A Review of Neuronal Damage in Human Immunodeficiency Virus Infection: Its Assessment, Possible Mechanism and Relationship to Dementia

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Abstract. Over the past decade it has been realized that HIV affects the central nervous system, and various investigations have illuminated the spectrum of neuropathology in AIDS. One major advance has been the demonstration that there is substantial neuronal loss, which appears independent of the HIV-associated inflammatory lesions. Quantitative studies on neuronal populations, while fraught with methodological difficulties, are essential to the understanding of the mechanism of this neurotoxic damage. This article will review, firstly, the modern stereological procedures available for quantitative investigations; secondly, the pattern, degree and time scale of HIV-associated neuronal loss; thirdly, other morphological evidence of neuronal damage; and finally, the pathological and clinical implications of these findings.

Key Words: Dissector; HIV encephalitis; Neuronal loss; Stereology.

INTRODUCTION

It has been realized, early in the acquired immune deficiency syndrome (AIDS) epidemic, that the nervous system is frequently affected (1). In addition to opportunistic infections, neoplasms and vascular complications in the central nervous system (CNS), a group of diseases has been identified which is thought to be caused primarily by HIV-1. These new diseases have been defined in a consensus report as HIV encephalitis (HIVE), HIV leukoencephalopathy, vacuolar leukoencephalopathy and vacuolar myelopathy, diffuse poliodystrophy, lymphocytic leptomeningitis and cerebral vasculitis, including granulomatous angiitis (2). Multinucleated giant cells, pathognomonic of HIV-1 infection, have been observed in the brain (3); immunocytochemistry, in situ hybridization, polymerase chain reaction and electron microscopy have all demonstrated the presence of HIV-1 (4).

Clinically, a frequent consequence is a neuropsychiatric disorder originally termed AIDS dementia complex (5, 6) and more recently referred to as HIV-1-associated cognitive–motor complex (7). This is characterized by cognitive impairment, personality change and motor dysfunction. In an early extensive clinicopathological review the severity of the dementia was related to the degree of inflammatory response in the brain. Patients with moderate to severe dementia all had multinucleated giant cells, and the virus could also be detected in the brain. Patients with a mild to moderate degree of dementia, however, did not have multinucleated giant cells in their brains at autopsy and viruses were also rarely demonstrated. Since this study, the concept and definition of dementia have been refined, nevertheless these early investigations substantially helped the understanding of the cerebral substrate of dementia.

It is therefore surprising that despite dementia being recognized so early after the beginning of the epidemic, very little has been known, until recently, about the effects of HIV-1 on neuronal populations. This initial lack of information on neuronal loss can be explained by the technical difficulties of neuronal quantitation in post-mortem brains. The aim of this review is to summarize the modern techniques for quantitation, to consider the evidence for neuronal loss, to discuss the possible mechanism of this neuronal damage and to assess its role in the cognitive impairment of AIDS patients.

OUTLINE OF STEREOLGICAL PROBES

Traditionally, prior to the modern stereological methods, the use of a two-dimensional test grid, of a defined area with a known number of points and lines, was a standard means of quantitation. A number of methods were also developed, adapting areal grids, for determining the number of neurons or other objects per unit volume (8). However, none of these methods overcomes the fact that data are acquired from a two-dimensional counting grid and extrapolated into three-dimensional data. The disadvantage of this extrapolation is that assumptions have to be made about the cells or other objects under investigation; for example, that there is uniformity of cellular shape and size. Obviously, neurons are not uniform. The last 10 years have witnessed new techniques to improve estimations in biological material. The most important developments are, firstly, the introduction of stereological probes of differing dimensions which includes the dissector principle; secondly, the concept of unbiased estimation; and thirdly, alternative methods of
estimating volume. Basically, there are four stereological probes: points, lines, planes and the dissector. They differ in being specifically sensitive to measuring a certain parameter. The law concerning the particular dimension of an object that a probe will be sensitive to measuring is given by \( d = n - k \), where \( d \) = measured dimensional characteristic of the object, \( n \) = the dimension of the object's embedding space, and \( k \) = the dimension of the probe. Hence, points, a zero-dimensional object, can in a three-dimensional embedding space be sensitive to measuring three-dimensional volume. Similarly, the only effective probe sensitive to estimating number, which is a zero-dimensional parameter, is the dissector, a three-dimensional probe in a three-dimensional embedding space.

Essentially, the dissector is a pair of identical section planes with test grids, a known distance apart. The reference space between them contains the objects to be sampled, hence objects can be counted in three dimensions. Practically, the procedure can be carried out by means of a physical dissector, where two physically separate consecutive sections are examined to determine the number of objects contained between, or by an optical dissector, where the two section planes are then obtained by optically dissecting within a relatively thick histological section. This second method eliminates the problems of optically lost caps and the Holmes effect. Furthermore, there is a statistical advantage in that objects are sampled in an unbiased manner, as each object has equal probability of being sampled regardless of size or shape. In contrast, previous techniques sampled an object directly proportional to its height. Techniques, such as orientating tissue blocks to produce "vertical sections," have also been recommended in order to overcome the bias of estimating cell size in an anisotropic tissue such as the brain. Consequently, previous methods have always resulted in biased estimates of taller or larger objects.

The dissector can be further adapted to provide estimates of cell volume. This is by use of the nucleator where all sampled neurons are measured directly, using a cubed ruler. The ruler is applied from nucleolus to cell membrane and repeated in the opposite direction. The average of these two estimates is an unbiased estimator of the volume. As it is used in conjunction with the dissector, the nucleator combines all the advantages of unbiased sampling together with object measurements along independent directions.

The new stereological probes outlined, the dissector and the nucleator, provide three-dimensional and sensitive methods of analyzing particle number and size. Furthermore, they have overcome inherent difficulties such as optically lost caps, the Holmes effect and estimations in anisotropic tissue. Thus, they are particularly appropriate for the investigation of neuronal loss in HIV infection, since they can detect subtle changes that could be missed by two-dimensional methods of estimation.

**NEURONAL POPULATION STUDIES**

Prior to our own stereological studies there was a preliminary morphometric analysis of neuronal density and perikaryal volume of Brodmann's area 11 of the fronto-orbital region in 18 unseleced AIDS brains. A significant neuronal loss, 18%, was found in the AIDS brains compared with controls. Moreover, the perikaryal volume fraction was stated to be decreased by 31%, although as the area of neuronal profiles did not change significantly, this volume decrease probably reflected the change in neuronal density. The authors speculated that the substantial neuronal loss could represent a part of the morphological substrate of dementia, although the interpretation of the results remains ambiguous. The brains had been unseleced and affected by the usual, wide range of opportunistic infections, malignant lymphomas and diseases thought to be primarily caused by HIV-1, including HIVE. Thus, they could not exclude the possibility that the multitude of pathologies in these brains was the cause of cortical changes.

Although not addressing the question of neuronal loss, a morphological and immunocytochemical study has drawn attention to other cortical changes in HIV infection. An increased activity of both astrocytes and microglial cells was found in the frontal lobe in all, including asymptomatic, HIV-infected cases. The degree of these glial changes could be correlated with the severity of lesions in the white matter. HIV core antigen (p24) was only present, as in previous studies, in the cells of microglia-macrophage lineage.

The report of a single case of a 25 year-old patient with AIDS who had progressive cognitive, motor and behavioral disturbance, and diffuse cerebral atrophy on CT scan revealed striking neuronal loss, which was not quantified, in the absence of severe pathology in the white matter and basal ganglia. This finding indicated that neuronal loss in the cortex is not necessarily associated with white matter damage, but might be caused directly by HIV. Interestingly, neuronal loss has also been observed in areas outside the cerebral cortex, including the substantia nigra and the cerebellum. However, these reports did not have the same systematic quantitative analysis as the cerebral cortex.

Thus, a growing body of evidence indicated that the cortex was damaged by HIV infection which could involve neuronal loss. For clarification, the stereological probes the dissector and the nucleator were used by our research group for the estimation of neuronal number and size changes in HIV-1-infected brains. Initially, the neuronal numerical density of the superior frontal gyrus was estimated in 11 symptomatically infected individuals and eight controls. Importantly, none in the HIV...
group had evidence of CNS opportunistic infections or neoplasms but did show features characteristic of HIV or only minimal pathology (mild perivascular cuffing or astrocytosis). There was a significant 38% reduction in the neuronal numerical density in the HIV-infected group and this occurred regardless of the presence of HIV. Further studies have demonstrated that there are varying degrees of loss in other neocortical areas (19): 30% loss in the primary visual area of the occipital lobe and 18% decrease in the superior parietal lobe, but no change in the inferior temporal gyrus. In all areas where the loss occurred this was noted even in the absence of HIV. Replication of the frontal and temporal areas using computer-assisted image analysis yielded very similar results (20).

Following the demonstration of neuronal loss in patients with symptomatic HIV disease it was important to clarify at what stage this loss occurred. An investigation was undertaken of a series of 29 drug-users who had died of opiate overdose or gunshot wounds. Fourteen were symptom-free HIV-infected, while the others were used as controls. Neuropathologically, none had evidence of HIV-induced disorders (21). Quantitative estimation of the neuronal numerical density of the superior frontal gyrus revealed no evidence of loss compared to controls (22). In contrast, the same area in the symptomatic series had demonstrated 38% neuronal loss (18).

Currently, a study of neuronal size, using the nucleator, has revealed that, apart from the inferior temporal area, there is a shift in the neuronal volume in the HIV group from smaller to larger neurons (19). This shift may be due to neuronal damage, possibly prior to loss. Other evidence of neuronal damage includes the 40–60% decrease in dendritic spine density in the frontal cortex (23).

**EXTENT OF NEURONAL DAMAGE**

Having demonstrated that neuronal loss is a feature of HIV infection, investigations have now turned toward clarifying the extent of this damage. In an extensive study neocortical damage was analyzed in 32 brains (24). The neocortical width in the brains with HIV was reduced by 20% when compared to HIV-seropositive controls. Moreover, there was a significant 30–50% decrease in the number of large neurons in the mid-frontal, inferior parietal and superior temporal areas. Immunostaining for synaptophysin showed focal areas of reduced granular reactivity in the frontal cortex, most obviously in layers 2, 3 and 5, in cases of HIV, with an average 25% decrease in presynaptic terminal staining. Dendritic abnormalities included loss of branching and tortuosity in the areas of fine vacuolation of HIV brains. While the validity of these findings is not in question, all these changes were associated with HIV, and the possibility that they have been caused by this inflammatory process rather than directly by the virus itself cannot be excluded. Immunocytochemistry for parvalbumin and neurofilament proteins has revealed selective vulnerability of neurons in HIV (25). In the frontal cortex density of such proteins was not influenced by the severity of HIV, while in the hippocampus there was significantly decreased density of parvalbumin-positive neurons in the CA3 area. This decrease in the hippocampus correlated well with the severity of HIV. These findings indicate that HIV may involve specific subpopulations of neurons. The mechanism of this damage remains to be established, but the authors stipulate that parvalbumin-positive neurons and their processes may be affected by cytokines released by infected microglial cells. To characterize further the dendritic abnormalities, a modified Golgi impregnation study in the frontal cortex revealed that in HIV brains the dendrites were dilated with vacuolated and tortuous apical dendrites whose length and branching had been reduced. Spine density was reduced by 40–60% along the entire length of the dendrites (23). While confirming a role for HIV in primary dendritic damage, further investigations by the same group (26) have shown strong correlation with the level of HIV gp41 envelope protein. Thus the presence of HIV in the cortex rather than multinucleated giant cells, which are indicative of HIV, may be responsible, either directly or indirectly, for the neuronal damage and loss. In fact, HIV has been detected both in the cortex and white matter more often than would be expected by immunocytochemistry (27).

**PATHOLOGICAL AND CLINICAL IMPLICATIONS**

There are both pathological and clinical conclusions to be drawn from the findings of the stereological studies. Neuropathologically, there is substantial neuronal loss in patients with HIV disease, the degree of this loss appears to be an independent process from the encephalitic disorder. The current nomenclature (2) only recognizes the possibility of neuronal loss within the descriptive category of diffuse poliodystrophy, which may occur in up to 43% of cases, while HIV is thought to be found in only 24% (2). Overall, it seems that the presence of multinucleated giant cells, or HIV, is not an accurate marker of neuronal loss.

The CNS is an early target of infection (28). However, it is not clear whether this reflects a limited meningeal involvement or whether there is also infection of the brain parenchyma (29). Either way, virus recovered at this stage is capable of only slow replication and low levels of viral RNA in infected cultured cells (30). The low level of virus replication may explain the lack of neutralizing antibodies in the cerebrospinal fluid at early stages of infection (31). Over time HIV changes to rapid replication and high titres, and at this stage the virus is thought to replicate in the brain (32). This is concomitant with progression

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to HIV-related disease and also heralds the emergence of HIV variants with increased virulence, including proliferation in monocytes and macrophages (33). It is at this symptomatic stage that inflammatory lesions are observed and virus can be identified within them.

The establishment within the brain of virulent viral strains during the progression of HIV-related disease provides the foundation for cytopathic events producing neurological abnormalities. Current models of neurotoxicity (34) suggest either direct neural injury by the virus or indirect injury mediated by the production of factors from either macrophages or microglial cells. In vitro studies have indicated that the envelope glycoprotein gp120 is neurotoxic (35) and that this may be linked to calcium channels (36), including those activated by glutamate through the N-methyl-D-aspartate (NMDA) receptor (37) and possibly neuronal vasoactive intestinal polypeptide receptors (35). In vitro, the calcium entry can be blocked by calcium channel antagonists (36). In the cerebrospinal fluid of patients with AIDS, elevated levels of quinolinic acid, a glutamate agonist, occur in the presence of cognitive and motor abnormalities (38), thus implying that an endogenous NMDA excitotoxin is involved in neuronal damage and death. Clinical evidence of the importance of gp120 in causing cognitive and motor impairments has been demonstrated in neonatal rats. Administration of purified gp120 caused dystrophic dendritic changes of pyramidal neurons in all cortical areas and the development of behavioral retardation (39).

Indirect mechanisms of neurotoxicity are postulated to involve macrophages and microglia, which following infection by HIV secrete immune mediators whose production are normally under strict control. These include cytokines, prostaglandins, oxidative radicals, and proteases, all of which are capable of neuronal damage (37) and have been demonstrated to be cytotoxic in vitro (40). In addition, astrocytes may also produce cytokines (41). Nonetheless, the involvement of cytokines in producing neuronal damage and therefore cognitive deficits has yet to be substantiated. They have been found to be increased in patients who died of AIDS, but there was no correlation with those who had dementing features (42). It has also been proposed that the load of the virus present in the brain may influence both the neuropathology and the clinical state of patients with AIDS. Higher levels of unintegrated HIV DNA have been detected in brains with HIVE (43). Pang et al (43) implied that there is a direct relationship between the load of viral DNA in the brain and the severity of the clinical dementia, but this hypothesis has not been substantiated.

Clinically, there are a number of considerations. Firstly, the symptom-free series indicates that neuronal loss is not a feature of asymptomatic infection. This is consistent with the findings that clinical cognitive abnormalities are very rare in asymptomatic HIV-infected individuals (44, 45). In addition, when neuropsychological abnormalities are found they are subclinical and their presence does not predict progression to clinical dementia (46). Structural neuroradiographic imaging studies, by MRI and CT, have not shown evidence of cerebral atrophy at such an early stage of disease compared to seronegative controls (44). Nonetheless, single positron emission tomography has indicated that even in early HIV infection there may be alterations in brain perfusion (47).

In contrast, CNS abnormalities in patients with AIDS are accepted as characteristic of symptomatic disease. The neuronal loss found in our investigation in the frontal cortex, the most severe of any cortical area, may be the pathological basis of impairments reported in neuropsychometric tests of the frontal area (7). Frontal release signs and other indicators of damage in this area are commonly observed in HIV-associated dementia and include saccadic eye movement abnormalities (48). These are thought to represent frontal lobe dysfunction, but considering that there is 30% loss of neurons in the occipital lobe, it is possible that disruption of saccadic eye movements occurs in parallel with the loss in the primary visual area. Moreover, approximately 40% axonal loss in the optic nerve (49) provides further evidence for HIV-associated damage to the visual system. General neocortical neuronal loss is supported by magnetic resonance spectroscopy (50). The atrophy noted on CT correlates well with quantitative brain measurements at postmortem (51). Moreover, MRI has demonstrated an increased number of lesions in patients with dementia (52).

CONCLUSION

Investigations of HIV-associated neuronal loss, reviewed in this article, demonstrate the following points. Firstly, quantitative assessments of neuronal populations are fraught with considerable difficulties and only the correct stereological approach will yield reliable results. Secondly, there is now unequivocal evidence of neuronal loss in HIV infection; this damage occurs independently of HIVE, develops only in manifest AIDS and affects various brain areas differently. Thirdly, although recent in vivo and in vitro investigations have begun to shed light on the pathogenesis of neuronal damage, the precise cellular and molecular mechanism remains largely unknown. This is not entirely surprising considering the variety of factors which may be operational at the same time in such a complex and diverse organ system as the brain. Fourthly, since neuronal loss apparently occurs without apparent infection of nerve cells, HIV infection may be considered as a viral model of neurodegeneration. Finally, it is important to correlate the neuronal loss with clinical symptoms of HIV-associated dementia and to clarify the mechanism and pathological substrate, since cognitively impaired individuals have a significantly increased risk of death (53). Most recently, two studies have
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attempted such a correlation. Weis et al (54), using traditional quantitation and relying on retrospective clinical data, failed to find any relationship, while our stereological study of prospective clinically verified individuals did in fact reveal a significant association between the presence of dementia and decreased neuronal density (55). Such work needs to be pursued further. Finally, our finding indicating that neuronal loss is not a feature in symptom-free individuals suggests that therapeutic agents, developed to prevent or reverse the neurotoxicity, could be given prophylactically to prevent future development of HIV-associated dementia.

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REFERENCES

40. Pulliam L, Herndier BG, Tang NM, McGrath MS. Human immunodeficiency virus-infected macrophages produce soluble fac-


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