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Neuronal apoptosis in human immunodeficiency virus infection

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Neuronal apoptosis has been shown to occur in HIV infection by a number of *in vivo* and *in vitro* studies, however, the cause of neuronal damage in AIDS is still unclear and its relationships with the cognitive disorders characteristic of HIV dementia remain a matter of debate. In this review, based on our experience, we analyse the techniques used to identify neuronal apoptosis on *post-mortem* AIDS brains and describe the relationships of neuronal apoptosis with the stage of disease, a history of HIV-dementia, the degree of productive HIV infection, microglial activation, blood-brain barrier involvement and axonal damage. We conclude that the severity of neuronal apoptosis in the cerebral cortex correlates with the presence of cerebral atrophy, but not with the cognitive disorders. There is no global quantitative correlation between neuronal apoptosis and HIV encephalitis, microglial activation or axonal damage. However we found some topographical correlation between these changes. We conclude that neuronal apoptosis and consequent neuronal loss, in HIV infected patients, are probably not related to a single cause. It seems likely that microglial activation, directly or indirectly related to HIV infection of the CNS, plays a major role in its causation possibly through the mediation of oxidative stress. Axonal damage, either secondary to microglial activation, or to the intervention of systemic factors may also contribute to neuronal apoptosis. *Journal of NeuroVirology* (2000) **6,** S38–S43.

Keywords: neuronal apoptosis; AIDS; HIV dementia; axonal damage; microglial activation

Introduction

Neurocognitive disorders are common in human immunodeficiency virus (HIV) infection. However, although the clinical characteristics of HIV dementia (HIVD) are now clearly established, its pathogenesis is unclear and its pathological basis remains a matter of debate. Indeed a variety of HIV-induced lesions of the central nervous system (CNS) have been described including HIV encephalitis (HIVE) due to productive infection of the CNS by the virus, HIV leukoencephalopathy mainly due to an involvement of the blood-brain barrier (BBB), and diffuse poliodystrophy affecting the grey matter, however, no clear correlation could be established between these changes and the cognitive disorders (Gray, 1998).

Neuronal loss has been demonstrated in AIDS patients by a number of morphometric studies (Everall *et al*, 1993) and it was postulated that HIV may cause cell depletion and tissue atrophy in the brain by inducing a programmed cell death (PCD) (Ameisen 1994), similar to its action on the immune system (Gougeon and Montagnier, 1993). This hypothesis was confirmed subsequently by several neuropathological studies in AIDS (Adle-Biassette *et al*, 1995; Gelbard *et al*, 1995; Petito and Roberts, 1995a; Shi *et al*, 1996; Vallat *et al*, 1998) and in pre-AIDS cases (An *et al*, 1996a). Experimental studies further tended to support these findings (Hery *et al*, 1997; Lannuzel *et al*, 1997).

The cause of neuronal damage in HIV infection is unknown. Local factors related to HIV infection of the brain are likely and the neurotoxicity of viral proteins, or substances produced by activated glial cells, or both acting in combination, has largely been demonstrated. On the other hand, involvement of the BBB has been shown to occur at different stages of HIV infection (Petito and Cash, 1992; Gray *et al*, 1996), and it was proposed that circulating factors, particularly cytokines, may also have an effect (Gray *et al*, 1998). Finally axonal damage was demonstrated in the brains of AIDS

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(Giometto *et al*, 1997) and, to a lesser extent, pre-AIDS (An *et al*, 1997) cases and it was proposed that it could play a causative role in neuronal apoptosis.

The initial studies failed to show any relationship between the severity of neuronal apoptosis and the presence of HIVE or a history of HIVD. We subsequently undertook a second more extensive study of AIDS cases who had been followed prospectively neuropsychologically, asymptomatic HIV positive individuals who died accidentally, and seronegative controls (Adle-Biassette *et al*, 1999). The aim of this study was to characterize the distribution and abundance of apoptotic neurons and their relationship to the stage of the disease, to any history of cognitive disorders, and to the degree of productive HIV infection, microglial activation and axonal damage in order to better understand the etiopathogenetic mechanisms of neuronal damage in HIV infection. This review is mostly based on the results from this study.

Results

Correlation of neuronal apoptosis with stage of disease

Neuronal apoptosis was identified in the vast majority of AIDS patients in the different studies and it was severe only in these cases. No significant neuronal apoptosis was found in the controls. This confirms the view that neuronal apoptosis is a feature of AIDS. Neuronal apoptosis seemed to correlate closely with neuronal loss and there was a positive correlation between neuronal apoptosis in the cerebral cortex, and cerebral atrophy (Figure 1).

Although no significant neuronal loss was found at that stage (Everall *et al*, 1992), occasional apoptotic neurons were also identified in a few pre-AIDS cases (Adle-Biassette *et al*, 1995,1999; An *et al*, 1996a). This suggests that neuronal damage is an early event in HIV infection and may be secondary to microglial activation or axonal damage which have been shown to occur already at this stage (An *et al*, 1996b,1997).

Correlation of neuronal apoptosis, HIVD and HIVE

In our last study (Adle-Biassette *et al*, 1999), changes characteristic of HIVE were found in six of 20 cases and there was a significant association with HIVD; indeed the only three patients with HIVD had HIVE. This confirms a previous finding of abundant viral load in the brains of patients with HIVD (Wiley and Achim, 1994). On the other hand, two patients with HIVE had presented with cognitive disorders that did not fulfill the criteria of HIVD and one had no recognizable cognitive disorder. This is in keeping with more recent studies which did not find significant correlation between the viral load evaluated by immunocytochemistry or quantitative PCR, and dementia (Glass *et al*, 1995; Johnson *et al*, 1996; Lazarini *et al*, 1997).

Microglial activation was identified both by major histocompatibility class 2 antigens (HLA-DR) and cytokines (tumor necrosis alpha and interleukine -1 (IL-1)) expression. In all the cases, the intensity and topography of HLA-DR and cytokine expression were remarkably superimposable. Microglial activation was constantly found in AIDS patients and severe microglial activation was only found in that group. All the cases with HIVE, which included all the cases with HIVD, invariably showed marked microglial activation, consistent with a previous report that the presence of macrophages and microglia is a good correlate for HIV dementia (Glass *et al*, 1995). However, severe

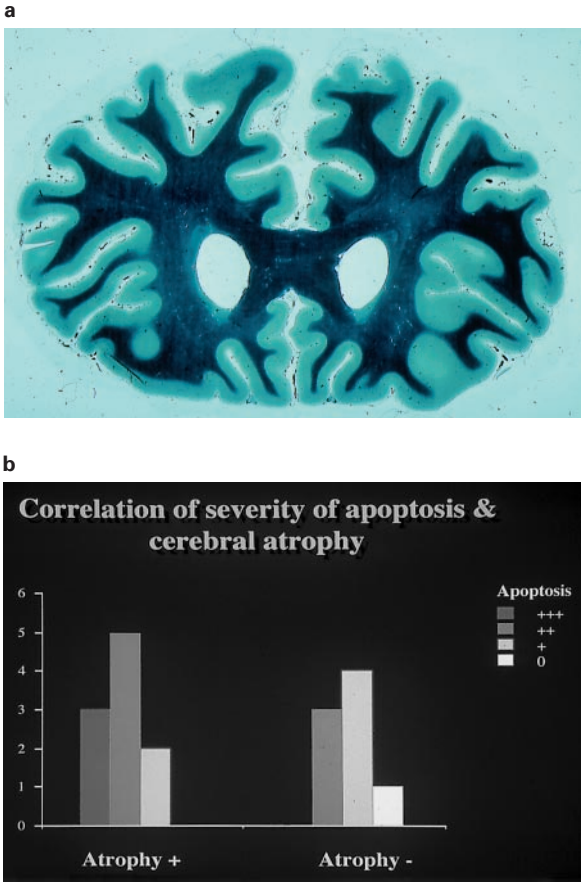

Figure 1 Neuronal apoptosis and cerebral atrophy. (**a**) Coronal section of both frontal hemispheres at the level of the rostrum of corpus callosum in a 35 year-old AIDS male without HIVE, opportunistic infection, or tumour in the CNS, showing marked frontal atrophy and ventricular dilatation (Loyez stain for myelin). (**b**) Correlation of the severity of neuronal apoptosis in the cerebral cortex (frontal cortex at the level of F1 and medial temporal cortex) with cerebral atrophy, in 18 AIDS cases without focal opportunistic infection or tumour in the CNS. Cerebral atrophy was evaluated semiquantitatively on myelin stains of large macroscopic sections of celloidin embedded specimens and correlated with premortem radiological examination as previously described (Gray *et al*, 1996).

microglial activation was not restricted to this group; it was also found in AIDS cases without HIVE, as in cases with atypical or no cognitive disorders. As already reported (An *et al*, 1996b), obvious microglial activation was also present in pre-AIDS cases.

Confirming previous studies, no correlation was found between the overall density of apoptotic neurons and a history of cognitive disorder, or the presence of HIVE. These results are consistent with morphometric demonstration that neuronal loss, in the cerebral cortex, is not restricted to patients with HIVE (Everall *et al*, 1993) and does not correlate with HIVD (Seilhean *et al*, 1993; Everall *et al*, 1994). In contrast, in the basal ganglia, there was some quantitative, and obvious topographic correlation between neuronal apoptosis and HIVE. This supports morphometric evidence that neuronal density, in the basal ganglia, is decreased in HIV infected patients, especially in those cases with HIVE (Everall *et al*, 1995). It was only in this area that we could identify immunologic NO synthase (iNOS) and superoxidismutase (SOD) expressing cells within foci of HIVE, consistent with recent demonstration that HIV protein and cytokine neurotoxicity may be mediated by oxidative stress and cause neuronal apoptosis (Adamson *et al*, 1996; Shi *et al*, 1998). It is also noteworthy that peroxide expression was only detected in our three demented patients. This is consistent with recent observation that expression of iNOS and SOD is significantly increased in demented AIDS patients compared to non-demented (Boven *et al*, 1999). Our findings also support the view that dysfunction of the basal ganglia is associated with HIVD (Berger and Nath, 1997).

Correlation of neuronal apoptosis and axonal damage

Confirming previous studies (Giometto *et al*, 1997; An *et al*, 1997) axonal damage was extremely frequent in AIDS cases and was also present in pre-AIDS cases. It was frequently associated with white matter pallor which is considered a consequence of BBB alteration (Power *et al*, 1993) and similar mechanisms may be proposed in their causation, particularly the role of proinflammatory cytokines. The role of locally produced cytokines was suggested (Giometto *et al*, 1997) and this is consistent with the frequent observation, in our cases, of axonal dilatation and spheroids within foci of HIVE and activated microglia. The role of systemic cytokines was also considered in the causation of diffuse BBB involvement and axonal damage in HIV infection (Gray *et al*, 1998).

Although no overall correlation was found between neuronal apoptosis and axonal damage, there was obvious topographical correlation. In the basal ganglia, apoptotic neurons were particularly abundant in cases with HIVE and there was a constant association with microglial activation, peroxide expression and axonal damage. In the brain stem, neuronal apoptosis was often topographically related to microglial activation and axonal damage. Axonal dilatations were present in the ponto-cerebellar (Figure 2a) whereas apoptotic neurons were identified in the surrounding pontine nuclei (Figure 2b). In the cerebral cortex, microglial activation and axonal damage were rarely found in the grey matter, close to apoptotic neurons. They were more often identified in the underlying subcortical white matter. In other instances, particularly pre-AIDS cases, neuronal apoptosis in the cerebral cortex seemed to be topographically

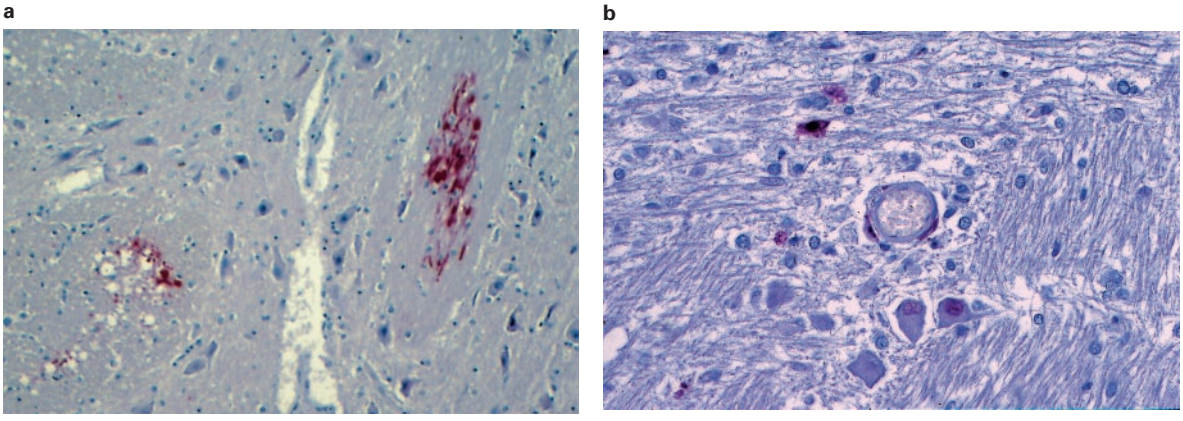

Figure 2 Topographic relationships between axonal damage and neuronal apoptosis in the pons of an AIDS patient. (**a**) Immunocytochemistry for βAPP (monoclonal, Boehringer, 1/200, revealed by an indirect immunoalkaline phosphatase method), showing axonal damage presenting as bundles of positive axonal dilatation in the ponto cerebellar fibres ($\times 250$). (**b**) *In situ* end labelling positively stains one neuron with morphological features of apoptosis and the nucleus of one normal-looking neuron in the pontine nuclei, in the same area (ApopTag kit, Oncor, $\times 400$).

related to axonal damage in the subcortical white matter, even in the absence of obvious microglial activation. These topographical correlations support the view that axonal damage could contribute to neuronal apoptosis either via retrograde degeneration (Groves *et al*, 1996) or by inducing deafferentation of neurons (Saji and Reis, 1987). This would be consistent with the description of synaptic and dendritic simplification in the brains of AIDS patients with severe HIVD (Masliah *et al*, 1997) as in those with mild to moderate neurocognitive disorders (Everall *et al*, 1998).

Conclusion

Our studies suggest that neuronal apoptosis and consequent neuronal loss, in HIV infected patients, is probably not related to a single cause. It seems likely that microglial activation, directly and/or indirectly related to HIV infection of the CNS, plays a major role in its causation, possibly through the mediation of oxidative stress. This mechanism is probably predominant in the basal ganglia where neurons are in close topographical relation with changes of HIVE and where HIV load has been shown to be higher than in the cerebral cortex (Wiley *et al*, 1998). However, in other instances when neuronal apoptosis occurs at a distance from, or in the absence of, changes of HIVE, different mechanisms may be postulated and it seems likely that axonal damage, either due to local microglial activation or to the intervention of systemic factors, also plays a causative role.

Finally, although massive neuronal loss may, in rare instances, be responsible for HIVD (Gray *et al*, 1991), neuronal apoptosis is certainly a late event and does not represent the main pathological substrate of the cognitive disorders. HIVD more likely reflects a specific neuronal dysfunction resulting from the confluence of several etiopathogenetic mechanisms, some of which may be reversible.

Methods

Demonstration of apoptotic neurons on postmortem brain tissue

The demonstration of apoptotic neurons on postmortem brain tissue was mostly based on the technique of *in situ* end labelling (ISEL) of the oligonucleosomes with labelled nucleotides (Gavrieli *et al*, 1992; Mighell *et al*, 1994) which is more appropriate than electron microscopy and gel electrophoresis of extracted DNA, the methods of reference, to assess apoptosis in tissue sections. ISEL has been shown to be a reliable and reproducible technique, not influenced by post-mortem delay under 72 h (Petito and Roberts, 1995b), and by formalin fixation under 5 weeks (Davison *et al*, 1995); it also allows interpretable clinico-pathological correlation on post-mortem material in spite of variations in agonal events (Adle-Biassette *et al*, 1998). In our first study, positive ISEL was correlated with electrophoresis of extracted DNA which showed the characteristic 'laddering pattern' (Adle-Biassette *et al*, 1995). In all our cases, positive *in situ* end labelling was frequently associated with morphological changes characteristic of apoptosis including shrunken cytoplasm, condensation of nuclear chromatin and its disintegration into apoptotic bodies. Endothelial cells which have a rapid turnover and therefore often undergo apoptosis, served as positive internal controls.

Acknowledgements

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