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In Vitro and Animal Models of Human Immunodeficiency Virus Infection of the Central Nervous System

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Initially, progressive dementia affected a large percentage (15 to 20%) of adults infected with human immunodeficiency virus (HIV). However, with the present use of potent antiretroviral therapies, its incidence has been reduced to approximately 8 to 12%. This dementia, known as HIV-associated dementia (HAD) or AIDS dementia complex, is characterized by a progressive deterioration of affective, cognitive, and motor skills. Patients show loss of the ability to concentrate. They also lose fine motor coordination and may display changes in behavior and personality. These symptoms become more pronounced in patients with end-stage disease, often progressing to memory loss, apathy, personality changes, and social withdrawal. Although potent antiretroviral therapies have significantly reduced other complications of HIV infections (e.g., opportunistic infections), the associated decrease in HAD has been proportionately less. Consequently HAD is becomingly a relatively more important complication of HIV infection.

HIV-infected children are also susceptible to HAD, with as many as 30 to 50% of this group displaying signs and symptoms of HAD (71). Unlike in adults, HAD in children is often an early event, manifest by microencephaly, failure to achieve both cognitive and motor developmental milestones, and/or frank regression of milestones once achieved. Indeed, it has been reported that children are more likely to develop dementia than to develop opportunistic infections (31).

Since the initial reports of AIDS in 1981, much time and energy has been devoted to determining how HIV enters the body, gains entry into cells, replicates, and then causes the immunosuppresion that is the hallmark of this disease. However, when we look specifically at the case of HIV and the central nervous system, it is obvious that there are important questions that remain to be definitively answered. For instance, how soon after infection does HIV enter the central nervous system (CNS)? It is believed to enter early after infection, but is this on the scale of hours or days? How does HIV cross the blood-brain barrier (BBB)? What is the mechanism by which cells in the brain are damaged? Why do not all HIV-infected patients develop HAD, and why do those that develop HAD display a wide range of symptoms? Why do some patients with extremely high viral loads in the CNS not develop HAD and others with relatively low viral loads develop frank dementia? Unfortunately, these types of questions are not easily answered. Ascertaining the answers to these and other similar questions would require access to tissues from individuals who have been very recently infected with HIV and from individuals at various points along the pathway to dementia. Recently infected individuals are very difficult to identify, and even when this is possible, only some types of samples are available for analysis. Brain tissue is generally unavailable unless the acutely infected patient dies by some other means shortly after infection. Another factor to consider is that individuals react differently to HIV infection. The pattern of disease in one person is often substantially different from that in the next. Although the end point is the same, progression to that point may follow markedly different paths. To overcome problems such as these, the research community must rely primarily on various in vitro and animal systems to model aspects of HIV infection of the CNS.

There are a number of in vitro systems and animal models that are being used to investigate aspects of HIV infection in the CNS. Each model has unique strengths and weaknesses. The purpose of this review is to briefly explain the most popular model systems and comment on the utility of each system with respect to CNS AIDS and HAD.

HIV IN THE CENTRAL NERVOUS SYSTEM

Shortly after the onset of the HIV epidemic, many infected patients were noted to be depressed. Initially this was thought to have been the result of being confronted with the news that they were infected with an enigmatic, incurable, fatal disease. Detailed neurological examination and neurocognitive testing soon revealed that many patients displayed discrete motor, cognitive, and affective deficits.

HIV apparently invades the CNS shortly after seroconversion (67), although how this occurs is not precisely known (reviewed in reference 40). HIV-infected macrophages, lymphocytes, and/or monocytes may carry the virus across the BBB (the so-called Trojan horse hypothesis). It is also likely that free virus is able to cross the BBB, as HIV gp120 is capable of binding to glycoproteins on the surfaces of endothelial cells and mediating absorptive endocytosis of viral particles and HIV-infected cells (4, 5). As these are not mutually exclusive scenarios, it is likely that more than one of these processes may be occurring at the BBB simultaneously.

The onset and progression of HAD are highly variable. Some patients display no signs of HAD despite high levels of viral RNA in the CSF, while other patients with lower levels of viral RNA are profoundly impaired. This is likely due to a combination of the viral strain with which the patient is infected, the evolution of that strain, BBB integrity, systemic HIV load, and as-yet-unknown genetic factors of the patient.

Pathologically, a number of different cell types are affected

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by HIV in the CNS. Interestingly, in the brain, only cells of the macrophage lineage are infected by HIV. Cells of the ectodermal lineage (astrocytes, oligodendrocytes, and neurons) are generally believed not to be infected by HIV, although restricted (non-virion-producing) infection of astrocytes has been reported to occur in pediatric patients (100). The presence of fused macrophages or macrophages fused with microglia, known as multinucleated giant cells (MGCs), generally serves as the pathological hallmark of the most severe form of HAD. This most severe form of HAD is usually correlated with HIV encephalitis (HIVE). In addition to MGCs, widespread activation and proliferation of macrophages and astrocytes is also observed. Neuronal damage and dropout are also common findings. Another pathological feature of HAD is myelin pallor, which is believed to be indicative of myelin or axonal injury, or BBB disruption. Glass et al. (38) report that about 50% of patients with HAD display MGCs and myelin pallor at the time of autopsy (reviewed in reference 40)

All of these pathological features, i.e., astrocytosis, gliosis, MGCs, and myelin pallor, are generally found in the subcortical regions of the brain such as the basal ganglia, brain stem, and deep white matter. It is not surprising that HAD thus displays features more characteristic of a subcortical dementia, such as Huntington's disease, than of a cortical dementia like Alzheimer's disease (for a review, see reference 81).

IN VITRO MODEL SYSTEMS FOR HIV IN THE CNS

Primary cultures of fetal cortical cells from both humans and rodents (rats and mice) have been used extensively by many investigators to study the effects of a number of neurotoxic factors, including gp120, arachadonic acid, platelet-activating factor, glutamate, quinolinic acid, chemokines, etc. (14, 26, 35, 47, 50, 55, 56, 68, 73, 86, 92). In addition, these model systems have also been used to examine potential neuroprotective agents.

The passage of nutrients, proteins, small molecules, and monocytes from the systemic circulation into the brain is regulated by the BBB, resulting in the so-called immunological privilege of the brain. The BBB is comprised of a monolayer of brain microvascular endothelial cells (BMVEC). Between the BMVEC are junctional complexes called tight junctions (see reference 25 for a review). These junctions, which stain positively for the zonula occludens-1 protein, are thought to prevent leukocytes from the peripheral circulation from invading the CNS by sliding between the BMVEC. The end feet of the astrocytes are in close approximation to the inner surface of the BMVEC, and the astrocytes are likely to assist in BBB function by secreting factors required by the BMVEC (49). In contrast to viewing the BBB as a static barrier, it may be best to view the BBB as a rather complex dynamic system which is likely to play an active role in HAD and other neurological diseases.

Various labs have developed in vitro model systems to study the BBB, the role it plays in regulating the passage of HIV and other infectious agents into the CNS, and factors which influence the permeability of the BBB. Generally these models have been one of three types: (i) primary cultures of BMVEC, (ii) cultures of endothelial cells from organs other than brain grown with astrocytes to induce BBB markers, or (iii) cocultures of BMVEC and astrocytes. Models such as these have been used to investigate various aspects of HIV infection, including the infectivity of the endothelial cells of the BBB by HIV and mechanisms of transendothelial migration of monocytes.

Using primary cultures of human microvascular endothelial cells from brain resections, Vinters et al. (108) reported the in vitro culture of cells from brain cortical microvessels which had been removed from patients undergoing lobectomy for intractable seizure disorder. Two cell populations arose from these cultures. One of these was a smooth muscle cell line, while the other demonstrated properties expected of an endothelial cell line except that it had only moderate positivity for factor VIII antigen. Dorovini-Zis et al. (23) used the same approach, isolating microvessels from cortical sections removed during surgery or at autopsy. These cells were factor VIII positive, displayed lectin-binding sites, and formed tight junctions when viewed by electron microscopy, suggesting that the cells are BMVEC.

A number of labs then proceeded to use BMVEC to construct in vitro models of the BBB. Hurwitz et al. (48) constructed a BBB model using human endothelial cells from umbilical cords and fetal astrocytes from human cerebrum. These cells were cocultured on opposite sides of a porous (3-μm-diameter pores) tissue culture support. The 3-μm-diameter pores allowed the astrocyte end feet to penetrate the barrier and contact the endothelial cell layer, inducing expression of the BBB proteins, brain-type glucose transporter (GLUT-1) and γ-glutamyltranspeptidase (γGT). When the pore size was small enough to prevent close contact of the end feet (0.45-μm diameter), the BBB markers were not expressed, supporting the notion that close approximation or contact of the astrocytic end feet and the microvascular endothelial cells is necessary for BBB formation. This work did not include any definitive electron microscopic evaluation of junctional complexes between the endothelial cells or any evaluation of the electrical resistance of the BBB model.

Hayashi et al. (43) used the same general type of model system in which endothelial cells are separated from astrocytes by a tissue culture barrier. However, in place of BMVEC and human astrocytes, they constructed their model using a heterologous coculture of human umbilical cord endothelial cells and rat fetal astrocytes. Cells were seeded onto culture inserts with two different sizes of pores, either 3.0 or 0.45 μm in diameter. They found that γGT is induced in the endothelial cells when they are contacted by astrocytes (3.0-μm-diameter pore). They also found that GLUT-1, P-glycoprotein, transferrin receptor, and γGT mRNAs were increased in the model with the 3.0-μm-diameter pore but not in the model with the 0.45-μm-diameter pore. They also found that the presence of astrocytes increased the impermeability of the barrier to [^{3}H]inulin. Transmission electron microscopy revealed regions resembling the zona occludens in areas where the endothelial cells were in contact with the astrocyte end feet.

Persidsky and Gendelman (89) constructed an in vitro BBB model system in which they used primary human BMVEC, human fetal astrocytes, and a collagen-coated tissue culture insert with 3-μm-diameter pores, allowing the astrocyte end feet to be in close approximation to the BMVEC layer. Analysis of the model showed that >95% of the BMVEC were

positive for von Willebrand factor and that >98% of the astrocytes showed glial fibrillary acid protein (GFAP) reactivity in the cytoplasm. Morphologically, the system appeared to be comprised of a monolayer of BMVEC with tight junctions between the BMVEC. On the other side of the membrane, astrocytes were found as flat cells with bundles of glial intermediate filaments in the perinuclear region. The model also displayed the high electrical resistance typical of the BBB, and the BMVEC-astrocyte layer was highly impermeable to [^{3}H]inulin (90). This model was then used to study the transendothelial migration of monocytes across the BBB. The morphology of the BMVEC changed with the application of monocytes, and the changes were comparable to those observed in activated endothelium (89).

Previous work had shown that BMVEC in patients with HIVE displayed an upregulation of the adhesion molecules vascular cell adhesion molecule 1 (VCAM-1) and E-selectin (83). This same upregulation was observed in this in vitro model and was attributed to increased expression of the proinflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), as the mRNAs for these cytokines were increased in stimulated monocytes but not in the control unstimulated cells (89). Utilization of models of this type will allow researchers to dissect the complex interactions and contributions of astrocytes, BMVEC, cytokines, monocytes, and HIV to transendothelial migration (see reference 84 for a review).

Other groups have used in vitro BBB models to investigate whether HIV can infect the cells of the BBB. There is some conflict as to whether HIV infects BMVEC grown in these in vitro systems. Moses et al. (76) demonstrated infection of microvascular endothelial cells with HIV_{LAV}, a T-lymphocyte-tropic (T-tropic) strain of HIV. They found that 40% of the endothelial cells were positive for HIV p24 antigen at 7 days after infection. They also reported that the infection was productive but noncytopathic. The infected cells shed virus into the culture medium. These cell-free supernatants could then be used to infect HeLa cells with HIV. Poland et al. (96) attempted to infect brain-derived microvascular endothelial cells with three T-tropic strains (MN, IIIb, and RF) and one macrophage-tropic (M-tropic) strain (SF162) of HIV. They showed that both M- and T-tropic HIV strains could infect the cultures at only a very low level. They were unable to demonstrate p24 antigen capture or positivity for HIV reverse transcriptase. The cultures were positive for HIV *env*, *gag*, and long terminal repeat by PCR, but passage of the infected lines resulted in loss of the ability to detect HIV sequences by PCR. They could recover HIV from these cultures by coculturing them with CEM-SS cells, a T-cell line permissive to infection. The results of both Moses et al. (76) and Poland et al. (96) suggest that HIV infection of BMVEC does occur, but the level of viral replication may be quite minimal. However, Nottet et al. (83) found that BMVEC are not permissive for infection by HIV-positive monocytes. They cocultured M-tropic $HIV\text{-}1_{ADA}$-infected monocytes with BMVEC and looked at synthesis of viral DNA, p24 antigen, and reverse transcriptase activity in the BMVEC for up to 5 days of coculture. They found no sign of infection by any of those methods. They also found no sign of infection 14 days after direct viral inoculation of $HIV\text{-}1_{ADA}$ into the MVEC. It may simply be that M-tropic HIV will not infect BMVEC, while T-tropic HIV will infect BMVEC with various efficiencies.

In a closely related area of research, Fiala et al. (33) used an in vitro BBB model with human BMVEC and astrocytes to look at the effect of cocaine on migration of monocytes across the BBB. The model, described by Fiala et al. (32), was assembled from tissue isolated from human brain. This was then cultured with primary fetal astrocytes. The two cell types were separated by a membrane with 3-μm-diameter pores. This model displayed endothelial cell markers (factor VIII and GLUT-1), BBB morphology, impermeability to macromolecules, and electrical resistance (32). The astrocytes were shown to be GFAP positive by immunocytochemistry. This work showed that treatment of the model with cocaine increased transmigration of monocytes across the BBB. They also demonstrated by enzyme-linked immunosorbent assay that cocaine upregulated intercellular adhesion molecule 1 and induced the expression of VCAM-1 and platelet/endothelial cell adhesion molecule 1. Assay of p24 antigen in the brain compartment of the model demonstrated that application of either exogenous TNF-α or IL-1β increased the migration of monocytes into the brain side of the chamber. Untreated BBB preparations permitted only minute amounts of cell-free HIV type 1 (HIV-1) to cross the membrane, while those treated with TNF-α were more permissive to the passage of cell-free $HIV_{JR\text{-}FL}$ (32).

Lyman et al. (65) described an organotypic culture system of the human fetal CNS. This system allows for the development of many of the same cell contacts that would be developed in vivo. They utilized explants of CNS tissue derived from 14- to 21-week fetuses that had been electively terminated. Explants were grown on collagen-coated coverslips in tissue culture plates. Using this system, they documented the differentiation of neurons, astrocytes, oligodendrocytes, and endothelial cells by using both biochemical and morphological criteria. This group went on to employ the organotypic system as a model for the interaction of HIV-1 with the developing CNS (66). They infected organotypic cultures with the T-tropic isolates RF and MN and the M-tropic isolates JR-FL and JR-CSF of HIV-1 and subsequently assayed the cultures for HIV-1 DNA, reverse transcriptase activity, p24 antigen, and the ability to form syncytia. Curiously their cultures were found to be productively infected by only the T-tropic strains of HIV for as long as 67 days after the initial infection. Double-label experiments indicated that microglia were the major cell type that was infected, with astrocytes infected to a lesser extent. More recently, this group (41) demonstrated that in this model HIV infection is mediated by gp120 binding to a cell receptor that is neither CD4 or galactocerebroside. They showed that either pretreatment of either HIV with soluble CD4 or pretreatment of organotypic CNS cultures with gp120 significantly inhibited infection by HIV. They also reported that pretreatment of the CNS cultures with deglycosylated gp120, anti-CD4, or antigalctocerebroside antibodies did not inhibit HIV infection. Taken together, these results suggest that gp120 is necessary for binding to CNS cells and that the receptor for this interaction is not CD4 or galactocerebroside. Recently, using cultured astrocytes, Hao and Lyman (42) showed that gp120 mediates HIV binding to astrocytes through an ~65-kDa receptor that is neither CD4 nor galactocerebroside. These findings contradict our present understanding of the mechanisms of HIV infection

of the CNS, and additional information is needed to resolve the apparent inconsistencies.

The use of in vitro BBB model systems and organotypic culture systems has greatly facilitated our understanding of the complex nature of interactions between HIV and the BBB, cytokines, cell surface receptors, and the various type of CNS cells. However, these systems have obvious disadvantages, and chief among these is that they allow us to look at only a small portion of the pathogenic processes of HIV infection of the CNS. To fully appreciate the complexities and mechanisms of neuropathology, animal model systems must be utilized. The following section outlines the development, utility, and limitations of the animal model systems presently in use.

ANIMAL MODEL SYSTEMS FOR HIV IN THE CNS

Ungulate lentiviruses. There are a number of ungulate lentiviruses that have been isolated and characterized. These include equine infectious anemia virus (EIAV), maedi-visna virus (MVV), bovine immunodeficiency virus (BIV), and caprine arthritis-encephalitis virus (CAEV). These ungulate lentiviruses should be considered to be only distantly related to the primate lentiviruses. Each has some utility, perhaps, but since they do not infect cells of lymphocytic origin and do not cause immunodeficiency, they are not likely to be of great use for modeling HIV infection of the CNS in humans.

EIAV is an economically important pathogen that naturally infects horses, mules, and donkeys and is transmitted by insects and iatrogenic contamination by means of dirty needles or instruments. Clinical manifestations of EIAV include hemolytic anemia, persistent viremia, weight loss, and fever. EIAV does provide a good model for the study of antigenic drift of lentiviruses, as different antigenic strains of EIAV predominate during each new bout of the disease and the predominant strain of the virus can change in as few as 2 weeks (reviewed in reference 74). EIAV infects cells of monocyte/macrophage lineage and does not infect lymphocytes, and since EIAV does not cause immunodeficiency, it is of only limited utility for the study of HIV in humans.

BIV was originally isolated in 1969 (107) and is perhaps the least well studied member of the lentivirus family. That herds of cattle worldwide are infected with BIV is not disputed; however, there is a paucity of evidence that BIV actually causes a clinical disease (reviewed in reference 19). Experimental infection results in a nonpersistent lymphocytosis that is typically mild. Heaton et al. (44) reported that BIV has a tropism for monocytes and macrophages, and they reported a reduction in $CD4^+$ cells in lymph nodes. There has been no clearly demonstrable immunodeficiency. Virus has been found to be present in tissues up to 3 years postinfection (16). Although BIV displays a genome organization similar to that of the other lentiviruses, it is clearly the least pathogenic member of this group and should not be labeled an immunodeficiency virus. Like EIAV, BIV has only limited use for modeling HIV in the human CNS.

MVV of sheep was first recognized as the etiological agent of two syndromes in sheep, maedi (interstitial pneumonia) and visna (demyelinating encephalomyelitis). As with all lentiviruses, there is a long delay between infection and development of clinical disease. Infection is widespread, but only a small portion of the infected animals actually progress to a clinical disease and then only 2 to 4 years after infection (87). This disease is characterized by a progressive pneumonia, encephalomyelitis, paralysis, and wasting. Some infections, both naturally occurring and experimentally induced, progress rapidly with short latency. These short-latency infections often cause a high mortality, especially in young (<6-month old) animals (87). MVV has been shown to infect cells of the monocyte/macrophage lineage only. The restricted nature of viral gene expression allows the virus to persist in the face of an active immune response mounted by the host. Productive viral replication occurs only after the monocyte matures into a macrophage and begins expressing factors necessary for viral replication (36, 79)

MVV and CAEV of goats are clearly distinct but closely related viruses, based on antigenicity and genome organization (see references 19 and 21 for reviews). CAEV causes progressive arthritis, encephalomyelitis, and interstitial pneumonia in a small population of animals infected with this virus. Encephalitis, characterized by ataxia and lameness in the hind legs, is most frequently seen in 2- to 4-month-old kids, while arthritis, respiratory impairment, and mastitis are seen in adult animals. Like MVV, CAEV infects cells of the monocyte/macrophage lineage and does not cause an immunodeficient state.

Despite causing diseases with some similarities to the disease caused by HIV, MVV and CAEV have shortcomings as ideal models. First, only cells of the monocyte/macrophage lineage, not lymphocytic cells, are infected. Second, they do not induce a state of immunodeficiency. Third, the CNS lesions produced by MVV and CAEV differ pathologically from those caused by HIV. Pathology caused by MVV and CAEV is characterized by changes mainly in white matter, while MGCs, which are frequently observed in cases of HIVE, have only rarely been reported with MVV and CAEV (37). The demyelination seen in MVV disease is usually a well-defined plaque accompanied by an area of extreme inflammatory reaction, while the pattern seen in HIV disease is more diffuse and accompanied by reactive astrocytosis (37). Vacuolar myleopathy, a pattern of myelin breakdown in the spinal white matter, is not seen with either MVV or CAEV.

Nonungulate lentiviruses: FIV. Feline immunodeficiency virus (FIV) could potentially offer a system that is more amenable to study and more closely approximates HIV infection in primates. FIV, the most recently discovered lentivirus, was first isolated from a cat in California in 1986 (85). FIV has been found in cats worldwide, with a prevalence of about 1 to 10%. FIV is spread very efficiently by biting; thus, wild male cats display the highest rate of infection (12). The monocyte/macrophage and lymphocyte ($CD4^+$, $CD8^+$, and B-lymphocyte) lineages are infected (15, 17).

Naturally occurring FIV infection displays three distinct phases. The initial infection is characterized by an acute flu-like illness accompanied by weight loss, fever, and a lymphoid hyperplasia that may last for months. This is followed by an asymptomatic period of variable length. This progresses to a stage of clinically active disease. This final stage is characterized by problems associated with immunodeficiency, such as opportunistic infections, weight loss, and neoplasia (11, 12, 23). Experimentally infected animals show the first two stages of infection, but it has been difficult to reproduce the immunod-

eficiency-associated problems. In a study using random-source (RS) and specific-pathogen-free (SPF) cats, English et al. (29) investigated the differences in the types of disease displayed by these two groups. Ten of 12 RS FIV-positive cats developed either chronic stomatitis or upper respiratory disease, while 4 of 7 FIV-positive SPF cats developed neurological disease or B-cell lymphoma rather than stomatitis or upper respiratory disease. Both groups displayed similar immunological changes, including a decrease in $CD4^+$ cells and the loss of normal mitogen-induced proliferative responses. The B-cell responses of these groups differed in that the RS FIV-positive cats, but not the SPF FIV-positive cats, displayed an increased immunoglobulin production in vitro, indicative of B-cell activation during FIV infection. This is an interesting facet of this model, as B-cell neoplasias are frequently seen in HIV-positive humans (61). Callanan et al. (18) and Beatty et al. (10) also reported B-cell lymphomas in FIV-positive cats.

As mentioned above, FIV-positive felines develop neurological disease. Subacute, quantifiable neurological disease has been documented in young cats as soon as 3 months postinfection with the neurotropic FIV MD strain. Podell et al. (93) demonstrated that experimentally infected cats developed immunodeficiency and delayed growth. Neurologically, three of six infected cats developed a compulsive roaming behavior as early as 1 month postinfection, and they displayed abnormal electroencephalogram recordings over the 16-month course of the study. Delays in visual evoked potentials (VEPs) (93, 94) and auditory evoked potentials (91) have been demonstrated. Researchers have also reported that some of the infected felines displayed behavioral changes that were manifest as greater aggression or reclusive behavior (91). As seen in HIV-positive humans, sleep patterns in FIV-positive cats are also altered (91, 97).

Despite neurological deficits, only relatively mild histopathological changes have been found in the brains of FIV-positive cats. These changes include diffuse gliosis, perivascular cuffing, glial nodules, and satellitosis, where four to five oligodendrocytes are seen to surround a single neuron (94). Meeker et al. (72) demonstrated neuronal loss in asymptomatic FIV-positive cats, finding that large neurons in the cortex and striatum were significantly decreased. This neuronal loss occurred in the absence of obvious neuropathological changes. Recently, Podell et al. (95), using single-voxel two-dimensional magnetic resonance spectroscopy, demonstrated that neurological functional disruption of the frontal cortex correlated strongly with neuronal injury or loss. The degree of neuronal injury was variable, but the cats with the highest levels of excitatory neurotoxic compounds had the most neuronal injury. These findings support the prevailing notion that neuronal injury and loss are the result of the production of diffusible neurotoxic compounds by activated macrophages. This same process is also believed to be the primary mechanism for neural damage in humans (see reference 70 for a review).

FIV is a useful model for the examining some of the neurological deficits caused by neurotropic lentiviruses. The cat is easy to handle and readily available, and FIV is not infectious to humans. There are obvious similarities of FIV to HIV, and yet this model has disadvantages. The observation of only minimal pathology is somewhat troubling, as is the long latency period between infection and clinical disease. The difficulty of experimentally reproducing the same type of immunodeficiency seen in natural infections is also a potential pitfall. Finally, the broader tropism exhibited by FIV and the fact that FIV does not utilize the CD4 receptor for infection (46, 82) may limit the utility of this model.

Murine models. Severe combined immunodeficient (SCID) mice harbor a genetic defect that prevents rearrangement of B- and T-cell receptor genes, precluding the development of immunologically competent B and T cells. Thus, these mice are unable to mount an immune response against transplanted foreign tissue, a fact that has been exploited by numerous studies in many areas of research. There are two basic types of SCID models that have been used to model the effects of HIV in the human CNS.

The first model consists of inoculation of second-trimester human fetal brain into the anterior eye chamber of SCID mice or immunosuppressed rats or injection of the fetal brain cells into the intrascapular fat pad of SCID mice (1, 30). In both of these systems, the brain cells engraft, increase in size, and develop a BBB. Neurons, astrocytes, and microglia can be identified. While infection of these xenografts by cell-free HIV was not possible, the neural tissue could be infected by inoculating the graft with HIV-positive human monocytes. After infection of the xenografts by HIV-positive monocytes, some features of HIV encephalitis were apparent, such as the presence of some MGCs and an increase in the number of astrocytes and loss of neurons (30). Since HIV does not infect murine cells, this is supportive of the ideas that neuronal loss and brain pathological changes are the result of neurotoxic compounds released from activated macrophages.

An improvement over the original model of Epstein et al. (30) was made by Achim et al. (1). This group used fetal brain tissue that had been dissociated by forcing it through a sieve and allowing the tissues to form aggregates while in a roller bottle. HIV-infected monocytes were then added to the brain tissue aggregates, allowing the monocytes to infiltrate the brain tissues. These grafts contain neurons, astrocytes, and blood vessels. Pathologically, numerous MGCs are formed, and both the MGCs and macrophages are positive for HIV. It is also likely that these cells are in an activated state, as the majority of these cells are major histocompatibility complex (MHC) class II positive. Although viral particles were detected in vivo up to 3 months after infection, the placement of the embryonic brain tissue in the intrascapular fat pad was unsatisfactory because the neural graft degenerated over time.

Recently Sanders et al. (101) modified the original SCID mouse model of Achim et al. (1). They cocultured macrophages derived from HIV-infected blood with second-trimester human fetal brain neuroglia. These aggregates were then injected into the brains of SCID mice. Grafts of this type differentiated into neurons, astrocytes, and microglia over a period of 4 months and survived for about 6 months. At 4 months, about 60% of the cells in the graft stained for the neuronal marker PGP9.5, and approximately 10% of those showed synapsin staining. Staining for HIV gp41 was positive throughout the experiment. No MGCs were observed in this system. Examination of the grafts by electron microscopy revealed the presence of synapses, suggesting that the use of mixed cell aggregates provides the necessary factors for neural cell growth and survival that were not present in the original

model of Achim et al. (1). Further development of this model should produce an even more useful tool for the evaluation of the effects of HIV on the human CNS in a SCID mouse host.

In the second type of SCID mouse model of CNS HIV, Tyor et al. (106) intracerebrally injected human peripheral blood mononuclear cells (PBMC) into SCID mice. Cell-free HIV was injected either at the same time as the PBMC or 1 day later. At 1 to 4 weeks postinjection, about 40% of the brains were found to contain human macrophages, 21% of which were p24 positive. The p24-positive macrophages were mostly found adjacent to the needle tract, but some were found in the uninjected hemisphere. These macrophages were MHC class II positive, most were TNF-α positive, and some were positive for IL-1 and VLA-4, suggesting that they were immunologically activated. The mice with the p24-positive macrophages, but not the PBMC- or HIV-only controls, displayed striking gliosis far removed from the needle tract, suggesting that it was not associated with injection trauma, and had significantly higher astrocyte counts. Cell cultures consisting of minced brain stem and cerebellum from these p24-positive mice cultured with human PBMC were also positive for HIV by enzyme-linked immunosorbent assay. This result demonstrated the presence of intact, infectious virus and not merely the presence of viral antigens (106).

The model of Tyor et al. (106) has since been refined by Persidsky et al. (88). The modifications consisted mainly of standardization of several of the parameters, such as stereotaxic injection of equivalent numbers of cells into the brain, injection of highly purified HIV-infected monocytes (identified by CD68 staining), and use of computer image analysis to more accurately quantify astrocytosis. Xenografts of HIV-infected monocytes remained viable for up to 5 weeks. At 3 days after injection, inspection of sections of the putamen revealed that about 40% of the human cells were HIV p24 positive. Over the next 5 weeks, the number of human cells per section declined from about 15 to 30 cells per section initially to 1 to 5 cells per section at 5 weeks. The percentage of cells staining for p24 remained essentially unchanged at about 40%, indicating that the virus was still being produced in these cells (88). A progressive inflammatory response was also seen in this experiment. This response consisted of a reactive astrocytosis. This inflammatory response of murine cells, apparently in reaction to the injected, activated human macrophages, consisted of morphological changes and increased IL-1β, IL-6, and VCAM-1 expression. This reaction occurred in areas of human monocyte infiltration and persisted throughout the 5 weeks of the experiment (88). Astrogliosis was also noted in the inoculated hemisphere. Inoculation itself causes some astrogliosis, but 1.6- to 2.2-fold more astrogliosis was noted in the animals receiving HIV-infected monocytes. Finally, neuronal damage was also noted in the areas around the injection site. Terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling assay revealed positive neurons and other cell types with morphological signs of apoptosis.

Recently, Avgeropoulos et al. (3) used a procedure based on that of Persidsky et al. (88) to model the effects of chronic HIVE on behavioral deficits. The mice were initially injected with HIV-positive monocytes and were then subsequently injected with more HIV-infected monocytes every month for 3 months to model a chronic state of infection. This study provides evidence for behavioral deficits and motor slowing in mice receiving HIV-infected monocytes compared to the control mice. Tests of cognitive function using a Morris water maze 3.5 months after the initial inoculation showed that the mice receiving HIV-infected monocytes performed more poorly than the controls and that the inoculated mice were less active than the controls, suggesting a motor slowing, perhaps similar to the type seen in patients with HAD. Pathological evaluation of the brains also revealed significant astrogliosis and the presence of more activated (MHC class II-positive) MGCs in the inoculated mice (3).

SCID mice inoculated with human monocytes infected in vitro with HIV offer an excellent model of HIVE. They develop pathology that is similar to that seen in humans with HIVE, including MGCs expressing p24 antigen, astrogliosis, increased cytokine expression and signs of activation of macrophages, neuronal injury, and behavioral deficits. An example of the type of experiments that can be undertaken using this model was reported by Limoges et al. (62).

Transgenic murine models have also shed some light on the pathogenesis of HIV (reviewed in reference 54). Various models have been constructed that express the complete HIV genome in neurons (103), gp120 in astrocytes (104), and *tat* (109) and *nef* (102) in T cells. These models have utility in that they have shown us how viral proteins may disrupt normal cellular functions. Indeed, these transgenic models have produced conditions similar to those seen in AIDS patients, such as the astrogliosis and neuronal loss observed in the gp120 mouse model (104) and a decline in $CD4^+$ T cells in the *nef* model (102). However, results of experiments using transgenic mice must be viewed cautiously for two reasons. First, HIV does not replicate in murine cells, and second, due to lack of gene specific transcriptional promoters, it is sometimes not possible to target expression of the transgene to the cell type in which it is usually expressed. Although astrogliosis and neuronal loss were observed in the gp120 transgenic mouse (104), astrocytes were the major source of the gp120, and astrocytes are not a site of active viral production in the human CNS. The end point, astrocytosis, and neuronal loss are the same, but we cannot say for certain that the mechanism is the same as that in humans.

Rat models. Rodent cells are not productively infected by HIV. However, rodent cell lines have been used to demonstrate the toxicity of HIV proteins such as gp120 and Tat (see reference 80 for a review). In vivo models of neurotoxicity of various HIV proteins have also been developed using rats. Rats either were injected with a single dose of protein being studied or were chronically cannulated and injected with multiple doses of protein.

Barks et al. (7) used a neonatal rat model to demonstrate a synergism between an HIV-derived peptide and *N*-methyl-D-aspartate (NMDA), an excitatory amino acid. This work used a synthetic HIV peptide, Env-Gag, consisting of an 80-amino-acid portion of gp41 (positions 560 to 639) and a 190-amino-acid fragment of p24 (position 87 to 276). This peptide had previously been shown to posses potent immunoregulatory activity in vitro (78). Stereotaxic injection of either Env-Gag or NMDA into the dorsal hippocampus of rats on postnatal day (PND) 7 produced little pathology when the brains from treated animals were assessed on PND 12. However, coinjec-

tion of 100 ng of Env-Gag along with 5 nM NMDA produced significantly more injury than injection of either compound alone. Severe injury was typified by hippocampal cell loss and atrophy and loss of cells in the CA3 region of the hippocampus. The animals with the most severe injury were those that had received the highest doses of NMDA plus Env-Gag (5 nM NMDA and 100 ng of Env-Gag) (7). More recently Barks et al. (8) showed that coinjection of gp120 along with NMDA into perinatal rats results in injury more severe than that seen with either of these agents alone. The increase in severity was blocked by injection of an NMDA antagonist, supporting the hypothesis that the neurotoxic effects of gp120 are at least partially mediated by NMDA receptor activation.

In a study of gp120-induced neuronal damage and developmental retardation, Hill et al. (45) demonstrated that systemic injection of gp120 (5 ng) from birth until PND 28 resulted in neuronal dystrophy of cortical neurons, including reduced branching of the neurons and reduced length of the dendrites. They also demonstrated that gp120 reaches the brain of the neonatal rat after systemic injection. Finally, they demonstrated that daily injection of 5 ng of gp120 from birth until PND 14 delayed several different complex developmental behaviors in the rat pups. Previous work by this group (39) had shown that administration of gp120 impaired the ability of rats to learn and remember as judged by performance in the Morris water maze.

Recently, Bansal et al. (6) investigated the ability of gp120 and Tat to cause striatal toxicity in the rat. Using a sterotaxic device, they injected doses of gp120, Tat, gp120 plus Tat, or saline into the striata of adult rats. Brain sections were evaluated for tissue loss and GFAP immunoreactivity 7 days later. Doses of 250 ng of gp120 per μl or higher and doses of 5 μg of Tat per μl or higher caused cell loss and increased GFAP immunoreactivity compared to the saline control. Combined injection of 100 ng of gp120 per μl and 1 μg of Tat per μl Tat also caused tissue loss, morphological changes, and increased GFAP immunoreactivity.

The studies described above and a number of others have used the rat model to investigate the neurotoxicities of various HIV proteins. While bearing in mind that rats do not naturally support productive HIV infection, it is obvious that this model has utility for investigating the neurotoxic effects of HIV proteins

Nonhuman primate models. Like HIV, simian immunodeficiency virus (SIV) is a lentiviral member of the *Retroviridae*. Both HIV and SIV infect cells of the macrophage/monocyte and lymphocyte lineages. They both gain entry into cells through the $CD4^+$ receptor and the receptor for the CC chemokines, CCR5 (20). Unlike some HIV strains, SIV does not use the CXCR4 chemokine receptor (27). HIV-1 and SIV are similar in both genomic organization and nucleic acid sequence (22). In its natural hosts, which include African green monkeys, sooty mangabeys, and mandrills, SIV causes an asymptomatic infection. However, when SIV is introduced into rhesus monkeys or macaques, an AIDS-like disease results (60). SIV-infected animals follow the same pattern of disease progression, albeit at an accelerated pace, as their HIV-infected human counterparts. An initial burst of viral replication, resulting in high viral loads in the blood, peaks by about 4 weeks. The drop in viral load coincides with the rise in both humoral and cellular immune responses (99). This is followed by a latency period (6 months to 2 years), which progresses to failure of immune function, opportunistic infections, and death. Infected animals show a depletion of $CD4^+$ lymphocytes that correlates with the progression of disease, neoplasias (typically B-cell lymphomas), lymphadenopathy, wasting, and various secondary infections, including cryptosporidiosis, candidiasis, and mycobacterial infections (58). A feature of simian immunodeficiency not seen in its human counterpart is disseminated giant cell disease. Lackner et al. (58) reported the presence of MGCs and foamy macrophages in the brains, eyes, lungs, gastrointestinal tracts, lymph nodes, and spleens of SIV-infected simians. As noted above, in humans, giant cells are found in the brains of about 50% of patients with HAD (38).

Neurologically, SIV-infected animals display features like those of HIV-infected humans. Infiltrates consisting of macrophages/monocytes, containing viral antigens, nucleic acid, and viral particles, are frequently seen in the white matter (57), as are MGCs, microglial nodules, and astrocytosis, with about 50% of terminally ill macaques displaying these lesions (9, 58). MGCs are more common in SIV-infected macaques, and, unlike HIV-infected humans, macaques do not display vacuolar myelopathy in the thoracic spinal cord (for a review see reference 110). Apoptosis of endothelial cells, perivascular inflammatory cells, and, most importantly, neurons is also seen in SIV-infected macaques with encephalitis (2). Neuronal loss and/or damage has been reported recently by two other groups. Tracey et al. (105), using proton magnetic resonance spectroscopy, reported that SIV-infected macaques have significantly less *N*-acetylaspartate (NAA), a neuronal marker, than uninfected controls. The loss of NAA correlated with histological analyses in that the animals with encephalitis or a mild inflammatory meningoencephalitis had significantly reduced NAA levels relative to control animals. Berman et al. (13), using the optical fractionator method, report that SIV-infected macaques had neuronal loss of up to 28% from the P layer of the lateral geniculate nucleus and that this loss correlated with neuropathological changes. The observed neuronal loss was most severe in animals with encephalitis in the subcortical white matter and brain stem, and loss was less severe in animals with mild encephalitis. Luthert et al. (64) had previously reported atrophy of hippocampal neurons in SIV-infected macaques. Neuronal loss in the cortex like that seen in HIV-infected humans (52) has not yet been reported for SIV-infected macaques. Interestingly, Tracey et al. (105) also found that of the nine chronically infected animals, two had NAA values similar to those of controls, and Berman et al. (13) reported that two macaques with changes in VEP had neuronal losses of 24 and 26%, while a third macaque with 28% neuronal loss showed a normal VEP. These observations suggest that the presence of CNS lesions does not necessarily correlate well with neurological impairment. Interestingly, neuronal damage with no obvious neurological impairment is also found in HIV-infected humans (28, 63, 77, 98).

Like HIV-infected humans, SIV-infected macaques develop cognitive and motor deficits during the disease (77, 98). Rausch et al. (98) reported on the cognitive and motor impairments of a group of monkeys infected with SIV. Prior to infection, the monkeys had been trained on a battery of tasks designed to detect impairments similar to those reported for

HIV-infected humans with HAD. At 10 months postinfection, seven of eight monkeys showed impairment relative to uninfected controls on a motor task, and three of eight monkeys were impaired on cognitive tasks. Interestingly, animals impaired on one task often performed well on another, suggesting that the poor performance was not simply a result of lethargy or poor health. The decline of the CD4/CD8 ratio was also unrelated to neurological impairment, as was an elevated plasma viral load. That the SIV model shows the same lack of correlation of neurological impairment and neuropathological findings seen in humans underscores the great utility of the model in investigating this puzzling facet of HIV infection.

Distinct molecular clones of SIV have been isolated and used to investigate the determinants of neurovirulence. Various groups have investigated the sequences in the *env* gene necessary for conferring dual tropism on a particular SIV strain (34, 59, 69, 75). Much work has also been done using molecular clones on the role played by the *nef* gene in immunopathogenesis (34, 51). All of these studies indicate that there are determinants of neurovirulence in regions of both *env* and *nef*. Additional studies comparing neurovirulent versus nonneurovirulent strains of SIV, and also comparing these SIV strains to various HIV strains, will shed more light on this important unsettled issue. These studies into the molecular determinants of neurovirulence also underscore the importance of using paired in vitro and in vivo studies. The ability to engineer SIV at the molecular level and observe the effect of the changes on the nature of the infection is very informative. Equally important is the ability afforded by this system to isolate virus from macaques experiencing neuropathological problems and observe the effect of the that strain of virus on culture systems of simian cells of various types.

Another advantage of the SIV model is the ability to investigate the specific pattern of disease progression in these animals. By euthanizing animals at specific times after infection or after the development of a particular neurological impairment, it is possible to correlate changes in brain chemistry and architecture with specific behavioral deficits. The possible confounding effects of various antiviral and palliative medications are also avoided in this system (see references 28 and 110 for discussions).

Despite having many obvious advantages, the SIV system is not the ideal system for investigating HIV in the CNS. Besides the ethical issues involved in research with nonhuman primates, monkeys are quite expensive to maintain and not easy to work with. As a result of this, it is difficult to run experiments with the large numbers of subjects needed to achieve reasonable statistical power. Another concern is that SIV infection of laboratory personnel has been reported (53). Although productive disease did not develop, one of the individuals described in that report had a sustained production of antibodies to SIV proteins, while the other showed an initial production of antibodies that waned and eventually disappeared over a 2-year period.

SUMMARY

Each of the model systems outlined in this review has applicability to HIV research. Obviously, some are more useful than others, and refinement of the presently available systems is occurring continually. Further development of these models will enable the research community to broaden our knowledge of the pathogenesis of AIDS encephalopathy. However, effective treatment of this major complication of HIV infection remains elusive. The further development of nonprimate animal systems to assess the efficacy of potential therapies for AIDS encephalopathy is an important goal.

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