HIV-1 coat protein gp120 stimulates interleukin-1β secretion from human neuroblastoma cells: evidence for a role in the mechanism of cell death

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1 The role of the pro-inflammatory cytokine interleukin-1β (IL-1β) in the mechanism of cell death induced by the human immunodeficiency virus type 1 (HIV-1) recombinant coat glycoprotein, gp120 IIIB, has been studied in the human CHP100 neuroblastoma cell line maintained in culture.

2 Death of neuroblastoma cells typically elicited by 10 pM gp120 or by human recombinant IL-1β (10 ng ml^{-1}) has been minimized by the antagonist of IL-1 receptor, i.e. IL-1ra (0.5 and 50 ng ml^{-1}, respectively), an endogenous molecule that antagonizes most of the biological actions of IL-1β, or by an antibody (5 and 50 ng ml^{-1}) which blocks the human IL-1 receptor type I (IL-1RI).

3 ELISA experiments have established that gp120 enhances immunoreactive IL-1β levels in the culture medium and this is prevented by exposure to the IL-1 converting enzyme (ICE) inhibitor t-butoxycarbonyl-L-aspartic acid benzyl ester-chloromethylketone [Boc-Asp(OBzl)-CMK] used at a concentration (2.5 μM) which significantly ($P < 0.001$) reduces cell death.

4 Death of CHP100 cells induced by gp120 is also prevented by acetyl-Tyr-Val-Ala-Asp-chloromethylketone (Ac-YVAD-CMK; 10–100 μM), a second inhibitor of ICE, supporting the concept that the viral protein stimulates the conversion of the 31 kDa pro-IL-1β in to the 17 kDa mature cytokine which is then secreted to cause death.

5 In conclusion, our present data demonstrate that gp120 stimulates the secretion of IL-1β which then triggers CHP100 neuroblastoma cell death *via* stimulation of IL-1 receptor type I.

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Abbreviations: Ac-YVAD-CMK, acetyl-Tyr-Val-Ala-Asp-chloromethylketone; Boc-Asp(OBzl)-CMK, t-butoxycarbonyl-L-aspartic acid benzyl ester-chloromethylketone; HIV-1, human immunodeficiency virus type 1; ICE, Interleukin-1 converting enzyme; IL-1β, interleukin-1β; IL-1ra, IL-1 receptor antagonist; IL-1 RI, IL-1 receptor type I

Introduction

Some 30% of patients suffering from AIDS develops a neurological syndrome, referred to as AIDS dementia complex, characterized by cognitive impairment, postural disorders and tremor which can culminate in paralysis (Price *et al.*, 1988). Loss of cortical neurones has been described at post mortem in the brain of AIDS patients (Everall *et al.*, 1991) and the human immunodeficiency virus type 1 (HIV-1) coat glycoprotein gp120 has been implicated in the mechanisms of the observed brain neuronal loss because it causes death of several types of neurones maintained in culture. In fact, *in vitro* exposure to gp120 causes death of rodent cortical (Dawson *et al.*, 1993) and hippocampal (Dreyer *et al.*, 1990) neurones, retinal ganglion cells (Lipton *et al.*, 1991), cerebellar granule cells (Savio & Levi, 1993) and human CHP100

neuroblastoma cells (Corasaniti *et al.*, 1995). The mechanism underlying cell death involves excessive Ca^{2+} entry into neurones *via* N-methyl-D-aspartate (NMDA) receptor associated cation channel and through voltage operated Ca^{2+} channels since NMDA antagonists and Ca^{2+} channels blockers prevent both the rise in the intracellular level of the cation and the cytotoxicity (Dreyer *et al.*, 1990; Lipton *et al.*, 1991) suggesting an excitotoxic, glutamate-mediated, type of death (see Choi, 1988). In agreement with the latter hypothesis, death of human CHP100 neuroblastoma cells induced by gp120 is prevented by selective, competitive (CGP37849 and LY274614) and non-competitive (MK801, 7-chlorokinurenic acid) antagonists of the NMDA receptor complex, by the dihydropyridine, nicardipine, an L-type Ca^{2+} channel blocker, and by removal of calcium ions from the culture medium (Corasaniti *et al.*, 1995).

Several experimental data demonstrate that excitotoxic stimuli increase the expression of the pro-inflammatory cytokine, IL-1β, and this has been implicated in neuronal

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damage (see Rothwell, 1999). In particular, enhanced expression of IL-1β mRNA has been reported in the brain of rats undergoing transient forebrain ischaemia (Minami *et al*., 1992) and of rats receiving systemic administration of chemical convulsants, such as kainic acid and pentylenetetrazole (Minami *et al*., 1990). Furthermore, focal intracerebral injection of NMDA, given at a dose which causes neuronal cell death, has been shown to enhance IL-1β expression in the brain of perinatal rats and this is prevented by a neuroprotective dose of the NMDA receptor antagonist MK801 (Hagan *et al*., 1996). In addition, intrastriatal injection of human recombinant IL-1β exacerbates brain damage caused by ischaemia in the adult rat (Stroemer & Rothwell, 1998). Further support for a role of IL-1β in excitotoxic neuronal damage stems from the evidence that inhibition of its biological actions by the naturally occurring IL-1 receptor antagonist (IL-1ra) reduces ischaemic brain damage (Relton & Rothwell, 1992; Loddick & Rothwell, 1996) and damage caused by brain trauma (Toulmond & Rothwell, 1995), excitotoxins (Relton & Rothwell, 1992) and by hypoxia-ischaemia insults (Martin *et al*., 1994). More recently, it has been reported that brain ischaemia induces the expression of IL-1ra mRNA and inhibition of its biological action markedly enhances ischaemic brain damage (Loddick *et al*., 1997). Altogether, these data strongly implicate abnormal expression of IL-1β in the mechanisms underlying neuronal cell death caused by excitotoxic stimuli. Here we now report experimental evidence demonstrating that the HIV-1 coat protein gp120 stimulates the secretion of IL-1β from neuroblastoma cells and this contributes to the mechanisms of cytotoxicity elicited by the viral coat protein because pharmacological manipulations reducing the biological actions of IL-1β prevent death of human CHP100 neuroblastoma cells.

Methods

Materials

Lyophilized, full-length glycosylated recombinant HIV-1 gp120 IIIB (>90% pure as by SDS–PAGE; tested randomly for endotoxin contamination), was from Intracel (London, U.K., Catalogue no 120011). Human recombinant IL-1β (hrIL-1β; biological activity, given as ED_{50} in a murine T cell line proliferation assay, is $5-10$ pg ml^{-1} and endotoxin content is <0.1 ng μg^{-1} of cytokine) and the anti-human IL-1 RI neutralizing antibody (lot n° MU03; cross-reactivity with rhIL-1 RII is $5-10\%$) were from R&D Systems (Milan, Italy). IL-1ra was a kind gift from Dr D. Boraschi (Dompè, Italy). ELISA reagents were from Endogen (MA, U.S.A.): coating antibody (mouse monoclonal anti-human IL-1β) specific for natural and recombinant human IL-1β (clone ILB1-H67); mouse monoclonal biotin-labelled anti-human IL-1β specific for natural and recombinant human IL-1β (clone ILB1-H6). The ICE inhibitors acetyl-Tyr-Val-Ala-Asp-chloromethylketone (Ac-YVAD-CMK) and t-butoxycarbonyl-L-aspartic acid benzyl ester-chloromethylketone [(Boc-Asp(OBzl)-CMK)] were from Bachem (Bubendorf, Switzerland).

Cell cultures and treatments

Human CHP100 neuroblastoma cells were cultured as previously described (Corasaniti *et al*., 1992) in a 1:1 mixture of MEM (Eagle's minimal essential medium with Earle's salts) and Ham's F-12 media, supplemented with 10% heat-inactivated foetal bovine serum, at 37°C in a 5% CO_2 atmosphere. Cells were subcultured from confluent 75 cm^2 flasks and seeded in 35 mm 6-well plates. Twenty-four hours after plating, the growth medium was replaced with fresh normal medium (control cultures) or with medium supplemented with gp120; exposure to gp120 was carried out for 24 h. The viral protein was used at 10 pM as this concentration has been shown to induce significant cytotoxic effects in CHP100 cells (Corasaniti *et al*., 1995; 1996; 1998; Maccarrone *et al*., 1998). In experiments involving IL-1ra, Ac-YVAD-CMK or Boc-Asp(OBzl)-CMK and the anti-human IL-1 RI neutralizing antibody, these were applied to CHP100 cells 30 min or 1 h before, respectively, the addition of the viral protein and they were present during the 24 h exposure time. Cell viability was assessed by cell exclusion of trypan blue (0.4% w v^{-1}) and cell death was reported as the percentage of stained (non viable) vs total cells counted (Corasaniti *et al*., 1995; 1996; 1998; Maccarrone *et al*., 1998). The data were expressed as mean ± s.e.mean percentage cell death.

IL-1β ELISA

For the determination of intracellular IL-1β concentrations, cells, treated as described above, were resuspended in phosphate buffer saline (PBS; 0.01 M phosphate buffer, 0.0027 M KC1, 0.137 M NaCl, pH 7.4) containing a cocktail of protease inhibitors (Sigma, Milan, Italy) and subjected to three freeze-thaw cycles, then the lysates were sonified and centrifuged at $30,000 \times g$ for 30 min to remove insoluble material. The resulting supernatants were assayed for protein content (Bio-Rad DC Protein Assay, Bio-Rad Laboratories, Milan, Italy) and employed for the determination of IL-1β levels by ELISA. Measurement of secreted IL-1β was carried out using aliquots of culture medium from control and treated cultures following centrifugation at $30,000 \times g$ for 20 min to sediment cell debris. The mouse monoclonal anti-human IL-1β coating antibody (1 μg ml^{-1}), the anti-human IL-1β mouse monoclonal biotin-labelled antibody (0.05 μg ml^{-1}) as well as the human recombinant IL-1β used to prepare the standard curve ($0.0-128$ pg ml^{-1}) were from Endogen (MA, U.S.A.). Poly-horseradish peroxidase-conjugated streptavidin (CLB, Amsterdam, Holland) was used at 1:5000 dilution and the colour developed by using the chromogen *o*-phenylenediamine (Sigma, Milan, Italy). Optical densities were read at 492 nm by using an automated plate reader (Multiscan MS, Labsystems) and cytokine levels calculated by interpolation from the standard curve ($0.0-128$ pg ml^{-1}). Data were corrected for protein concentration of the sample and the values expressed as picograms of IL-1β mg^{-1} of protein.

Statistical analysis

Data are expressed as mean ± s.e.mean and the resulting means evaluated statistically for differences using one-way

analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Values of $P < 0.05$ were considered significant.

Results

Blockade of IL-1 receptors prevents HIV-1 gp120-induced cell death

To characterize the role of IL-1β in gp120-induced cell death this has been studied in human CHP100 neuroblastoma cell cultures exposed to the IL-1 receptor antagonist (IL-1ra), an endogenous protein which binds IL-1 receptors and prevents most of the biological effects of the cytokine (Hannum *et al.*, 1990). The experiments were carried out by incubation with the viral protein at a concentration (10 pM) known to produce significant cytotoxic effects in human CHP100 cultures (Corasaniti *et al.*, 1995; 1996; 1998; Maccarrone *et al.*, 1998) and complete dose-effect relations are reported elsewhere (Corasaniti *et al.*, 1995). Under these experimental conditions, exposure to 10 pM gp120 ($n = 8$ experiments) yielded a $22.4 \pm 1.25\%$ cell death ($P < 0.001$ vs control cell death; $n = 8$ experiments) (Figure 1). As shown in Figure 1, IL-1ra (0.5–500 ng ml^{-1}) significantly reduced cell death elicited by the viral coat protein. In particular, an approximately 80% ($80.6 \pm 9.3\%$; $n = 4$ experiments) inhibition of gp120-induced cell death is produced by IL-1ra applied at a concentration of 0.5 ng ml^{-1}; no further protection is provided by higher concentrations of the antagonist (5–500 ng ml^{-1}; $n = 4$–8 experiments per concentration) whereas at 0.05 ng ml^{-1} ($n = 4$ experiments) IL-1ra was ineffective against gp120-induced cell death (Figure 1). IL-1ra given alone (0.05–500 ng ml^{-1}; $n = 4$ experiments per concentration) does not affect the viability of CHP100 cultures (Figure 1).
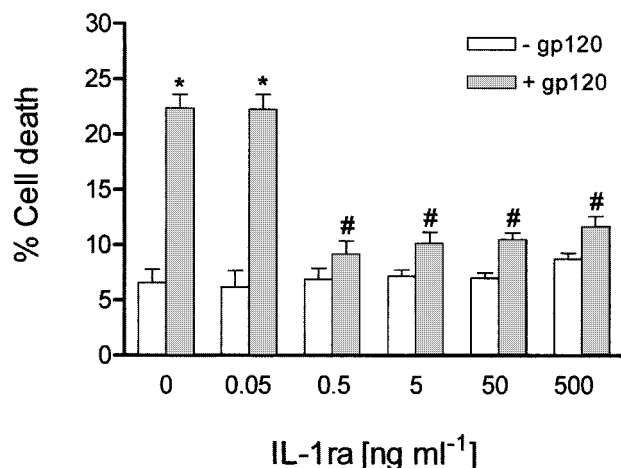
Prevention of gp120 (10 pM)-induced cell death is also afforded by an antibody which blocks the human IL-1 receptor type I (IL-1 RI) (Figure 2). This neutralizing antibody is effective at concentrations of 5 and 50 ng ml^{-1} ($n = 4$–6 experiments per concentration) whereas no prevention of gp120-induced cell death is conferred by a lower concentration (0.05 ng ml^{-1}; $n = 6$ experiments) of the antibody (Figure 2). As shown in Figure 2, the anti-IL-1 RI neutralizing antibody given alone does not affect CHP100 viability ($P > 0.05$ vs control cell death).

Recombinant IL-1β induces death of CHP100 cells and this is reduced by IL-1ra

To further evaluate the cytotoxic potential of IL-1β in CHP100 cultures, these have been exposed to human recombinant IL-1β (hrIL-1β) for 24 h alone or in the presence of IL-1ra. As reported in Figure 3, hrIL-1β (10 ng ml^{-1}; $n = 4$ experiments) has cytotoxic effects ($P < 0.001$ vs control) that are significantly ($P < 0.01$) reduced by IL-1ra (50 ng ml^{-1}; $n = 4$ experiments). A lower concentration (0.1 ng ml^{-1}; $n = 4$ experiments) of the human recombinant cytokine does not affect cell viability ($P > 0.05$ vs control; Figure 3).

HIV-1 gp120 enhances the secretion of IL-1β from CHP100 cells

The finding that hrIL-1β likewise gp120 causes neuroblastoma cell death and that the latter is minimized by blockade of cell surface IL-1β receptors strongly supports the deduction that the viral protein stimulates the secretion of IL-1β from CHP100 cells. To test the latter hypothesis, measurement of IL-1β levels by ELISA was carried out in cellular lysates and in the extracellular medium of control- and gp120-treated cultures. In comparison with untreated, control, cultures treatment with
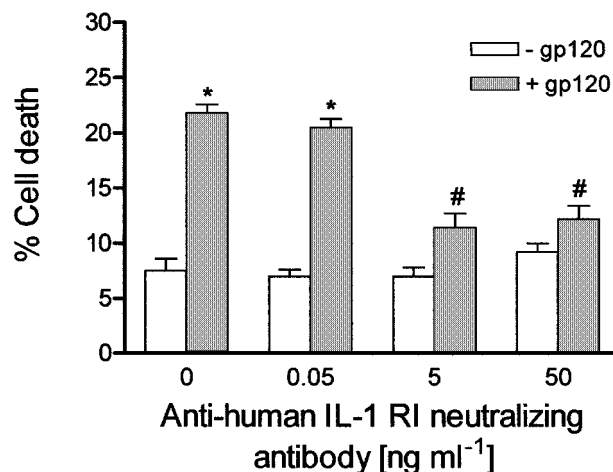

Figure 1 Inhibition of HIV-1 gp120-induced death of human CHP100 neuroblastoma cells by IL-1 receptor antagonist. Incubation of neuroblastoma cells with 10 pM gp120 for 24 h in the presence of IL-1 receptor antagonist (IL-1ra; 0.5–500 ng ml^{-1}) prevents the cell death induced by the viral protein. A lower concentration of IL-1ra (0.05 ng ml^{-1}) is ineffective. Each value is the mean ± s.e.mean of 4–8 experiments. *Denotes $P < 0.001$ vs control cell death; # denotes $P < 0.001$ vs cell death produced by gp120 given alone (one-way ANOVA followed by Tukey–Kramer multiple comparison test).

Figure 2 Blockade of IL-1 receptor type I (IL-1 RI) protects against HIV-1 gp120-induced death of human CHP100 neuroblastoma cells. Incubation of neuroblastoma cells with 10 pM gp120 for 24 h in the presence of an anti-human IL-1 RI neutralizing antibody (5 and 50 ng ml^{-1}) prevents the cell death induced by the viral protein. Each value is the mean ± s.e.mean of 4–8 experiments. *Denotes $P < 0.001$ vs cell death of untreated, control, cultures; # denotes $P < 0.001$ vs gp120 given alone (one-way ANOVA followed by Tukey–Kramer multiple comparison test).
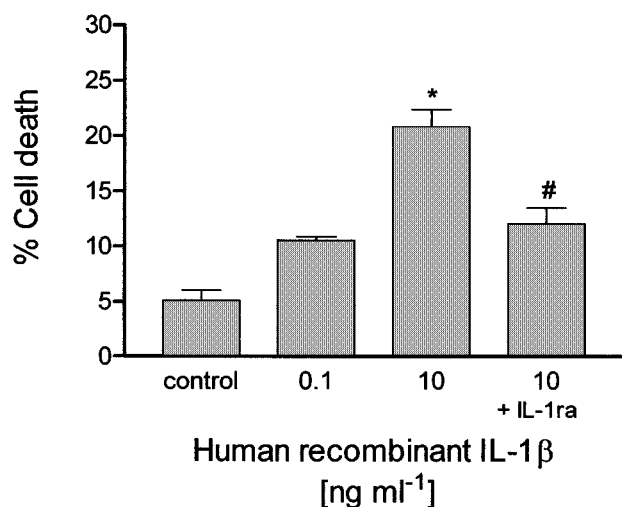
Figure 3 Human recombinant IL-1β (hrIL-1β) induces death of human CHP100 neuroblastoma cells and this is reduced by IL-1 receptor antagonist. Exposure of neuroblastoma cultures to hrIL-1β (10 ng ml^{-1}) for 24 h induces significant cytotoxic effects, as estimated by trypan blue uptake assay. At a lower concentration (0.1 ng ml^{-1}), hrIL-1β does not affect significantly CHP100 cell viability ($P > 0.05$ vs control cell death). The cell death induced by 10 ng ml^{-1} hrIL-1β is significantly ($P < 0.01$) reduced by IL-1 receptor antagonist (IL-1 ra; 50 ng ml^{-1}). Each value is the mean $\pm$ s.e.m. of four experiments. *Denotes $P < 0.001$ vs control cell death; # denotes $P < 0.01$ vs cell death produced by 10 ng ml^{-1} hrIL-1β given alone (one-way ANOVA followed by Tukey–Kramer multiple comparison test).

10 pM gp120 significantly ($P < 0.05$) increases IL-1β levels in the culture supernatant (from 18.0 $\pm$ 4.8 to 39.9 $\pm$ 5.9 pg mg^{-1} of protein; $n = 5$ experiments) but does not affect intracellular content of the cytokine (3.37 $\pm$ 0.77 and 3.49 $\pm$ 0.59 pg mg^{-1} of protein, respectively; $n = 5$ experiments). Thus, exposure to gp120 stimulates CHP100 neuroblastoma cells to produce and release IL-1β.

Inhibition of ICE minimizes gp120-induced cell death via *reduction of secreted IL-1β*

IL-1β is synthesized as a 31 kDa inactive precursor, pro-IL-1β, that to yield the mature, 17 kDa biologically active form of the cytokine has to be cleaved by IL-1 converting enzyme (ICE) (Black *et al.*, 1988; Thornberry *et al.*, 1992), also known as caspase-1, a member of the caspase family of proteases that are known to be implicated in cell death (Yuan & Yankner, 2000). To assess whether blockade of IL-1β production from its inactive precursor could affect the induction of cell death by gp120, we examined the ability of Ac-YVAD-CMK, an irreversible inhibitor of ICE (see Milligan *et al.*, 1995), to prevent CHP100 neuroblastoma cell death caused by the viral protein. Incubation of CHP100 cells with 10 pM gp120 for 24 h in the presence of Ac-YVAD-CMK (10 and 100 μM; $n = 5 - 7$ experiments per concentration) significantly ($P < 0.001$) reduces the per cent cell death elicited by the viral protein (Figure 4); a lower concentration (1 μM; $n = 4$ experiments) of Ac-YVAD-CMK resulted ineffective (Figure 4). At concentrations of 10 and 100 μM, Ac-YVAD-CMK given alone ($n = 4$ experiments per concentration) induces a slight but statistically significant ($P < 0.05$
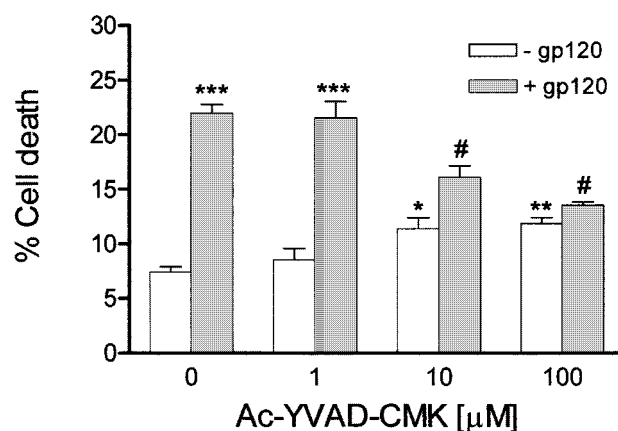

Figure 4 The inhibitor of ICE, Ac-YVAD-CMK, reduces gp120-induced death of human CHP100 neuroblastoma cells. Exposure of CHP100 cells to 10 pM gp120 for 24 h in the presence of Ac-YVAD-CMK (10 and 100 μM) significantly reduces the cell death caused by the viral protein. No protection is afforded by the enzyme inhibitor applied at a lower concentration (1 μM). Each value is the mean $\pm$ s.e.mean of 4–6 experiments. *,** and ***denote $P < 0.05$, $P < 0.01$ and $P < 0.001$ vs control cell death, respectively; # denotes $P < 0.001$ vs control cell death and $P < 0.001$ vs cell death induced by gp120 given alone (one-way ANOVA followed by Tukey–Kramer multiple comparison test).

and $P < 0.01$, respectively) increase over control cell death, a finding this that may explain why this enzyme inhibitor reduces but does not prevent cell death induced by gp120.

Protection from gp120-induced cell death was also afforded by another inhibitor of ICE, Boc-Asp(OBzl)-CMK (Boudreau *et al.*, 1995). In particular, a significant ($P < 0.001$) reduction of gp120-induced death was provided by Boc-Asp(OBbzl)-CMK applied at a concentration of 2.5 μM ($n = 8$ experiments; Figure 5), whereas at a lower concentration (0.25 μM, $n = 5$ experiments) the ICE inhibitor was ineffective. As illustrated in Figure 5, the ICE inhibitor given alone (0.25 and 2.5 μM; $n = 5$ experiments per concentration) does not affect cell viability.

To strengthen the concept that protection afforded by Boc-Asp(OBzl)-CMK relates to inhibition of ICE and consequent reduction of the secreted, mature cytokine, IL-1β expression was studied in gp120-treated cells and in cells exposed to the viral protein in the presence of a concentration (2.5 μM) of the ICE inhibitor known to rescue neuroblastoma cells from gp120-induced cytotoxicity. As shown in Figure 6, exposure of CHP100 cultures to Boc-Asp(OBzl)-CMK ($n = 5$ experiments) prevents gp120-stimulated release of IL-1β.

Discussion

Whilst the present experiments have confirmed that exposure of human CHP100 neuroblastoma cultures to the HIV-1 coat protein gp120 causes cell death (Corasaniti *et al.*, 1995) they have established that the pro-inflammatory cytokine IL-1β is involved. In fact, a pretreatment with IL-1ra, a molecule which inhibits most of the biological actions of IL-1β (Dripps *et al.*, 1991; Hagan *et al.*, 1996; Hannum *et al.*, 1990), or with a neutralizing antibody for IL-1 RI, the receptor that mediates IL-1 signalling (Sims *et al.*, 1993), abolished the
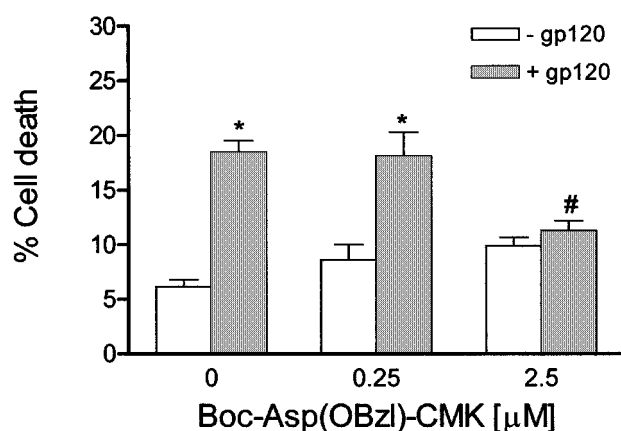
Figure 5 The inhibitor of ICE, Boc-Asp(OBzl)-CMK, reduces death of human CHP100 neuroblastoma cells induced by HIV-1 coat protein gp120. Incubation of CHP100 cells with 10 pM gp120 for 24 h in the presence of Boc-Asp(OBzl)-CMK (2.5 μM), significantly reduces death caused by the viral protein. No protection is afforded by the enzyme inhibitor applied at a lower concentration (0.25 μM). Each value is the mean±s.e.mean of 5–8 experiments. *Denotes $P<0.001$ vs control cell death; # denotes $P<0.001$ vs cell death induced by gp120 given alone (one-way ANOVA followed by Tukey–Kramer multiple comparison test).

cytotoxic effects of the viral coat protein suggesting that CHP100 neuroblastoma cells exposed to gp120 secrete IL-1β. This hypothesis is confirmed by the results of ELISA experiments carried out on aliquots from the extracellular medium of neuroblastoma cell cultures exposed to gp120; under these experimental conditions, in fact, IL-1β immunoreactive levels were significantly enhanced by gp120 as compared to control. Incubation of CHP100 cells with inhibitors of group 1 caspases (see Alnemri *et al*., 1996), e.g. Ac-YVAD-CMK or Boc-Asp(OBzl)-CMK (Milligan *et al*., 1995; Boudreau *et al*., 1995; Nicholson, 2000), prevents cell death; interestingly, Boc-Asp(OBzl)-CMK also prevented IL-1β release elicited by the viral coat protein suggesting that cleavage of the 31 kDa inactive precursor, pro-IL-1β, to yield the mature, 17 kDa biologically active form of the cytokine (Black *et al*., 1988; Thornberry *et al*., 1992) is important. However, it cannot be excluded that inhibition of IL-1β release by the relatively low selective group 1 caspase inhibitor Boc-Asp(OBzl)-CMK may stem from reduced cell death attributable to blockade of other members of the caspase family of proteases (see Nicholson, 2000 for further discussion). Collectively, these data, together with the observation that human recombinant IL-1β causes death of neuroblastoma cells sensitive to the blockade of IL-1ra, suggest that gp120 activates an autocrine loop leading to secretion of IL-1β which then stimulates IL-1 RI to trigger human CHP100 neuroblastoma cell death.

Previous *in vitro* studies have shown that exposure to HIV-1 or to the viral coat protein, gp120, induces IL-1β production in blood monocytes (Wahl *et al*., 1989) and in brain microglia and/or astrocytes (Merril *et al*., 1992) and this has been implicated in the mechanisms of death of co-cultured neuronal cells (Genis *et al*., 1992). Interestingly, increased expression of IL-1β mRNA (Ilyin & Plata-Salaman, 1997) and gene product (Bagetta *et al*., 1999) have also been reported in the brain of adult rat following administration of
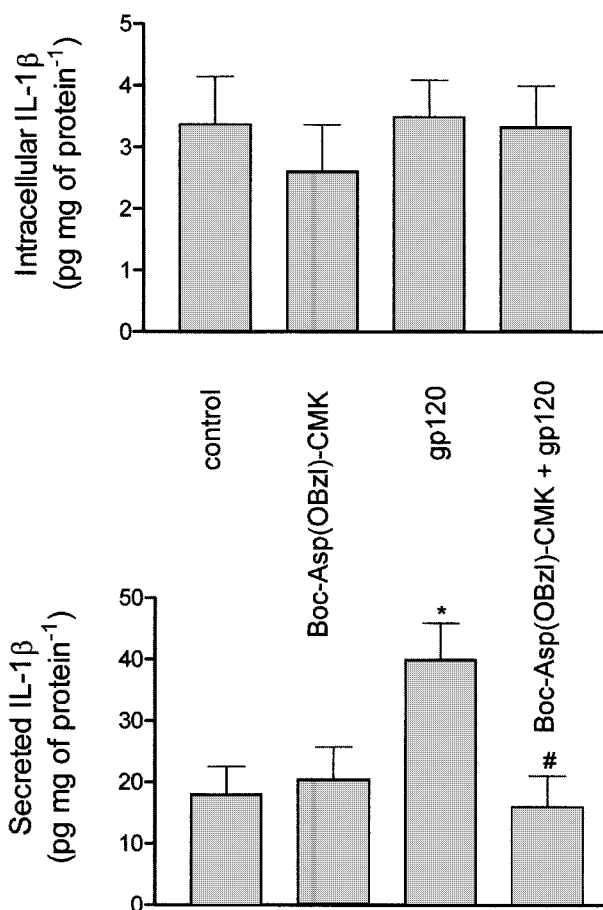
Figure 6 The inhibitor of ICE, Boc-Asp(OBzl)-CMK, prevents gp120-stimulated release of IL-1β in human CHP100 neuroblastoma cells. CHP100 cell cultures were incubated with 10 pM gp120 for 24 h alone or in the presence of Boc-Asp(OBzl)-CMK applied at a concentration (2.5 μM) able to rescue neuroblastoma cells from death caused by the viral protein. At the end of the incubation time, IL-1β levels were measured by ELISA in cell lysates (intracellular IL-1β) and in the corresponding culture medium (secreted IL-1β) (see Methods). Under these conditions, the inhibitor of ICE prevents the enhancement of IL-1β release induced by gp120. Each value is the mean±s.e.mean of five experiments. *Denotes $P<0.05$ vs control; # denotes $P<0.05$ vs gp120 given alone (one-way ANOVA followed by Tukey–Kramer multiple comparison test).

gp120. In particular, double-labelling immunofluorescence experiments have established that neuronal as well as non-neuronal cells, very likely microglial cells, represent the main source for enhanced IL-1β expression which then is secreted in the extracellular space (Bagetta *et al*., 1999). These data in conjunction with the present demonstration that gp120 enhances IL-1β and causes cell death in a neuroectodermal tumour cell line, e.g. in the absence of other, contaminating, cell types, support the hypothesis that cells of the myelomonocytic lineage and neuronal cells are both important sources for the expression of the pro-inflammatory cytokine, IL-1β. In addition, it is conceivable that this cytokine might be the signal through which neurones contribute to recruitment and activation of immune cells and to their own demise.

The mechanism through which gp120 stimulates the secretion of IL-1β from CHP100 cells is not known; however,

the observation that in P388D1 murine macrophage and human Jurkat T cell lines (Siders & Mizel, 1995; Siders *et al.*, 1993) and in human activated monocytes (Rubartelli *et al.*, 1990) the secretion of mature IL-1β is enhanced by calcium ionophores raises the possibility that elevation of intracellular calcium may play a role in the secretory process. We have previously reported that L-type voltage-dependent Ca^{2+} channel blockers and the NMDA receptor associated cation channel blocker, MK801, prevent gp120-induced neuroblastoma cell death (Corasaniti *et al.*, 1995). More recently, we have established that, at least in part, gp120 causes death of CHP100 cells *via* activation of CXCR4 and CCR5 chemokine receptors which are constitutively expressed by this human neuroblastoma cell line (Catani *et al.*, 2000) and, like gp120, are known to affect the regulation of Ca^{2+} signalling in neurones (Meucci *et al.*, 1998). Therefore, it can be assumed that different cellular mechanisms contribute to increase $[Ca^{2+}]_i$ which in turn enhances IL-1β availability in CHP100 cells exposed to the viral protein.

It is well established that in the mammalian brain IL-1β is endowed with neurotrophic as well as neurotoxic properties (see Strijbos & Rothwell, 1995). Under our experimental conditions, increased IL-1β availability has a clear neurotoxic significance though, the lack of a glial cell component in our culture system may conceivably limit the extent of cytotoxicity. Interestingly, increased IL-1β levels have been reported in the CNS of patients affected by AIDS dementia complex (Perrella *et al.*, 1992; Tyor *et al.*, 1992), Alzheimer disease and Down syndrome (Griffin *et al.*, 1989) though its neuronal origin and pathophysiological role in these chronic neurodegenerative diseases remain to be established.

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