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Neuronal damage – recent issues and implications for therapy

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The spectrum of structural damage in the brain associated with HIV is now more fully understood. Such changes include inflammatory disorders (such as HIV encephalitis, leukoencephalopathy, and diffuse poliodystrophy), dendritic and synaptic damage, and neuronal loss. However, the relationship between neuronal damage and loss and clinical variables is still not clear. In my laboratory my research group has been addressing three separate areas, which will be the subject of this presentation. (1) The relationship of neuronal damage and loss to various clinical features such as cognitive symptoms, and risk group. (2) The cellular site of production of neurotoxic factors in the HIV-infected brain. (3) Assessing potential neuroprotective strategies using appropriate in-vitro models. *Journal of NeuroVirology* (2000) **6,** S103–S105.

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Clinicopathological correlations of neuronal damage and loss

With regard to the clinical relevance of neuronal damage and loss, ours and others work, has highlighted the possible temporal evolution of this pathology with the development of cognitive impairments. Neuronal pathology appears to commence with synaptic and dendritic damage followed by neuronal loss. Recently it was observed that cortical dendritic damage (Masliah *et al*, 1997) and synaptic loss (Everall *et al*, 1999) occurs in individuals with early cognitive impairment, and at this stage there is equivocal observable neuronal loss. Furthermore, the severity of this damage is greater in those brains with higher viral loads. Interestingly, Wiley *et al* (1998) have shown that viral load can vary amongst various brain regions. Thus, the relevance of brain region to the development of cognitive impairment needs to be clarified. In contrast, the development of dementia is accompanied by frank cortical neuronal loss. Asare *et al* (1996) reported that the development of mild dementia is associated with an alteration in pyramidal neurons, while progression to severe dementia also involved small interneurons. The characteristics of these affected neuronal populations is still under investigation. Currently, Eleanor Roberts and Sixolelwe Magena are investigating the relationship between density of calbindin and parvalbumin containing neurons with severity of dementia. These neuronal populations have been demonstrated to be affected in the HIV-infected brain (Masliah *et al*, 1992). Thus, we are assessing these neuronal populations in the frontal cortex and basal ganglia regions.

Recently, Eleanor Roberts has found that neuronal loss cannot be observed in intravenous drug users (IVDU), who either died of other causes while asymptomatically infected, or died of a HIV-related illness (Roberts *et al*, 1997). The reliability of this finding is supported by the fact that this observation has been noted in two separate brain tissue series of IVDUs, one from Edinburgh, donated by Jeanne Bell, and the other from Francoise Gray in Paris. This finding is important as it raises a number of questions. It suggests that neuronal loss is not necessarily a universal event, and that its occurrence may be linked to other factors, such as stage of HIV disease when dying. It is unclear as to whether IVDUs have a higher risk of dying earlier in the course of their HIV infection than other risk groups (Chaisson *et al*, 1995; Selwyn *et al*, 1992), but if this was the case this would indicate that neuronal loss

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may be a later event. Finally, a later occurrence of irreversible neuronal loss has therapeutic implications in that there may be a window of opportunity to prevent or delay the onset of neuronal loss and thus arrest the clinical development of HIV-associated dementia. At present such treatment is confined to antiretroviral therapy, which can penetrate the blood-brain barrier, and will be able to minimise the brain viral burden and protect against pathological events that would accompany the development of dementia. Clinically, the relationship between the lack of observable neuronal loss in IVDUs and the development of cognitive impairments needs to be addressed. While some studies have suggested that there are higher rates of cognitive impairment in IVDUs with HIV (Grassi *et al*, 1995; Starace *et al*, 1998) the rate of development of these deficits may not be higher than in HIV-infected gay men (Concha *et al*, 1997; Selnes *et al*, 1997). It may be that in the IVDUs the cognitive deficits are more closely related to viral burden than neuronal loss as Bell *et al* (1998) recently reported a correlation between neocortical viral productivity and cognitive impairments in IVDUs and also noted viral immunopositive staining was higher in the IVDUs compared to the gay men.

Cellular site of HIV neurotoxic factors in HIV-infected brain

The second aim that we are investigating is to identify which cell *in vivo* is responsible for producing toxic proteins that result in neuronal death. To date many putative toxic agents have been proposed, which may be produced from either astroyctes or microglia and macrophages, and *in vitro* tissue culture experiments have shown that these cells are capable of producing neurotoxic products (Giulian *et al*, 1996; Lipton *et al*, 1997). However, in order to demonstrate the relevance of these findings to the *in vivo* situation we have examined the spatial pattern of arrangement of neurons across the cortical ribbon in cases donated by Justin McArthur's group, in which the severity of the dementia was rated during life. This neuronal pattern is then compared to the spatial pattern of arrangement of astrocytes in the same cortical region. To date we have not found any relationship between these two cellular patterns. If astrocytes were the site of neurotoxin production, then a 'neuron-free halo' would be expected around the astrocytes. No such halo was detected (Roberts *et al*, 1999). While this finding indicates that astrocytes overall are not toxin reservoirs, it may be that the 1–3% of astrocytes which are infected with HIV are toxin-producing. We are proposing to investigate this infected subpopulation, and we are comparing the pattern of microglia/macrophages with neurons to assess their role *in vivo* with toxin production.

Potential neuroprotective strategies

Finally, Gusta Trillo-Pazos, in my group, has established a stationary human aggregate model to assess neuroprotective strategies against HIV. This is an important area for two reasons. Firstly, there is very little information on the neuro effectiveness of currently available antiretroviral agents, although clinical improvements in cognitive performance have been observed following combination antiretroviral therapy. Secondly, therapeutic strategies, which are independent of antiretroviral agents need to be developed which do not suffer from the problem of developing viral resistance. We have established a stationary human brain aggregates system similar to the rotating method devised by Pulliam *et al* (1988). This model provides a human *in vitro* brain tissue system which may be the closest approximation to the *in vivo* human brain, and thus relevant to investigating methods of neuroprotection. The aggregates have been characterised and the proportion of neurons, astrocytes, oligodendrocytes, microglia/macrophages have been determined (Trillo-Pazos and Everall, 1999). Currently, Gusta Trillo-Pazos and Apsara Kandanearatchi are exposing aggregates to HIV and we will test the ability of several anti-retroviral agents to prevent viral replication and neuronal damage. Other recent work in our laboratory has shown that nef, tat and gp120 are directly toxic to human primary neuronal cultures (Trillo-Pazos *et al*, 1999). In collaboration with Eliezer Masliah we have also shown that in human primary neuronal cultures, fibroblast growth factor (FGF) 1 and 2 protect against gp120-mediated damage. This work is being furthered by Chris Bell using both primary neuronal cultures and an aggregate system in my group.

Through the use of these different approaches, we aim to understand the consequence of neuronal damage, the *in vivo* mechanisms that are responsible for this damage, and the means to prevent or ameliorate this process by appropriate neuroprotective strategies.

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