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Evidence that increases of mitochondrial immunoreactive IL-1β by HIV-1 gp120 implicate *in situ* cleavage of pro-IL-1β in the neocortex of rat

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Abstract

Immunoelectron microscopy analysis of brain tissue sections and rat-specific sandwich ELISA allowed the localization of interleukin-1β (IL-1β) immunoreactivity in the mitochondria and cytosol of neocortical tissue preparations from the brain of naive, untreated, rats and rats receiving a single daily injection into one lateral cerebral ventricle (i.c.v.) of bovine serum albumin (BSA; 100 ng/day) for seven consecutive days. Interestingly, seven days i.c.v. treatment with the HIV-1 coat protein gp120 (100 ng/day) enhances IL-1β immunoreactivity in the cellular fractions studied. Elevation of mitochondrial immunoreactive IL-1β levels seems to originate from the conversion operated by the interleukin converting enzyme (ICE) of mitochondrial pro-IL-1β; in fact, IL-1β increases reported in the ELISA experiments were paralleled by a decrease of the mitochondrial pro-IL-1β 31-kDa band in conjunction with enhanced expression of the p20 component of activated ICE. In conclusion, the present results demonstrate that gp120-enhanced neocortical expression of IL-1β originates, at least in part, from *in situ* cleavage of mitochondrial pro-IL-1β and suggest that this, together with the central role of the mitochondrion in the expression of programmed cell death, may be important for apoptosis induced by the viral coat protein in the brain of rats.

Keywords: apoptosis, human immunodeficiency virus type-1 glycoprotein 120, interleukin-1β, interleukin converting enzyme, pro-IL-1β, mitochondria.

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Some 50% of the paediatric patients and 25% of the adult patients suffering from AIDS develop a neurological syndrome referred to as AIDS dementia complex, which is characterized by motor and cognitive dysfunctions that can culminate in paralysis and dementia (for a review see Spencer and Price 1992 and Price and Perry 1994). The loss of neurones, dendrites and synapses has often been described at *post-mortem* in the brain neocortex of AIDS patients (Everall *et al*. 1991). Neuropathological features similar to those described in the brain of patients suffering from AIDS dementia complex have been reported in the brain of transgenic mice expressing the HIV-1 envelope glycoprotein gp120 (Toggas *et al*. 1994). A large body of evidence demonstrated that gp120 causes degeneration and death of several types of neurones maintained in culture (for a review see Lipton and Gendelman 1995). In the adult rat, daily injection of gp120 into one lateral cerebral ventricle (i.c.v.) for seven consecutive days, but not for 1 or 3 days, causes DNA fragmentation in the brain neocortex, but not in the hippocampus, leading to the suggestion that the viral protein may induce apoptosis (Bagetta *et al*. 1995, 1996a).

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Abbreviations used: BSA, bovine serum albumin; Grp75, 75-kDa glucose-regulated protein; HIV-1 gp120, human immunodeficiency virus type-1 glycoprotein 120; interleukin converting enzyme, ICE; i.c.v., intracerebroventricular; IL-β, interleukin-1β; PBS, phosphate-buffered saline; SDS–PAGE, sodium dodecyl sulfhate–polyacrylamide gel electrophoresis; OD, optical densities.

The latter hypothesis has then been confirmed by electron microscopy analysis showing the occurrence of nuclear chromatin marginalization, compaction and other ultrastructural changes (Bagetta *et al*. 1996b, 1998, 1999) typical of early and late apoptosis (Kerr *et al*. 1987). These data, together with the observation of DNA fragmentation in cells from the neocortex of AIDS subjects (Petito and Roberts 1995), strongly implicate apoptosis induced by gp120 in the mechanisms underlying neuronal loss in the brain of patients suffering from AIDS dementia complex (for a review see Price and Perry 1994; Lipton and Gendelman 1995; Spencer and Price 1992).

The mechanisms through which gp120 causes apoptosis in the brain of rat has yet to be discovered, although a series of recent experimental data does implicate the pro-inflammatory cytokine interleukin-1β (IL-1β). In fact, immunohistochemical experiments show that treatment with gp120 enhances the expression of IL-1β in the neocortex area of the brain of rat, where the coat protein causes apoptosis (Bagetta *et al*. 1999). Double-labelling immunofluorescence experiments have established that neuronal and, possibly, microglial cells are the main source for gp120-enhanced IL-1β expression in the neocortex of rat (Bagetta *et al*. 1999). Interestingly, antagonism studies have shown that combined treatment with gp120 and the inhibitor II (Ac-Tyr-Val-Ala-Asp-chloromethylketone) (Milligan *et al*. 1995) of interleukin converting enzyme (ICE), the protease that processes pro-IL-1β in biologically mature IL-1β (Yuan *et al*. 1993; Walker *et al*. 1994; Martins and Earnshow 1997), or 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), minimizes apoptotic cell death caused by the viral protein (Corasaniti *et al*. 1998; Bagetta *et al*. 1999) further implicating IL-1β.

Using immunoelectron microscopy, ELISA and western blotting techniques, we have now discovered that IL-1β increases in the mitochondria where a parallel decrease in pro-IL-1β is seen in conjunction with *in situ* activation of ICE.

Methods and materials

Subjects

Male Wistar rats (250–280 g), housed in a temperature (22°C) and humidity (65%) controlled colony room, were anaesthetized with chloral hydrate (400 mg/kg i.p.) for chronic implantation of a guide cannula (25 gauge) into one lateral cerebral ventricle under stereotaxic guidance (Paxinos and Watson 1982), as previously described (Bagetta *et al*. 1995). The animals were allowed 4 days recovery before treatment. Then, a single dose (100 ng) of gp120 or bovine serum albumin (BSA; control) was administered daily for up to seven consecutive days to each individual rat with a 5-μL Hamilton syringe (1 μL volume; 1 μL/min rate) connected via a Teflon tube to an injector, which exceeded by 2 mm the length of the guide cannula. The experimental procedures used meet the guidelines of the Ministry of Health (GU n°40, February 18, 1992).

Subcellular fractionation

Individual brain ($n = 3$ per group) cortical tissue samples were homogenized in 1 : 6 volumes (w/v) of ice-cold homogenization buffer [320 mM sucrose, 10 mM Tris-HCl, pH 7.4, containing a cocktail of protease inhibitors (Sigma, Milan, Italy)] and the homogenates were centrifuged twice at 1300 *g* for 3 min to pellet nuclei. The supernatants were centrifuged at 17 000 *g* for 10 min to obtain the crude mitochondrial pellets (Lai and Clark 1979). The post-mitochondrial supernatants were centrifuged at 100 000 *g* for 1 h and the high-speed supernatant retained as cytosolic fraction. The crude mitochondrial pellets were resuspended in 0.3 mL of sucrose buffer, subjected to three freeze–thaw cycles and then sonified for 30 s on ice; the mitochondrial suspensions were then centrifuged at 40 000 g for 30 min to remove insoluble material. This procedure gives rise to an enriched mitochondrial fraction and Fig. 1 shows western blots of the mitochondrial matrix proteins, e.g. the constitutive 75-kDa glucose-regulated protein (Grp75), a member of the hsp70 family (Manning-Krieg *et al*. 1991), and complex III, as markers for this subcellular compartment. Protein concentration was determined by using the Bio-Rad DC protein assay (Bio-Rad Laboratories, Milan, Italy). All procedures were performed at 4°C.

Western blotting

For western blot analysis of pro-IL-1β and IL-1β subcellular localization, 20 μg of protein (mitochondrial or cytosolic) were resolved by 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), transferred to Hybond-ECL-nitrocellulose membranes (Amersham, Milan, Italy) and probed overnight

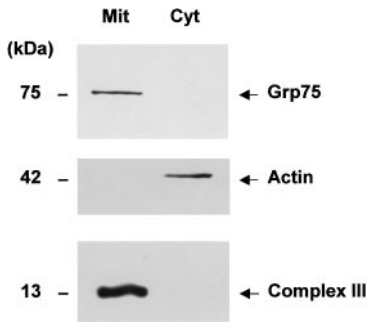


Fig. 1 Western blot analysis to show appropriate fractionation of mitochondrial and cytosolic proteins isolated from the brain neocortex of a Wistar adult rat. Mitochondrial (Mit) and cytosolic (Cyt) proteins (20 μg/lane) were separated by 15% SDS–PAGE and transferred to nitrocellulose membrane that was then cut into three strips: the first, from the origin to the 50-kDa position, the second, encompassing the region between 50 and 30 kDa and the third, from the 30-kDa position to the front. The three strips (from top to bottom) were probed, respectively, with anti-Grp75, anti-actin and anti-complex III antibodies (for details see Materials and methods). Note the immunoreactive bands for Grp75 (75 kDa) and complex III (13 kDa) in the mitochondrial, but not cytosolic, fraction. Similarly, note the immunoreactive band for actin (42 kDa) in the cytosolic fraction. The positions of the molecular mass standards (in kDa) are indicated on the left.

at 4°C with a polyclonal sheep anti-rat antibody, which recognizes both the precursor and mature form of this cytokine [S1002/BM, National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire, UK; 7.5 μg/mL]. The membranes were then incubated with horseradish peroxidase-conjugated anti-sheep IgG (1 : 5000 dilution; Chemicon International, Inc., CA, USA) for 1 h at room temperature (25°C). Immunoreactivity was visualized by chemiluminescent detection (Amersham).

To study ICE expression, mitochondrial fractions (20 μg of protein), resolved by 15% SDS–PAGE and transferred to Hybond-ECL-nitrocellulose membranes (Amersham), were probed overnight at 4°C with a rabbit polyclonal anti-human ICE antibody cross-reacting with rat ICE and recognizing both the p45 (45 kDa) pro-enzyme and the p20 (20 kDa) component of the active enzyme (Upstate Biotechnology, NY, USA; 1 μg/mL). The membranes were then incubated with horseradish peroxidase-conjugated anti-rabbit IgG (1 : 8000 dilution; Pierce, Rockford, IL, USA) for 1 h at room temperature and the immunoreactivity visualized by chemiluminescent detection (Amersham). The Grp75 and complex III were used as markers for the mitochondrial enriched fractions whereas actin has been used as a marker for the cytosolic fraction (Fig. 1). To this end, proteins (20 μg) from subcellular fractions were resolved by 15% SDS–PAGE transferred to nitrocellulose membranes and blots were cut to yield strips individually immunoprobed with mouse monoclonal anti-Grp75 (clone 30A5, 1 : 1000 dilution; StressGen Biotechnologies Corp., Victoria, BC, Canada), mouse monoclonal anti-actin (clone AC-40, 1 : 2000 dilution; Sigma, St Louis, MO, USA) or rabbit polyclonal anti-bovine complex-III (1 : 1000 dilution; from R. Bisson, University of Padova, Italy) antibodies. The latter antibody recognizes all of the polypeptides of the complex III (Borgese *et al*. 1996); however, the polypeptide with an apparent molecular mass of 13 000 was chosen because of its convenient position on the gel.

Autoradiographic strip films were scanned and analysed as previously reported (Bagetta *et al*. 1999) using a computer-assisted program (QUANTISCAN, Biosoft, Cambridge, UK).

IL-1β ELISA

Mitochondrial and cytosolic fractions, isolated from individual brain cortical tissue samples (see above) and diluted in high-performance ELISA buffer (Central Laboratorium Bloedtransfusiendienst (CLB), Amsterdam, the Netherlands), were assayed for immunoreactive IL-1β levels by an established, rat specific, sandwich ELISA (Hagan *et al*. 1996). The immunoaffinity-purified polyclonal sheep anti-rat IL-1β coating antibody (1 μg/mL), the biotinylated, immunoaffinity-purified polyclonal sheep anti-rat IL-1β detecting antibody (1 : 1000 dilution), as well as the recombinant rat IL-1β standard were kindly provided by Dr Stephen Poole (National Institute of Biological Standards and Controls (NIBSC), Hertfordshire, UK). Poly-horseradish peroxidase-conjugated streptavidin (CLB) was used at 1 : 5000 dilution and the colour was developed by using the chromogen *o*-phenylenediamine (Sigma). Optical densities (OD) were read at 492 nm by using an automated plate reader (Multiscan MS; Labsystems, Helsinki, Finland) and cytokine levels calculated by interpolation from the standard curve (0.0–1000 pg/mL). Data were corrected for protein concentration and the results expressed as pg of IL-1β per mg of protein. Under our experimental conditions, the assay has a sensitivity of 2 pg/mL [lowest cytokine concentration that gives OD values ≥ two standard deviations above those yielded by the background (0 pg/mL cytokine)]. Recovery of rat IL-1β added to mitochondrial or cytosolic fractions was 84.5 ± 2.3%.

Preparation of brain tissue sections for immunoelectron microscopy

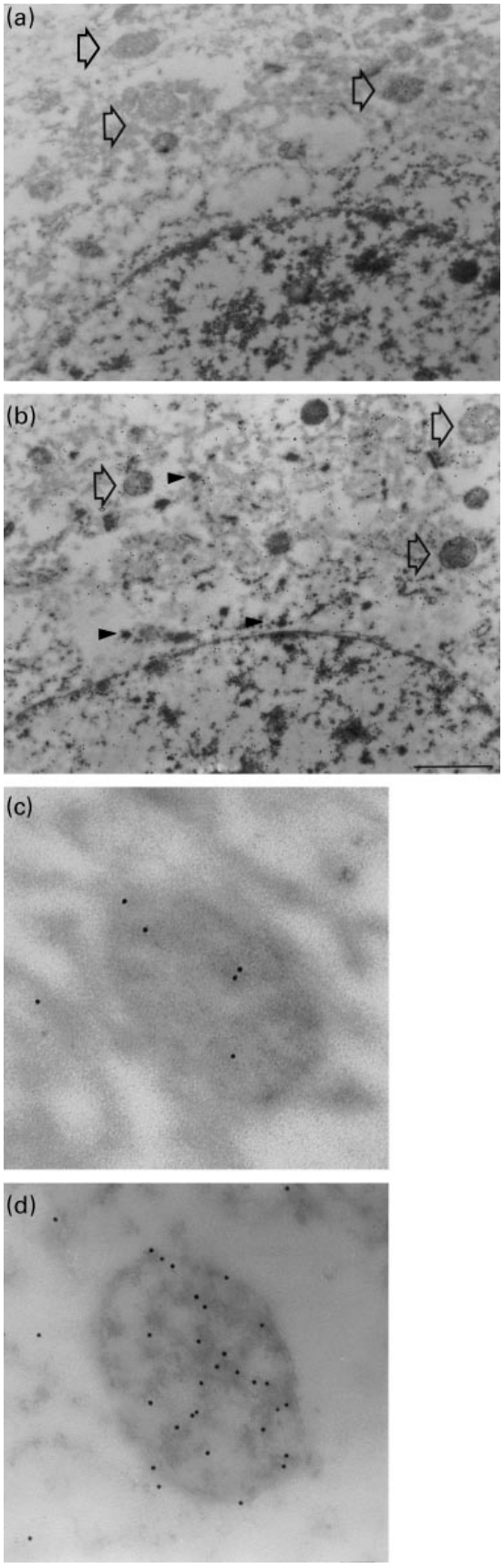
Brain ($n = 6$ per group) cortical tissue samples were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) by perfusion method, and were dehydrated in ethanol gradient and embedded in Lowicryl HM20 (Electron Microscopy Sciences, Fort Washington, PA, USA). The resin was polymerized under UV light for 2 h at 4°C and for 22 h at room temperature. For immunolocalization in post-embedding procedure ultra thin sections, obtained with a LKB ultramicrotome (Ultratome Nova; Bromma, Sweden), were collected on gold grids and treated for 5 min with PBS containing 0.15% (w/v) glycin. After washing by quickly floating the grids on PBS drops containg 0.4% (w/v) gelatin and 0.1% (w/v) BSA, the sections were incubated overnight at 4°C with a polyclonal goat anti-rat antibody (R & D Systems Inc., Minneapolis, MN, USA) to detect IL-1β (1 : 10). After washing by floating the grids on PBS drops containing 0.1% (w/v) BSA for 1 h at room temperature, the sections were pre-incubated with PBS plus 0.4% (w/v) gelatin and 0.1% (w/v) BSA for 10 min, washed in PBS containing 0.1% (w/v) BSA, and then labelled with anti-goat IgG 10-nm gold conjugate (diluted 1 : 10; Sigma, Milan, Italy) for 20 min, then washed in PBS containing 0.1% BSA for 20 min at room temperature. Controls included: (i) omission of the primary antibody; (ii) substitution of the specific primary antibody with an irrelevant goat polyclonal IgG. Ultra thin sections were stained with uranyl acetate and lead citrate for examination with a transmission electron microscope (Philips 208 model).

Materials

Lyophilized, full-length glycosylated recombinant HIV-1 gp120 IIIB (> 90% pure as by SDS–PAGE; tested randomly for endotoxin contamination), dissolved in PBS, aliquoted on ice upon receipt, stored at −20°C and used in concentrations of 100 ng/μL (quantified by enzyme-linked immunosorbent assay), was from INTRACEL (Catalogue n°120011; DBA, Milan, Italy). For immunoelectron microscopy, the anti-rat IL-1β antibody (lot number YR01, rat IL-1β specific goat IgG) was from R & D Systems Inc. (Minneapolis, MN, USA).

Results

Immunoelectron microscopy analysis of brain tissue sections from control, BSA-treated, rats ($n = 6$) showed a very low expression of IL-1β immunoreactivity in the neocortex. Subchronic treatment ($n = 6$ rats) with gp120 (100 ng given i.c.v. once daily for seven consecutive days) induced an increase of immune labelling (see Fig. 2b for a typical example) with a localization of the antigen in cell structures such as mitochondria, lysosomes and nucleus (Fig. 2b). In particular, gold labelling of mitochondria showed apparent differences in the expression of IL-1β immunoreactivity; an increased labelling, mainly localized in the inner mitochondrial membrane and mitochondrial cristae (compare Figs 2c and d), was detected.



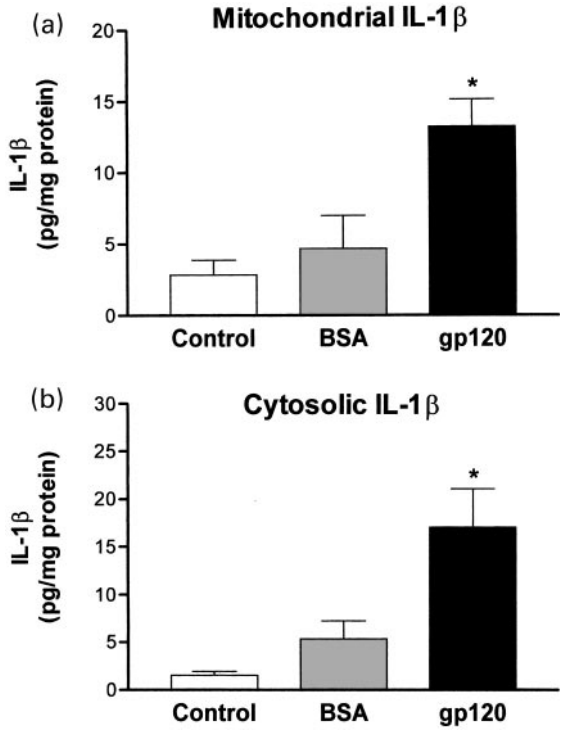


Fig. 3 Immunoreactive IL-1β levels are increased in mitochondrial and cytosolic fractions from the neocortex of rats receiving a single daily injection of the HIV-1 coat protein gp120 (100 ng) given into one lateral cerebral ventricle (i.c.v.) for seven consecutive days. Immunoreactive IL-1β levels were assayed in mitochondrial and cytosolic fractions, isolated from individual brain ($n = 3$ per group) cortical tissue samples, by an established, rat specific, sandwich ELISA (see Materials and methods). Interleukin-1β levels were corrected for protein concentration and the results expressed as pg of IL-1β per mg of protein. Data are expressed as mean ± SEM (bars) values ($n = 3$ per group). The resulting means were evaluated statistically for differences using ANOVA followed by Tukey–Kramer test for multiple comparisons. * Denotes $p < 0.05$ vs. BSA.

Fig. 2 Transmission immunoelectron microscopy photographs to illustrate the subcellular localization of IL-1β the expression of which is enhanced in the brain neocortex of a rat treated with the HIV-1 coat protein gp120. Ultra thin tissue sections from the brain of a control (a), BSA-treated (100 ng/die given i.c.v. for 7 consecutive days) rat and from a rat (b and d) treated with gp120 (100 ng/die given i.c.v. for 7 consecutive days) immunolabelled with a polyclonal antibody to detect IL-1β. Note (i) the widespread presence of gold labelling visible at low magnification in sections from gp120-exposed rat (b) as compared with the labelling shown in a control section (a), (ii) the presence of a marked microsome associated positivity detected in vesicular bodies (b, probably corresponding to lysosomal compartments, arrowheads), mitochondria (arrows) and nucleus (iii) the scarse presence of gold particles in mitochondria obtained from BSA-treated samples (shown in c) if compared with (iv) the massive increase of gold particles essentially localized along the mitochondrial cristae (d). Bars correspond to 10 μm (a and b) and 0.1 μm (c and d).

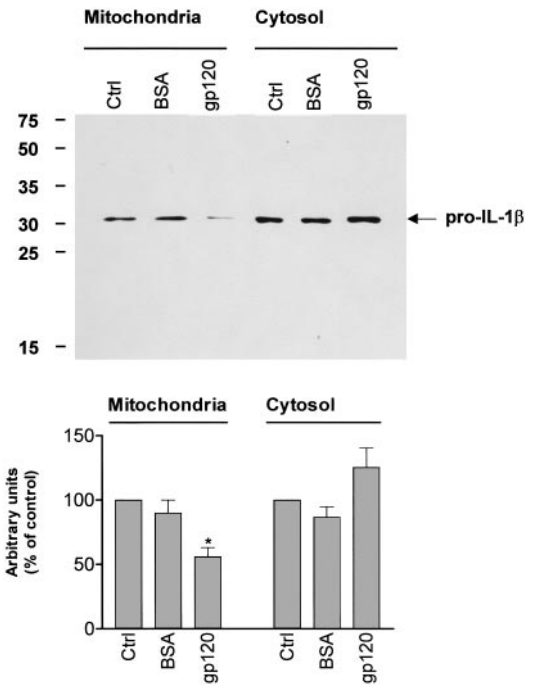


Fig. 4 (Top panel) Western blot analysis showing reduction of the intensity of the 31-kDa band corresponding to pro-IL-1β in the mitochondrial but not cytosolic fraction obtained from the neocortex of gp120-treated rat. Individual brain neocortical tissue samples from a naive, untreated rat (Control), a rat treated for seven consecutive days with bovine serum albumin (BSA; 100 ng/die i.c.v.) or from gp120 (100 ng/die i.c.v.) were fractionated into mitochondrial (Mitochondria) and cytosolic (Cytosol) fractions, resolved (20 μg of protein/lane) by 15% SDS–PAGE and immunoblotted with sheep anti-rat IL-1β polyclonal antibody (see Materials and methods). Note the lack of reduction of the 31-kDa band in the high speed supernatant (cytosolic fraction) from the neocortex of gp120-treated rat as compared with both control (Control) and BSA-treated rats. The western blot represents a typical example of three independent experiments ($n = 3$ rats per group) yielding similar results. The positions of the molecular mass standards (in kDa) are indicated on the left. For comparison, relative intensity values (arbitrary units) of the autoradiographic bands were determined by computer-assisted densitometric analysis as previously described (Bagetta *et al.* 1999). The data from quantitative analysis of three independent experiments are described in the histogram shown in the bottom panel and the resulting means (± SEM) evaluated statistically for difference by ANOVA followed by Tukey–Kramer multiple comparisons test. *Denotes $p < 0.05$ vs. BSA.

In agreement with the above described observation, ELISA experiments demonstrate that subchronic treatment with gp120 (100 ng given i.c.v. once daily for seven consecutive days) increases immunoreactive IL-1β levels in the mitochondrial and cytosolic fractions obtained from rat brain neocortical tissue as compared with the levels measured in identical subcellular fractions from the brain of naive, untreated, rats and rats treated with BSA (100 ng given i.c.v. once daily for seven consecutive days) (Fig. 3).

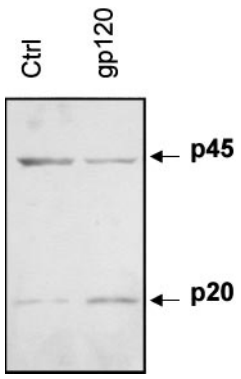


Fig. 5 The HIV-1 gp120 (100 ng, given i.c.v. 6 h beforehand) reduces the expression of the p45 pro-form of the IL-1β converting enzyme (ICE, also referred to as Caspase-1) and enhances the expression of the p20 component of the active enzyme in the mitochondrial fraction of the neocortex of rat. Note the basal expression of either p45 and p20 ICE in the mitochondrial fraction of control rat (Ctrl). The western blot represents a typical example of three independent experiments ($n = 3$ rats per group) yielding similar results.

To gain insights in the origin of basal and gp120-stimulated mitochondrial IL-1β, aliquots (20 μg) of the same mitochondrial and cytosolic fractions processed for IL-1β ELISA, were resolved by 15% SDS–PAGE, transferred to nitrocellulose membranes and probed with a sheep anti-rat IL-1β antibody (S1002/BM, 7.5 μg/mL) that also recognizes the 31-kDa precursor protein of this cytokine (Dr Stephen Poole, National Institute of Biological Standards and Controls, Hertfordshire, UK; personal communication). As shown in the top panel of Fig. 4, pro-IL-1β is normally expressed into the mitochondria from neocortical brain tissue of control (naive), untreated rats and this is significantly ($p < 0.05$) reduced by subchronic treatment with gp120 ($n = 3$ rats) (Fig. 4, top and bottom panels). The latter observation together with the evidence that mitochondrial fractions from gp 120-treated animals do express the 45-kDa form of ICE (the inactive pro-enzyme, p45-ICE) as well as the p20 component of activated ICE (Fig. 5) supports the deduction that the viral protein is able to induce *in situ* activation of ICE with consequent processing and diminution of the 31-kDa pro-IL-1β band (Fig. 4, top and bottom panels) accompanied by enhancement of the biologically mature form of the cytokine (Fig. 3). Similarly, the 31 kDa pro-IL-1β signal is observed in the cytosol of control and gp 120-treated animals (Fig. 4a); however, subchronic treatment with the viral coat protein failed to reduce the expression of the precursor protein in the cytosolic fraction (Fig. 4b).

Discussion

It has been previously shown by immunoelectron microscopy and TUNEL staining techniques that the neocortex, but not the hippocampus, is the area of the rat brain

susceptible to the detrimental action of the HIV coat protein (Bagetta *et al*. 1995, 1996b) and this formed the basis for studying the expression of IL-1β in the former brain region. To this end, immunoelectron microscopy and ELISA techniques allowed the expression of the pro-inflammatory cytokine IL-1β to be studied in the neocortex of rat. In particular, the use of an established subcellular fractionation procedure allowed the detection of IL-1β at the subcellular level by ELISA (see Materials and methods). The validity of the fractionation procedure used here is confirmed by western blotting analysis showing the expression of proteins specific for the mitochondrial matrix or for the cytosol without contaminating components (Fig. 1). Furthemore, application of this subcellular fractionation procedure, in conjunction with immunoelectron microscopy, has previously allowed the successful mitochondrial localization of other proteins in the rat brain (Bates *et al*. 1995) strengthening the above deduction.

Using this experimental approach here we have shown that IL-1β is expressed in the mitochondria and in the cytosol of brain neocortical cells of naive, untreated, rats and of rats treated for seven consecutive days with a single daily i.c.v. injection of BSA. The mitochondrial and the high speed supernatant (i.e. cytosol) fractions also express a 31-kDa band corresponding to the IL-1β precursor protein (Black *et al*. 1988; Kostura *et al*. 1989). Interestingly, the mitochondrial fraction from control, untreated, rats also showed the occurrence of the p20 band of ICE demonstrating that this enzyme is active (Thornberry *et al*. 1992; Walker *et al*. 1994) and suggesting that, under normal conditions, mitochondrial IL-1β immunoreactivity may originate from *in situ*, ICE-mediated, cleavage of pro-IL-1β. The latter mechanism seems to account for enhanced IL-1β levels reported in the mitochondrial fraction from the brain neocortex of rats treated with gp120. In fact, under these conditions elevation of mitochondrial IL-1β was accompanied by a parallel decrease of the pro-IL-1β 31 kDa band together with enhanced expression of the p20 component of activated ICE (Thornberry *et al*. 1992). However, in the cytosolic fraction of gp120-treated animals the lack of obvious changes in the 31-kDa band was accompanied by elevated, instead of reduced, IL-1β and the underlying mechanism remains unknown.

Interleukin-1β-like immunogold positive material has also been observed in the nucleus of neocortical cells from gp120-treated rats (Fig. 2) and this localization, although intriguing, appears to be genuine; in fact, high levels of ICE have been recently described in the nucleus of neuroblastoma cells undergoing apoptosis (Ikeda *et al*. 1997; Nakagawara *et al*. 1997).

At variance with the lack of information concerning the ultrastructural localization of IL-1β and of pro-IL-1β in resting and stimulated mammalian brain tissue, this has often been described in human monocytes isolated from peripheral blood samples (see, for example, Singer *et al*. 1988 and Beesley *et al*. 1990). Thus, in agreement with our present data, immunoelectron microscopy and immunoprecipitation experiments have previously shown the mitochondrial localization of pro-IL-1β in human monocytes isolated from peripheral blood samples and stimulated *in vitro* with lipopolysaccharide (LPS) (Beesley *et al*. 1990). In agreement with our results Beesley *et al*. (1990) failed to demonstrate a clear 17-kDa band, corresponding to mature IL-1β, in any of the subcellular fractions studied and this may conceivably reflect a low level of sensitivity of the immunoblotting technique. In fact, control experiments demonstrate that the antibody used here recognizes recombinant rat IL-1β when it is loaded at 2 ng/lane in the gel as a positive control (data not shown). However, under our experimental conditions, western blotting analysis does not reach the sensitivity required to detect the low levels of mature IL-1β (femtograms of IL-1β per 20 μg of protein loaded into each lane of the gel, as determined by ELISA) present in brain samples.

The results of our original double labelling immunohistofluorescence experiments strongly support the concept that gp120 enhances IL-1β expression in neuronal and microglial cells but not astrocytes (Bagetta *et al*. 1996a, 1999). More importantly, enhanced expression of this cytokine is an early event (24 h from gp120 administration; Bagetta *et al*. 1999) as compared with the expression of apoptosis (seven days; Bagetta *et al*. 1995, 1996a,b, 1998, 1999) and this may conceivably stem from the time required for the death programme to be executed under the detrimental stimulus provided by each individual gp120 injection.

In conclusion, our present results represent the first detailed description of the distribution at the ultrastructural level of the pro-inflammatory cytokine, IL-1β, in the brain neocortex of control and gp120-treated rats. In addition, the data support the concept that gp120-enhanced mitochondrial IL-1β expression may originate from cleavage of pro-IL-1β operated by ICE activated in the mitochondrion, an important cellular structure implicated in the expression of apoptosis (see Susin *et al*. 1999).

Finally, our present and previous (Bagetta *et al*. 1999) data suggest that abnormal expression of IL-1β, together with the gene products of which expression can be induced by IL-1β (e.g. cyclo-oxygenase type 2; see Bagetta *et al*. 1998; Corasaniti *et al*. 2000), may be the target for the development of novel therapeutical strategies to treat chronic neurodegenerative diseases other than AIDS dementia complex and including Alzheimer's and Huntington's diseases, in which excess IL-1β in the CNS has been documented (Griffin *et al*. 1989; Genis *et al*. 1992; Lipton and Gendelman 1995).

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