHIV), are generally accepted as the most practical NHP models for human HIV-infection and AIDS and they are widely used to evaluate vaccine strategies against AIDS (reviewed in Letvin *et al.,* 2002; McChesney *et al.,* 1999; McMichael and Hanke, 2003; Robinson, 2002).

Virus challenge: viral properties and mucosal transmission

It is well-established that the capacity of a primate lentivirus to cause immunodeficiency disease, in Asian macaque species, is associated with its ability to replicate to high levels after establishing systemic infection (McChesney *et al.,* 1999). Although several biological properties, including co-receptor use and growth in transformed T cell lines and primary macaque blood cells, were proposed to account for virulence of SIV and SHIV isolates, it has been demonstrated that none of these reliably predict the ability to induce AIDS in infected macaques (Table 34.2). Persistently high viral replication, as measured by high levels of viral RNA or core antigen in plasma, is also the best predictor of the reliability of transmission by mucosal exposure for SIV or SHIV isolates (Table 34.2).

NHP models for HIV breast milk transmission

In developed countries, where infant formula is readily available and breastfeeding is not essential to infant survival, vertical HIV transmission can be prevented effectively by antiretroviral treatment during pregnancy and delivery combined with a short course of antiretroviral drugs given to the newborn. However, in most developing nations, even with some prenatal care or limited access to antiretroviral drugs, there are few alternatives to breast feeding because formula and clean water are rarely available or affordable (Mbori-Ngacha *et al.,* 2001). In these settings, exposure to breast milk remains a major risk for HIV infection and a neonatal vaccine, that can protect against HIV breast milk transmission, is urgently needed.

To evaluate strategies that may prevent HIV infection by breast feeding, HIV breast milk transmission has been modeled in infant rhesus macaques. The most direct model is SIV-infection of pregnant macaques who are allowed to suckle their infants. Early studies using this model found low or unreliable SIV transmission to infants (McClure *et al.,* 1991). Recently Amedee and colleagues (Amedee *et al.,* 2003) reported efficient breast milk transmission of SIV to infants of SIV-infected dams. A limitation of this model is that the duration of breast feeding, until SIV infection of infants, varied from a few weeks to months and was not associated with levels of viral RNA in milk (Amedee *et al.,* 2003).

To conserve adult female macaques needed for breeding and to better control viral and host variables, an alternative macaque model of HIV breast milk transmission is oral SIV or SHIV inoculation of newborn and infant macaques that may be nursery-reared. Advantages of experimental oral virus inoculation include the ability to use a specific dose of a well-characterized virus inoculum, to vary the total number of virus exposures and the intervals between virus exposures, and to assess how infant development may modulate outcome of virus exposure. Disadvantages are the need for labor-intensive nursery rearing of SIV-exposed infant macaques in specialized BSL-2

TABLE 34.1: Comparison of available nonhuman primate models to human HIV infection

NHP host species /virus	Similarities	Differences
Chimpanzee/HIV-1	NHP species most like humans, and only NHP species in which HIV-1 establishes persistent infection Genital mucosal exposure to high doses of some HIV-1 isolates has resulted in infected chimpanzees	Virus replicates only after adaptation by passage in PBMC of this species. Viral levels in blood are low in most, and transiently detected in many, HIV-1 infected animals Infected animals rarely develop disease; only after serial animal passage of virus
Macaque/HIV-2* or Baboon/HIV-2*	HIV-2 establishes persistent infection in multiple macaque species and in baboons Some macaque species develop AIDS after long-term infection with an HIV-2 adapted to that species	Viral levels in blood are low in many HIV-2 infected animals, except after virus is adapted to a host species
Macaque/SHIV*	SHIVs are chimeric viruses made by molecular biology techniques which encode some HIV-1 or HIV-2 genes (e.g. envelope) in an otherwise SIV genome	Virus replicates to high levels only after adaptation by passage in a species; viremia controlled spontaneously in many animals Biological properties of SHIVs in NHP differ substantially from HIV in humans and SIV in macaques. Pathogenic SHIVs induce rapid, severe, though often transient, reduction in CD4+ T cells in peripheral blood; AIDS results from sustained CD4+ T cell loss (observed in 25–40% of animals infected with pathogenic SHIVs) Mucosal transmission of most SHIVs is inefficient and unreliable
Macaque/SIV*	SIVs that infect macaques are most similar genetically to HIV-2 (SIV from wild chimpanzees most similar to HIV-1) Efficiency of pathogenic SIV transmission by mucosal exposure (genital, rectal, oral) is route and dose dependent Pathogenic SIV isolates reliably cause a spectrum of immunodeficiency disease remarkably similar to AIDS in HIV-1 infected humans SIV isolates with deletions in accessory genes are attenuated and induce slow progression to AIDS as seen in humans infected with HIV-1 containing nef gene deletions	Most pathogenic SIV isolates cause simian AIDS within 6 to 12 months after infection by parenteral or mucosal exposure to high (100,000 TCID50%) doses of virus

*Multiple virus isolates with distinct biological properties.

TABLE 34.2: Biological properties of SIV and SHIV isolates in rhesus macaques

Virus isolate '	Growth in macrophage	Co-receptor use		Replication in macaques (after IV inoc.)	Induction of AIDS	Reliability of mucosal virus transmission	
		CXCR4	CCR5			Vaginal	Oral
SIVmac251	+	–	+	High	+	High	High
SIVmac239	–	–	+	High	+	High	High
SHIV89.6PD	+	+	+	High	+	High	Not done
SHIV33A	+	+	–	High	+	High	High
SHIV89.6	+	+	+	Moderate	–	Moderate	High
SHIV33	–	+	–	Moderate	–	Low	None
SIVmac1A11	+	–	+	Low	–	Low	Low
SHIVHxb2	+	+	–	Low	–	Low	Not done

containment facilities, increased clinical care, by veterinary staff, of SIV-infected infants and the absence of anti-microbial factors normally present in macaque breast milk, which may more closely mimic conditions found in breast milk of HIV-infected women (Farquhar *et al.,* 2002). Studies using oral inoculation of infant macaques, with SIV or SHIV, have demonstrated that antiretroviral drugs (Van Rompay *et al.,* 2001), HIV neutralizing monoclonal antibodies (Baba *et al.,* 2000; Hofmann-Lehmann *et al.,* 2001), and passively acquired SIV-specific, non-neutralizing antibodies (Van Rompay *et al.,* 1998) can prevent infection if given before or, in some cases, soon after virus exposure. Recently, Van Rompay and colleagues (Van Rompay *et al.,* 2003) have shown that active immunization of infant rhesus macaques, with live-attenuated SIV and recombinant poxvirus vectors expressing SIV structural proteins, can protect against high viremia and delay onset of AIDS after oral inoculation with virulent SIV. Collectively, the results of these studies, using the SIV/infant macaque model, suggest that both passive and active immunization strategies may be successful in reducing breast milk transmission of HIV.

Influence of immunogenetics on NHP models for AIDS and HIV vaccine development

The major histocompatibility complex (MHC) plays an essential role in immune responses to viral infections and the enormous variation observed, for human MHC genes, is thought to reflect selective pressure by microbial, especially viral, pathogens. There is consensus that some MHC alleles (Carrington and Bontrop, 2002) and haplotypes can significantly modulate the rate of progress to AIDS in HIV-infected people (Carrington, 2003; Carrington and O'Brien, 2003; Cullen *et al.,* 2002; Cullen *et al.,* 2003; Trachtenberg *et al.,* 2003). In addition, concordance between MHC class I alleles of HIV-infected women and their infants is associated with increased risk of vertical HIV transmission (Polycarpou *et al.,* 2002). Further, there is evidence that specific human MHC alleles influence both the response to HIV-infection and immunization with HIV vaccines (Kaslow *et al.,* 2001). It has even been proposed that the relatively low genetic variation at MHC class I in chimpanzees is a direct result of long-term selection by an ancient SIV during a devastating pandemic in wild chimpanzees (de Groot *et al.,* 2002).

In contrast to the chimpanzee, recent studies of the MHC of Asian macaques, indicate that the allelic and haplotype variation among individual animals is similar to, or greater than, MHC variation observed for humans (Doxiadis *et al.,* 2000). Also, as shown for HIV-infected humans, some rhesus MHC alleles and haplotypes have been reported to influence the rate of disease progression in SIV-infected animals (Carrington and Bontrop, 2002; O'Connor *et al.,* 2003; Pal *et al.,* 2002; Sauermann *et al.,* 2000) and may modulate vaccine protection against SIV challenge (O'Connor *et al.,* 2003; Pal *et al.,* 2002). These observations underscore the need to identify more MHC alleles and to define complete MHC halplotypes in NHP species used for AIDS vaccine development (Friedrich and Watkins, 2003; Sauermann, 2001).

Ability of NHP studies to predict outcomes of human clinical HIV vaccine trials

The ultimate test of the relevance and utility of NHP models of AIDS, for HIV vaccine development, is their accuracy in predicting efficacy of specific vaccine strategies in human clinical trials. To date, only results of a single clinical trial of HIV vaccine efficacy are available for comparison with efficacy observed in NHP models. This double-blind, placebo controlled human trial, jointly sponsored by the NIH and Vaxgen, found no overall efficacy of a clade B HIV recombinant gp120 protein vaccine in groups at high-risk for HIV exposure in the USA; i.e. there were no statistically significant differences between individuals, receiving placebo or vaccine, in either the proportion of individuals who become HIV-infected, or in viral load after infection (Cohen, 2003a; Cohen, 2003b). This outcome was mirrored in prior NHP efficacy trials of recombinant HIV envelope (gp120 or 160), for chimpanzees challenged with non-homologous laboratory isolates of HIV-1 (Girard *et al.,* 1996), and for macaques immunized with recombinant HIV envelope (gp120 or gp160). Both examples showed little or no protection against infection by parenteral or mucosal challenge with pathogenic SIV or SHIV isolates (reviewed in Robinson, 2002).

Results are expected to be available, by the end of 2003, of a second large human clinical trial to assess the efficacy of recombinant canarypox vector, expressing HIV envelope, and other structural and regulatory proteins given as primary immunization followed by recombinant HIV envelope protein as a booster immunization. Preclinical studies in macaques immunized with recombinant canarypox expressing SIV and/or HIV antigens found that, despite only modest levels of virus-specific immune responses, vaccinated animals had reduced viral load and slower disease progression compared to unvaccinated controls after challenge (reviewed in Robinson, 2002). If the results of NHP efficacy studies consistently reflect those for human trials of similar HIV vaccine strategies, this will reinforce the value and relevance of NHP models of AIDS for HIV vaccine development, especially to identify immune correlates and perhaps, to understand mechanisms of HIV vaccine efficacy.

Simian cytomegalovirus

Use of NHPs as models for human cytomegalovirus (HCMV) has expanded dramatically in the last few years because of the increasing appreciation of the impact of HCMV infection on human health. HCMV is a member of the *Herpesviridae* family of viruses (Mocarski, 1993). It has been recognized for over 30 years as a serious threat to the developing fetus where it can cause a wide spectrum of pathological outcomes (Weller, 1971). HCMV is also a major cause of morbidity and mortality in those with either AIDS or an immunosuppressed immune system (Alford and Britt, 1993). There is no licensed vaccine for HCMV, although there has been a long-standing recognition of the medical need for one (Committee to Study Priorities for Vaccine Development and Medicine, 1999).

HCMV is species-specific and will not grow in the NHPs available for study. This limits experimental investigations to tissue culture settings and natural history studies in humans, leaving many experimental questions unanswered. However, the NHP models are ideally suited to fill the experimental void concerning many critical aspects of HCMV natural history and to develop novel immunological and chemotherapeutic strategies that can either prevent infection or limit disease.

The vast majority of studies have involved infection of rhesus macaques with rhesus CMV (RhCMV). However, important observations have also been made with CMV of other macaque species, the African green monkey, baboon and chimpanzee. All of these simian systems are also relevant surrogates for HCMV. For this review, a prototypical simian CMV phenotype is presented, based on studies with the different NHP CMV. Comparative studies between different NHP CMV are extremely limited. It should be stressed that each CMV representative has co-evolved with its host species. Accordingly, the evolution of virus-host relationships may have led to important and, as yet, undiscovered distinctions between the different CMV isolates.

CMV has been isolated from multiple genera and species of old and new world NHP hosts (Asher *et al.,* 1974; Black *et al.,* 1963; Eizuru *et al.,* 1989). It is probable that every NHP species has an associated species-specific CMV. Analyses of the genomes, protein expression and serological cross-relatedness demonstrate that CMV isolates from different NHP species are unique to each host (Davison, A.J. *et al.,* 2003; Eizuru *et al.,* 1989; Gibson, 1983; Hansen *et al.,* 2003; Kilpatrick *et al.,* 1976; Minamishima *et al.,* 1971; Tinghitella *et al.,* 1982). There is no evidence to date that the CMV of one host species can grow in another host species following natural exposure. However, simian CMV replication *in vitro* is not restricted to cells of the host species. The CMV of African green monkeys (Black *et al.,* 1963) and Rh CMV (Alcendor *et al.,* 1993) can

productively infect human and NHP cells in culture. Further, AGMCMV can productively infect rhesus macaques and replicate for years (Swack *et al.,* 1971). With the expansion of NHP CMV sequences on GenBank, it is now possible to confirm the species identity of a primary isolate by limited sequence analysis.

Like HCMV, simian CMV is a common infectious agent in NHP populations (Andrade *et al.,* 2003; Black *et al.,* 1963; Eizuru *et al.,* 1989; Kessler *et al.,* 1989; Minamishima *et al.,* 1971; Swack and Hsiung, 1982; Swack *et al.,* 1971; Vogel *et al.,* 1994). Virtually 100% of monkeys in breeding facilities are seropositive by one year of age and 50% of infants are seropositive by 6 months of age (Vogel *et al.,* 1994). It is likely that multiple strains of CMV are present within each breeding facility (Alcendor *et al.,* 1993). Comparable rates of seroprevalence have been reported in monkeys trapped in the wild (Eizuru *et al.,* 1989; Minamishima *et al.,* 1971; Ohtaki *et al.,* 1986; Swack *et al.,* 1971). The routes of transmission are not known, but it is likely that virus is horizontally transmitted from mother to infant via breast milk and saliva, similar to identified modes in humans. Virus is also normally excreted in urine, adding another route of virus spread. There is no evidence consistent with transmission in utero, although low rates cannot be excluded (Vogel *et al.,* 1994). Maternal-fetal transmission is a critical component of HCMV congenital transmission, and this is one aspect of HCMV natural history that cannot be modeled in NHP at this time. It is most likely that absence of congenital infection, in NHP, has to do with the high seroprevalence of HCMV in breeding age females.

CMV seroprevalence changes dramatically if animals are reared in smaller cohorts from birth. When infants are separated from the dam at, or soon after, birth, and hand reared in a nursery, the animals remain essentially CMV-free well past the age of sexual maturity (Barry, unpublished; Minamishima *et al.,* 1971).

Primary infection

Simian CMV infection in immunocompetent hosts, either experimental or following natural exposure, does not result in any clinical signs of disease. This is similar to the vast majority of HCMV infections. Mononucleosis, a relatively rare outcome of primary HCMV infection, has never been associated with CMV infection in the NHP. In addition, there have been no published reports, or anecdotal observations, of CMV disease in monkeys that were culled or died of reasons other than acquired immunodeficiency or immunosuppression. It can be concluded, from the absence of disease, that host immune responses to primary CMV infection are highly protective.

The general course of primary infection in a seronegative host involves rapid dissemination from the site of infection to distal sites throughout the body (Lockridge *et al.,* 1999). In monkeys naturally exposed to CMV, virus is probably transmitted across the oral or genital mucosa. Experimental inoculations have been done by the oral, intravenous and subcutaneous (sc) routes. Viral DNA can generally be detected in the blood within 7 days of infection and in multiple tissues within two weeks (Lockridge *et al.,* 1999). Antiviral immune responses are rapid and increase in intensity as viral plasma DNA loads decrease. Cellular antiviral responses follow the same course of development as the humoral responses. A variety of hematological changes follow experimental IV inoculation, although no consistent pattern is observed (Lockridge *et al.,* 1999).

Persistent infection

There are two prominent hallmarks of the persistent phase of RCMV infection: chronic viral shedding and stability of the antiviral immune responses. These characteristics are identical to HCMV. Infected monkeys can remain viuric for years following primary infection, probably for the life of the host (Asher *et al.,* 1974; Swack and Hsiung, 1982). The frequency of CMV shedding is variable between animals, although some monkeys appear to be constantly shedding infectious virus at the oral and genital mucosa. Historically, shedding has been assayed by culturing virus. More recently, sensitive molecular techniques, such as real-time PCR, have been used to detect and quantify CMV DNA purified from mucosal swabs (Huff *et al.,* 2003). Approximately 50% of seropositive monkeys are DNA-positive in mucosal fluids at any one time. Thus, there is active and ongoing virus replication at mucosal surfaces within a persistently infected host. Occasionally, antigen-positive cells can be detected by immunohistochemistry in other tissues, although the tissues are usually histologically normal without an accompanying inflammatory response.

The relative constant exposure to CMV antigens probably explains the pattern of antiviral immune responses observed in long-term infected monkeys. Both antibody titers and cellular responses stay relatively stable over time. End-point antibody titers, to total viral antigen preparations, hover around the plateau level achieved at the end of the primary infection. Cellular responses to RhCMV antigens also exhibit little fluctuation (Kaur *et al.,* 2002).

The maintenance of a stable virus-host relationship (i.e., no disease) requires a considerable expenditure of the immunological repertoire of the infected host. Up to 5.8% and 5.3% of memory $CD4^+$ and $CD8^+$ T-lymphocytes, respectively, have been shown to be CMV-specific in healthy, CMV-positive rhesus macaques (Kaur *et al.,* 2002; Pitcher *et al.,* 2002). Comparably high frequencies of CMV-specific, memory T cells have been observed in persistently infected humans (Waldrop *et al.,* 1998; Waldrop *et al.,* 1997).

The three conditions in which HCMV is a serious pathogen (immunodeficiency, immunosuppression, and intrauterine infection) have strong parallels in NHP. These conditions are summarized below.

Immunodeficiency

The first published descriptions of CMV disease in NHP occurred in the context of rhesus macaques coinfected with the Type D simian retrovirus (SRV) and a lentivirus, the simian immunodeficiency virus (SIV) (Henrickson *et al.,* 1983; King *et al.,* 1983; Lerche *et al.,* 1984; Letvin *et al.,* 1983a; Letvin *et al.,* 1983b; Osborn *et al.,* 1984). The acquired immunodeficiency disease, caused by both SRV and SIV, was characterized by persistent lymphadenopathy, severe wasting, chronic diarrhea, high morbidity and mortality and multiple opportunistic infections, including activated CMV. Activated CMV disease is generally characterized by an abundance of cytomegalic cells containing cytoplasmic and/or intranuclear inclusions and often associated with tissue necrosis and neutrophilic infiltration in tissues. This pattern of CMV infection in immunodeficient monkeys is distinct from the paucity of viral inclusions in healthy macaques and is strikingly similar to CMV disease in human AIDS patients. The incidence of CMV disease in monkeys with SAIDS caused by SIV or SRV is variable. Some studies have reported that up to one-third to one-half of CMV seropositive animals have evidence of CMV disease at necropsy (Baskin *et al.,* 1988; Kaur *et al.,* 2003; King *et al.,* 1983; Kuhn *et al.,* 1999; Osborn *et al.,* 1984). Similar to HCMV, activated simian CMV can be observed in multiple tissues, (Baskin, 1987). However, CMV disease is not always systemic and can occur in only a single tissue (Sequar *et al.,* 2002). CMV retinitis, an important clinical problem in some human AIDS patients, has not been described in a SAIDS monkey.

There are multiple distinctions between the course of RhCMV infection in an immunodeficient and an immunocompetent host. Notable changes include an increased frequency of detectable RhCMV DNA in blood, elevated genome copy numbers in tissues and declining measures of anti-CMV immune functions, such as CTL activity, cytokine secretion, and neutralizing antibody titers (Kaur *et al.,* 2003; Sequar *et al.,* 2002). The kinetics of these changes are variable. If SIV infection occurs during the primary phase of RhCMV infection, the onset of RhCMV disease and SAIDS can be rapid (10–27 weeks post SIV). If the monkeys are inoculated with SIV during the persistent phase of RhCMV infection, the time of death can be greatly extended (10 weeks – >1 year). One study observed a statistically significant decrease in the time of death in animals with RhCMV end-organ disease compared to animals without histological evidence of activated RhCMV (Kaur *et al.,* 2003).

Transplantation

CMV sequelae have also been observed in immunosuppressed NHP receiving either allografts or xenografts. Although there are not many references in the literature, the occurrence of activated CMV in transplant-recipients appears to be related to the use of intense immunosuppression regimes, such as cyclophosphamide, corticosteroids and/or anti-thymocytic globulin, designed to prevent graft rejection. A transplant recipient can develop CMV histopathology, similar to CMV disease in human allograft recipients, including pneumonitis and vasculopathies (Ghanekar *et al.,* 2002; Mueller *et al.,* 2002; Ohtaki *et al.,* 1986; Ohtaki *et al.,* 1988; Teranishi *et al.,* 2003). Further studies are required to determine the utility of NHP as an experimental model to study transplantation-associated CMV disease, primarily because the frequency of CMV reactivation in this setting is not known.

Fetal infection

RhCMV can cause a range of developmental defects in experimentally inoculated rhesus macaque fetuses that are almost identical to those observed in human infants congenitally infected with HCMV. It should be stressed that fetal infection in this NHP is a model of intrauterine pathogenesis and not transplacental transmission. No studies have been reported documenting either natural or experimental maternal-fetal CMV transmission in NHP. The published studies of CMV-induced fetal disease required direct *in utero* inoculation of fetuses with virus (Chang *et al.,* 2002; London *et al.,* 1986; Tarantal *et al.,* 1998). Using ultrasound guidance, needles can be directed through the abdominal wall of the dam to deliver virus to precise locations within the

developing fetus at defined stages of gestation. Growth and developmental outcomes can be prospectively monitored, by ultrasound, and fetal samples (blood, amniotic fluid, and tissue) can be obtained by needle biopsy.

Rhesus macaque fetuses have been inoculated with RhCMV by the intramniotic (IA), intracranial (IC), and intraperitoneal (IP) routes from late in the first trimester (gestation day 50) through mid-gestation (day 80) (Chang *et al.,* 2002; London *et al.,* 1986; Tarantal *et al.,* 1998). Mild to severe fetal outcomes have been observed in approximately 50% of inoculated fetuses. No RhCMV sequelae have ever been observed in the dams, although there has been suggestive evidence of retrograde transmission of the virus across the placenta (London *et al.,* 1986).

The developing brain is highly sensitive to CMV disease with a spectrum of developmental abnormalities. These include microcephaly, lissencephaly, ventricular dilatation, leptomeningitis, encephalitis and periventricular calcifications. All of these are hallmarks of congenital HCMV infection (Alford and Britt, 1993). RhCMV histopathology is not limited to the brain and systemic effects such as intrauterine growth restriction, disseminated RhCMV disease and isolation of virus in blood and tissues have been seen (Chang *et al.,* 2002; Tarantal *et al.,* 1998). Placental abnormalities (deciduitis, infarction, calcification, and lymphocytic infiltration) have been seen in some of the inoculated fetuses (London *et al.,* 1986).

Cercopithecine herpesvirus 1 (Herpes B virus)

Cercopithecine herpesvirus 1, generally referred to as herpes B virus (BV), is an alphaherpesvirus of the rhesus macaque with strong genetic, virological and immunological relatedness to herpes simplex virus (HSV) of humans. BV infection of the rhesus monkey seems to accurately model HSV infection of humans, but the deadly zoonotic potential of BV has prevented its use in an animal model. However, it was recently listed as a categorical pathogen by the U.S. Public Health Service and could be an agent of bioterror. Thus a brief discussion of BV is included here.

BV is endemic in Asian macaque populations (Weigler, 1992), and closely related alphaherpesviral variants are present in African NHP (Eberle and Hilliard, 1995). BV deservedly bears the most attention of the NHP alphaherpesviruses because of its pathogenic potential in humans. Zoonotic infection of humans with B virus is almost invariably fatal (>70%) in the absence of antiviral chemotherapies, and severe, non-fatal infections can result in encephalomyelitis or severe neurological impairment (Huff and Barry, 2003). BV is the only simian herpesvirus that is known to cause disease in humans. The most salient feature of BV natural history, in terms of its occupational risk, is that an overwhelming majority of macaques shed B virus without overt signs of disease (Huff, J.E. *et al.,* 2003; Weigler *et al.,* 1993; Zwartouw and Boulter, 1984). Thus, every BV-seropositive macaque must be considered as a potential source of infectious BV, whether through its bodily fluids or its tissues.

Despite the preceding risk assessment, there have only been approximately 40 documented cases of human infection since the first reported transmission to humans in 1932 (Huff and Barry, 2003). The disproportionality between the number of zoonotic infections and the high seroprevalence of BV in breeding age animals is a function of the BV life cycle. The biology of BV infection in macaques is characterized by a lifelong persistence with infrequent, and usually subclinical, shedding at mucosal surfaces. Although the natural history of BV has not been described in detail, the replication cycle of HSV serves as a precedent (Roizman and Sears, 1993). Virus is transmitted across a mucosal surface, such as the oral or genital mucosa. Following localized replication in mucosal epithelial cells, the virus is transmitted directly to sensory nerve endings. The prevailing thought is that there is no associated bloodborne stage of infection, except in rare systemic infections (Simon *et al.,* 1993). The virus particle is carried, by axonal transport, to the dorsal root ganglia where the virus establishes a true latent infection in neurons.

Latency is noted for a lack of viral replication and an extremely limited pattern of viral transcription. Periodic reactivation from latency results in the production of progeny virions which transport back down the axon to mucosal epithelial cells, where they replicate and the infectious virus is released from the mucosal epithelium. For BV, most episodes of recurrent viral shedding are asymptomatic (Huff *et al.,* 2003; Weigler *et al.,* 1993; Zwartouw and Boulter, 1984), and represent a constant risk of zoonoses. Clinical signs of either primary or recurrent infection (oral herpetic lesions such as gingivostomatitis, oral and lingual ulcers, and conjunctivitis) are the exception (Carlson *et al.,* 1997; Keeble *et al.,* 1958; Weigler, 1992), and usually require immediate euthanasia of the animal. Virus isolation and molecular detection of BV DNA indicate that the frequency of shedding in a population is low (1–5%), although more studies are needed to establish a true rate. There is evidence to suggest that shedding frequency

may go up during breeding season (Huff *et al.,* 2003; Weigler *et al.,* 1993).

There is a strong correlation between the seroconversion to BV and the age of the animal. Seroconversion rates increase sharply when monkeys reach the age of sexual maturity, with prevalence rates of 80–100% in adult populations (Weigler, 1992; Weigler *et al.,* 1993). There is no evidence for vertical transmission of BV.

Human B virus infections have generally involved direct contact with macaques or their tissues or fluids (Huff and Barry, 2003). Methods of contact have included a bite, scratch, contact of a mucosal surface with a macaque body fluid or tissue, or a contaminated needle puncture or cage scratch (Huff and Barry, 2003). There has only been one documented case of human-to-human transmission, although the potential for secondary transmission is probably low (Holmes *et al.,* 1990). BV disease can begin within a few days to a month, although disease progression is variable in terms of the kinetics and clinical signs. Guidelines for reducing potential exposure and treatment of suspected BV infections in humans have recently been published (Holmes *et al.,* 1995).

Hepatitis viruses

Hepatitis B virus

Hepatitis B virus (HBV), a small double-shelled hepadenavirus virus that contains a partially double-stranded DNA genome of approximately 3200 bases, is found in several species, including woodchuck, ground squirrel, a range of bird species such as duck, goose and grey heron (Marion *et al.,* 1980; Mason *et al.,* 1980; Summers *et al.,* 1978), and the NHPs chimpanzee (*Pan troglodytes*), woolly monkey (*Lagothrix lagothrica*), orangutan (*Pongo pygmaeus*), gibbon (*Hylobates sp.*) and gorilla (*Gorilla gorilla*) (Grethe *et al.,* 2000; Lanford *et al.,* 1998; Mimms *et al.,* 1993; Vaudin *et al.,* 1988; Warren *et al.,* 1999). HBV, isolated from gibbons and chimpanzees, share an early phylogenetic lineage, indicating that these viruses were indigenous to their respective hosts (Norder *et al.,* 1996). Despite the species specificity of natural HBV isolates, experimental infection of chimpanzees with human and gibbon HBV can be accomplished (Gallagher, 1991). Gibbons can be infected through experimental exposure to human saliva containing HBV (Bancroft *et al.,* 1977; Scott *et al.,* 1980). Replication of human HBV in a number of primate species suggests that natural HBV cross-transmission can occur. HBV can be present in the blood and other body fluids, including saliva/nasopharyngeal fluids, semen, cervical secretions and leukocytes (Alter *et al.,* 1977; Davison, F. *et al.,* 1987). However, HBV transmission from gibbon or chimpanzee to human has never been documented. CD8+ T cells mediate viral clearance during acute HBV infection in chimpanzees (Thimme *et al.,* 2003).

Hepatitis C virus

Hepatitis C virus (HCV) is a member of the Flaviviridae family and has a single-stranded positive sense RNA genome. The chimpanzee is the only experimental animal susceptible to infection with hepatitis C virus (HCV). The chimpanzee model of HCV infection was instrumental in the initial studies on non-A, non-B hepatitis, including observations on the clinical course of infection, determination of the physical properties of the virus and eventual cloning of the HCV nucleic acid (reviewed in Lanford and Bigger, 2002). Other NHP models of HCV have been developed using surrogate viruses such as GB virus-B (reviewed in Beames *et al.,* 2001). GB virus-B virus is closely related to HCV and it is hepatotropic. In addition, the level of GBV-B viremia observed in infected tamarins, the animal model for GBV-B, is greater than 1000-fold higher than for HCV. Tamarins are much easier to house than chimpanzees and a tissue culture system for GBV-B, using primary tamarin hepatocytes, is available (Beames *et al.,* 2000). Thus, primates are likely to play an important role in developing effective HCV vaccines.

Conclusion

Early success in developing effective antibiotics and vaccines provided some hope that modern medical advances would minimize the impact of infectious diseases on human health and that research effort could shift to meet the challenges of chronic degenerative diseases of humans. However, the AIDS epidemic, the emergence of multi-drug resistant tuberculosis and the numerous emerging diseases of viral etiology, including SARS, hantavirus, West Nile virus, etc., have made it very clear that infectious diseases still have the capacity to alter human society dramatically. Thus, infectious disease research remains a very high priority and NHP models of human viral diseases will remain a critical tool in understanding pathogenesis and in developing vaccines and therapies to viral agents that are a major public health challenge.

Correspondence

Any correspondence should be directed to Dr. Christopher J. Miller, California National Primate Research Center, University of California, Davis, CA 95616. Telephone: (530) 752-0447. Fax: (530) 752-2880. E-mail: cjmiller@ucdavis.edu

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