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Mechanisms of HIV envelope-induced T lymphocyte apoptosis

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Abstract: Infection by the human immunodeficiency virus (HIV) is characterized by a progressive depletion of CD4 T lymphocytes, which leads to dysfunction of the immune system. Although a variety of mechanisms may contribute to the gradual T cell decline that occurs in HIV-infected patients, abnormal apoptosis of infected or bystander T lymphocytes is an important event leading to immunodeficiency. The HIV envelope glycoprotein plays a crucial role in HIV associated apoptosis through both death receptor-mediated and mitochondria-dependent pathways. This review summarizes current knowledge of Env-mediated T lymphocyte apoptosis.

Key words: HIV; Envelope glycoprotein; T lymphocyte; Apoptosis

The global epidemic of acquired immunodeficiency syndrome (AIDS) has become one of the most pressing public health emergencies. According to the latest statistics provided by the Joint United Nations Programme on HIV/AIDS (UNAIDS), there were an estimated 33 million people living with HIV and 2.0 million deaths due to AIDS in 2007[62]. Since the emergence of HIV, intensive studies have been done to understand the disease and find effective therapies. Highly active antiretroviral therapy (HAART) is considered to be the standard therapy formula and has been proven to be very successful in controlling viral replication and disease progression. However, toxicity, drug-resistance and inability to eliminate the virus associated with the antiretroviral therapy make it urgent to find new therapies. Thus, further understanding the pathogenesis of HIV is critically important.

There are two types of HIV, HIV-1 and HIV-2, both of which are capable of causing AIDS. Given that HIV-2 progresses more slowly and is less aggressive than HIV-1, the majority of studies have been done on HIV-1. HIV preferentially infects cells expressing CD4 molecules, including CD4 T lymphocyte, macrophages and dendritic cells. After acute infection, HIV-1 establishes a chronic infection that is marked by the progressive depletion of CD4 T lymphocytes and increased susceptibility to opportunistic infections and malignancies. Although the exact mechanisms by which this depletion arises remain a matter of controversy, overwhelming evidence show that the main cause of T lymphocyte depletion is abnormal increased T-cell apoptosis[9].

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Thus, understanding of the mechanisms of HIV infection-induced lymphocyte apoptosis can help understand the HIV/AIDS pathogenesis. Research on HIV-associated lymphocyte apoptosis may lead to new and more effective therapies for HIV/AIDS.

Apoptosis is a key event in biological homeostasis but is also involved in the pathogenesis of many viral diseases. On one hand, the virus may utilize the mechanism to efficiently disseminate progeny while limiting inflammatory and other immune responses[41]. On the other hand, during evolution, the immune system has developed several strategies to fight viral infections. Programmed cell death may play a role in limiting propagation of certain viruses[15].

HIV INFECTION AND APOPTOSIS

Apoptosis is an energy-dependent process of cell suicide in response to a variety of stimuli, such as chemical attacks or viral infections[59]. The mechanisms of apoptosis are highly complex, involving an energy dependent cascade of molecular events. So far, research in this field has shown that there are two main apoptotic pathways: the extrinsic pathway in response to death signals and the intrinsic pathway in response to cellular stress signals[23]. The extrinsic and intrinsic pathways both end at the point of the execution phase, an irreversible process.

The extrinsic apoptosis pathway is initiated by death ligand/death receptor interaction. The involved transmembrane death receptors are members of the tumor necrosis factor (TNF) receptor gene superfamily, which includes Fas, TNFR1, death receptor 3/4/5 and TRAIL death receptors[8,37]. After the binding of ligand with receptor, cytoplasmic adapter proteins with a corresponding death domain are recruited. Subsequently, with the procaspase-8 being recruited via dimerization of the death effector domain, the death-inducing signaling complex (DISC) is formed. The formation of the DISC leads to the auto-catalytic activation of procaspase-8[34]. Once activated, caspase-8 can directly activate caspase-3 and other executioner caspases that lead to apoptosis. At this point, the execution phase of apoptosis is triggered. Otherwise, caspase-8 cleavage of the proapoptotic Bcl-2 family member Bid to activate p15 truncated Bid (tBid), which in turn triggers apoptosis through the intrinsic pathway[36].

The intrinsic apoptosis pathway is independent of death receptors, and is initiated by cellular stress signals and regulated by members of the Bcl-2 family[20]. During the initiation phase, pro-apoptotic signals accumulate in the cell and finally act on the mitochondria to increase the mitochondria membrane permeability[15]. This change in the mitochondria membranes results in the opening of the mitochondrial permeability transition (MPT) pore, loss of the mitochondrial transmembrane potential($\Delta\Psi m$) and release of pro-apoptotic proteins from the intermembrane space into the cytosol[50]. These pro-apoptotic proteins lead to apoptosis in a caspase-dependent manner. The Bcl-2 family governs mitochondrial membrane permeability, so the release of these proapoptosis proteins is regulated by the Bcl-2 family of proteins, which function in pro-apoptotic manner or in anti- apoptotic manner.

Infection by HIV is characterized by gradual CD4 T lymphocyte depletion, which leads to immunodeficiency. However, the particular mechanism by which HIV causes T lymphocyte depletion is still uncertain. T cell apoptosis had been proposed as the

mechanism responsible for T cell depletion in patients infected with HIV in 1991[6,35]. Since then, intensive investigations both *in vivo* and *ex vivo* have confirmed the apoptosis hypothesis. First, compared with healthy persons, a greater portion of cultured T cells from HIV-infected individuals underwent spontaneous apoptosis, and cell death could be increased by activation stimuli such as ionomycin, mitogen, superantigen, and anti-TCR antibodies [27,39]. Furthermore, the increased susceptibility to apoptosis of CD4 and CD8 T cells from infected persons was correlated with lymphocyte activation and with disease progression[26]. Second, the intensity of apoptosis in immune organs, such as lymph nodes and tonsillar tissue, was increased in HIV-infected individuals [40,48]. Third, a significant correlation between CD4 T lymphocyte depletion and apoptosis has been reported in a number of different animal models of AIDS. Viral infection did not increase apoptosis in chimpanzees, in which viral replication does not result in AIDS. All these studies evidenced that inappropriate apoptosis induced by HIV is central to the pathogenesis of AIDS.

HIV infection may induce apoptosis of both infected cells and uninfected cells through various mechanisms, which include: (i) direct role of HIV-specific proteins, (ii) activation-induced cell death (AICD), (iii) direct infection of T lymphocytes, (iv) autologous cell-mediated killing of uninfected T cells and (v) dysregulation of cytokine/chemokine production[50]. However, considering the degree of cell loss largely exceeds the number of infected cells in HIV-1-infected patients, HIV-1-induced apoptosis in uninfected bystander cells is probably the key to the depletion of T lymphocytes. Furthermore, apoptosis occurs predominantly in bystander cells but not in productively infected cells of HIV-infected lymph nodes[25]. So, it is reasonable to assume that HIV infection induces bystander lymphocyte apoptosis, which in turn leads to immune system dysfunction.

MECHANISMS OF ENV INDUCED LYMPHOCYTE APOPTOSIS

HIV-1 encodes several apoptogenic proteins including envelop glycoprotein (Env), virus protein R (Vpr), transactivator (Tat), negative regulator factor (Nef), virus protein U (Vpu) and protease[2,12,51,54,55,63]. Although the exact mechanism of HIV-1-induced apoptosis *in vivo* is far from clear, it is considered that among all the products encoded by HIV-1 genome, the principle apoptosis-inducing protein is the envelope glycoprotein[44]. Thus, this review will focus on the role played by the glycoprotein in HIV-associated lymphocyte apoptosis.

The mature HIV-1 envelope glycoproteins (gp160) are composed of gp120, the surface glycoprotein, and gp41, the transmembrane glycoprotein. During HIV entry process, the gp120 subunit of Env functions to recognize HIV's primary and secondary cellular receptors, CD4 and CCR5/CXCR4 respectively, on target cells. Gp41, which contains a transmembrane anchor domain that anchors Env into the lipid membrane, is responsible for fusion of the viral and cellular membranes[21].

Actually, soluble gp120, secreted from infected cells, Env expressed on virions or on the cell surface of infected cells, are able to induce apoptosis of uninfected T cells. Soluble gp120 resulting from shedding of the surfaces of viral particles or infected cells can act as a ligand of CD4 and coreceptor molecules (CCR5/CXCR4) and transmits death

signals through these receptors. Infected cells expressing Env at their surface can interact with uninfected T cells presenting CD4 and coreceptor molecules and leads to the following events: (i) an apoptotic signaling through receptor and/or coreceptor, (ii) a hemifusion event leading to target cell death or (iii) syncytium formation [1].

Gp120-induced lymphocyte apoptosis

Both soluble gp120 and surface-presented gp120 produced within the infected lymphoid tissue can directly kill or sensitize single T cell to subsequent death. In fact, *in vitro* dose-response studies of soluble gp120 showed that concentrations as low as 500 ng/mL were sufficient to mediate significant T-cell death[60]. And *ex vivo* examination results showed that gp120 at 120-960 ng/mL may exist in lymph nodes of HIV-infected individuals [43,56]. Thus, soluble gp120 may play a role in T lymphocyte depletion in HIV positive patients.

Considering that gp120 is a natural ligand of CD4 and coreceptors (CXCR4/CCR5)，the death signals of gp120 induced T cell apoptosis may transmitted by these receptors. Numerous studies have been done to elucidate the mechanisms of these different death signaling pathways.

Gp120-CD4 interaction

In lymphocytes, Activationinduced cell death (AICD) is an important physiological mechanism that regulates the capacity of immune responses to maintain both tolerance to self-antigens and homeostasis of the immune system [1]. AICD can be achieved by repeated antigenic stimulation, such as antigen, mitogen and CD3, and this process is mediated by the interaction of the cell death receptor Fas and its ligand (Fas-L) [61,65].

Compared with normal individuals, the apoptosis level of peripheral blood mononuclear cells (PBMCs) from human HIV-infected patients is elevated [42]. It has been suggested that increased apoptosis is caused by excessive immune activation in response to HIV infection through Fas/FasL[4]. Both *in vivo* and *in vitro*, HIV infection is associated with T cell activation, increased expression of Fas, enhanced susceptibility to Fas-mediated killing, and increased FasL expression level after T-cell receptor stimulation[9]. For example: Fas expression on $CD4^+$ T cells from HIV-infected patients increases with disease progression[7,22]; peripheral blood $CD4^+$ T lymphocytes from HIV-infected individuals undergo apoptosis in response to stimulation of Fas antigen at a much higher frequency than from healthy individuals[33]. Furthermore, blocking the HIV-induced Fas/FasL death pathway may be benefitial in AIDS therapy. PBMC from HIV positive individuals exhibited decreased *ex vivo* apoptosis after taking all-trans retinoic acid[64], which prevents upregulation of FasL and inhibits activation-induced T cell apoptosis[65]. High levels of Fas-susceptibility found in peripheral $CD4^+$ T cells before HAART are significantly reduced after treatment, coinciding with an increase in peripheral $CD4^+$ T lymphocyte count[1]. Thus, what is the mechanism by which HIV infection renders the T lymphocyte more susceptible to Fas-mediated cell death? An *in vitro* study showed that cross-ligation of CD4 molecules prior to T cell receptor (TCR) stimulation triggers an up-regulation of Fas on purified T cells and expression of FasL upon antigenic stimulation, making the T cells more susceptible to Fas-mediated apoptosis[58]. As a natural ligand of CD4, recombinant gp120 can render cells more sensitive to activation-induced apoptosis through

cross-linking with CD4[14]. Generally speaking, gp120-CD4 interaction results in the increased susceptibility of the $CD4^+$ cells to Fas-induced apoptosis via two mechanisms: (1) upregulation of Fas and Fas-ligand; and (2) downmodulation of a caspase inhibitor, the FLICE-like inhibitory protein (FLIP)[53].

However, some other studies indicated that the molecular signal responsible for elevated levels of AICD in HIV-infected patients is not just Fas, for the administration of TRAIL/APO 2-L or TNF antagonists can also reduce levels of apoptosis after mitogenic stimulation or TCR cross-linking[32]. This suggests that Fas, TNF, and TRAIL/APO 2-L mediated cell death may all be involved in the process.

Besides regulating AICD, some other mechanisms of gp120-induced apoptosis have also been demonstrated. It appears that gp120 binding to CD4 may lead to regulation of the Bcl-2 protein family, which in turn regulates the mitochondrial apoptosis pathway. Interaction of gp120 with CD4 induces downregulation of Bcl-2, an anti-apoptosis protein, and upregulation of Bax, a pro-apoptosis protein, leading to mitochondria-dependent apoptosis [28,53].

Gp120-CXCR4 interaction

Other than CD4 signaling, HIV-1 Env-mediated single cell apoptosis can also regulated by CD4 independent mechanisms. CXCR4 is a seven transmembrane G-Protein Coupled Receptor (GPCR) expressed on the cell surface lymphocytes. The physiological ligand for CXCR4 is the chemokine stromal cell-derived factor-1 (SDF-1) [16]. As a natural coreceptor of HIV Env, CXCR4 can also transduce a death signal when bound to Env.

In a well-designed experiment with human T cell lines, where the cytoplasmic part of CD4 was missing and unable to transduce a signal, infectious X4 isolates of HIV-1 could still induce cell apoptosis[30]. In another study, it was shown that macrophage associated gp120 may mediate CD8 T-cell apoptosis through interaction with CXCR4[29]. Furthermore, SDF-1 and CXCR4 antagonists were capable of blocking Env-induced cell death[10]. More importantly, *ex vivo* experiment showed the evolution of HIV toward increased pathogenicity through CXCR4-mediated killing of uninfected CD4 T cells[31]. All these underline the role of CXCR4 in Env-associated cell apoptosis.

Env-induced apoptosis through CXCR4 is not mediated by Fas death signal, but is associated with the mitochondrial intrinsic apoptosis pathway. The process is independent of Fas signaling, and does not involve activation of the stress- and apoptosis-related mitogen-activated protein kinases (MAPKs) p38 and JNK[10]. Binding of gp120 to CXCR4 induces mitochondrial transmembrane depolarization, cyto-chrome-C release and activation of the caspases-9, which are the hallmarks of mitochondrial intrinsic apoptosis[47].

Gp120-CCR5 interaction

CCR5/M tropic (R5) HIV strains interact with CD4 and CCR5, infecting only primary CD4 T cells and macrophages[5]. Activation of CCR5 by membrane-anchored or soluble R5 Env causes apoptosis of both infected and uninfected CD4 T cells. The binding of R5 Env to CCR5 leads to both *de novo* expression of FasL and induction of susceptibility to Fas-mediated apoptosis in resting primary CD4 T cells[3]. Thus, CCR5 signaling may also play a role in HIV-induced T cell apoptosis. However, given that only about 15 to 30% of the $CD4^+$ T lymphocytes express detectable levels of CCR5 on the cell surface[13], the role of CCR5 may be very minor. This is consistent with the

observations that high $CD4^+$ T cell depletion occurs after the emergence of X4 isolate in HIV-infected patients[18].

Env(gp120/gp41)-induced cell fusion

CXCR4-tropic HIV isolates were generally found in the later stages of HIV infection. Env (gp120/gp41) of CXCR4-tropic HIV isolates on the plasma membrane surface of infected cells drives cell-to-cell fusion with adjacent uninfected $CD4^+$ T cells[52]. This phenomenon may be associated with T cell depletion in HIV infection[38]. *In vitro*, syncytium formation is the principal cause of HIV-1mediated T cell destruction[57]. *In vivo*, transmission of HIV-1 generally results from R5 viruses. After several years, X4 viruses can be detected in at least 50% of individuals infected with HIV-1, and this shift is strongly associated with disease progression [19]. Furthermore, a positive correlation between CD4 T-cell decline and infection by syncytium-inducing HIV-1 has been established[11]. All these confirmed that HIV Env-induced cell fusion plays a role in HIV disease. Actually, there are two models of HIV Env induced apoptosis after cell fusion: (i) a hemifusion event leading to single cell death, (ii) syncytium formation leading to apoptosis of associated cells.

Hemifusion

After gp120 function, gp41 mediates close cell-to-cell contacts, thereby triggering cell death in single uninfected cells in the absence of detectable cell-to-cell fusion. In this process, gp41-mediated transfer of lipids from the membrane of Env- expressing cells to the target cell occurs, but not with detectable cytoplasm mixing[12].

Syncytium formation

Although the interaction of gp120/41 with CD4/CXCR4 can signal for apoptosis via a transient cell-to-cell contact (hemifusion)[12], in most cases, this interaction induces cellular fusion and syncytium formation[45]. Syncytia have a short lifespan, so it is not surprising that syncytium can hardly be detected in HIV-infected patients.

In vitro models using co-culture systems showed that syncytium undergoes apoptosis through the mitochondrial intrinsic pathway [24,49]. However, whether caspase activation is involved in the process is still a matter of controversy. Some studies indicated that nuclear apoptosis of syncytia is caspase- dependent[24], while others showed the caspase inhibition has no or little cytoprotective effect in the process, indicating that syncytia undergo apoptosis in a caspase-independent manner[46]. *In vitro*, the cascade of events during syncytial apoptosis includes: cell fusion, activation of Cdk1/cyclin B, activation of the mammalian target of rapamycin (mTOR), p53 phosphorylation on serine 15 and serine 46 by mTOR and p38 MAPK, transcriptional activation of pro-apoptosis proteins (Bax, Puma) by p53, Bax translocation to mitochondria with consequent MMP, AIF release and Cyt-c release, nuclear apoptosis [17,44,45,49].

CONCLUSION

Accumulating studies evidenced that the T lymphocytes depletion in HIV-infected patients is due to increased apoptosis, and HIV Env plays a central role in the disease progression. The different mechanisms of HIV envelope-induced T cell apoptosis were reviewed in this article. Soluble and membrane-anchored gp120 induce both Fas-dependent (upregulation of Fas/FasL, decreased FLIP) and Fas-independent (increased Bax, decreased Bcl-2)

apoptotic pathways, through cell receptors such as CD4, CXCR4 and CCR5. HIV-1 Env (gp120/gp41) expressed on the plasma membrane surface of infected cells drives cell-to-cell fusion with adjacent uninfected $CD4^+$ T cells, the fused cells undergo apoptosis through mitochondrial intrinsic pathway.

Considering the abnormal apoptosis is the dominating cause of immune system dysfunction, intervention in this process might be clinically beneficial. However, to date, only a few pioneer clinical experiments have been done and there is no enough evidence to support this notion. As knowledge of mechanisms of HIV-associated apoptosis accumulates, we may find some new therapies for HIV/AIDS.

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