

## Review

# The development of animal model systems for HIV-1 encephalitis and its associated dementia

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The human immunodeficiency virus (HIV) is neuroinvasive and can be neurovirulent. Indeed, 20–30% of individuals with the acquired immune deficiency syndrome (AIDS) develop cognitive and motor dysfunction (termed the AIDS dementia complex or HIV dementia) coincident with advanced immunosuppression. Despite massive research efforts to discern viral neuropathogenic mechanisms, much remains incompletely understood. Recently, we and others developed animal model systems to elucidate how HIV infection within the brain can lead to impairment of central nervous system function. In this report, we evaluate each of the published animal models for their ability to mirror HIV dementia. Ease of handling and expense were also under consideration. Ultimately, studies in animal systems should permit a better understanding of the nature of HIV-1-induced neurological injury and aid in the development of effective treatments for this dreaded complication of HIV infection.

**Keywords:** acquired immune deficiency syndrome; human immunodeficiency virus; neurological manifestations of HIV infection; animal models; lentivirus infections

## Clinical and neuropathological manifestations of the HIV dementia

A range of clinical and pathological manifestations follow human immunodeficiency virus (HIV) infection of the central nervous system (CNS). These are collectively termed, the HIV-1-associated cognitive/motor complex or HIV dementia. HIV dementia is a prominent clinical manifestation that occurs in 20–30% of patients with the acquired immune deficiency syndrome (AIDS) (Janssen *et al*, 1991). Initially, clinical neurological findings are subtle and include mental and physical slowing. These symptoms may progress, usually in months, to forgetfulness and behavioral changes. Frank memory loss, difficulties with carrying out simple day to day tasks, apathy and loss of spontaneity usually follow. Inevitably there is a social withdrawal, alterations in personality and an inability to perform even daily living activities (including dressing, eating,

and ambulating) (Navia *et al*, 1986). Progression to a florid dementia with incontinence, hallucinations, seizures, then coma is characteristic at or near the time of death (Navia *et al*, 1986; Price *et al*, 1988; Kiebertz and Schiffer, 1989).

The most severe forms of HIV dementia manifest themselves pathologically in a multinucleated giant cell (MGC) encephalitis (also called HIV encephalitis) and in a vacuolar myelopathy. The neuropathologic features include a proliferation and activation of astrocytes (termed astrogliosis) and macrophages (collectively microglia, brain macrophages and MGCs) in subcortical white and grey matter. An increase in both astrocyte number and size usually precedes macrophage parenchymal infiltration. Non-specific white matter pallor and a variable degree of vacuolation of myelin can also occur. Neuronal injury and death often accompany high levels of HIV replication (reviewed by Gendelman *et al*, 1994a and Lipton and Gendelman 1995).

A seemingly important aspect of HIV neuropathogenesis is the selective localization of virus to

perivascular and parenchymal blood-derived brain macrophages and microglia (Koenig *et al*, 1986; Wiley *et al*, 1986). Indeed, a pathological hallmark of HIV-1 brain infection is the macrophage-derived MGCs that arise from fused infected and/or uninfected cells (Michaels *et al*, 1988). A unique feature of HIV-1 infection of the CNS is that primary brain ectodermal-derived cells (including neurons, oligodendrocytes and astrocytes) and microvascular endothelial cells are rarely if at all infected *in vivo* (Koenig *et al*, 1986; Wiley *et al*, 1986). When infection is demonstrated in astrocytes, it is highly restricted, and likely present only in pediatric subjects (Blumberg *et al*, 1994; Saito *et al*, 1994). The nearly exclusive viral localization to cells of the macrophage lineage makes HIV-1 encephalitis particular. In contrast, other viral infections of brain occur with distinctive tropism for neurons and/or glia (for example herpes simplex virus type 1 and 2, JC virus and cytomegalovirus infections). In HIV-1 encephalitis, infected macrophages/microglia predominately localized in cerebral white matter, deep gray matter (basal ganglia and thalamus), and the brainstem rather than in the cortex.

Viral infection of brain macrophages and microglia is accompanied by neuronal loss in deep grey matter and cortex. Loss of cortical neurons with concomitant losses in the complexity of dendritic arborization and reductions of perikaryon volume was described previously (Ketzler *et al*, 1990; Everall *et al*, 1991). Morphometric studies revealed up to a 50% decrease in the number of large neurons in the frontal, parietal, and temporal lobes within HIV encephalitic brain tissue (Wiley *et al*, 1991). Not all studies, however, support such a dramatic loss of neurons in HIV dementia. This suggests that CNS symptoms might also occur as a consequence of subtle abnormalities in neuronal function (Seilhean *et al*, 1993).

Virtually all demented patients with HIV infection and advanced immunosuppression have high levels of virus in brain (Wiley and Achim, 1994). Although there is a 100% correlation between neurological disease and HIV expression the converse is not always true. Indeed, high level viral gene expression does not always predict the levels of neurological impairment (Kure *et al*, 1990; Dickson *et al*, 1991; Gendelman *et al*, 1994a; Wiley and Achim, 1994). These findings underlie the complexity of HIV neuropathogenesis. In support of this, our own laboratory works recently demonstrated a requirement for both HIV-1 infection and cellular activation in order for the macrophage to produce neurotoxic activities (see below). Ample evidence exists for diffuse CNS activation during the evolution of the HIV-1-associated cognitive/motor complex (Tyor *et al*, 1992; Wesselingh *et al*, 1993; Nottet *et al*, 1995b). Thus, an interplay between viral and host factors likely predicts the development of neurological dysfunction in AIDS.

## In vitro model systems for HIV-1-induced neurological disease

### The role of virus infected macrophages

Laboratory cell assays that mimic neuropathological events associated with HIV infection were recently developed. Using these assays we observed that: (1) secretory products from macrophages induce neural damage; (2) the *in vitro* reconstitution of primary human brain cells together with HIV infected macrophages mirror aspects of human pathology and (3) assays of cerebrospinal fluids (CSF) and brain tissue for neurotoxic activities validate many of the observed laboratory experiments. Our *in vitro* model for HIV dementia demonstrated that viral infection primes monocytes for activation (Nottet *et al*, 1995a). As a consequence, virus-infected monocytes overexpress a variety of putative neurotoxins, for example eicosanoids, platelet activating factor (PAF), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and nitric oxide (NO) (Genis *et al*, 1992; Nottet *et al*, 1995a; Bukrinsky *et al*, 1995). These factors are potent neuromodulators and their overexpression results in altered neuronal function then death. For example, NO can precipitate severe neuronal degeneration depending on its redox state (Lipton *et al*, 1993). PAF and leukotrienes (LTB<sub>4</sub>, LTD<sub>4</sub>, and LXA<sub>4</sub>) have excitatory effects on neurons (Clark *et al*, 1992; Kato *et al*, 1994). PAF, NO and arachidonic acid increase intracellular neuronal Ca<sup>2+</sup> leading to enhanced neurotransmission (Kornecki and Ehrlich, 1988; Miller *et al*, 1992; Khurana and Bennett, 1993). Although the rise in intracellular Ca<sup>2+</sup> may not by itself cause neuronal injury, this seems to be critical for neuronal death. Cytokines may participate in CNS injury. Like PAF and NO, TNF- $\alpha$  may contribute to neural damage by increasing voltage-dependent Ca<sup>2+</sup> currents (Soliven and Albert, 1992). TNF- $\alpha$  and interleukin-1 $\beta$  (IL-1 $\beta$ ) stimulate astrocytosis. TNF- $\alpha$  can produce myelin damage and lysis of oligodendrocyte (Selmaj and Raine, 1988). Lastly, TNF- $\alpha$  can upregulate NO in HIV-infected monocytes (Bukrinsky *et al*, 1995) and provoke oxidant stress (Talley *et al*, 1995).

We and others validated the pathobiological importance of the HIV-induced neurotoxins by assay of their levels in the CSFs and brains of patients with progressive CNS disease. TNF- $\alpha$ , IL-1 $\beta$ , PAF, NO and eicosanoids were present in the CSF and/or brain tissue of AIDS patients with neurological impairment (Tyor *et al*, 1992; Wesselingh *et al*, 1993; Gelbard *et al*, 1994; Griffin *et al*, 1994; Bukrinsky *et al*, 1995; Nottet *et al*, 1995b). Placement of individual neurotoxins (for example TNF- $\alpha$  and PAF) into cultivated human/rat neurons resulted in neural injury and death (Gelbard *et al*, 1994).

The secretion of neurotoxins by HIV-infected macrophages is likely regulated by a complex series of intercellular interactions between several differ-



**Table 1** Comparative aspects of animal models for HIV-1 encephalitis

Animal model for HIV-1 encephalitis	Relevance to HIV encephalitis		
	Pathology	Pathogenesis	Ease/utility
Visna virus and CAEV	+	++	Fair
FIV	+	++	Good
SIV in rhesus macaques	+++	++++	Fair
Transgenic mouse (gp120)	++	+	Excellent
Human neural xenograft	+	++	Good/excellent
MuLV encephalitis	+	+/-	Excellent
SCID mouse model	++	++	Good/Excellent

0: none or few similarities seen

+: some similarities, overall several important distinctions

++: many similarities, some distinctive aspects

+++ : very analogous system

++++: mirrors almost exactly HIV encephalitis

CAEV: caprine arthritis encephalitis virus; FIV: feline immunodeficiency virus; SIV: simian immune deficiency virus; MuLV: murine leukemia virus

ent brain cell types including macrophages, astrocytes, endothelial cells and neurons. HIV primes macrophages for production of neurotoxic factors. The secondary activation factors required for macrophages to synthesize neurotoxins may include brain-specific transcriptional/regulatory elements and/or opportunistic infections to trigger CNS disease. Moreover, cell-cell interactions may serve in a regulatory capacity to limit neurotoxin production and give rise to slowly progressive clinical disease. In this way, we recently investigated the intercellular interactions between activated HIV-infected macrophages and astrocytes (Nottet *et al*, 1995a). Astrocytes are neural regulatory cells within the CNS. They outnumber neurons by ten to one, occupy about one-third of the volume in the cerebral cortex and are critical for the maintenance of a balanced neuronal homeostatic microenvironment (Norenberg, 1994). Our studies demonstrate that primary human astrocytes decrease neurotoxin production from activated macrophages (Nottet *et al*, 1995a). The demonstration of restricted HIV-1 infection of astrocytes *in vivo* may support a functional breakdown of astrocytes later in the course of disease (Blumberg *et al*, 1994; Saito *et al*, 1994). Increasing numbers of HIV-infected brain macrophages could break a balanced homeostatic microenvironment for neurons created by astrocytes. Indeed, we observed that addition of primary human astrocytes to unactivated HIV-infected macrophages produce low, but significant, levels of eicosanoids and PAF. Others have described that high concentrations of gp120 alters ion transport in astrocytes (Benos *et al*, 1994). These findings, taken together, suggest a dual role for astrocytes in HIV

encephalitis. Future investigation of macrophage astrocyte-interactions may provide valuable new insights into the mechanism of HIV dementia.

#### *Viral penetration of the blood-brain barrier*

To gain further insights into mechanisms for HIV brain disease, we investigated the intercellular interactions between HIV-infected macrophages and human brain microvascular endothelial cells (BMVEC). These studies explored the mechanisms for HIV-1 entry and monocyte infiltration into brain. HIV-infected monocytes selectively induced E-selectin expression on BMVEC (Nottet *et al*, 1995b). Immune activation of the HIV-infected monocytes induced even higher levels of E-selectin and vascular cell adhesion molecule-1 (VCAM-1) on BMVEC. To explore the *in vivo* relevance of these findings, the relationships between adhesion molecules, proinflammatory cytokines (for example TNF- $\alpha$  and IL-1 $\beta$ ) and HIV gene expression in brain tissue was evaluated (Nottet *et al*, 1995b). E-selectin and to a lesser degree VCAM-1 expression on endothelial cells closely paralleled the levels of HIV-1 gene products and proinflammatory cytokines in brain tissues (Nottet *et al*, 1995b). As E-selectin and VCAM-1 mediate transendothelial migration of monocytes these data suggest that HIV-infected monocytes have a selective advantage for entry into the CNS (Carlos *et al*, 1991; Hakkert *et al*, 1991; Chuluyan and Issekutz, 1993). Parallel studies done in the simian immunodeficiency virus (SIV)-infected monkeys (see below) support the works performed in human tissue (Sasseville *et al*, 1992). For example, animals with histologic evidence of SIV-encephalitis had higher expression of VCAM-1 on



BMVEC than did either SIV-infected or control uninfected animals without encephalitis. These findings suggest that monocytic cell infiltrates may accumulate as a result of interactions between VCAM-1 on endothelial cells and its receptor, very late antigen 4 (VLA-4), on blood monocytes. Furthermore, BMVEC expressing VCAM-1 in encephalitic brains caused selective adherence of human monocytic cell lines *in vitro*. This cell-cell interaction was blocked with antibodies to VLA-4 (Sasseville *et al*, 1994).

## Animal models for HIV dementia (an overview)

The tissue culture systems described have significant limitations in adequately reflecting the complexities of cell to cell interactions *in vivo* or the intricacies of brain tissue function. The pathogenesis of virus-induced tissue damage can be studied comprehensively only *in vivo*. This is especially true for the pathology that underlies HIV dementia. The central question here is how relatively small numbers of infected macrophages produce widespread devastating neurologic dysfunction. This can be addressed only by animal models. To these ends several animal systems mimicking HIV infection of the CNS have been proposed. Each model, notably, has both advantages and limitations in studies of HIV dementia (Table 1). We will review each system in order of their discovery and critique their individual utility for studies of HIV dementia.

## Visna and caprine arthritis-encephalitis virus (CAEV)

Visna and CAEV are prototypes of a taxonomic group of lentiviruses which include HIV-1, HIV-2, SIV and feline immunodeficiency virus (FIV) (Narayan *et al*, 1988). These viruses are linked to HIV by their genetic structure, long incubation periods, unique tropism for cells of the monocyte/macrophage lineage and induction of inflammatory brain disease. All lentiviruses infect the CNS and elicit a progressive defect predominantly in motor function. In CAEV and visna virus-infected animals, motor dysfunction includes a progressive loss of balance/coordination, leg weakness and/or tremor closely resembling that seen in humans with HIV dementia. The neurological impairments are most frequent in kids (goats younger than 6 months) but can occur in adult ruminants (both sheep and goats) (Narayan and Cork, 1985).

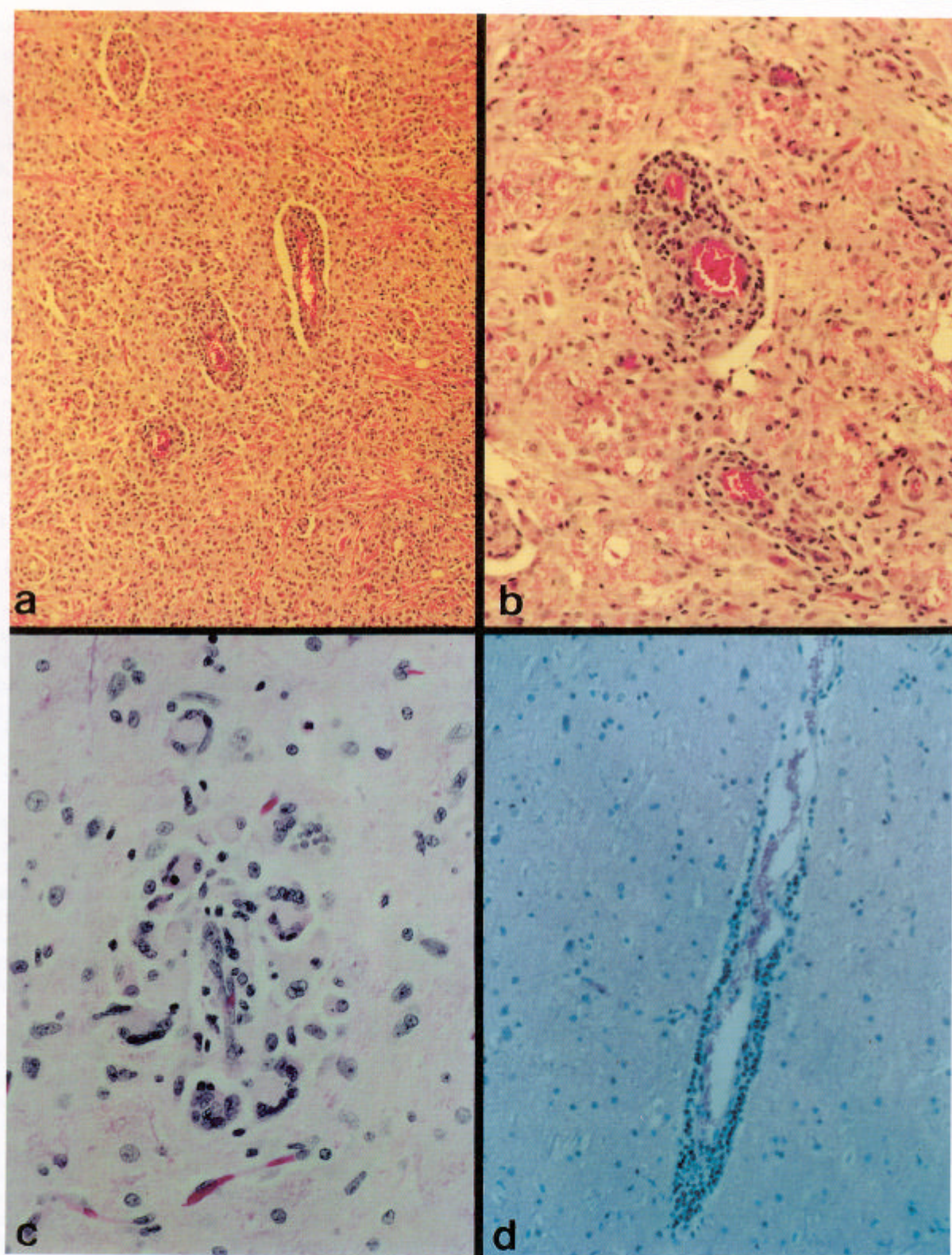
Visna virus was first recognized as the etiological agent of a chronic wasting sheep disease (called visna-maedi) in the 1950s (Sirgusson *et al*, 1958). Clinical signs of visna infection include a long incubation period (measured in months to years) and a slowly progressive paralysis (Narayan and Cork,

1985). A related clinical syndrome of goats, called CAEV, was first described in the 1980's (Crawford *et al*, 1980). Both viruses have similar morphology, genomic size (of approximately 9.6 kb) and share antigenic cross-reactivity (Pyper *et al*, 1984; Pyper *et al*, 1986). *In vitro* and *in vivo*, the viruses infect cells of monocyte-macrophage lineage (Zink *et al*, 1990). Their life cycle is regulated by factors involved in maturation of monocyte precursor cells in bone marrow to tissue macrophages which enable the viral cycle to proceed to completion with production of infectious particles (Gendelman *et al*, 1985). The histopathological lesions observed in sheep and goats are active chronic inflammation with infiltration and proliferation of mononuclear cells in specific target tissues (the CNS, the lungs, the synovia and mammary gland) (Figure 1a). The susceptibility of these tissues to pathological process has been traced to permissiveness of local macrophage populations for productive viral replication (Gendelman *et al*, 1984; Zink *et al*, 1990). Although some viral RNA-positive cells are in almost all animal tissues, organs showing inflammatory changes consistently contain more virus-infected cells. Inflammation in lentiviral disease has been attributed to the expression of viral proteins in association with Ia (class II MHC) antigens on macrophages and other inflammatory cells (Kennedy *et al*, 1985). In perivascular spaces this likely results in persistent antigenic stimulation and recruitment of additional inflammatory cells. The recent demonstration that visna and CAEV inflammatory lesions are driven by cytokines (secreted by immune activated cells) has been important in directing research into HIV disease (Johnson, 1994).

Although CNS pathology is similar in both sheep and goats, clinical disease, as in humans, occurs in only a portion of infected animals. Microscopically, multifocal inflammatory and demyelinating lesions are found within the brain and spinal cord. Cellular infiltrates consisting of large numbers of lymphocytes, macrophages and plasma cells are located perivascularly or diffusely in the neuropil (Figure 1a, b) (Narayan *et al*, 1988). In the most severe lesions, inflammatory cells are distributed throughout the white matter of the brain and spinal cord and occasionally extend also to gray matter. In mild lesions, focal accumulation of mononuclear cells is accompanied by microglial and astrocyte reactions. Axons are preserved but myelin is lost completely. Severe destruction of myelin may cause cavitation frequently seen in the spinal cord. Meningitis is common. Myelin loss and intensive astrogliosis with few mononuclear cells are prominent features of chronic lesions.

The clinical picture of neurologic disease of goats is posterior ataxia or lameness progressing to weakness then paralysis of one or more hind limbs. This may occur in as short a time as 1 week. Additional clinical signs include circling, facial twitching and





**Figure 1** CNS histopathology in visna, SIV and FIV infections. CNS tissue infected with lentivirus was fixed in formalin and paraffin embedded. Six micron sections were cut and stained with hematoxylin and eosin. Visna virus infected spinal cord tissue (a and b). (a) Marked mononuclear cell infiltration in Virchow-Robin spaces with loss of architecture in spinal cord  $\times 100$ , and (b) prominent perivascular accumulation of macrophage in white matter  $\times 180$  is seen within the spinal cord; (c) SIV infected brain tissue. A parenchymal brain infiltrate of macrophages and multinucleated cells is seen surrounding a capillary in the thalamus of an SIV-infected rhesus monkey  $\times 280$ ; and (d) FIV infected brain tissue. Perivascular lymphocytes are present in white matter of the frontal cortex of a cat inoculated intracerebrally for 1 month with FIV  $\times 175$ .



head tremor. Nearly all affected animals are afebrile and mentally alert. Goat kids with mild pathology may recover some degree of their lost neurological function. Sporadic neurologic disease in adults manifests by ataxia in hind limbs and stumbling gait. These signs worsen over course of weeks to months leading to paralysis of hind limbs and occasionally quadriplegia. The clinical neurological findings associated with visna virus infection in sheep is similar to that of adult goats (Sundquist *et al*, 1981).

Nevertheless, there are notable differences between the pathogenesis of visna or CAEV and HIV brain infections. CAEV and visna cause a chronic and recurrent leukoencephalitis and do not infect CD4<sup>+</sup> T lymphocytes or induce an immunodeficient state. In contrast HIV induces a MGC encephalitis and a progressive loss of CD4<sup>+</sup> T cells. The pathological changes of recurrent demyelinating lesions in visna and CAEV are localized mainly in white matter. Paralytic disease with motor tract and spinal cord involvement are usually seen in lentivirus-infected ruminants and are not prominent in HIV-dementia (Gendelman *et al*, 1994a). The size of the animals and its costs coupled with the marked differences in neuropathology strongly suggest that visna and CAEV infections of the brain may not be suitable models for studies of HIV CNS disease.

## SIV

SIVs are African non-human primate lentiviruses which are the closest relatives of HIV (Desrosiers, 1990). Their natural hosts include African green monkeys (*Cercopithecus aethiops*), sooty mangabeys (*Cercocebus atys*), and mandrills (*Papio sphinx*). Importantly, SIV infection of rhesus monkeys and macaques outside their natural habitat produce an AIDS-like disease (Letvin *et al*, 1985). The SIV-infected animals show progressive depletion of CD4<sup>+</sup> T lymphocytes with concomitant opportunistic infections, neoplasms, wasting and death. SIV is similar to HIV in genomic organization and gene sequence (genomic homology to HIV-2 is 75% and to HIV-1 is 40%) (Desrosiers, 1990). There is antigenic cross reactivity between HIV and SIV and both utilize CD4 as the viral-cell receptor (Desrosiers, 1990). Moreover, the CD4<sup>+</sup> T lymphocyte and the monocyte/macrophage are the major cellular targets for both HIV and SIV (Desrosiers *et al*, 1991). SIV infection of CD4<sup>+</sup> T lymphocytes results in a qualitative dysfunction and a quantitative loss of these lymphocytes in peripheral blood and lymphoid tissues. Tropism of SIV strains for monocyte/macrophages correlates with pronounced inflammatory and degenerative changes in the CNS, lung, skin and other organs independent from those pathologies associated with secondary opportunistic pathogens. Interestingly, SIV macrophage tro-

pism is conferred to a lymphotropic strain, SIVmac 239, by as few as three amino acid changes in *env* gene. These amino acid changes occur in about 30% of SIV infected rhesus monkeys (Desrosiers *et al*, 1991).

Neurologic disease is present in 30–60% of SIV-infected macaques with the simian form of AIDS (Lackner *et al*, 1994). The primary histologic lesion in SIV-infected brain tissue is perivascular infiltrates of macrophages and MGCs (Figure 1c). In addition there are occasional CD8<sup>+</sup> T lymphocytes within the lesions (Lackner *et al*, 1991). Cellular infiltrates are disseminated throughout the grey and white matter. Many of the macrophages and MGCs comprising the infiltrates contain SIV nucleic acid, antigen, and budding viral particles within the cisternae of smooth endoplasmic reticulum or in cell vacuoles (Ringler *et al*, 1988; Lackner *et al*, 1991). Viral gene products and histopathologic lesions within the CNS occur predominately in the white matter early after experimental infection of rhesus monkeys with pathogenic SIV (Ringler *et al*, 1988; Chakrabarti *et al*, 1991; Lackner *et al*, 1994). Meningitis, lymphocytic perivascular cuffs and glial nodules are found in brains of animals beginning 2 weeks post inoculation. Signs of meningitis are accompanied by perivascular cuffs of lymphocytes in cerebral white matter (Sharer, 1994; Lackner *et al*, 1994). These lesions contain SIV RNA and are found together with plasma and CSF viral gene products. Reactive astrocytes usually surround macrophage/MGCs infiltrates, however, white matter astrocytosis (identified by immunocytochemical labeling for glial fibrillary acidic protein (GFAP) for astrocytes) is difficult to evaluate since there is also a prominent GFAP-positive astrocytosis in the white matter of uninfected animals. Rarely, vacuolization of the neuropil and perivascular mineralization of basal ganglia are observed in SIV encephalitis. Myelin pallor is usually restricted to areas of macrophage infiltrates (Sharer, 1994). Decreased number of neurons in cerebral cortex shown in HIV-1 encephalitis (Wiley *et al*, 1991) has not yet been shown in the SIV model system.

A correlation exists between the numbers of macrophages/MGCs, the severity of brain lesions and levels of SIV gene expression (Lackner *et al*, 1991). Interestingly, the humoral response to SIV antigens, the duration of survival following viral challenge, and the age and sex of the animals do not affect the distribution and level of SIV expression in the CNS. However, the lack of humoral response to SIV may predispose animals to early invasion and persistence of virus in brain (Chakrabarti *et al*, 1991; Lackner *et al*, 1994).

SIV-infected rhesus monkeys, like HIV-infected humans, develop cognitive and motor impairments during AIDS (Murray *et al*, 1992). Animals exhibit significant behavioral deficits well before either evidence of opportunistic infection or signs of progres-



sive clinical disease. Although the varying degrees of CNS involvement by SIV are detected in these monkeys at necropsy, there is no obvious correlation between the severity or location of the lesions and the degree of neurological impairments (Rausch *et al*, 1994). Importantly, these exact pathogenic features are in HIV dementia (Lipton and Gendelman, 1995).

SIV-infected rhesus monkeys, therefore, are an excellent animal model for examining the pathogenesis of HIV encephalitis. Nevertheless, some important differences exist between SIV and HIV CNS lesions. SIV-infected monkeys show minimal myelin pallor and gliosis which are prominent features in human brains infected with HIV (Sharer, 1994; Lipton and Gendelman, 1995). Also absent in SIV are significant myelopathy and peripheral neuropathy commonly observed in HIV-infected subjects. Difficulties arise with utilizing the SIV model for quantitative analyses of CNS disease. The animals are difficult to maintain and costly. The most comprehensive study for SIV encephalitis included only 204 animals (28% of them have morphological signs of encephalitis) (Baskin *et al*, 1992). Such difficulty with numbers may preclude performance of experiments that require statistical significance either for uncovering mechanisms for disease pathogenesis or for drug testing and other interventional therapies.

## FIV

Cats infected by FIV could provide substantial advantages over visna, CAEV or SIV as animal models for HIV dementia because of animal size and ease of use. FIV is a lentivirus that shares several common disease patterns with HIV. For example, soon after FIV infection animals develop an acute retroviral syndrome characterized by a flu-like illness. This is followed by a variable length clinically asymptomatic stage of disease ultimately resulting in a variety of opportunistic infectious complications associated with immune deficiency (Beebe *et al*, 1994). Moreover, FIV has many biologic, molecular and biochemical features shared with HIV. For example, CD4<sup>+</sup> T lymphocytes and monocyte/macrophages are the major cellular targets for both FIV and HIV (Beebe *et al*, 1994).

To some extent FIV-infected cats develop an encephalopathy that mirrors HIV dementia. Seroepidemiological surveys have identified a subset of FIV infected animals with behavioral abnormalities (compulsive roaming, motor disturbances, seizure activity). This suggested viral infection of the brain with involvement of cortical and subcortical structures (Podell *et al*, 1993). Indeed, FIV was recovered from CSF and brain tissue of naturally and experimentally infected cats (Yamamoto *et al*, 1988; Dow *et al*, 1990). Like HIV, FIV, CNS disorders have distinct neuropathological features

(Hurtrel *et al*, 1992). CNS lesions occur as early as 1 month following intracerebral FIV inoculation and 2 months after intravenous infection (Hurtrel *et al*, 1992). Pathological examination of brain tissue of naturally infected cats show a diffuse gliosis, rare perivascular infiltrates, meningitis and meningeal calcifications (Hurtrel *et al*, 1992). Mild focal inflammatory changes (perivascular accumulation of mononuclear cells) (Figure 1d), diffuse gliosis, discrete glial nodules and white matter pallor occur in experimentally infected animals (Dow *et al*, 1990; Hurtrel *et al*, 1992; Beebe *et al*, 1994). Recently, investigators (Podell *et al*, 1993) showed a satellitosis (accumulation of 4–7 oligodendrocytes around affected neurons) without astrogliosis or myelin loss in cats 16 months following FIV inoculation. Histopathological lesions of FIV thus differ from HIV encephalitis in that they are less severe. Nevertheless, *in vitro* both FIV and HIV infect primary cultures of microglia, astrocytes (Dow *et al*, 1990) and brain microvascular endothelial cells (Steffan *et al*, 1994). In addition, FIV also produces neurological deficits including anisocoria, alterations in pupillary reflexes, delayed auditory and visual evoked potentials, and abnormal sleep patterns (Phillips *et al*, 1994). Similar findings were reported for HIV (Norman *et al*, 1992; Jabbari *et al*, 1993).

Despite the obvious benefits, the FIV model has several pitfalls as a model for HIV CNS disease. Most importantly, minimal histopathological lesions are in brains of FIV-infected cats. There are no features of granulomatous inflammation. Moreover, neuronal loss, a prominent feature of HIV dementia has not been documented for FIV. Some of the neurological functions studied in FIV-infected animals return to normal spontaneously. The duration of the asymptomatic stage in FIV infection is very long and the immunodeficient stage has been studied only on naturally infected cats (Hurtrel *et al*, 1992). These factors limit the FIV model for use in studying neurological complications of HIV disease.

## Murine leukemia virus (MuLV)

Difficulties in working with non-human primates and the limitations described with other models of HIV dementia urged scientists to search for smaller more reproducible animal systems. One readily available naturally occurring neurologic disease of feral mice is caused by an ecotropic type-C retrovirus, MuLV (Kay *et al*, 1991). Several neurotropic strains of MuLV induce hind limb paralysis. The underlying pathology is a spongiform myeloencephalopathy and chronic non-inflammatory CNS degeneration (Czub *et al*, 1994). Microscopic lesions in the CNS of infected mice consist of small vacuoles distributed along grey and white matter junctions. The lesions are observed in the spinal cord 4



weeks after virus inoculation. As disease progresses, the vacuoles coalesce, and pathologic changes expand to the brainstem and to the deep cerebellar grey matter. Spongiform lesions occur occasionally in cerebral white matter but only at the most advanced stages of disease. Importantly, inflammatory infiltrates are not seen in the brain parenchyma or present perivascularly in vacuolated areas. Astrocytosis only follows the spongiform transformation. Interestingly, diffuse gliosis can be present without vacuolar lesions in grey matter of spinal cord, brainstem and/or deep cerebellar nuclei. Usually, however, the intensity of gliosis and extent of CNS vacuolation correlates with the severity of neurologic signs (Nagra *et al*, 1992). The major cell types infected by virus are microvascular brain endothelial cells, oligodendrocytes and neurons (Nagra *et al*, 1993). Expression of MHC class I and class II antigens on endothelial cells are found within or near the lesion (Nagra *et al*, 1993). Subsequently, infected oligodendrocytes appear in regions with/out spongiform changes. Ultrastructural study demonstrates budding virions from cell membrane of oligodendrocytes. Neuronal infection can be detected in the cerebellar cortex, hippocampus and olfactory bulb without any evidence of cytopathology by light or electron microscopy (Lynch *et al*, 1991). Decrease in immunostaining intensity for neuronal markers is often present in the brainstem and neocortex of infected mice (Nagra *et al*, 1993). Quantitation of neocortical thickness in these animals shows thinning when compared to non-infected mice or those infected with non-neurotropic viruses (Nagra *et al*, 1994). Diminished neocortex thickness and loss of neurons are not strongly associated with viral replication in neurons. Release of neurotoxins by brain macrophages and microglial cells may also influence neuronal dropout since infected microglia do occur commonly in sites of spongiform degeneration (Bazsler and Zachary, 1991; Gravel *et al*, 1993; Lynch *et al*, 1995). The importance of microglia in neurological disease was recently confirmed. Intracerebral transplantation of infected mouse microglia cells precipitated significant ipsi- and contralateral CNS infection and focal spongiform degeneration (Lynch *et al*, 1995). These data, taken together, support a role for the MuLV system in studies of HIV neuropathogenesis. However, along with clear advantages, this model has obvious pitfalls. (1) Infection is not limited to macrophages. Neurons and oligodendrocytes are also infected with MuLV and glial activation and inflammatory responses influence retrovirus-induced spongiform degeneration. Indeed, the pathogenesis of type-C retrovirus is very different from that of lentiviruses in that these viruses have a broader cell tropism and brain pathology. (2) Inflammatory infiltration is never observed in spongiform lesions following MuLV infection. MuLV primarily involves the motor centers of the CNS, in

contrast to HIV encephalitis which is characterized by perivascular and parenchymal macrophage and MGC infiltration. (3) There are wide variations in results and pathologies obtained by different groups studying the MuLV model system.

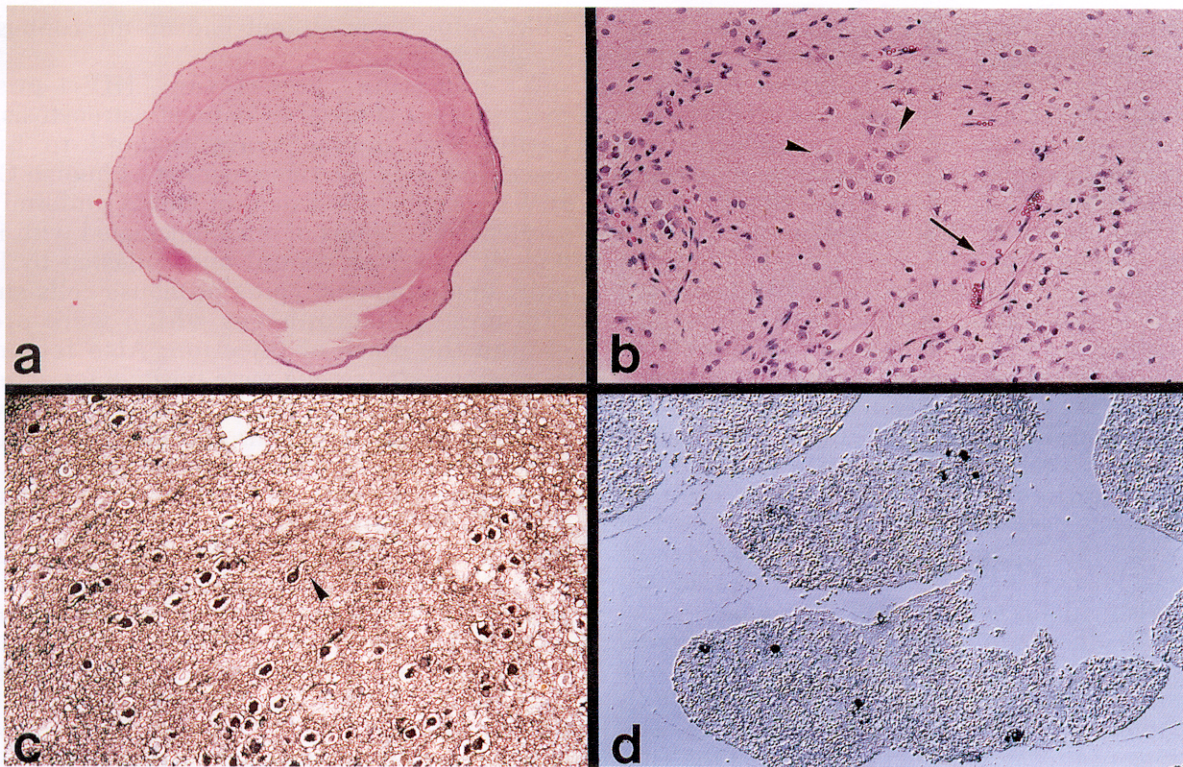
### Transgenic mouse models

Transgenic mice expressing subgenomic fragments or the entire genome of HIV-1 were generated to study the pathogenic mechanisms of HIV. Initial experiments were performed on transgenic animals containing integrated copies of the HIV-1 long terminal repeat (LTR) linked to the bacterial gene chloramphenicol acetyltransferase (CAT). Mice were constructed to assay HIV LTR-directed gene expression in monocytes during differentiation into macrophages (Gendelman *et al*, 1988; Leonard *et al*, 1989). Bone marrow-derived macrophages of HIV-1 LTR containing transgenic mice expressed low levels of CAT activity but after treatment with immune factors causing monocyte/macrophage differentiation a 2–3 fold increase in CAT expression was observed (Leonard *et al*, 1989). In a survey of CAT activity from a variety of tissues HIV-1 LTR directed gene expression was found only in skin, thymus, eye, heart and spleen. Importantly, no CAT activity was seen in brain tissue in any of the animals. This demonstrated that neurons and glia (at least in the murine system) lack the necessary transcriptional factors to support HIV gene expression. Further experiments performed by Corboy *et al*, (1992) generated transgenic mice that contained LTRs from CNS-derived strains and a T cell tropic strain of HIV-1. Only mice constructed with CNS-derived HIV LTRs directed gene expression in neurons. Thus, select strains of HIV-1 may have some advantage for expression within the CNS. The ultimate significance of these findings remains in question as neurons and astroglia do not support sustained HIV replication *in vivo*.

Transgenic mice have now been generated that express whole HIV genomes in neurons (Thomas *et al*, 1994). HIV gene expression in neurons was put under the transcriptional control of the neurofilament promoter (NF-L gene) (Thomas *et al*, 1994). The transgene was predominantly expressed in anterior thalamic and spinal motor neurons. Animals developed a neurological syndrome characterized by hypoactivity and weakness. Axon degeneration was found in sciatic nerves that showed a reduction in nerve fiber density. Decrease in myelinated fiber density and axon degeneration were confirmed by transmission electron microscopy. The lesions described in this model resemble sensorimotor neuropathy in patients with AIDS but not CNS disease.

Toggas *et al* (1994) generated transgenic mice in which expression of HIV-1 gp120 was placed under regulatory control of the murine GFAP promoter. In





**Figure 2** Histopathology of human neural xenografts. (a) A 15 week human fetal brain grafted to the anterior chamber of the eye of a SCID mouse, 7 months post transplantation. Hematoxylin and eosin  $\times 30$ ; (b) the xenograft, higher magnification of panel (a), shows a mixed cell population of neurons (arrowheads) with large nuclei and prominent nucleoli, astrocytes and neovascularization (arrow)  $\times 160$ ; (c) neuronal cell bodies and processes stained by neuronal cell marker PGP 9.5 (adjacent section to panel (a)). Neurons are stained with antibody against PGP 9.5  $\times 160$ ; and (d) human fetal brain aggregate after co-culture with HIV-1<sub>ADA</sub>-infected monocytes prior to transplantation. HIV-1 p24 stain identifies infected macrophages within the aggregate  $\times 80$ .

this case astrocytes would likely express large levels of HIV-1 gp120. A 40% reduction in the number of neurons larger than  $100 \mu\text{m}^2$  was found in neocortex of such transgenic mice. The loss of neuronal subpopulation was combined with widespread neuronal dendritic vacuolation. Reactive astrogliosis was prominent in transgenic animals showing high level of gp120 expression. Immunostaining for mouse macrophage marker F4/80 revealed increased numbers of microglial cells in animals with high gp120 production. Although this study demonstrated that expression of gp120 within the mouse CNS was sufficient to induce pathological effects, it cannot be directly extrapolated to HIV encephalitis. Indeed, the HIV-1 envelope protein was expressed selectively in astrocytes which are not a site for productive viral replication in human brain. Nevertheless, several features of HIV encephalitis are recapitulated by this model including reactive gliosis and neuronal death. The pathogenic mechanisms in support of the findings in the gp120 transgenic mice remain to be determined.

### Human neural xenografts

A small animal model of human neuronal xenograft

has been established recently in order to study HIV infection of human CNS (Epstein *et al*, 1992; Cvetkovich *et al*, 1992). Second–third trimester fetal human brain (11–17.5 weeks) or neural retina are transplanted into the anterior eye chamber of immunosuppressed adult rats or severe combined immunodeficiency (SCID) mice (Figure 2a). The site was chosen based on previous experience in use of the anterior chamber as immune privileged site, similarity of anterior chamber fluid to cerebrospinal fluid, and ability to perform serial examinations of the graft *in situ* (Figure 2b). These grafts survive for several months, vascularize, form a blood–brain barrier and increase in size as the neuropil develops (Figure 2c). Immunohistochemistry performed for brain cell specific markers identified neurons (Figure 2c), astrocytes and few microglia cells. Transmission electron microscopy showed growth of axonal growth cones and synaptic junctions. These xenografts served as targets for HIV-1 infection (cell-free or by co-transplantation with HIV-1-infected monocytes). Infection of the xenografts with cell-free HIV-1 proves difficult; however, injection of HIV-1-infected human monocytes results in pathological changes including the formation of syncytial giant cells, neuronal loss, and astroglia



proliferation, supporting the hypothesis that virus-infected macrophages can mediate neurotoxicity (Cvetkovich *et al*, 1992; Epstein *et al*, 1992; Gendelman *et al*, 1994a). In order to introduce HIV-1-infected and uninfected human monocytes in a uniform ratio, experimental methods were recently modified by adding an intermediate step in which human fetal brain is first dissociated and then allowed to reaggregate. After 1–2 weeks the brain aggregates can be co-cultivated with HIV-1-infected (or uninfected) human monocytes (Figure 2d). These monocytes enter the aggregates and are then placed in the anterior chamber where they coalesce and vascularize. The integrity of blood–brain barrier in such a model (Achim *et al*, 1993) may remain intact. This system could facilitate the study of direct and indirect effects of HIV-1 infection of human neural tissue and the interactions between HIV-1 and other opportunistic pathogens such as cytomegalovirus (Epstein *et al*, 1994). The advantage of this model is avoidance of extrapolation necessary with other lentiviral infections. Furthermore, it uses relatively inexpensive laboratory rats or SCID mice and should be useful in testing of antiviral therapies that target HIV-1 infection of CNS. The limitations of this system include that: (1) the fetal brain placed into mice while reflecting the developing CNS does not recapitulate adult brain tissue; (2) the dissociation step in xenograft preparation does not allow the reconstruction of regional cytoarchitecture; and (3) the model relies on human fetal abortus tissue, an often highly variable tissue source.

### Severe combined immunodeficiency (SCID) mice inoculated intracerebrally with HIV-1-infected human macrophages

A small animal model has been recently developed (Tyor *et al*, 1993) that closely parallels main features of HIV encephalitis. The model uses SCID mice lacking functional B and T cells due to a defect in T-cell receptor and immunoglobulin variable chain rearrangement. They can accept human xenografts without rejection, and cells of human immune system can survive for months. In the original study (Tyor *et al*, 1993), peripheral blood mononuclear cells (PBMC) were inoculated intracerebrally to SCID mice followed by cell-free HIV-1 injection. CD68-labeled human macrophages were found primarily along the needle tract in the brains of 39% animals sacrificed 1–3 weeks post inoculation. 21% of them were HIV-1 p24-positive, and occasional giant cells also contained viral protein. Macrophages were also labeled with anti-TNF $\alpha$ , MHC class II, IL-1 and VLA-4 antibodies showing that they were immune activated. Occasional T lymphocytes were also detected but they did not correspond to HIV-1-containing cells. Most of control mice (59%) which received PBMC only or

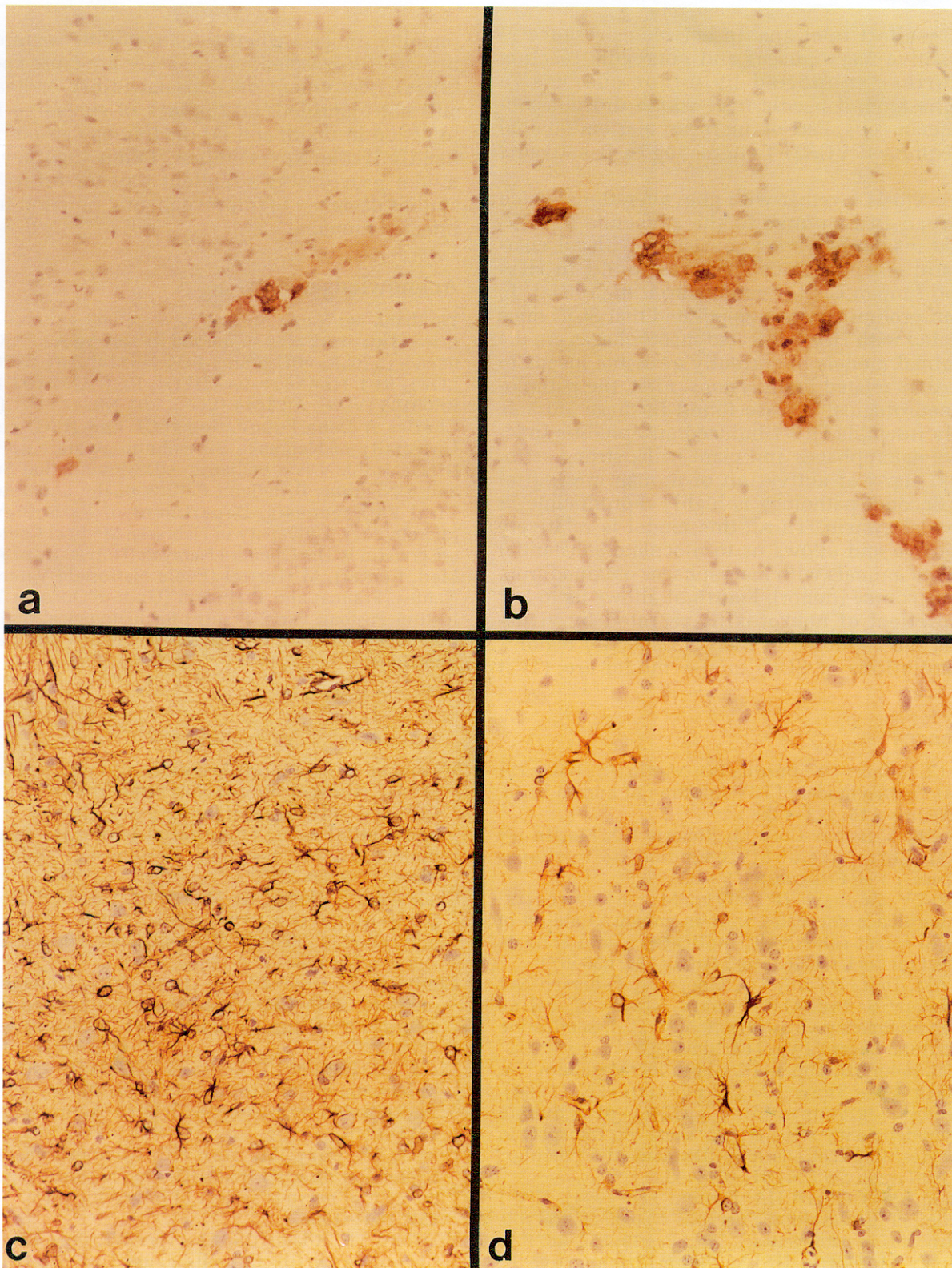
PBMC plus virus diluent had no macrophages or T cells visible.

The brains containing HIV-1-positive macrophages showed prominent astrogliosis indicating that mouse brain reacted to human macrophages in the same manner as human CNS to HIV-1 encephalitis. Extent of gliosis uniformly was greater in the brains of mice inoculated with PBMC and HIV-1 *versus* control mice injected by PBMC only. Immunophenotyping of mouse cells showed rare mouse macrophages which were present around the place of injection. Also few mouse macrophages/microglia were positive for class II in meninges, perivascularly, subependimally and intraparenchymally. Brains of SCID mice inoculated cell-free virus only had no p24-positive cells and minor astrocyte reaction attributed to trauma. Co-culture of brain tissue with permissive cells revealed HIV only in mice showing virus-containing cells. Tyor *et al* (1993) repeated experiments several times and showed that results were reproducible. However, a very low number of animals contained infected macrophages at time of sacrifice.

In order to further improve reproducibility of the model, we have decided to inject SCID mice with HIV-1-infected macrophages since such mode of inoculation would increase the number of infected cells in mouse brain. Human monocyte-derived macrophages were infected with macrophage-tropic strain of HIV-1<sub>ADA</sub>. These cells were injected into SCID mouse brains at different times post infection, and animals were sacrificed 0.5 h – 35 d after inoculation. HIV-1 p24-positive macrophages and multinucleated cells were found in putamen, neocortex and ventricles (Figure 3a). Number of CD68-stained cells varied from 3–5 to 50–60 per section (Figure 3b). Human macrophages were detected along the needle tract and on certain distance from it depending on the time postinoculation, in 17 out of 24 animals (71%). More than 50% of the macrophages were HIV-1 p24-positive. Immunolabeling for cytokine production and MHC antigen expression showed that part of injected cells were in the state of immune activation.

Serial coronal sections revealed reactive astrogliosis which was widely spread comparing to mouse brains injected with the same amount of non-infected macrophages from the same donor (Figure 3c, d). Strong astrocyte reaction included increase in number and size of GFAP-expressing astrocytes with numerous processes. Different time of sacrifice allowed us to unravel the dynamics of astrocyte reaction. An important positive correlation existed between number of infected cells and astrogliosis. Alterations of mouse microglia were detected by immunolabeling with mouse macrophage marker F4/80 and lectin RCA-1. Microglia showed signs of activation both by changed morphology (increased branching and cytoplasmic distentions) and cytokine production.





**Figure 3** Histopathological features of the SCID mice inoculated intracerebrally for 1 week with HIV-infected macrophages. Tissue preparations were counterstained with hematoxylin. (a) Viral p24-positive multinucleated cell and macrophage are in putamen of mouse brain inoculated for 1 week with  $10^5$  HIV-1<sub>ADA</sub>-infected human macrophages. Tissue was immunostained with antibodies against HIV-1 p24 antigen  $\times 400$ ; (b) CD68-positive macrophages (immunostained with KP-1 antibody) are within the cerebral cortex  $\times 400$ ; (c) pronounced astrocytosis is in the putamen of a mouse injected with HIV-1<sub>ADA</sub>-infected macrophages; and (d) a mild astrocyte reaction is seen in the identical region of brain tissue of a mouse inoculated with control uninfected macrophages. Astrocytes on panel b and c were immunostained with anti-GFAP antibody  $\times 200$ .



A cause-effect relationship between presence of HIV-positive macrophages and microglial reaction was further supported by minor changes in microglia around needle tract which did not contain human cells. Part of microvascular endothelial cells expressed VCAM-1 suggesting their activated state.

Astrocytosis together with glial alterations which developed in response to the presence of HIV-infected macrophages are consistent with all characteristic morphological features found in HIV-infected human CNS (Gendelman *et al*, 1994a). *In toto*, our preliminary results pointed that SCID mice inoculated with HIV-1-infected macrophages are the best animal models of currently available means to study AIDS related brain lesions. First, the pathology of neurological disease found in these animals strongly resemble HIV-1-induced encephalitis (p24-positive macrophages and giant cells, strong astrocytosis, signs of activation of mouse microglial cells). Second, pathology is highly reproducible in this model system. Third, this model is relatively inexpensive and allows us to inoculate substantial number of animals with macrophages obtained from the same source (donor). Fourth, it allows us to test *in vivo* effect of neurotoxins secreted by virus-infected macrophages in the CNS specific regions. The limitations of SCID mouse model are: (1) it

remains unclear whether neurotoxins secreted by human HIV-infected macrophages are cross species; (2) the component of neuronal injury or death is still not identified; (3) the lack of spreading HIV-1 infection in brain; and (4) the trauma caused by the needle track. Nevertheless, taken together, this model provides an excellent tool for future drug studies designed to improve mental function in HIV infected humans.

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