Human Immunodeficiency Virus (HIV) Proteins in Neuropathogenesis of HIV Dementia

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Human immunodeficiency virus (HIV) infection of the nervous system is unique when compared with other viral encephalitides. Neuronal cell loss occurs in the absence of neuronal infection. Viral proteins, termed “virotoxins,” are released from the infected glial cells that initiate a cascade of positive feedback loops by activating uninfected microglial cells and astrocytes. These activated cells release a variety of toxic substances that result in neuronal dysfunction and cell loss. The virotoxins act by a hit and run phenomenon. Thus, a transient exposure to the proteins initiates the neurotoxic cascade. High concentrations of these proteins likely occur in tight extracellular spaces where they may cause direct neurotoxicity as well. The emerging concepts in viral protein-induced neurotoxicity are reviewed as are the neurotoxic potential of each protein. Future therapeutic strategies must target common mechanisms such as oxidative stress and dysregulation of intracellular calcium involved in virotoxin-mediated neurotoxicity.

Before the advent of human immunodeficiency virus (HIV) infection, it was typically believed that the neurologic manifestations of viral encephalitis were due to direct viral infection of brain cells. For example, herpes simplex infects neurons in the frontotemporal lobe, rabies infects neurons in the limbic system, poliovirus infects the anterior horn cells, and JC virus infects oligodendrocytes and astrocytes in a multifocal manner. The clinical manifestations correlate well with the site of infection. However, in HIV encephalitis, there is widespread neuronal loss but the neurons themselves are rarely infected. Only perivascular macrophages and some microglia are productively infected while astrocytes have a restricted infection. Hence it was postulated that soluble products released from these cells must be toxic to neurons. Here I discuss several new and developing concepts about the role of viral proteins in cerebral dysfunction and review the role of each in neurotoxicity.

Terminology: Virotoxins and Neurotoxins

Many microorganisms release toxic substances including bacteria, fungi, and parasites. Some of the best studied include Clostridium botulinum, Clostridium tetani, cholera, pertussis, and anthrax. Thus, it should come as no surprise that viruses too are capable of producing toxic proteins. The terminology used to describe these toxins reflects their source of origin. For example, botulinum toxin and tetanus toxoid are released by C. botulinum and C. tetani, respectively, and toxic viral proteins are termed “virotoxins.” In contrast, neuroscience terminology has evolved from the target cells. Thus, “neurotoxins” are substances that injure neurons but are not necessarily produced by neurons. I will use both terms—virotoxins and neurotoxins.

Mechanisms of Release of Proteins into the Extracellular Environment

There are several mechanisms by which viral proteins may become available to the extracellular environment.

Release of viral proteins from a cytopathic infection. During a cytopathic infectious process, all viral proteins within the infected/disrupted cell become available to the extracellular environment. This occurs in HIV-infected lymphocytes.

Formation of viral proteins during a restricted viral infection. There may be a restricted expression of viral genes whereby some proteins are overexpressed but a nonreplicative state of the viral genome is maintained. For example, in HIV-infected astrocytes, the regulatory genes tat, nef, and rev are overexpressed. Furthermore, during the normal course of viral replication, not all structural proteins formed within infected cells become incorporated into the virus structure. These proteins are either degraded by the cells or are available for extracellular release.

Formation of defective viral particles. Not all viral particles formed by infected cells are replication competent. Thus, structural proteins of the virus in the form of defective viral particles may have access to and affect uninfected cells [1]. In fact, for animal and plant viruses, most viral particles produced are defective and not infectious.

Active release of viral proteins. Viral proteins, such as the HIV Tat protein, may be actively secreted by the cell [2].

Shedding of viral coat. When a virus infects cells, the core
particle enters the cell while the envelope either becomes incorporated into the cell membrane of the infected cell or may be shed outside [3].

**Interaction with viral proteins by cell-to-cell contact.** HIV proteins may interact with surrounding uninfected cells by cell-to-cell contact. For example, gp41 is a transmembrane protein that is expressed on the surface of infected cells, which may induce neuronal injury to cells in close proximity [4].

**Extracellular Space, Degradation, and Transport of Virotoxins**

*Extracellular space is a dynamic space.* Once viral proteins are released extracellularly, they have the opportunity to interact with uninfected cells to cause cellular dysfunction or toxicity. Their distribution in the extracellular space would be expected to be highest in close vicinity to infected cells. The extracellular space in the brain comprises nearly 20% of the total brain volume and the size of the space varies between regions. For example, extracellular space in the CA1 subregion of the hippocampus is one-half that in layer VI of the sensorimotor cortex. Furthermore, the extracellular space is dynamic and its volume is greatly influenced by the ionic composition of the surrounding cells. For example, increase in potassium following neuronal depolarization, decrease in pH, or alteration in sodium concentrations can lead to decreases in the size of the extracellular space by as much as 50% [5].

Factors such as tortuosity and glial cell swelling can also impact upon extracellular volumes. This is particularly germane for patients with HIV infection because astrocytosis and hypertrophy of astrocytes are early and prominent findings [6]. Therefore, the relative concentrations of neurotoxic substances can increase significantly without a corresponding increase in the total amount of toxin present in that area. Hence, techniques that involve the use of tissue extracts for determining viral protein concentrations likely underestimate the relevant in vivo concentrations.

**Factors influencing half-life of viral proteins.** The half-life of HIV-1 proteins in extracellular spaces will affect their neurotoxic effects. However, the actual half-life of HIV proteins in brain or in circulation has not been well studied. Several factors may control the half-lives of these substances: those that regulate its release into the extracellular space, inactivation by proteases, and cellular transport. To date, only the HIV-1 protein Tat has been shown to be actively secreted from HIV-1-infected cells by an energy-dependent process [2]. Once secreted into the extracellular space, HIV-1 proteins may be released into the circulation, may bind to cell surface receptors on uninfected cells, or may be taken up by infected or uninfected cells. gp120, Tat, and Vpr have been measured in the serum of HIV-infected patients [7–9]. These proteins bind to uninfected cells and Tat may also be taken up by uninfected cells [10]. The cellular uptake of Tat appears to be governed by the region formed by its second exon [10]. Of interest, Tat sequences from brains of patients with HIV dementia show glutamate substitutions in the second exon [11]; these likely decrease its ability to be taken up by cells and increase its extracellular concentrations.

**Transport of viral proteins across the blood-brain barrier (BBB).** Typically the BBB is impervious to large molecules and actively pumps out a number of small molecules and drugs. However, in HIV-infected persons there is a breach in the BBB [12] that may permit the influx of HIV proteins. Tat [13] and gp120 [14] can be found by immunostaining in the perivascular regions within the brain. Tat can be transported very efficiently across the intact BBB—so much so that investigators are now using Tat-derived peptides to deliver other large molecules to the brain [15]. gp120 crosses the BBB by absorptive endocytosis [16].

**Hit and Run Phenomenon with Amplification of Virotoxin Effects by Positive Feedback Loops**

Prolonged continuous exposure to the virotoxins is not necessary to disrupt neuroglial relationships or induce neurotoxicity. Rather, a transient exposure may be sufficient to trigger a cascade of events that eventually results in neuronal damage [17]. These virotoxins may cause neurotoxicity either by direct action on the neuronal cells or by activating glial cells to cause the release of cytokines, chemokines, or neurotoxic substances. These substances initiate several positive feedback loops. For example, release of chemokines such as monocyte chemotactic protein (MCP)-1 would lead to an influx of monocytes, which upon activation would lead to further release of cytokines, chemokines, and neurotoxic substances. Thus, virotoxins are able to amplify their neurotoxic potential and cause damage at distant sites. For example, injection of Tat in the striatum can lead to neuronal cell loss and gliosis in the substantia nigra [18].

The recognition that viral proteins may significantly amplify their toxic potential by interaction with other uninfected cells was a major advancement in our understanding of the pathogenesis of HIV dementia. Earlier, several investigators noted the dilemma that neuropathology of HIV dementia showed the presence of increased glial cell activation with only a few infected cells. The numbers of infected cells were thought unlikely to account for the widespread neuronal loss and the clinical manifestations of HIV dementia.

**Detection of Viral Proteins in the Brain**

There is no current technology for real-time measurement of viral proteins in central nervous system (CNS) tissues, and measurement or detection of viral proteins in autopsy tissue has several limitations. The tissue represents end-stage disease with cross-sectional analysis in time. Since the brain is exposed to viral proteins over months to years, these proteins likely have long been degraded, metabolized, or transported from CNS.
tissues. Further, viral proteins may rapidly degrade in autopsy tissue, especially in extracellular compartments. Earlier studies were also limited by cross-reactivity of antisera to viral proteins such as Tat, Rev, and Nef with normal brain tissue [19]. However, those technical problems were overcome by development of new reagents and refinement of immunostaining techniques. We have demonstrated the presence of both gp120 [14] and Tat in patients with HIV encephalitis, and the latter was confirmed by Western blot analysis and mRNA detection in the brain [13]. Tissue levels of gp41 [4] and Vpr are also elevated in patients with HIV encephalitis. Nef and Rev are expressed preferentially in HIV-infected astrocytes [20].

HIV Virotoxins: Evidence for and Mechanisms of Action (table 1)

gp120

HIV-1 gp120 is a potent neurotoxin (reviewed in [21]) with neurotoxicity selective for certain neuronal populations: Calbindin-containing neurons are resistant to gp120 toxicity [22], while dopaminergic neurons may be susceptible [23]. The neurotoxic actions of gp120 appear to be mediated indirectly via its actions on microglia and astrocytes [24], although direct action on neurons has been suggested [25].

gp120 can act on astrocytes to stimulate the inducible form of nitric oxide synthase (iNOS), inhibit β-adrenergic function, induce tyrosine kinase activity, induce the expression of adhesion molecules on glial cells, and produce cytoskeletal changes (reviewed in [21]). gp120 stimulates the Na+/H+ exchange system, which allows Na+ to enter the cell and H+ to flow from the cell, with a net result of intracellular alkalization [26]. Also, a decreased Na+ gradient leads to inhibition of Na+-dependent glutamate influx in astrocytes, which normally have high uptake capacities for glutamate [26]. gp120 also increases the release of arachidonic acid from astrocytes, which then inhibits the reuptake of glutamate by neurons and astrocytes [27]. The net result is an increase in the extracellular concentration of glutamate, which, through activation of excitatory amino acid (aa) receptors on neurons, could lead to neurotoxicity. The effects of gp120 on the Na+/H+ exchanger can be blocked by the widely available drug amiloride [26]. The key molecules on the astrocyte cell surface involved in interactions with gp120 need to be determined. However, a unique gp120 binding site with a molecular mass of ~260 kDa and Kd of 26 nM has been identified [28]. Other molecules implicated include a 65-kDa protein that is likely a monomeric form of the 260-kDa protein, CXCR4 expression, which can be induced on astrocytes by cytokines, and a transmembrane protein APJ.

Effect on astrocytes. The presence of increased amounts of glutamate leads to the excitation of the excitatory aa receptors and subsequently of the opening of voltage gated calcium channels. Calcium may also enter the cells through some excitatory aa receptors. Increased amounts of calcium in the cytoplasm cause disruption of mitochondrial function and activation of a number of cellular and apoptotic pathways. However, a clinical trial that used nimodipine to block the l-type calcium channels failed to show significant efficacy in patients with HIV dementia [29], although an earlier study showed that nimodipine could block gp120-mediated neurotoxicity [30]. My colleagues and I observed that although nimodipine could block gp120-mediated increases in calcium in neurons, it was unable to block similar calcium changes in astrocytes [31]. Another compound, memantine, a nonspecific glutamate channel antagonist, blocked gp120- and Tat-mediated calcium changes in neurons and astrocytes [31] and prevented neuronal cell death [32]. A clinical trial with this drug is underway in patients with HIV dementia.

Effect on monocytes and macrophages. gp120 induces the production of tumor necrosis factor (TNF)-α, interleukin (IL)-1, and arachidonic acid metabolites of the cyclooxygenase and lipooxygenase pathways [33]. Some of these effects on monocytes are mediated via gp120-CD4 interactions, and CXCR4 involvement has also been implicated [34]. Arachidonic acid metabolites and TNF-α are both implicated in producing neurotoxicity and in the pathogenesis of HIV encephalopathy (reviewed in [35]). gp120 can cause oxidative damage to monocytes as well [36]. Of interest, the cytokine tumor growth factor-b1 is protective against gp120-induced neurotoxicity [37].

Table 1. HIV gene products and their neurotoxic potential.

<table>
<thead>
<tr>
<th>Structural</th>
<th>Protein products</th>
<th>Neurotoxicity</th>
<th>Mechanism</th>
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<tbody>
<tr>
<td>env</td>
<td>Envelope proteins (gp120, gp41)</td>
<td>Yes</td>
<td>Acts on microglia and astrocytes, blocks glutamate uptake, releases cytokines and induces iNOS</td>
</tr>
<tr>
<td>gag</td>
<td>Core protein (p24)</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>pol</td>
<td>Reverse transcriptase, protease, endonuclease</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Regulatory</td>
<td>Transactivator of transcription (Tat)</td>
<td>Yes</td>
<td>Directs excitation of neurons; activates host genes after cellular uptake by glial cells, release of cytokines, and chemokines</td>
</tr>
<tr>
<td>rev</td>
<td>Regulator of viral RNA splicing and transport (Rev)</td>
<td>Yes</td>
<td>Basic region interacts with cell membrane causing its disruption</td>
</tr>
<tr>
<td>vpu</td>
<td>Viral protein U (Vpu)</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>vpr</td>
<td>Viral protein R (Vpr)</td>
<td>Yes</td>
<td>Inserts in cell membrane to form ion channels</td>
</tr>
<tr>
<td>nef</td>
<td>Negative factor (Nef)</td>
<td>Yes</td>
<td>Sequence similarity to scorpion neurotoxins; inhibits K channels</td>
</tr>
<tr>
<td>vif</td>
<td>Viral infectivity protein (Vif)</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: iNOS, inducible nitric oxide synthase.
gp41

gp41 is the transmembrane protein that links gp120 to the envelope of the virion. Patients with severe HIV dementia have significantly elevated gp41 levels in the brain. Further, gp41 causes elevations of iNOS in mixed neuronal-glial cultures leading to neurotoxicity [38] that requires the presence of glial cells. A peptide, corresponding to the carboxy-terminus of gp41, causes neurotoxicity via a depletion of glutathione and disruption of the mitochondrial function [39]. gp41 also stimulates the release of glutamate from astrocytes [40] and might contribute to excitotoxicity in neurons.

Tat

Tat is a nonstructural viral protein of 86–101 aa that is formed from 2 exons. The first exon contributes to the initial 72 aa and is a transacting nuclear regulatory protein that is essential for viral replication. Tat is also an important mediator of neurotoxicity (reviewed in [21]). Of interest, Tat sequences isolated from the brain of patients with HIV dementia are poor transactivators of the HIV long-terminal repeat but are potent inducers of the host genome (Widghal B, Power C, personal communication). However, unlike other HIV-1 virotoxins, Tat is the only viral protein actively released extracellularly by infected lymphoid [2] and glial cells [41]. Tat causes loss of selective populations of neurons in vitro and in vivo [18, 42]. Regions particularly susceptible to Tat neurotoxicity include the striatum [18], dentate gyrus, and the CA3 region of the hippocampus [43].

Effect on neurons. Tat can depolarize neurons by directly interacting with the neuronal cell membrane [42, 44]. In contrast, neurotoxicity induced by gp120 is mediated primarily by indirect mechanisms (see above). A unique feature of the electrophysiologic property of Tat is that it does not show any evidence of desensitization upon repetitive applications [44]. The degree of desensitization of glutamate receptors is inversely predictive of agonist toxicity [45]. Tat induces dramatic increases in levels of intracellular calcium in neurons [46]. There is an initial brief burst of intracellular calcium release through IP3 (inositol 1,4,5-triphosphate) sensitive pools followed by prolonged increases in cytoplasmic calcium resulting from an influx of extracellular calcium [47]. This is followed by mitochondrial calcium uptake, generation of reactive oxygen species, activation of caspases, and eventually apoptosis [48]. Tat-induced neuronal cell death can be prevented by excitatory aa receptor antagonists [42], inhibitors of voltage-dependent calcium channels, INOS and caspases, antioxidants, agents that stabilize mitochondrial membrane permeability, and IP3 pools of intracellular calcium [47]. Tat also inhibits nephrilysin, an enzyme on the neuronal cell membrane essential for degradation of amyloid b peptide (Pulliam L, personal communication). These findings may have relevance for the aging population with HIV infection.

Effect on microglial/macrophages and astrocytes. Tat has a number of effects on glial cell function. It stimulates the production of proinflammatory cytokines in the brain [49] and induces TNF-α and IL-1 in monocytes and macrophages and a milieu of cytokines and chemokines in astrocytes, including IL-8, RANTES, MCP-1, and TNF-α [50]. Most significant among these are TNF-α and MCP-1. In fact, Tat is more potent than even lipopolysaccharide in inducing TNF-α production [49]. Cytokine induction in both cell types is NFkB dependent [51]. Tat-induced TNF-α can mediate neurotoxicity [52, 53]. MCP-1 is a highly potent chemotaxant for monocytes. Levels of this chemokine are elevated in the cerebrospinal fluid (CSF) and brains of patients with HIV dementia [54]. Tat also induces matrix metalloproteinases (MMP) expression in astrocytes, which also facilitates monocyte transmigration by degradation of the extracellular matrix [55]. Together, these studies suggest that Tat may be an important mediator of the inflammatory response in the brain.

Tat can act as a substrate for adhesion and induces aggregation of neurons in culture [56]. The second exon of Tat contains a conserved Arg-Gly-Asp cell adhesion motif that binds to integrin receptors. The basic region of Tat in the first exon also contributes to its cell adhesive properties [57]. Tat may interfere with the normal development and migration of neurons as well as remodeling after a traumatic insult to the accessory cells by competing with extracellular matrix proteins. Tat also can also be taken up by uninfected cells [58] and may bind to a cellular transcription factor Pur-α [59], altering cellular function.

Nef

Nef is an important nonstructural protein required for proper budding of virions from infected cells. Nef expression has been detected in HIV-infected brains with neuronal damage. This expression is abundant within astrocytes of brains with HIV encephalitis where it is tightly bound to the cytoskeleton [60]. The presence of Nef sequences can be detected in neurons of the hippocampus. Nef can cause toxicity to neurons and glial cells [61], and intracellular expression of Nef can cause inactivation of a large-conductance potassium channel [62]. These properties have been likened to those of scorpion neurotoxins [62] with which it has significant sequence homology [63]. Nef can also induce the production of MMP and thus play a role in disruption of the BBB [64].

Vpr

Vpr is an important accessory protein of HIV that may exist in a soluble form. It is found both in serum and CSF [65]. Higher levels of Vpr are found in the CSF of AIDS patients with neurologic disorders. Extracellular exposure of neurons and astrocytes to Vpr may induce apoptosis [66]. Vpr also has the ability to insert into lipid bilayers, forming cation-selective ion channels.
In neurons this causes an inward sodium current that depolarizes the neurons [9]. The region in Vpr identified as a 40-peptide domain is necessary to form this channel [67] and might lead to neuronal dysfunction and cell death. In contrast, intracellularly expressed Vpr has strong antiapoptotic effects [68].

Rev

Like Tat, Rev is a transactivator of certain gene expression within the HIV genome. However, unlike Tat, Rev activity is mostly posttranscriptional. Extracellular Rev also has neurotoxic properties. Intracerebroventricular injection of a synthetic peptide spanning the basic Rev region was neurotoxic, leading to the animal’s death. The basic region of the Rev protein might interact with acidic phospholipids, similar to certain natural venoms, and form α-helical conformations. This interaction and conformational change of Rev might then be able to disrupt the membrane sufficiently to induce neurotoxicity [69].

Conclusion

Several new concepts have emerged in the neuropathogenesis of HIV infection, which collectively suggest a critical role for viral proteins in mediating the inflammation and neurodegeneration that accompany HIV encephalitis. Common mechanisms of neurotoxicity would be good targets for development of therapeutic approaches. The wide array of effects of viral proteins on the brain also suggests that both structural and regulatory proteins should be essential components of future therapeutic vaccine strategies.

References


