Apoptosis in AIDS

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Acquired immunodeficiency syndrome (AIDS) is a complex disease induced by human immunodeficiency virus (HIV) infection. We have acquired an extensive knowledge of the molecular characteristics of the virus in the 10 years since its first isolation (*1*). Nevertheless, the pathophysiological basis of the profound and irrevers ible immune depression that follows this retrolentiviral infection is disappointingly obscure. A number of mechanisms have been proposed to account for the defects and depletion of helper CD4$^+$ cells. These include syncytium formation between infected and noninfected cells, selective infection and destruction of memory T helper cells, inappropriate immune killing of uninfected cells, and autoimmune responses (*2*). Now, new findings suggest another potential mechanism. In HIV-infected patients, the loss of CD4$^+$ cells is associated with lymphocyte activation (*3–5*), but this activation does not result in cell proliferation, as it does normally, but rather in cell death by a mechanism known as programmed cell death (*6*).

Programmed cell death, or apoptosis, is a physiological suicide mechanism that preserves homeostasis, in which cell death naturally occurs during normal tissue turnover (*6*). In general, cells undergoing apoptosis display profound structural changes, including a rapid blebbing of the plasma membrane and nuclear collapse, which are associated with extensive damage to chromatin and DNA cleavage into oligonucleosomal length DNA fragments. In most cases, apoptosis occurs after activation of a calcium-dependent endogenous endonuclease (*7*).

Apoptosis is essential in many physiological processes, including maturation of the immune system. Apoptotic cell death is part of the normal development of intrathymic T cells and of self-tolerance. Indeed, apoptosis can be triggered in immature thymocytes by glucocorticoids, radiation, calcium ionophores, and specific activation of the T cell receptor—CD3 complex (*8*). Mature T cells are resistant to these stimuli and respond to T cell receptor stimulation by cell proliferation and cytokine secretion. However, under some circumstances, antigen-receptor signaling may lead to the abnormal induction of a cell death program in mature T cells (*9*).

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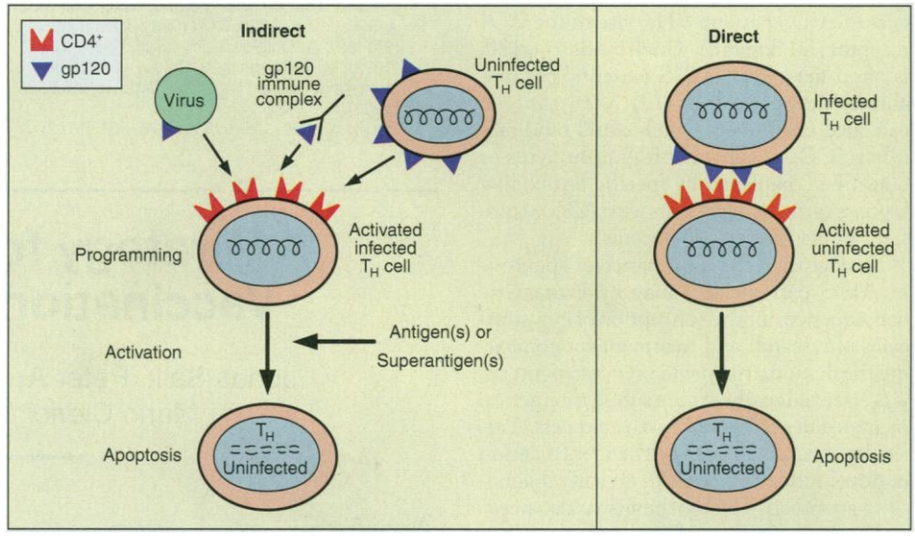
A cell death program is triggered in patients with AIDS. Indeed, in vitro activation of mature T cells from asymptomatic, HIV-infected individuals by polyclonal activators such as calcium ionophore or antibodies to the T cell receptor induces apoptosis in a fraction of CD4$^+$ T cells and also in CD8$^+$ T cells (*4, 5*). Moreover, specific cell death of CD4$^+$ T cells is observed upon activation of T cells by major histocompatibility com plex (MHC) class II–dependent bacterial superantigens (*3, 4*). In the absence of activating agents, a high background amount of apoptosis occurs in cultured lymphocytes of HIV-infected patients, which is not dependent on protein synthesis. However, T cell receptor activation–induced apoptosis of these patients' T cells requires protein synthesis, indicating that this form of apoptosis could potentially be suppressed. Indeed, addition of a second stimulus in the form of an antibody to CD28 (*3*) or addition of cytokines [interleukin-1α (IL-1α) and IL-2] (*4*) prevents T cell suicide and restores antigen-dependent T cell activation.

Several mechanisms have been proposed to account for these ex vivo phenomena. The external glycoprotein gp120 of the virus is a good candidate for causing apoptosis in AIDS. The binding of CD4 receptors by gp120 followed by clustering induced by antibody to gp120 primes normal human CD4$^+$ T cells for apoptosis in response to subsequent T cell receptor stimulation (*10*). Thus, envelope protein expressed on the surface of HIV-infected cells may prime neighboring uninfected cells for apoptosis by cross-linking the CD4 molecule and therefore induce in these uninfected cells a cell death program triggered by a subsequent activation with antigen. Furthermore, the complex of gp120-gp41 (the HIV transmembrane protein) on infected cells is necessary and sufficient to induce apoptosis inactivated noninfected cells. During this process, the antiviral agent azidothymidine (AZT) blocks cell-to-cell spreading of HIV infection without having any apparent effect on apoptosis (*11*). Consequently, by virtue of their expression of the gp120-gp41 complex, HIV-infected cells can prime for pathological programmed cell death but can also trigger apoptosis in uninfected, activated cells.



What turns on the cell death program? Apoptosis might be triggered indirectly **(left)** or directly **(right)** in patients with AIDS.

Additional mechanisms may contribute to programmed cell death induction in HIV infection, including defects in activation signaling. Optimal activation of T cells requires T cell receptor stimulation and a second signal delivered from an antigen-presenting cell. When there is an inappropriate cosignal, delivered by an infected antigen-presenting cell for example or T cell preactivation by IL-2 (*12*), consecutive T cell receptor stimulation induces T cell deletion by apoptosis.

The full relevance of these observations for the in vivo situation remains to be determined. CD8$^+$ as well as CD4$^+$ T cells from HIV-infected individuals undergo apoptosis in vitro. Do CD8$^+$ T cells die in vivo but then are replaced? Is the CD4$^+$ T cell deletion the consequence of an impairment in T cell renewal rather than that of a massive depletion? Recent studies with simian models of lentiviral infections indicate

that programmed cell death induction is related to AIDS pathogenesis (*4*). Chimpanzees that have been infected with HIV-1 are productively infected but do not develop disease and do not show any immune deficiency,and simian immunodeficiency virus (SIV)–infected macaque rhesus monkeys show a rapid CD4 cell decline leading to AIDS-like disease. Programmed cell death did not occur in T cells from HIV-1–infected chimpanzees and, by contrast, cell death by apoptosis was observed upon activation of T cells from SIV-infected macaques. The absence of apoptosis in HIV-infected chimpanzees suggests that T cell programmed cell death is not an obligatory consequence of HIV infection, but reflects a more complex array of interactions between HIV and the immune system.

Is there in vivo evidence that binding of CD4 receptors on lymphocytes by gp120 occurs in AIDS patients? The site of the CD4 receptor (D1 domain), which binds to gp120, is often masked in AIDS patients, presumably by the gp120 itself (*13*). Viral particles can shed free gp120, which could bind rapidly to CD4 receptors on lymphocytes or could be complexed by specific antibodies. Upon treatment of patients with AZT, a transient unmasking of CD4 occurs.

Recognizing the importance of apoptosis in AIDS pathogenesis may have dramatic consequences for the conception of new strategies of research and treatment for combating the disease. In order to prevent apoptosis, it is essential to dissect the subtle interaction of molecules involved in the process. Particularly important will be the identification of novel surface markers for an early diagnosis of apoptosis. The biochemistry also needs to be clarified. The role of oxidative stress and mitochondrial alterations should be analyzed. Antioxidants such as *N*-acetylcystein, vitamins C and E, and superoxide dismutase should be included in clinical trials in combination with antiviral therapy.

The masking of CD4 receptor by gp120 may be used as a surrogate marker for the clinical evaluation of antivirals, as suggested by the rapid, although transient, unmasking of this receptor by AZT. Soluble CD4 could prevent the binding of gp120 to the CD4 receptor. As a viral inhibitor, soluble CD4 has been rather disappointing, but it could have a beneficial effect on the course of the disease by preventing the abnormal signaling of the CD4 lymphocytes due to the binding of the viral glycoprotein. We suggest therefore that the pharmaceutical industry reenter this neglected field and make sufficient amounts of soluble CD4 for clinical trials.

Finally, it would be very important to identify the superantigens, if any, that activate $CD4^+$ and $CD8^+$ T cells. Because of the existence of superantigens in animal retroviruses (*14*), it has been proposed that HIV encodes a superantigen. However, there is no convincing evidence at the present time in favor of this suggestion. Another explanation is that there is more than one superantigen coming from a microorganism such as mycoplasma (*15*). Association of some mycoplasma species with HIV and AIDS has been described (*16*, *17*).

Antiviral therapy of AIDS patients or of HIV-infected individuals has shown little success. The time has come to fight this complex disease by combinations of several treatments including antivirals, antibiotics, and anti-apoptotic drugs. The validation of the efficacy of these drug combinations by appropriate clinical trials will be difficult, but in our opinion this is the only way to respond correctly to the challenge of AIDS.

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A Strategy for Prophylactic Vaccination Against HIV

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A prophylactic vaccine against human immunodeficiency virus (HIV) infection represents the best hope for controlling the continuing and devastating worldwide AIDS epidemic. In this commentary, we outline evidence suggesting that the goal of immunization to prevent or control HIV infection should be activation of the cell-mediated, rather than the antibody-mediated, arm of the immune system. Accordingly, we propose a vaccination strategy intended to ensure that a stable cell-mediated response occurs after exposure to HIV.

During the past few years it has become clear that apparently harmless, and possibly protective, encounters with HIV can occur. Some individuals who have been exposed to the virus and are therefore at high risk for HIV infection remain apparently uninfected; they do not have antibodies to HIV in their blood, and neither HIV nor its nucleic acids can be detected in blood samples. Nevertheless, in one study of 97 such individuals seronegative for HIV, 49% exhibit cell-mediated immunity to HIV (their T cells respond to HIV peptides in vitro), whereas only 2% of 163 individuals not known to be exposed to HIV exhibit responses to these peptides (*1*, *2*). Such HIV-specific, cell-mediated responses have been seen in gay men with known sexual exposure, intravenous drug users, health care workers exposed by accidental needle stick, and newborn infants of HIV-positive mothers (*1*, *2*). HIV-specific lymphoproliferation or cytotoxic T lymphocyte (CTL) activity, hallmarks of cell-mediated responses, have also been observed by other investigators in some exposed, but apparently uninfected, subjects (*3*).

These findings have been extended by monitoring HIV-exposed individuals over time (*1*, *2*). HIV status was followed in six gay men who exhibited cell-mediated immunity in the absence of antibody to HIV or detect-

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