Journal of Neurochemistry, 2001, **79**, 1–8

REVIEW

Exploitation of the HIV-1 coat glycoprotein, gp120, in neurodegenerative studies *in vivo*

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Abstract

Neuronal loss has often been described at post-mortem in the brain neocortex of patients suffering from AIDS. Neuroinvasive strains of HIV infect macrophages, microglial cells and multinucleated giant cells, but not neurones. Processing of the virus by cells of the myelomonocytic lineage yields viral products that, in conjunction with potentially neurotoxic molecules generated by the host, might initiate a complex network of events which lead neurones to death. In particular, the HIV-1 coat glycoprotein, gp120, has been proposed as a likely aetiologic agent of the described neuronal loss because it causes death of neurones in culture. More recently, it has been shown that brain neocortical cell death is caused in rat by intracerebroventricular injection of a recombinant gp120 coat protein, and that this occurs via apoptosis. The latter observation broadens our knowledge in the pathophysiology of the reported neuronal cell loss and opens a new lane of experimental research for the development of novel therapeutic strategies to limit damage to the brain of patients suffering from HIV-associated dementia.

Keywords: apoptosis, cyclooxygenase type-2 (COX-2), HIV-associated dementia (HAD), HIV-1 gp120, interleukin-1β, neocortex.

J. Neurochem. (2001) **79**, 1–8.

Cognitive impairment, postural disorders and tremor are among the most common symptoms encountered in patients suffering from HIV-associated dementia (HAD), a neurological syndrome described in some 20% of AIDS patients (Everall *et al*. 1994). The neuropathological features of the brain described at post-mortem are myelin pallor, appearance of multinucleated giant cells, infiltration by blood-derived macrophages, astroglial cell reaction and brain cortical neuronal cell loss (Everall *et al*. 1991; Price and Perry 1994). The syndrome has been attributed to infection of the brain caused by the human immunodeficiency virus type 1 (HIV-1) because it is observed in patients free from opportunistic infections or concomitant cancer in the brain (Price and Perry 1994), although neuroinvasive strains of HIV infect macrophages, microglial cells and multinucleated giant cells, but not neurones (Mucke *et al*. 1995). Processing of the virus by cells of the myelomonocytic lineage yields host and viral products known to initiate a complex network of events, which may lead neurones to death and to the development of cerebral atrophy in AIDS patients (Gray *et al*. 2000). In particular, the HIV-1 coat protein, gp120, has been proposed as a likely aetiologic agent of the described neuronal loss because it causes the death of neurones in culture (Lipton and Gendelman 1995). More recently, brain cortical cell death has also been reported following intracerebroventricular injection of gp120 in rat, and this occurs via apoptosis.

Received November 14, 2000; revised manuscript received May 9, 2001; accepted May 19, 2001.

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Abbreviations used: COX-2, cyclooxygenase type-2; HAD, HIV-associated dementia; HIV-1, human immunodeficiency virus type-1; GFAP, glial fibrillary acidic protein; ICE, interleukin-converting enzyme; i.c.v., lateral cerebral ventricle; IL-1β, interleukin-1β; NGF, nerve growth factor; NO, nitric oxide; PGE_2, prostaglandin E_2.

Here, we summarize *in vivo* data supporting a role for the HIV coat protein, gp120, in the mechanisms of neuronal cell loss often described in the brain cortex of patients suffering from HAD (Everall *et al*. 1994).

gp120 causes apoptosis in the neocortex of rat

Transgenic animals overexpressing gp120 in astrocytes display 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 (Toggas *et al*. 1994). Retardation in behavioural development has been described in neonatal rats treated systemically with gp120 (Glowa *et al*. 1992; Hill *et al*. 1993), demonstrating that this is capable of causing cognitive impairment along with neuronal damage. More recently, Barks *et al*. (1997) 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, and also failed to cause neuronal damage other than a subtle focal pyramidal cell loss immediately adjacent to the injection track. In these animals, however, the same authors have shown that focal intrahippocampal co-injection of gp120 and NMDA brought 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 subtype of glutamate receptor (Barks *et al*. 1997). Lack of gross hippocampal damage and of statistically significant neuronal cell loss has been previously reported in adult rats receiving focal injection of gp120 (Bagetta *et al*. 1994a,b), and this is in line with the data reported by Barks *et al*. (1997). More recently, using the terminal-transferase (terminal fluorescein 12-dUTP nick-end labeling, TUNEL) technique (Gavrieli *et al*. 1992), we have shown 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.; Bagetta *et al*. 1995, 1996a), suggesting that neuronal death caused by the HIV-1 coat protein may be of the apoptotic type. The latter deduction has been confirmed by transmission electron microscopy (TEM) analysis of brain tissue sections obtained from rats treated with gp120 that revealed compaction and marginalization of nuclear chromatin along the inner surface of the nuclear envelope, and convolution of the nuclear margin in brain cortical cells (Bagetta *et al*. 1996b) (Fig. 1), unequivocal signs of early and late apoptosis (Kerr *et al*. 1987). 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

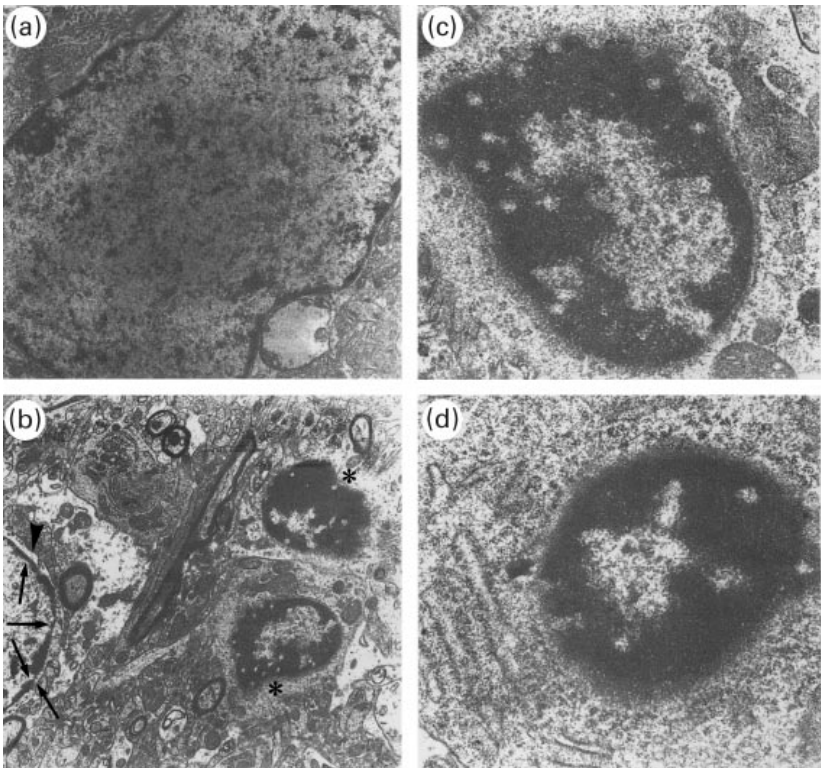

Fig. 1 (a) Nucleus of a neurone from a control, BSA-treated rat (100 ng given i.c.v. once daily for seven consecutive days) with normally dispersed chromatin (×6880). (b–d) Microphotographs showing apoptotic nuclei from the cortex of a rat receiving a single daily injection of gp120 (100 ng/day) for seven consecutive days. At low magnification (×540, b), two apoptotic nuclei (asterisks) and an injured cell with dilated nuclear envelope (arrows) can be seen. Chromatin aggregation, and pore dilation and clustering, typical of apoptotic cell death, are easely detectable at high magnification (×17 000). Note the change in mitochondrial integrity in (c) and endosplamic reticulum dilation in (d). Taken from Bagetta *et al.* (1996b).

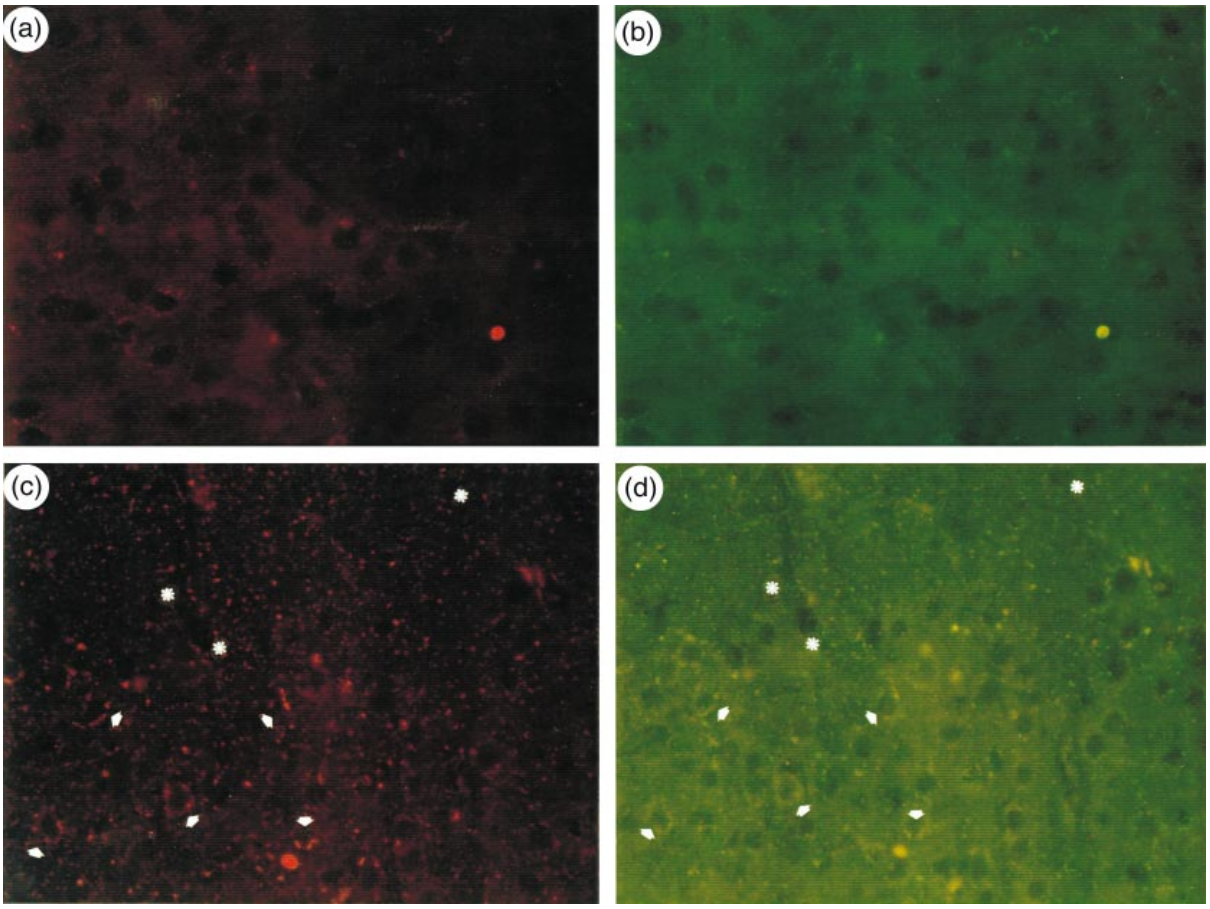

Fig. 2 Photomicrographs to show neurofilament-triplet (NF-T) and IL-1β double-immunoreactivity in brain tissue coronal sections (30 μm) obtained from a rat killed 24 h after a single i.c.v. injection of gp120 (100 ng), and processed for immunohistofluorescence. NF-T-immunopositivity (red fluorescence) is evident throughout panel (b); the specificity of NF-T immunostaining is confirmed by the lack of immunoreactivity in an adjacent section (a), incubated in the absence of the primary antibody for negative control. Green fluorescence, indicating specific IL-1β immunoreactivity (see panel c for negative control; the same section was shown in panel a), is shown in panel (d; the same section was shown in panel b). Arrowheads in (d) indicate cells double-immunopositive (see yellow dots) for IL-1β and NF-T (see panel b for comparison). Asterisks indicate cells positive for NF-T (b) and negative for IL-1β. Green immunofluorescence is also evident (panel d) in areas of the tissue section lacking cell bodies, and this may conceivably represent secretory IL-1β. Magnification: 40×. Reprinted from Bagetta *et al.* (1999) with permission from Elsevier Science.

appearing mitochondria. Immunoelectronmicroscopy analysis of brain neocortical cells bearing ultrastructural features typical of apoptosis revealed that these are immunopositive for the neurofilament (Fig. 2), a typical neuronal marker (Bagetta *et al*. 1999); by contrast, glial fibrillary acidic protein (GFAP) immunopositive cells appeared normal, suggesting that under the present experimental conditions astroglial cells may not undergo apoptosis (data not shown; Bagetta *et al*. 1999).

Neuronal apoptosis by gp120 was minimized in rats receiving (1 h beforehand) a single daily injection (0.25 pmoles given i.c.v. for seven consecutive days in all instances) of the β-chemokines RANTES, MIP-1α (natural ligands for the CCR5 chemokine receptor) or the α-chemokine SDF-1α (natural ligand for CXCR4 chemokine receptor) (Meucci and Miller 1999); likewise gp120, a higher dose (2.5 pmoles) of SDF-1α caused *in situ* DNA fragmentation (Corasaniti *et al*. 2000a). Collectively, these data support the concept that neuronal and microglial mechanisms, downstream CCR5 and CXCR4 receptors, coreceptors for gp120 binding and HIV-1 penetration into macrophages and T cells, respectively (Meucci and Miller 1999), may be responsible for neuronal apoptosis caused by the HIV-1 coat protein in the neocortex of rat.

gp120 causes abnormal expression of interleukin-1β in the neocortex

The mechanisms through which gp120 causes apoptosis in the brain of rat has yet to be discovered, although a series

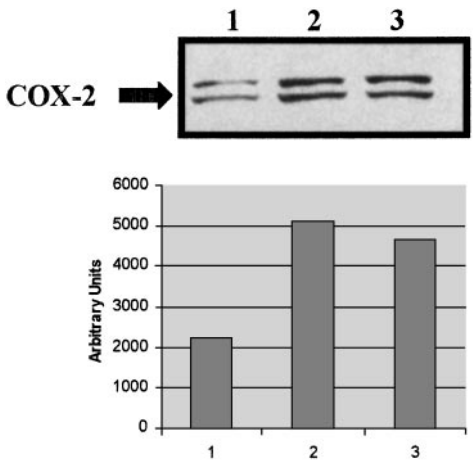

Fig. 3 A single i.c.v. dose of the HIV-1 coat protein gp120 causes a rapid enhancement of COX-2 expression in the brain neocortex of rat. This is a typical example of western blotting analysis to show COX-2 expression in neocortical brain tissue homogenate obtained from two independent rats treated 6 h previously with a single dose of bovine serum albumin (BSA, 100 ng/i.c.v.; lane 1) or gp120 (100 ng/i.c.v.; lane 2), respectively. Lane 3 shows the effect of MK801 (0.3 mg/kg given i.p. 30 min before gp120) on gp120-induced enhanced expression of COX-2 (lane 2). For comparison, the histograms show relative intensity values of the autoradiographic bands (see above) as determined by computer-assisted densitometric analysis (QUANTISCAN, Biosoft, Cambridge, UK). Note that gp120 almost doubles the expression of COX-2 as compared with control (BSA treated), and this is unaffected by treatment with MK801, a selective antagonist of the NMDA subtype of glutamate receptor. Taken from Corasaniti *et al.* (2000a).

of recent experimental data does implicate the pro-inflammatory cytokine interleukin-1β (IL-1β). In fact, immunohistochemical and western blotting experiments show that treatment with gp120 enhances the expression of IL-1β in the neocortex, and double-labelling immunofluorescence experiments have established that neuronal and, possibly, microglial cells are the main source of IL-1β (Bagetta *et al*. 1999) (Fig. 3). Immunoelectron microscopy and enzyme-linked immunosorbent assay (ELISA) data have established that IL-1β is expressed, although at very low levels, in the mitochondria of brain neocortical cells of naive untreated rats; more importantly, subchronic administration (i.c.v.) of gp120 enhances the mitochondrial expression of the pro-inflammatory cytokine, and this implicates *in situ* activation of interleukin-converting enzyme (ICE) (Corasaniti *et al*. 2001). In agreement with the latter deduction, antagonism studies have shown that combined treatment with gp120 and the inhibitor II (Ac-Tyr-Val-Ala-Asp-chloromethylketone) of ICE (Milligan *et al*. 1995), the protease (also known as caspase 1) that processes pro-IL-1β in biologically mature IL-1β (Black *et al*. 1988; Kostura *et al*. 1989; Yuan *et al*. 1993; Walker *et al*. 1994; Martins and Earnshow 1997), minimizes apoptotic cell death induced by the viral protein in the neocortex of rat (Bagetta *et al*. 1999). Quite importantly, treatment with the antagonist of IL-1 receptor (IL-1ra), the receptor species that mediates most of the biological actions of IL-1β (Dripps *et al*. 1991; Hagan *et al*. 1996), prevents apoptotic cell death caused by the viral protein (Corasaniti *et al*. 1998; Bagetta *et al*. 1999) and, likewise for gp120, subchronic i.c.v. administration of murine recombinant IL-1β causes apoptosis in the neocortex of rat (Bagetta *et al*. 1999), further implicating this cytokine in the mechanism of gp120-induced neocortical cell death.

Cyclooxygenase-2 induction by gp120 triggers apoptosis via an excitotoxic, glutamate mediated, mechanism

The mechanism through which IL-1β mediates gp120-induced apoptosis in the neocortex of rat is not known. In the mammalian brain this pro-inflammatory cytokine represents a physiological signal for secretion of nerve growth factor (NGF), and this could enhance the survival of injured neurones (Strijbos and Rothwell 1995). Interestingly, i.c.v. injections of gp120 enhanced IL-1β expression (Bagetta *et al*. 1999) but failed to elevate NGF production in the neocortex (Bagetta *et al*. 1996a), and this might contribute, at least in part, to cell death (Bagetta *et al*. 1995, 1996b) because of the lack of adequate trophic support (see Corasaniti *et al*. 1998 for further discussion).

It is well established that IL-1β can also affect the expression of inducible enzymes, such as nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), the terminal products of which may be highly cytotoxic (Merrill *et al*. 1993). However, at variance with several *in vitro* data (Lipton and Gendelman 1995), in rats treated with gp120 we failed to observe significant changes in brain cortical citrulline (Bagetta *et al*. 1996a, 1997, 1998a), the coproduct of NO synthesis (Knowles and Moncada 1994). Although these data do negate the occurrence of excessive NO production in the neocortex of gp120-treated rats, it cannot be excluded that physiological levels of NO can interact with other radical species that may originate from activated brain cortical microglial cells (Bagetta *et al*. 1999) to produce peroxynitrite, known to spontaneously decompose to yield the hydroxyl radical, a species even more cytotoxic than NO and known to be involved in apoptosis (Coyle and Puttfarcken 1993).

Instead, more recent data do support an important role for COX-2 in the mechanism of gp120-induced apoptosis. In fact, we have reported immunohistochemical evidence demonstrating that subchronic i.c.v. treatment with gp120 enhances the expression of COX-2 in the neocortex of rat (Bagetta *et al*. 1998b). More importantly, a single dose of gp120 causes an increase of COX-2 expression, which is

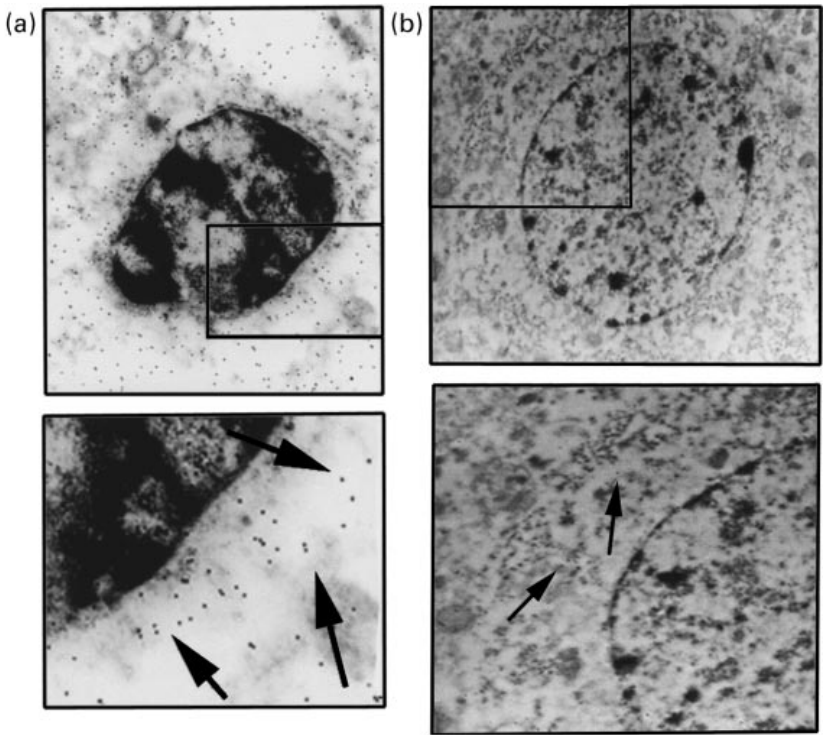

Fig. 4 Ultrastructural evidence of apoptosis caused by the HIV-1 coat glycoprotein gp120 in a rat brain neocortical cell immunopositive for the neurofilament, cytoskeletal, proteins and typical neuronal markers. Ultrathin tissue sections from the brain neocortex of a rat treated for seven consecutive days with a single daily dose (100 ng, given i.c.v.) of gp120 have been processed for electron microscopy detection of neurofilament protein immunogold positive cells (see Bagetta *et al.* 1999 for antisera properties). In (a), the upper panel (11 000× magnification) shows masses of condensed chromatin typical of late apoptosis; the lower panel shows, at a higher magnification (33 000×) of the area indicated by the box in the upper panel, a typical filament distribution (see arrows) of alligned gold particles. In (b), the lower panel shows rare gold particles (see arrows; 13 000× magnification). These are indistinguishable in the boxed area at lower magnification (6000×) of the upper panel in a neocortical brain tissue section adjacent to the one shown in (a) and processed for negative control.

apparent 6 h after the injection of the viral protein (Fig. 4), and this is paralleled by a significant accumulation of prostaglandin E_2 (PGE_2) in the neocortex (Corasaniti *et al.* 2000b; Maccarrone *et al.* 2000) and a significant increase in body temperature (Bagetta *et al.* 1999) in the rat. Experimental evidence suggests that in the mammalian CNS, enhanced expression of COX-2, and accumulation of products of the arachidonic acid cascade, including tromboxan B_2 and PGE_2, may be implicated in the pathophysiology of brain damage that follows exposure to

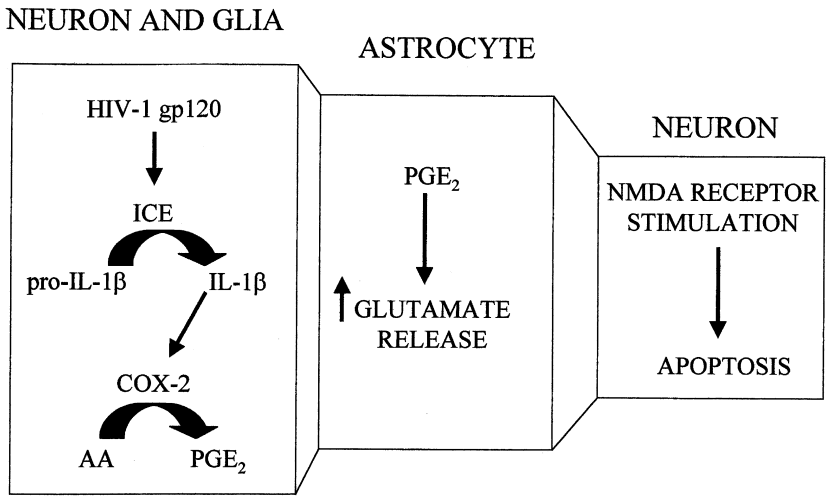

Fig. 5 Schematic representation of a unifying hypothesis on the mechanisms underlying neuronal apoptosis induced by gp120 in the neocortex of rat. Administration of recombinant HIV-1 gp120 IIIB enhances neuronal and microglial expression of IL-1β (Bagetta *et al.* 1999), an event that requires the conversion of the pro-IL-1β in the mature form of this cytokine via the intervention of interleukin converting enzyme (ICE) (Corasaniti *et al.* 2001). IL-1β may enhance the expression of COX-2 (it is established that in the mammalian brain COX-2 is located in neuronal cells; Yamagata *et al.* 1993) to convert arachidonic acid (AA) into prostaglandin E_2 (PGE_2) which than accumulate (Bagetta *et al.* 1998b; Maccarrone *et al.* 2000). Elevated PGE_2 stimulate Ca^{2+}-dependent release of glutamate from astrocytes (Bezzi *et al.* 1998) and this may be responsible for excitotoxic neuronal apoptosis in the neocortex of rat (Corasaniti *et al.* 2000a).

excitotoxic stimuli (Gaudet *et al*. 1980; Fostermann *et al*. 1982; Baran *et al*. 1987; Seregi *et al*. 1987; Planas *et al*. 1995; Nogawa *et al*. 1997). Therefore, it is conceivable that the observed abnormal expression of COX-2 and that accumulation of PGE_2 may be implicated in the mechanisms of apoptosis caused by gp120 in the neocortex of rat. In agreement with the latter hypothesis is the observation that apoptosis induced by gp120 is reduced by a systemic pretreatment with indomethacin (Bagetta *et al*. 1998b), a specific but non-selective inhibitor of COX activities, and by NS398, a selective COX-2 inhibitor (Corasaniti *et al*. 2000a).

Under physiologic conditions, the level of expression of COX-2 gene product appears to correlate well with the state of activation of excitatory, glutamate-mediated, synaptic transmission (Yamagata *et al*. 1993). *In vitro* and *in vivo* data suggest that gp120 enhances glutamate transmission via the release from astroglial cells of not yet well-identified excitotoxins acting at the NMDA, but not non-NMDA, receptors in the mammalian brain (Lipton and Gendelman 1995). Altogether, these data support the concept that the enhanced expression of COX-2 and the accumulation of PGE_2 observed here may be the consequence of abnormal activation of glutamate neurotransmission in the neocortex of 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 h after treatment with the viral coat protein (Corasaniti *et al*. 2000a) (Fig. 4). However, systemic pretreatment 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 neocortex of rat (Corasaniti *et al*. 2000b), supporting an excitotoxic glutamate-mediated mechanism of death (Choi 1988). Bezzi *et al*. (1998) have previously demonstrated that products of the arachidonic acid cascade (PGE_2 being among 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 patho-physiological 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, we suggest that IL-1β may be responsible for the gp120-evoked rapid induction of COX-2 and accumulation of PGE_2, which may elevate, possibly through a mechanism similar to that described by Bezzi *et al*. (1998), synaptic glutamate; this would then trigger a vitious loop leading the cell to oxidative stress and apototic death via an excitotoxic mechanism (Choi 1988). The series of events initiated by gp120 and leading to apoptotic cell death are schematically reported in Fig. 5.

In conclusion, the observation that gp120 induces apoptotic cell death in the rat neocortex *in vivo*, together with the recent evidence of DNA fragmentation reported at post-mortem in the brain of AIDS patients (Petito and Roberts 1995), suggests that this mechanism may underlie the well-established cortical neuronal loss described in the brain of AIDS patients. The recent immunolocalization of gp120 in human brain tissue with the neuropathological correlates of HIV-1 encephalitis and pre-mortem diagnosis of HAD provides the missing link in the understanding of HIV neuropathogenisis (Jones *et al*. 2000); gp120 may, in fact, be present in sufficient quantity during HIV infection to cause neuronal damage (Jones *et al*. 2000), although other viral components, such as Tat, may also contribute (Bansal *et al*. 2000).

Here, we would like to speculate that confirmation of the neuroinflammatory steps we have partly dissected in the brain of gp120-treated rats may prove useful for the study of the underlying pathophysiological mechanisms of neuronal death. Finally, demonstration at the 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 that interfere with mediators of neuro-inflammation and the crucial steps involved in the activation of the death programme.

Acknowledgements

This work was funded by: the II AIDS Project to AFA and WM, Istituto Superiore di Sanità, Rome, Italy; the MURST-DAE Cofin '1998' (D.M. 1451/97); the European Fund for Regional Development (Calabria Region, P.O.P. 94/99). Our thanks go to Mr N. Fico and to Mr G. Politi for excellent technical assistance.

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