Letter to the Editor

Neuroprotection by the caspase-1 inhibitor Ac-YVAD-(acyloxy)mk in experimental neuroAIDS is independent from IL-1 β generation

Cell Death and Differentiation (2005) 12, 999–1001. doi:10.1038/sj.cdd.4401644
Published online 22 April 2005

Dear Editor,

The human immunodeficiency virus type-1 (HIV-1) coat protein, gp120, has been implicated in the progression of central nervous system (CNS) damage caused by HIV-1 infection (see Corasaniti *et al.*¹). Intracerebral (i.c.v.) injection of gp120 in adult rats causes neuropathological alterations, such as microglial cell activation, enhanced expression of the proinflammatory cytokine IL-1 β and selective neuronal apoptosis in the brain neocortex which resemble those reported in HIV encephalitis (see Corasaniti *et al.*¹). In particular, expression of neuronal damage in the rat brain neocortex by gp120 is preceded by microglial cell activation and by an early (at 6 and 24 h from i.c.v. injection of a single dose of gp120) enhancement of IL-1 β expression with more enhanced expression detectable after 7 days of treatment (see Corasaniti *et al.*¹). IL-1 β is synthesised as an inactive 31 kDa precursor molecule (pro-IL-1 β) that is processed by caspase-1 (formerly referred to as interleukin-1 converting enzyme, ICE), to yield the mature, 17 kDa biologically active form of the cytokine.²

Pharmacological manipulations indicate that abnormally elevated IL-1 β plays a causative role in the expression of neuronal apoptosis yielded by HIV-1 gp120 in the brain neocortex of rat.³ Thus, neuronal cell loss was minimised by Ac-Tyr-Val-Ala-Asp-chloromethylketone (Ac-YVAD-cmk), a specific inhibitor of caspase 1 (see Corasaniti *et al.*¹). In addition, protection from cell death was afforded by the endogenous antagonist of IL-1 receptor type I (IL-1ra), a molecule which inhibits most of the biological actions of IL-1 β .³

We report here that HIV-1 gp120 elevates cytochrome *c* immunoreactivity into the cell cytoplasm of rat brain neocortex and that this is an early event that occurs 6 h following a single i.c.v. injection of the viral protein. Interestingly, acetyl-Tyr-Val-Ala-Asp-2,6-dimethylbenzoyloxy-methylketone (Ac-YVAD-(acyloxy)mk), an inhibitor of caspase-1,⁴ was able to counteract gp120-induced cytosolic cytochrome *c* elevation and this without affecting IL-1 β levels. Collectively, these observations suggest that Ac-YVAD-(acyloxy)mk prevents cytochrome *c* expression into the cytosol through a mechanism independent from inhibition of IL-1 β generation.

Ultrathin sections of rat brain neocortex labelled with anti-cytochrome *c* antibody were observed by transmission electron microscopy to study cytochrome *c* localisation. In tissue sections of brain neocortex from BSA (100 ng given i.c.v. 6 h before killing)-treated rats, anti-cytochrome *c*

reactive epitopes are mainly localised into the mitochondria (see Figure 1a for a typical example). A quite different intracellular localisation of cytochrome *c* immunoreactivity is observed in brain neocortical sections from rats treated 6 h before with a single dose of gp120 (100 ng i.c.v.) in which the gold particles are visible in the mitochondria and throughout the cell cytoplasm (Figure 1b). Likewise, enhanced cytochrome *c* immunoreactivity is also seen in cytosolic fractions obtained from individual brain cortical tissues of rats treated 6 h before with a single dose (100 ng i.c.v.) of gp120 and processed by Western blot technique (Figure 1d). Interestingly, the latter effect is reduced by the caspase-1 inhibitor Ac-YVAD-(acyloxy)mk (50 pmol given i.c.v. 1 h before gp120) (Figure 1c and d) and this does not appear to be mediated via inhibition of IL-1 β generation. In fact, Ac-YVAD-(acyloxy)mk is unable to affect cleavage of pro-IL-1 triggered by gp120 (Figure 1f); consistently, Ac-YVAD-(acyloxy)mk also failed to reduce gp120-increased IL-1 β levels (Figure 1e).

The original observation that gp120 induces cytochrome *c* translocation in the brain neocortex of rats treated 6 h before with a single dose of the viral protein, that is, at a time when there is no evidence of cell loss (see Corasaniti *et al.*¹), suggests that release of cytochrome *c* is an early event in the pathway of neuronal cell demise triggered by the HIV-1 coat protein *in vivo*. To the best of our knowledge, this is the first *in vivo* evidence that an inhibitor of caspase-1 prevents cytochrome *c* translocation.

Cytochrome *c*, a member of the mitochondrial electron-transport chain, once released from the mitochondrial inter-membrane space into the cytosol functions as a proapoptotic factor (see Kroemer and Reed⁵). *In vitro*, cytochrome *c* translocation and caspase-8- and caspase-9-dependent activation of caspase-3 has been described in cerebrocortical neurons exposed to gp120⁶; under these experimental conditions, relatively specific inhibitors of caspase cascade prevented gp120-induced apoptosis. Caspase-3-like immunoreactivity is elevated in cerebrocortical neurons from patients with HAD.⁶ These observations suggest that caspases may represent potential targets for the prevention of neuronal damage induced by HIV-1.

Quite importantly, cytochrome *c* elevation into the cell cytosol of brain cortical tissue of gp120-treated rats is prevented by Ac-YVAD-(acyloxy)mk and this does not seem

to occur through inhibition of IL-1 β generation (i.e. caspase-1 inhibition).

In a rat model of permanent middle cerebral artery occlusion, i.c.v. injection of Ac-YVAD-cmk afforded neuro-

protection and reduced brain levels of IL-1 β .⁷ By contrast, in a rat model of endotoxaemia, Ac-YVAD-cmk reduced lethality induced by intravenous administration of LPS but it was unable to affect increased blood levels of IL-1 β .⁸ The ability or

not of caspase-1 inhibitors to decrease the generation of IL-1 β may be related to the fact that more than one proinflammatory caspase, that is, caspase-11 and caspase-5, may be involved in IL-1 processing (see Burns *et al.*⁹). In addition, caspase-1 inhibitors are not selective for caspase-1, and proteases, other than caspases, involved in cell death, may be inhibited¹⁰; then, it cannot be excluded that in our rat model of experimental neuroAIDS, prevention of cytochrome *c* translocation (present data) and minimisation of apoptotic neurons³ by caspase-1 inhibitors may be unrelated to caspase-1 inhibition. Incidentally, *in vitro* inhibition of cathepsins by 'caspase-specific inhibitors', including Ac-YVAD-cmk, has been described¹⁰ and there is evidence that inhibition of cathepsin B is implicated in the mechanisms through which Ac-YVAD-cmk rescues neurons from death induced by oxidative stress and oxygen/glucose deprivation.¹¹

In view of the established link between inhibition of cytochrome *c* translocation and neuroprotection, further experiments are in progress to characterise the molecular targets involved in the mechanisms underlying the observed inhibition of cytochrome *c* translocation by caspase-1 inhibitors in experimental neuroAIDS.

Acknowledgements

This work has been carried out in collaboration with and with the financial support of the Istituto Superiore di Sanità (ISS, Rome, Italy) in the frame of 'Progetti di ricerca su neoplasie, patologie cardiovascolari, malattie respiratorie, salute della donna, neuroscienze, riabilitazione, malattie

infettive, tabagismo'. Partial financial support from the IV National Programme on AIDS, ISS, and MIUR (FIRB 2001, RBNE01E7YX_005) is gratefully acknowledged.

MT Corasaniti^{*1}, R Russo¹, D Amantea², M Gliozzi¹, E Siviglia¹, AR Stringaro³, W Malorni⁴, G Melino⁵ and G Bagetta²

¹ Department of Pharmacobiological Sciences, University 'Magna Graecia' of Catanzaro, Catanzaro, Italy;

² Department of Pharmacobiology, University of Calabria, Rende, Italy;

³ Department of Technology and Health, ISS, Rome, Italy;

⁴ Department of Drug Res and Eval, ISS, Rome, Italy;

⁵ Biochemistry Laboratory, IDI-IRCCS, c/o University of Rome Tor Vergata, Rome, Italy

* Corresponding author: MT Corasaniti, Department of Pharmacobiological Sciences, University 'Magna Graecia' of Catanzaro, Catanzaro, Italy.

Tel: 39 0961 391157; Fax: 39 0961 391490; E-mail: mtorasa@unicz.it

1. Corasaniti MT *et al.* (2003) Toxicol. Letter 139: 199–206

2. Thornberry NA *et al.* (1992) Nature 356: 768–774

3. Bagetta G *et al.* (1999) Neuroscience 89: 1051–1066

4. Thornberry NA *et al.* (1994) Biochemistry 33: 3934–3940

5. Kroemer G and Reed JC (2000) Nat. Med. 6: 513–519

6. Garden GA *et al.* (2002) J. Neurosci. 22: 4015–4024

7. Rabuffetti M *et al.* (2000) J. Neurosci. 20: 4398–4404

8. Mathiak G *et al.* (2000) Br. J. Pharmacol. 131: 383–386

9. Burns K *et al.* (2003) Curr. Opin. Immunol. 15: 26–30

10. Rozman-Pungercar J *et al.* (2003) Cell Death Differ. 10: 881–888

11. Gray Y *et al.* (2001) J. Biol. Chem. 276: 32750–32755

Figure 1 The HIV-1 envelope glycoprotein gp120 causes cytochrome *c* translocation to the cytosol in brain neocortical tissue of rats treated with a single dose of the viral coat protein: prevention by a pretreatment with Ac-YVAD-(acyloxy)mk, a caspase-1 inhibitor unable to affect the conversion of pro-IL-1 β into the mature form of the cytokine caused by the viral coat protein. Transmission electron microscopy photographs to illustrate ($\times 20\,000$ magnification) subcellular localisation of cytochrome *c* immunoreactivity in ultrathin tissue sections of rat brain neocortex labelled with a mouse monoclonal anti-cytochrome *c* antibody and with anti-mouse gold-labelled IgG. Note in (a) the mitochondrial localisation of gold particles in a brain section from BSA-treated (100 ng given i.c.v. 6 h beforehand) rat; note in (b) the widespread presence of gold labelling throughout the cell cytoplasm in a section from gp120-treated (100 ng given i.c.v. 6 h beforehand) rat and reduction (c) by a pretreatment with Ac-YVAD-(acyloxy)mk (50 pmoles given i.c.v. 1 h before gp120). Likewise, the Western blot in the top panel to (d) represents a typical example of three independent experiments ($n=3$ rats per group) to show enhanced cytochrome *c* immunoreactivity in brain neocortex cytosolic fractions of a rat treated 6 h before with a single dose (100 ng i.c.v.) of the viral protein and minimisation by a pretreatment (1 h beforehand) with Ac-YVAD-(acyloxy)mk (YVAD; 50 pmoles, i.c.v.). For comparison, relative intensity values (arbitrary units) of the autoradiographic bands were determined by computer-assisted (Quantiscan, Biosoft, UK) densitometric analysis and the data from quantitative analysis of three independent experiments are shown in the bottom panel as mean $\pm$ S.E.M. (*denotes $P<0.05$ versus control (Ctrl)-, Ac-YVAD-(acyloxy)mk-, and Ac-YVAD-(acyloxy)mk + gp120-treated rats (one-way ANOVA followed by Tukey–Kramer multiple comparisons test)). Panel (e) demonstrates that the caspase-1 inhibitor Ac-YVAD-(acyloxy)mk (YVAD) does not reduce increased (* $P<0.05$ versus YVAD-treated rats; one-way ANOVA followed by Tukey–Kramer multiple comparisons test) mitochondrial and cytosolic, respectively, immunoreactive IL-1 β levels ($n=3$ per group) as assayed by an established sandwich ELISA in the cerebral cortex of rats treated 6 h earlier with gp120. In (f), the latter effect is paralleled by gp120 reduced expression of pro-IL-1 β in the neocortex of rats pretreated with the caspase-1 inhibitor Ac-YVAD-(acyloxy)mk. Pro-IL-1 β immunoreactivity was assayed by Western blot analysis by using the same cytosolic fractions used for IL-1 β ELISA (see e). Note the reduction of the intensity of the 31 kDa band corresponding to pro-IL-1 β in the cytosolic fractions obtained from the neocortex of Ac-YVAD-(acyloxy)mk + gp120-treated rats as compared to Ac-YVAD-(acyloxy)mk-treated animals (upper panel). The panel shows the results of Western blot analysis obtained in individual animals in three independent experiments ($n=3$ rats per group). Actin immunoreactivity from the same gels is shown as an internal control. Densitometric analysis of autoradiographic bands corresponding to pro-IL-1 β have been normalised to the values yielded for actin and the resulting values (mean $\pm$ S.E.M.; $n=3$) are summarised in the bottom panel. *Denotes $P<0.05$ versus Ac-YVAD-(acyloxy)mk (paired Student's *t* test)