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AIDS Dementia and HIV-1-Induced Neurotoxicity: Possible Pathogenic Associations and Mechanisms

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Abstract
AIDS Dementia Complex (ADC) is a syndrome of cognitive, behavioral, and motor deficits resulting from HIV-1 infection within the brain. ADC is characterized by variable degrees of neuronal cell death and gliosis that likely result, at least, in part from release of metabolic products, cytokines, and viral proteins from infected macrophages, although a unifying explanation for the neurological dysfunction has yet to be established. Major unanswered questions include: (i) do neurologic symptoms result from neuronal cell death and/or dysfunction in surviving neurons?; (ii) are viral genomic sequences determinants of neurotoxicity?; (iii) is HIV infection of neurons and astrocytes relevant to pathogenesis?, and (iv) what circulating factors within the brain affect neuronal cell survival and function? This review addresses the association between HIV-1 replication within the brain, production of potential neurotoxins and possible mechanisms of induction of neurotoxicity and neuronal dysfunction contributing to the pathogenesis of ADC.

Central nervous system (CNS) dysfunction in AIDS includes cognitive, motor and behavioral manifestations which in part define a neurological syndrome commonly known as AIDS dementia complex (ADC) or HIV-associated dementia complex [111]. Although much is known about possible mechanisms of neuronal cell death and damage induced by HIV-1 infection within the brain, the pathogenesis of ADC remains controversial. It is clear, however, that invasion and replication of HIV-1 within the CNS is responsible for the development of ADC [129, 204, 207, 232, 242, 287]. This review discusses how observations derived from both in vitro and in vivo model systems have been assembled into theories explaining the pathological and neurological features of ADC.

Clinical Features

The striking feature of ADC which distinguishes its presentation from the dementia of Alzheimer's disease (AD) is the tendency for memory impairment to occur much later in the course, after evidence for 'subcortical' features of inattention, indifference, and psychomotor slowing [156, 166, reviewed in 7]. Patients often present with apathy and inattentiveness, which is often misinterpreted as memory dysfunction. In addition, slowing of motor movements and dyscoordination, with the legs affected earlier and more severely than the arms, often accompanies the mentation changes. Generally, subtle abnormalities of gait and strength may then appear, fol-

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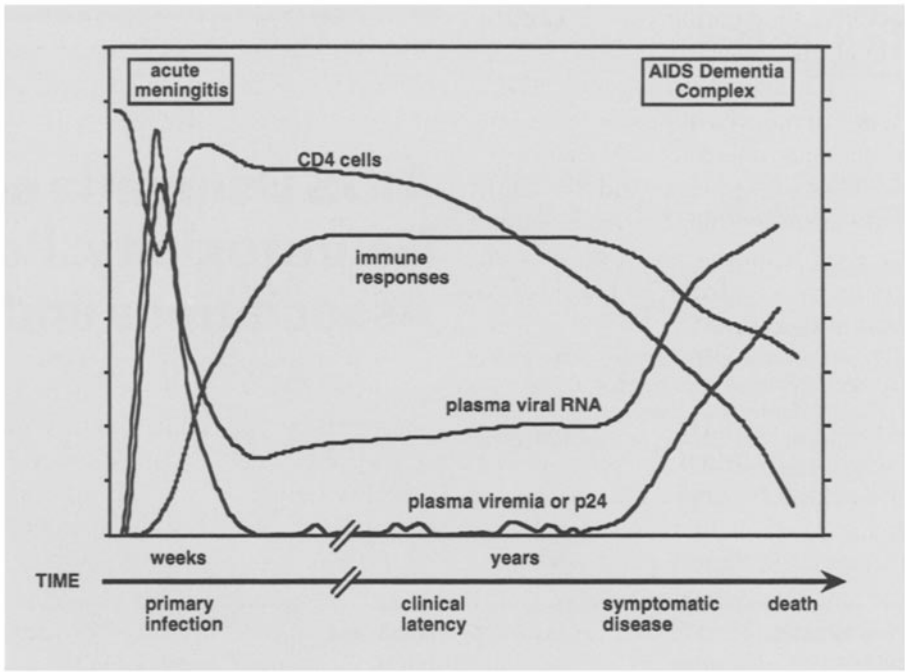

Fig. 1. Clinical, immunological, and viral spectrum of AIDS. The time course of $CD4^+$ T lymphocyte depletion, plasma viral load, and immune responsiveness in relation to the clinical prevalence of CNS dysfunction is illustrated. Acute HIV-1-induced meningitis is an early manifestation (within weeks) of infection, while the AIDS dementia complex typically appears years after infection when systemic manifestations are apparent and $CD4^+$ T lymphocyte counts are reduced.

lowed by major intellectual difficulties with memory, language, and problem solving. End-stage ADC evolves into a vegetative state with rudimentary comprehension, mutism, paraplegia, and sphincter incontinence [204]. Significant cognitive and motor impairments resembling many features of ADC in humans are also seen in simian immunodeficiency virus (SIV)-infected rhesus macaques [177]. Notably, acute HIV-1 seroconversion is often associated with a self-limited meningitis in 25–50% of cases [182], while ADC occurs generally late in the course of AIDS [86, 111, 129] when $CD4^+$ T lymphocyte counts are declining and plasma viral load is increasing, as indicated in figure 1.

The incidence of ADC is estimated at approximately 20% of all AIDS patients [112, 166]. The Center for Disease Control reports that ADC is actually reported in about 7% of AIDS patients and is the initial clinical symptom in approximately 3% [112]. Notably the mean survival after the diagnosis of dementia is approximately 6 months, which is less than half the average survival of nondemented AIDS patients [165, 216]. Early on, the diagnosis of ADC may be difficult, because of confusion of symptoms with depression, anxiety disorders, and drug-related effects [195, 261]. Furthermore, agitation or mania may be present [166]. One expects the ADC patient at diagnosis to be alert and free from fevers, headaches or focal neurological deficits, typically. One must be alert for the presence of opportunistic infections of which the most easily mistaken for ADC is CMV encephalitis [166]. This is distinguished by coincidence neuroradiologic abnormalities of ventriculitis and associated clinical findings of CMV retinitis.

The diagnosis of ADC depends upon demonstration of central nervous system neurological deficits with the exclusion of other neurological and/or psychiatric problems, in the setting of documented HIV-1 infection. Formal psychiatric tests as well as bedside neurological evaluations are directed towards assessment of psychomotor skills. The Trail Making, Grooved Peg Board, and Single Digit tests are the most useful formal screening tests for ADC [66]. Bedside testing focuses on performance of tasks of attention and concentration as well as tests of gait and coordination, all of which are generally impaired early in the course. Relative sparing of language and memory functions early in the course are characteristic. Several excellent reviews on the subject of clinical and formal neuropsychiatric testing for the diagnosis of ADC have been published [166, 240].

Viral Invasion of the CNS in ADC

Overwhelming evidence implicates direct invasion by HIV-1 into the brain as an essential initiator of ADC [129, 204, 207, 232, 242, 287]. Brain invasion by HIV-1 has been demonstrated within weeks of accidental peripheral

vascular inoculation of the virus and CNS invasion is thought to be nearly universal in AIDS [37, 40, 47, 88, 115, 202, 242]. Furthermore, ADC is not seen in other causes of immunosuppression and presents in the absence of demonstrable alternative pathogens. Also, animal lentiviruses such as visna and SIV similarly invade the CNS and cause neurological dysfunction [204]. In fact, with rigorous repetitive isolation procedures infectious virus can be isolated from the brains of ADC patients in up to 80% of cases and 50% of nondemented patients [40, 212, 246]. The total 'viral burden' in the brain, defined by PCR amplification of DNA and RNA or immunohistochemical detection of HIV-1 antigens, has been correlated in some studies with the severity of dementia [24], although other studies claim no clear association [115]. Recent evidence indicates that levels of viral RNA detected in the cerebrospinal fluid may be correlated with the incidence and severity of the dementia [87].

Considerable debate still exists concerning the mechanism of HIV-1 entry into the brain. Support for monocyte-mediated entry is provided by the early presence of infected perivascular macrophages in brain parenchyma and the presence of macrophage-tropic isolates in peripheral blood throughout the course of infection, as evidence for this 'Trojan horse'-mediated entry [27, 54, 140, 234, 235, 277]. Nottet et al. [188] recently demonstrated a correlation between macrophage infiltration and increased endothelial cell adhesion molecule (E-selectin and VCAM-1) expression in HIV encephalitis. However, the requirement for LPS stimulation of monocytes to maximally induce expression of these molecules raises questions as to in vivo mechanisms of induction [171]. Interestingly, exogenous Tat protein may also facilitate human monocyte-endothelial cell adhesion and transmigration through the induction of monocyte expression of β_2 integrins, ICAM-1, and metalloproteinase (MMP-9) [142].

An argument for monocyte-mediated SIV entry into the CNS of macaques has been presented by Sasseville et al. [224], who demonstrated the adhesion of the human monocytic cell lines U937 and THP-1, and the human B-lymphocyte line, Ramos, to vascular endothelium in animals with SIV encephalitis. This adhesion was mediated by VCAM-1/$\alpha_4\beta_1$ integrin interactions, which in view of the induction of VCAM-1 expression in SIV encephalitis [225] argues strongly in favor of endothelial adhesion as a means of monocyte and lymphocyte recruitment into the CNS during infection [223]. Finally, Shrikant et al. [243] proposed that monocyte entry into the CNS might also be facilitated by exogenous gp120-induced ICAM-1 expression on astrocytic end feet, which in turn would allow adherence of monocytes via integrin (LFA-1) expression as they traversed the endothelial cell barrier. In all, these reports implicate the induction of adhesion molecule expression on both capillary endothelial cells and peripheral blood monocytes by HIV-1 infection or viral gene products as a common pathway for facilitation of HIV-1-infection monocyte entry into the CNS.

Others have suggested that direct infection of vascular endothelial cells may also lead to direct seeding of the CNS through virion release on the parenchymal endothelial membrane surface. This has clearly been demonstrated in endothelial cell culture systems and in some instances in vivo through in situ hybridization techniques [9, 176]. Infection of cells within the choroid plexus has also been demonstrated, both in choroid plexus cells and macrophages within the stroma [9, 66, 196]. Of note, the virus could enter CSF spaces by trafficking of infected macrophages across the choroid plexus epithelial cell barrier or via secondary infection of these epithelial cells [196]. Entry of cell-free virus directly into interstitial spaces through defects in the blood/brain barrier at the level of the tight junctions of the intraparenchymal brain capillaries and/or the blood/CSF barrier at the level of the capillary epithelial cells are other hypothetical possibilities, though this has yet to be demonstrated [196]. An interesting in vivo model for endothelial infection by HIV-1 was presented by Frumkin et al. [73], who demonstrated HIV-1 infection within cerebral vascular endothelia in pigtailed macaques *(Macaca nemestrina)* 60 weeks after intravenous inoculation with the T lymphocyte-tropic strain LAI [3]. A broader survey of suspectibility utilizing primary macrophage-tropic strains will be necessary to further validate this model system; nonetheless, this approach may prove invaluable for defining the mechanism(s) of HIV-1 entry into human brain.

Finally, lymphocyte-mediated HIV-1 entry into the CNS has received some considerable support [291]. Activation of $CD4^+$ T lymphocytes by HIV-1 infection may enhance expression of cell adhesion molecules such as the integrins LFA-1 and VLA-4 which, in turn, interact with endothelial cell ligands ICAM-1 and VCAM-1, promoting lymphocyte/endothelial adhesion [244, 281, 291]. Expression of basement membrane-degrading enzymes by lymphocytes may enable transendothelial migration of infected cells [244, 291, Narayan, pers. commun.]. Support for both lymphocyte- and monocyte-mediated SIV entry into the CNS is provided by the SIV model system [42, 223, 225]. Clements et al. [42] have shown that peripheral inoculation of brain homogenates from an animal in-

fected intracerebrally with a macrophage-adapted SIVmac 239 virus derivative into naive susceptible macaques resulted in establishment of infection in the brain. In addition, peripheral inoculation of marrow monocytes infected with an SIVmac239 chimera containing an envelope sequence from an encephalitic brain derivative resulted in neuroinvasiveness but not neurovirulence [114].

More recently, Stephens et al. [249] verified the persistence of the lymphocyte-tropic phenotype of SIVmac239 within the brains of animals inoculated intravenously 2 years earlier. Interestingly, in all but one animal, sequence variability was lower in virus recovered from the brain in comparison to lymphoid tissues in these animals, suggesting a slower replication rate reflected in a lower mutation rate [249]. However, an important distinction between SIV and HIV-1 infection in the brain is the relative paucity of lymphocytic infiltrates in HIV-infected brain and the abundance of such infiltrates in SIV-infected brain, making correlative conclusions from the animal model to human infection difficult [235]. It thus seems likely that trafficking of infected $CD4^+$ T lymphocytes as well as monocytes into the CNS may occur under certain circumstances, and that further studies will be necessary to confirm the mode of HIV-1 entry into human brain.

Viral Replication within the CNS

Macrophages/Microglia

With rare exception, viruses isolated from the CNS of patients with ADC are macrophage-tropic, consistent with pathological evidence supporting productive infection within the brain in cells of the macrophage lineage (perivascular macrophages and intrinsic microglia [56, 57, 64, 129, 162, 164, 180, 234, 252, 287]. While it is clear that infection of monocyte-derived macrophages is CD4-dependent [43], the presence of CD4 on microglia in vivo remains controversial and likely reflects their state of activation [54, 55]. Despite the predominance of macrophage-tropic HIV-1 strains from isolation attempts with CNS-derived tissues, some paired CNS/peripheral blood isolates from ADC patients do show differential tropism, with peripheral isolates showing ability to infect CD4-negative glioma cell lines [38, 137]. In general, isolation procedures for harvesting virus from brain tissue are likely to amplify the more abundant and/or growth-advantaged viral species and not necessarily those representing the highly restricted infections demonstrated in other brain cell lineages. While it is generally accepted that the replication of macrophage-tropic isolates within the brain is necessary but not sufficient for the development of ADC, analysis of the phenotype(s) of strains infecting other cell types within the brain and their possible role in induction of ADC awaits investigation. A discussion of recent studies addressing neurovirulence and neuroinvasiveness of CNS-derived HIV-1 strains is presented in a later section concerning neurotoxicity of HIV-1 viral proteins.

Infection of Neurons, Astrocytes, Endothelial Cells, and Oligodendrocytes

Many autopsy case studies have demonstrated that only 5–20% of macrophages are productively infected, while recent in situ PCR studies have demonstrated that, in addition, similar numbers of astrocytes and neurons may be infected, albeit in a restricted manner with little expression of viral structural proteins [9, 189]. The recent study by Bagasra et al. [9] utilized triple-labeling technique involving in situ DNA PCR, reverse transcriptase-initiated in situ PCR, and immunohistochemistry to identify cell types harboring HIV-1 DNA, RNA, and proteins, respectively, within 22 autopsied brains of AIDS patients. In agreement with previous studies microglia and macrophages were the most commonly infected cell type (range, 1–22%) and showed expression of HIV-1 DNA, RNA, and proteins. In addition, significant numbers of neurons (range 0–16%), both in gray and white matter, were found to be infected. Neuronal infection was seen in 17 out of 22 cases (77%), and the clinical severity of ADC was roughly correlated with the percentage of neurons infected with HIV-1. Furthermore, 44% of the neurons containing HIV-1 provirus expressed HIV-1 mRNA, and <0.02% of these cells expressed abundant levels of unspliced HIV-1 RNA, indicating highly restricted infection. This study thus raises important questions as to the potential role of restricted neuronal infection in the induction of neuronal death and/or cellular dysfunction in ADC, which still remains largely unexplored.

An additional observation made by these authors was the significant percentages of HIV-1-infected microvascular endothelial cells (MVEC; 0–11%), astrocytes (0–7%), and choroid plexus cells (30–50%). Notably, over 65% of the MVEC expressed HIV-1 mRNA, suggesting that infection of such cells may be an important avenue of HIV-1 entry into the CNS. Other studies have confirmed the susceptibility of MVEC to cell-free infection in vitro and in vivo [287], although such infection may be highly restricted under these conditions [198]. Similarly high levels of HIV-1 mRNA expression were seen in cells of the

choroid plexus (40% of those cells expressing HIV-1 DNA), also suggesting that productive infection of the choroid plexus might facilitate entry of HIV-1 into the CNS, as previously suggested [67, 94, 152]. Finally, this study confirmed earlier reports of HIV-1 infection of astrocytes in vivo in both pediatric [260] and adult brain tissues [222] and in human fetal organotypic brain cultures [98]. This is especially important, since the astrocyte is the most abundant cell type within the brain, and maintains direct contact with neurons, oligodendrocytes, microglia and macrophages, and endothelial cells. Thus, cell-to-cell spread of HIV-1 via endothelial cells to astrocytes is an important potential mechanism of early HIV-1 transmission to and within the CNS [9]. Several other studies using in situ hybridization without PCR amplification have also demonstrated restricted infection in astrocytes, but not neurons, with evidence for overexpression of the regulatory protein Nef and the absence of expression of structural proteins [130, 210, 222, 260]. The potential significance of Nef expression in such circumstances is discussed in a following section.

Oligodendrocyte infection in vivo has been much more difficult to demonstrate. An early report of oligodendrocyte infection by HIV-1 [93] was refuted as being a misidentification of cell types infected [28, 233]. In the first published in situ PCR report from autopsied brains from HIV-1-infected individuals, Nuovo et al. [189] demonstrated infection in colabeled microglia (1–11%), astrocytes (1–5%), and neurons (1–17%), but in 'rare' instances in cells which morphologically resembled oligodendrocytes. Bagasra et al. [9] showed infection in 0–6% of oligodendrocytes in 59% of patients in their series of 22 autopsied brains described above. In addition, recent evidence also suggests that oligodendrocytes may be infected via cell-free viral inoculation in vitro by certain HIV-1 strains [4]. In this study, Albright et al. [4] used highly purified oligodendrocytes from temporal lobe biopsy specimens and demonstrated low-level (pg/ml) $p24_{gag}$ antigen and infectious virus release up to 4 weeks in cultures inoculated with the T lymphocyte-tropic HIV-1 strain IIIB, and monocyte-tropic strains Bal, and 89.6. Moreover, oligodendrocytes that were cocultured with (but physically separated from) infected microglia became infected, suggesting release of virions from the microglia with subsequent infection of the oligodendrocytes. Thus, although HIV-1 infection of oligodendrocytes in vivo appears to be rarer than infection of other cell types, such infection must be considered as a potential contributing factor in the pathogenesis of ADC.

The mechanism(s) of HIV-1 entry and transcriptional regulation of such restricted viral replication in brain cells of lineages distinct from macrophages and the potential consequences of such events are areas of intense interest [36, 39, 95, 108, 125, 147, 170, 219, 238, 263, 266, 270]. It is generally accepted that HIV-1 entry into cells other than macrophages and lymphocytes is CD4-independent [278], although some evidence exists for the expression of CD4 on neurons within the adult brain, particularly in the basal ganglia [74]. A number of studies have identified possible alternate receptors for HIV-1 in neuronal and astrocytic cells and among these the best studied to date is galactosylceramide [18, 96, 227]. It is expressed to high levels on oligodendrocytes and to a lesser degree on glioblastoma and other neurally derived cell lines [113, 126, 209]. Antibodies against galactosylceramide partially inhibit entry of HIV-1 into neuroepithelioma and glioblastoma cells, suggesting a role for this glycolipid as an independent receptor for HIV-1 in the brain [96, 97].

These and other studies have indeed indicated that infection in astrocytes, neurons, and oligodendrocytes is highly restricted, and recent evidence suggests that infection is limited not only by lack of CD4 expression in these cells, but also by inherent blocks in viral expression [183, 184, 263, 266]. Neumann et al. [183] showed that restriction of HIV-1 expression in the TH4-7-5 astrocytoma cell line was likely due to a block in Rev function by demonstration of failure to accumulate unspliced mRNA transcripts in a manner that could not be overcome by overexpression of Rev protein. In neuronal cells, differentiation may be associated with a decrease in HIV-1 expression, although the mechanism for this is unknown [263, 266]. Niikura et al. [184] has demonstrated that restriction of HIV-1 transcription in the astrocytoma line U-87 MG may be due in part to a deficiency in the molecular machinery for generation of short mRNA transcripts, lack of an undefined cellular factor, or presence of a cell-specific inhibitor. It should be noted that this cell line supports TAR-independent transactivation through a unique NF-κB-like transcriptional factor that may bind directly to Tat, unlike prototypic NF-κB [254].

Other in vivo experiments support a role for restricted retroviral expression in neuronal cells in neurodegeneration in several model systems. For example, abortive infection by the murine retrovirus Cas-Br-E in neurons results in spongiform degeneration in the brain and spinal cord in infected mice [237]. This is likely associated with deficient posttranscriptional events resulting in decreased envelope expression specifically in the target neurons [237]. Furthermore, Thomas et al. [256] demonstrated

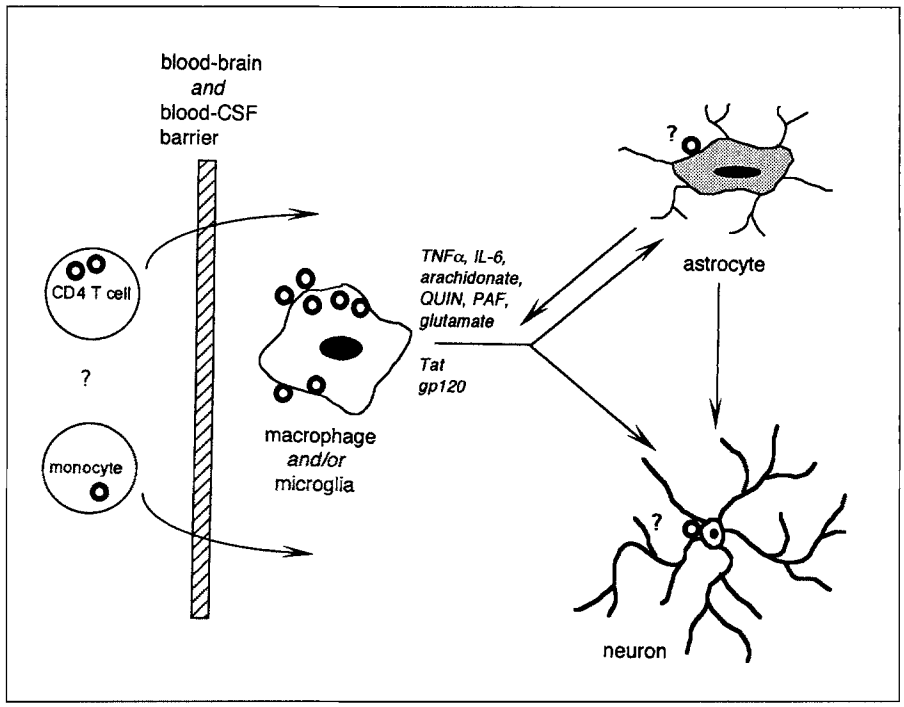

Fig. 2. Proposed model for HIV-1-induced neurotoxicity within the brain. Experimental evidence suggests entry of virus into the CNS via infection of circulating $CD4^+$ T lymphocytes and monocytes. Productive replication within macrophages results in release of both viral and cellular products which may act directly upon neurons as well as astrocytes to produce neuronal cell death and/or dysfunction. Recent evidence suggests that direct restricted infection of neurons and astrocytes also occurs in a significant percentage of cases [see 9, 189]. TNFα = Tumor necrosis factor-alpha; QUIN = quinolinic acid; PAF = platelet activating factor.

that transgenic mice expressing a full-length HIV-1 genome in spinal and brainstem motor neurons underwent axonal degeneration in these areas after 7–12 months in the absence of detectable HIV-1-specific protein (p24, p17, gp120, gp160) expression. This suggested that restricted expression of the viral genome (independent of the insertion site) in neurons could induce chronic neurodegeneration [256]. Taken together, these data suggest that, since regulation of HIV-1 expression in astrocytes (and perhaps neurons) may be unique in comparison to productively infected cell types, an understanding of the regulation of viral expression in these cells and the consequences thereof is critical to fully understanding the potential role of such infection in the pathogenesis of neuronal dysfunction of ADC [45].

Since productive HIV-1 infection occurs in a minority of the total number of macrophages within the brain, pathogenic mechanisms of neurological dysfunction are presumed to reflect 'amplification' steps involving release of cellular metabolites, cytokines, toxins, and viral gene products which then interact directly or indirectly through glial cells upon neurons [55, 81, 157, 168, 204]. However, neither the presence of virus within the brain nor the prevalence of macrophage infection are predictors of the incidence or severity of dementia, suggesting that host responses (cellular?, immune?) and/or viral characteristics contribute significantly to the development of neurological dysfunction [24, 86]. A simplified model of viral/cell interactions thought to be involved in the pathogenesis is presented in figure 2, with specific features of this model discussed in turn in the following sections.

Neuropathological Correlates of ADC

Perhaps the most intriguing question concerning the pathogenesis of ADC is how infection within the brain induces profound cognitive and behavioral dysfunction without obviously consistent neuropathological abnormalities. Neuroimaging studies indicate that cortical atrophy is the most common abnormality found, and in particular, volume loss in the basal ganglia correlates with the presence of dementia [8, 191]. Importantly, the basal ganglia also harbors the greatest density of infected macrophages in ADC brains, suggesting a causal relationship to the tissue volume loss [8, 24, 141]. Possible explanations for this local volume loss include a loss of neurons and/or other cell types that are particularly vulnerable to HIV-1-induced 'neurotoxins' or loss of cells due merely to high local concentrations of released toxic factors [162].

The neuropathological hallmark of HIV encephalitis is the multinucleated giant cell, which is presumably formed by fusion of infected macrophages via gp120/CD4 interactions at the cell membrane. However, MGCs are

present in only approximately 50% of patients with the clinical diagnosis of ADC in some series [122, 290]. Furthermore, among all ADC patients it is estimated that on average only 50–70% show specific histological abnormalities related to HIV-1 infection in the brain [29, 234, 267]. Thus one cannot accurately predict either the presence or severity of ADC by histopathological criteria. Among other histological changes that may be seen in addition to MGCs are neuronal loss, neuronal dendritic alterations, white matter pallor (which may represent edema as well as myelin destruction), and astrocytosis (see table 1).

Neuronal dropout in various areas has been demonstrated by a number of studies [65, 111, 124, 159] but not in others [230], and its relationship to ADC remains unclear. Seilhean et al. [230] failed to see neuronal loss in the neocortex of 6 patients with ADC, while Ketzler et al. [124] demonstrated an 18% loss in neurons (type unspecified) and a 31% reduction in cell body volumes in the frontal cortex of 18 unselected AIDS brains. Wiley et al. [288] showed a 30–50% loss in the number of large cortical neurons (200–500 μm^2) in the frontal, parietal, and temporal lobes of AIDS patients wtih HIV encephalitis. Neither of these studies was designed to correlate severity of dementia with neuronal loss. Everall et al. [65] demonstrated neuronal cell loss in the frontal and temporal lobes in all patients (from a series of 32) dying of AIDS, but the degree of change did not correlate with the presence or severity of dementia. Masliah et al. [159] demonstrated selective neuronal vulnerability in the frontal cortex and hippocampous of patients with HIV encephalitis which in total did not correlate with the severity of the encephalitis. However, there was a disproportional loss of a subset of neurons (parvalbumin-positive interneurons) in the CA3 region of the hippocampus, suggesting for the first time differential neuronal sensitivity in HIV-1 infection, although correlation to clinical deficits was not made. This is in contradistinction to the dementia of AD in which this subset of neurons is relatively resistant to injury [159].

Recent evidence suggests that neurons within the cortex and basal ganglia undergo apoptotic cell death in both pediatric and adult cases of ADC [2, 29], and that in some cases greater numbers of affected neurons colocalize with HIV-1-infected perivascular infiltrates [19]. In a series of 16 HIV-1-infected patients, Adle-Biassette et al. [2] demonstrated apoptosis in the cortical neurons of the frontal and temporal lobes in all 12 symptomatic patients (regardless of the presence or absence of neurological symptoms) and in none of 4 seropositive asymptomatic controls. These studies thus implicate apoptotic neuronal cell death as the mechanism of neuronal loss during the course of HIV-1 infection.

Table 1. Prevalence of pathological changes in HIV-1-infected brains at autopsy

Pathological changes	Prevalence, %
Neuronal loss/dendritic changes	up to 100
White matter (myelin) pallor	70–90
Multinucleated giant cells	25–60
Astrocytosis	30–60
Microglial nodules	20–50

These data are summarized from references 89, 122, 124, 161, 180, 234, 288 and 290. They represent average estimates of each type of abnormality, derived from multiple series of autopsied cases of patients with HIV-1 infection of the brain. Since not all cases had confirming clinical data presented to specify the presence of ADC, these estimates undoubtledly include some patients with only histological abnormalities in the brain and not neurological dysfunction.

In addition to neuronal dropout, Masliah et al. [159] demonstrated morphological alterations in the neuritic processes of neurons in both the frontal cortex and hippocampus, presumably from indirect effects of HIV-1 infection of other cells (macrophages/microglia). Recent anatomical analyses of neuronal cell structure throughout the cortex and subcortex have provided solid evidence that damage to dendritic projections in cortical pyramidal neurons may be a predictor of neurological dysfunction in patients with ADC [64, 100, 159, 160, 161, 288]. Neuronal morphological analysis of the large cortical pyramidal neurons in the frontal cortex in one series of autopsies revealed a 40–60 % decrease in the dendritic spine density, along with swelling and vacuolization [161]. Such a loss of dendritic spines has also been reported in other neurodegenerative disorders including Down's syndrome, Pick's disease, Creutzfeld-Jakob disease, and AD, though to a lesser extent [161]. This study furthermore suggested that primary dendritic pathology distinguishes HIV encephalitis from AD, where the pathological changes are primarily presynaptic. The significance of such consistent dendritic abnormalities should not be underestimated, since dendrites subserve critical biochemical functions in neurons including expression of calcium uptake mechanisms [128, 229], neurotransmitter receptors [128], and neurotransmitter enzyme expression [150]. It thus seems likely that these consistent dendritic abnormalities would result in functional disturbances in these cortical neurons and play

an important role in the genesis of ADC. Understanding the pathogenesis of these dendritic alterations may thus be a key to understanding the pathogenesis of ADC.

Putative Neurotoxins and Their Potential Role in ADC

Since cells of the macrophage lineage are the only focus of productive HIV-1 infection within the brain in ADC, much effort has been directed towards identification of potential 'neurotoxins' produced or induced by macrophages. Furthermore, identification of candidate neurotoxins has generally been approached through the assessment of the ability of cellular cytokines, metabolites, and other soluble factors released by monocytic cells to cause neuronal cell death in a variety of human and nonhuman neuronal cell culture systems [252]. Such an approach, while certainly straightforward and potentially fruitful, presupposes that induction of neuronal cell death is a primary mechanism of induction of neurological dysfunction in ADC, yet pathological studies have failed to demonstrate close correlation with the degree of neuronal dropout and the severity of dementia [65, 159].

Among the candidate 'neurotoxins' induced by HIV-1 infection within the brain are a variety of cytokines released by glial cells, including macrophages, microglia, and astrocytes [reviewed in 16 and 168]. A number of studies have demonstrated elevations of IL-6 [75, 194], IL-1β [75], IL-1α [194] and TNFα [91, 194] in the CSF of HIV-1-infected patients, and particularly in those with ADC. Others have also demonstrated elevations in levels of mRNA expression in HIV-1-infected brain for TNFα, IL-1β, and TGFβ [276, 283], although others have disputed some of these findings [76]. Each of these cytokines has been examined in one or more in vitro neuronal cell culture systems for evidence of neurotoxicity with (expectedly) varying results. Tables 2 and 3 summarize the published effects of viral and cellular products induced by HIV-1 infection and are discussed in turn in the following sections.

TNFα

TNFα has been implicated both in oligodendrocyte damage and myelin destruction [231] and in direct neurotoxicity [34]. Selmaj and Raine [231] demonstrated that recombinant human TNFβ applied to organotypic cultures of mouse spinal cord resulted in ballooning of myelin sheaths and degeneration of oligodendrocytes with infrequent neuronal death after 18 h of exposure. In addi-

Table 2. Neurotoxicity of HIV-1 gene products

Viral product	Action/mechanism	Reference No.
gp120	killing of fetal rodent/human embryonic neurons by NMDA-receptor-mediated excitotoxicity leading to increased intracellular calcium	23, 60, 149
	neuroprotection by NMDA receptor blockade and Ca^{2+} channel blockers	60, 148
	neuroprotection via Na^+ channel blocker	58
	neuroprotection via neuronal expression of calbindin	59
	damage to neuritic processes and impaired dopamine transport in dopaminergic neurons from rat midbrain	13
	activation of tyrosine kinase in glioma cells	227
	increased intracellular Ca^{2+} in type II astrocytes from rat brain	41
	stimulation of NOS, release NO from human astrocytoma cells	172
	release of PGE_2 from human astrocytoma cells	173
	release of NO from macrophages	197
	induction of neuronal (pyramidal cell) loss, astrocytosis, and dendritic vacuolizations in transgenic mice expressing gp120 mRNA	257
	inhibition of myelin formation in rat oligodendrocyte cultures	127
	inhibition of rat and human astrocytic Na^+/H^+ ion exchange	14, 15
Tat	killing of rat neuroblastoma cells in vitro and lethality in mice via intracerebral injection	221
	killing of rat pheochromocytoma (PC 12) cells	280
	killing primary human fetal cortical neurons by direct Tat interaction with NMDA receptors, unlike gp120, and blocking of toxicity by NMDA receptor antagonists	151, 181
	interference with neuron/extracellular matrix adhesion with RGD sequence involvement	131, 190
Nef	overexpression in astrocytes in pediatric and adult brain	210, 222, 260
	sequence similarity with scorpion neurotoxin	282

Published data on potential in vivo and in vitro neurotoxic effects of gp120 and Nef are summarized.

Table 3. Neurotoxicity of HIV-1-induced cellular products

Cellular product	Action/mechanism	Reference No.
TNFα	killing of oligodendrocytes and ballooning of myelin sheaths in organotypic cultures of mouse spinal cord	231
	killing of cultured primary human oligodendrocytes by culture supernatants from HIV-1-infected human microglia containing low levels (<32 pg/ml) of TNFα	292
	potentiation of glutamate-mediated killing of cultured primary human fetal neurons by high levels (40 ng/ml) of TNFα	34
	killing of cultured primary human fetal neurons by low levels (200 pg/ml) of TNFα	78
	killing primary human fetal neurons at 'subtoxic' levels (50 pg/ml) in combination with non-NMDA receptor (AMPA) agonist	78
	killing of cultured human NTera 2 neurons by high levels (50 ng/ml) of TNFα	285
Arachidonic acid	inhibition of high-affinity glutamate uptake in primary rodent astrocytes and brain synaptosomes	10, 262, 271, 272
	depression of non-NMDA receptor currents	136
NO	killing of cultured primary embryonic rodent neurons, produced by activated rodent microglia	33, 298
	mediates neuronal cell death induced by NMDA receptor stimulation	49
	low-level constitutive NOS activity in endothelial cells and neurons and inducible activity in reactive astrocytes in multiple sclerosis plaques	20, 26
	low-level induction of NO by HIV-1 infection of macrophages, stimulated with LPS, TNFα, and contact with astrocytes	30
	low-level nitrite release from macrophages stimulated with high levels of gp120	30, 197
	failure to detect NOS expression in brains of 22 patients dying with AIDS	199
QUIN	killing of cultured primary embryonic rodent neurons by activation of NMDA receptors	70, 123, 228, 286
	chronic (months) killing of neurons in organotypic cultures of rat striatum at physiological (100 n*M*) levels	286
	induction of axon-sparing dendritic lesions in rodent brain	70, 228, 286
	killing of rodent hippocampal pyramidal neurons and striatal interneurons in vivo	70
	CSF levels correlated to degree of neurological dysfunction in SIV-infected macaques and HIV-1-infected humans	1, 103, 105, 116, 286
PAF	killing of neurons in mixed human fetal brain cell cultures and rat retinal ganglion cell cultures at low (≤850 pg/ml) levels, partially blocked by NMDA receptor antagonists	80
	killing of NTera 2 human neuronal cells at high (5 ng/ml) levels	285
IL-6	enhancement of intracellular calcium responses to NMDA in developing cultured rat cerebellar granular neurons	208
	increased firing rates of hypothalamic neurons in guinea pigs with induction of fever	293
IL-1β (no known neurotoxicity)	increased density of IL-1β receptors on cortical neurons in brains with HIV-1 encephalitis	163
	may induce astrocytosis and enhance neuronal cell survival through induction of astrocytic-derived neuronal trophic factors	85
TGFβ1 (no known neurotoxicity)	increased TGFβ1 receptor expression on dendritic processes of cortical neurons in HIV encephalitis	163

Published data on potential in vivo and in vitro neurotoxic and neuropathological effects of cytokines and cellular metabolites that are associated with HIV-1 infection in the brain are summarized. NO = Nitric oxide; QUIN = quinolinic acid; PAF = platelet activating factor.

tion, Wilt et al. [292] demonstrated that conditioned medium from HIV-1-infected microglia containing ≤32 pg/ml of TNFα was toxic to human oligodendrocytes and that this toxicity was neutralized with anti-TNFα antibodies, while purified recombinant human TNFα was toxic only at concentrations of ≥40 ng/ml. Chao and Hu [34] showed also that high levels of TNFα (40 ng/ml) potentiated glutamate-mediated neurotoxicity in human fetal brain cell cultures. Notably, TNFα levels in CSF in ADC patients are generally much lower (pg/ml) [92].

In what may be considered more physiologically attainable conditions, Gelbard et al. [78] demonstrated that low levels of TNFα (200 pg/ml) were neurotoxic to primary human fetal neurons in mixed cultures, and that 'subtoxic' TNFα doses (50 pg/ml) were rendered toxic when applied in combination with the glutamate receptor agonist AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid). In one series of 45 patients with AIDS, CSF TNFα levels were found in this range (10–90 pg/ml) in 55%, and 72% of those patients had clinical neurological involvement [91]. In addition, elevated levels of TNFα expression are seen in autopsied brains of patients with ADC, particularly in the basal ganglia [1, 265]. However, in other disease states such as multiple sclerosis where neuronal cell death is not a common pathological finding, elevated TNFα expression in brain and CSF have also been documented [236]. Thus, these in vitro studies suggest that under specific conditions a high concentration of soluble purified TNFα may have direct toxicity to neurons and oligodendrocytes, but that other factors are likely necessary to mediate toxic effects and neurological symptoms in vivo.

Besides potential toxicity from soluble TNFα, some authors have demonstrated that TNFα may also act as a potent neurotoxin when presented on the surface of the effector cell membrane [reviewed in 144, 296]. TNFα may mediate apoptotic as well as necrotic forms of cell lysis, and in each case toxicity appears to depend upon the expression of TNFα receptors on the target cells [144]. Zajicek et al. [296] demonstrated that paraformaldehyde-fixed rat microglia could lyse oligodendrocytes by direct cell-cell contact in a manner inhibited by anti-TNFα antibodies. Furthermore, it has been shown that paraformaldehyde-fixed human monocytes expressing TNFα on their surface may directly lyse target cells, presumably through the interaction of a transmembrane form of TNFα with the target cell surface [138]. Since binding of HIV-1 to macrophages may induce expression of TNFα [167], it is interesting to speculate whether this is a potential pathway for macrophage-mediated neurotoxicity in the brain. Although it has been shown that an HIV-1-infected monocytoid cell line (U937) may kill neurons through cell/cell contact in primary neocortical cell cultures, the mechanism of such killing has not been determined [251].

It is unlikely that the concentrations (ng/ml) of recombinant TNFα necessary to mediate neuronal and oligodendrocyte death in vitro are achieved in vivo, and it seems likely that any such TNFα toxicity in vivo results from synergistic (or additive) effects of other agents as well. Importantly, TNFα may act upon astrocytes to induce production of TNFα, IL-6, GM-CSF, G-CSF, and M-CSF which may in turn influence leukocyte recruitment, inflammatory responses, and HIV-1 replication [16]. Analysis of the effects of such secondary factors in addition to the primary TNFα effects on neurons and glia will be necessary to determine any potential role for TNFα in the pathogenesis of ADC.

gp120

The envelope glycoprotein, gp120, is perhaps the most widely investigated (and promoted) HIV-1 'neurotoxin' to date. Its effects on neuronal/glial cell viability and glial cell function have been studied in a variety of vertebrate and invertebrate cell culture systems, along with in vivo studies in transgenic and intracerebrally injected rodents [5, 12, 14, 15, 23, 32, 41, 50, 58–60, 83, 118, 127, 149, 172, 173, 192, 206, 248, 255, 257, 295]. We will now summarize the salient features of these studies.

In one of the earliest published studies of gp120 neurotoxicity, Brenneman et al. [23] demonstrated that recombinant gp120 from lymphocyte-tropic strains LAV, IIIB and RF2 killed embryonic rat hippocampal neurons (age 7 days in culture) at picomolar concentrations. This effect was inhibited by vasoactive intestinal peptide, a naturally occurring neurotrophic peptide which contains some sequence homology with gp120, as well as by antimouse CD4 antibodies. Thus, this initial observation suggested that soluble released gp120 may act as a neurotoxin within the CNS through interference (directly or indirectly) with normal neuronal neurotrophic factors, possibly by receptor blocking, but whether such an effect may relate to the pathogenesis of ADC remains as an intriguing unanswered question.

Subsequent studies of gp120 neurotoxicity have focused upon induction of excitotoxicity, i.e. glutamate-receptor-mediated cell death, as another pathway triggered by soluble gp120 exposure. Dreyer et al. [60] subsequently extended the earlier finding of Brenneman et al. of gp120 (IIIB, RF)-induced hippocampal neurotoxicity

by demonstrating that the toxicity may be secondary to induction of increased intracellular calcium levels, which could be blocked by selective calcium channel antagonists. This study also included postnatal rat retinal ganglion cell cultures, indicating that more mature neurons were also susceptible. The resulting neuronal death was prevented by anti-gp120 antibodies, but not by antirat CD4 antibodies [118], suggesting that CD4/gp120 binding was not involved.

Important additional follow-up experiments indicated that gp120 might act indirectly through activation of NMDA receptors, which suggested a possible practical means of pharmacologic NMDA receptor blockade as a means of protection against gp120 toxicity [148, 149]. Lipton et al. [148] concluded that gp120 did not act alone but rather synergistically with endogenous glutamate to induce neurotoxicity, and subsequently found that the NMDA antagonist memantine prevented such toxicity in vitro [156]. One additional report also suggested that Na^+ channel blockade with tetrodotoxin may also protect neurons from gp120-induced toxicity [159]. This same group also showed that neuronal expression of calbindin, a calcium-binding protein that buffers intracellular calcium fluxes, was protective and that expression of heat shock protein 70 was not neuroprotective against gp120 [172]. Thus, this report offered some experimental evidence for selective neuronal vulnerability (lack of calbindin protein expression) to toxic effects of gp120. A more recent study demonstrated gp120-induced damage to neuritic processes and impaired dopamine transport in dopaminergic neurons cultured from rat midbrain, which was prevented by NMDA receptor antagonists [175]. The common theme from these studies, therefore, was that indirect activation of NMDA receptors by gp120 results in calcium-mediated neuronal cell death, which may be blocked with NMDA receptor antagonists. Lipton [149] has proposed this as the 'final common pathway' of gp120-induced neuronal cell death in ADC and a clinical trial of NMDA receptor blocking agents has been undertaken.

A number of subsequent studies elucidated some possible additional secondary effects of gp120 in neuronal and glial cell cultures [157, 160, 163, 164, 166–173]. Dawson et al. [50] showed that glutamate-dependent gp120 toxicity in neocortical embryonic rat cell cultures is mediated in part through production of nitric oxide (NO), probably from subsets of NO synthase (NOS)-expressing neurons. Several other groups have focused upon gp120 effects on astrocytes as another potential indirect means of inducing neurotoxicity. Activation of a tyrosine kinase in human glioma cells by exposure to gp120 has been proposed as a means of signal transduction in CD4-negative brain cells [97]. In addition, specific subsets of type II astrocytes from rat brain may show increases in intracellular calcium in response to gp120, which may in turn affect other astrocytes as well as neurons by intercellular signaling [169]. Pietraforte et al. [199] demonstrated that gp120 induced release of NO from human macrophages although in general human macrophages produce a much lower amount of NO than murine macrophages [197]. In studies paralleling those of Dawson et al., others have shown that stimulation of NOS activity in human astrocytoma cells by gp120 may also result in the release of NO, with potential neurotoxic results [172]. This same group later showed that such stimulation also resulted in the release of prostaglandin E_2 (PGE_2) [173], a breakdown product of arachidonic acid metabolism, which is known to be elevated in the CSF of patients with ADC [90]. Release of arachidonic acid and PGE_2 may also be induced by HIV-1 infection of macrophages [186]. The production of arachidonic acid and such metabolites by HIV-1-infected macrophages has been shown to be a possible pathway of induction of neurotoxicity in mixed neuronal/glial cell cultures [82, 187], though the mechanism(s) of such toxicity are unknown.

The potential neurotoxicity of gp120 in vivo has been also examined by utilizing a transgenic mouse model system [257]. In this study, a gp120 sequence from strain LAV was expressed by a glial fibrillary acidic protein promoter which is specific to astrocytes. These mice clearly demonstrated expression of glial fibrillary acidic protein mRNA, which was highest in the neocortex, olfactory bulb, hippocampus, and midbrain, but gp120 protein was not detectable. In the higher expressing transgenic lines, neuropathological changes included loss of pyramidal neurons, astrocytosis, and dendritic vacuolizations, similar to changes seen in ADC brains [159, 161]. These authors concluded that a cause/effect relationship between gp120 and histological damage in the mouse brain was likely.

Several other in vitro studies have addressed possible effects of gp120 in altering normal glial cell functions (instead of induction of neurotoxin release from glia) as a means of inducing histological damage and cell death. Kimura-Kuroda et al. [127] recently showed that gp120 may inhibit myelin formation by oligodendrocytes in rat neocortical brain cultures and postulated this as an explanation in part for myelin loss in ADC. An intriguing report by Benos et al. [14, 15] recently emphasized the concept of disturbances in normal glial cell functions induced by gp120 as a means of affecting homeostatic

mechanisms in the brain, which may in turn impact on neuronal cell function. In these studies, alterations in the Na^+/H^+ ion transporter in astrocytes induced by gp120 resulted in stimulation of glutamate and potassium release through the activation of tyrosine kinase, which may ultimately lead to excitotoxic neuronal cell death. However, it should be noted that gp120 concentrations of 25 n*M* were utilized in these experiments, again raising the question as to extrapolation of these in vitro observations to in vivo conditions. Nonetheless, the importance of such observations lies in the potential for pharmacologic targeting of such ionic transport processes in the brains of patients with ADC [14].

Recent evidence has suggested sequence specificity in the envelope region of SIV and HIV-1 brain isolates as a determinant of their potential for induction of neurotoxicity [53, 114, 135, 143, 174, 201, 211, 218, 239, 264]. Power et al. [201] cloned and sequenced *env* sequences from postmortem brains of 14 demented and 8 nondemented AIDS patients and demonstrated that both groups showed a macrophage-tropic consensus sequence within the V3 loop. Furthermore, two mutations, one within V3 (305 Pro→His) and one just outside of V3 (329 Ile→Leu), were common in demented patients. In addition there was an average of 16–24 differing amino acids in the envelope region including the C2 and V3 regions between brain and spleen isolates within the same individual, and an average of 4–5 such changes between brain sequences from the same individual. These observations indicate that there may be little sequence divergence within the CNS compartment and suggest also the presence of viral determinants for neuroinvasiveness and/or neurovirulence within *env*.

A subsequent study by Korber et al. [135] analyzed *env* sequences from stereotactic brain biopsy specimens from 6 AIDS patients and confirmed a clustering of *env* sequences (V3 to V5) in comparison to blood viral sequences, again suggesting brain compartmentalization of HIV-1. Furthermore, V3 loop sequences from brain tissue showed a tendency towards a neutral or negative net charge in comparison to blood isolates, and a brain V3 *env* 'signature pattern'. However, these patients were not diagnosed specifically with ADC; all patients had either other opportunistic infections or CNS lymphoma, and so no conclusions could be made about a role for these sequences in the pathogenesis of ADC. However, the implication from this analysis nonetheless is that V3 loop determinants may influence neuroinvasiveness and/or confer selective advantage for HIV-1 growth in the brain, which in turn may ultimately confer neurotoxicity.

Several other studies, however, argue against the existence of 'brain specific' HIV-1 *env* sequences [53, 139, 211, 241]. In contrast to the Korber and Power studies [135, 201], Kuiken et al. [139] found that paired CSF/blood isolates showed little V3 sequence variation within either ADC or non-ADC patients, but that differences existed between ADC and non-ADC AIDS groups. One must interpret this result with caution, however, since these HIV-1 isolates reflect CSF sampling and not isolates derived directly from brain parenchyma. Reddy et al. [211] found no brain-specific unique V3 sequences in multiple clones derived from brain tissue of 3 patients dying with HIV encephalitis in comparison to spleen-derived HIV-1 sequences. Finally, Di Stefano et al. [53] found the V3 (Pro→His) mutation identified as brain-specific in the Power study [201] to be common in a majority (16 of 25) of nondemented patients as well. Again in contradistinction to Power et al., this study also failed to demonstrate the presence of the V3 305 Pro residue in nondemented patients [53]. Thus, the question of brain-specific *env* sequences, and more specifically, brain-specific 'neurotoxic' *env* sequences remains in doubt. Nonetheless, it is interesting to speculate as to whether direct sampling of HIV-1 sequences from infected astrocytes and neurons within the brain would yield additional information about viral (*env* or other) determinants of HIV-1 neurotropism and neurotoxicity.

In summary, an abundance of in vitro evidence implicates gp120 as both a neurotoxin and a neural/glial cell modulator, but whether levels of gp120 necessary to achieve these effects are expressed in vivo remains unanswered. Furthermore, the presence of a brain-specific *env* signature sequence also remains speculative. Pathological abnormalities are indeed seen in transgenic mice expressing gp120 mRNA signals within the brain, but the existence of free gp120 in the CSF or interstitial fluid within the brains of these animals or in brain tissue of autopsied cases of ADC has not been documented. It is assumed, not unreasonably, that infected macrophages and microglia release monomeric gp120 in vivo during the course of infection, and it is further assumed that 'local' levels of released protein are sufficiently high to induce toxic responses in neurons and glia. However, demonstration of such elevations of locally released viral proteins (gp120, Tat, Nef) in vivo remains a major experimental challenge in further supporting the in vitro evidence of the neuropathogenic potential of such viral proteins in vivo.

Arachidonic Acid and Its Metabolites

Arachidonic acid has been proposed as an important neuromodulator within the CNS by means of modulation of neuronal NMDA- and non-NMDA receptor currents [51, 61, 136, 169], and recently as a mediator of neurotoxicity in ADC [82, 90, 178, 187]. Arachidonic acid may be released from the surface membrane of a variety of cells (macrophages, astrocytes, endothelia, neurons) through the action of phospholipase A_2, and may be further metabolized in two distinct enzymatic pathways [90, 185]. One pathway, prostaglandin formation, depends upon the actions of cyclooxygenase, while the second pathway, leukotriene formation, proceeds via the action of lipooxygenase, and each of these pathways is operational within the CNS [90]. Activation of NMDA receptors by glutamate during brain ischemia or by other glutamate receptor agonists has been shown to release arachidonic acid from neurons which will in turn potentiate NMDA receptor currents in the same or neighboring neurons [61, 169]. The result may be a further stimulation of arachidonic acid release, resulting in an amplification cascade of increasing neuronal calcium accumulation during glutamate activation [51, 169]. Arachidonic acid may also depress some non-NMDA receptor currents [136].

An important functional consequence of arachidonic acid release within the CNS is the capacity of free arachidonic acid to impair high-affinity glutamate transport in astrocytes and brain synaptosomes [262, 271, 272]. Glial cells (astrocytes, macrophages/microglia, and oligodendrocytes) as well as neurons and epithelial express high-affinity transport mechanisms for exogenous glutamate [6, 35, 69, 119–121, 134, 215], and in the case of astrocytes this mechanism allows for the scavenging of extracellular glutamate which may be toxic to neurons when it accumulates to high levels in the CNS [34, 213, 258, 273]. Exposure to high levels (m*M*) of free arachidonic acid quickly (minutes) inhibits this transport process in astrocytes in vitro and this effect is maintained for at least 20 min after removal of arachidonic acid [10, 272]. In addition, chronic inhibition of glutamate transport by a variety of inhibitors is a well-recognized means of inducing glutamate-mediated neurotoxicity in cell culture systems as well as in organotypic tissue preparations from hippocampus and spinal cord and defective glutamate transport if thought to be a factor in anterior horn cell neurodegeneration in amyotrophic lateral sclerosis [214, 273]. It is thus interesting to speculate that release of arachidonic acid within the CNS with resultant glutamate transport inhibition could result in either acute or chronic neurodegeneration, but this awaits further experimental support. Since arachidonic acid is highly lipophilic and membrane-associated, it may not exist in free form in the extracellular space and has not been demonstrated in the CSF of patients with ADC. Whether concentrations sufficient to significantly inhibit glutamate transport may accumulate at the membrane surface is unkown, but nonetheless arachidonic acid-mediated inhibition of glutamate transport remains an interesting potential 'indirect' means of impairing neuronal function in HIV-1-infected brain.

Breakdown products of the cyclooxygenase pathway of arachidonic acid metabolism, prostaglandins, are elevated in the CSF of patients with ADC, and in vitro studies suggest that these metabolites may be neurotoxic [82, 90, 187]. Griffin et al. [90] demonstrated that elevations of PGE_2, prostaglandin $F_{2\alpha}$, and thromboxane B_2 (TxB_2) were associated with elevations of cyclooxygenase-1 mRNA in ADC; however, no evidence for activation of the lipooxygenase pathway was found. Nokta et al. [186] demonstrated production of PGE_2 (cyclooxygenase pathway) from HIV-1-infected monocytes and macrophages, with higher levels seen with more highly differentiated cells. In contrast, Genis et al. [82] demonstrated that HIV-1 infection of macrophages resulted in significant levels of products of the lipooxygenase pathway (leukotrienes and hydroxyeicosatetraenoic acids) but very low levels of products of the cyclooxygenase pathway. In this study, release of TNFα, IL1β, and platelet activating factor (PAF) from HIV-1-infected macrophages was also reported. Moreover, neurotoxicity was markedly enhanced by cocultivation of the infected macrophages with astrocytic cell lines, although direct involvement of arachidonic acid metabolites in the neurotoxicity was not demonstrated. This same group had earlier demonstrated that HIV-1 virions alone or culture fluids from HIV-1-infected monocytes grown in the absence of astrocytes were not directly neurotoxic unless LPS or mycoplasma contaminated the cultures [17]. Thus, the conditions under which cells are grown (single-cell or mixed-culture) influence the production of putative neurotoxins, emphasizing the importance of cell-cell interactions within the brain (and in cell culture models) for induction of neurotoxicity.

A subsequent study from the same group demonstrated that maximal stimulation of TNFα, PAF and arachidonic acid metabolite release from infected macrophages required stimulation with LPS, and that addition of primary human astrocytes actually *reduced* the levels of these products [187]. Thus, the roles of HIV-1 infection, LPS activation, and astrocyte interactions with macrophages in the production of arachidonic acid and other

potential neurotoxins remain enigmatic, and astrocytes have been proposed as mediators of neurotoxicity under some conditions [82] and as attenuators of neurotoxicity under other conditions [187]. It is apparent that further studies will be necessary to determine the role of arachidonic acid metabolites produced by both the cyclooxygenase and lipooxygenase pathways in neurotoxicity, and the role of astrocytes in modulating these responses.

Nitric Oxide

Perhaps more so than any other metabolite, the role of nitric oxide (NO) in the induction of neuronal cell death in ADC remains the most controversial. Since its identification as a neuronal messenger molecule within the brain by Snyder et al. [48, 245], and the localization of specific isozymes of its synthetic enzyme nitric oxide synthase (NOS) to discrete neuronal populations, endothelial cells, and macrophages within the brain [22], NO has received considerable attention as a potential neurotoxin. Importantly, NMDA and non-NMDA receptor activation by glutamate is a major trigger for NO formation in several areas within the brain [48] and NMDA receptor-mediated neurotoxicity in rat brain cell cultures may be abrogated by neuronal NOS inhibitors [49]. Purified murine microglia produce large amounts of NO upon stimulation with LPS or γ-interferon, yielding the metabolite nitrite [298], which is directly neurotoxic [33].

There is little evidence to support the consistent presence of NOS activity in human macrophages, however [20, reviewed in 26]. In human brains low-level constitutive NOS activity has been demonstrated in endothelial cells and neurons, and inducible high-level NOS activity in reactive astrocytes [20, 26]. Bo et al. [20] showed that this astrocytic NOS is expressed to high levels around the areas of active demyelination in multiple sclerosis, perhaps in response to local production of cytokines by macrophages/microglia, suggesting a response to nonspecific stimulation. A study by Bukrinsky et al. [30] demonstrated low-level production of NO by HIV-1-infected human monocyte-derived macrophages in comparison to uninfected cells, with slight further stimulation by LPS, TNFα, and contact with astrocytes. Interestingly, NOS activity was not detected in the macrophage cultures until at least 6 days after HIV-1 infection, when reverse transcriptase activity was dramatically decreased. These authors also demonstrated the production of nitrite in these cells and confirmed an earlier report of gp120 induction of low-level nitrite release [197], although this effect required very high levels of gp120 (up to 40 μg/ml). The authors also reported detection of inducible NOS activity in the autopsied brain of a pediatric patient dying with severe HIV encephalitis, with failure of detection in five less severely affected HIV-1-infected adult brains. A recent survey of 22 brains from autopsied adult HIV-1-infected patients also failed to demonstrate elevated levels of NOS activity [199]. In total, these data suggest that NO induction is unlikely to be a major factor in neurotoxicity in most cases of ADC.

Quinolinic Acid

Quinolinic acid (QUIN) is a product of *L*-tryptophan metabolism produced by human macrophages in response to a variety of stimuli [106], including HIV-1 infection [25]. QUIN which may act as an NMDA receptor agonist and induce neurotoxicity in vitro [70, 123, 228, 286]. Importantly, QUIN may act either as an acute or chronic neurotoxin under certain conditions and cause axonal-sparing lesions which often involve dendritic processes in vivo [70, 228, 286]. In organotypic cultures of rat striatum QUIN exposure (at levels found in the CSF of ADC patients, 100 n*M*) induced neurotoxicity over 6–7 weeks in culture [286]. In addition, others have shown that pyramidal cells of the hippocampal cortex as well as striatal (basal ganglia) interneurons are susceptible to both the neurotoxic effects of QUIN in vivo [70] and the neurotoxic effects of HIV-1 [159, 161].

Several studies have now demonstrated that QUIN levels in the CNS in patients with ADC as well as rhesus macaques infected with SIV are closely correlated with the degree of neurological dysfunction, thus directly implicating QUIN in the pathogenesis of ADC [1, 103, 105, 116]. In an early study, Heyes et al. [102] demonstrated elevated QUIN levels in the CSF of patients with AIDS but without neurological symptoms. Subsequently, sustained increases in the CSF of rhesus macaques naturally infected with simian retrovirus type-D were demonstrated and proposed to reflect inflammatory responses within the brain [103]. Several of these animals showed CNS gliosis with macrophages and pervascular cuffing and one showed evidence for macrophage activation, thus providing in vivo evidence for the production of QUIN by macrophages throughout the course of retroviral infection in the CNS. In an important follow-up study, Jordan and Heyes [116] showed that CSF and serum QUIN levels were elevated within weeks of peripheral inoculation of macaques, and that symptoms of immunodeficiency, shortened survival, and consistent peripheral blood mononuclear cells viral isolation were associated with marked elevations of CSF and serum QUIN. This longitudinal study thus linked viral persistence and clinical

symptoms with persistence of QUIN elevations in the animal model.

A significant relationship has been demonstrated between QUIN expression and neurological status in AIDS patients as well [1, 105]. In a study of 126 HIV-1-infected patients, Heyes et al. [105] found persistently elevated CSF QUIN levels, particularly in patients with ADC, opportunistic infections and meningitis, reflecting the elevation of CSF QUIN levels in inflammatory conditions in general [104, 107]. Notably, treatment of the underlying conditions with either zidovudine or other specific antimicrobial therapy resulted in a decrease in the CSF QUIN levels and a parallel improvement in neurological status. The severity of the dementia correlated with the degree of elevation of CSF QUIN levels as well as the persistence of these elevations, suggesting a chronic neurotoxic effect in these patients [286]. In a quantitative analysis of HIV-1 burden, activation markers, and QUIN levels, Achim et al. [1] demonstrated high levels of HIV-1 antigen as well as QUIN within the basal ganglia of autopsied brains of ADC patients, again implicating virally induced local release of this neurotoxin. It is important to note that this regional expression of QUIN [and TNFα; 1] in the basal ganglia in association with high levels of HIV-1 suggests a direct causal relationship to the clinical expression of 'subcortical' dementia, including psychomotor slowing and prolongation of reaction times [156, 166].

Thus, perhaps more than any other potential HIV-1-induced neurotoxin identified to data, QUIN's role in the pathogenesis of ADC seems most broadly supported. This is based upon the demonstration of elevations of CSF and tissue levels of QUIN in ADC patients, in vitro release studies in macrophage cultures, in vitro and in vivo neurotoxicity studies with primary neural tissues, and in vivo correlates demonstrated in rhesus macaques.

Other Cytokines (TGFβ, IL-6, IL-1β, PAF)

Among the cytokines which have been demonstrated to be expressed at high levels in CSF and brain tissue from patients with ADC, TGFβ, IL-6, and IL-1β have the strongest experimental support for a role in ADC. Each will be briefly discussed in turn with respect to in vitro and in vivo evidence for its association with ADC.

TGFβ1. TGFβ is synthesized by nearly all cell types and comprises a gene family of at least three members: TGFβ1, TGFβ2, and TGFβ3 [175]. The prototype, TGFβ1, is a homodimeric protein of 25 kD whose sequence shows a marked conservation in mammals [247]. TGFβ1 shows a remarkable variety of effects on gene expression in vitro, including: (1) stimulation of extracellular matrix protein expression, adhesion molecules, and matrix receptors [117, 217, 220], (2) regulation of cell proliferation [247], (3) regulation of IFNγ, and (4) promotion of motor neuron survival [158]. Little is known about the intrinsic physiological role of TGFβ in vivo, although it may be important in embryonal differentiation as well as wound repair [reviewed in 247]. TGFβ1 is known to be expressed by astrocytes and macrophages, which may be the source within the brain [276, reviewed in 16]. Importantly, it may be a chemoattractant for macrophages and may inhibit HIV-1 replication in macrophages [275].

Elevations of TGFβ1 have been identified in the autopsied brains of patients dying with AIDS but not in control brains [276]. In this study, expression was detected in macrophages and astrocytes in areas of tissue pathology, although expression of TGFβ1 was not limited to infected cells, and the authors also demonstrated the release of TGFβ1 by both HIV-1-infected macrophages and cocultured astrocytes in vitro. Notably, however, none of these patients were known to be suffering from ADC. Because of the known variety of regulatory effects of TGFβ1 it was concluded that secondary effects of such expression in the brain may contribute to the neuropathogenesis of HIV-1 infection. In a provocative later study, Masliah et al. [163] demonstrated increased binding of labeled TGFβ1 to dendritic processes of neurons in the neocortex of autopsied brains with HIV encephalitis in comparison to controls, and suggested that these neurons might be targets for cytokine-mediated damage due to an abnormal pattern of TGFβ1 receptor expression. However, much work remains to define the primary and/or secondary effects of TGFβ1 in the CNS in ADC and a role for TGFβ1 in the pathogenesis of ADC currently remains purely speculative.

IL-6. IL-6 is another pleiotropic cytokine that is released by a variety of cell types including lymphocytes, monocytes, astrocytes, endothelial cells, and microglia [16, 268]. Both primary human and rodent astrocytes and astrocytic cell lines release IL-6, which in turn has been demonstrated to stimulate HIV-1 replication in macrophages [200, 268].

A number of studies have demonstrated elevations in CSF IL-6 levels in conjunction with other cytokines in patients with ADC [75, 145, 194]. Perrella et al. [194] found levels of 20–320 pg/ml in 25 of 30 ADC patients with varying degrees of neurological involvement ranging from stages 0.5 to 2–3, according to the criteria of Price and Brew [203] while IL-6 was undetectable in control patients with other neurological illnesses. Gallo et al. [75] found elevations of CSF IL-6 in 16 of 38 (42%) HIV-1-

infected patients and within this group, 4 of 5 patients with ADC had CSF IL-6 elevations. Laurenzi et al. [145] examined IL-6 levels in the CSF in 90 patients with inflammatory and noninflammatory neurological diseases, including 38 patients with HIV-1 infection at various stages. This group found levels up to 300 pg/ml in 80% of symptomatic AIDS patients [versus 50 pg/ml maximum in asymptomatic carriers (44%)] with similarly high levels found in other inflammatory conditions including meningoencephalitis, neuroborelliosis, and neurosyphilis.

Direct neurotoxic effects of IL-6 have not been described and its potential role in the pathogenesis of ADC thus also remains unknown. Qiu et al. [208] demonstrated that IL-6 could selectively enhance the intracellular calcium responses to NMDA in developing cultured rat cerebellar granule neurons by altered calcium influx and release from intracellular stores. Xin and Blatties [293] showed that firing rates of hypothalamic neurons in guinea pigs, with the consequence being induction of fever. Whether these preliminary results suggest neurotoxic responses in humans is speculative. One such scenario could be the stimulation of HIV-1 replication in macrophages by IL-6 [200, 268] with subsequent release of produce neurotoxins within the CNS, although direct evidence is lacking. It thus remains unclear whether IL-6 has a significant role in the pathogenesis of ADC.

IL-1β. IL-1β may also be increased in the CSF and brain tissue and cortical neurons may have increased levels of IL-1β receptors in patients with ADC [163, 193, 265, 289]. It is produced primarily by endothelial cells and microglia within the brain and by monocyte-derived macrophages infected with HIV-1 in vitro [82, 167, 294], and may induce proliferation of astrocytes [85]. It has been proposed to exert indirect neurotrophic effects through induction of release astrocytic factors and to our knowledge has not been clearly demonstrated to have neurotoxic effects. Its potential role in the pathogenesis of ADC remains undefined [193].

PAF. PAF has been demonstrated in the CSF of HIV-1-infected patients with neurological dysfunction ($\leq$850 pg/ml) and may also be released by HIV-1 infection of monocyte-derived macrophages [80]. Although a correlation between its presence and the development of ADC remains to be established, it may have neurotoxic effects at high concentrations. Gelbard et al. [80] demonstrated neurotoxicity in mixed primary human fetal brain cell cultures at concentrations ranging from 300 to 6,000 pg/ml in a dose-dependent manner, with partial blocking by NMDA receptor antagonists. Westmoreland et al. [285] demonstrated neurotoxicity in the NTera 2 human neuronal cell line at concentrations of 5 ng/ml in cultures containing only these neurons and not astrocytes or microglia. Thus, PAF may be considered another candidate neurotoxin in the pathogenesis of ADC for which direct and indirect mechanisms of toxicity may be functional.

Other Viral Proteins (Tat, Nef)

Tat. The viral transcriptional transactivator gene product, Tat, has been proposed as potential mediators of in vivo neurotoxicity based upon a number of in vitro studies in primary and transformed neuronal cell culture systems, as well as a limited number of in vivo rodent studies [77, 99, 131, 146, 151, 181, 221, 280]. Great interest in the potential of Tat protein as a neurotoxin was prompted by several reports demonstrating the ability of cells to take up exogenous recombinant Tat protein in a biologically active form [71, 153] and the ability of a release of biologically active Tat protein from HIV-1-infected cells [62, 63]. Ensoli et al. [62] demonstrated the release of Tat from acutely infected H9 cells (8–16 days postinfection) and Tat-transfected COS-1 cells under conditions that reportedly discounted the likelihood of cell death as the mechanism of release. The absolute levels of Tat released into the culture medium were not determined, however.

Many prior and subsequent reports demonstrated that extracellular recombinant Tat protein could be taken up by a variety of cell types and be transported to the nucleus in a form capable of transactivating the HIV-1 LTR [68, 71, 72, 132, 153]. However, achieving detectable levels of transactivation required either mechanical disruption of the target cells by scrape loading or the concomitant addition of the lysosomotropic agent chloroquine [71, 132, 133], which was postulated to prevent intracellular proteolytic digestion of Tat [71]. The release and uptake of biologically active Tat protein thus prompted a number of investigations into the potential modulating and toxic effects of Tat on a variety of neuronal and nonneuronal cell types. Tat has been shown to stimulate promoters for TGFβ1 [46, 297], TNFα [31, 226], G6PD [274], and IL-2 [284] and repress MHC class promoter activity [110]. In addition it may directly or indirectly stimulate expression of IL-4 receptors [205], procollagen and fibronectin [253], and inhibit antigen-specific T cell responsiveness [250]. In addition, Tat has been shown to transcellularly transactivate the HIV-1 long terminal repeat by direct cell/cell contact [101, 154, 155]. Thus, the variety of potential modulatory as well as toxic effects on a variety of lymphoid and nonlymphoid cell types has raised interest in

Tat as a potential effector protein within the CNS during HIV-1 infection.

Sabatier et al. [221] first reported the neurotoxic potential of recombinant Tat (IIIB, 86 amino acids) on murine glioma and neuroblastoma cell lines, cockroach giant interneurons, and in vivo in injected mice. These authors reported that micromolar concentrations of Tat killed the murine cell lines after several days of exposure, and that intracerebral injections of microgram quantities of Tat intraventricularly into mice resulted in tremors, seizures, paralysis, and death. Neurotoxicity was mapped to the basic region (Tat residues 49–57). Nonspecific membrane depolarization effects were demonstrated in cockroach giant interneurons and frog muscle fiber preparations and proposed to be a possible mechanism of induction of neurotoxicity. Finally, binding to rat brain synaptosomes was demonstrated, and this binding was also mapped to the basic region. This study thus established Tat as a potential neurotoxin within the CNS and prompted a number of independent studies.

A subsequent study by Weeks et al. [280] demonstrated that a Tat peptide containing the basic region (Tat 49–58) blocked the neurotoxic effects of full-length Tat (Tat 1–86), suggesting that the basic region of Tat was necessary for cellular binding and uptake and subsequent toxic effects. Magnuson et al. [151] found that Tat depolarized fetal rat and adult human neurons and was toxic to fetal human neurons at micromolar levels. Interestingly, NMDA receptor antagonists blocked the neurotoxic effects without having any effects on depolarization. These authors also suggested that Tat is directly toxic to neurons by activation of NMDA receptors, whereas gp120 indirectly activates such receptors probably via induction of release of metabolites from macrophages [148, 149]. Finally, Nath et al. [181] demonstrated that Tat at high doses (17 μM) were mildly toxic to human fetal neurons, and at very high doses (100 μM) Tat induced increases in the intracellular levels of Ca^{2+}. Again, these effects were mapped to the first Tat exon, which extends through amino acid residue 72 and includes the basic region. In both of these studies, the human brain cultures were at least 4 weeks old at the time of testing with Tat.

Our own studies of Tat effects in developing brain cell cultures contrasted with these studies in showing no direct neurotoxic effects in young (3 days old) embryonic cortical rat brain cultures, but rather striking effects on the neuronal and glial architecture [131]. We demonstrated that Tat induced neuronal aggregation and neuritic fascicle formation over 24–72 h in such cultures up to 2 weeks of age (during which time neuronal and glial migration normally occurs) and that this effect depended upon the RGD sequence within the second Tat exon. The changes were seen at submicromolar concentrations of Tat and were not associated with neuronal cell death. These effects were eliminated with mutations in the RGD (arginine-glycine-aspartic acid) sequence which substituted KGE (lysine-glycine-glutamic acid) and preserved the native charge ratio. These morphological alterations were consistent with effects on neuronal cell/substrate adhesion, consistent with Tat's ability to bind to certain types of integrin receptors or other cell surface proteins [11, 279].

A subsequent study by Orsini et al. [190] confirmed the neuronal aggregation-promoting effect of extracellular Tat utilizing primary embryonic rodent cerebellar neuronal cultures. In this study, the RGD sequence, cysteine-rich region, and the basic regions all contributed to these aggregation-promoting effects of Tat. Thus, Tat may have direct toxic effects or more subtle sublethal effects on developing neurons, and susceptibility appears to be largely dependent upon the age of the neurons. These two studies [131, 190] along with other reports of Tat interactions with integrin receptors and other surface proteins suggest that Tat may modulate neuronal cell function through interference with normal surface receptor function in addition to its well-described ability to transactivate promoters within the cell nucleus [44, 63, 71, 132, 153]. However, the requirement for high concentrations of Tat for neurotoxic effects in vitro and in vivo via cerebral injection again raises the question of how one might surmise that such Tat levels may be achieved in vivo during the course of infection. This central issue of Tat protein levels in vivo, as for pg120 levels in vivo, still awaits experimental confirmation.

Nef. Great interest in the possible role of the viral regulatory protein, Nef, in induction of neurotoxicity within the CNS began with the demonstration of Nef protein expression within the CNS of patients with AIDS [210, 222, 260]. Tornatore et al. [260] and Saito et al. [222] demonstrated that subcortical astrocytes in pediatric brain tissue were infected with HIV-1 and that such infection was marked by high levels of expression of Nef protein. Since Nef has some sequence similarities to scorpion toxin, it has been suggested to have potential neurotoxic effects in vivo and a possible role in the pathogenesis of ADC [282]. In the two aforementioned pathological studies, the prevalence of astrocyte infection ranged from <1 to 20%. Less than 1% of the astrocytes in 4 of 12 brains examined in the Tornatore study revealed evidence for HIV-1 infection, and only two of these showed Nef expression in the astrocytes [260]. Up to 20% of astro-

cytes in selected brain regions were found to be infected with HIV-1 in 3 of 8 brains in the Saito study, with one brain showing Nef expression in astrocytes without evidence for HIV-1 infection [222]. Finally, in a series of 14 autopsied brains from adult AIDS patients Rani et al. [210] found Nef expression in astrocytes in seven cases, six of whom suffered from moderate to severe dementia. Surprisingly, four cases showed 'late' gene expression *(Gag/pol* or *env)*, suggesting productive infection in the astrocytes. Importantly, this study showed no association between the expression of Nef protein or HIV-1 mRNA in astrocytes with the presence of multinucleated giant cells, microglial nodules, myelin loss or gliosis, implying that Nef expression in astrocytes itself might be a predictor of dementia. Furthermore, Blumberg et al. [19] demonstrated that *nef* gene sequences amplified from brain tissue showed a rate of 92% of sequences with open reading frames, again suggesting potential expression of Nef protein in a majority of infected brains in vivo.

These in vivo findings support the notion that HIV-1 infection in astrocytes is highly restricted, resulting in expression of multiply spliced mRNAs (including *nef*) due perhaps to a deficiency of Rev function in these cells [183, 259]. The consequences of such restricted infection in astrocytes remain largely unexplored, and it is interesting to speculate whether overexpression of Nef results in subtle alterations in astrocytic functions which normally subserve important neuronal cell functions and which may ultimately result in neuronal dysfunction and/or cell death [84, 109, 192]. Such sublethal alterations of neuronal and glial cell functions induced by expression of HIV-1 proteins within the CNS will likely remain the object of many future investigations [81].

Conclusions and Future Directions

It seems clear that the pathogenesis of neurological dysfunction in ADC is multifactorial, with no clinically and pathologically completely unique features other than the presence of HIV-1 within the brain. Perturbations in levels of endogenous cytokines and cellular metabolites, especially TNFα, IL-6, arachidonic acid, and QUIN and their effects on neuronal cells may be important to the pathogenesis of ADC. It is critical to correlate in vitro findings with in vivo evidence for expression of putative neurotoxins, and such evidence exists for each of these. Among these the currently most thoroughly supported is QUIN, based upon in vitro as well as in vivo evidence in a variety of model systems, although it is doubtful that any one (these or others not specified) is solely responsible.

The role of retroviral proteins (gp120, Tat, Nef) still awaits demonstration of a clear correlation between in vivo expression and potential neurotoxic effects, either clinically or pathologically. Because gp120 has been demonstrated to have a wide range of effects on neuronal and glial cells, either directly or indirectly, it has received the most attention and support among the viral proteins as a mediator of neurotoxicity, perhaps largely through the induced expression of the aforementioned cytokines and metabolites. Tat's role as a neurotoxin is also largely supported by in vitro evidence, with levels of expression in vivo remaining as a major question in its role in ADC. The recent demonstration of Nef expression in infected astrocytes in vivo has generated enthusiasm for Nef's potential role in ADC as well, although, unlike gp120, in vitro evidence for Nef neurotoxicity or neuromodulation is scanty. This will likely be a target of intense investigation in the future.

While the role of infection of macrophages/microglia in the brain in ADC seems secure, the possible role of restricted infection in other elements (astrocytes, neurons, and oligodendrocytes) remains controversial. Until recently, the generally accepted notion was that only macrophages and microglia were infected and this was based upon the current level of sensitivity of techniques for viral detection in vivo. More recent evidence has suggested astrocytic and neuronal cell infection, although detection of neuronal infection appears to require the sensitivity of in situ PCR amplification. Nonetheless, the final answer to the question of which cells in addition to macrophages/microglia are infected in vivo will depend upon systematic utilization of such techniques in autopsied ADC brains.

Finally, the natural history of ADC suggests that it is indeed a chronic neurodegenerative condition in many cases, and that model systems for study of its pathogenesis should provide for long-term analysis of neuronal and glial responses to HIV-1 infection. SIV infection in macaques provides a reasonable long-term model, although many in vitro cell culture systems fail to do so. As such, most in vitro neurotoxicity studies determine short-term endpoints of neuronal cell death which may not extend into a true in vivo parallel for chronic (months to years) neurodegeneration. With the exception of transgenic mouse models, virtually all cell and tissue culture model systems utilized to date in studies of HIV-1-induced neurotoxicity have targeted short-term (hours to days) effects on neuronal cell survival to identify potential neurotoxins

and mechanisms of neurotoxicity to explain the pathogenesis of ADC. Cell culture systems which may be extended for weeks and months must be developed to provide additional clues to long-term effects of HIV-1 replication within the brain which may induce distinct mechanisms of neuronal cell dysfunction and death that are beyond analysis in currently utilized systems. Only then can comprehensive rational therapeutic approaches for prevention and treatment of ADC be fully developed.

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