Journal of NeuroVirology, 14: 292–300, 2008

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280802074539

informa
healthcare

Review

Nonhuman primate models of NeuroAIDS

Rachel Williams,[1] Sirosh Bokhari,[2] Peter Silverstein,[2] David Pinson,[3] Anil Kumar,[4] and Shilpa Buch[1]

[1]Departments of Molecular and Integrative Physiology, [2]Microbiology, Molecular Genetics, and Immunology, and [3]Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, Kansas, USA; [4]Division of Pharmacology, School of Pharmacy, University of Missouri, Kansas City, Missouri, USA

Human Immunodeficiency virus (HIV), the virus that causes acquired immunodeficiency syndrome (AIDS), also manifests neurological complications. HIV-associated dementia (HAD) is the most severe form of HIV-induced neurocognitive disorders. HIV encephalitis (HIVE), the pathological correlate of HAD, is characterized by the formation of multinucleated giant cells and microglial nodules, astrocytosis, and neuronal damage and loss. Pathological evaluation of HAD disease progression in humans is not possible, with the only data collected being from individuals who have succumbed to the disorder, a snap shot of end-stage disease at best. Therefore, pertinent animal models have been developed to alleviate this gap of knowledge in the field of neurovirology and neuroinflammation. In general, the most widely used animal models are the simian immunodeficiency virus (SIV) and the chimeric simian/human immunodeficiency virus (SHIV) macaque model systems. Although both SIV and SHIV model systems are able to potentiate neuroinvasion and the concomitant neuropathology similar to that seen in the human syndromes, the innate differences between the two in disease pathogenesis and progression make for two separate, yet effective, systems for the study of HIV-associated neuropathology. *Journal of NeuroVirology* (2008) **14,** 292–300.

Keywords: HIV; macaque; SHIV; SIV

Human immunodeficiency virus (HIV) is a retrovirus that belongs to the family of lentiviruses (meaning "slow viruses") (Joag and Narayan, 1996; Hirsch and Curran, 1996). One of the peculiarities of this family of viruses is their ability to infect cells of the host's immune system, causing persistent and lifelong infection (Narayan and Clements, 1989). It is now well established that the pathogenesis of HIV infection and the complex disease patterns caused by the virus revolve around the dystrophic effects of the infection first in $CD4^+$ T cells and then later, in cells of the macrophage lineage (Rosenberg and Fauci, 1991; Pantaleo *et al,* 1993; Ho *et al*, 1995). The initial stages of viral pathogenesis comprise the highly productive infection in, and elimination of, the $CD4^+$ T-cell population, leading to a loss of cell-mediated immune (CMI) responses and Th1 cytokines, such as interleukin (IL)-2 and interferon (IFN)-γ (Dalgleish, 1995). The lack of these responses is associated with two phenomena: first, development of selected tumors and proliferation of opportunistic pathogens in different organ systems of the body (Barker, 1995; Orenstein *et al*, 1997; Liu *et al*, 1999), and second, progressive viral replication in specific tissues such as the brain (Gendelman *et al*, 1994; Orenstein *et al*, 1997; Igarashi *et al*, 2001). Unlike other viral infections in the brain, HIV-1 infection in the central nervous system (CNS) is centered around viral replication in cells of macrophage lineage (Gendelman *et al*, 1994), which correlates with development of encephalitis and dementia.

The early phase of HIV infection is dominated by highly productive viral replication in the lymph nodes. This results in massive viremia and loss of cells bearing the CD4 phenotype (Levy, 1993; Schnittman and Fauci, 1994). At this stage mononuclear cells in the blood are activated and

Address correspondence to Shilpa Buch, PhD, Department of Molecular and Integrative Physiology, Kansas University Medical Center, Kansas City, 5031 Wahl Hall West, 3901 Rainbow Boulevard, Kansas City, KS 66103, USA. E-mail: sbuch@kumc.edu

This review is dedicated to the memory of the late Professor Bill Narayan (1936–2007).

This work was supported in part by NIH grant R01 MH068212 and NIDA grants R01 DA024442 and DA020392-01 to S.B.

$CD4^+$ T cells are productively infected in the presence of Th1 cytokines (Clerici *et al*, 1994; Clerici and Shearer, 1993). Large numbers of monocytes are also infected in this stage, but macrophages derived from these cells show only minimal viral productivity. Although HIV appears in the cerebrospinal fluid (CSF) during this phase of the infection (Gabuzda and Sobel, 1987), the nature of the infection in the CNS is not known. Whether the presence of virus in the CSF/CNS results from trafficking activated, infected $CD4^+$ T cells, monocytes, or dendritic cells (DCs) from periphery is not known. Activated, infected $CD4^+$ T cells are probably important for this event because molecularly cloned macrophage-tropic SIV_{mac} viruses are poorly neuroinvasive (Lackner *et al*, 1994). Further, the prototypic lentivirus infection—visna virus infection—in sheep, which is strictly macrophage tropic, is not neuroinvasive (Chebloune *et al*, 1998), even though the virus is highly neurovirulent when injected intracerebrally (Craig *et al*, 1997).

Following the early phase of activation and infection, which is accompanied by increased virus production by the $CD4^+$T cells, subsequent progressive elimination of these cells occurs due to the viral cytopathic effects. Antiviral CMI responses of the Th1 type develop and initially reduce virus replication, but fail to eliminate the agent. The continued loss of $CD4^+$T cells inevitably results in the loss of the T-cell response required for driving the CMI responses. This leads to the collapse of the cellular arm of the immune response and the loss of immunocompetence. The Th2 arm of the immune system remains intact and frequently becomes activated when opportunistic pathogens begin to proliferate in the void of Th1 responsiveness. These pathogens include certain protozoa, viruses, mycotic agents, and intracellular bacteria, such as the mycobacterium species. Macrophages induced by these pathogens often become the target for viral replication in whatever tissue the parasites proliferate; this includes the CNS.

Productive viral replication in the CNS coincides with the severe loss of T-cell function. HIV-associated dementia (HAD) is a clinical disorder characterized by progressive cognitive, motor, and behavioral abnormalities (Navia *et al*, 1986) caused by HIV-1 infection. Prominent neurological disease occurs in 15% to 20% (McArthur *et al*, 1999) of infected individuals and is associated with a marked depletion of $CD4^+$T lymphocytes (Glass *et al*, 1995; Michaels *et al*, 1988; Wiley *et al*, 1986). HIV-1 encephalitis (HIVE), a common pathological manifestation of HAD, includes the infiltration of macrophages into brain where they become productively infected with the virus. This is accompanied by massive cytokine (Poli *et al*, 1995) and chemokine dysregulation in the brain, which often culminates into the unique neuropathology belonging to this syndrome. Characteristic pathological changes of HIVE include perivascular accumulations of mononuclear cells, formation of microglial nodules and multinucleated giant cells, activation and proliferation of astrocytes, and neuronal dysfunction and loss (Bell, 1998; Gendelman *et al*, 1994; Nath, 1999). Productive infection of macrophages and microglial cells is believed to contribute to pathological changes in neurons through secretion of viral (gp120, Tat, and Nef) and cellular (cytokines, chemokines, and nitric oxide) neurotoxic products (Kolson and Pomerantz, 1996; Lipton and Gendelman, 1995).

The mechanism of late onset of the neurological complications in HIV-infected individuals is puzzling because the virus can enter the CNS within days of primary infection. It remains unclear (a) whether neurological complications are due to virus reactivation during the late stage of infection; (b) whether there is a renewed phase of viral neuroinvasion in late stage disease; or (c) whether the virus is continuously replicating in the CNS at low levels. Although studies on humans with HIV-CNS disease have contributed significantly to our understanding of the pathogenesis of HAD, they only generate snapshots of the disease end point. This creates a need for an animal-model system that will accurately reproduce the pathogenesis of HIV infection. However, HIV does not cause disease in animals, therefore the questions of neuropathogenesis have to be relegated to studies in macaques infected with simian immunodeficiency virus (SIV) and chimeras of SIV and HIV—SHIVs (Joag *et al*, 1997; Lackner *et al*, 1991, 1994). SIV infection in macaques has provided excellent models of HIV encephalopathy (Joag *et al*, 1997; Lackner *et al*, 1991, 1994). Pathogenic chimeric SHIVs, which have the envelope of HIV-1 (HXB2) on a background of $SIV_{mac}239$, have also provided additional working models of HIV neurological disease. These chimeric viruses provide a unique opportunity to examine the effect of the HIV-1 envelope on the biology of the virus in the brain within the context of neurological disease. In the following sections we will discuss each of the model systems and their advantages and/or limitations as it relates to the study of NeuroAIDS in humans.

SIV infection in macaques

Most studies on the neuropathogenesis of SIV infection in macaques have been performed with the strains $SIV_{mac}239$ and $SIV_{mac}251$ (Lackner *et al*, 1994; Luciw *et al*, 1992; Smith *et al*, 1995).These viruses use both the CD4 and CCR5 receptors for entry into host cells (Edinger *et al*, 1997; Kirchhoff *et al*, 1997; Weissman *et al*, 1997). Both SIVs cause acute infections characterized by cell-associated and cell-free viremia. Virus-infected cells can be found in the CSF throughout the acute phase, which can last for 2 to 10 weeks (Joag *et al*, 1994; Sharma *et al*, 1992a, 1992b). During this phase, infection in the CNS is characterized by meningitis and accumulations of mononuclear cells in the Virchow-Robin spaces in the brain. Virus-infected macrophages have also been identified

in these exudate cells (Lackner *et al*, 1991; Lane *et al*, 1996). Lesions caused by $SIV_{mac}239$ are less severe and do not involve the neuropil. Instead, meningitis disappears at the end of the acute, systemic phase of infection (Joag *et al*, 1994; Lackner *et al*, 1994), and viral DNA, demonstrable by polymerase chain reaction (PCR), provides the only record of the antecedent infection in the brain (Stephens *et al*, 1995). The failure of the virus to cause progressive CNS infection could be attributable to its inability to replicate in macrophages (Stephens *et al*, 1997).

Studies on SIV infection in macaques have shown that in all cases of encephalopathy, viral replication in macrophages in brain (identified by *in situ* hybridization and immunohistochemistry) correlated with the ability of the virus to replicate productively in cultured macrophages derived from peripheral blood of animals of the affected species. Examples of these are SIV/17E-Fr (Zink *et al*, 1997) and SIVsmmFGb infection in pig-tailed macaques (Bucur *et al*, 1998). These assays confirmed that the potential for causing encephalitis required that the virus be capable of replicating in activated $CD4^+$ T cells and in macrophages, and that viruses incapable of replication in macrophages were incapable of causing encephalitis (Lackner *et al*, 1994; Sharma *et al*, 1992b). These viruses all utilize the CCR5 coreceptor for entry into susceptible cells, but it is of interest that other strains of SIV that also use the CCR5 coreceptor and were incapable of causing encephalopathy were also incapable of replicating productively in peripheral blood mononuclear cell (PBMC)-derived macrophages (Stephens *et al*, 1997).

$SIV_{mac}251$ is a dual-tropic virus, capable of replicating productively in $CD4^+$ T lymphocytes and macrophages (Stephens *et al*, 1997; Wu *et al*, 1996). During the acute phase of infection caused by this virus, viral neuroinvasion and meningitis develop in a pattern identical to that observed with $SIV_{mac}239$ (Chakrabarti *et al*, 1991; Lackner *et al*, 1994; Luciw *et al*, 1992; Smith *et al*, 1995); however, the $SIV_{mac}251$ infection progresses to involve the neuropil. Similar to the human syndrome, astrocytes and microglia also become activated, the latter frequently undergoing proliferation, resulting in nodular accumulations (Blumberg *et al*, 1994; da Cunha *et al*, 1997; Saito *et al*, 1994; Sharer *et al*, 1994, 1996). Encephalitic changes, involving dense infiltrations of mononuclear cells, develop in both gray and white matter with the spread of mononuclear inflammatory cells from perivascular locations into the brain substance (Raghavan *et al*, 1999). Multinucleated giant cells occur frequently among the inflammatory cells and there is extensive viral replication in macrophages. Unlike the human syndrome, in $SIV_{mac}251$-infected macaques, inflammatory lesions in the meninges and neuropil occur in the presence of relatively normal numbers of $CD4^+$ T lymphocytes in peripheral blood, although $CD4^+$ T cells frequently "crash" during progressive neurological disease (Raghavan *et al*, 1999). These histopathological changes progress continuously in animals that fail to develop antiviral immune responses. Furthermore, unlike the human CNS disease, SIV lesions in the CNS are much more severe compared with those seen in HIV-infected brains. These differences can be attributed to the natural history of HIV infection in which $CD4^+$ T cells are lost prior to development of neurological symptoms, whereas in macaques, the $CD4^+$ T cells may contribute to the development of lesions.

As opposed to infected animals that fail to develop immune responses and succumb to neurological disease, some infected macaques that develop CMI responses to the virus following acute phase of infection will also control systemic and CNS viral replication (Kuroda *et al*, 1999). However, treatment of such animals with antibodies to $CD8^+$ T cells abolished the cytotoxic T-lymphocyte (CTL) response, resulting in the onset of neurological disease accompanied by productive virus replication in the brain (Matano *et al*, 1998; Schmitz *et al*, 1999a). These findings led to the suggestion that lesions that might have developed during acute infection underwent resolution, concomitant with CTL-mediated retrenchement of viral replication in the lymphoid system and the brain. Following elimination of CTLs with antibodies, viral recrudescence developed in the brain.

Macaques infected with $SIV_{mac}182$ have also aided in our understanding of how virus-host interactions contribute to the related CNS functional defects. In this model, SIV-specific $CD8^+$ CTLs were discovered in the CSF of infected macaques very early after infection and in the brain at necropsy (Marcondes *et al*, 2007). This model has been used to explore the impact of immune responses in mediating CNS injury and was exploited to evaluate the brain infiltrate and the phenotype of T cells in the CNS compared with those in the periphery, at a relatively early stage of SIV-induced disease. It has been elegantly demonstrated that $SIV_{mac}251$-infected macaques have altered evoked potentials, a drastic increase in the number of activated $CD8^+$T cells expressing predominantly a memory and a cytolytic phenotype and a unique cytokine milieu in the brains during early SIV infection. These findings suggest that SIV infection induces a chronic immune response in the CNS that mediates injury to the brain (Marcondes *et al*, 2001, 2003, 2007).

Although the SIV-infected macaque model of neuro AIDS best recapitulates HIV neuropathogenesis, there are limitations with this model. For one, similar to the human scenario, not all infected macaques predictably develop CNS disease, in fact the percentage of animals developing SIV encephalitis (SIVE) is only 25%. Secondly, the prolonged progression (1 to 3 years) towards development of AIDS further limits its usefulness. This has led to the development of two additional accelerated macaque models that overcome the barriers of low incidence of SIVE and prolonged time to disease pathogenesis (Schmitz

et al, 1999b; Zink *et al*, 1997, 1999). One of these models developed by Zink *et al* uses pigtailed macaques coinoculated intravenously with a combination of two viruses: SIV/17E-Fr, a neurovirulent molecular clone, and SIV/DeltaB670, an immunosuppressive and neurovirulent virus swarm (Clements *et al*, 2002; Craig *et al*, 1997; Zink *et al*, 1999). Infection with these viruses leads to predictable $CD4^+$ T-lymphocyte depletion and highly efficient replication in CNS macrophages within 3 months post inoculation in almost 90% of inoculated macaques. This highly reproducible and predictable model of neuro AIDS has proven extremely valuable in exploring the host and viral events in the CNS during acute infection and during the subsequent development of SIVE. In this model, viral load in the CSF during terminal infection and the CSF:plasma ratio of monocyte chemoattractant protein (MCP)-1 are strong predictors of the severity of encephalitis. Many key questions pertaining to persistence of latent viral DNA in the CNS in the asymptomatic phase of infection and selective replication of macrophage-tropic, neurovirulent viral strains during terminal stage of CNS disease have been answered using this accelerated model.

The other accelerated model of SIV infection involves the use of antibody-mediated depletion of $CD8^+$ T lymphocytes, which results in the accumulation of monocytes/macrophages in the CNS early after infection, rapid disease progression, and severe SIVE (Clements *et al*, 2002; Craig *et al*, 1997; Zink *et al*, 1999). This accelerated model of SIV infection in rhesus macaques eliminated $CD8^+$ T lymphocytes during chronic SIV infection, resulting in a rapid and marked increase in viremia that was again suppressed coinciding with the reappearance of SIV-specific $CD8^+$ T cells. (Schmitz *et al*, 1999a).

SHIV infection in macaques

Although SIV-infected macaques have been critical in our understanding of lentiviral neuropathogenesis, the genetic relatedness of these viruses is more to HIV-2 than HIV-1, especially in the *env* gene, which raises the question of whether these models accurately mimic the neuropathogenesis of HIV-1 infection. The construction of the chimeric of SHIV has led to significant advances in the field of AIDS, particularly in the study of immune response and vaccine development. It was necessary to establish an animal model system of AIDS that not only reflected the variety of cells, organs, and tissues afflicted, but could exhibit the immune responses typical to HIV-1 infection in humans (Joag, 2000). In general, SHIVs are created by combining the HIV-1 envelope gene *env* and accessory genes *tat, rev,* and *vpu* with the SIV_{mac} genome, including the *gag* and *pol* SIV genes (Joag, 2000). When the SHIV/macaque model first started being utilized, there was a discrepancy between animal infectability and the development of disease. The macaques inoculated with SHIV, though infected, failed to show clinical or pathological signs of the disease (Joag, 2000; Joag *et al*, 1996). The discovery that serial passaging of SHIV in the bone marrow of infected macaques, and/or animal-to-animal passaging, could produce pathogenic SHIV revolutionized exploitation of the SHIV/macaque model (Joag *et al*, 1996). Such innovation has led to the development of several strains and variations of SHIV, each one with specific characteristics in regard to pathogenic phenotype that make it convenient for studying certain aspects of AIDS progression.

SHIV infection in macaques creates a model system closely resembling the disease course of human AIDS. In most cases, within a number weeks the infected macaque will demonstrate a depletion of $CD4^+$ T lymphocytes, leading to the occurrence of AIDS in a time frame of a few weeks to 2 years (Joag, 2000). Another key feature of the SHIV/macaque model is that the pathology of certain tissues and organ systems, such as the CNS, are compatible with the corresponding pathological changes seen in human AIDS. This underscores the utility of SHIV in examining specific HIV-1–induced disease pathology, such as encephalitis, making SHIV an essential model for the study of neuro AIDS (Joag, 2000).

There are two main categories of SHIVs partitioned into coreceptor usage: CXCR4 (X4)- or CCR5 (R5)-tropic, and dual (X4/R5)-tropic, depending on the specificity of the HIV-1 clone used in the creation of the chimera. It has been reported that X4- and R5-tropic SHIVs have differential pathogenic routes, with X4-tropic viruses causing a substantial loss of T lymphocytes in the periphery, whereas R5/dual-tropic viruses negatively impact the T-cell count first in the gut, then gradually deplete the numbers in the periphery (Harouse *et al*, 1999). The most widely recognized SHIVs with X4-tropic HIV-1 genes are the KU-1, KU-2, SIV_{agm}/HIV-1, and RT-SHIV. Both $SHIV_{KU-1/2}$ were constructed on a SIV_{mac293} genomic background with the *env*, *tat*, *rev*, and *vpu* genes of HIV-1 HXBc2, an X4-tropic virus (Joag *et al*, 1996, 1997; Liu *et al*, 1999). The $SHIV_{KU-1}$ and $SHIV_{KU-2}$ pathogenic viruses can cause a decrease in the $CD4^+$ T-lymphocyte count and the development of AIDS in macaques in 1 year with associated diseases such as pneumonia, encephalitis, and other opportunistic pathogens (Joag *et al*, 1996, 1997; Liu *et al*, 1999). Examination of archival tissues from macaques infected with $SHIV_{KU-2}$ showed that of 14 infected rhesus macaques that developed AIDS, 10 had neuropathological changes with clear evidence of virus replication in macrophages in the brain. In contrast, of 22 infected pig-tailed macaques that developed AIDS, 21 had no evidence of $SHIV_{KU-2}$ replication in the brain. This difference in susceptibility to CNS disease caused by $SHIV_{KU-2}$ in the two macaque species provides a novel system to further explore

mechanisms of lentiviral neuropathogenesis (Joag *et al*, 1996, 1997; Liu *et al*, 1999).

Different from the KU-1 and KU-2 SHIVs, the SIV_{agm}/HIV-1 construct was created with the HIV-1 molecular clone NL4.3 (and variations thereof) in combination with SIV_{agm}(Shibata *et al*, 1990, 1991) instead of SIV_{mac}293. The reverse transcriptase (RT)-SHIV system is atypical from other SHIVs in that it replaces only the SIV_{mac239} reverse transcriptase with that of the HIV-1 clone HXBc2, without inclusion of any of the other HIV-1 genes. This model system is essentially used for testing the effectiveness of pharmaceutical drugs targeting the reverse transcriptase activity (North *et al*, 2005; Soderberg *et al*, 2002; Uberla *et al*, 1995).

In the pool of R5/dual-tropic SHIVs, there are two main model systems, $SHIV_{89.6P}$ and $SHIV_{SF162}$. Both constructs are based on a SIV_{mac239} background; however, $SHIV_{89.6P}$ (and its variants) use an isolate of HIV-1 from an AIDS patient ($HIV\text{-}1_{89.6}$) (Karlsson *et al*, 1997; Reimann *et al*, 1996), whereas $SHIV_{SF162}$ has a $HIV\text{-}1_{SF162}$ counterpart (Luciw *et al*, 1995; Harouse *et al*, 1999, 2001). These models are pertinent systems when examining NeuroAIDS due to their enhanced ability to infect macrophages and microglia, the only known resident cell types within the CNS capable of sustaining productive HIV-1 infection.

In studying a model system for HIVE, the development of neuropathology is the rate-limiting feature, and not all SHIVs are created equal when using this criteria as a delimiting factor. For example, in examining pig-tailed macaques infected with $SHIV_{KU-1}$, no viral infection or pathology was found in the CNS (Buch *et al*, 2002; Raghavan *et al*, 1997). These results are in contrast to the findings of rhesus macaques subjected to $SHIV_{KU-2}$ infection, where a majority of the animals displayed productive SHIV replication in the brain associated with focal multinucleated giant cells and nodular, demyelinating lesions—neuropathology associated with HIVE (Buch *et al*, 2002; Raghavan *et al*, 1997).

In a head to head study comparing $SHIV_{KU}$ to $SHIV_{89.6P}$ in respect to the development of neuropathogenesis, results pointed towards $SHIV_{89.6P}$ for more consistently leading to the development of neuropathology linked to HIV-1–associated CNS disease (Buch *et al*, 2000). The $SHIV_{89.6P}$-infected macaques that developed encephalitis displayed activated astrocytes and microglia, multinucleated giant cells, nodular lesions of microglia, and macrophages harboring viral RNA. The $SHIV_{KU}$-infected macaques sustained a progressive, yet slow, infection of the CNS; however, they lacked typical neuropathology and productively infected macrophages were not found (Buch *et al*, 2000). However, baboons infected with either $SHIV_{KU}$ or $SHIV_{89.6P}$ demonstrated productive infection of microglia (Kanmogne *et al*, 2002). Another study has demonstrated that the presence of IL-4 is necessary for the induction of neuropathology in X4-tropic strains of SHIV (Buch *et al*, 2004b).

The inherent differences between strains of SHIV could lead to the differential development of CNS disease. Although both viruses can take advantage of the CXCR4 coreceptor, $SHIV_{89.6P}$ is also adept at utilizing the CCR5 coreceptor, a receptor found on macrophages and microglia, perhaps enhancing its innate neurovirulence (Buch *et al*, 2000). Such results underscore the importance of host species and viral construct in the study of specific aspects of a disease.

After the confirmation of SHIV-dependent neurological disease in the macaque animal model, the mechanisms by which SHIV exerts its neurovirulence were investigated. One SHIV/macaque model demonstrated blood-brain barrier (BBB) perturbation after 2 weeks of SHIV infection. Specifically, the expression of the tight junction protein zonula occludens, which plays an integral role in BBB integrity, was significantly decreased (Stephens *et al*, 2003).

The current thinking on HIV-1–associated CNS disease is that it is the consequence of the interplay between HIV-1/viral proteins and host immune factors, resulting in the dysregulation of cytokines/chemokines, which in turn leads to the neuropathological manifestation of the disease (Buch *et al*, 2004a; Sui *et al*, 2003). Therefore, much emphasis has been placed on determining which host immune factors in SHIVE macaques are imbalanced as compared to those in control animals. A microarray analysis of brain tissue from $SHIV_{89.6P}$ encephalitic macaques revealed a significant up-regulation of several cytokines and chemokines, including IL-4, MCP-1, and interferon-inducible peptide-10 (IP-10/CXCL10) (Buch *et al*, 2004a; Sui *et al*, 2003). These genes all have the capability to regulate immune cell influx to the CNS, and when up-regulated can lead to the neuroinvasion typical of HIVE pathology (Sui *et al*, 2003). On the opposite spectrum, growth factors, such as brain-derived growth factor (BDNF), necessary for maintaining the sensitive neuronal environment, were down-regulated in SHIVE macaques (Sui *et al*, 2003). However, the expression of another growth factor, the platelet-derived growth factor B chain (PDGF-B), was found to be increased in the brains of $SHIV_{KU-2}$ encephalitic macaques in a separate study (Potula *et al*, 2004).

Additionally, a neurovirulent $SHIV_{KU-1}$ variant derived from a macaque that developed neurological disease has been used in several studies (Singh *et al*, 2001, 2002, 2003; Stephens *et al*, 2006). This strain of SHIV, with a nonfunctional *vpu* gene and mutations in the *env* and *nef* genes, is capable of causing astrocytosis (Singh *et al*, 2002, 2003), along with up-regulating several genes, including IL-6 and cripto-1, in the brains of the infected macaques (Singh *et al*, 2001). IL-6 can have immunoregulatory properties concerning microglia and astroglia activation, which contribute to neuroinflammation in the diseased brain. Cripto-1, which has neuroprotective properties, has been shown to colocalize with neurons

during viral assault on the CNS, perhaps representing a neuronal survival mechanism (Stephens *et al*, 2006).

Such a wide range of host responses that are species and viral strain dependent only emphasize the delicate balance between disease states and normal CNS functioning in the complex battle between host and virus during SHIVE. To delineate such complexities for the use of therapies, vaccine or otherwise, is of the foremost importance and the reason why the SHIV/macaque model system is so valuable.

Conclusion

In summary, the SIV and SHIV macaque models of HIV infection have shown that the virus invades the brain early during the systemic infection, and that CNS infection is mediated by infected mononuclear cells and T cells that cross the blood brain barrier. Though the study of HAD is ongoing, the exact cellular and molecular mechanisms by which the disease progresses are still uncertain. The SIV and SHIV animal model systems have shed light on a multitude of viral and host factor functions and interactions during both active and latent infection. More recently, the SIV-infected macaques continue to enhance our understanding of how drugs of abuse can synergize with the virus to accelerate the development of SIV-induced encephalitis (Marcario *et al*, 2008). With key differences in disease progression and neuropathology, selection of the appropriate animal model for the hypothesis in question is of critical importance. Already, studies using pertinent macaque model systems have demonstrated both the effectiveness and the shortcomings of therapies targeting immune activation in the CNS. With the use of both the SIV and the SHIV macaque model systems, scientists are advancing the fields of Neuro Virology and neuroimmune pharmacology in their quest to unlock the mysteries of HIV-associated neurocognitive disorders.

References

Bell JE (1998). The neuropathology of adult HIV infection. *Rev Neurol (Paris)* **154:** 816–829.

Blumberg BM, Gelbard HA, Epstein LG (1994). HIV-1 infection of the developing nervous system: central role of astrocytes in pathogenesis. *Virus Res* **32:** 253-267.

Buch S, Pinson D, Hou Y, Adany I, Li Z, Mukherjee S, Jia F, Mackay G, Silverstein P, Kumar A, Narayan O (2000). Neuropathogenesis of chimeric simian human immunodeficiency virus infection in rhesus macaques. *J Med Primatol* **29:** 96–106.

Buch S, Sui Y, Dhillon N, Potula R, Zien C, Pinson D, Li S, Dhillon S, Nicolay B, Sidelnik A, Li C, Villinger T, Bisarriya K, Narayan O (2004a). Investigations on four host response factors whose expression is enhanced in X4 SHIV encephalitis. *J Neuroimmunol* **157:** 71–80.

Buch S, Sui Y, Potula R, Pinson D, Adany I, Li Z, Huang M, Li S, Dhillon N, Major E, Narayan O (2004b). Role of interleukin-4 and monocyte chemoattractant protein-1 in the neuropathogenesis of X4 simian human immunodeficiency virus infection in macaques. *J Neuro Virol* **10(Suppl 1):** 118–124.

Buch SJ, Villinger F, Pinson D, Hou Y, Adany I, Li Z, Dalal R, Raghavan R, Kumar A, Narayan O (2002). Innate differences between simian-human immunodeficiency virus (SHIV)(KU-2)-infected rhesus and pig-tailed macaques in development of neurological disease. *Virology* **295:** 54–62.

Bucur SZ, Lackey DA, 3rd, Adams JW, Lee ME, Villinger F, Mayne A, Bray RA, Winton EF, Novembre F, Strobert EA, De Rosayro J, Dailey PJ, Ansari AA, Hillyer CD (1998). Hematologic and virologic effects of lineage-specific and non-lineage-specific recombinant human and rhesus cytokines in a cohort of SIVmac239-infected macaques. *AIDS Res Hum Retroviruses* **14:** 651–660.

Chakrabarti L, Hurtrel M, Maire MA, Vazeux R, Dormont D, Montagnier L, Hurtrel B (1991). Early viral replication in the brain of SIV-infected rhesus monkeys. *Am J Pathol* **139:** 1273–1280.

Chebloune Y, Karr BM, Raghavan R, Singh DK, Leung K, Sheffer D, Pinson D, Foresman L, Narayan O (1998). Neuroinvasion by ovine lentivirus in infected sheep mediated by inflammatory cells associated with experimental allergic encephalomyelitis. *J Neuro Virol* **4:** 38–48.

Clements JE, Babas T, Mankowski JL, Suryanarayana K, Piatak M Jr, Tarwater PM, Lifson JD, Zink MC (2002). The central nervous system as a reservoir for simian immunodeficiency virus (SIV): steady-state levels of SIV DNA in brain from acute through asymptomatic infection. *J Infect Dis* **186:** 905–913.

Clerici M, Levin JM, Kessler HA, Harris A, Berzofsky JA, Landay AL, Shearer GM (1994). HIV-specific T-helper activity in seronegative health care workers exposed to contaminated blood. *JAMA* **271:** 42–46.

Clerici M, Shearer GM (1993). A TH1LTH2 switch is a critical step in the etiology of HIV infection. *Immunol Today* **14:** 107–111.

Craig LE, Sheffer D, Meyer AL, Hauer D, Lechner F, Peterhans E, Adams RJ, Clements JE, Narayan O, Zink MC (1997). Pathogenesis of ovine lentiviral encephalitis: derivation of a neurovirulent strain by in vivo passage. *J Neuro Virol* **3:** 417–27.

da Cunha A, Mintz M, Eiden LE, Sharer LR (1997). A neuronal and neuroanatomical correlate of HIV-1 encephalopathy relative to HIV-1 encephalitis in HIV-1-infected children. *J Neuropathol Exp Neurol* **56:** 974–987.

Edinger AL, Amedee A, Miller K, Doranz BJ, Endres M, Sharron M, Samson M, Lu ZH, Clements JE, Murphey-Corb M, Peiper SC, Parmentier M, Broder CC, Doms RW (1997). Differential utilization of CCR5 by macrophage and T cell tropic simian immunodeficiency virus strains. *Proc Natl Acad Sci U S A* **94:** 4005–4010.

Gabuzda DH, Sobel RA (1987). HIV antigen in brains of patients with AIDS. *Ann Neurol* **22:** 668.

Gendelman HE, Lipton SA, Tardieu M, Bukrinsky MI, Nottet HS (1994). The neuropathogenesis of HIV-1 infection. *J Leukoc Biol* **56:** 389–398.

Glass JD, Fedor H, Wesselingh SL, McArthur JC (1995). Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia. *Ann Neurol* **38:** 755–762.

Harouse JM, Gettie A, Eshetu T, Tan RC, Bohm R, Blanchard J, Baskin G, Cheng-Mayer C (2001). Mucosal transmission and induction of simian AIDS by CCR5-specific simian/human immunodeficiency virus SHIV(SF162P3). *J Virol* **75:** 1990–1995.

Harouse JM, Gettie A, Tan RC, Blanchard J, Cheng-Mayer C (1999). Distinct pathogenic sequela in rhesus macaques infected with CCR5 or CXCR4 utilizing SHIVs. *Science* **284:** 816–819.

Joag SV (2000). Primate models of AIDS. *Microbes Infect* **2:** 223–229.

Joag SV, Adams RJ, Foresman L, Galbreath D, Zink MC, Pinson DM, McClure H, Narayan O (1994). Early activation of PBMC and appearance of antiviral CD8+ cells influence the prognosis of SIV-induced disease in rhesus macaques. *J Med Primatol* **23:** 108–116.

Joag SV, Li Z, Foresman L, Pinson DM, Raghavan R, Zhuge W, Adany I, Wang C, Jia F, Sheffer D, Ranchalis J, Watson A, Narayan O (1997). Characterization of the pathogenic KU-SHIV model of acquired immunodeficiency syndrome in macaques. *AIDS Res Hum Retroviruses* **13:** 635–645.

Joag SV, Li Z, Foresman L, Stephens EB, Zhao LJ, Adany I, Pinson DM, McClure HM, Narayan O (1996). Chimeric simian/human immunodeficiency virus that causes progressive loss of CD4+ T cells and AIDS in pig-tailed macaques. *J Virol* **70:** 3189–3197.

Kanmogne GD, Kennedy RC, Grammas P (2002). Infection of baboon microglia with SIV-HIV recombinant viruses: role of CD4 and chemokine receptors. *AIDS Res Hum Retroviruses* **18:** 557–565.

Karlsson GB, Halloran M, Li J, Park IW, Gomila R, Reimann KA, Axthelm MK, Iliff SA, Letvin NL, Sodroski J (1997). Characterization of molecularly cloned simian-human immunodeficiency viruses causing rapid CD4+ lymphocyte depletion in rhesus monkeys. *J Virol* **71:** 4218–4225.

Kirchhoff F, Pohlmann S, Hamacher M, Means RE, Kraus T, Uberla K, Di Marzio P (1997). Simian immunodeficiency virus variants with differential T-cell and macrophage tropism use CCR5 and an unidentified cofactor expressed in CEMx174 cells for efficient entry. *J Virol* **71:** 6509–6516.

Kolson DL, Pomerantz RJ (1996). AIDS dementia and HIV-1-induced neurotoxicity: possible pathogenic associations and mechanisms. *J Biomed Sci* **3:** 389–414.

Kuroda MJ, Schmitz JE, Charini WA, Nickerson CE, Lifton MA, Lord CI, Forman MA, Letvin NL (1999). Emergence of CTL coincides with clearance of virus during primary simian immunodeficiency virus infection in rhesus monkeys. *J Immunol* **162:** 5127–5133.

Lackner AA, Smith MO, Munn RJ, Martfeld DJ, Gardner MB, Marx PA, Dandekar S (1991). Localization of simian immunodeficiency virus in the central nervous system of rhesus monkeys. *Am J Pathol* **139:** 609–621.

Lackner AA, Vogel P, Ramos RA, Kluge JD, Marthas M (1994). Early events in tissues during infection with pathogenic (SIVmac239) and nonpathogenic (SIV_{mac}1A11) molecular clones of simian immunodeficiency virus. *Am J Pathol* **145:** 428–439.

Lane JH, Sasseville VG, Smith MO, Vogel P, Pauley DR, Heyes MP, Lackner AA (1996). Neuroinvasion by simian immunodeficiency virus coincides with increased numbers of perivascular macrophages/microglia and intrathecal immune activation. *J Neuro Virol* **2:** 423–432.

Levy JA (1993). Pathogenesis of human immunodeficiency virus infection. *Microbiol Rev* **57:** 183–289.

Lipton SA, Gendelman HE (1995). Seminars in medicine of the Beth Israel Hospital, Boston. Dementia associated with the acquired immunodeficiency syndrome. *N Engl J Med* **332:** 934–940.

Liu ZQ, Muhkerjee S, Sahni M, McCormick-Davis C, Leung K, Li Z, Gattone VH 2nd, Tian C, Doms RW, Hoffman TL, Raghavan R, Narayan O, Stephens EB (1999). Derivation and biological characterization of a molecular clone of SHIV(KU-2) that causes AIDS, neurological disease, and renal disease in rhesus macaques. *Virology* **260:** 295–307.

Luciw PA, Pratt-Lowe E, Shaw KE, Levy JA, Cheng-Mayer C (1995). Persistent infection of rhesus macaques with T-cell-line-tropic and macrophage-tropic clones of simian/human immunodeficiency viruses (SHIV). *Proc Natl Acad Sci U S A* **92:** 7490–7494.

Luciw PA, Shaw KE, Unger RE, Planelles V, Stout MW, Lackner JE, Pratt-Lowe E, Leung NJ, Banapour B, Marthas ML (1992). Genetic and biological comparisons of pathogenic and nonpathogenic molecular clones of simian immunodeficiency virus (SIVmac). *AIDS Res Hum Retroviruses* **8:** 395–402.

Marcario JK, Riazi M, Adany I, Kenjale H, Fleming K, Marquis J, Nemon O, Mayo MS, Yankee T, Narayan O, Cheney PD (2008). Effect of morphine on the neuropathogenesis of SIVmac infection in Indian rhesus macaques. *J Neuroimmune Pharmacol* **3:** 12–25.

Marcondes MC, Burdo TH, Sopper S, Huitron-Resendiz S, Lanigan C, Watry D, Flynn C, Zandonatti M, Fox HS (2007). Enrichment and persistence of virus-specific CTL in the brain of simian immunodeficiency virus-infected monkeys is associated with a unique cytokine environment. *J Immunol* **178:** 5812–9.

Marcondes MC, Burudi EM, Huitron-Resendiz S, Sanchez-Alavez M, Watry D, Zandonatti M, Henriksen SJ, Fox HS (2001). Highly activated CD8(+) T cells in the brain correlate with early central nervous system dysfunction in simian immunodeficiency virus infection. *J Immunol* **167:** 5429–5438.

Marcondes MC, Phillipson CA, Fox HS (2003). Distinct clonal repertoire of brain CD8+ cells in simian immunodeficiency virus infection. *AIDS* **17:** 1605–1611.

Matano T, Shibata R, Siemon C, Connors M, Lane HC, Martin MA (1998). Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. *J Virol* **72:** 164–169.

McArthur JC, Sacktor N, Selnes O (1999). Human immunodeficiency virus-associated dementia. *Semin Neurol* **19:** 129–150.

Michaels J, Price RW, Rosenblum MK (1988). Microglia in the giant cell encephalitis of acquired immune deficiency syndrome: proliferation, infection and fusion. *Acta Neuropathol (Berl)* **76:** 373–379.

Nath A (1999). Pathobiology of human immunodeficiency virus dementia. *Semin Neurol* **19:** 113–127.

Navia BA, Jordan BD, Price RW (1986). The AIDS dementia complex: I. Clinical features. *Ann Neurol* **19:** 517–524.

North TW, Van Rompay KK, Higgins J, Matthews TB, Wadford DA, Pedersen NC, Schinazi RF (2005). Suppression of virus load by highly active antiretroviral therapy in rhesus macaques infected with a recombinant simian immunodeficiency virus containing reverse transcriptase from human immunodeficiency virus type 1. *J Virol* **79:** 7349–7354.

Poli G, Vicenzi E, Ghezzi S, Lazzarin A (1995). Cytokines in the acquired immunodeficiency syndrome and other infectious diseases. *Int J Clin Lab Res* **25:** 128–134.

Potula R, Dhillion N, Sui Y, Zien CA, Funa K, Pinson D, Mayo MS, Singh DK, Narayan O, Buch S (2004). Association of platelet-derived growth factor-B chain with simian human immunodeficiency virus encephalitis. *Am J Pathol* **165:** 815–824.

Raghavan R, Cheney PD, Raymond LA, Joag SV, Stephens EB, Adany I, Pinson DM, Li Z, Marcario JK, Jia F, Wang C, Foresman L, Berman NE, Narayan O (1999). Morphological correlates of neurological dysfunction in macaques infected with neurovirulent simian immunodeficiency virus. *Neuropathol Appl Neurobiol* **25:** 285–294.

Raghavan R, Stephens EB, Joag SV, Adany I, Pinson DM, Li Z, Jia F, Sahni M, Wang C, Leung K, Foresman L, Narayan O (1997). Neuropathogenesis of chimeric simian/human immunodeficiency virus infection in pig-tailed and rhesus macaques. *Brain Pathol* **7:** 851–861.

Reimann KA, Li JT, Veazey R, Halloran M, Park IW, Karlsson GB, Sodroski J, Letvin NL (1996). A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate env causes an AIDS-like disease after in vivo passage in rhesus monkeys. *J Virol* **70:** 6922–6928.

Saito Y, Sharer LR, Epstein LG, Michaels J, Mintz M, Louder M, Golding K, Cvetkovich TA, Blumberg BM (1994). Overexpression of nef as a marker for restricted HIV-1 infection of astrocytes in postmortem pediatric central nervous tissues. *Neurology* **44:** 474–481.

Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, Racz P, Tenner-Racz K, Dalesandro M, Scallon BJ, Ghrayeb J, Forman MA, Montefiori DC, Rieber EP, Letvin NL, Reimann KA (1999a). Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* **283:** 857–860.

Schmitz JE, Simon MA, Kuroda MJ, Lifton MA, Ollert MW, Vogel CW, Racz P, Tenner-Racz K, Scallon BJ, Dalesandro M, Ghrayeb J, Rieber EP, Sasseville VG, Reimann KA (1999b). A nonhuman primate model for the selective elimination of CD8+ lymphocytes using a mouse-human chimeric monoclonal antibody. *Am J Pathol* **154:** 1923–1932.

Schnittman SM, Fauci AS (1994). Human immunodeficiency virus and acquired immunodeficiency syndrome: an update. *Adv Intern Med* **39:** 305–355.

Sharer LR, Saito Y, Da Cunha A, Ung PC, Gelbard HA, Epstein LG, Blumberg BM (1996). In situ amplification and detection of HIV-1 DNA in fixed pediatric AIDS brain tissue. *um Pathol* **27:** 614–617.

Sharer LR, Saito Y, Epstein LG, Blumberg BM (1994). Detection of HIV-1 DNA in pediatric AIDS brain tissue by two-step ISPCR. *Adv Neuroimmunol* **4:** 283–285.

Sharma DP, Anderson M, Zink MC, Adams RJ, Donnenberg AD, Clements JE, Narayan O (1992a). Pathogenesis of acute infection in rhesus macaques with a lymphocytetropic strain of simian immunodeficiency virus. *J Infect Dis* **166:** 738–746.

Sharma DP, Zink MC, Anderson M, Adams R, Clements JE, Joag SV, Narayan O (1992b). Derivation of neurotropic simian immunodeficiency virus from exclusively lymphocytetropic parental virus: pathogenesis of infection in macaques. *J Virol* **66:** 3550–3556.

Shibata R, Kawamura M, Sakai H, Hayami M, Ishimoto A, Adachi A (1991). Generation of a chimeric human and simian immunodeficiency virus infectious to monkey peripheral blood mononuclear cells. *J Virol* **65:** 3514–3520.

Shibata R, Sakai H, Kiyomasu T, Ishimoto A, Hayami M, Adachi A (1990). Generation and characterization of infectious chimeric clones between human immunodeficiency virus type 1 and simian immunodeficiency virus from an African green monkey. *J Virol* **64:** 5861–5868.

Singh DK, Chouduri R, Pacyniak E, Berman NE, Stephens EB (2003). Infection of human astrocytoma cells with simian-human immunodeficiency virus results in up-regulation of gene expression and altered growth properties. *Neurosci Lett* **340:** 201–204.

Singh DK, McCormick C, Pacyniak E, Griffin D, Pinson DM, Sun F, Berman NE, Stephens EB (2002). Pathogenic and nef-interrupted simian-human immunodeficiency viruses traffic to the macaque CNS and cause astrocytosis early after inoculation. *Virology* **296:** 39–51.

Singh DK, McCormick C, Pacyniak E, Lawrence K, Dalton SB, Pinson DM, Sun F, Berman NE, Calvert M, Gunderson RS, Wong SW, Stephens EB (2001). A simian human immunodeficiency virus with a nonfunctional Vpu (deltavpuSHIV(KU-1bMC33)) isolated from a macaque with neuro AIDS has selected for mutations in env and nef that contributed to its pathogenic phenotype. *Virology* **282:** 123–140.

Smith MO, Heyes MP, Lackner AA (1995). Early intrathecal events in rhesus macaques (Macaca mulatta) infected with pathogenic or nonpathogenic molecular clones of simian immunodeficiency virus. *Lab Invest* **72:** 547–558.

Soderberg K, Denekamp L, Nikiforow S, Sautter K, Desrosiers RC, Alexander L (2002). A nucleotide substitution in the tRNA(Lys) primer binding site dramatically increases replication of recombinant simian immunodeficiency virus containing a human immunodeficiency virus type 1 reverse transcriptase. *J Virol* **76:** 5803–5806.

Stephens EB, Galbreath D, Liu ZQ, Sahni M, Li Z, Lamb-Wharton R, Foresman L, Joag SV, Narayan O (1997). Significance of macrophage tropism of SIV in the macaque model of HIV disease. *J Leukoc Biol* **62:** 12–19.

Stephens EB, Jackson M, Cui L, Pacyniak E, Choudhuri R, Liverman CS, Salomon DS, Berman NE (2006). Early dysregulation of cripto-1 and immunomodulatory genes in the cerebral cortex in a macaque model of neuro AIDS. *Neurosci Lett* **410:** 94–99.

Stephens EB, Liu ZQ, Zhu GW, Adany I, Joag SV, Foresman L, Berman NE, Narayan O (1995). Lymphocyte-tropic simian immunodeficiency virus causes persistent infection in the brains of rhesus monkeys. *Virology* **213:** 600–614.

Stephens EB, Singh DK, Kohler ME, Jackson M, Pacyniak E, Berman NE (2003). The primary phase of infection by pathogenic simian-human immunodeficiency virus results in disruption of the blood-brain barrier. *AIDS Res Hum Retroviruses* **19:** 837–846.

Sui Y, Potula R, Pinson D, Adany I, Li Z, Day J, Buch E, Segebrecht J, Villinger F, Liu Z, Huang M, Narayan O, Buch S (2003). Microarray analysis of cytokine and chemokine genes in the brains of macaques with SHIV-encephalitis. *J Med Primatol* **32:** 229–239.

Uberla K, Stahl-Hennig C, Bottiger D, Matz-Rensing K, Kaup FJ, Li J, Haseltine WA, Fleckenstein B, Hunsmann G, Oberg B, *et al* (1995). Animal model for the therapy of acquired immunodeficiency syndrome with reverse transcriptase inhibitors. *Proc Natl Acad Sci U S A* **92:** 8210–8214.

Weissman D, Rabin RL, Arthos J, Rubbert A, Dybul M, Swofford R, Venkatesan S, Farber JM, Fauci AS (1997). Macrophage-tropic HIV and SIV envelope proteins induce a signal through the CCR5 chemokine receptor. *Nature* **389:** 981–985.

Wiley CA, Schrier RD, Nelson JA, Lampert PW, Oldstone MB (1986). Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. *Proc Natl Acad Sci U S A* **83:** 7089–7093.

Wu Z, Qian G, Zhen QL, Narayan O, Stephens EB (1996). Neutralization of SIVmac239/17E in lymphocyte cultures involves virus strain-specific linear and conformational epitopes encoded by different regions of the env gene including the "V3" domain. *Virology* **222:** 184–192.

Zink MC, Amedee AM, Mankowski JL, Craig L, Didier P, Carter DL, Munoz A, Murphey-Corb M, Clements JE (1997). Pathogenesis of SIV encephalitis. Selection and replication of neurovirulent SIV. *Am J Pathol* **151:** 793–803.

Zink MC, Suryanarayana K, Mankowski JL, Shen A, Piatak M, Jr., Spelman JP, Carter DL, Adams RJ, Lifson JD, Clements JE (1999). High viral load in the cerebrospinal fluid and brain correlates with severity of simian immunodeficiency virus encephalitis. *J Virol* **73:** 10480–10488.