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Review

SIV infection of macaques – modeling the progression to AIDS dementia

MC Zink[1], JP Spelman[1], R Bedno Robinson[2] and JE Clements[1]

[1]*Division of Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205;* [2]*MCM, 11718 Bowman Green Drive, Reston, Virginia 20190, USA*

AIDS dementia complex affects 15–20% of HIV-infected adults and a greater percentage of HIV-infected children. Whether or not an HIV-infected individual develops neurological disease and how early in infection the clinical signs appear is most likely the net result of both viral virulence factors and host factors. Important viral factors include cell tropism and sequences that determine neurovirulence. The host factors include the cellular expression of viral co-receptors and maintenance of competent immune responses. The pathogenesis of AIDS dementia complex is difficult to study in the human host because of the difficulty in identifying acutely infected individuals and the inaccessibility of human brain tissue for examination during infection. The SIV/macaque model is excellent for the study of viral virulence factors and host responses to infection. This review outlines how the SIV/macaque model has been used to identify viral factors that are important for the development of neurological disease, to determine when HIV enters the brain, and to characterize the host immune responses affecting virus entry to the CNS and the development of neurological disease.

Keywords: HIV; pathogenesis; CNS; brain

Introduction

AIDS dementia complex is a syndrome that encompasses motor and cognitive deficits affecting 15–20% of HIV-infected individuals and a greater percentage of HIV-infected children (McArthur *et al*, 1997). AIDS dementia complex is most often diagnosed in the terminal stage of virus replication, when the infected individual is immunosuppressed, although some individuals manifest clinical signs of neurological disease early in infection, prior to immunosuppression (Ellis *et al*, 1997; McArthur *et al*, 1997). Whether or not an HIV-infected individual develops neurological disease and how early in infection the clinical signs appear is most likely the net result of both viral virulence factors and host factors such as expression of viral co-receptors and immune responses.

There are many gaps in our understanding of the pathogenesis of AIDS dementia. Some unanswered questions include: Are all strains of HIV potentially neurovirulent? When does HIV enter the brain? What viral factors are important for the development of neurological disease? Does the host immune response affect virus entry to the CNS or modulate the expression of neurological disease? Does the CNS constitute a virus reservoir? These questions are difficult to answer in the human host because of the difficulty in identifying acutely infected individuals and the inaccessibility of the human brain for sampling during infection. Animal models are a necessary tool to allow us to identify viral virulence factors and to detail the host's responses to infection.

SIV infection of macaques results in neurological abnormalities that are clinically and pathologically similar to those of AIDS dementia (Desrosiers *et al*, 1989; Gardner, 1990; Ringler *et al*, 1988; Sharer *et al*, 1988). Macaques can be inoculated with well-characterized virus strains or molecular clones of SIV to identify specific viral genes that are important in the development of organ-specific disease. Further, body fluids can be repeatedly sampled and tissues (e.g., lymph nodes) biopsied at different stages of disease to measure virus replication and evaluate the host's immune re-

Correspondence: MC Zink, 720 Rutland Avenue, Traylor G-60, Baltimore, Maryland, 21205 USA
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sponses. Finally, infected animals can be euthanized at different stages of infection, enabling the reconstruction of a complete picture of the interrelationships between the viral virulence mechanisms and the host's defenses. Recent studies from our laboratory as well as others using the SIV model are reviewed here.

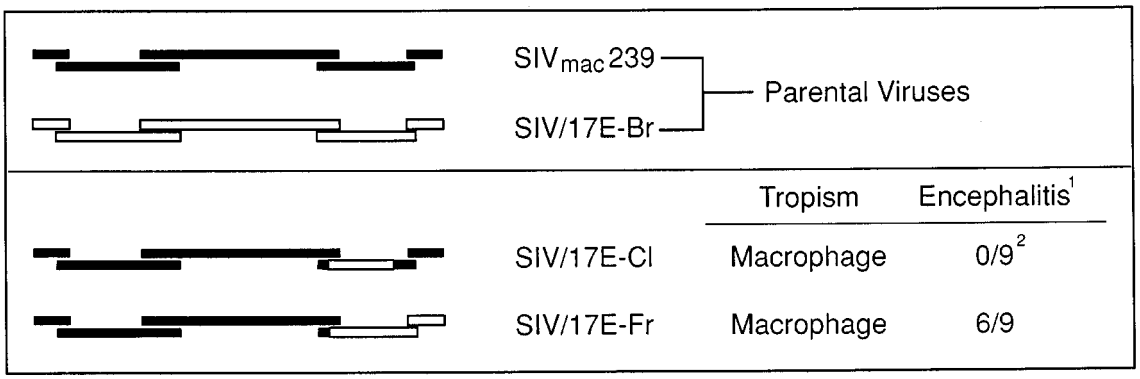
Viral contributions to AIDS dementia

Question: Are all strains of HIV potentially neurovirulent?

Productive replication of HIV in the brain occurs mainly in cells of macrophage lineage. Viral antigen and RNA are regularly identified in perivascular and meningeal macrophages and in microglial cells within the brain parenchyma, suggesting that the ability of HIV to replicate in monocytes/macrophages is a prerequisite for infection in the brain and viral neurovirulence (Koenig *et al*, 1986; Michaels *et al*, 1988; Price *et al*, 1988). Studies of the molecular determinants for neurovirulence of HIV strains are, however, limited by the fact that few primary HIV isolates from the brain have been studied, and brain isolates are frequently cultured extensively *in vitro*, often in cell lines. This results in selection of strains which may not retain the tropism and neurovirulent phenotype of the predominant virus(es) replicating within the brain parenchyma. One seminal study, which examined viruses from brain tissue and cerebrospinal fluid, showed that virus isolated from the frontal lobe of the brain (HIV$_{JR-FL}$) was macrophage-tropic and clearly distinct from the lymphocyte-tropic virus detected in the CSF of the same infected individual (Koyanagi *et al*, 1987; Li *et al*, 1991; Pang *et al*, 1991).

Studies using the SIV model of AIDS dementia have shed light on the relationship between viral tropism and neurovirulence. The relationship between macrophage tropism and neurovirulence of SIV has been examined in a number of studies (Kodama *et al*, 1993; Sharma *et al*, 1992; Zink *et al*, 1997). In one of these studies, macaques were inoculated with two recombinant hybrid viruses derived from SIVmac239, a lymphocyte-tropic nonneurovirulent infectious molecularly cloned virus, and SIV/17E-Br, a macrophage-tropic, neurovirulent virus strain. The first recombinant, SIV/17E-Cl, contains the portion of the *env* gene that encodes the surface glycoprotein and a short segment of the transmembrane glycoprotein of SIV/17E-Br in the backbone of SIVmac239 (Figure 1) (Anderson *et al*, 1993). Unlike SIVmac239, SIV/17E-Cl replicates productively in macrophages, demonstrating that sequences in the surface portion of *env* determine macrophage tropism. None of five macaques inoculated with SIV/17E-Cl developed SIV encephalitis (Mankowski *et al*, 1997). These findings were confirmed by another study in which 0/4 macaques inoculated with SIV/17E-Cl developed encephalitis (Joag *et al*, 1995). In both studies of SIV/17E-Cl, viral DNA could be detected in the brains of macaques, yet no evidence of viral replication or histological lesions was seen.

A second recombinant, SIV/17E-Fr, which contained the entire *env* and *nef* genes and the 3′ LTR of SIV/17E-Br in the SIVmac239 backbone, was also macrophage-tropic (Flaherty *et al*, 1997). Six of nine macaques inoculated with SIV/17E-Fr developed SIV encephalitis ranging from mild to moderate in severity, indicating a significant (P=0.031) difference in the neurovirulence of the two recombinants (Figure 1) (Mankowski *et al*, 1997). This study



[1]Number of macaques that developed neurological lesions.
[2]Combined results of two studies (Mankowski et. al. 1997 & Joag et. al. 1995).

Figure 1 Two recombinant molecular clones were made to examine the relationship between tropism and neurovirulence. SIV/17E-Cl was made by inserting the surface portion and a small segment of the transmembrane portion of the *env* of SIV/17E-Br into the backbone of SIV mac239. SIV/17E-Fr was constructed by inserting the entire *env* and *nef* genes and the 3′ LTR of SIV/17E-Br into the backbone of SIVmac239, SIV/17E-Fr was neurovirulent, but SIV/17E-Cl was not.

demonstrated that macrophage tropism alone is not sufficient for the development of neurological disease. In addition, it showed that while sequences in the surface portion of the envelope gene determine macrophage tropism, additional sequences derived from the transmembrane portion of envelope and/or *nef* confer neurovirulence (Flaherty *et al*, 1997; Mankowski *et al*, 1997). Further, while viral DNA was detected in the brain of macaques infected with SIV/17E-Cl, in only the CNS of macaques infected with SIV/17E-Fr were viral DNA, RNA and antigen detected, demonstrating productive virus replication in the brain. Thus, the presence of viral DNA alone in the CNS does not indicate viral replication and has not been found in the SIV model to correlate with the presence of lesions in the CNS. In contrast, the presence of viral RNA and viral antigen correlate strongly with the development of CNS lesions.

Question: When does HIV enter the brain?
Recent studies suggest that regardless of the route of transmission (mucosal, intravenous, drug use, transfusion of blood products), the HIV that is transmitted is genotypically fairly homogenous (Cichutek *et al*, 1991; McNearney *et al*, 1992; Pang *et al*, 1992) and phenotypically macrophage-tropic. This is in direct contrast to the lymphocyte-tropic viruses that predominate later in infection (Cichutek *et al*, 1991; McNearney *et al*, 1992; Pang *et al*, 1992; Wolfs *et al*, 1992; Wolinsky *et al*, 1992; Zhang *et al*, 1993; Zhu *et al*, 1993). Since macrophage-tropism appears to be a prerequisite for the development of neurological disease, this suggests that the virus that is transmitted and replicating during acute infection has the capability of entering and potentially replicating in the brain quite early in infection. There is one report demonstrating replication of HIV in the brain of a man who was accidentally inoculated with HIV-1 18 days prior to his death from cancer, suggesting that the virus can enter the brain and replicate during early infection (Davis *et al*, 1992). Whether this is true in the majority of HIV-infected individuals is not known. Whether or not the virus remains in the CNS or is cleared after the development of immune responses is also not known. In one study, HIV was not detected in the brains of HIV-infected individuals dying of other causes during the clinically quiescent stage of disease (months after the initial round of viral replication but prior to terminal immunosuppression and the development of AIDS (Chiodi *et al*, 1992). This study did not use PCR technology to detect virus; more sensitive tests may have revealed latent virus infection. Thus, the question of whether virus present in the CNS early in infection may later be cleaved, remains unresolved.

Studies of early SIV infection in macaques support the findings of HIV-infected humans. In macaques inoculated with two pathogenic strains of SIV and euthanized at 2, 8, 13 and 23 weeks post-inoculation, virus was readily isolated from the CSF during the first 4 weeks after inoculation, but only occasionally afterward. Then, when the animals approached terminal disease (21 to 23 weeks post-inoculation) the virus was again readily isolated. Macaques euthanized at 2 weeks post-inoculation had encephalitis, and viral RNA was detected in the CNS and other tissues by *in situ* hybridization (Smith *et al*, 1995). Later in infection, there were no lesions in the brain and *in situ* hybridization was negative. Again, however, the most sensitive method to detect virus, PCR on brain homogenates, was not used, leaving open the issue as to whether virus persists in very low numbers or in a latent form after the initial replicative stage.

Question: Is there selection for replication of certain virus strains in the CNS?
Although it appears that HIV and SIV enter the CNS early in most individuals, many infected individuals never develop AIDS dementia. One possible reason for this resistance to disease is that multiple strains enter the CNS but selection of neurovirulent strains of virus is required for continued replication and the development of lesions. This possibility has been examined in the SIV/macaque model.

In one study, nine macaques were intravenously co-inoculated with SIV/DeltaB670, a primary isolate of SIV and SIV/17E-Fr, a neurovirulent recombinant clone (Zink *et al*, 1997). SIV/Delta B670 is a complex virus swarm that contains at least 21 different genotypes including both macrophage-tropic and T cell-tropic viruses. Further SIV/DeltaB670 alone has been shown to cause CNS disease (Sharer *et al*, 1988). Seven of the nine macaques (78%) developed typical SIV-associated neurological lesions classified as severe (four macaques), moderate (two macaques) or mild (one macaque) with a mean time to euthanasia of 7 months (Figure 2). The SIV *env* V1 region was amplified from homogenates of saline-perfused brain and from lysed PBMC to compare the genotypes present in brain and blood. SIV/17E-Fr was detected in brain homogenates from all four macaques with severe encephalitis and in three of these animals, SIV/17E-Fr was the only genotype identified in the CNS. In two of the three macaques with moderate or mild encephalitis, single clones of SIV/DeltaB670 were identified; mixed SIV/DeltaB670 clones were detected in the third. In contrast, SIV/17E-Fr and a variety of strains of SIV/DeltaB670 were detected in PBMC throughout infection. This study demonstrated that neurovirulent strains within the virus swarm can selectively enter and become established in the CNS.

In another study, a macaque (#183) was inoculated intravenously with the virus swarm, SIVmac251. Its microglia were isolated and used for intravenous inoculation of another macaque, #230

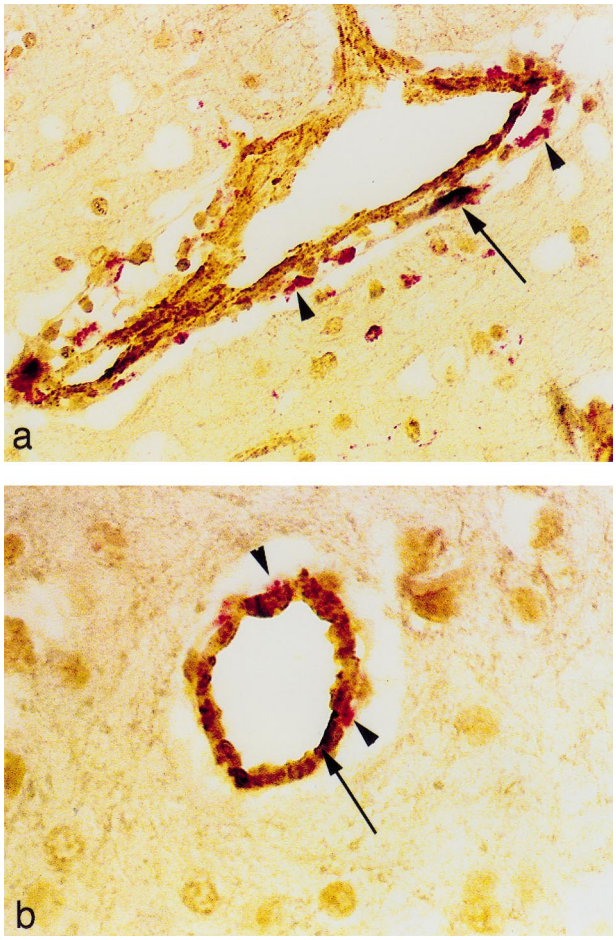


Figure 2 Seven of nine macaques co-inoculated with SIV/17E-Fr and SIV/DeltaB670 developed neurological lesions. (**a**) Tissue sections triple-labeled using immunohistochemistry demonstrated macrophages (Ham 57; red; arrow heads), some of which expressed viral gp41 (blue; arrow) in the perivascular spaces (endothelial cells stained with antibody to FVIII-related antigen; brown). (**b**) Occasional blood vessels expressed viral antigen (blue; arrow) on putative endothelial cells lining the vessel lumen.

(Lane *et al*, 1995). The microglial passage protocol was then repeated, with three additional macaques (181, 182, 185) being inoculated with microglia from macaque #230. All of these second passage macaques developed CNS disease with lesions typical of SIV encephalitis. The V1 through V4 regions of the virus demonstrated a convergence of sequences during passage, resulting in a homogenous population in the final three macaques. Comparison of multiple *env* clones from the brain and lymph nodes of macaque 181 revealed much more heterogenous sequences in the lymph nodes than in the brain. This study suggested that CNS lesions are caused by a distinct and limited set of viruses.

Question: What viral components are important for the development of neurological disease?
Studies of the molecular determinants of AIDS dementia require the molecular analysis of primary viral isolates from the brains of large numbers of HIV-infected individuals with and without AIDS dementia. The majority of primary HIV isolates from the brain are macrophage-tropic, a phenotype for which sequences in the V3 loop are at least partially responsible. Distinct envelope sequences in V3 and flanking this region have been shown to be associated with HIV dementia (Power *et al*, 1994; Reddy *et al*, 1996). Whether or not macrophage-tropism is sufficient for the development of neurological disease or whether additional determinants are required is not known.

Studies of the SIV model demonstrated that the two closely related recombinant viruses, SIV/17E-Fr and SIV/17E-Cl, both of which are macrophage-tropic, differed in their ability to replicate in brain cells derived from microvessel endothelium (BDME) and in their ability to cause neurological lesions. In contrast to SIV/17E-Cl, SIV/17E-Fr replicated in BDME and caused neurological lesions (Flaherty *et al*, 1997; Mankowski *et al*, 1994, 1997). Since the two recombinant viruses differed only in the transmembrane portion of *env* and in *nef*, this suggested that sequences in one or both of these areas are important determinants of neurovirulence (Flaherty *et al*, 1997). To identify the amino acids that accounted for these differences in neurovirulence, additional infectious molecular clones were constructed. Analysis of these recombinant viruses revealed that a single amino acid change in the TM portion of the envelope protein and a full-length Nef protein were required for replication in BDME (Figure 3) (Flaherty *et al*, 1997). Thus, changes in the TM and *nef* genes between SIV/17E-Cl and SIV/17E-Fr account for differences in tropism *in vitro* and neurovirulence *in vivo*.

In another study, sequences were identified in V1 through V4 that were shared by neurovirulent strains of SIV from three macaques infected with microglial cell-passaged virus. However, TM and *nef* sequences were not examined in that study (Lane *et al*, 1995). Molecular studies of additional strains of SIV, and comparative studies of SIV and HIV brain isolates are needed to determine whether there are additional determinants for neurovirulence and whether the molecular determinants in macaque viruses have parallels in HIV.

Question: How does the virus enter the CNS?
For SIV or HIV to enter the brain, it must first traverse the blood-brain barrier, the anatomic and biochemical barrier that prevents indiscriminate accession of molecules and cells to the brain parenchyma (Goldstein and Betz, 1986). There are several routes by which viruses cross the blood-brain barrier. The virus may infect brain endothelial cells, the main anatomic component of the blood-brain barrier. Alternatively, the virus may break down the blood-brain barrier, either by direct

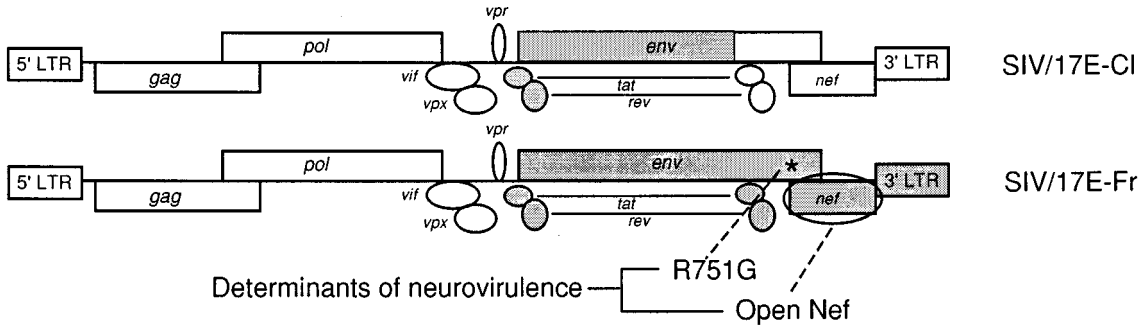


Figure 3 A single R to G change in the TM portion of *env* and an open *nef* are responsible for the neurovirulent phenotype of SIV/17E-Fr.

infection or by immune-mediated damage, thus permitting free virus and virus-infected cells to enter the brain. Finally, the virus may enter the CNS in infected cells which traverse the blood-brain barrier during trafficking (Figure 4) (Johnson, 1982). There are lines of evidence indicating that the virus may use all three of these mechanisms for entry into the CNS.

Viral replication in endothelial cells would not only provide a mechanism for initial viral entry to the CNS, but virus-induced changes in endothelial cells could potentially alter the integrity or function of the BBB. Several early reports demonstrated HIV RNA or proteins in brain endothelial cells in autopsy material from patients with AIDS dementia but this finding has not been universal (Kure *et al*, 1990; Rostad *et al*, 1987; Smith *et al*, 1990; Wiley *et al*, 1986). Moses *et al* have demonstrated productive infection of cultured human brain microvessel endothelial cells by HIV-1 (Moses *et al*, 1996, 1993; Moses and Nelson, 1993). Further, these investigators demonstrated that viral infection of endothelial cells was not mediated by the CD4 molecule.

The SIV/macaque model has been used to determine whether SIV infects CNS endothelial cells. SIV RNA was detected in capillary endothelial cells in brain sections from animals parenterally inoculated with a neurovirulent strain of SIV by double immunohistochemistry and *in situ* hybridization, and by reverse transcriptase *in situ* polymerase chain reaction (see also Figure 2) (Mankowski *et al*, 1994). These *in vivo* observations were extended by examining whether SIV replicated productively in cultured macaque brain endothelial cells (MBEC). The neurovirulent strain, SIVmac293/17E-Br, replicated productively in MBEC as determined by the presence of viral cytopathic effect (syncytia), viral DNA by PCR, viral RNA by *in situ* hybridization, and viral antigen by immunohistochemistry, and by the production of high titers of cell-free virus. Virus replication was confirmed by electron microscopy (Mankowski *et al*, 1994). This correlation between replication in brain endothelial cells and neurovirulence has been extended to other viruses that are neurovirulent. Thus, SIV/DeltaB670, SIVmac251, and the microglia-derived virus from Lane *et al* all replicate in these cells (Clements and Zink, 1996; J Nelson, personal communication). In contrast, a non-virulent virus, SIVmac239, does not infect MBEC. As with the findings in HIV, infection of the endothelial cells was not blocked by soluble CD4 or antibodies to CD4 (Mankowski *et al*, 1994). Thus, it appears that at least some strains of SIV and HIV may enter the CNS by infecting endothelial cells using a CD4-independent mechanism.

Several studies have demonstrated the presence of serum proteins in the brain parenchyma of patients with AIDS dementia complex, providing evidence of alterations in the integrity of the BBB in HIV infection (Petito and Cash, 1992; Rhodes, 1991). The mechanism for damage to the blood-brain barrier has not yet been determined. If the microvessel endothelial cells comprising the major anatomical component of the blood-brain barrier are grossly damaged, a possibility supported by studies showing increased apoptosis in endothelial cells in the brains of macaques with SIV encephalitis, then both virus-infected cells and cell-free virus would have ready access to the brain parenchyma (Adamson *et al*, 1996). In the perivascular milieu, cell-free virus would soon infect perivascular macrophages, one of the major host cells for virus replication in the CNS.

Cell trafficking provides another mechanism for entry of virus-infected cells into the CNS. Studies of expression of cell adhesion molecules during SIV infection have demonstrated increased expression of VCAM-1 on blood vessels in the brain parenchyma of macaques infected with pathogenic but not non-pathogenic strains of SIV (Sasseville *et al*, 1992, 1995). Adherence of peripheral blood mononuclear cells to blood vessels in frozen sections of brain parenchyma demonstrated that the VCAM-1 expressed on the endothelial cells was functionally active (Sasseville *et al*, 1994). The

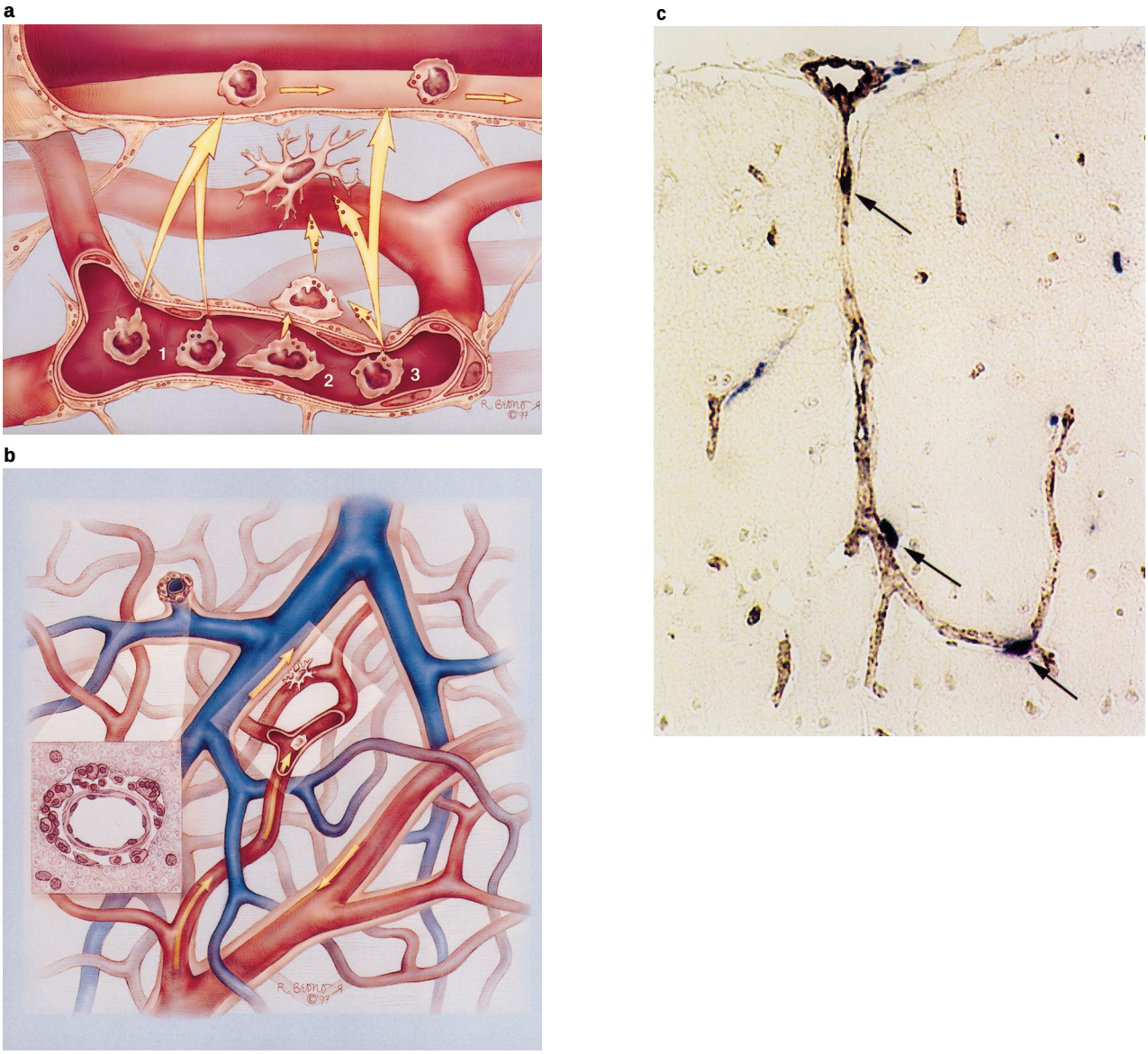


Figure 4 Trafficking of infected cells through brain parenchyma. (**a**) Cells infected with lymphocyte-tropic strains of virus may traverse the brain parenchyma without transmitting the virus (1). Infected macrophages may enter the brain and become perivascular macrophages (2). Trafficking lymphocytes or macrophages infected with macrophage-tropic strains of virus may transmit the virus to resident macrophages or microglia during their passage through the brain (3). (**b**) Trafficking cells enter the brain from the vascular lumen, cross the blood-brain barrier, traverse the brain parenchyma, then enter the perivascular spaces. In the perivascular spaces, cells flow into the subpial space and from there enter the CSF. (**c**) A tissue section double-labeled immunohistochemically shows several virus-infected cells (blue; arrows) within the perivascular space on their way to the meninges. Endothelium is labeled with antibody to FVIII-related antigen (brown).

majority of cells trafficking through the CNS in HIV are CD4+ lymphocytes, yet the main infected cells in the CNS are cells of macrophage lineage. This suggests that trafficking of virus-infected lymphocytes may be just as important as trafficking macrophages for viral entry to the CNS. Presumably the lymphocytes transmit the virus to susceptible perivascular macrophages and microglia during their journey through the brain parenchyma.

Question: What receptor(s) does the virus use to enter cells in the CNS?

A major advance in understanding the molecular basis of HIV cell and organ tropism resulted from studies that identified chemokine receptors as co-receptors for viral entry into cells. Chemokines are a group of low molecular weight proteins which direct leukocyte migration by interacting with chemokine receptors on the cell surface of leukocytes. The chemokine receptors are G protein-coupled with

seven transmembrane domains (Horuk, 1994). Cell surface CD4 has long been known to serve as the primary receptor for HIV entry, engaging viral envelope protein (gp120), and subsequently triggering a conformational change in the viral transmembrane protein (gp41) leading to fusion (McDougal *et al*, 1985). Expression of CD4, however, is insufficient to permit viral entry, as transfecting CD4 into non-human cells or resistant human cell lines does not always confer susceptibility to infection (Chesebro *et al*, 1990). The observation that the β-chemokines RANTES, MIP-1α, and MIP-1β, which are all ligands of the chemokine receptor CCR5, inhibit infection by HIV (Cocchi *et al*, 1995) was closely followed by reports demonstrating that CCR5 served as a co-receptor with CD4 for HIV entry (Alkhatib *et al*, 1996; Choe *et al*, 1996; Deng *et al*, 1996; Doranz *et al*, 1996; Dragic *et al*, 1996; Samson *et al*, 1996). Interestingly, this use of CCR5 appears to be limited to macrophage-tropic strains of HIV while lymphocyte-tropic HIV strains utilize another receptor, CXCR4 (Bleul *et al*, 1997). Studies identifying CXCR4 as a co-receptor actually preceded those implicating CCR5, and also contributed to the studies that targeted CCR5 as a co-receptor candidate (Bleul *et al*, 1996; Feng *et al*, 1996).

Studies of co-receptor use by SIV have demonstrated that all simian immunodeficiency virus strains appear to use CCR5 as a co-receptor and none, including lymphocyte-tropic SIV strains, use CXCR4 (Chackerian *et al*, 1997; Chen *et al*, 1997; Edinger *et al*, 1997a; Marcon *et al*, 1997). Other co-receptors for SIV exist, as SIV also infects CCR5 negative cells. Recently identified orphan chemokine receptors STRL33, GPR1, and GPR15 function as co-receptors for SIV with CD4 (Alkhatib *et al*, 1997; Deng *et al*, 1997; Farzan *et al*, 1997; Liao *et al*, 1997). Studies suggest that macrophage-tropic and lymphocyte-tropic SIV may interact with CCR5 in a different structural context, which may account for SIV cell tropism. Macrophage-tropic SIV env binds to the CCR5 amino terminus while the *env* of lymphocyte-tropic SIV binds to the second extracellular loop (Edinger *et al*, 1997a).

Of particular importance for infection of cells in the CNS, where there is minimal expression of CD4, is the recent finding that neurovirulent strains of SIV use CCR5 as a receptor to infect brain-derived microvessel endothelial cells in a CD4-independent manner (Edinger *et al*, 1997b). Recent studies have identified immunoreactivity for CCR5 on neurons, astrocytes, and neurons in addition to endothelium and vascular smooth muscle in the cerebrum and hippocampus of both humans and macaques (Rottman *et al*, 1997), and confirmed that HIV uses CCR3 and CCR5 as co-receptors for infection of cultured microglia (He *et al*, 1997). Further studies emphasizing the *in vivo* expression of chemokine receptors will be of great value in elucidating their role in pathogenesis.

Host contributions to AIDS dementia

Question: Is the development of AIDS dementia associated with a failure of the immune system to contain virus replication in the CNS?

Although some individuals develop AIDS dementia prior to the onset of immunosuppression, it has long been observed that the neurological manifestations of HIV infection most often occur during the terminal stages of infection, when the individual is immuno-suppressed (McArthur *et al*, 1997). Whether this correlation between AIDS dementia and immunosuppression is causative or coincidental remains to be determined. This question is difficult to study in humans because it is difficult to control for variations in the neurovirulence of the infecting and evolving strains of virus.

Several studies of SIV infection in macaques have suggested an association between immunosuppression and neurological disease. In one early study of 21 macaques inoculated with SIVmac251, animals with typical giant cell encephalitis were hypogammaglobulinemic, possibly reflecting immune suppression, in contrast to those without lesions in the brain parenchyma (Dean *et al*, 1993). Another study of macaques inoculated with a virus derived from SIVmac251 demonstrated SIV replication in the CNS during the acute stage of infection that declined coincident with increases in CSF IgG and quinolinic acid, indicating intrathecal immune activation (Smith *et al*, 1995).

In another study, nine macaques were intravenously co-inoculated with two viruses of different phenotypes (Zink *et al*, 1997). One virus, SIV/DeltaB670, was a primary isolate of SIV consisting of at least 21 different genotypes. This virus had been shown in previous studies to cause both immunosuppression and neurological disease. The other virus, SIV/17E-Fr, was a recombinant clone which was neurovirulent but not immunosuppressive. The macaques co-inoculated with SIV/DeltaB670 and SIV/17E-Fr were thus administered both neurovirulent virus and immunosuppressive viruses. The immune status of the macaques was monitored by measuring CD4+ T cell counts throughout infection. Viral replication was assessed by measuring plasma p27 viral antigen. Macaques showing clinical signs of neurological disease or immunosuppression were euthanized and complete necropsies were performed. Virus gene expression in the CNS and other tissues was evaluated by performing *in situ* hybridization and immunohistochemistry to detect viral RNA and antigen, respectively.

Seven of the nine co-inoculated macaques (78%) developed typical SIV-associated neurological lesions classified as severe (four macaques), moderate (two macaques) or mild (one macaque). Macaques with severe neurological lesions progressed significantly more rapidly to AIDS, with a mean time to

euthanasia of 3.6 months; those with mild or no neurological lesions had a mean time to euthanasia of 11 months. Macaques with severe neurological lesions had the most precipitous declines in CD4+ cell counts, the highest levels of virus in the blood, and the greatest expression of viral RNA and protein in the CNS. This study demonstrated a significant relationship between the development of host immunosuppression and the presence of neurological lesions accompanied by high levels of virus replication in the CNS and periphery, suggesting that host immune suppression is an important factor in the development of AIDS dementia.

Question: What component(s) of the immune response are responsible for down-regulating virus replication in the CNS?

HIV- and SIV-specific CTL have been isolated from peripheral blood and from lymphoid tissues early after infection and are associated with the control of primary viremia (Borrow *et al*, 1994; Hadida *et al*, 1992; Koup *et al*, 1994; Pantaleo *et al*, 1994; Reimann *et al*, 1994; Safrit *et al*, 1994; Yasutomi *et al*, 1993). CTL have also been identified in the CSF of HIV-infected individuals, but it is not known whether these cells actually traverse the brain parenchyma and drain to the CSF or are made in the meninges. Further, what role, if any, they play in down-regulating virus replication in the CNS is not known.

CTL have been detected in the blood and CSF of SIV-infected macaques as early as 1 week post-inoculation, and their appearance correlated with a decline in virus in plasma. In addition, SIV-specific CTL were isolated from the brain parenchyma (von Herrath *et al*, 1995). Interestingly, CTLs from these different compartments often recognized different complements of SIV proteins, suggesting that the CTLs were produced in response to SIV replication in each of these compartments. In this small study (six animals), no relationship was demonstrated between the presence of CTL in the brain or CSF and viral load in the brain. However, the presence of lesions in the CNS was not reported. It may be that CTLs in the brain parenchyma are ineffective at reducing viral load and may, in fact, contribute to CNS damage via immunopathological mechanisms. Whether other cellular immune mechanisms, such as NK cells, or humoral immune responses contribute to control of virus replication in the CNS is an important issue that needs to be addressed in the SIV model.

Summary

There is still much to be learned about the pathogenesis of HIV encephalitis and dementia. The question of the existence of neurovirulent strains has been addressed in the SIV model. These strains exist, selectively enter and replicate in the CNS, and replication is closely correlated with the development of CNS lesions. The role of neurovirulent strains of HIV will be more difficult to precisely characterize. However, the use of primary CNS cells such as microvessel endothelial cells and microglia may provide one approach.

The issue of HIV/SIV entry into the CNS is still an important question, requiring further studies in the SIV model. If the virus enters the CNS early does it elicit immune responses in the brain or recruit immune cells? The role of specific cellular immune cells such as NK and CTL in the brain during acute infection will be important to investigate. Virus clearance from the CNS is an important and unresolved issue in both HIV and SIV. Further, the role of the CNS as a possible reservoir of virus-infected cells in humans treated with anti-viral drugs is a crucial issue that has not been adequately addressed in either HIV or the SIV model (Lipton, 1998).

The role of immune responses in the CNS is a key issue since there is a strong correlation between the development of CNS disease and immunosuppression in both HIV and SIV infected individuals. The role of these immune responses during acute infection and in the later stages of disease can be examined in the SIV model. The SIV model has made substantial contributions to the understanding of the pathogenesis of AIDS and AIDS CNS diseases. This model is poised to further elucidate the molecular basis for the complex events that lead to CNS disease with contributions from both the virus and the host.

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