[Frontiers in Bioscience 2, d527-537, November 1, 1997]

CELLULAR MECHANISMS OF FELINE IMMUNODEFICIENCY VIRUS (FIV)-INDUCED NEUROPATHOGENESIS

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TABLE OF CONTENTS

1. ABSTRACT

The high incidence of neurologic dysfunction from human immunodeficiency virus (HIV) infection has heightened interest in neuropathogenesis of other lentiviruses, including that associated with feline immunodeficiency virus (FIV). Both HIV and FIV efficiently enter the central nervous system and cause primary neurological disease that is not attributable to opportunistic infections or systemic disease. Cells in the brain infected by FIV are similar to those observed in HIV infection, both viruses infect macrophages, microglia, and astrocytes. Although substantial neuronal loss can occur in the cortex of HIV- or FIV-infected patients, most studies agree that neurons are not infected and indirect mechanisms of neurotoxicity are postulated. This review describes recent information on the neuropathogenesis of FIV and how this information correlates with what is known about the neuropathogenesis of HIV. Although the pathogenesis of neurological dysfunction in HIV- and FIV-infected patients is far from clear, it is becoming increasingly evident that the relationship between lentivirus presence in the brain and neurological signs is not straightforward and cannot be explained by simple cytolytic infection. The observed neurologic dysfunction is likely multifactorial and complex involving an intricate web of subcellular pathways and neurotoxic factors interacting with multiple cell types.

2. INTRODUCTION

Among the clinically notable and biologically intriguing aspects of lentiviruses are their effects on the central nervous system (CNS). In addition to opportunistic CNS infections and neoplasms associated with immunodeficiency, there is also a unique syndrome of neurologic impairment which appears to result from a more fundamental effect of the lentivirus. The lentiviruses feline immunodeficiency virus (FIV) and human immunodeficiency virus (HIV) are clinically relevant neuropathogens. The syndrome of neurologic dysfunction associated with HIV infection that includes cognitive and motor impairment in both adults and children has been termed acquired immunodeficiency syndrome (AIDS) dementia complex, HIV-1 encephalopathy, or simply NeuroAIDS.

The high incidence of neurologic dysfunction from HIV infection has heightened interest in neuropathogenesis associated with feline retrovirus infections. Feline immunodeficiency virus causes a disease syndrome in cats remarkably similar to that associated with HIV infection in people (1, 2, 3) and is therefore not only an important feline pathogen but also an invaluable small animal model of HIV. Although there has been a surge of new information during the last several years, many questions regarding lentivirus-associated neuropathogenesis still exist. Despite nearly a decade of research, the CNS target cells and alterations responsible for neurologic dysfunction from FIV are yet to be definitively identified. This review highlights major research findings in the area of cellular mechanisms of FIV-mediated neuropathogenesis.

Although not recognized as frequently as in HIV-infected people, a wide range of neurologic deficits has been described in FIV-infected cats and neurologic disease represents an important sequela to naturally- and experimentally-acquired FIV-infection. The nature of FIV-associated neurologic abnormalities tends to be behavioral rather than motor in nature. Reported clinical behavioral abnormalities in adult cats encompass subtle to overt changes including docility, agitation, confusion, loss of

Received 10/7/97 Accepted 10/23/97
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litterbox training, dementia or psychotic behavior (hiding, rage, excessive aggression), and compulsive (i.e., repetitive, purposeless) motor movement or roaming (4, 5). Specific neurologic defects have included anisocoria, nystagmus, delayed pupillary reflex, ataxia, paresis, paralysis, delayed or absent righting and other abnormal postural reflexes, intention tremors, and focal or generalized seizures (5, 6).

The seemingly lower incidence of neurologic manifestations of FIV compared to HIV may be a real difference in disease expression between these viruses or may simply represent a decreased ability to detect subtle behavioral or functional alterations in cats. Although a relatively insensitive diagnostic indicator, magnetic resonance imaging reveals subtle but distinct abnormalities in some FIV-infected cats including ventricular enlargement and focal white-matter lesions. A significant decrease in N-acetylaspartate, a marker of neuronal dysfunction has been detected using proton magnetic resonance spectroscopy (MRS) in FIV-infected cats (7). Neuroelectrodiagnostic evaluation has proven to be a sensitive means of detecting neurologic dysfunction in both overtly affected as well as seemingly asymptomatic FIV-infected cats as early as 3 months postinfection (6, 8, 9, 10, 11). Delayed visual evoked potentials (VEP) and brain stem auditory evoked potentials (BAEP), decreased retinocortical times, and decreased evoked spinal potentials suggesting a demyelinating process and/or selective fiber dropout have been detected in many FIV-infected cats. Abnormalities detected by electroencephalography (EEG) range from asymmetrical or diffuse high-amplitude activity to marked alterations in sleep patterns with predominant slow-wave activity. The EEG findings correlate with abnormal behavior in some cats and parallel findings reported in HIV-infected patients.

Although the majority of FIV-infected cats do not manifest clinically observable neurologic dysfunction, a much higher proportion have microscopic CNS lesions. However, as occurs with HIV as well, often there is poor correlation between the histopathologic lesions and detected clinical neurologic abnormalities. Experimental infection with the Petaluma (FIV-Pet) or Pisa-M2 (FIV-Pisa-M2) consistently causes moderate to pronounced gliosis of both gray and white matter, vacuolar myelopathy and inflammatory cell infiltration in the CNS (4, 12, 13). However, these lesions are not associated with clinical disease. In contrast, experimental infection with the phylogenetically distantly related Maryland strain of FIV (FIV-MD) causes rapid onset of neurological signs but only mild CNS lesions (6, 14). Importantly, FIV can be isolated from areas virtually devoid of histologic lesions and extent of virus distribution seems to correlate well with severity of neurologic dysfunction. With neurovirulent strains of FIV, there is significant loss of cortical neurons and evidence of compensatory increases of synaptic terminal densities suggesting that neurodegeneration begins after the early viremia during the asymptomatic stage of disease (9, 15). Compensatory changes likely mask slow and progressive loss of neurons which only becomes clinically significant during AIDS or when there are other factors which accelerate the neurodegenerative process.

It is widely accepted that HIV and FIV gain access to the brain via migration of infected hematogenous cells. However, definitive proof is yet to be brought forward demonstrating this phenomenon. The potentiality of free FIV, instead of or in addition to cell-associated virus, entering the CNS has been suggested by results of studies demonstrating disrupted blood-brain barrier (BBB) integrity during acute FIV infection (7, 9). Increased BBB permeability is correlated temporally with peak viremia and CD4/CD8 ratio inversions (7).

In vivo and *in vitro* studies suggest that neuroglia, the support cells of the CNS, rather than neurons are the principle cellular targets for lentiviruses. Although neurons are for all intents not infected, a substantial degree of neuronal loss can occur in the cortex (16, 17) and indirect viral effects are presumably responsible for clinical and pathological findings (18, 19, 20, 21). The observed neurologic dysfunction likely involves an intricate web of subcellular pathways and neurotoxic factors affecting neurons but produced by infected neuroglia, specifically microglia and astroglia.

3. A ROLE FOR MICROGLIA AND/OR MACROPHAGES?

Microglia, macrophages, and the derivative multinucleated cells have been identified as the major HIV expressing cells in the brain. However, the number of these cells is small relative to the widespread neuropathology. There have been few investigations of effects of FIV on microglia. *In vitro* FIV infection of microglia is relatively noncytopathic. FIV does not induce a significant release of inflammatory cytokines tumor necrosis factor-alpha (TNF-alpha), intereukin 1 (IL1), or IL 6 from infected microglia, although increased major histocompatibility complex (MHC) class II expression has been demonstrated (personal communication, S. W. Dow, University of Colorado Health Sciences Center).

These findings parallel the results of experiments examining similar parameters in HIV infected microglia. Interferon was not examined and cannot be ruled out as a contributor to the neurotoxicologic process because of its importance in viral infections and MHC class II expression. The neurotoxicologic contribution of upregulated MHC class II in the brain, outside upregulation of the immune response, is uncertain. Although a purported requirement for neurotropic lentiviruses, macrophage tropism is frequently determined to be lacking for FIV isolates from brain tissue (personal communication, T.W. Vahlenkamp, Utrecht University). Infections with the FIV-PPR molecular clone or a specific FIV-PPR mutant which has decreased macrophage tropism (DU-FIV-PPR) produce similar neurologic dysfunctions (8) and similar neuropathological profiles are observed in FIV-infected cats treated with a drug delivery system that prevents viral replication in macrophages (22). Despite widespread belief in the importance of macrophages and/or microglia in

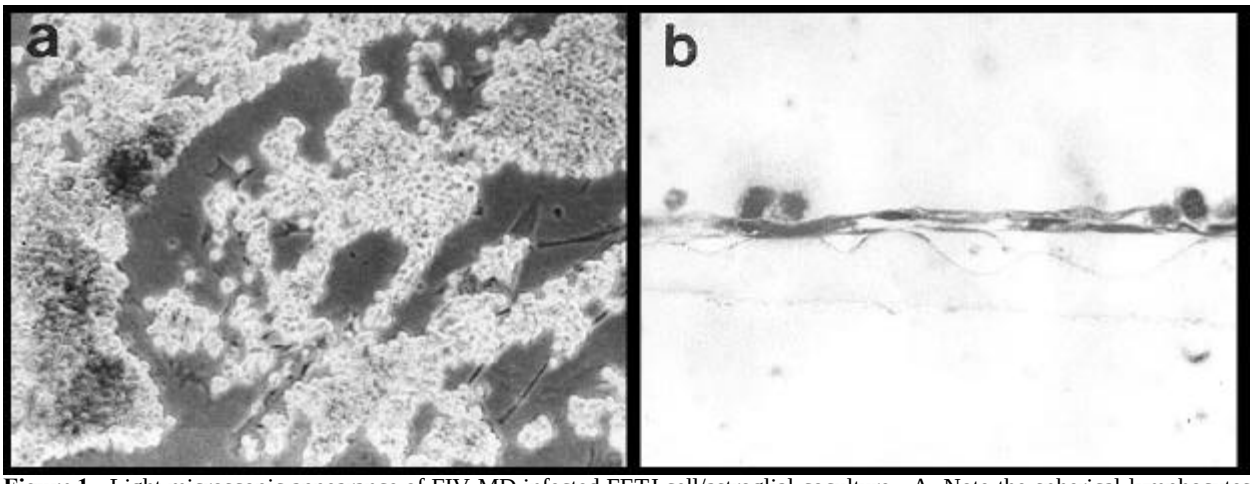

Figure 1. Light microscopic appearance of FIV-MD infected FETJ cell/astroglial coculture. A. Note the spherical lymphocytes specifically attached to the barely visible nonconfluent monolayer of feline astroglial cells but not to the plastic of the tissue culture flask. (x250). B. Cross-section of FIV-MD-infected FETJ cell/astroglial coculture fixed in paraformaldehyde. (x500).

lentivirus-associated neuropathogenesis, there are likely to be other players of at least as much significance (19).

4. A ROLE FOR ASTROGLIA?

The cellular basis for the neurobehavioral deficits associated with HIV infection have not been identified. Because of the paradox between the relatively small number of productively infected glia and the magnitude of clinical dysfunction (23, 24), indirect mechanisms of neurological impairment are suspected. Astroglia, which are interposed between neuronal cells and brain capillaries, with astroglial foot plates ensheathing these capillaries (25), are the most numerous cells in the brain and are essential for maintenance of normal neuronal function. Astrocytes play immunologic roles by secreting cytokines and serving as facultative antigen presenting cells and maintain the extracellular fluid homeostasis by regulating levels of certain electrolytes and neurotransmitters. Because of their unique relationship with cerebral blood vessels and neurons, and importance in the maintenance of the CNS environment, astrocytes are likely to play a very strategic role in the progression of neurological disease in lentiviral-infected patients. Although *in vivo* productive infection of astroglia seems to be rare, extensive persistent restricted and latent HIV infection occurs in astroglia and can be reactivated under certain conditions including cytokine activation (26, 27, 28). Elucidation of functional impairment of cells essential to neuronal function, such as astroglia, may provide insight into how low numbers of productively infected cells can elicit progressive and devastating neurological impairment.

4.1. Lymphocyte facilitated infection of astroglia by neurovirulent FIV

Recent studies have demonstrated that persistent astroglial infection by HIV *in vivo* may be extensive (28, 29), supporting early reports of the presence of viral particles in astrocytes (21, 30, 31). Because astroglial foot-processes surround the majority of the vascular surfaces in the mammalian brain (25) and widespread infection of astroglia may contribute to the neurologic deficit (28), the hypothesis that astroglia are the initial sites of lentiviral replication and source of infectious viral particles for other brain cells is compelling. Primary feline astroglia and the feline G355-5 astroglial cell line are readily infected with cell-free stocks of FIV-Pet but not with cell-free stocks of FIV-MD. Whether feline T-cells infected with FIV-MD might transmit the virus more effectively than exposure to cell-free virus was investigated (32). Astroglial infection is very efficient by this method, with virtually 100% of the astroglia demonstrating positive staining for FIV by immunofluorescence assay (IFA) after coculture. The importance of cell-cell transmission of HIV is well established (33, 34, 35, 36, 37). Recently, a situation similar to that we described was reported with lymphocytotropic but not monocytotropic strains of HIV (34). In this section, we describe our recent findings concerning a mechanism of FIV infection of astroglia that requires lymphocyte-facilitation.

To facilitate infection of feline astroglia with FIV-MD, FETJ cells, a feline IL-2 independent T cell line were first infected and then added to established astroglial cell cultures. FETJ cells infected with FIV-MD rapidly and specifically adhered to the astroglial cells (figure 1). Evaluation of reverse transcriptase and p26 in supernatants at intervals ranging from 20 minutes to 24 hours immediately following coculture with astroglial cells revealed no increase in FIV protein release from FIV-MD-infected FETJ cells suggesting direct viral transfer or a release of virions into the confined space between contacting cells. Further, FIV-MD infection of astroglia was non-productive, a situation that may be analogous to that observed for nonproductive infection of glial cells by HIV *in vitro* (26, 27, 28, 38) and *in vivo* (26, 29). Maryland strain FIV-infected FETJ cells in contact with astroglial cells demonstrated striking microvillus formation close to

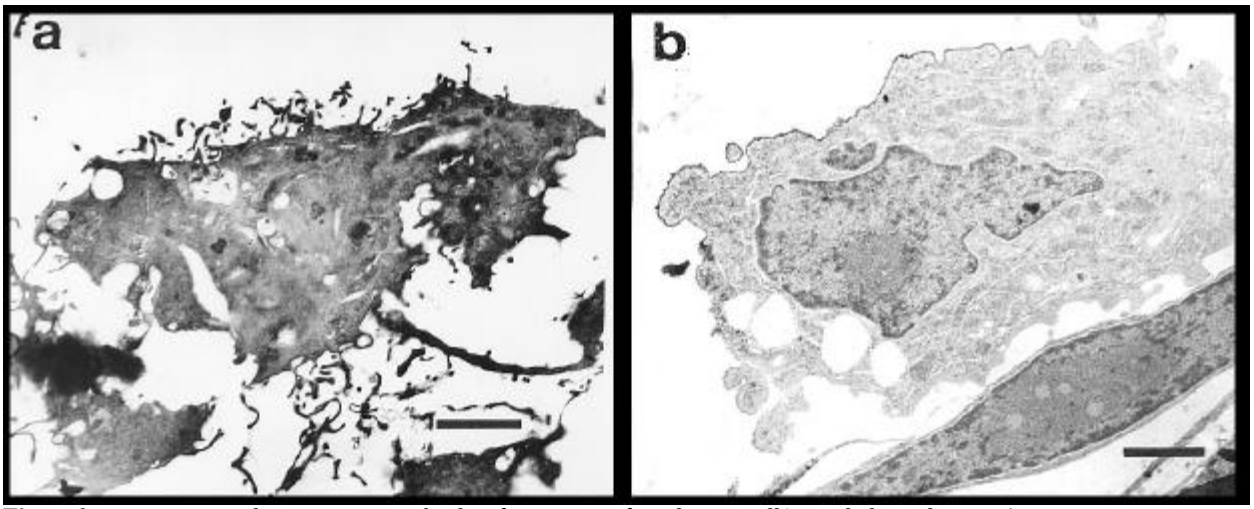

Figure 2. Transmission electron micrographaphs of FIV-MD-infected FETJ cell/astroglial cocultures. A. Transverse section cut though an FIV-MD-infected FETJ cell at a level close to its association with the astroglial monolayer. Note the striking microvillus formation at this level. B. Cross-section through both cell types showing an FIV-MD-infected FETJ cell (top) tightly attached to the surface of an astroglial cell (lower right). Bar = 2 µm.

the level of contact with the astroglial cell monolayer (figure 2) but not at levels opposite this area.

In the absence of reagents that recognize feline adhesion molecules, adherence assays were performed to investigate the mechanism of cell-cell adhesion by evaluating the degree of cell-cell interaction between uninfected and FIV-infected FETJ cells with other cells. For comparison of adhesion to feline astroglial G355-5 cells with adhesion to other cell types, similar experiments were performed with cocultures of FIV-MD-infected or uninfected FETJ cells with primary feline astroglial cells, primary astroglial cells from rats, CRFK, C-6, and SY5Y cell lines. Adherence of uninfected or FIV-MD infected feline PBMC and FIV-Pet-infected FETJ cells to G355-5 cells was also examined. Despite similar levels of infection, feline leukocytes infected with the neurovirulent Maryland strain, but not the non-neurovirulent Petaluma strain, adhered tightly and specifically to astroglial cells of feline origin but not to neural cells from other species nor non-astroglial feline cells, suggesting specific cell-cell interaction between these FIV-MD-infected FETJ cells and astroglia. In contrast, uninfected and FIV-Pet infected FETJ cells adhered only loosely, if at all. Results of experiments with primary cells (primary feline astroglia or primary feline lymphocytes) were similar to those with established cell lines. The role of specific proteins expressed on the cell surface or free in the cell culture medium of FIV-MD-infected lymphocytes in the observed interaction with astroglia was investigated by preincubation of FIV-MD-infected FETJ cells with specific polyclonal or monoclonal antibodies. Although the anti-FIV antibodies used are neutralizing for FIV-MD in other systems (e.g., infection of feline primary lymphocytes), preincubation of FIV-MD-infected lymphocytes with these antibodies did not block adhesion in the described experiments. Similarly, preincubation with antibodies to CD18 or CD69 did not prevent adhesion.

The BBB is a highly selective barrier whose structure and control is intricately linked to astrocytes (39, 40). The presence of this selective barrier between the blood and the neuropil that impedes the passive diffusion of solutes and yet allows the entry of selected substances and cells necessitates a specific transport mechanism for neurotropic viruses entering the CNS. Little is know about lymphocyte/astroglial interaction *in vivo* although it has been demonstrated that the CNS is continuously patrolled by small numbers of T cells and monocytes (41). These hematogenous cells may serve as a source of infectious virions for resident glia. Our findings are consistent with this generally accepted belief. We have demonstrated that infection of astrocytes via FIV-MD-infected lymphoblastoid cells is much more efficient than by free virus, a finding perhaps more meaningful in terms of viral pathogenesis than the specific cell-cell adhesion we reported (32). Immune surveillance mechanisms may be evaded by FIV-MD by direct transmission of virus across the confined space between infected lymphocytes and astroglia. Although macrophage tropism has long been a purported requirement for tissue dissemination of lentiviruses (18, 42, 43, 44, 45, 46, 47, 48), recent studies contradict this widely held belief (38, 49). Our findings and those recently reported by others necessitate investigation into the role of lymphocytes in CNS infection by neurotropic lentiviruses. Although the specific mechanism of cell-cell interaction and viral transmission is yet to be elucidated, our observations may offer insight into the cellular sequence of events which may occur during transmission of FIV from blood to brain tissue and may further the understanding of FIV- and HIV-associated neuropathogenesis.

4.2. FIV-associated cytotoxicity and neurotoxicity

Astroglial functions in the maintenance of CNS homeostasis include regulation of ion concentrations, uptake and metabolism of certain neurotransmitters such as the excitotoxic amino acid glutamate, and development and

maintenance of the BBB. Although *in vivo* productive infection of astroglia seems to be rare, nonproductive infection of astroglial cells may play a major role in the development and progression of lentiviral-associated neurologic dysfunction. Prominent nonexclusive hypotheses to explain possible indirect mechanisms of retrovirus-associated, neuroglial cell-mediated neuronal dysfunction include direct toxicity of viral proteins, excitotoxicity from accumulation or potentiation of excitatory neurotransmitters such as glutamate, and immunologically-mediated damage from toxic products such as TNF-alpha, nitric oxide, or free radicals (23, 27, 50).

4.2.1. Laser cytometric analysis of FIV-associated cytotoxicity

The importance of astrocyte functions in neuronal homeostasis suggests that virally-induced changes in astroglia might ultimately impair neuronal function. This postulate, if proven to be true, may have direct relevance to neuropathology of lentiviral-infection in cats and humans. In consideration of this hypothesis, we performed experiments to investigate the mechanism of FIV-induced alterations in primary feline astroglial cell cultures via vital fluorescence bioassays which examine specific indicators of cellular dysfunction. The indicators of cellular dysfunction investigated and found to be abnormal in FIV-Pet-infected astroglia are mitochondrial membrane potentials, cell-cell communication via gap junctions, calcium homeostasis, plasma membrane fluidity, and intracellular glutathione concentration (3, 51).

Envelope proteins of HIV and FIV have been implicated in causing cytotoxicity and cytopathic effects (CPE) (52, 53, 54, 55, 56). Interactions between the host cell and viral proteins are postulated to cause changes in cellular membranes, interfering with normal homeostasis (57, 58). Interestingly, the primary toxic effects of a number of more classical toxins such as mycotoxins, heavy metals, and polychlorinated biphenyls (PCBs) also appear to involve cellular membranes (59, 60, 61, 62). It seems that although cellular insults are diverse, the ways that a cell can react to injury are limited (61). Direct lipid peroxidation has been implicated in cytotoxicity due to many cellular insults. Alternatively, cellular thiols may be depleted directly, sensitizing the cell to oxidative stress. In either instance, altered membrane structure may lead to release of calcium from internal stores, activation of membrane phospholipases, altered membrane permeability barriers, depletion of mitochondrial membrane potential and cellular ATP, and a decrease of cell-cell communication. Results of cytotoxicity assays suggest that membrane changes may occur very early following exposure to FIV, prior to detectable virus production, and that there are alterations of vital cellular constituents and functions associated with established persistent infection when high titers of FIV-Pet are produced. Petaluma strain FIV is cytotoxic based on all parameters tested. Interestingly, although exposure of astroglia to non-infectious cell-free neurovirulent FIV-MD causes evidence of cytotoxicity, infection per se does not. An *in vivo* relationship may exist when considering that there is CNS inflammation following FIV-Pet but not with FIV-MD. With FIV-Pet, astroglia may be cytopathically infected and die causing microfoci of inflammation and gliosis. If FIV-MD is not cytopathic, no inflammation would be expected. Although not conclusive, this correlation is at least consistent with the paradoxical findings of inversely related lesions and severity of clinical neurologic dysfunction. The next section discusses a functional abnormality that strengthens the evidence for this scenario.

4.2.2. Excitotoxicity in the neuropathogenesis of FIV

An excess of glutamate can be toxic to neurons. Under normal conditions, the post synaptic action of glutamate is quickly terminated by its rapid uptake by neurons and astrocytes surrounding the synaptic cleft. Glial glutamate transporters provide the majority of this uptake. Astroglial play a critical role in maintaining low extracellular glutamate and controlling the balance between physiologic excitatory transmission and excitotoxicity (63). In particular, overstimulation of NMDA receptors by glutamate leads to a series of neurotoxic events including excessive influx of calcium, generation of nitric oxide, superoxide anion, and peroxynitrite (64, 65). Glutamate also competitively inhibits neuronal uptake of cysteine, which is the limiting precursor for glutathione (66), rendering neurons more vulnerable to oxidative injury. Infusion of glutamate into the mesopontine area reduces REM sleep in cats similar to FIV-associated sleep alteration mentioned in section 2 (10). Exposure to FIV enhances sensitivity to the excitotoxic effects of glutamate in primary feline mixed neuronal-glial cultures (67). Whether this effect results from impaired glutamate uptake by astroglia or a synergistic effect of the virus and the excitatory amino acid is uncertain and may actually be a combination of both. In this section results of experiments demonstrating impaired astroglial uptake of glutamate following exposure to FIV are described. A mechanism of neurotoxicity whereby FIV infection may promote an increase in extracellular glutamate within the brain, thereby leading to neuronal excitotoxicity and increased neuronal sensitivity to oxidative stress is suggested.

To study the effects of FIV on glutamate uptake, astroglia were either exposed to cell-free virus stocks of FIV-Pet or FIV-MD for a short time period (24 hours), or infected and analyzed after persistent infection was established. Glutamate uptake into feline astroglia, both primary and the G355-5 cell line, was linear over the uptake interval studied. Because of the greater variability of response and the more heterogeneous nature of the primary cells, the G355-5 established cell line was used for most of these studies. Figure 3 demonstrates the extremely significant ($p< 0.0001$) inhibition of glutamate uptake by FIV-Pet within 1 day of infection compared to uninfected G355-5 cells. Effects of one day of exposure to FIV-MD are also shown in figure 3. Although there is also a statistically significant ($p=0.013$) decrease in glutamate uptake in G355-5 cells exposed to FIV-MD, the drop is not nearly as profound as that observed with FIV-Pet. Figure 4 illustrates the persistent defect in glutamate uptake in FIV-Pet-infected G355-5 cells through 10 days of observation postinfection.

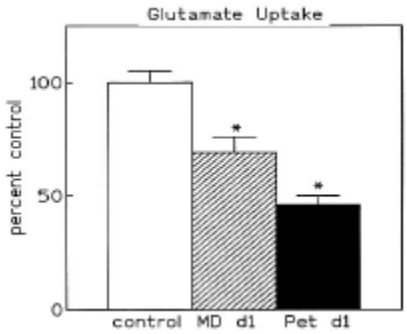

Figure 3. The effect of one day of exposure to FIV-MD or FIV-Pet on uptake of L-[G-^{3}H]glutamate by feline G355-5 glial cells. The bars are the mean uptake at 5 minutes ± S.D. for each group. *Significantly different from control: control differs from FIV-MD exposed (p=0.013) and FIV-Pet exposed (p=0.004), and FIV-MD exposed differs from FIV-Pet exposed (p=0.0107).

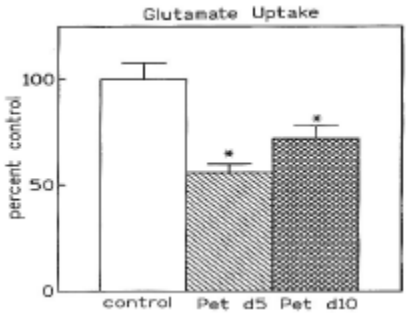

Figure 4. The effect of FIV-Pet infection on uptake of L-[G-3H]glutamate by feline G355-5 glial cells. The bars are the mean uptake at 5 minutes ± S.D. for each group. *Significantly different from control: control differs from FIV-PET day 5 (p=0.0001) and day 10 (p=0.01) and FIV-Pet infected day 5 differs marginally from FIV-PET day 10 (p=0.0888).

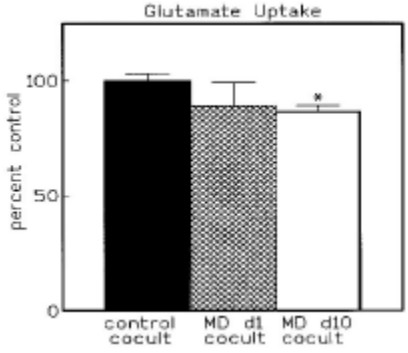

Figure 5. The effect of FIV-MD-infection on uptake of L-[G-3H]glutamate by feline G355-5 glial cells. "Control cocult" represents uptake by G355-5 cells cocultured with uninfected FETJ cells as per the procedure described for infection of G355-5 cells with FIV-MD by coculture FIV-MD-infected FETJ cells. The bars are the mean uptake at 5 minutes ± S.D. for each group. *Significantly different from control: control does not differ from day 1 but differs significantly from day 10 (p=0.002).

To study glutamate uptake in G355-5 cells infected with, rather than just exposed to, FIV-MD, coculture FIV-MD infected G355-5 cells were prepared as described above. Additionally, G355-5 cells were mock infected by coculture with uninfected FETJ cells and results of glutamate uptake were compared between untreated G355-5 cells, G355-5 cells mock infected via coculture with uninfected FETJ cells, and G355-5 cells infected via coculture with FIV-MD-infected FETJ cells. Infection of G355-5 cells with FIV-MD via coculture (as opposed to exposure to free virus) did not cause a significant decrease in glutamate uptake early (day one postinfection), but did cause a statistically significant decrease (p=0.0085) later in infection (day 10 postinfection, see figure 5). Although statistically significantly different, the glutamate uptake of cells infected with FIV-MD was still approximately 85% of control levels compared to a drop of as much as 55% for FIV-Pet infection.

The mechanism by which FIV induces neurologic disease remains poorly understood but is believed to involve, at least in part, dysregulation of the intricate balance of intercellular signals between glia and neurons. The data presented herein indicate that FIV is able, at least *in vitro*, to alter the glutamate cycle. Astroglia infected by FIV have impaired glutamate uptake capabilities but the less neurovirulent FIV-Pet strain causes more profound effects than the more neurovirulent FIV-MD strain. Excitotoxicity associated with glutamate has generally been induced by high levels of glutamate but it has recently been demonstrated that even modest increases in extracellular glutamate can have deleterious effects (68). Therefore, it is very likely that the subtle decrease in glutamate uptake in FIV-MD-infected astroglia may relate to neuropathogenesis.

The mode of neuronal cell death after damage initiated by excitotoxins has been controversial, with some groups finding features of necrosis and others reporting characteristics of apoptosis. Both these mechanisms of cell death may be initiated by the same factors depending on the initial severity of the insult and the microenvironment present at the time of injury. For example, otherwise non-toxic levels of glutamate may be potentiated by reactive oxygen species (ROS), peroxynitrite, or other toxic substances, potentially including viral envelope proteins. A major difference between the two types of cell death is the generalized involvement of neighboring cells. To prevent leakage of excitatory amino acids, proteolytic enzymes, DNA, and oxidized lipids, apoptotic cells condense their chromatin, shrink, and shield their intracellular milieu. In a sense, apoptosis is "silent" cell death. On the other hand, necrosis is caused by catastrophic injury leading to membrane lysis, release of cellular constituents, and resulting inflammation. Depending on the intensity of the initial insult, excitotoxic insults can result in either apoptotic or necrotic neuronal cell damage (68). Because FIV-MD-infected cats have evidence of neuron death without appreciable inflammation detected, it is possible that a modest decrease in glutamate uptake by noncytopathically infected astroglia leads to mild rather than large increases in extracellular glutamate with subsequent neuronal apoptosis rather than necrosis. Widespread neuronal apoptosis certainly would cause much more profound clinical disease than small focal areas of necrosis and inflammation associated with FIV-Pet.

Analysis of glutamate uptake by FIV-Pet infected astroglia showed that day one infected cells displayed the most dramatic drop in uptake and the uptake seemed to improved over the 10 days of infection. These results parallel the lipid mobility data mentioned in section 4.2.1. and cannot be correlated with the level of extracellular virus. The extracellular fluid at day one contained only one tenth the p26 present at 10 postinfection which contained the highest level of p26. This result might imply that disruption of the cell membrane structure associated with viral entry, ROS generated during infection, or some other process associated with disruption of the plasma membrane integrity is at work rather that a straightforward toxic effect of the virus or it's associated proteins. However, cells exposed to FIV-MD and astroglia infected with FIV-MD for 10 days also display decreased uptake of glutamate. Cell-free FIV-MD is not infectious for astroglia and astroglia infected via coculture as described above do not produce detectable virus in the extracellular fluid so the mechanism of glutamate uptake inhibition is not readily apparent and requires further study.

The consequences of altered glutamate uptake by FIV-infected astroglia are likely important in the understanding of AIDS associated neuropathogenesis. Excitotoxic injury of neurons, either by necrosis or apoptosis, is one possible sequela and is consistent with the reported data about these two strains of FIV. Clinical trials with NMDA antagonists are underway in the hope that blocking the receptors associated with excitotoxicity may abrogate the neurologic manifestations of HIV infection.

5. PERSPECTIVES

The lentiviruses HIV and FIV are able to efficiently enter the CNS and cause primary neurological disease. The characterization of the early stages of CNS infection by HIV, and its progression to the terminal phase, are fundamental in understanding physiopathology, and can only be analyzed in a non-human model. The FIV-infected cat serves as an excellent animal model for human AIDS and AIDS-associated neurological abnormalities. *In vitro* models of viral infection are of great interest as a means of studying possible mechanisms by which lentiviruses may impair neural cells, although these models cannot reproduce all cellular interactions within the CNS *in vivo*. This review describes several *in vitro* experiments designed to investigate the effects of FIV at the cellular level.

Although the exact role of astroglia in the neuropathogenesis of NeuroAIDS is yet to be clearly established, results of experiments described herein suggest that altered astroglial function may likely relate to the neurologic signs observed. For both FIV and HIV infections, there is a paradox between the magnitude of clinical signs observed and the paucity of CNS lesions. The converse situation is also seen. Several authors have commented on the surprising lack of lesions based on the severity of dementia or the lack of dementia based on the severity of lesions with human NeuroAIDS. Study of FIV strains that fit these extremes of clinical expression may contribute to the understanding of this paradox. Differences in neurovirulence observed *in vivo* may relate to specific differences in the mechanism of astroglial infection, viral expression, tendency to cause cytotoxicity, and ability to cause alteration of astroglial function.

Certain viruses which have the ability to persistently infect neural cells spare host cell vital functions but subtly interfere with its ability to perform specialized functions (69). Based on findings discussed, the pattern of astroglial infection by FIV varies with the strain being studied. Petaluma strain FIV, which is associated with significant neuropathology but minimal to no neurologic deficits, is cytotoxic to astroglia. Maryland strain FIV, which is associated with profound neurologic deficits, but minimal neuropathology, does not cause altered cell morphology or detectable cytotoxicity. It is possible that FIV-Pet infection in the CNS is arrested by rapid cell death of infected astroglia and ensuing inflammation, whereas FIV-MD may trigger a process or processes that, once initiated, may lead to disturbances in CNS homeostasis, eventually resulting in disease. One can expect that FIV-MD may thusly perturb complex interactions between astrocytes and neurons.

As information has accrued, it is becoming increasingly evident that the relationship between neurological signs and lentiviral infection of the CNS are not straight forward and cannot be explained by simple cytolytic brain infection. The observed neurologic dysfunction is likely multifactorial and complex, involving an intricate web of subcellular pathways and neurotoxic factors interacting with microglia, astroglia, and neurons. The importance of astroglia in the maintenance of CNS functions suggests that virally induced changes in infected astroglia may be essential in the progression of NeuroAIDS. A substantial degree of neuronal loss can occur in the cortex of HIV- or FIV-infected patients. There are three ways that neurons may die following lentivirus infection of the brain: murder, manslaughter, or suicide. Based on the accumulated evidence, murder is not very likely but one could make the case for manslaughter by substandard excitatory neurotransmitter uptake or suicide in the face of an unfavorable environment.

Among the many unresolved issues about lentivirus-mediated neuropathogenesis is how the lentivirus is able to penetrate an intact BBB. Despite definitive evidence, the most widely held belief is that the HIV and FIV gain access into the brain via infected hematogenous cells. Although the BBB is generally a formidable barrier to cells in circulation, activated lymphocytes readily traffic through the CNS. The cell-cell adhesion described in section 4.1. may be cytokine driven and integrin mediated, although neither of these factors have been adequately investigated because of limited reagent availability. The results of the experiments examining the cell-cell interaction and virus transmission, albeit mostly negative, provide fascinating suggestions to further explore the mechanism of brain infection by neurovirulent lentiviruses.

Astrocytes are crucial to the normal homeostatic regulation of the neuronal microenvironment, in large part

because of their ability to selectively regulate extracellular levels of glutamate (70). Impaired astroglial uptake of glutamate following exposure to FIV suggests a mechanism of neurotoxicity whereby FIV infection may promote an increase in extracellular glutamate within the brain, thereby leading to neuronal excitotoxicity and increased neuronal sensitivity to oxidative stress. There was a difference noted in the severity of the glutamate uptake defect between the two strains of virus studied. Petaluma strain FIV is associated with much more profound drop in glutamate uptake whereas FIV-MD is associated with a subtle drop. The typical morphologic characteristics of excitotoxic injury (i.e., cell swelling) are consistent with a necrotic type of death. However, recent studies have provided evidence that exposure of neurons to relatively short durations or low concentrations of NMDA induces a delayed form of neurotoxicity predominated by apoptotic features (71). Brains from cats infected with FIV-Pet consistently demonstrate microfoci of inflammation and gliosis on postmortem examination (4), a finding that is consistent with the scenario of excessive extracellular glutamate and associated neuronal necrosis. Brains from cats infected with FIV-MD display very subtle postmortem lesions consisting primarily of white matter pallor (6, 9). This lesion is consistent with neuronal death without inflammation, a situation compatible with apoptosis. These proposed scenarios are certainly quite speculative and require much study for confirmation. However, results of these experiments are at least provocative and may provide a thread with which we may begin the unraveling of this obviously very complex mystery.

6. ACKNOWLEDGEMENTS

This work was supported by grants NIAID 1K11AI01221-01A1 and 5RO1AI32360-03 from the National Institutes of Health. We gratefully acknowledge Miles Frey for transmission electron microscopy.

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