INTRODUCTION

Some 30% of patients suffering from AIDS develop a neurological syndrome that is referred to as AIDS dementia complex (1) and characterized by cognitive impairment, postural disorders and tremor. Neuropathological characteristics of the brain described at post mortem are myelin pallor, the appearance of multinucleated giant cells, infiltration by blood-derived macrophages, astroglial cell reaction and brain cortical neuronal cell loss (1,2). It is well established that neuronal cells are normally resistant to neuroinvasive HIV-1 strains whereas macrophages, microglial cells and multinucleated giant cells are infected (3). These data together with experimental evidence originating mainly from in vitro studies have led to the hypothesis that a complex series of events initiated by infected or activated cells (e.g., macrophages, microglia and astrocytes) interacting with viral products shed in the brain by HIV-1 infected myelomonocytic cells may be involved in the pathogenesis of the AIDS associated neurological syndrome (4). Among other viral products, the HIV-1 coat protein gp120 has been proposed as a likely aetiological agent of the neuronal loss observed in the brains of...
AIDS patients (2) because it causes death of neurones in culture (4). In fact, gp120 has been reported to produce death of rodent hippocampal neurones, retinal ganglion cells (4) and cerebellar granule cells (5). Furthermore, in transgenic animals, overexpression of gp120 in astrocytes causes a pattern of neuropathological changes reminiscent of those described in subjects with AIDS thus supporting a role for the HIV-1 coat protein in the pathophysiology of the associated neurological syndrome (6). It has been suggested that activated macrophage/microglial cells play an obligatory role in the expression of gp120-induced neuron death. Thus, it has been shown that cortical brain neurones undergo cell death when co-cultured with HIV-1 infected macrophages (7). Similarly, it has been shown that gp120 kills retinal ganglion cells when the latter are cultured in the presence of microglial cells (8). The mechanism underlying gp120-induced neuronal damage appears to involve the release of excitotoxins (e.g., quinolinic acid, glutamate etc.) from non-neuronal cells with consequent abnormal Ca\(^{2+}\) entry into neurones via N-methyl-D-aspartate (NMDA) receptor associated cation channel and through voltage operated Ca\(^{2+}\) channels, since NMDA receptor antagonists and Ca\(^{2+}\) channel blockers prevent neuronal death (4). Quite importantly, it has been shown that gp120 enhances NMDA-evoked exocytotic neurotransmitter release from human and rat cortical and hippocampal nerve ending preparations (9) and, in a cultured human neuroblastoma cell line, causes cell death, preventable by antagonists of the NMDA receptor complex (10). These data implicate an as yet not fully understood positive modulatory action of gp120 on the NMDA receptor complex, which may activate an autocrine excitotoxic loop leading to death of neuronal cells. Interestingly, in vitro exposure of cortical neurones (11) and of human neuroblastoma cells (10) to gp120 stimulates Ca\(^{2+}\)-dependent nitric oxide (NO) synthase (NOS), via NMDA receptor gated Ca\(^{2+}\) entry, to yield abnormal levels of NO, a highly reactive radical species (12), and this has been implicated in the mechanism of neuronal death caused by the viral protein because inhibitors of NOS abolished cytotoxicity (10, 11). Altogether, these data support the concept that an excitotoxic, glutamate-mediated, type mechanism (4) may underlie neuronal death caused in vitro by the coat protein.

**MECHANISMS OF NEURONAL APOPTOSIS INDUCED BY gp120 IN VIVO**

In vivo studies have shown that retardation in behavioural development occurs in neonatal rats treated systemically with gp120 (13, 14) demonstrating that this can cause cognitive impairment and neuronal damage. Barks et al. (15) have reported that in P7 neonatal rats focal injection of gp120 into the CA1 area of one dorsal hippocampus failed to produce, five days later (P12), hippocampal atrophy nor did it cause neuronal damage other than a subtle focal pyramidal cell loss immediately adjacent to the injection track. However, in these animals, the same authors have shown that focal intra-hippocampal coinjection of gp120 and N-methyl-D-aspartate (NMDA) took the reduction of hippocampal volume caused by the latter excitotoxin from 19% to 26.4%; this effect was prevented by antagonists of the NMDA receptor complex thus providing direct evidence of neurotoxic synergism between the HIV-1 coat glycoprotein gp120 and excitatory amino acids in vivo in the immature brain and confirming that this interaction may occur at the level of the NMDA glutamate receptor subtype (15). Lack of gross hippocampal damage has previously been reported in adult rats receiving focal injection of gp120 (16) and this agrees with the data reported by Barks et al. (15). Using the terminal-transferase (TUNEL) technique (17) we showed the occurrence of DNA fragmentation in brain cortical tissue sections of adult rats receiving injections of the viral protein into one
lateral cerebral ventricle (i.c.v.; 18,19) suggesting that neuronal death caused by the HIV-1 coat protein may be of the apoptotic type. The latter deduction was then confirmed at the ultrastructural level; in fact, transmission electron microscopy (TEM) analysis of brain tissue sections obtained from rats treated with gp120 revealed compaction and marginalisation of nuclear chromatin along the inner surface of the nuclear envelope and convolution of the nuclear margin in brain cortical cells (20), unequivocal signs of early and late apoptosis (21). In these animals, ultrastructural changes indicative of late apoptosis, such as masses of condensed chromatin and clumping of the nuclear envelope, have also been seen along with enlargement of the endoplasmic reticulum and normally appearing mitochondria.

Interestingly, apoptosis by gp120 is minimized in rats receiving 1 hour beforehand a single daily injection (0.25 pmoles given i.c.v. for seven consecutive days in all instances) of the α-chemokine SDF-1α (natural ligand for CXCR4 chemokine receptor) (22); like gp120, the latter chemokine, however, given at a higher dose (2.5 pmoles), caused in situ DNA fragmentation (23). Collectively, these data support the concept that neuronal and microglial mechanisms downstream stimulation of CXCR4 and possibly CCR5 receptors [receptor species through which gp120 binds to macrophages and T cells, respectively, and that function as co-receptors for HIV-1 entry into the cell (22)] may be responsible for neuronal apoptosis caused by the HIV-1 coat protein in the neocortex of rat.

The mechanisms through which gp120 causes apoptosis in the brain of the adult rat are not known. Quite importantly, i.c.v. injection of the viral protein in rat causes microglial cell activation and enhances IL-1β expression in the neocortex (24). In the mammalian brain this inflammatory cytokine represents a physiological signal for secretion of NGF and this could enhance survival of injured neurones (25). Apoptosis is an active process underlying cell death which occurs during development and adult life (26,27) and is also implicated in the pathogenesis of several neurodegenerative disorders (28). Nerve growth factor (NGF) and related neurotrophins seem to play an important role in apoptosis during development, adult life and in some pathological conditions (29). In addition, spontaneous and drug-induced apoptotic cell death has often been described in cultured neurones upon removal of NGF from the culture medium (29,30). However, in the neocortex of rats treatment with gp120 does not produce any apparent increase in NGF but causes apoptosis (19). IL-1β has often been reported to cause opposite, e.g., neuroprotective and neuropathological, effects in vitro (25) and this seems also to occur in vivo. IL-1β can affect the expression of enzymes such as inducible NOS (iNOS) and COX (COX-2) whose terminal products may be highly cytotoxic (31). However, at variance with several in vitro data, in rats treated with gp120 we have failed to observe significant changes in brain cortical citrulline, the co-product of NO synthesis (19). Whilst these data negate the occurrence of excessive NO production in the brain cortex of gp120-treated rats it cannot be excluded that physiological levels of NO can interact with other radical species which may originate from activated brain cortical microglial cells to produce peroxynitrite known to be involved in apoptosis, although further experiments are needed in order to confirm this.

At variance with the above data, recent evidence does support an important role for COX-2 in the mechanism of gp120-induced apoptosis. Thus, a single dose of gp120 causes an increase of COX-2 expression which is apparent 6 hours after the injection of the viral protein and this is paralleled by a significant accumulation of PGE₂ in the neocortex (32,33) and a significant increase in body temperature (24) in the rat. Experimental evidence suggests that in the mammalian central nervous system (CNS) enhanced expression of COX-2 and accumulation of products of
the arachidonic acid cascade, including trombox-
an B2 and PGE2, may be implicated in the patho-
physiology of brain damage which follows exci-
totoxic stimuli (34-39). Therefore, it is conceiv-
able that the observed abnormal expression of
COX-2 and accumulation of PGE2 may be im-
plied in the mechanisms of apoptosis caused by gp120 in the neocortex of the rat. In agree-
ment with the latter hypothesis is the observation
that apoptosis induced by gp120 is reduced by a
systemic pretreatment with indomethacin (40), a
specific but not selective inhibitor of COX activ-
ities, and by the selective COX-2 inhibitor
NS398 (32).

It is well established that under physiologi-
ical conditions, the level of expression of
COX-2 gene product appears to correlate well
with the state of activation of excitatory, glutam-
ate-mediated, synaptic transmission (41). In
vitro and in vivo data suggest that gp120 en-
hances glutamate transmission via release from
astroglial cells of as yet poorly identified exci-
totoxins acting at the NMDA, but not non-NM-
DA, receptors in the mammalian brain (4). Al-
together, these data support the concept that the
enhanced expression of COX-2 and accumula-
tion of PGE2 observed here may be the conse-
quence of abnormal activation of glutamate
neurotransmission in the neocortex of the gp120-treated rat. However, this does not appear to be the case because under our experimental conditions a systemic pretreatment with MK801, a selective antagonist of the NMDA receptor complex, failed to counteract gp120-enhanced COX-2 expression observed 6 hours after treatment with the viral coat protein (32). However, systemic pre-treatment with competitive and non-competitive NMDA receptor antagonists or with U-74389G, a free radical scavenger of the 21-aminosteroid family, reduced gp120-induced apoptosis in the rat neocortex (32) supporting an excitotoxic, glutamate-mediated, mechanism of death (42). Bezzi et al. (43) have previously demonstrated that products of the arachidonic acid cascade (PGE$_2$ being the most potent) stimulate the Ca$^{2+}$-dependent release of glutamate from astroglial cells leading to the suggestion that this mechanism may have physiological as well as pathophysiological consequences in the mammalian brain. Therefore, to rationalize the observed lack of MK801 effect on COX-2 expression with the neuroprotection afforded by the NMDA receptor antagonists and by the 21-aminosteroid, U-74389G, it can be assumed that IL-1$\beta$ is responsible for gp120-evoked fast induction of COX-2 and accumulation of PGE$_2$ which may elevate, possibly through a mechanism similar to that described by Bezzi et al. (43), synaptic glutamate; this would then trigger a vicious circle leading the cell to oxidative stress and apoptotic death via an excitotoxic mechanism (42). The series of events initiated by gp120 and leading to apoptotic cell death are schematically reported in Figure 1.

In conclusion, the observation that gp120 induces apoptotic cell death in the rat cortex in vivo, together with the recent immunolocalization of gp120 (44) and the evidence of DNA fragmentation reported at post-mortem in the brain of AIDS patients (45), suggest that this mechanism may underlie the well established brain cortical neuronal loss described in AIDS patients. Finally, confirmation at ultrastructural level of the occurrence of apoptosis in the brain cortex of AIDS patients will validate the usefulness of the rat model we have developed for the characterization of the neuroprotective profile of drugs which interfere with crucial steps involved in the activation of the death programme.

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