Available online at www.sciencedirect.com

SCIENCE DIRECT®

Virology 344 (2006) 468 – 479

VIROLOGY

www.elsevier.com/locate/yviro

Point mutations in the C-terminus of HIV-1 gp160 reduce apoptosis and calmodulin binding without affecting viral replication

Keith J. Micoli [a], Olga Mamaeva [a], Sabine C. Piller [c,d,1], Jennifer L. Barker [a], George Pan [a], Eric Hunter [d,2], Jay M. McDonald [a,b,*]

[a] *Department of Pathology, University of Alabama at Birmingham, Birmingham, AL 35294, USA*
[b] *Veteran's Administration Medical Center, Birmingham, AL 35233, USA*
[c] *Westmead Millennium Institute, University of Sydney, Westmead, NSW 2145, Australia*
[d] *Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA*

Received 25 March 2005; returned to author for revision 1 June 2005; accepted 22 August 2005

Abstract

One hallmark of AIDS progression is a decline in CD4$^+$ T lymphocytes, though the mechanism is poorly defined. There is ample evidence that increased apoptosis is responsible for some, if not all, of the decline. Prior studies have shown that binding of cellular calmodulin to the envelope glycoprotein (Env) of HIV-1 increases sensitivity to fas-mediated apoptosis and that calmodulin antagonists can block this effect. We show that individual mutation of five residues in the C-terminal calmodulin-binding domain of Env is sufficient to significantly reduce fas-mediated apoptosis in transfected cells. The A835W mutation in the cytoplasmic domain of gp41 eliminated co-immunoprecipitation of Env with calmodulin in studies with stably transfected cells. Four point mutations (A835W, A838W, A838I, and I842R) and the corresponding region of HIV-1 HXB2 were cloned into the HIV-1 proviral vector pNL4-3 with no significant effect on viral production or envelope expression, although co-immunoprecipitation of calmodulin and Env was decreased in three of these mutant viruses. Only wild-type envelope-containing virus induced significantly elevated levels of spontaneous apoptosis by day 5 post-infection. Fas-mediated apoptosis levels positively correlated with the degree of calmodulin co-immunoprecipitation, with the lowest apoptosis levels occurring in cells infected with the A835W envelope mutation. While spontaneous apoptosis appears to be at least partially calmodulin-independent, the effects of HIV-1 Env on fas-mediated apoptosis are directly related to calmodulin binding.

Keywords: HIV-1; Calmodulin; Apoptosis

Introduction

While increased apoptosis during HIV infection is a well-documented phenomenon both in vitro (Katsikis et al., 1995) and in vivo (Badley et al., 1998), its role in the pathogenesis of AIDS remains controversial (Bell and Dockrell, 2003). Although lymphocyte turnover during the asymptomatic stage of HIV infection is thought to be as high as 10^9 cells/day (Ho et al., 1995), the fate of these cells remains undefined. Hypotheses for this event include apoptosis (Alimonti et al., 2003) direct cell killing by the virus (Cao et al., 1996) and altered homing of lymphocytes to different immune compartments (Alimonti et al., 2003; Cloyd et al., 2000) It is likely that these all occur in vivo, and they may turn out to be related events. Peripheral blood mononuclear cells (PBMCs), which include CD4$^+$ lymphocytes, from HIV-infected patients undergo more spontaneous apoptosis than PBMCs from uninfected donors (Pan et al., 1998). This increase in apoptosis correlates with stage of disease (Cotton et al., 1997) and with fas (Silvestris et al., 1996) and fas ligand (Badley et al., 1998) expression, two proteins responsible for regulating physiological apoptosis in lymphocytes (Mountz et al., 1994).

---

[*] Corresponding author. Department of Pathology, University of Alabama at Birmingham, 701 19th Street South LHRB 509 Birmingham, AL 35294-0007, USA. Fax: +1 205 975 9927.

E-mail address: jmcdonald@path.uab.edu (J.M. McDonald).

[1] Current address: Westmead Millennium Institute, University of Sydney, Westmead, NSW 2145, Australia.

[2] Current address: Emory University, Emory Vaccine Research Center, 954 Gatewood Rd, Atlanta, GA 30329, USA.

0042-6822/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2005.08.033

Fas is a transmembrane receptor in the tumor necrosis factor receptor (TNFR) superfamily that transduces an apoptotic signal following binding to fas ligand, (for review, see Peter et al., 1999). This binding induces recruitment of proteins to the death inducing signaling complex (DISC) (Scaffidi et al., 1999). Included in the DISC are upstream initiator caspases, including caspases 8 (Boatright et al., 2003) and 10 (Cohen, 1997), which are activated upon recruitment. This causes activation of downstream effector caspases, including caspases 3, 6, and 7, release of cytochrome C from the mitochondria (Green and Reed, 1998), cleavage of survival-promoting proteins (Geng et al., 1998; Janicke et al., 1998), externalization of phosphatidyl serine on the cell membrane (Denecker et al., 2000), cell and nuclear shrinkage, DNA cleavage, and cell death (Cidlowski et al., 1996). This simplified view of apoptosis includes events that do not occur in every type of apoptosis. Additionally, there is an opposing survival pathway in operation at the same time (Gomez-Angelats et al., 2000), and the relative strength of the two signals determines the ultimate fate of the cell.

Within this expanding concept of apoptotic signaling is a growing body of evidence that calcium (Krebs, 1998) and calmodulin (Ahn et al., 2003) play a role in mediating apoptosis. A slow, sustained increase in intracellular calcium concentration has been observed in many types of apoptosis, with calcium coming from both extracellular and intracellular sources. Calcium channel blockers inhibit apoptosis (Ares et al., 1997), and calcium release by thapsigargin can induce apoptosis in certain cell types (Tombal et al., 2000). Calpain, a calcium-dependent protease, has been shown to be active during apoptosis (Ghibelli et al., 2003), and the endonuclease responsible for the chromatin cleavage that occurs in most forms of apoptosis is calcium-dependent (Yakovlev et al., 2000). Death-associated protein (DAP) kinase is both calcium- and calmodulin-dependent and functions in fas- and TNFα-induced apoptosis downstream of receptor ligation but upstream of effector caspases (Cohen et al., 1997). Other calmodulin-dependent enzymes have proposed roles in apoptosis, including calmodulin-dependent protein kinases (CaM kinase) II (Wright et al., 1997) and IV (Means et al., 1997) and the calmodulin-dependent phosphatase, calcineurin (Ankarcrona et al., 1996). Calcineurin has been shown to induce apoptosis by dephosphorylating the pro-apoptotic bcl-2 homologue, bad (Wang et al., 1999), which subsequently translocates to the mitochondria. Overexpression of calcineurin alone can induce apoptosis (Shibasaki and McKeon, 1995), and cyclosporine and FK506, which inhibit calcineurin (Wiederrecht et al., 1993), can prevent many types of apoptosis (Higashigawa et al., 1997). Recently, Ahn et al. demonstrated that calmodulin binds to the cytoplasmic domain of Fas and that this binding is altered during fas-mediated apoptosis (Ahn et al., 2004).

HIV attachment and entry into cells occur through the action of the viral envelope glycoproteins, gp120 and gp41. The surface subunit of this Env complex is gp120, and the transmembrane subunit (TM) is gp41. The binding and entry process is multi-step and involves first the binding of gp120 to the host CD4 molecule which allows gp120 to then bind the chemokine receptor, CXCR4, on T cells (Choe et al., 1998). The binding of the external subunit of envelope to the host cell causes a conformational change that exposes the external fusion domain of gp41 and allows viral entry (Hunter, 1997). The cytoplasmic region of gp41 appears to play an important role in viral assembly and infectivity, by directing the glycoprotein to the correct subcellular location (Nguyen and Hildreth, 2000) and by mediating interactions between Env and the assembling capsid (Murakami and Freed, 2000). The cytoplasmic tail of gp41 is approximately 150 amino acids long and is fairly well-conserved in different strains of HIV-1 (Modrow et al., 1987). Especially well-conserved are two calmodulin-binding domains near the C-terminus of gp41 (Tencza et al., 1997). These regions are predicted to form amphipathic helices, and peptides corresponding to these regions bind to calmodulin (Srinivas et al., 1993) and have been called lentiviral lytic peptides 1 and 2 (LLP1 and 2) based on their ability to lyse cells (Miller et al., 1991). Expression of Env increases sensitivity to fas-mediated apoptosis (Micoli et al., 2000) and has also been reported to induce apoptosis in the absence of external stimuli (Ishikawa et al., 1998).

Cells expressing full-length HIV Env, in contrast to those expressing gp120 alone, display increased intracellular levels of calcium that can be prevented by treatment with calmodulin antagonists (Sasaki et al., 1996). The two highly conserved calmodulin-binding regions of gp160/41 (Tencza et al., 1997) were identified by homology to the calmodulin-binding domain of myosin light chain kinase and confirmed by peptide binding studies (Micoli et al., 2000; Srinivas et al., 1993). Calmodulin has also been shown to associate with gp160/gp41, but not with gp120 or various truncation mutants of gp160/gp41 (Ishikawa et al., 1998; Radding et al., 1996). Calmodulin expression increases in cells expressing Env with intact calmodulin-binding domains, but not in cells expressing truncated forms of Env or a point mutant that disrupts calmodulin binding (Micoli et al., 2000; Radding et al., 1996).

Expression of Env alone has been shown to increase apoptosis induced by fas (Ishikawa et al., 1998), and this could be blocked by calmodulin antagonists or by point mutation of the C-terminal calmodulin-binding domain of Env (Micoli et al., 2000; Pan et al., 1996). Additionally, calmodulin antagonists reduce spontaneous apoptosis of infected PBMCs cultured from AIDS patients (Pan et al., 1998). Therefore, apoptosis may play a key role in the progression of AIDS and one of the apoptotic triggers may be the interaction between Env and calmodulin. However, the precise molecular mechanism underlying the apoptotic events, including upregulation of calmodulin by Env, remains to be determined and represents a potentially useful area for the development of new therapies for HIV-infected patients. Furthermore, it is not known whether calmodulin binding to Env plays a role in viral infectivity (Dubay et al., 1992) and/or replication (Srinivas et al., 1994), processes clearly critical in the pathogenesis of AIDS.