HIV induces lymphocyte apoptosis by a p53-initiated, mitochondrial-mediated mechanism

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SPECIFIC AIMS

HIV-1 induces apoptosis and leads to CD4+ T lymphocyte depletion in humans. It is still unclear whether HIV-1 kills infected cells directly or indirectly. In this study we provide a mechanistic view on how HIV-1 induces apoptotic death of infected primary human CD4+ T lymphocytes.

PRINCIPAL FINDINGS

1. HIV damages mitochondria, leading to cytochrome c release and caspase activation

The time course of activation of the extrinsic and intrinsic pathways of apoptosis was examined after HIV-1 infection of primary CD4+ T lymphocytes. The percentage of cells undergoing apoptosis was quantified by flow cytometry and measuring the proportion of cells with sub-G1 DNA content and correlating it with HIV p24 production. Caspase activities were measured at multiple time points after infection by using specific fluorometric substrates (Fig. 1). The pattern of caspase activation strongly suggested that the intrinsic pathway of apoptosis induction was operational. This suggestion was borne out by the following observations. The catalytic activity of the apical caspase-2 and -9 increased after 2 days, concomitantly with the activation of the executioner caspase-3. Activated caspase-6 was detectable only 72 h after infection. No activity of the death receptor-associated apical caspase-8 was observed during the infection (up to 72 h). Caspase enzymatic activities were corroborated by immunoblotting. Cleavage of the proform of caspase-3 was observed in all samples, but in HIV-1 treated cells the ratio of the cleaved and uncleaved pro-caspase product was increased as early as 24 h and remained elevated up to 72 h relative to the control. The levels of the pro-caspase-9 and -6 products were reduced 48 and 72 h after infection, which suggests processing to their active forms.

2. HIV induces p53 phosphorylation and activation

To study the role of p53 in the initiation of the apoptotic pathway during HIV-1 infection, we measured the total level of p53 and its phosphorylation at residue Ser15 by using a phospho-specific antibody. Total and phosphorylated p53 levels increased 24 h after HIV-1 infection. This HIV-triggered phosphorylation of p53 peaked at 48 h and remained detectable for up to 72 h (Fig. 2). To confirm that phosphorylation and induction of p53 resulted in the activation of the p53 pathway, we analyzed the protein and mRNA levels of p53-induced genes, p21/CIP1/WAF1 (p21), HDM2, and Bax. Both Bax and p21 levels increased 48 h after HIV-1 infection, and HDM2 increased after 72 h compared to uninfected controls.

3. HIV up-regulates Fas ligand but only as a late event

To investigate whether HIV would activate an extrinsic apoptotic pathway involving Fas ligand binding to Fas receptor, we analyzed the expressions of signaling proteins involved in the Fas signaling pathway. In primary cells, the protein levels of Fas receptor and of Fas ligand were not modulated by HIV infection (data not shown), but the expression of surface Fas ligand measured by flow cytometry was increased significantly at 72 h. However, the Fas binding proteins FADD, DAXX, and RIP were all down-regulated, which would be expected to inhibit FasL-Fas-induced apoptosis.

CONCLUSIONS AND SIGNIFICANCE

We demonstrated that the intrinsic mitochondrial pathway of apoptosis is the primary mechanism that induces
CD4+ T cells to undergo apoptosis. Mitochondrial membrane permeabilization may be a consequence of the activation of the p53 pathway. Once phosphorylated, p53 induces up-regulation of Bax, which may translocate to the mitochondrial membrane and promote cytochrome c and AIF release.

Figure 1. Caspase activation in HIV-1-infected CD4+ T cells. At the times indicated, 2 x 10^6 PBL were washed with PBS and lysed in caspase buffer. A) Caspase activity of 10–20 μg of total protein was measured with specific substrates for caspase-2 ( ), 3 ( ), 6 ( ), 8 ( ), and 9 ( ) (100 μM) after 1 h incubation at 37°C. The cleavage of the fluorometric AMC/AFC was monitored fluorometrically at 400/380 nm excitation and 505/460 nm emission. Activity is represented in relative units to the control without HIV.

Figure 2. Activation of the p53 pathway by HIV-1 infection. Immunoblot analysis was performed on total cell lysates of 5 x 10^6 cells in 100 μl lysing buffer as described. The same membranes were stripped and reblot with specific antibodies, including a tubulin antibody that served as a control for loading.

Figure 3. HIV-1 enters the cells, and its genome is reverse-transcribed and integrated in host DNA. Less than 24 h after the infection, phosphorylation of p53 at residue Ser15 is observed. This p53 phosphorylation leads to its transcriptional activation, either by increasing its protein levels or by a conformational modification. Activation of the p53 pathway increases the expression of the protein Bax. Bax multimerizes and generates pores in the mitochondrial membranes and allows the release of the pro-apoptotic protein cytochrome c and apoptosis-inducing factor (AIF). The released cytochrome c will bind to apaf-1 and, in the presence of dATP, sequester and activate caspase-9 and caspase-3, which leads to activation of the caspase proteolytic cascade and results in apoptosis. The released AIF will localize to the nucleus and promote chromatin condensation.

This phenomenon may be especially relevant in primary acute infection when high levels of virus are present and no potent mechanisms of viral control are yet fully operational. The primary infection stage has the highest proportion of CD4 lymphocyte infected during the course of HIV infection. This p53-mediated apoptosis may be responsible for the precipitous drop in CD4+ T cells seen in primary acute HIV-infected patients, with eventual stabilization of both CD4 and viral loads. These events set the stage for determining the propensity for progression to AIDS. In summary, CD4 T cell death as a result of HIV-1 infection is mediated by the activation of p53 and the intrinsic mitochondrial apoptotic pathway. The mechanism by which HIV mediates this process remains to be further clarified.