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News and Commentary

# Apoptosis of HIV-specific CD8 + T cells: an HIV evasion strategy

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The failure of HIV-specific CD8 + T cells to control HIV has suggested a functional defect for these cells in HIV infection. Recently, HIV-specific CD8 + T cells from HIV-infected patients were shown to exhibit reduced levels of Bcl-2 and Bcl-$x_L$ and to be highly prone to CD95/Fas-induced apoptosis. We hypothesize that this apoptosis of HIV-specific CD8 + T cells may affect their maturation and/or impair effector function. In this review, we discuss the apoptotic defect, the intracellular mediators of this proapoptotic state and the potential mechanisms that result in the priming of HIV-specific CD8 + T cells to apoptosis. Thus, HIV may employ a counterattack mechanism in which HIV-specific CD8 + T cells undergo apoptosis upon engagement of CD95L/FasL on apoptosis-resistant HIV-infected cells. Understanding the mechanisms governing the survival of HIV-specific CD8 + T cells is a prerequisite both for therapeutic interventions and vaccines targeting an effective long-lasting immunoprotective cytotoxic T lymphocyte (CTL) response.

Multiple host factors and their interaction with HIV contribute to the complexity of HIV pathogenesis and disease progression. The urgency to understand the biology of HIV infection emerges from the necessity to develop safe and effective treatments and vaccines. Despite the great advances in this field, over the past few years, critical questions concerning the mechanisms employed by HIV to evade the immune system still remain unanswered. HIV can establish a persistent infection characterized by continuous viral replication, ultimately leading to destruction of the immune system.[1] The CTL response is a major effector arm of the immune defense against HIV virus;[2,3] however, this response eventually fails to control virus and prevent AIDS progression. Recent data suggest that accelerated apoptosis of CTLs in HIV infection constitute one of the mechanisms HIV employs to escape immune control. Such apoptosis of CTL may constitute an important obstacle in developing effective therapeutics and vaccines. An ideal HIV vaccine should be able to elicit a robust long-lasting immunity, capable of controlling a wide variety of HIV isolates.[4] Thus, reconstitution and reinforcement of the CTL function against multiple HIV epitopes is a major goal of therapeutic strategies and vaccines against HIV. Understanding and overcoming the apoptotic defect of naturally occurring and vaccine-elicited HIV-specific CD8 + T cells therefore is an important goal in the quest for novel cellular immunity-based therapies, and therapeutic and preventive vaccines.

## CTL Response in HIV Infection

The critical role of CD8 + T-lymphocytes in the immune response against various chronic and acute viral pathogens in humans is well established.[5,6] The inhibitory activity of CD8 + T cells on HIV replication in autologous CD4 + T cells has been known since 1986,[7] while the detection of HIV-specific CD8 + T cells followed shortly after.[8] A vigorous oligoclonal CD8 + T-cell response develops at a very early stage after acute infection.[9,10] This response reaches a peak at the same time when primary viremia starts to fall.[6,11] Following viral load decline, CD8 + T cells are rapidly removed, presumably by apoptosis, while an HIV-specific CD8 + T-cell population approximately 1% of total CD8 + T cells finally remains in circulation.[12–14]

A large body of evidence supports the importance of CD8 + T-cell-mediated immunity in HIV control. CTL activity seems to be the primary immune-defense mechanism at early stages of infection, when neutralizing antibodies have not yet appeared.[6,15] Clinical studies with long-term nonprogressors (LTNPs) who can control the infection revealed the presence of high levels of HIV-specific CTL activity.[16] Furthermore, slow disease progression was found to correlate with HIV-specific CD4 + and CD8 + T-cell responses.[17,18] The rapid appearance of CTL-escape viral mutants is indicative of the strong pressure exerted by CD8 + T cells on virus.[2,19] Direct *in vivo* evidence of the importance of the CTL response comes from SIV-infected non-human primates. SIV viremia is dramatically increased, while disease progression is accelerated in SIV-infected macaques after *in vivo* depletion of CD8 + T cells with monoclonal antibodies, directly implicating these cells in virus control.[20,21]

Treatment with HAART results in a decline of HIV-specific CD8 + T cells, indicating that CTL expansion or survival is supported by high viral loads.[22,23] On the other hand, interruption of HAART therapy was found to suppress the percentage of functionally responsive HIV-specific CD8 + T cells.[24] Interestingly, a more recent study demonstrated that structured treatment interruption results in recovery of the HIV-specific CD8 + T-cell response that was correlated with improved viral control.[25] These studies indicate that antigen exposure may be important not only for regulating the

numbers of HIV-specific CD8+ T cells but also affect their functionality.

## Apoptotic Defect of HIV-Specific CD8+ T Cells

### Apoptosis affects both CD4+ and CD8+ T cells in HIV infection

HIV infection results in progressive loss of both CD4+ and CD8+ T cells that has been associated with disease progression.[26–28] Thus, apoptosis of T cells has been widely considered as a strategy used by HIV to evade the immune system.[29] Early *in vitro* studies made apparent that both CD4+ and CD8+ T cells from HIV-infected individuals are sensitive to spontaneous apoptosis, activation-induced cell death (AICD) and CD95/Fas-mediated apoptosis.[27,30–33] *In vivo*, this cell death occurs primarily in noninfected bystander cells and rarely in HIV-infected cells.[34,35] Furthermore, CD4+ and CD8+ T-cell apoptosis was detected in tonsillar tissues,[36] while a generalized apoptotic phenotype including also B cells and dendritic cells was observed in lymph nodes of HIV patients.[37]

Loss of CD4+ T cells is the hallmark of the HIV infection.[38–40] On the other hand, potent HIV-specific CD4+ T-cell response is associated with efficient control of HIV.[41] Various mechanisms have been proposed to explain CD4+ T-cell dynamics, including direct destruction by HIV virus,[42,43] impaired generation[39] and indirect mechanisms of CD4+ T-cell loss.[44–46] HIV proteins can directly activate T cells in a TCR-independent manner,[47] while cytokines released by antigen-presenting cells (APCs) or other activated T cells can indirectly activate T cells.[48,49] This activation can then contribute to the elimination of CD4+ T cells by AICD.[50,51] CD95/Fas-induced apoptosis may also play an important role in CD4+ T-cell depletion.[26,27,52] Thus, apoptosis may be an important contributor if not the major one for the loss of CD4+ T cells.

Several clinical studies have established a positive correlation between the magnitude of apoptosis and disease progression.[53,54] On the other hand, LTNPs are characterized by low frequency of apoptotic CD4+ and CD8+ T cells[28,55] and lesser mitochondrial membrane potential disruption.[28] In patients with positive response to antiviral therapy, it was found that inhibition of CD8+ and not CD4+ T-cell apoptosis resulted in CD4+ T-cell rescue.[56] Rapid decreases of T-cell apoptosis after treatment with HIV protease inhibitors has been reported by several investigators.[57,58] Additional evidence supporting the critical role of apoptosis in AIDS progression emerges from studies in SIV-infected macaques where lymphocyte apoptosis was found to be associated with the pathogenicity of lentiviral infection.[31,32] Furthermore, an extensive apoptosis in peripheral lymphoid organs during primary SIV infection was previously found to be predictive of the ability of the immune system to control viral replication and disease progression.[59] In summary, elevated T-cell apoptosis (reviewed elsewhere in this issue) is well established in HIV infection, correlates with disease progression and response to therapy, suggesting a potential important role in the pathogenesis of HIV disease.

### HIV-specific CD8+ T cells are highly sensitive to apoptosis

Cytotoxic CD8+ T cells function as serial killers that can eliminate multiple infected cells. Most of the known apoptotic pathways have been proposed as a mechanism of general CD8+ T-cell loss in HIV infection. Increased *in vitro* spontaneous, AICD and CD95/Fas-induced apoptosis have been reported in early studies.[26,27,30,31] The involvement of TNFR1 and TNFR2 in apoptosis of CD8+ T cells from HIV-infected individuals was shown recently.[60] Furthermore, TNF-related apoptosis-inducing ligand (TRAIL) can mediate in part AICD of CD4+ and CD8+ T cells.[33] It is apparent that multiple mechanisms that can potentially crosstalk are involved in general CD8+ T-cell apoptosis in HIV infection. Clarification of the contribution of each of these mechanisms in different stages of disease progression would significantly add to our knowledge of HIV pathogenesis. Despite, however, our knowledge of CD8+ T-cell apoptosis in HIV infection for more than 10 years,[26,27,30,31] if and how this apoptosis may affect HIV-specific CD8+ T cells was unknown till recently.[61]

We recently demonstrated that HIV-specific CD8+ T cells are highly susceptible to CD95/Fas-mediated apoptosis.[61] CMV-specific CD8+ T cells from the same patients were found to be less susceptible to this apoptosis, indicating that this sensitivity was not a general feature characterizing virus-specific CD8+ T cells in HIV infection.[61] In fact, we found that HIV-specific CD8+ T cells were three-fold more prone to CD95/Fas-induced apoptosis compared to CMV-specific CD8+ T cells, while both virus-specific CD8+ T-cell populations were found to be similarly susceptible to spontaneous apoptosis and AICD.[61] Intriguingly, no correlation was found between CD95/Fas expression on HIV- and CMV-specific CD8+ T cells and their sensitivity to CD95/Fas-induced apoptosis as these virus-specific cells are nearly 100% CD95/Fas positive.[61] These data suggest that altered CD95/Fas intracellular signaling or defects in the intrinsic antiapoptotic machinery of HIV-specific CD8+ T cells may be responsible for their high susceptibility to apoptosis. The involvement of both caspase-dependent and caspase-independent pathways in the apoptosis of T cells from SIV-infected primates has been recently described.[62] In agreement with these studies, we have found increased sensitivity to spontaneous and CD95/Fas-induced apoptosis of SIV-specific CD8+ T cells in SIV-infected rhesus macaques (YM Mueller, C Petrovas and PD Katsikis, unpublished data). This is consistent with previous data showing that blocking of CD95/FasL system results in the regeneration of SIV-specific CD8+ T cells in infected animals, while the failure of SIV-specific CD8+ T cells to clear the virus was found to be associated with the FasL upregulation by Nef protein on infected cells.[63]

The above-described increased apoptosis sensitivity of HIV-specific CD8+ T cells may affect their ability to deal with HIV-infected cells. Indeed, HIV-specific CD8+ T cells are *in vitro* killed by HIV-infected macrophages and this killing is mediated, at least in part, by the CD95/Fas system.[61] Similarly, these cells could be killed by CD95/Fas ligand expressed on activated CD4+ T cells.[64,65] *In vitro* treatment with a noninfectious HIV particle upregulates CD95/Fas and

FasL in general CD4 + and CD8 + T cells, and this is followed by their apoptosis and such fratricide could also kill HIV-specific CD8 + T cells.[66] HIV therefore can be detrimental to HIV-specific cytotoxic CD8 + T-cell function in two manners: through inhibition of CD95/Fas-mediated apoptosis of HIV-infected cells,[67] which makes these cells resistant to killing, and the induction of HIV-specific CD8 + T-cell apoptosis upon contact with CD95L/FasL-expressing HIV-infected cells[68–70] (Figure 1). Interaction of HIV gp120 with the chemokine receptor CXCR4 on macrophages can induce apoptosis of CD8 + T cells by a TNFR-mediated mechanism,[71] providing another 'fighting back' mechanism for HIV virus if HIV-specific CD8 + T cells are sensitive to such TNFR-mediated apoptosis. A similar phenomenon has been recently described for HCMV, where the virus can induce apoptosis of virus-specific CD8 + T cells through the action of CD95/Fas and TRAIL.[72] Despite the strong *in vitro* data, however, it is important to note that the significance of such HIV-mediated counterattack processes *in vivo* remains to be determined.

A critical question to be addressed is the relation between the high apoptosis sensitivity of HIV-specific CD8 + T cells and their *in vivo* dynamics. A recent study described high *ex vivo* sensitivity of HIV-specific CD8 + T cells to apoptosis during primary infection, followed by a chronic asymptomatic phase where they form a stable pool of resting cells.[73] Interruption of HAART revealed that recurrence of viral load is accompanied by sequential patterns of HIV-specific CD8 + T-cell activation and distinct populations of these cells with different levels of TCR expression and susceptibility to apoptosis.[74] The effect of antiretroviral therapy, however, on the HIV-specific CTL response is depended on the time course of the therapy. Early start of treatment results in the preservation of HIV-specific CD8 + T cells in the blood,[75] while the opposite result was observed after delayed initiation of therapy.[23,76] This positive response to HAART is accompanied by reduction of CD8 + T-cell apoptosis and preservation of the HIV-specific CD8 + T-cell response.

The *in vivo* T-lymphocyte dynamics, however, in HIV-infected patients remain controversial. Studies in SIV-infected monkeys have shown a faster turnover of both CD4 + and CD8 + T cells in infected compared to noninfected animals.[77,78] In humans, an increased proliferation and death rate in CD4 + T cells was found, while the CD8 + T-cell compartment was characterized by elevated proliferation but only moderately increased apoptosis in HIV-infected patients compared to healthy subjects.[79] The perturbed turnover rate

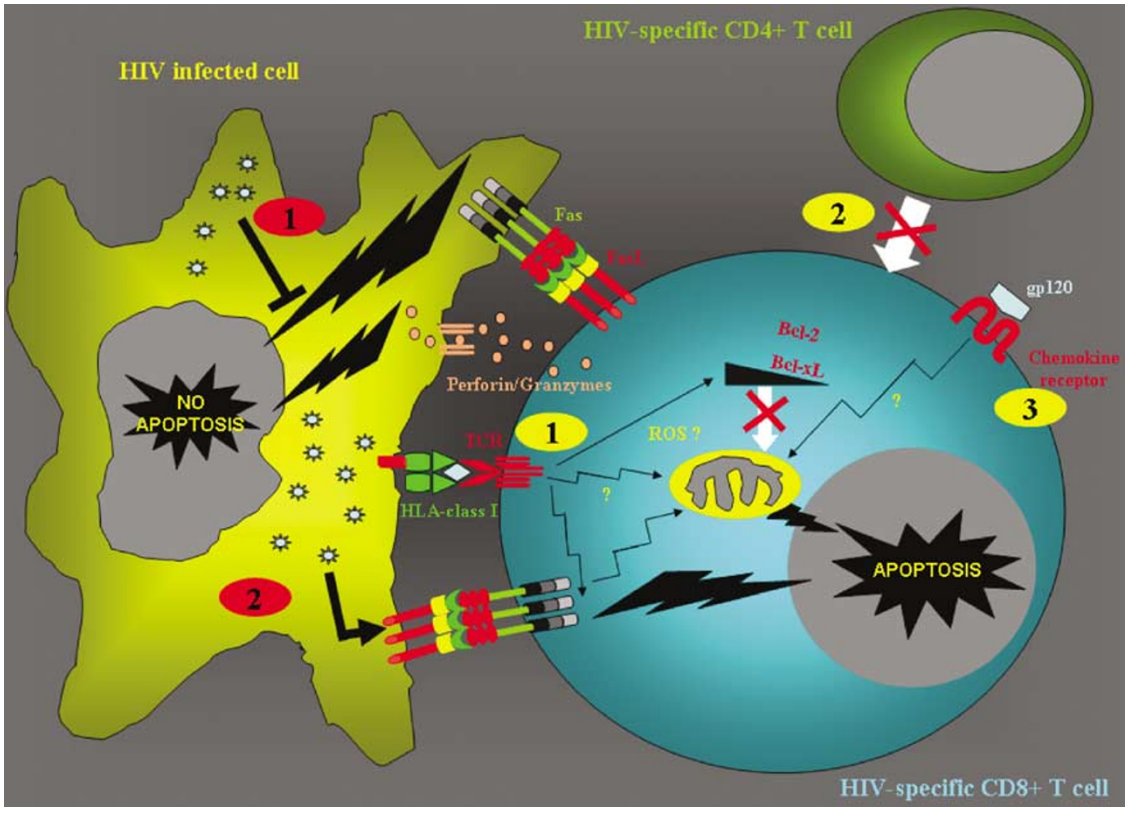


**Figure 1** HIV virus can counterattack the CTL response through apoptosis. In chronically infected HIV patients, HIV-specific CD8 + T cells are primed to undergo CD95/Fas-mediated apoptosis and exhibit reduced levels of Bcl-2 and Bcl-$x_L$. HIV virus, on the other hand, mounts a counterattack on the CTL response. This is achieved by promoting resistance to CD95/Fas-mediated apoptosis in HIV-infected cells (red circle number 1) and by upregulating CD95L/FasL on the surface of infected cells (red circle number 2), both actions that have been attributed to HIV Nef protein. Numbers in yellow circles depict possible mechanisms responsible directly or indirectly in the priming HIV-specific CTLs to apoptosis: (1) chronic antigen-specific TCR activation, (2) loss or lack of HIV-specific CD4 + T-cell help and (3) aberrant or inappropriate chemokine receptor signaling following interaction of chemokine receptors (i.e. CCR5) with HIV protein gp120. The ultimate combined effect of HIV on virus-infected cells and HIV-specific CD8 + T cells is that HIV-specific CD8 + T cells lose their ability to act as serial killers and clear or control the virus. Therefore, manipulation of apoptosis may be a critical mechanism employed by HIV to subvert the immune response

was found to be corrected after successful HAART in one study,[79] while it remained high in another.[80] By using bromodeoxyuridine (BrdU) labeling, Kovacs and colleagues[81] found distinct proliferating CD4+ and CD8+ T-cell subpopulations that are differentially affected by HAART. A shift toward the rapidly proliferating compartment as a mechanism of CD4+ and CD8+ T-cell loss in HIV infection was proposed in the same study.[81] Most if not all of the studies, however, have evaluated the fate of total CD8+ T cells and one has to be careful in extending these findings to HIV-specific CD8+ T-cell populations for which we have no direct data yet. From the above, it is clear that HIV- and SIV-specific CD8+ T cells are very susceptible to apoptosis. This increased apoptosis would obviously accelerate their turnover, but may also affect the efficiency of these cells to clear virus-infected cells by altering the differentiation or function of these cells. Although *in vivo* studies of total CD8+ T cells have not shown increased turnover of CD8+ T cells, the turnover of HIV-specific CD8+ T cells is not known. The rapid loss of this population, however, upon HAART initiation in patients[76] indirectly suggests that their turnover is increased. Finally, it should be noted that although the *in vitro* data as well *in vivo* apoptosis at the tissue level[34,37] suggest an important role for apoptosis in the biology of CD8+ T cells in HIV infection, whether or not this 'fighting back' mechanism is important for the disease progression needs to be addressed *in vivo*, where the crosstalk and the impact of particular death-inducing ligands is yet unknown.

## Priming of HIV-Specific CD8+ T Cells to Apoptosis

To date, several mechanisms have been proposed to explain the sensitivity of CD8+ T cells to apoptosis in HIV infection. Such generalized mechanisms, however, may not apply to HIV-specific CD8+ T cells, which are selectively primed to apoptosis compared to other virus-specific, that is, CMV- or EBV- CD8+ T-cell populations in HIV-infected individuals.[61] Thus, elucidation of the HIV-specific interactions that affect HIV-specific CD8+ T cells, but spare other virus-specific populations in patients, is a critical step to understanding the preferential apoptosis of these cells. Below we will discuss some of the potential mechanisms that may preferentially sensitize HIV-specific CD8+ T cells during infection (Figure 1).

### Chronic activation of the immune system

Chronic activation of the immune system has been considered as a major mechanism of T-cell sensitization to apoptosis.[40,46] This activation can be mediated by chronic antigen-specific stimulation or as a nonantigen-specific consequence of virus infection such as altered cytokine milieu. The sensitivity to apoptosis of peripheral total CD8+ and CD4+ T cells in HIV infection was found to be in correlation with their activation status and disease progression.[82,83] Additional evidence coming from a recent study in SIV-infected sooty mangabey monkeys, where no generalized immune activation or excessive apoptosis was found, implicates activation in the immunopathology of at least bystander T cells.[84] The inter-relation between chronic immune activation, cell-cycle deregulation and excessive apoptosis has been proposed to play a significant role in the pathogenesis of AIDS.[85,86] A correlation between responsiveness to HAART and correction of cell-cycle deregulation was recently reported.[87] Furthermore, cell-cycle correction was proposed as a 'prognostic marker' to identify patients that could benefit from immunotherapy in addition to standard HAART.[87] A recent study revealed that HIV-specific CD8+ T cells were skewed toward a CD38+CD8+ phenotype, which was characterized by high levels of spontaneous and CD95/Fas-mediated apoptosis,[88] while a strong correlation between the levels of HIV-specific CD8+ T cells and the percentage of total CD8+ T cells expressing CD38 was found in the same study.[88] High activation accompanied by low expression of the Bcl-2 antiapoptotic molecule during primary infection likely primes HIV-specific CD8+ T cells to apoptosis.[73] However, we have found no difference in the expression of activation markers (CD38, CD69, CD25, HLA-DR) between HIV- and CMV-specific CD8+ T cells during chronic infection, indicating that, in addition to activation levels, other factors contribute to the priming of HIV-specific CD8+ T cells to apoptosis.[61] Furthermore, HIV-specific CD8+ T cells are characterized by limited proliferative ability,[89] which supports the idea of a perturbed cell cycle in these cells. Repeated exposure of HIV-specific CD8+ T cells to antigen could lead to chronic antigen-specific activation, which results in enhanced apoptosis, reduced proliferative capacity and increased activation. This mechanism obviously could explain the preferential apoptosis of HIV-specific CD8+ T cells compared to other virus-specific CD8+ T cells in HIV-infected patients.[61] Chronic antigen-specific TCR stimulation could also result in deregulated expression of death mediators, that is, Fas/FasL[90] or alternatively lead to cell senescence through sequential divisions; disturbances that have been both described for HIV-specific CD8+ T cells.[89,91] Persistent exposure to viral antigens results in clonal exhaustion (anergy and/or deletion) of virus-specific memory CD8+ T cells in mouse models,[92,93] a process mediated by the cytolytic function of molecules like perforin, Fas and TNFR-1.[94] The level and kinetics of CTL exhaustion in infected lymphoid tissues seem to be critical parameters for the progression of the infection (acute or chronic) in the LCMV mouse model.[95] This could be of particular interest in HIV infection where high viral load is present in lymph nodes during the onset of the infection.[96] However, studies in mouse viral models have revealed a differential tissue-specific regulation of virus-specific CD8+ T cells,[95,97,98] underscoring the difficulty of a comprehensive analysis of the *in vivo* regulation of the HIV-specific CD8+ T-cell response. Overall, persistent exposure of HIV-specific CD8+ T cells to their targets could result in selective sensitization of these cells. This mechanism, however, is challenged by the lack of a correlation between HIV-specific CD8+ T cells apoptosis sensitivity and viral load.[61] Such an analysis, however, does not take into account the cumulative viral load over time, which may be more important in determining apoptosis sensitivity than the snapshot of a single bleed. Therefore, further longitudinal studies are required to test the correlation of such apoptosis

sensitivity in patients with higher viral loads to determine whether this leads to a specific increased apoptosis sensitivity of HIV-specific CD8+ T cells.

## Loss of HIV-specific CD4+ T-cell help

HIV-specific CD4+ T cells are believed to be deleted early during infection as they are preferentially targeted by HIV virus.[99] The loss of these cells early on may therefore result in a 'helpless' environment for HIV-specific CD8+ T cells, which ultimately contributes to their apoptosis sensitivity. Altered TCR signaling has been described in T-lymphocytes from HIV patients. Impaired TCR signaling can be attributed to the downregulation of the CD3ζ chain[100] in both CD4+ and CD8+ T cells from HIV-infected patients. Reduced levels of CD3ζ chain correlate with disease progression[101] and are restored following HAART therapy.[101] HIV-specific CD8+ T cells are characterized also by decreased expression of the essential costimulatory molecule CD28,[102,103] which is considered indicative of disease progression.[104] Conversely, expression of CD28 after *in vitro* transduction results in the reconstitution of IL-2 production by CD28− HIV-specific CD8+ T cells and their autocrine proliferation upon peptide recognition.[105] This finding underlines the importance of CD28 in the regulation of responding HIV-specific CD8+ T cells. Given that these cells lack the α-chain of IL-2 receptor (CD25) and cannot produce IL-2,[100,106] CD28 loss enhances their dependence on HIV-specific CD4+ T-cell help. Overall, one can assume that altered TCR signaling and costimulation could make HIV-specific CD8+ T cells more dependent on HIV-specific CD4+ T-cell help, but may also be the result of a lack of such help. Thus, loss of HIV-specific CD4+ T cells during the course of HIV infection could provide a mechanism for the selective apoptosis of HIV-specific CD8+ T cells. In support of this idea are findings from both human studies and mouse models of viral infection. Early studies in murine models revealed an impaired generation of anti-viral CD8+ T-cell response when mice were treated with a CD4 depleting monoclonal antibody.[107] Particularly, long-term control of persistent infection seems to be depended on the CD4+ T-cell help.[108,109] More recent studies described the requirement of CD4+ T-cell help for the secondary expansion of CTLs upon reencountering virus antigens.[110] The precise nature of CD4+ T-cell help in the *in vivo* generation of a CTL response is not fully understood. Indirect mechanisms such as APC activation through CD40/CD40L interaction[111] or direct action of cytokines and growth factors produced by CD4+ T cells, that is, IL-2[112] could mediate this help to activated CD8+ T cells *in vivo*. In HIV infection, progression to AIDS was found to be associated with loss of gag-specific CD8+ T cells, decline of CD4+ T cells and severe weakening of T-cell function.[113] On the contrary, control of viremia was associated with a polyclonal, vigorous HIV-specific CD4+ T-cell response accompanied by a strong CTL response.[114] As mentioned, reconstitution of CD28 expression in CD28 HIV-specific CD8+ T cells results in the restoration of autocrine production of IL-2 and antigen-specific proliferation, suggesting an increased dependence on CD4+ T-cell help in the absence of CD28 and impaired ability for IL-2 production.[105] Furthermore, diminished IL-2 could further deteriorate the CTL response through the downregulation of proliferation of HIV-specific CD4+ T cells.[115] As mentioned, increased susceptibility to infection by HIV provides a specific mechanism for selective loss of HIV-specific CD4+ T cells compared to CMV-specific CD4+ T cells,[99] resulting in selective loss of help and potentially contributing to the increased apoptosis, which characterizes HIV-specific CD8+ T cells, compared to other virus-specific CD8+ T cells.[61] Nevertheless, the precise role of HIV-specific CD4+ T-cell help in supporting the maintenance of HIV-specific CTL responses is controversial. A strong correlation between CTL precursors and HIV-specific CD4+ T helper responses has been described,[116] while the requirement of autologous CD4+ T cells for the *in vitro* expansion of HIV-specific CD8+ T cells was shown recently.[117] In contrast, similar levels of HIV-specific CD8+ T cells were found in both progressors and nonprogressors,[118,119] and no correlation was found between the frequency of HIV-specific CD4+ and CD8+ T cells in HIV patients under HAART therapy using overlapping peptide pools encompassing all products of HIV-genome.[120] Thus, more studies are needed to clarify the role of HIV-specific CD4+ T-cell help for a persistent and effective HIV-specific CD8+ T-cell response as it has been previously proposed.[121] This is further supported by recent data from murine studies emphasizing the importance of CD4+ T-cell help in clonal expansion and survival of CTL responses.[122]

## Proapoptotic activity of HIV proteins

A large body of literature has been focused on the role of HIV proteins in priming or inducing apoptosis of T-lymphocytes.[29,123] These proteins could potentially induce apoptosis of HIV-specific CD8+ T cells either through a direct or an indirect mechanism. Physical interaction between HIV proteins and chemokine receptors expressed in CD8+ T cells could be such a direct mechanism. HIV gp120 expressed on infected cells or shredded protein from viral particles and the surface of infected cells[124,125] could interact with these receptors. Particularly, the gp120 protein expressed on infected cells could preferentially interact with HIV-specific CD8+ T cells providing a selective mechanism for their apoptosis compared to other virus-specific CD8+ T cells. CXCR4 activation by X4/gp120 appears to induce a caspase-independent death of both uninfected CD4+ and CD8+ T cells and infected CD4+ cells *in vitro*.[124] Blockade of CXCR4 was able to inhibit the *in vitro* apoptosis of CD8+ T cells from HIV-infected individuals, again underscoring the role of this receptor in the elimination of CD8+ T cells.[126] As the disease progresses, T-tropic (X4) virus strains that bind CXCR4 become predominant, potentially contributing to CD8+ T-cell loss in late stages of the disease.[71,127] In contrast to CXCR4, CCR5 engagement by gp120 induced *in vitro* apoptosis only in CD4+ T cells.[124] However, the contribution of such a mechanism to the *in vivo* apoptosis of HIV-specific CD8+ T cells cannot be excluded. One would expect that HIV-specific CD8+ T cells would preferentially interact with their antigen-loaded targets, that is, HIV-infected cells, which will also express membrane gp120. Cell to cell contact between HIV-specific CTL and their targets would thus result in engagement of CXCR4 or CCR5 with gp120, and aberrant or

inappropriate chemokine receptor signaling result in apoptosis priming of these cells.

Other soluble proteins like Tat and Vpr could also contribute to apoptosis of HIV-specific CD8+ T cells. Tat can prime T cells for AICD apoptosis,[128] a process that can be inhibited by growth factors and is associated with enhanced activation of cyclin-dependent kinases.[51] Vpr, a cell-permeable protein, could also contribute to apoptosis of uninfected CD8+ T cells by dissipating the mitochondrial membrane potential.[129,130] Circulating HIV proteins, however, should equally affect all virus-specific CD8+ T cells. Consequently, this mechanism cannot explain the selective sensitivity of HIV-specific CD8+ T cells to apoptosis.

### Role of cytokine milieu in HIV-specific CD8+ T-cell apoptosis

Excessive cytokines or lack of cytokines may potentially prime for T-cell apoptosis. HIV-specific CD4+ and CD8+ T-cell responses were found to be associated with increased *in vitro* production of IL-4, but an absence of IL-2, during AIDS progression.[131] In contrast, a balanced cytokine profile was found in LTNPs.[131] On the other hand, *in vitro* treatment with IL-2 corrects the cell-cycle deregulation observed in CD8+ T cells from HIV-positive patients, indicating a role of cytokine deficiency in apoptosis of CD8+ T cells.[132] Exogenous cytokines like IL-2, IL-10, IL-12 and IL-15 can inhibit apoptosis of CD8+ T cells *in vitro*[133–136] and augment *in vitro* the immune functions of peripheral blood mononuclear cells (PBMCs) from HIV-infected patients,[137] further supporting the involvement of cytokines in the CD8+ T-cell loss in HIV infection. An altered cytokine profile toward a Th2 type (IL-4, IL-5, IL-6, IL-10) has been suggested;[138] however, this has been challenged in other studies where an intracellular staining assay was used to determine cytokine production in HIV-infected patients.[139] Generalized immune activation due to cytokine deficiency or inflammatory cytokine milieu could potentially explain the tendency of total CD8+ and CD4+ T cells from HIV-infected patients to undergo apoptosis. However, it lacks the specificity to explain the selective sensitivity of HIV-specific CD8+ T cells to apoptosis compared to other virus-specific CD8+ T cells from the same HIV-infected individuals.[61] As is the case with most generalized mechanisms, an inflammatory cytokine milieu at the microenvironment level, for example, an HIV-infected lymph node, could however preferentially be exposed to HIV-specific CD8+ T cells due to their selective trafficking to these sites.

Independent of the changes in the cytokine milieu, defective cytokine signaling intrinsic to HIV-specific CD8+ T cells may also contribute to their apoptosis sensitivity. A selective reduction in STAT5 molecules has been reported in peripheral T cells from HIV-infected individuals and *in vitro* after HIV infection,[140] potentially contributing to the loss of T-cell function in HIV disease. Interestingly, IL-2 treatment was not able to activate the STAT5a and STAT5b molecules in CD8+ T cells from untreated HIV-positive patients, while HAART could restore Jak/STAT signaling in such patients.[141] Furthermore, the IL-7Ralpha chain (CD127) is downmodulated in CD8+ T cells from HIV-infected patients,[142] while IL-2 or antiretroviral treatment of patients resulted in the restoration of this expression.[142,143] These observations were restricted to general CD8+ T cells and therefore studies are needed to elucidate the cytokine signaling status in HIV-specific CD8+ T cells. However, such defects in cytokine-induced signaling could further deteriorate the function and survival of HIV-specific CD8+ T cells, especially in an environment where production of cytokines promoting CTL responses is altered.[138]

### Cells mediating HIV-specific CD8+ T-cell apoptosis

Given the increased apoptotic potential of HIV-specific CD8+ T cells, it is important to understand which cells express FasL and are potentially mediating this apoptosis *in vivo*. Cells directly infected with HIV virus (direct mechanism) or cells indirectly affected by virus proteins (indirect mechanism) can express FasL, and therefore may contribute to this apoptosis. HIV infection of CD4+ T cells and macrophages has been shown to result in FasL upregulation.[63,69] The interaction of HIV-specific CD8+ T cells in the lymph nodes of patients with such HIV-infected cells would obviously trigger the CD95/Fas apoptotic pathway in these CTL, resulting in their elimination. An indirect mechanism could also be mediated through the action of HIV proteins on immune cells other than CD8+ T cells. Early studies have demonstrated the upregulation of CD95/FasL on CD4+ T cells induced by Tat[144] or Nef proteins.[70] CD4 crosslinking on CD4+ T cells was found to induce CD8+ T-cell apoptosis through Fas/FasL interaction.[65] A physical interaction between CD4+ and CD8+ T cells was required for this apoptosis.[65] Such CD4 crosslinking could obviously be mediated by gp120 in HIV infection. Furthermore, Nef induces apoptosis of CD8+ T cells indirectly by upregulating the expression of CD95/FasL and TNF-alpha on dendritic cells.[145] The contribution of such indirect mechanisms, however, in the selective apoptosis of HIV-specific CD8+ T cells remains to be determined. Since HIV-CTL are expected to home to sites where HIV-infected cells reside, one could envision that HIV proteins released in the microenvironment could result in FasL expression by uninfected bystanders. Therefore, it is possible that both direct and indirect mechanisms of induction of FasL expression may be operative in infected lymph nodes.

## Toward Understanding the Molecular Mechanism of HIV-Specific CD8+ T-Cell Apoptosis Sensitivity

What is the molecular basis underlying the sensitivity of HIV-specific CD8+ T-cell to apoptosis is currently unknown. This question is critical to our understanding the apoptotic defect of HIV-specific CD8+ T cells, since the molecular changes in these cells that predispose or control proapoptotic signaling may point to the mechanisms responsible for priming, and also indicate potential therapeutics. A large body of studies has focused on the molecular mechanisms mediating apoptosis of infected as well as uninfected CD4+ T cells

revealing the importance of HIV-specific proteins in this process.[123,146] In the case of CD8+ T cells, however, little information is available and one should be careful in extrapolating conclusions from studies concerning total CD8+ T-cell apoptosis to the mechanisms that control HIV-specific CD8+ T-cell apoptosis.

Cell apoptosis is mediated by two major mechanisms: signaling through surface death receptors, that is, Fas and TNFR (extrinsic pathway) and signaling that involves members of the Bcl-2 family and the mitochondrial function (intrinsic pathway).[147,148] In both mechanisms, caspases are central mediators of initiation (i.e. caspase-8) and execution (i.e. caspase-3) of apoptosis.[149] Cells have been classified to type I and II according to the mechanism leading to caspase-3 activation; directly activated by caspase-8 in type I cells and indirectly by caspase-9 and proapoptotic factors released from mitochondria in type II cells.[150] Since most of the studies have been carried out in cell lines, the impact of these intracellular pathways in primary human T-cell apoptosis is not fully understood. It should be noted that the type I/type II classification may not be that clear cut or mutually exclusive in primary cells. Technical reasons such as the difficulty for efficient transfection of primary T cells are responsible for the limited knowledge we have for the apoptotic mechanisms in human primary lymphocytes. In the case of HIV-specific CD8+ T cells, the small size of the cell population that can be isolated from blood and lymph nodes limits even more the assays one can perform.

We took advantage of a flow cytometry assay to investigate the protein expression levels of antiapoptotic or proapoptotic factors in HIV-specific CD8+ T cells. By combining HLA-class I peptide-loaded tetramers and intracellular stains, we were able to measure the levels of Bcl-2 and Bcl-$x_L$ molecules in virus-specific and total CD8+ T cells from HIV-infected individuals. A remarkably reduced expression of Bcl-2 in HIV-specific CD8+ T cells compared to CMV-specific and total CD8+ T cells from HIV-infected individuals were found.[151] Furthermore, the downregulation of Bcl-2 was accompanied by a failure of HIV-specific CD8+ T cells to upregulate Bcl-$x_L$ to the same extent as CMV-specific CD8+ T cells. A vital issue in the apoptosis field is the relative ratio between pro- and antiapoptotic factors that critically affect a cell's survival.[151] Similarly, we found downregulated levels of Bcl-2 in SIV-specific CD8+ T cells from SIV-infected rhesus monkeys, which also exhibit increased apoptosis (YM Mueller, C Petrovas and PD Katsikis, unpublished data).

A reciprocal feedback regulatory mechanism is likely present between Bcl-2 and Bcl-$x_L$,[152] while distinct roles in T-cell survival have been proposed for each molecule, with Bcl-2 mediating the survival of resting T cells and Bcl-$x_L$ being important for activated cells.[153] Our data revealed that such a compensatory mechanism is impaired in HIV-specific CD8+ T cells,[151] potentially priming them to apoptosis. On the other hand, CMV-specific CD8+ T cells from HIV-infected patients are characterized by increased levels of Bcl-$x_L$,[151] likely making them less sensitive to apoptosis, in agreement with our previous data.[61] Interestingly, total CD4+ T cells from HIV-infected patients express comparable Bcl-2 levels with CD4+ T cells from healthy individuals.[151] Therefore, it is possible that Bcl-2 is regulated in a different manner in CD4+ T cells or alternatively Bcl-2 low CD4+ T cells primed to apoptosis are rapidly removed from the circulation and cannot be detected *ex vivo*. Since both CD4+ and CD8+ T cells are sensitive to CD95/Fas-induced apoptosis in HIV infection,[26,27] an interesting question is whether these two cell populations employ different intracellular pathways to execute the death process.

The involvement of Bcl-2-like molecules in apoptosis of total CD8+ T cells from HIV-infected individuals has been proposed by previous studies.[83,154] Additionally, *in vitro*-activated PBMCs from asymptomatic HIV-infected individuals failed to upregulate the levels of Bcl-$x_L$.[155] Various reasons like growth factor/cytokine deficiency and chronic activation could be responsible for the observed dramatic reduction of Bcl-2 levels in HIV-specific CD8+ T cells. Reactive oxygen species (ROS) production, which mediates apoptosis in various cell types, appears to be a critical regulator of Bcl-2 levels in activated T cells.[156] This is an attractive hypothesis, since elevated ROS production has been described in T cells from HIV-infected patients.[157] Alternatively, the absence of HIV-specific CD4+ T-cell help,[114] together with impaired costimulatory signals, that is, CD28,[106] could be responsible for the inability of HIV-specific CD8+ T cells to upregulate the expression of Bcl-$x_L$.

Differentiation level, activation status and apoptosis sensitivity are closely related in HIV infection.[83,158] Increased apoptosis in CD38+ HIV-specific CD8+ T cells was recently reported.[88] In agreement with this, we have found a significant reduction of Bcl-2 in activated CD38+ HIV-specific and total CD8+ T cells from HIV-infected patients (C Petrovas and PD Katsikis, unpublished data). Furthermore, increased levels of apoptosis were found to be associated with deregulated expression of cell-cycle proteins in CD4+ and CD8+ T cells from HIV-infected individuals.[85,132] Bcl-2 is a molecule that mediates both apoptosis and cell-cycle regulation,[159,160] something described also for other Bcl-2 family molecules.[161] We hypothesize that a potential deregulation of Bcl-2-like molecules in HIV-specific CD8+ T cells could be the linker between a cell-cycle deregulation, activation and apoptosis sensitivity.

A critical question regarding the apoptotic process is whether the commitment to death takes place upstream or downstream of mitochondria, the main organelle where Bcl-2 family molecules exert their antiapoptotic function.[149] Therefore, several studies have investigated the mitochondrial dysfunction in HIV pathogenesis.[157,162,163] Most of them have been focused on the investigation of mitochondrial membrane potential ($\Delta\Psi_m$) in treated lymphocytes from HIV-infected individuals.[91,157,163] Importantly, LTNPs have low numbers of cells with reduced ($\Delta\Psi_m$) compared to patients that developed AIDS, which correlates with lower sensitivity to spontaneous apoptosis and higher frequencies of CD4+ T cells.[28] HIV-encoded proteins induce permeabilization of the mitochondrial membrane and sensitize infected T cells from HIV patients to apoptosis.[130,164] Alternatively, cell-permeable HIV proteins like Vpr could potentially be involved in killing of both infected and uninfected cells. The role of mitochondria, however, in early steps of apoptosis, especially the CD95/Fas-induced one, of HIV-specific and total CD8+ T cells from HIV-infected individuals is unexplored. The crosstalk between

mitochondria and CD95/Fas signaling has been shown in certain experimental systems.[165–167] Upon CD95/Fas cross-linking, the ganglioside GD3, a ceramide metabolite, targets mitochondria in a Bcl-2-controlled manner.[168] The involvement of ceramide in the apoptosis of HIV-specific CD8+ T cells from HIV-infected individuals is an attractive hypothesis since elevated ceramide levels have been found in HIV-infected patients.[169,170] As a consequence, one could hypothesize that a deregulated expression of antiapoptotic factors along with an impaired mitochondrial function could condemn HIV-specific CD8+ T cells to death.

Unfortunately, little if any is known on the role of other intracellular mediators acting upstream to mitochondria in CD8+ T-cell apoptosis from HIV-infected individuals. A previous study failed to show different expression of c-FLIP in purified CD4+ and CD8+ T cells from HIV-infected patients.[57] The expression of this molecule, however, in HIV-specific CD8+ T-cell populations is not known. Similarly, the role of other factors regulating the CD95/Fas-induced apoptosis like FADD or Bid that has been described as the link between the Fas signaling and mitochondria[171] is also not known. Further studies are obviously needed in order to elucidate the molecular mechanisms that interplay to result in apoptosis sensitivity of HIV-specific CD8+ T cells.

## Apoptosis and Altered Phenotype/ Function of HIV-Specific CD8+ T Cells

As mentioned above, HIV-specific CTL response is a critical effector arm of the immune system against HIV. Despite the presence of such a response even in late stages of the disease, HIV-specific CD8+ T cells ultimately fail to clear the virus and prevent disease progression. Various strategies employed by HIV could be responsible for this failure. The virus can manipulate the infected cell to avoid the adaptive immune response as is the case with the increased resistance of HIV-infected cells to CD8+ T-cell cytolytic function, a resistance mediated by HIV proteins like Nef[67,172] or the emergence of viral escape mutants[173] (reviewed elsewhere in this issue). On the other hand, the virus can manipulate the adaptive immune response itself by inducing intrinsic defects of HIV-specific CD8+ T cells that can potentially affect their function as serial killers and their ability to eliminate infected cells. These intrinsic defects involve perturbed differentiation, reduced effector function or increased apoptosis. A hypothetical model is that this increased apoptosis could potentially be a major defect that affects, at least in part, both differentiation and effector function and explain the defects observed in these cells.

### Apoptosis and skewed HIV-specific CD8+ T-cell differentiation

Human naive T cells express CD45RA and the lymph node homing receptors CD62L and CCR7. After exposure to antigen, a downregulation of CD62L and CCR7 is accompanied by an upregulation of integrins that allows CD8+ T cells to migrate from the lymph nodes to peripheral tissues.[174] By using surface markers together with intracellular stains for cytokine production, human memory CD8+ T cells can be categorized into three subpopulations: a central memory ($T_{CM}$), which is $CD45RA^-$ $CCR7^+$ $CD62L^+$ $CD28^+$ IL-$2^+$ IFN$\gamma^-$,[175–177] and two effector memory populations, the $CD45RA^-$ $CCR7^-$ $CD62L^-$ and the terminally differentiated $CD45RA^+$ $CCR7^-$ $CD62L^-$ that express perforin and can secrete IFN$\gamma$ and TNF-$\alpha$. These effector memory CD8+ T cells exert rapidly their cytolytic function upon antigen stimulation.[175–178] A linear model has been suggested: $CD45RA^-$ $CCR7^+$ $CD62L^+ \rightarrow CD45RA^-$ $CCR7^-$ $CD62L^- \rightarrow$ $CD45RA^+$ $CCR7^-$ $CD62L^-$.[176] However, the relationship between the memory populations is still not fully understood. Studies in murine models of viral infection have shown that the CD8+ T-cell differentiation program can be altered by the stimulating conditions where the quality of activation, that is, strength of TCR engagement as well as the cytokine microenvironment can lead to different functional outcomes.[179,180]

Recent findings suggest that peripheral blood HIV-specific CD8+ T cells lack terminal differentiation. In contrast to CMV- and EBV-specific CD8+ T cells, the majority of HIV-specific CD8+ T cells have the preterminally differentiated $CD45RA^-$ $CD62L^-$ phenotype.[61,181,182] This phenotype is also seen when different combinations of surface markers (CD45RA with CCR7 and/or CD62L) are used.[181,182] Similarly, SIV-specific CD8+ T cells also express this skewed maturation status both in peripheral blood and lymph nodes of SIV-infected monkeys.[183,184] The reasons for the altered differentiation program of HIV-specific CD8+ T cells are currently unknown. A block in differentiation has been proposed.[181] We recently demonstrated that the accumulation of HIV-specific CD8+ T cells in the $CD45RA^-$ $CD62L^-$ compartment is accompanied by an increased sensitivity to spontaneous and CD95/Fas-induced apoptosis of these cells.[61] If one assumes that transition from preterminally to terminally differentiated HIV-specific CD8+ T cells requires antigen recognition and therefore interaction with a FasL expressing infected cell, apoptosis may be affecting differentiation. We hypothesize that $CD45RA^-CD62L^-$ HIV-specific CD8+ T cells undergo apoptosis instead of differentiating into $CD45RA^+$ $CD62L^-$ terminally differentiated effector cells upon interaction with HIV-infected target cells. Thus, we hypothesized that apoptosis may be a critical mechanism leading to defective maturation and subsequently to impaired function or migration of HIV-specific CD8+ T cells. Since most of the studies have been carried out *in vitro* with circulating HIV-specific CD8+ T cells, the *in vivo* validation of this hypothesis remains to be provided. We should stress here, however, that the true meaning of this skewed differentiation of HIV-specific CD8+ T cells and how it relates to function or migration is completely unknown.

### Effector function of HIV-specific CD8+ T cells

Upon recognition of MHC-I/peptide complex on infected cells, CD8+ T-lymphocytes exert their effector functions including cytotoxicity and cytokine production.[185] Cytotoxicity is mediated by the release of cytolytic granules containing perforin and granzymes or through surface death receptors, that is, CD95/Fas and TRAIL.[186] On the other hand, synthesis

of cytokines (IL-2, IFN$\gamma$, TNF$\alpha$) and chemokines (MIP-1$\alpha$,$\beta$) takes place within a few hours after CD8+ T-cell activation.[187] Previous data support the notion that antigen concentration is a major factor governing the nature of antiviral CTL response, with IFN$\gamma$ secretion requiring a stronger signal compared to cytotoxicity.[188] In agreement with these findings, primary human HIV- and CMV-specific CD8+ T cells were found to exhibit both granule exocytosis and produce IFN$\gamma$, depending on the antigenic peptide concentration.[189]

However, the cytotoxic activity of HIV-specific CD8+ T cells is still debatable. Plethora of studies has shown that PBMCs from HIV-infected individuals possess cytotoxic function,[8,11,190,191] which is correlated with the frequency of tetramer-positive cells.[11] More recently, the preferential ability for cytotoxicity of HIV-specific CD8+ T cells secreting both IFN$\gamma$ and TNF$\alpha$ was shown.[192] We have previously shown that HIV- and CMV-specific CD8+ T cells exert similar cytotoxicity.[61] On the other hand, others have described an impaired cytotoxicity for HIV-specific CD8+ T cells.[193,194] Various reasons such as antigen concentration, incubation times, target cell lines used as well as the disease stage of the patients tested[195] could be responsible for this discrepancy. Since HIV-specific CD8+ T cells are highly sensitive to CD95/Fas-induced apoptosis, it is important to note that the use of EBV-infected cell lines overexpressing FasL,[196] as targets in cytotoxicity assays, may result in the underestimation of their cytotoxic function. Furthermore, HAART was found to impair selectively the granule-dependent CTL function in a recent study,[197] adding to the complexicity of estimating this function as potent treatment may be affecting cytotoxicity.

The ability of HIV-specific CD8+ T cells to produce IFN$\gamma$ is also controversial. IFN$\gamma$ production by HIV- and SIV-specific CD8+ T cells from the rectal mucosa was recently described[198,199] in agreement with a plethora of previous reports[181,194,200,201] including our own studies.[61] However, IFN$\gamma$ production may be impaired in late-stage patients.[202–204] The upregulation of iNKR immunoglobulin-like transcript-2 (ILT2/CD85j), an inhibitor of IFN$\gamma$ production, could contribute to low cytokine production in chronically infected late-stage patients.[205] Concerning the production of other anti-viral cytokines (MIP-1, RANTES) by HIV-specific CD8+ T cells, no defect has been found.[194,206]

The effector function and differentiation stage of CD8+ T cells are closely related. As mentioned above, $T_{CM}$ cells produce IL-2, while effector memory populations produce IFN$\gamma$ and TNF$\alpha$, with the terminally differentiated memory CD8+ T cells expressing the highest levels of perforin.[175,176] Thus, the skewed maturation of HIV-specific CD8+ T cells could be responsible for lower perforin expression and cytotoxic activity as it has been described previously.[181,194,207] Fully differentiated HIV-specific CD8+ T cells expressing high levels of perforin were recently detected in LTNPs and structured treatment interruption patients, who can control viral replication.[25] Thus, it is critical to discover the mechanisms governing the maturation of HIV-specific CD8+ T cells in order to understand the dynamics of these cells, and also to evaluate the efficacy of antiretroviral treatments and vaccine protocols. Although not directly proven, one can hypothesize that increased apoptosis could be, at least in part, the cause of the skewed differentiation of these cells, leading potentially to perturbation of their dynamics and affecting their function.

## Apoptosis and the Detection of HIV-Specific CD8+ T Cells

Apoptosis of HIV-specific CD8+ T cells will directly affect their measurement in a number of assays. Peptide-loaded HLA-class I tetramers has allowed the direct visualization and quantitative analysis of HIV-specific CD8+ T cells by flow cytometry.[13] Most importantly, it has been instrumental in our understanding the functional defects associated with HIV-specific CD8+ T cells. Before the tetramer era, the frequency of the HIV-specific CD8+ T cells was determined by limiting dilution assay (LDA). Using this technique, a frequency of HIV-specific CD8+ T cell ranging between 0.0001 and 0.003 (1/10 000–1/3000) was reported.[208–210] LDA is a very laborious and variable assay detecting only a subset of memory effector cells that can survive, proliferate and retain their cytotoxicity after prolonged stimulation *in vitro*. Proliferative or apoptotic defects of HIV-specific CD8+ T cells will obviously directly influence the measurements of the LDA assay, leading to an underestimation of these cells.

Although tetramer stains have been invaluable in detecting antigen-specific CD8+ T cells, it is important to remember that not all tetramer-positive CD8+ T cells exert measurable effector function.[211,212] Therefore, the combination of tetramer staining with other functional assays such as cytotoxicity ($^{51}$Cr) and cytokine production assays (ELISPOT) is necessary in order to have a comprehensive assessment of HIV-specific CD8+ T cells. Finally, the combination of tetramers with reagents that detect phosphatidylserine surface exposure (annexin V), plasma membrane integrity (EtB, propidium iodide, 7AAD), mitochondrial membrane potential (i.e. $DiOC_3$) and caspase activity (FAM-DEVD-fmk, FAM-IEDT-fmk) have allowed for the direct estimation of the apoptotic potential of HIV-specific CD8+ T cells and uncovered defects not previously recognized in these important anti-viral cells. Tetramer stains in combination with intracellular stains of anti- or proapoptotic molecules, that is, Bcl-2, Bcl-$x_L$, Bax, etc. or phosphorylated kinases will potentially permit us to understand the molecular pathways that control the susceptibility of these cells to apoptosis.

Apoptosis of HIV-specific CD8+ T cells may also affect their measurement in cytotoxicity assays and peptide-stimulated cytokine production assays, that is, ELISPOT and intracytoplasmic cytokine stains. Most studies to date have used frozen samples to measure or evaluate the HIV-specific CD8+ T-cell response in the blood of patients; however, the use of such frozen samples may result in a preferential loss of HIV-specific CD8+ T cells or subpopulations of these proapoptotic cells thus introducing a bias in these studies. Therefore, when assessing the numbers or function of HIV-specific CD8+ T cells, one needs to be careful to take into account the propensity of these cells to die and how this death may confound the findings.

## Conclusions

From what has been so far discussed, one can hypothesize that apoptosis sensitivity could critically influence HIV-specific CD8+ T-cell biology at multiple levels. Under normal conditions, CTL cells deliver death signals to virus-infected cells, mainly through perforin–granzyme and CD95/Fas–FasL mechanisms. In HIV infection, the opposite phenomenon seems to take place: FasL is expressed on the surface of HIV-infected cells and engages CD95/Fas on HIV-specific CD8+ T cells, which in turn are induced to undergo CD95/Fas-mediated apoptosis. Thus, HIV virus becomes the hunter rather than the hunted. This high apoptosis sensitivity likely contributes to the skewed maturation of HIV-specific CD8+ T cells by abrogating the generation or increasing turnover of terminally differentiated HIV-specific CD8+ T cells, something that may affect the effectiveness or migration of these cells. Most importantly, the ability of HIV-specific CD8+ T cells to function as serial killers will be seriously compromised due to their propensity to die. This increased apoptosis of HIV-specific CD8+ T cells should be taken into account when measuring these cells or their cytotoxic capacity as both may be affected by increased death. Unfortunately, most of the studies to date have been carried out in circulating HIV-specific CD8+ T cells.[61,88,151] As mentioned above, detection of virus-specific CD8+ T cells at the tissue level and investigation of their apoptosis sensitivity would add significantly to our understanding of their *in vivo* dynamics and the relationship with other components of the immune system. The study of SIV-specific CD8+ T cells in rhesus macaques, which share the apoptotic defect of HIV-specific CD8+ T cells will allow for the study of such *in vivo* dynamics.

An important question for investigation regards the evolution of the HIV-specific CD8+ T-cell apoptosis sensitivity during the progression of HIV infection. From this point of view, the study of a possible interrelationship between the apoptosis sensitivity of HIV-specific CD8+ T cells and the appearance of viral escape mutants is of particular significance. Emergence of viral mutants has been considered as a major CTL evasion mechanism and a central determinant of disease progression.[213,214] Recent data have shown that impaired CTL responses generated in the absence of CD4+ T-cell help result in the appearance of viral escape mutants.[215] SIV-infected non-human primates will prove an invaluable tool for the investigation of the possible coevolution of HIV-specific CD8+ T-cell apoptosis and the development of CTL-escape mutants.

Finally, understanding the biology of HIV-specific CD8+ T cells in HIV infection is a prerequisite for the evaluation of the current therapeutic interventions and the development of new antiviral strategies. Most, if any, of the current vaccines target the development of an effective long-lasting virus-specific CD8+ T-cell response.[4] The possibility, however, that the vaccine-induced CD8+ T cells will also be sensitive to apoptosis cannot be excluded. This possibility is even higher in the case of therapeutic vaccines where HIV infection has established already an environment that favors the apoptosis of CTLs. With that said, development of immunotherapies that enhance the survival of the HIV-specific CD8+ T cells would be a critical step in the fight against HIV. We have recently demonstrated the ability of IL-15 to enhance the effector function and increase the *in vitro* survival of HIV-specific and total CD8+ T cells from HIV-infected individuals,[52,136] making it a potential candidate for immunotherapy. Therapeutic interventions targeting the survival of HIV-specific CD8+ T cells in combination with vaccination or antiretroviral therapy could result in effective long-lasting immunoprotective CTL responses, something of a holy grail in our efforts to reestablish the balance in favor of antiviral adaptive immune responses in HIV infection.

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