

# Pathogenic mechanisms of neuronal damage in the AIDS dementia complex

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It has become clear that infection with HIV-1 is capable of causing a progressive syndrome of neurological disease that is not strictly dependent upon the state of immunosuppression in the infected individual.<sup>1</sup> Although the central nervous system (CNS) is often the target of opportunistic infections in the later stages of AIDS related complex (ARC) or in AIDS itself, the AIDS dementia complex (ADC), or the HIV associated cognitive motor complex as it is otherwise known, is by far the most common cause of neurological dysfunction.<sup>2</sup> Neuropsychiatric abnormalities become most prevalent in the late stages of HIV-1 infection, and occur in 40–50% of adults and as many as 70–80% of children with clinically defined AIDS.<sup>3</sup> Rarely, the sole indication of HIV-1 infection is the involvement of the CNS.<sup>4,5</sup>

Patients with the neurological syndrome associated with HIV-1 infection portray a slow, progressive degeneration of both cognitive and motor functions that does not remit.<sup>6</sup> Frequently, it begins with mild symptoms of impaired concentration and difficulty in performing simple tasks that gradually increase in severity, leading to a major loss of intellectual capacity and complete motor disability. Finally, patients enter a nearly vegetative state that is terminal.<sup>7</sup> The time course for progression through the disease is variable and probably dependent upon unknown host or viral factors<sup>3</sup> but significant deterioration can occur in the course of two months to one year following the appearance of symptoms.<sup>6</sup>

## Neuropathological changes

The more severe cases of ADC are characterised pathologically by prominent microgranulomatous foci of multinucleate giant cells (MGC) that are initially found in the cerebral white matter and, as the disease progresses, these abnormalities are found more frequently in the grey matter.<sup>3,8</sup> The occurrence of MGC is accompanied by reactive gliosis, a term for an increase in both the size and number of astrocytes and the infiltration by cells of the monocytoid lineage, the brain derived microglial cells, and blood derived macrophages<sup>3</sup>; this condition is referred to as HIV encephalitis. Vacuolar myelopathy can also be a common pathological observation in affected brain but is more frequently found in the spinal cord.<sup>8</sup> The earliest lesions of vacuolar myelopathy consist of intramyelin swelling causing vacuolation, while severe cases also show evidence of gross demyelination and axonal loss. These lesions are characterised by a minimal inflammatory response with macrophages only occa-

sionally observed around sites of vacuolation.<sup>5</sup> The occurrence of vacuolar myelopathy is not predicted by the presence of HIV encephalitis as the two may occur independently, or concurrently, although MGC are found more frequently in patients with severe vacuolar myelopathy.<sup>9</sup> There is a third pathological condition identified by diffuse damage to the cerebral and cerebellar white matter that creates a general white matter pallor and has the characteristic traits of reactive gliosis, demyelination, and disseminated, perivascular infiltration by monocytic cells.<sup>10</sup> This is referred to as progressive diffuse leucoencephalopathy as it shows evidence of an intensifying, generalised degeneration of the white matter without gross inflammation.<sup>8,10</sup>

Radiological methods of assessing CNS damage detect some gross changes in affected patients. Computed tomography shows a rise in brain atrophy and ventricular enlargement with an increased signal from the white matter as the disease progresses.<sup>1</sup> The degree of cerebral atrophy, as judged by magnetic resonance imaging (MRI), correlates with the symptoms and progression of ADC,<sup>11</sup> indicating that the clinical features of dementia may parallel atrophy of specific regions of the brain.<sup>12</sup> MRI can also show abnormalities in additional regions of the CNS not clinically implicated by the symptoms and provide evidence of inflammatory changes.<sup>5</sup> Overall, ADC affects the subcortical regions before it affects the cortical regions of the brain and, therefore, clinically it is referred to as a subcortical dementia that leads to neuronal loss in selective regions of the brain.<sup>12-14</sup> Neurons are depleted in the orbitofrontal region of the cortex<sup>15</sup> and neuronal losses of 50–90% occur in the interneurons of the hippocampus.<sup>16</sup> However, neuropathology, neuronal loss, and clinical symptoms are not absolutely correlated as has been shown by postmortem examination of the brains of some patients suffering from AIDS or ADC.<sup>17</sup>

## Viral invasion of the CNS and the cellular targets of HIV-1 replication

The time at which HIV-1 gains entry into the CNS remains unclear. Aseptic meningitis is a dominant symptom of HIV-1 infection at the time of seroconversion.<sup>5</sup> Several reports document both an early intrathecal HIV-1 specific antibody synthesis<sup>18</sup> and an early recovery of the virus from cerebrospinal fluid (CSF),<sup>13,19</sup> suggesting viral entry recently after infection. This may be confirmed by one tragic case of accidental infection with HIV-1, where virus

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Accepted for publication  
4 February 1997

was detected in the brain after 15 days at post-mortem examination.<sup>17</sup>

By analogy to another lentivirus, Visna, free virus could cross the blood brain barrier following replication in cells of the choroid plexus<sup>20</sup> but a more widely held view is that HIV-1 is carried across by infected macrophages.<sup>5, 14</sup> This mechanism of infection is possibly facilitated by the enhanced ability of HIV-1 infected macrophages to induce adhesion molecules on the surfaces of capillary endothelial cells of the blood brain barrier, thus allowing their binding and ultimate penetration.<sup>14</sup> Brain capillary endothelial cells can be infected in a non-cytolytic fashion by HIV-1 and may also contribute to CNS invasion by producing virus for the infection of normal leucocyte traffic.<sup>21</sup> Once infected with HIV-1, macrophages produce high levels of the chemoattractant cytokines, macrophage inflammatory proteins 1 $\alpha$  and  $\beta$ , which might recruit uninfected T cells and monocytes to sites within the CNS and amplify the infection.<sup>22</sup> Despite the evidence indicating early entry into the CNS, ADC most frequently occurs late in the disease, raising the possibility that productive infection of the brain commonly occurs after a considerable time,<sup>13</sup> or that the CNS is reseeded, probably as the levels of circulating virus in the body increase late in the disease.<sup>14</sup> It can be speculated that the late appearance of the symptoms of ADC is due to a minor evolution of viral tropism for the CNS. The tropism of HIV-1 is determined primarily by the V3 region of the envelope glycoprotein, gp120, with all primary isolates of HIV-1 being able to infect both macrophage and activated T lymphocyte cell cultures. Two reports document CNS derived HIV-1 as possessing an altered V3 region in patients suffering from dementia.<sup>23, 24</sup>

Following the discovery of a close association between neurological dysfunction and HIV-1 infection, many studies were undertaken to determine which elements of the CNS became infected during ADC. Shaw *et al.*,<sup>25</sup> studying necropsy tissue, first reported that the brain harboured considerable integrated and unintegrated HIV-1 sequences with an abundance often exceeding that seen in lymphoid tissues, such as lymph nodes and spleen. The major infected cell type in the CNS supporting viral replication was found to be the macrophage or microglial cell.<sup>26-28</sup> The MGC found in HIV-1 encephalitis are similarly macrophage in origin<sup>10</sup> and probably result from the fusion of infected and uninfected cells.<sup>29</sup> Only in severe cases of HIV-1 encephalopathy are HIV-1 structural antigens found in a few neurons and astrocytes,<sup>29, 30</sup> although significant numbers of astrocytes support limited HIV-1 replication with the production of only the non-structural Nef and Rev proteins in patients who have suffered dementia.<sup>31</sup>

The consensus, however, is that there is insufficient viral replication within macrophages and not enough detectable virus in the CNS to account for the neurological dysfunction.<sup>5</sup> Generally, although the presence and frequency of infected cells correlates with

the neuropathological findings<sup>29</sup> and the clinical stage of ADC, the level of HIV-1 infection is still often less pronounced than the symptoms of dementia.<sup>13, 32</sup> These findings indicate that although productive brain infection, assessed by the CSF levels of the viral capsid antigen, is the closest correlate to the degree of neurological damage,<sup>33</sup> and antiretroviral therapy can delay or mitigate the symptoms of ADC,<sup>34</sup> the cause of CNS pathology and ultimately dementia while irrefutably linked to the presence of HIV, is only indirectly dependent upon the virus.<sup>1, 2, 5, 29</sup>

### Mechanisms of nervous system damage

Since HIV-1 infection of the CNS shows no direct replication associated pathology within neuronal, astrocytic, or oligodendroglial cells,<sup>26, 29</sup> what can the indirect methods of neuronal damage be? Research has demonstrated that one (or more) viral gene product is either directly or indirectly toxic to neuronal and astrocytic cells. In addition, brain macrophages and microglial cells that are virally infected become immunologically activated to produce high levels of cytotoxic, proinflammatory cytokines and metabolites with similar toxic properties that act on neurons, oligodendrocytes, and astrocytes.

#### VIRAL PROTEINS

Individual viral gene products of HIV-1, particularly the envelope glycoprotein, gp120, an extracellular protein shed from virions and infected cells, have profound effects on neuronal and astrocytic cell function and cause neurotoxicity around infected macrophages producing viral antigens.<sup>35-42</sup> The binding of free gp120 to neurons in culture causes their death via an interaction with receptors that also bind the neurotrophic factor, vasoactive intestinal peptide, that is implicated in promoting neuronal survival.<sup>35</sup> gp120, in the context of mixed neuronal-astroglial cultures, exerts potent and selective killing of hippocampal neurons<sup>40</sup> which also become depleted in the brains of ADC patients.<sup>16</sup> Only fragments of gp120 are required for neurotoxicity and their effects can be ameliorated by competition with an artificial peptide, peptide T.<sup>40</sup> In primary cortical cultures, the neurotoxicity of gp120 operates in a nitric oxide dependent manner that requires Ca<sup>2+</sup> and glutamate, the primary excitatory amino acid in the brain,<sup>41</sup> and occurs through the activation of voltage sensitive Ca<sup>2+</sup> channels and glutamate sensitive N-methyl-D-aspartate channels (NMDA), which leads to an unregulated Ca<sup>2+</sup> influx and neuronal death or dysfunction via an excitotoxic mechanism.<sup>41, 43</sup> Superoxide anions that are damaging to cells are also induced by gp120 in primary cortical cultures and play a role in mediating neurotoxicity.<sup>41</sup> Furthermore, the intracerebral expression of gp120 in transgenic mice produces a range of neuronal and astrocytic changes resembling those found in the human brain, the extent of which correlates with the level of gp120 expression.<sup>39</sup>

Astrocytes also possess receptors for VIP and gp120 may exert deleterious effects on their

physiology<sup>36</sup> or cause their death directly.<sup>37</sup> Astrocytes primarily regulate the ionic and solute concentrations of the extracellular space, including the levels of neurotransmitters, the alteration of which will disrupt neuronal function.<sup>44</sup> Indeed, exposure of astrocytes to gp120 stimulates Na<sup>+</sup>/H<sup>+</sup> antiport, K<sup>+</sup> conductance, and glutamate efflux.<sup>38</sup> Astrocytic Na<sup>+</sup>/H<sup>+</sup> exchange leads to intracellular alkalinisation which activates the glutamate efflux and K<sup>+</sup> channel activity in excess of the Na<sup>+</sup>,K<sup>+</sup>-ATPase that reabsorbs extracellular K<sup>+</sup>. Excessive K<sup>+</sup> in the extracellular space surrounding neurons activates their voltage dependent Ca<sup>2+</sup> channels and the increased glutamate levels activate NMDA channels.<sup>38-42</sup> The resulting elevation in neuronal Ca<sup>2+</sup> leads to depolarisation that, if prolonged, causes cellular dysfunction and death in a manner similar to the direct action of gp120 on neurons.

The viral transactivator protein, Tat, is toxic to neurons in vitro and intracerebral injection of Tat is lethal to mice.<sup>45</sup> The protein, like gp120, is secreted from expressing cells<sup>46</sup> and acts on neurons directly, causing their depolarisation and death.<sup>45-47</sup> In the case of Tat, the activation of both NMDA and non-NMDA excitatory amino acid receptors is implicated in neuronal toxicity.<sup>47</sup> Two other virion proteins, the envelope transmembrane protein, gp41, and the *gag* gene product, matrix, may be a further cause of CNS injury due to antibody cross-reactivity with surface epitopes on astrocytes.<sup>48-50</sup> The immunodominant epitope of gp41 is shared by astrocytes and astrocyte reactive antibodies are present in some patients with neurological complications.<sup>49</sup>

#### THE HIV-1 INFECTED, ACTIVATED MACROPHAGE

The productive infection of macrophages with HIV-1 primes the cell for immunological activation probably through an IFN $\gamma$ -like pathway even though there are relatively low numbers of IFN $\gamma$  secreting lymphocytes within the CNS.<sup>14</sup> The viral matrix protein has structural similarities with IFN $\gamma$ <sup>51</sup> and HIV may have developed mechanisms to mimic the action of the cytokine. Subsequent immunological activation of the primed, infected macrophage then occurs via direct interactions with astrocytes<sup>52-53</sup> (facilitated by the ability of gp120 to induce adhesion molecule expression on the surface of the astrocyte),<sup>54</sup> exposure to endogenous brain chemicals, such as endorphins,<sup>55</sup> or interaction with opportunistic infections already present in the CNS.<sup>14</sup> Once activated, HIV-1 infected macrophages produce significant amounts of the cytokines, TNF $\alpha$  and IL-1 $\beta$ , and the bioactive metabolites, eicosanoids, quinolinic acid, platelet activating factor (PAF), and nitric oxide, which are directly or indirectly neurotoxic.<sup>14-33-56-62</sup> The levels of most of these potential neurotoxins (TNF $\alpha$ , IL-1 $\beta$ , quinolinic acid, PAF, eicosanoids and the inducible form of nitric oxide synthetase) are known to be elevated in the CSF or brain tissue of patients with neurological disease.<sup>14-56-63-64</sup>

Of the cytokines, TNF $\alpha$  mediates neuronal damage and dysfunction at several levels. It is

known to upregulate HIV-1 replication and may act on infected macrophages in an autocrine manner<sup>55</sup> to increase the production of neurotoxic viral proteins, while at the same time it is cytotoxic to both oligodendrocytes and neurons by an apoptotic mechanism.<sup>60-61</sup> At subcytotoxic doses, but in the presence of a glutamate receptor agonist, it exerts neurotoxicity by activation of the neuronal  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) subtype of glutamate receptor channels,<sup>53</sup> and it has been noted that there is a loss of AMPA receptor protein in the brains of AIDS patients.<sup>66</sup> TNF $\alpha$  stimulates Na<sup>+</sup>/H<sup>+</sup> exchange in the membranes of astrocytes, in a manner similar to gp120, with identical neurotoxic potential<sup>57</sup> but, furthermore, it inhibits astrocytic glutamate reuptake, compounding the processes leading to unregulated Ca<sup>2+</sup> influx and neuronal damage via voltage dependent Ca<sup>2+</sup> channels and NMDA operated receptor channels.<sup>62</sup> IL-1 $\beta$  can also contribute to the degeneration of oligodendrocytes in conjunction with TNF $\alpha$ <sup>67</sup> and alone it promotes Na<sup>+</sup>/H<sup>+</sup> exchange in astrocytes.<sup>57</sup> In addition, IL-1 $\beta$  is the cause of astrocyte proliferation.<sup>68</sup> Quinolinic acid exerts direct neuronal toxicity via stimulation of the NMDA receptor channels and the subsequent increase in intracellular Ca<sup>2+</sup>.<sup>69</sup> Nitric oxide similarly produces neurotoxicity via NMDA receptors, although in itself it does not appear to do so directly, it first combines with the superoxide anion to form a toxic intermediate.<sup>14</sup> PAF is a lipid mediator that is also a potent neurotoxin which again acts via NMDA receptors<sup>58</sup> causing glutamate mediated excitotoxicity and inducing a rise in neuronal intracellular Ca<sup>2+</sup>.<sup>70</sup> PAF could further potentiate neurotoxicity indirectly as it is a potent inducer of TNF $\alpha$  and IL-1 $\beta$  production from macrophages.<sup>71</sup> Of the eicosanoids, prostaglandins E2, F2 $\alpha$ , and thromboxane B2 are elevated in the CNS of demented patients and are potent neuromodulators that promote cell injury.<sup>63</sup> Finally, arachidonic acid can also inhibit astrocyte reuptake of glutamate, promoting the potential for neuronal excitotoxicity via NMDA receptors.<sup>72</sup>

#### Conclusions

Current research has identified the infected macrophage or microglial cell as the initiator of neuronal dysfunction and death in ADC through the production of viral gene products, notably gp120, and diffusable, cellular neurotoxins, the synthesis of which becomes enhanced after interaction with resident astrocytic cells. Astrocytes serve a dual role in ADC, initially preventing neuronal damage by maintaining the extracellular microenvironment but, increasingly, as the numbers of infected macrophages rises amplifying the excitotoxic mechanism of neuron damage. The majority of viral or macrophage produced neurotoxins act directly or indirectly via voltage dependent Ca<sup>2+</sup> channels and NMDA receptor operated channels, a mechanism that is not unique to ADC, but also is believed to occur in other neurodegenerative diseases, such as Hunting-

ton's disease, Parkinson's disease and amyotrophic lateral sclerosis.<sup>43</sup> Clinically tolerated antagonists of these pathways, such as memantine, are undergoing trials as potential therapies for ADC.<sup>17, 43</sup> In addition, drugs that limit the synthesis of specific macrophage derived inflammatory products, PAF and eicosanoids, are also being developed<sup>14</sup> in the hope that they will ameliorate the symptoms of ADC more effectively than antiretroviral drugs alone.

I am grateful to Dr Alan Morris for helpful discussions and critical reading of a draft of this paper.

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