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News and Commentary

To kill or be killed: how HIV exhausts the immune system

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Viruses have evolved numerous mechanisms to evade the host immune system and one of the strategies developed by HIV is to activate apoptotic programs that destroy immune effectors. HIV infects CD4 T cells, preferentialy HIV Ag-specific T cells,[1] and it induces profound alterations on CD8T cells and B cells, two populations of lymphocytes that are not direct targets for productive HIV replication.[2,3] Early reports demonstrated the role of premature lymphocyte apoptosis in CD4 T cell loss during HIV infection.[4–7] Although our understanding of the causes of CD4 T cell lymphopenia is still incomplete and controversial, involving altered T-cell renewal and increased turnover rates of T cells, increasing evidence points out to HIV-driven lymphocyte apoptosis as an important contributor to the destruction of the immune compartment. Not only does the HIV genome encode proapoptotic proteins, which kill both infected and uninfected lymphocytes through either the members of the tumor necrosis factor family or the mitochondrial pathway, but it also creates a state of hyperactivation induced by ongoing viral replication and responsible for the exacerbation of physiological mechanisms of clonal deletion. This review discusses recent advances on our understanding of the role of apoptosis on the impairment of HIV-specific immunity and how new therapeutics might counteract this process.

How HIV Perturbs T-Cell Dynamics

HIV infects cells of the immune system, and particularly T helper cells, which express CD4 molecule, the receptor for HIV envelope. In the absence of antiviral treatment, HIV infection is characterized by the gradual loss of $CD4^+$ T cells and a progressive immune deficiency that leads to opportunistic infections, and ultimately death. As mature $CD4^+$ T helper cells are key effectors of antiviral immunity, it was initially proposed that HIV-associated immunodeficiency results from the direct virus-mediated killing of $CD4^+$ T cells (reviewed in Levy[8]). However, the mechanisms by which $CD4^+$ T cell loss arises remain a matter of controversy. Recent attempts to understand how HIV disrupts T-cell homeostasis suggested that chronic immune activation, due to persistent expression of viral particles, results in high turnover rates of T cells, leading to increased T-cell proliferation that is physiologically controlled by increased apoptosis. Since lymphocyte homeostasis is achieved by a balance between the production and death of lymphocytes, it was proposed that HIV interferes with T-cell renewal, both at the level of progenitors and thymic differentiation, preventing appropriate replacement of prematurely destroyed mature T cells (reviewed in Douek[9]).

During acute infection

Exposure to HIV is primarily through the mucosal route, and the establishment of infection is dependent on the target cell's expression of CD4 and a chemokine receptor (CCR). CCR5 and CXCR4 are the major receptors used by HIV *in vivo*, with CCR5 almost always being the initial target coreceptor for naturally transmitted virus.[10] Little is known about the primary pathophysiological events of lymphocyte and viral dynamics in acute, as opposed to chronic HIV infection in humans. SIV models of rhesus macaque infection showed that, after establishment of infection in the mucosa, SIV is rapidly disseminated over the next 2 weeks, infecting increasing numbers of $CCR5+CD4+$ T cells, a memory T-cell subset that accounts for almost all $CD4+$ T cells in tissues including the mucosal surfaces of the intestinal, respiratory and reproductive tracts.[11] Inflammatory cytokines and HIV gene products, such as Nef, Tat, Vpr, induce TCR-independent T-cell activation and contribute to efficient production and propagation of the virus from infected cells to adjacent $CCR5^+CD4^+$ T cells. The consequence is the selective depletion by apoptosis of these memory CD4 T cells through direct or indirect cytopathicity and CD8-mediated destruction of infected CD4 T cells.[12] Gene expression profiling of macaques acutely infected with SIV/HIV-1 chimeric virus (SHIV) demonstrated a differential expression of 10 apoptosis regulators during the first 2 weeks of infection, including TNFRSF6 (tumor necrosis factor receptor superfamily, member 6 (FAS)), BCL2, BCL2L1, DAD1,TIAL1 and PDL2.[13] Accordingly, pathogenic SHIV was shown to induce an accumulation of apoptotic cells during the second week of infection in both lymph nodes and thymus, which colocalized to sites of both virus replication and CD4 T-cell loss.[14]

During chronic infection

In contrast to acute infection, characterized by rapidly increasing and then decreasing viral load and rapid CD4 T-cell depletion, the chronic phase of the infection is associated with lower but persistent plasma viral load and a slow decrease of CD4 T cell counts over a period of 10 years before the onset of AIDS. *In vivo* studies of T-cell dynamics in chronic HIV infection, using methods of direct DNA labeling of

proliferating cells with stable deuterium isotope, have shown a state of high turnover of both CD4 and CD8 T cells and a decrease in memory CD4 and CD8 T cell half-life.[15,16] This was associated with persistent high viral load and was markedly reduced after initiation of HAART.[16–18] This concept of high turnover involves increased T-cell activation, expansion, shift from naive to memory/effector stage and activation-induced cell death. Analysis of Ki67 expression as a marker of cell proliferation showed that CD4 and CD8 proliferating T cells increase in both naive and memory subsets, and decrease with HAART.[19–21] Quantitative image analysis of lymph nodes from HIV-1-infected persons has revealed CD4 T-cell depletion in an environment of increased T-cell proliferation and apoptosis.[22] The interpretation of these data in terms of cause and effect has been the subject of controversy: does the virus cause massive CD4 T-cell death for which the immune system attempts to compensate with increased homeostatic proliferative response, or does the virus cause massive T-cell activation and proliferation, with death being the natural immunological consequence?[2]

Lymphocyte homeostasis is achieved by a balance between the production and death of lymphocytes, T-lymphocyte progenitors differentiate and mature in the thymus and are released into the peripheral circulation, where they can migrate to sites of encounter with antigen.[24] Several studies have suggested that $CD4^+$ T-cell lymphopenia might be linked to interference with T-cell renewal by HIV, both at the level of progenitors and thymic differentiation, preventing appropriate replacement of prematurely destroyed mature T cells. In accordance with this, $CD34^+$ progenitors from individuals progressing to AIDS have a dramatic loss in T-cell development capacity.[24] The contribution of thymic output to T-cell homeostasis can be evaluated by measurement of recent thymic emigrants (naive T cells) that harbor T-cell receptor excision circles (TREC) formed during T-cell rearrangement in the thymus. The number of TREC is thought to correlate with thymic function.[23] TREC number is reduced in the blood within a few months of HIV infection,[25] and it may have prognostic value, as individuals with low numbers of TRECs progress to AIDS at a faster pace.[26] A number of factors besides thymic production may determine TREC content of the T-cell population, including cell division (TRECs do not replicate during mitosis) and cell death, intracellular degradation of TRECs and reversion of memory cells to a naive phenotype, complicating the interpretation of TREC data.[27] However, an essential role for the thymus for T-cell renewal is suggested in early HIV-infected individuals,[28] and in patients on highly active antiretroviral therapy (HAART) in whom suppression of the viral load is accompanied with concomitant enhanced numbers of naive T cells and increased TREC content.

How HIV Exploits the Apoptotic Machinery

Early studies revealed that HIV induces premature lymphocyte apoptosis in lymphoid organs, affecting all lymphocyte subsets, including B cells, CD4 and CD8 T cells, dendritic cells,[29,30] and independently of the cytopathic effect of HIV since apoptosis occured mainly in uninfected cells rather than in the HIV-infected cells.[31] Parallel studies showed that a marked fraction of both infected and uninfected T cells from HIV-positive individuals die by apoptosis *ex vivo*, either spontaneouly following a short-term incubation, or after TCR-triggering,[3–7] as do HIV-infected T cells *in vitro*.[32–34] The mechanisms that are involved in HIV-associated apoptosis of lymphocyte include: direct killing of infected target cells following virus-gene expression and cytopathicity, death of bystander cells by proapoptotic viral proteins released by infected cells, killing of HIV-specific effectors following their recruitment to infected lymphoid tissues, and altered expression of cellular apoptosis regulatory molecules on lymphocytes and antigen-presenting cells (APCs) as a consequence of HIV-mediated immune activation.[35]

Apoptosis in HIV-infected cells and influence of HIV products

There are several mechanisms by which HIV can induce cell death directly in the cell it infects. CXCR4 tropic HIV isolates generally found in the later stages of HIV infection, preferentially infect T cells and induce membrane fusion between cells forming a giant multinucleated cell called a syncytium. The relevance of fusion-induced apoptosis to AIDS pathogenesis is suggested by the positive correlation between infection by syncytium-inducing HIV or SIV isolates and the decline in $CD4^+$ T cell numbers *in vivo*.[36–38] In lymph nodes from HIV^+ individuals, syncytia express markers of early apoptosis such as tissue transglutaminase.[30] Syncytia arising from the fusion of cells expressing the HIV envelope protein with cells expressing the CD4/CXCR4 complex undergo apoptosis through a mitochondrion-dependent pathway involving mTOR-mediated phosphorylation of p53, upregulation of Bax and activation of the mitochondrial pathway leading to apoptosis.[39] When this apoptotic pathway is initiated in HIV envelope-expressing cells, coculture with a noninfected $CD4^+$ fusion partner results in apoptosis of the syncytium following mitochondrial alterations and caspase-dependent nuclear pyknosis. This cell-to-cell transmission of the lethal signal was only observed when the nuclear pyknosis from donor cells exhibited preapoptotic chromatin condensation.[40] Notably, when peripheral blood $CD4^+$ T cells are highly infected with HIV *in vitro*, the cytopathic killing is associated with necrosis rather than apoptosis, and expression of neither Env nor Nef is required for this nonapoptotic cell death.[41]

HIV-encoded products can trigger *in vitro* cell death pathways. As summarized in Table 1, crosslinking of $CD4^+$ T cells by envelope gp120 activates the CD95/CD95L pathway,[75] and Nef-expressing T cells coexpress CD95L,[70] thus becoming potential killers of uninfected CD95-expressing T cells. Similarly, Tat secreted from infected cells upregulates CD95/CD95L on uninfected cells and enhances susceptibility to CD95-induced apoptosis.[76,59] Bystander T-cell killing can also be mediated by macrophages since ligation of the chemokine receptor CXCR4 by HIV gp120 or its ligand SDF-1 on macrophages induces membrane expression of TNF-α, which triggers apoptosis on TNFRII-expressing $CD8^+$ T cells.[45] Extracellular Nef protein targets $CD4^+$ T

Table 1 HIV gene products expressing pro-apoptotic activity

HIV Effector	Proapoptotic mechanism	References
Env	CXCR4-dependent CD4 T cell and hepatocyte death	42–44
	CXCR4/TNFRII-dependent death of CD8 T cells	45
	Upregulation of Fas expression on lymphocytes	46
	Macrophage-dependent apoptosis mediated by FasL	47,48
	Activation of caspase-3 and FAK cleavage	49
	Apoptosis of CD4+CD34+ progenitor cells	50
	Disruption of neuronal calcium homeostasis and apoptosis	51
	Apoptosis in cardiomyocytes through mitochondrion pathway	52
	Decreased Bcl-2 expression	53
	Activation of AP-1	54
	mTOR-mediated p53 phosphorylation	39
	Activation of the Ras pathway	54
Tat	Downregulation of caspase-10 and upregulation of c-FLIP	55
	Bax-dependent apoptosis	56
	Upregulation of TRAIL by macrophages	57
	TRAIL-dependent killing of CD4 T cells	58
	Functional upregulation of Fas/FasL pathway	59,46
	NO-mediated apoptosis of brain microvascular endothelial cells	60
	Increased caspase-8 activity	61
	Downregulation of Bcl-2	62
	Inhibition of superoxide dismutase	46
Vpr	Cell cycle arrest in G2, nuclear BRCA1 and gamma-H2AX focus formation	63,64
	Dissipation of the mitochondrial potential and cytochrome *c* release	65,66
	Procaspase-8 and -3 cleavage	63,67
	Caspase-9 activation and apoptosis of primary cells	68
	Caspase-independent MMP	69
Nef	Functional upregulation of Fas/FasL pathway	70
	Caspase activation	71
	Downmodulation of CD4 and MHC class I	72
Vpu	Enhanced susceptibility to Fas-induced apoptosis	73
Protease	Activation of caspase-8 and proteolytic degradation of Bcl-2	74

cells for apoptosis by interacting with CXCR4 surface receptors. Two apoptotic motifs were identified on Nef, the removal of which completely eliminated the ability of Nef to induce apoptosis while retaining Nef ability to enhance viral infectivity.[77,78] Like many other viruses, HIV codes for proteins that act on mitochondria and control apoptosis of infected cells.[79] For example, Vpr causes a rapid dissipation of the mitochondrial transmembrane potential in intact cells, as well as the release of cytochrome *c* and cellular apoptosis.[65] Like other viral proapoptotic proteins, such as HBx coded by HBV, Vpr contains amphipatic α helices that are necessary for the proapoptotic effect and seem to have pore-forming properties.[79] Tat induces apoptosis by downregulating Bcl-2[62] and upregulating caspase-8,[61] and the binding of HIV gp120 to CD4 receptor on previously activated cells induces the downregulation of Bcl-2[53] promoting the release of cytochrome *c*.[80] Proteolytic degradation of Bcl-2 can also be the consequence of the activation of caspase-8 by HIV protease.[74]

In order to efficiently disseminate, HIV also needs to inhibit cellular apoptosis of the host cell, at least until high levels of progeny virus are produced, like many other viruses do.[72] HIV strategies to protect lymphocytes from apoptosis include: (1) the upregulation of Bcl-2 and downmodulation of Bax by Vpr;[81] (2) the promotion of cell cycle progression, and subsequent increased virus production, by Tat-dependent inhibition of p53 transcription;[82] (3) the downregulation by Nef and gp120 of expression of the CD4 receptor by infected cells, thereby preventing subsequent gp120-CD4-mediated apoptosis;[83–85] and (4) the downmodulation by Vpu and Nef of the expression of MHC class I molecules and the upregulation of CD95L expression on infected cells, a strategy that might function to protect infected cells from cytolysis by CTL or NK cells.[86,87] These *in vitro* data are in line with *in vivo* observations showing that, in lymph-node biopsies from HIV-infected humans and SIV-infected monkeys, productively infected cells are not apoptotic, indicating that they are relatively resistant to direct HIV-induced killing *in vivo*.[31] Collectively, these data indicate that HIV can manipulate cellular apoptotic machinery, destroying the immune system through the activation of apoptotic programs in lymphocytes, but ensuring viral survival by manipulating the apoptotic machinery to its advantage in infected cells.

Apoptosis in bystander cells

Activated T-cell autonomous death

Apoptosis is required during an immune response to foreign antigen to eliminate most activated antigen-specific T

lymphocytes and thereby prevent autoimmunity. It occurs via two main pathways: activation-induced cell death (AICD), which is mediated by death receptors, and activated T-cell autonomous death (ACAD), which is mediated by Bcl-2-related proteins.[88–90] In the context of a persistent viral antigen expression, one may hypothesize that apoptosis regulation is perturbed, leading to the progressive elimination of activated lymphocytes. This is illustrated by the observation that peripheral T cells from HIV$^+$ individuals, which undergo spontaneous apoptosis after a short-term culture,[5,7,91,92] exhibit an activated phenotype. In addition, they show *ex vivo* the downregulation of Bcl-2,[93] also detected *in vivo* in patients' lymph-node cells.[94] Cells that have low levels of Bcl-2 express activation markers, they belong to the CD8 T-cell compartment and they express cytotoxicity granules.[93,94] A recent study demonstrated that Bcl-2 expression was markedly decreased in HIV-specific CD8$^+$ T cells compared with CMV-specific and total CD8$^+$ T cells from HIV-infected individuals, and lower levels of Bcl-$x_{(L)}$ were also found in HIV-specific CD8$^+$ T cells.[95] Interestingly, the nonpathogenicity of HIV-1 infection in chimpanzees is associated with the lack of immune activation, a very low level of spontaneous T-cell apoptosis and normal expression of Bcl-2.[96] Altogether, these observations suggest that, in pathogenic situations, uncontrolled retroviral replication is responsible for hyperimmune activation associated with downmodulation of survival molecules, such as Bcl-2 and Bcl-x_L, further leading to apoptosis of HIV-specific cytotoxic CD8 T cells. ACAD is normally prevented by cytokines and, accordingly, IL-2 and IL-15 can promote *in vitro* the survival of patients' T cells by upregulating the expression of Bcl-2.[97,98] As discussed below, immunotherapy with these cytokines are actually under evaluation to restore HIV-specific immunity.

Activation-induced cell death

Peripheral T cells from HIV$^+$ individuals show increased level of AICD, as compared with T cell from healthy donors, when activated *ex vivo* through the T-cell receptor (TCR) for antigen.[5–7] AICD involves the CD95 pathway and it is normally required to eliminate activated cells in excess following antigenic response.[99] A number of studies demonstrate that CD95/CD95L are upregulated during the chronic phase of HIV infection and may be involved in the destruction of T cells: (1) Elevated levels of soluble CD95 and CD95L are detected in the serum of HIV$^+$ individuals, and CD95 level is a predictive marker for progression to AIDS.[100,47,101] (2) CD95L-expressing macrophages can kill CD95-sensitive target T cells in an MHC-unrestricted and CD95/TNFR-dependent manner.[48] Notably, macrophage-mediated killing is selective for uninfected bystander T cells,[102] and. a correlation is found between macrophage-associated CD95L in lymph nodes from HIV-infected patients and the degree of tissue apoptosis;[103] (3) Crosslinking of CD4 molecule by gp120 leads to CD95 and CD95L upregulation on CD4 T cells, which are then primed for apoptosis upon CD3 stimulation. CD4 crosslinking induces sensitization for apoptosis of CD8 T cells as well, which is associated with Bcl-x downmodulation, and both CD4 and CD8 T cells die of apoptosis following cell–cell contact with CD95L$^+$ CD4 T cells.[104] (4) HIV-specific CTL are also potential effectors of the destruction of CD95-expressing activated lymphocytes, and it was shown that an MHC class I restricted CTL clone specific for Nef, derived from an HIV$^+$ subject, is able to mediate both perforin- and CD95-dependent cytotoxic activities on either Nef-presenting target cells or CD95-expressing cells, respectively.[105] (5) CD95 and CD95L expression is increased on both CD4$^+$ and CD8$^+$ T cells from HIV$^+$ individuals, and this is associated with increased susceptibility of these cells to CD95-induced apoptosis, a marker for disease progression;[106–108] (6) T-cell susceptibility to CD95-induced apoptosis is not observed in the nonpathogenic model of lentiviral infection, such as chimpanzees infected with HIV.[96] The molecular mechanisms involved in the susceptibility of T cells from HIV-infected individuals to AICD have not been clearly identified yet. However, T-cell priming for apoptosis is associated with the *in vivo* expression in both CD4$^+$ and CD8$^+$ T cells of the active forms of caspase-8 and caspase-3,[109] which can be induced *in vitro* by a number of HIV proteins, such as Tat, Env, Nef or Vpr.[62,49,71,63] Since T-cell activation is downmodulating the level of the apoptosis inhibitory protein FLIP (FLICE-like inhibitory protein), it was hypothesized that susceptibility of patients' T cells to AICD was associated with decreased c-FLIP expression. However, the levels of c-FLIP in purified CD4$^+$ and CD8$^+$ T cells from HIV$^+$ donors do not differ from that of noninfected control donors.[110]

The TNF/TNF receptors death pathway is also activated in HIV$^+$ individuals, and both CD4$^+$ and CD8$^+$ T cells are susceptible to TNFR1- and TNFR2-induced apoptosis.[109] Susceptibility of patients' T cells to TNFR-induced apoptosis is associated with the downregulation of Bcl-2 protein, while there is no increased expression of the two TNFRs on the surface of T cells, and TNFR1-associated death domain (TRADD), receptor interacting protein (RIP) or TNF receptor-associated factor 2 (TRAF-2) expression is not altered by HIV-infection.[109] The ligand for TNFRs, TNF-α, is detected at increased levels in the serum of symptomatic individuals and elevated levels of soluble TNFR2 were found to be predictive of HIV disease progression.[111] The TNFR pathway may be involved in the destruction of CD8 T cells since it has been shown that ligation of the CXCR4 coreceptor by HIV gp120 upregulates the expression of TNF-RII on CD8$^+$ T cells, which become susceptible to apoptosis induced by membrane TNF expressed on macrophages.[45] The involvement of another member of the TNF family, TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) to the bystander destruction of T cells is suggested by experiments performed in the humanized murine model, the hu-PBL-NOD-SCID mice. Following infection with HIV, TRAIL is released by macrophages, and exogenous HIV Tat protein upregulates TRAIL production by primary macrophages *in vitro*.[112] This suggests that a TRAIL-dependent mechanism of destruction of bystander CD4$^+$ T cells occurs *in vivo*, which may be triggered by Tat produced by HIV-infected cells. As a corollary, AICD is partially inhibited by antagonistic TRAIL-specific antibodies, and T cells from HIV$^+$ individuals are susceptible to TRAIL-mediated killing, in contrast to cells from control donors.[113]

Viral evasion of apoptosis

Before destroying the immune system through the activation of apoptotic programs in lymphocytes, HIV may ensure viral survival by manipulating the apoptotic machinery to its advantage in infected cells. HIV-1 Tat downregulates caspase-10 activity and concomitantly upregulates c-FLIP in lymphoid T cells lines stably transfected with a plasmid-expressing *tat* gene, rendering these cells resistant to TRAIL cytotoxicity.[55] Tat also decreases transcription of p53,[114] so promoting cell cycle progression, inhibiting apoptosis and allowing the cells to increase virus production. Nef down-modulates the expression of MHC class I molecules and upregulates CD95L expression on infected cells, a strategy that may function to protect infected cells from cytolysis by CTL or NK cells.[115,116] Nef, gp120 and Vpu contribute to the downregulation of CD4 receptor on infected cells, preventing subsequent gp120-CD4-mediated apoptosis.[117–119] The relative resistance to direct HIV-induced killing of infected cells is confirmed by i*n vivo* studies on lymph node biopsies from HIV^+ individuals, showing that productively infected cells are not apoptotic, in contrast to uninfected cells.[31]

How Apoptosis Impairs HIV-Specific Immunity

Destruction of HIV-specific CD4 T-helper cells

One of the most striking features of HIV infection is the loss of HIV-specific $CD4^+$ T-helper cells early in the infection (reviewed in Noris and Rosenberg[120]). However, a vigorous HIV-specific $CD4^+$ T-cell proliferative response can be found in a small subset of individuals that are able to control viral load either naturally[121] or after antiretroviral treatment early in primary infection.[122] These observations suggest an important role for $CD4^+$ T-cell effectors in HIV control, and they argue for the rapid loss of HIV-specific $CD4^+$ T-cell response during the acute phase. The destruction of HIV-specific naive CD4 T cells may occur following their recruitment into infected lymphoid sites, where they may be killed directly after their specific priming and infection by HIV-infected dendritic cells.[123,124] Accordingly, during acute infection, rapidly proliferating HIV-specific memory $CD4^+$ T cells are highly susceptible to HIV infection, and they were found to contain more HIV viral DNA than other memory $CD4^+$ T cells,[123] indicating their preferential infection *in vivo* and consequently their preferential loss. Langherans cells are likely initial targets for HIV following sexual exposure to virus and, following their infection, they were shown to preferentially transmit HIV to proliferating autologous memory $CD4^+$ T cells, further dying of apoptosis after antigenic stimulation.[125] The *in vivo* destruction of HIV-specific $CD4^+$ T-cell precursors may also occur in lymph nodes following HIV binding[126] and ligation of the homing receptor CD62L, as suggested.[127] Failure to detect HIV-specific $CD4^+$ T cells *ex vivo* might also be due to either their *in vivo* inhibition by high levels of viremia,[128] their anergy resulting from interaction with peripheral blood dendritic cells,[129] or their suppression by $CD4^+CD25^+$ regulatory T cells[130,131] (Figure 1).

Impairment of HIV-specific CD8 T cells

The effectiveness of protection conferred by $CD8^+$ memory T cells is determined by both their quality and their quantity. HIV stimulates strong CTL responses in infected people[132,133] and the importance of these cells in the control of HIV viremia in the acute phase of the infection is suggested by monkey experiments in which *in vivo* depletion of CD8 T cells leads to the lack of control of viremia following SIV infection.[134] During primary HIV infection, virus-specific CD8 T cells initially follow the virus rise in the blood, and when that response reaches a peak, the virus levels fall, suggesting that CTL contribute to the control of HIV viremia are early stage of the infection.[135] Virus-specific CD8 T cells, after clearance of the infection, reduce their number in lymphoid organs by apoptotic death and by migration into peripheral tissues. In the LCMV murine model, many virus-specific CD8 T cells in lymphoid organs are in a preapoptotic state (Annexin-V^+), which was shown to preclude the development of functional memory.[136] In HIV infection, downregulation of Bcl-2 and Bcl-x_L was detected on peripheral blood[93,95] and lymph node CD8 T cells,[94] priming these cells for autonomous cell death,[93] and contributing to the deletion of HIV-specific memory T cells.[95] Bcl-2 down-regulation and CTL apoptosis may also be induced by high doses of virus during the primary viral infection, causing the rapid deletion of high avidity virus-specific CTLs, as suggested in a murine model.[137] Since a recent study showed that the deletion of antigen-specific effectors, but not that of memory T cells, is dependent on selective increase in caspase-3 activation,[138] one may hypothesize that *in vivo* upregulation of caspase-3 in CD8 T cells from HIV-infected persons[109] contributes to the destruction of this subset. In addition to be primed for apoptosis, virus-specific CTL taken *ex vivo* from HIV-infected individuals have functional defects that could undermine their ability to control viral infections. For example, although producing antiviral molecules (IFN-γ, MIP-1ß) on contact with their cognate antigen, most HIV-specific CTL express low levels of perforin and consequently poorly kill appropriate target cells *ex vivo*.[135,139] This may be related to a defect in HIV-specific CTL maturation, as suggested by their phenotype, which is not characteristic of fully mature effectors, in contrast to CMV-specific effectors in the same donors, but corresponds to an intermediate-differentiated subset.[140] Altered differentiation of CTL might also be linked to an unfavorable cytokine environment, as rescue from apoptosis of the Bcl-2 low CD8 T cell subset, detected in the lymph nodes and blood of individuals chronically infected with HIV, can be induced by exogenous IL-2 and IL-15.[95,97,98]

Defective help

$CD4^+$ T cells are the helper cells (T_h) of the immune system that facilitate the generation of antibody and CTL responses. After encountering the antigen, naive T_h cells can differentiate into at least two functional classes of cells during an immune response – T_h1 cells, which secrete IFN-γ, and T_h2 cells, which secrete IL-4.[141] Among the many factors that influence the decision to become a T_h1, cytokines such as IL-12, derived from pathogen-activated macrophages or appropriately activated DC, and IFN-γ, derived from

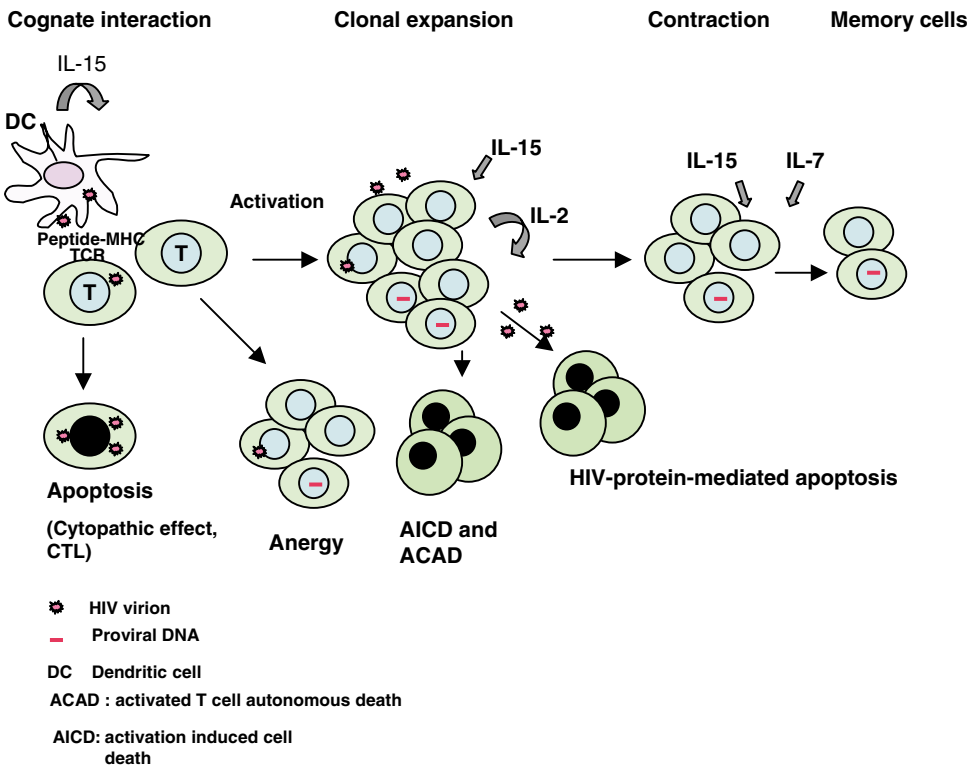

Figure 1 Mechanisms of depletion of HIV-specific CD4 T helper cells. Activation of CD4 T cells requires cognate interaction involving TCR ligation of peptide–MHC complex at the surface of dendritic cells (DC). At this stage, HIV-infected DC may infect HIV-specific CD4 T cells leading to the subsequent apoptosis of this subset, induced either by HIV-driven cytopathogenicity or by CTLs. IL-15 may be involved in DC activation. After T-cell receptor ligation, substantial T-cell clonal activation and expansion occurs, driven in part by IL-2. As a result of their activation state, clonally expanded CD4 T cells are susceptible to infection and destruction by HIV virions, and by the proapoptotic effects of HIV products, such as gp120, Tat, Nef, Vpr or Vpu. In addition, AICD and ACAD occur, a physiological process of contraction that may be amplified by, on the one hand HIV proteins, and on the other hand deficient amount of survival factors such as IL-2 or IL-15. In HIV infection, part of HIV-specific CD4 T cells are found anergic. This may occur following ligation of CD4 receptor by gp120. The massive cell death that normally occurs during the contraction phase results in the loss of most antigen-specific T cells. Both IL-7 and IL-15 may rescue T cells from cell death at this stage, thereby allowing memory T-cell generation. Memory T cells are maintained long-term under the survival effect of IL-7 and by undergoing a low level of proliferation, which depends on IL-15. In the case of HIV infection, a fraction of memory CD4 T cells may contain proviral DNA, constituting a reservoir invisible from the immune system

pathogen-activated NK cells, provide important signals for this differentiation.[141] T_h1 pattern is altered by HIV infection. So, antigenic simulation of PBMCs from HIV-infected patients is characterized by a reduced production of IL-12, IFNγ and IL-2 and an increased production of IL-4 and IL-10, as compared with PBMC from healthy donors.[142] As T_h1 immunity is considered to be crucial for appropriate anti-viral CTL response, which in turn contributes to the control of viral load, it is important to consider that alteration of T_h1 pattern is indicative of the level of $CD4^+$ T-cell loss and disease progression.[142] *Ex vivo* single cell quantification of the frequency of T_h1/T_h2 subsets derived from peripheral T cells stimulated in short-term cultures showed that HIV infection is associated with a differential alteration in the frequency of Th1 subsets: a marked decrease in the IL-2-producing T cells was observed, whereas the frequency of IFNγ-producing T cells was preserved throughout HIV infection, and this was directly related to the contraction of the naive $CD45RA^+$ $CD4^+$ T-cell compartment, which occurred as infection progressed.[143] The contribution of AICD to the alteration of T_h1 pattern was demonstrated by the concomitant detection of apoptosis in cytokine-producing T cells following activation. It appeared that, among the different Th1 subsets, there is a gradient of susceptibility to activation-induced cell death (IL-2 producing T cells being the less susceptible to AICD and TNF-α-producing T cells the most), which is regulated by the expression of Bcl-2, and which contributes to the deregulation of T_h1 cells.[143] Interestingly, HIV infection is associated with the progressive decrease in the proportion of IL-2-producing T cells, which correlates with their susceptibility to apoptosis and with disease progression. Preservation of HIV-specific CD4 T helper effectors in chronically HIV-infected individuals may be obtained following administration of IL-2.[144] Thus, exacerbated activation-induced cell death in peripheral T-helper cells may be responsible for the impairment of HIV-specific immunity in HIV disease (Figure 1).

Cytokine control of memory T-cell survival

Evidence has accumulated that cytokines have a fundamental role in the differentiation of memory T cells. Members of the common cytokine-receptor γ-chain-cytokine family, in particular IL-7 and IL-15, act at each stage of the immune response to promote proliferation and survival (reviewed in

Khaled and Durum[145]). IL-15, primarily produced by macrophages, exhibit many activities in common with IL-2, it is required for survival of memory $CD8^+$ T cells, natural killer (NK) cells and NK T cells, and it increases NK cell cytotoxicity and enhances T-cell proliferation to mitogens and opportunistic antigens in PBMC from HIV^+ individuals.[98,146] The stimulating effect of IL-15 on NK function is mediated through the upregulation of TRAIL, and IL-15-treated NK cells have an antiviral effect, reducing the burden of replication-competent HIV, and causing undetectable HIV-DNA in autologous PBMCs.[147] In addition, IL-15 is a potent survival factor in the prevention of autonomous cell death, acting by upregulating the expression of Bcl-2 and Bcl-x_L, both in total[98] and HIV-specific $CD8^+$ T cells from HIV^+ individuals.[95] IL-15 enhances the effector function of memory CD4 and CD8 T cells increasing the secretion by these cells of the effector cytokines IFN-γ and TNF-α, [148] and it inhibits CD95-induced apoptosis of HIV-specific CD8 T cells.[149] However, IL-15 does not rescue a particular subset of HIV-specific CD8 T cells, which coexpress CD57, accumulate as replicative senescent cells and die of apoptosis following a short-term culture.[150] Finally IL-15 was found to inhibit caspase activation and to increase Bcl-2 expression in a subset of $CCR5^+$ Bcl-2 low T cells, which proliferate during primary HIV infection and are highly susceptible to spontaneous apoptosis.[151] IL-15 could thus be an effective promoter of innate and adaptative immune responses, contributing to the development and survival of T_h1 cells and HIV-specific $CD8^+$ T cells, and recent attention has turned towards IL-15 as a possible alternative immunotherapy in HIV^+ individuals.

IL-7 is a critical component of thymopoiesis and it has recently been shown to play an important role in T-cell homeostasis. *In vitro,* IL-7 provides potent antiapoptotic and proliferative signals to early thymocyte progenitors, mainly single positive ($CD4^+CD3^+CD8^-$ and $CD4^-CD3^+CD8^+$).[152] The contribution of IL-7 to thymic T-cell regeneration has been indicated by experiments using thymic organ culture systems, in which exogenous IL-7: (1) increased the frequency of TRECs in fetal, as well as infant, thymi, indicating that increased rearrangement of $\alpha\beta$TCRs had occurred;[153] (2) together with SDF-1, it enhanced the viability of $CD34^+$ T-cell precursors, by modulating the expression of Bcl-2 and Bax, and stimulated their proliferation.[153] In macaques infected with SIV, IL-7 induces both central renewal and a peripheral expansion of T lymphocytes associated with cell activation, without increasing the viral load.[154] However, comparison of pathogenic and nonpathogenic models of SIV infection showed that elevated levels of IL-7 are associated with disease progression.[155] Similarly, in HIV-infected subjects, falling $CD4^+$ T-cell counts are associated with increased circulating levels of IL-7.[156] IL-7 may have deleterious effects and contribute to the destruction of $CXCR4^+CD4^+$ progenitors since it was found to favor HIV replication in thymocytes by inducing expansion of mature $CD27^+$ thymocytes expressing the CXCR4 coreceptor.[157] In addition, IL-7 pretreatment of peripheral naive T cells mediates their expansion and enhances their susceptibility to primary isolates of HIV.[158] These data have to be considered when evaluating IL-7 as therapeutic immunomodulator for HIV^+ individuals.

Strategies to Enhance T-Cell Reconstitution

The availability of potent antiretroviral therapies (HAART), which combine nucleoside inhibitors of HIV-reverse transcriptase (NRTIs) and HIV-protease inhibitors (PIs), and capable of limiting HIV replication, has challenged strategies to preserve or restore immune functions in HIV-infected patients. Potential immune strategies include the use of IL-2, human growth factor, IL-7 and IL-15.

Following HAART, a rise in the number of $CD4^+$ T cells is generally observed in treated HIV^+ patients, in parallel with suppression of HIV replication, which corresponds to several phases of immune reconstitution. The first phase is attributable to increased lymphocyte survival, as demonstrated both in lymphoid organs *in vivo* and in blood lymphocytes *ex vivo*.[159] A decrease in both spontaneous apoptosis and AICD is observed in T-cell subsets from most of the treated individuals and this is related to decreased immune activation.[110,160–162] The acquired resistance to CD95-induced apoptosis is correlated with an increased rate of $CD4^+$ T-cell production.[160] Decreased apoptosis is likely to depend on many factors, including the downregulation of proapoptotic HIV proteins and the reduction of virus-driven immune activation. The antiretroviral drugs may also play a role, as some of them exhibit antiapoptotic properties,[162] while others exhibit a proapoptotic activity *in vitro*,[163] possibly contributing to the persistence of high levels of lymphocyte apoptosis in some patients.[163] The second phase of quantitative $CD4^+$ T-cell restoration is due to the recirculation and peripheral proliferation of memory/activated $CD4^+$ T cells that were sequestered in the lymphoid tissues. The third phase, which occurs after several months, corresponds to the reconstitution of the naive T-cell pool due to increased thymic output, indicated by an increase in the level of TRECs levels.[2,3]

Long-term potent antiretroviral therapies (HAART) are often associated with a syndrome of lipodystrophy, characterized by hyperlipidemia, insulin-resistance and changes in body fat distribution (peripheral lipoatrophy and/or central fat accumulation).[164] The mechanisms involved in these metabolic complications are poorly understood but some anti-HIV drugs and cytokines were shown to induce functional alteration and apoptosis of peripheral adipocytes and lymphocytes, possibly contributing to these complications.[165] For example, PIs inhibit *in vitro* adipocyte differentiation, trigger apoptosis, and induce an insulin resistant state in differentiated adipocytes, as found *in vivo* in atrophic adipose tissue of patients with lipodystrophy.[166] NRTIs induce mitochondrial toxicity on adipocytes, leading to depletion of cellular mtDNA content and apoptosis.[167] Cytokines can also affect adipocyte functions, and in particular TNF-α inhibits adipogenesis in preadipocytes, and it stimulates lipolysis and promotes insulin resistance.[168] Altered homeostasis of TNFα synthesis is observed in the blood of HIV^+ patients with lipodystrophy, which is correlated with increased serum levels of triglycerides, and cholesterol,[161] and TNF-α expression is upregulated in patient's adipose tissue.[166] Also, hyperlipidemia in HAART-treated patients is related to both increased production of IFNα and hormonal perturbations, and positive correlations are

found between serum IFN-α level or cortisol/DHEA ratio and atherogenic lipid levels.[169]

IL-2 treatment of HIV infection has been evaluated in a large number of studies. In the first trials, IL-2 treatment was assessed in patients receiving NRTIs, and its use was associated with consistent improvement in $CD4^+$ T-cell counts and, in the long-term, IL-2 was able to preserve an HIV-specific CD4 T-cell response, which was not detected in HAART patients.[144] In more recent studies, IL-2 administration along with initiation of HAART induced a consistent increase in CD4 T-cell counts, even in patients with initial low count.[170] The gains in CD4 T cells consist predominantly of increases in naïve CD4 + T cells *versus* memory cells. The expansion of the naïve CD4 T-cell pool appears to be attributable primarily to the proliferation and increased survival of existing naïve cells in the peripheral circulation.[171] The possible influence of IL-2 on the generation of new naive cells by the thymus is still a matter of debate. However, a question is still open: is immune reconstitution aided by the use of IL-2? Human growth hormone (GH) was recently tested in HIV-infected patients to determine if GH treatment enhances T-cell production. It was found that GH might enhance thymic function by stimulating bone marrow progenitors and facilitating their engraftment in the thymus.[172] However, GH treatment is associated with significant toxicity, including edema, arthralgias and diabetes. IL-7 therapy has not yet been administered to humans. However, IL-7 has been shown to stimulate expansion and export of progenitor cells from the bone marrow, to inhibit apoptosis and enhance proliferation of developing thymocytes, and to enhance cytotoxicity of mature T cells. It is also believed to play an important role in T-cell homeostasis. Supporting line of evidence include the findings that circulating IL-7 levels are increased in lymphopenia, and that cellular production of IL-7 is increased in lymphocyte-depleted lymph nodes.[173] The potent effect of IL-7 on T-cell development and homeostasis have made it an attractive therapeutic candidate to restore T cells in HIV infection, but its stimulating effect on HIV replication *in vitro* indicate that its use in HIV disease will need to be pursued with caution. An advantage of IL-15 therapy over IL-2 therapy is that IL-15 does not induce increased HIV replication within CD4 T cells, thus inducing cellular immune responses without affecting virus replication within the infected cell. In addition, this cytokine may have a special value in therapy by enhancing the development and function of NK cells, stimulating IFN-γ production by these cells and having a beneficial effect on the CD8 T cell noncytotoxic anti-HIV response, and finally enhancing effector functions of HIV-specific CD4 and CD8 T cells.

Conclusion

Understanding the viral strategies involved in the destruction of HIV-specific effectors is particularly important since no currently available therapies can efficiently restore virus-specific immunity. HAART limits HIV replication and retards disease progression but drug toxicity and the emergence of drug-resistant variants preclude long-term control in HIV-infected persons. In addition to the immune-based strategies discussed above, structured treatment interruption (STI) is currently evaluated, aiming at inducing 'auto-vaccination' (a concept based on the theory that increased exposure to autologous virus could stimulate HIV-specific immune responses and attenuate viral rebound) while reducing dependence on antiretroviral drugs.[174] This strategy may be used in patients treated immediately after HIV infection since HIV-specific effectors are preserved and viral escape mutants have not been selected,[122] but it may not be applied to chronically infected patients, who have lost most of the HIV-specific effectors. Use of therapeutic vaccines to increase the strength and breadth of HIV-specific cellular immune responses may be more successful. This is suggested by a recent study showing that immunization of HIV-infected patients with dendritic cells loaded with chemically inactivated HIV could induce, in the absence of antiretroviral therapy, a prolonged suppression of HIV viral load, which was positively correlated with virus-specific CD4 and CD8 T cells.[175] This study shows for the first time that improved immune control of HIV infection is possible and it offers new hopes of the possibility of therapeutic vaccines to modulate the course of HIV disease.

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