Biochemical Pharmacology, Vol. 56, pp. 153–156, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(98)00044-6

COMMENTARY

The HIV Envelope Protein gp120 in the Nervous System

Interactions with nitric oxide, interleukin-1β and nerve growth factor signalling, with pathological implications *in vivo* and *in vitro*

M. Tiziana Corasaniti, Giacinto Bagetta,†‡‖ Domenicantonio Rotiroti* and Giuseppe Nisticò§*

*Chair of Pharmacology, Faculty of Pharmacy and IBAF-CNR, 88100 Catanzaro; †Department of Pharmacobiology, Calabria University at Cosenza, Cosenza; ‡Experimental Neurobiology Center "Mondino-Tor Vergata" at IRCCS Santa Lucia, Rome; and §Department of Biology, University of Rome, "Tor Vergata," Rome, Italy

ABSTRACT. The neuronal loss often described at *post-mortem* in the brain neocortex of patients suffering from AIDS has been proposed to be responsible for the development of the AIDS dementia complex. Neuroinvasive strains of the HIV virus infect macrophages, microglial cells, and multinucleated giant cells, but not neurones. Processing of the virus by cells of the myelomonocytic lineage yields viral products known to initiate a complex network of events that may lead to the death of neurones and to the development of AIDS-associated neurological syndrome. The HIV-1 coat protein gp120, in particular, has been proposed as a likely etiologic agent of the described neuronal loss because it causes the death of neurones in culture. More recently, it has been shown that brain cortical cell death caused in rats by intracerebroventricular injection of gp120 occurs via apoptosis. This observation broadens our knowledge of the pathophysiology of the reported neuronal cell loss and opens a new avenue of experimental research for the development of novel therapeutic strategies for the treatment of patients suffering from AIDS-associated neurological syndrome. BIOCHEM PHARMACOL **56**;2: 153–156, 1998. © 1998 Elsevier Science Inc.

KEY WORDS: AIDS dementia complex; apoptosis; NGF; nitric oxide

Some 30% of adult and 50% of pediatric patients suffering from AIDS develop neurological disorders, which are collectively referred to as neurological syndrome called AIDS dementia complex [1]. Cognitive impairment, postural disorders, and tremor are among the most common symptoms encountered in patients suffering from AIDS dementia complex. 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 [1, 2]. The syndrome can be attributed to infection of the brain caused by HIV-1¶ because it is observed in patients free from opportunistic infections of, or concomitant cancer in, the brain (see Ref. 1). It is well established that macrophages, microglial cells, and multinucleated giant cells are infected by HIV-1, whereas neuronal cells normally are resistant to neuroinvasive HIV-1 strains [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 viral products, the HIV-1 coat protein gp120 has been proposed as a likely etiologic agent of the observed neuronal loss 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 in 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]. Activated macrophage/microglial cells have been proposed to play an obligatory role in the expression of gp120-induced neurone death. Thus, it has been shown that cortical brain neurones undergo cell death when co-cultured with HIV-1-infected

‖ Corresponding author: Prof. Giacinto Bagetta, Department of Pharmacobiology, University of Calabria at Cosenza, 87030 Arcavacata di Rende, Cosenza, Italy. Tel. 39-984-493965; FAX 39-984-493763; E-mail: Bagetta@mail.csdim.unical.it.

¶ *Abbreviations:* HIV-1, human immunodeficiency virus type 1; IL-1β, interleukin 1β; NGF, nerve growth factor; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOS, NO synthase; and TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labelling.

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 and glutamate) from non-neuronal cells, with consequent abnormal Ca^{2+} entry into neurones via the 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 causes cell death in a cultured human neuroblastoma cell line, which is prevented by antagonists of the NMDA receptor complex [10]. These data implicate a not yet 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 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 abolish cytotoxicity [10, 11]. Altogether, these data support the concept that an excitotoxic, glutamate-mediated type of mechanism (see Ref. 4) may underlie neuronal death caused *in vitro* by the coat protein.

Retardation in behavioural development and neuronal damage have been described in neonatal rats treated systemically with gp120 [13]. More recently, Barks *et al.* [14] have reported that in P7 neonatal rats, focal injection of gp120 into the CA1 area of one dorsal hippocampus failed to produce hippocampal atrophy 5 days later (P12), nor did it cause neuronal damage other than a subtle focal pyramidal cell loss immediately adjacent to the injection track. In these animals, however, the same authors demonstrated that focal intrahippocampal coinjection of gp120 and NMDA augmented from 19 to 26.4% the reduction of hippocampal volume caused by the latter excitotoxin, and this was prevented by NMDA receptor complex antagonists, 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 receptor complex [14]. Lack of hippocampal damage and of pyramidal cell loss has been reported previously in adult rats receiving focal injection of gp120 [15], and this is in line with the data reported by Barks *et al.* [14]. However, at variance with the latter authors, we failed to show any interaction of gp120 with excitatory amino acid neurotransmission in the hippocampus of adult rats [15].

More recently, using the TUNEL technique [16], we have shown the occurrence of DNA fragmentation in brain cortical tissue sections of adult rats receiving intracerebroventricular injection of the viral protein [17, 18], suggesting that neuronal death caused by the HIV-1 coat protein may be of the apoptotic type. In agreement with the latter deduction, transmission electron microscopy analysis of brain tissue sections obtained from rats treated with gp120 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, unequivocal signs of early and late apoptosis [19]. 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 obvious enlargement of the endoplasmic reticulum (Fig. 1). The mechanisms through which gp120 causes apoptosis in the brain of adult rats are not known. Apoptosis is an active process underlying cell death, which occurs during development and adult life [20, 21], and also is implicated in the pathogenesis of several neurodegenerative disorders [22]. NGF and related neurotrophins seem to play an important role in apoptosis during development, adult life, and in some pathological conditions [23]. In addition, spontaneous and drug-induced apoptotic cell death has often been described in cultured neurones upon removal of NGF from the culture medium [23, 24]. Intracerebroventricular injection of the viral protein in rats caused microglial cell activation and enhanced IL-1β expression in the neocortex and hippocampus.* In the mammalian brain this inflammatory cytokine represents a physiological signal for the secretion of NGF, and this could enhance survival of injured neurones [25]. Interestingly, in rats receiving i.c.v. injections of gp120 a concomitant increase of the NGF level has been observed in the hippocampus [18], an area of the rat brain where no signs of neuronal degeneration or death were seen [17, 18]. The latter effect was accompanied by a decrease in the number of p75NGFR-immunopositive cells of 45–120 μm^2 in the septal nucleus whose nerve fibers terminate in the hippocampus, and by an increase in the p75NGFR immunoreactivity of 200–300 μm^2 neurones in the septum, where neuronal death was not apparent [18, 26].

These data suggest that enhanced expression of IL-1β may play a neuroprotective role in the hippocampus via accumulation of NGF, which, in turn, may prevent or delay the onset of neuronal cell death in this region of the rat brain. Increased expression of IL-1β is also seen in the brain cortex of rats treated likewise with gp120;* however, in this region of the rat brain, gp120 does not produce any apparent increase in NGF but causes apoptosis [18]. Interestingly, combined i.c.v. administration of gp120 with IL-1 receptor antagonist (IL-1ra) prevents the occurrence of apoptosis in the brain cortex,* supporting a neuropathological role for IL-1β in this region. IL-1β has often been reported to cause opposite effects, e.g. neuroprotective and

* Bagetta G, Corasaniti MT, Berliocchi L, Costa N and Nisticò G, Role IL-1β in gp120-induced apoptosis in the rat brain. In: *Drug Targets in Heart and Brain Ischaemia, Florence (Italy) July 11–14, 1997*. Abstract book p. 30.

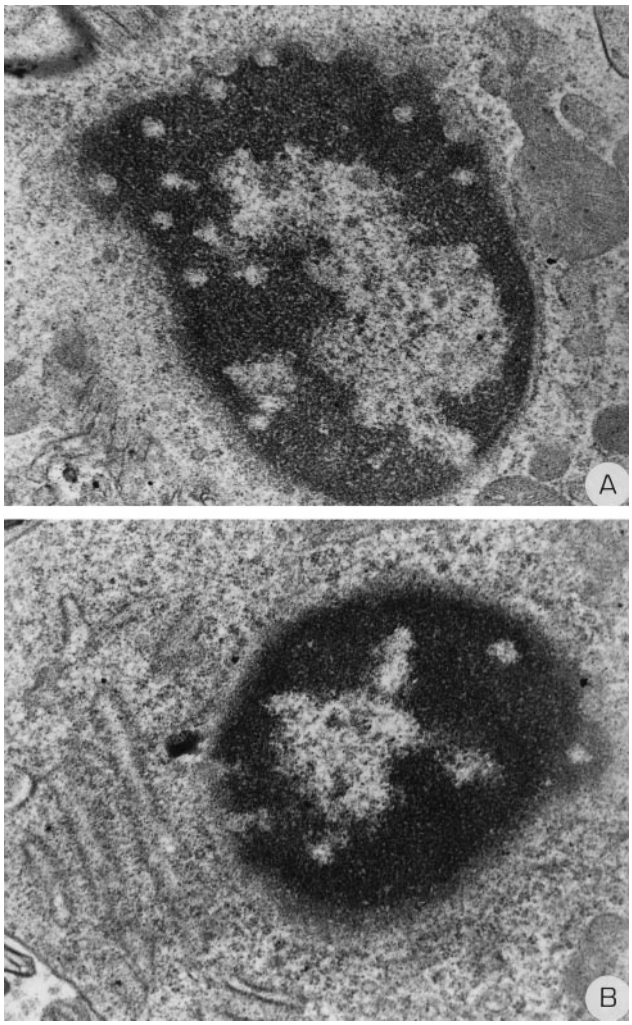

FIG. 1. Microphotographs of two apoptotic nuclei from the cortex of a rat receiving a single daily intracerebroventricular injection of gp120 (100 ng/day) for 7 consecutive days. Note in both panels chromatin aggregation and pore dilation and clustering, typical of apoptotic cell death (magnification 17,000×). Endosplamic reticulum dilatation is shown in panel B.

neuropathological, *in vitro* [25], and this seems to happen also *in vivo*. IL-1β can affect the expression of enzymes, such as inducible NOS, whose terminal products may be highly cytotoxic [27]. However, at variance with *in vitro* data, we have failed to observe significant changes in brain cortical citrulline, the co-product of NO synthesis, in rats treated with gp120 [18, 28]. While 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 that may originate from activated brain cortical microglial cells to produce peroxynitrite, which is known to be involved in apoptosis, although this requires confirmation by further experimentation. Quite surprisingly, gp120 yielded a two-fold decrease of neuronal NOS mRNA expression [18] and reduced neuronal NOS immunoreactivity [26] in the dorsal hippocampus, an area of the rat brain where the viral protein does not cause apoptosis [17, 18]. The effect of gp120 on neuronal NOS mRNA and enzyme protein expression was paralleled by a significant decrease of Ca^{2+}-dependent [^{3}H]citrulline formation in hippocampal brain tissue homogenates [18]. In the mammalian brain, NO mediates important physiological processes including long-term potentiation of synaptic transmission [29], the proposed electrophysiological substrate of learning and memory [30]; furthermore, behavioural and electrophysiological data suggest an important role for the hippocampus in memory formation [30]. Therefore, regardless of the mechanism through which gp120 reduces the expression of hippocampal neuronal NOS, *per se* this effect should be considered a neurotoxic event that, along with the described apoptotic brain cortical cell death, may underlie the cognitive dysfunctions often reported in AIDS patients.

In conclusion, the observation that gp120 induces apoptotic cell death in the rat cortex *in vivo* and the recent evidence of DNA fragmentation reported at *post mortem* in the brain of AIDS patients [31] suggest that this mechanism may underlie the well established brain cortical neuronal loss described in AIDS patients. In addition, the observed reduction of NOS activity in the rat hippocampus, an important area for memory formation, suggests that this may contribute to the associated dementia syndrome. Finally, confirmation 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 crucial steps involved in the activation of the death program.

This work was funded by the X AIDS Project, Istituto Superiore di Sanità, Rome (Italy), by the CNR (Progetto Strategico: Ciclo Cellulare ed Apoptosi), and by the European Fund for Regional Development (Calabria Region, P.O.P. 94/99). Our thanks go to Ms. R. Baboro for typing the manuscript and to Mr. G. Politi for technical assistance.

References

1. Price RW, and Perry SW, *HIV, AIDS, and the Brain*. Raven Press, New York, 1994.
2. Everall IP, Luthert PJ and Lantos PL, Neuronal loss in the frontal cortex in HIV infection. *Lancet* **337:** 1119–1121, 1991.
3. Mucke L, Masliah E and Campbell IL, Transgenic models to assess the neuropathogenic potential of HIV-1 proteins and cytokines. *Curr Top Microbiol Immunol* **202:** 187–205, 1995.
4. Lipton SA and Gendelman HE, Dementia associated with the acquired immunodeficiency syndrome. *N Engl J Med* **332:** 934–940, 1995.
5. Savio T and Levi G, Neurotoxicity of HIV coat protein gp120, NMDA receptors, and protein kinase C: A study with rat cerebellar granule cell cultures. *J Neurosci Res* **34:** 265–272, 1993.
6. Toggas SM, Masliah E, Rockenstein EM, Rall GF, Abraham CR and Mucke L, Central nervous system damage produced by expression of the HIV-1 coat protein gp120 in transgenic mice. *Nature* **367:** 188–193, 1994.
7. Giulian D, Wendt E, Vaca K and Noonan CA, The envelope

glycoprotein of human immunodeficiency virus type 1 stimulates release of neurotoxins from monocytes. *Proc Natl Acad Sci USA* **90:** 2769–2773, 1993.
8. Lipton SA, Requirement for macrophages in neuronal injury induced by HIV envelope protein gp120. *Neuroreport* **3:** 913–915, 1992.
9. Pittaluga A, Pattarini R, Severi P and Raiteri M, Human brain *N*-methyl-D-aspartate receptors regulating noradrenaline release are positively modulated by HIV-1 coat protein gp120. *AIDS* **10:** 463–468, 1996.
10. Corasaniti MT, Melino G, Navarra M, Garaci E, Finazzi-Agrò A and Nisticò G, Death of cultured human neuroblastoma cells induced by HIV-1 gp120 is prevented by NMDA receptor antagonists and inhibitors of nitric oxide and cyclooxygenase. *Neurodegeneration* **4:** 315–321, 1995.
11. Dawson VL, Dawson TM, Uhl GR and Snyder SH, Human immunodeficiency virus type 1 coat protein neurotoxicity mediated by nitric oxide in primary cortical cultures. *Proc Natl Acad Sci USA* **90:** 3256–3259, 1993.
12. Knowles RG and Moncada S, Nitric oxide synthases in mammals. *Biochem J* **298:** 249–258, 1994.
13. Hill JM, Mervis RF, Avidor R, Moody TW and Brenneman DE, HIV envelope protein-induced neuronal damage and retardation of behavioral development in rat neonates. *Brain Res* **603:** 222–233, 1993.
14. Barks JDE, Liu X-H, Sun R and Silverstein FS, gp120, human immunodeficiency virus-1 coat protein, augments excitotoxic hippocampal injury in perinatal rats. *Neuroscience* **76:** 397–409, 1997.
15. Bagetta G, Finazzi-Agrò A, Palma E and Nisticò G, Intracerebral injection of human immunodeficiency virus type 1 coat glycoprotein GP120 does not produce neurodegeneration in rat. *Neurosci Lett* **176:** 97–100, 1994.
16. Gavrieli Y, Sherman Y and Ben-Sasson SA, Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. *J Cell Biol* **119:** 493–501, 1992.
17. Bagetta G, Corasaniti MT, Berliocchi L, Navarra M, Finazzi-Agrò A and Nisticò G, HIV-1 gp120 produces DNA fragmentation in the cerebral cortex of rat. *Biochem Biophys Res Commun* **211:** 130–136, 1995.
18. Bagetta G, Corasaniti MT, Aloe L, Berliocchi L, Costa N, Finazzi-Agrò A and Nisticò G, Intracerebral injection of human immunodeficiency virus type 1 coat protein gp120 differentially affects the expression of nerve growth factor and nitric oxide synthase in the hippocampus of rat. *Proc Natl Acad Sci USA* **93:** 928–933, 1996.
19. Bagetta G, Corasaniti MT, Malorni W, Rainaldi G, Costa N, Berliocchi L, Finazzi-Agrò A, and Nisticò G, The HIV-1 gp120 causes ultrastructural changes typical of apoptosis in the rat cerebral cortex. *Neuroreport* **7:** 1722–1724, 1996.
20. Hamburger V and Levi-Montalcini R,, Proliferation, differentiation and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions. *J Exp Zool* **111:** 457–469, 1949.
21. Vaux DL, Toward an understanding of the molecular mechanisms of physiological cell death. *Proc Natl Acad Sci USA* **90:** 786–789, 1993.
22. Pollard H, Charriaut-Marlangue C, Cantagrel S, Represa A, Robain O, Moreau J and Ben-Ari Y, Kainate-induced apoptotic cell death in hippocampal neurons. *Neuroscience* **63:** 7–18, 1994.
23. Raff MC, Barre BA, Burne JF, Coles HS, Ishizaki Y and Jacobson MD, Programmed cell death and the control of cell survival: Lessons from the nervous system. *Science* **262:** 695–700, 1993.
24. Lindholm D, Heumann R, Meyer M and Thoenen H, Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. *Nature* **330:** 658–659, 1987.
25. Strijbos PJLM and Rothwell NJ, Interleukin-1β attenuates excitatory amino acid-induced neurodegeneration *in vitro*: Involvement of nerve growth factor. *J Neurosci* **15:** 3468–3474, 1995.
26. Bagetta G, Corasaniti MT, Costa N, Berliocchi L, Finazzi-Agrò A and Nisticò G, The human immunodeficiency virus type 1 (HIV-1) glycoprotein gp120 reduces the expression of neuronal nitric oxide synthase in the hippocampus but not in the cerebral cortex and medial septal nucleus. *Neurosci Lett* **224:** 1–4, 1997.
27. Merrill JE, Ignarro LJ, Sherman MP, Melinek J and Lane TE, Microglial cell cytotoxicity of oligodendrocytes is mediated through nitric oxide. *J Immunol* **151:** 2132–2141, 1993.
28. Bagetta G, Berliocchi L, Costa N, Palma E, Aloe L and Nisticò G, Role of nitric oxide and NGF in the mechanisms of neurotoxicity induced by the HIV-1 coat protein gp120 in rat. In: *Nitric Oxide and the Cell: Proliferation, Differentiation and Death* (Eds. Moncada S, Nisticò G, Bagetta G and Higgs AE), Portland Press, London, in press.
29. Schuman EM and Madison DV, Nitric oxide as an intercellular signal in long-term potentiation. *Semin Neurosci* **5:** 207–215, 1994.
30. Bliss TVP and Collingridge GL, A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature* **361:** 31–39, 1993.
31. Petito CK and Roberts B, Evidence of apoptotic cell death in HIV encephalitis. *Am J Pathol* **146:** 1121–1130, 1995.