Antigen stimulation of resting mature lymphocytes in the peripheral lymphoid organs can induce expansion of the reactive lymphocyte pool, or anergy in the absence of co-stimulatory signals. In previously activated lymphocytes, triggering of the antigen receptor may lead to proliferation and memory or to death by apoptosis. Apoptosis in the peripheral lymphoid organs may serve several functions. It may be a second safeguard to assure self-tolerance and control auto-reactive cells that arise in the periphery from cells that have escaped tolerization in the central lymphoid organs. In addition, apoptosis may prevent uncontrolled expansion of specific, antigen-reactive lymphocytes and may be one mechanism of immunosuppression. For example, in viral infections such as those induced by Epstein-Barr virus and Varicella Zoster virus, apoptosis is thought to contribute to the rapid clearance of activated T-cell blasts in vivo and thus act as a homeostatic mechanism ensuring the clearance of primed lymphocytes that are no longer required [1]. Nevertheless, as detailed below, this normal process of cellular elimination might be detrimental for the immune system in the case of a chronic infection such as that induced by human immunodeficiency virus (HIV).

### Apoptosis in AIDS. Relation with lymphocyte activation

Primary HIV infection is initiated with a stage of virus dissemination in lymphoid organs followed by a systemic dissemination of HIV to the other lymphoid tissue, as evidenced by a peak in viremia within 3 weeks of infection. In spite of powerful cell-mediated and humoral HIV-specific responses, which contribute to the down-regulation of viremia, a complete clearance of HIV is never observed. Failure of the immune response to terminate HIV infection is probably related to the ability of HIV to escape the immune response, partly because latently HIV-infected cells cannot be eliminated by HIV-specific cytotoxic lymphocytes. A large reservoir of latently infected cells is found in peripheral blood cells as well as in lymph-node cells. Furthermore a dichotomy is found in viral burden and viral replication between peripheral blood and lymph nodes because of a sequestration of infected cells and trapping of extracellular virions in the follicular dendritic cell network. Thus infection with HIV is extensive, even during the asymptomatic phase, and much of its activity is within secondary organs [2].

A general state of immune activation is rapidly observed in the asymptomatic phase of HIV infection. This is reflected by follicular hyperplasia and extension of the follicular dendritic cell network in lymph nodes. Moreover, activation markers such as HLA-DR, CD38, CD57 and CD45R0 are found on patients' lymphocytes [2]. This cellular activation is required for productive infection, and HIV fully subverts the activation machinery for its own devices. However, the persistent and unbalanced activation of patients' lymphocytes leads to immune dysfunction, including anergy to T-cell-receptor-mediated stimulation and apoptosis.

Indeed, T-cells from HIV-infected persons are highly prone to in vitro spontaneous and activation-induced apoptosis [3]. Although not detected in freshly isolated peripheral blood lymphocytes from patients [4], apoptosis can be induced following a short-term culture in both CD4 and CD8 T-cells [4-8] and is increased after in vitro activation by mitogens, super-antigens or following triggering of the T-cell receptor [6,7,9]. Importantly, recent studies demonstrated that apoptosis occurs in vivo in lymph nodes of HIV-infected persons [10-12], and it was detected not only in CD4 T-cells but also in B-cells and CD8 T-cells [10,11]. In addition, concomitant analysis of apoptosis and viral RNA in lymph-node tissues of HIV-infected children and simian immunodeficiency virus (SIV)-infected macaques revealed that apoptosis occurred predominantly in bystander cells rather than in productively infected CD4 T-cells [12].

Recent studies in lymph nodes suggested that apoptosis detected in B- and T-cell subsets was related to and caused by the continuous stimulation of the immune system, observed...
throughout HIV infection [10,11]. By using quantitative flow-cytometric methods, we characterized apoptosis in peripheral blood lymphocytes of a large cohort of HIV-infected persons at various stages of the disease and compared it with that of control individuals. Apoptosis was readily detected in lymphocytes from both HIV-negative and HIV-positive persons following a short-term culture. In both groups of donors, the same cell subsets were involved in the process of apoptosis, i.e. CD4 T-cells, CD8 T-cells, B-cells and natural killer cells, but the degree of apoptosis was significantly higher in CD4, CD8 and B-cells from HIV-infected persons as compared with their counterparts in healthy donors. Phenotypic characteristics of apoptotic cells indicated that the majority of them were in an activated state, although naive and non-activated cells were also involved in this cell-death process. Moreover the intensity of activation-induced apoptosis in T-cells was found to correlate with the degree of immune activation in the patients, evaluated by in vitro expression of T-cell-activation markers. Finally, the extent of spontaneous and activation-induced apoptosis in CD4 and CD8 T-cell subsets clearly correlated with disease progression [4]. Altogether, these studies support the hypothesis that the chronic activation of the immune system occurring throughout HIV infection is the primary mechanism responsible for the increased susceptibility to apoptosis of peripheral T-cells in lymph nodes [11] and blood [4,13] from HIV-infected persons.

**Genetic control of apoptosis in AIDS. Regulation by Bcl-2 and Fas molecules**

**In vivo down-regulation of Bcl-2 and up-regulation of Fas in patients' CD8 T-cells**

In mammals, the proto-oncogene bcl-2 has been found to function as a repressor of apoptosis in multiple cell types. It encodes a 26 kDa protein localized in mitochondria, endoplasmic reticulum and perinuclear membranes, and it inhibits programmed cell death by enhancing cell survival rather than by accelerating the rate of cellular proliferation [14]. bcl-2 and its product play a key role in the control of cell death of T- and B-cell lineages during lymphoid development, ensuring their appropriate selection. Recent findings also suggest that regulation of bcl-2 expression in mature T-lymphocytes might be crucial for the development and persistence of a memory T-cell response following an immune activation [15].

The intracellular expression of Bcl-2 protein was compared within ex vivo CD4 and CD8 peripheral T-cells, isolated either from healthy donors or from HIV-infected individuals at various stages of the disease. While no modification of Bcl-2 expression could be detected within the CD4 T-cell subset from seropositive donors, striking variations in Bcl-2 expression were found within the CD8 T-cell subpopulation during HIV infection. In healthy individuals, most of the CD8+ T-lymphocytes showed a homogeneous expression of the Bcl-2 molecule. In contrast, in HIV-infected persons, three distinct CD8 subpopulations could be distinguished, according to the level of Bcl-2 expression. Indeed, 10–50% of CD8 T-lymphocytes showed a reduced level of Bcl-2 protein, and, in parallel, emergence of high Bcl-2-expressing cells, never detected in controls, was observed [16]. Interestingly, the in vivo down-regulation of Bcl-2 expression rendered the CD8 T-cells highly susceptible to in vitro spontaneous apoptosis [16]. These low-Bcl-2 T-cells harboured the phenotype of activated (CD45R0+, HLA-DR+ CD38+ cytotoxic (TAl+) but anergic (CD28-) T-cells, and similar observations were recently reported in situ in lymph nodes from HIV-infected persons [10]. Interestingly, the relationship between Bcl-2 and Fas appeared to be reciprocal in this T-cell subset, since the down-regulation of Bcl-2 was associated with an up-regulation of Fas, and such cells were highly sensitive to apoptosis triggered through ligation of Fas [16]. Altogether, these observations suggest that the cellular dysfunction of the CD8 subset and the further loss of CD8+ cytotoxic activity in HIV-positive subjects might be related to a down-regulation of Bcl-2 associated with an up-regulation of Fas in these lymphocytes, following a persistent immune stimulation and the gradual loss of growth factors in the course of infection.

**Influence of Fas–Fas-L interaction on apoptosis of CD4 T-cells in HIV infection**

Fas is highly expressed on peripheral blood mononuclear cells from HIV-infected children [16a] and on a fraction of T-cells from HIV-infected adult patients [4]. At the AIDS stage, around 100% of CD4 and CD8 T-cells are Fas+. Ligation of Fas by a specific antibody [17,18] or by its recently identified ligand (Fas ligand, Fas-L) [19,20] has been shown to transduce in vitro
We have compared the influence of Fas-L or a potent apoptotic signal in sensitive target cells, and it is thought that this Fas-mediated cell death might play a significant role in the development and function of the immune system [21]. We have compared the influence of Fas-L or agonist anti-Fas monoclonal antibodies on apoptosis of CD4 and CD8 T-cells from control and HIV-infected persons. It appeared that, although anti-Fas monoclonal antibodies had no effect on CD4 or CD8 T-lymphocytes from control donors, they induced apoptosis on patients’ T-cells and they appeared to be more efficient on CD4 T-cells than CD8 T-cells, particularly for patients at the AIDS stage (M. L. Gougeon, H. Lecoeur and Y. Sasaki, unpublished work). Similar observations were reported elsewhere [22].

It is thus likely that in vivo encounter of Fas+ T-cells with Fas-L would result in their apoptosis, which might occur either as a ‘fratricide’ phenomenon if they interact with neighbouring cells expressing Fas-L [23] or as an autocrine suicide through a cell autonomous Fas–Fas-L interaction [24,25], provided these Fas+ cells co-express Fas-L on their surface following their activation through the T-cell-receptor–CD3 complex [26]. Moreover, the virus itself might influence this Fas-mediated cell-death pathway, since Westendorp et al. [27] recently reported that two HIV proteins, Tat and gp120, accelerated Fas-mediated activation-induced T-cell apoptosis, through an increased expression of Fas-L.

Relevance of apoptosis for AIDS pathogenesis
An important question still unresolved concerns the relevance of apoptosis for AIDS pathogenesis, and whether activation-induced cell death is the cause of AIDS or the consequence. Increased lymphocyte apoptosis is not restricted to human HIV infection, since it is detected in several animal models of AIDS, including macaques infected with SIV [7], cats infected with feline immunodeficiency virus [28], and murine AIDS [29]. A correlation between the degree of apoptosis and disease evolution was previously reported in studies comparing apoptosis in pathogenic and non-pathogenic primate lentiviral infections [7,30,31]. Indeed, while the degree of apoptosis was high in lymphocytes from SIV-infected macaques, which develop AIDS, a very low level of apoptosis was detected in non-pathogenic SIV infection of African green monkeys [7,31], or in chimpanzees inoculated with HIV [7,30] or naturally infected with chimpanzee immunodeficiency virus [30]. Moreover, a recent analysis on the control by Bcl-2 and Fas molecules of apoptosis in peripheral blood mononuclear cells from HIV-infected chimpanzees revealed a normal expression of Bcl-2 in CD8 T-cells and insensitivity to Fas-mediated apoptosis of both CD4 and CD8 T-cells from infected chimps. In fact the low level of apoptosis in these animals is correlated with the lack of general activation of the immune system (M. L. Gougeon, H. Lecoeur and J. Heeney, unpublished work).

Several mechanisms can be evoked arguing for apoptosis as a consequence of HIV infection, such as: (i) the chronic production of viral antigens would contribute to apoptosis either directly by inducing a cell-death signal or indirectly by influencing the activation of the immune system; (ii) the suggested rapid turnover of CD4 T-cells in HIV-infected persons [32], due to an active lymphocyte regenerative process, may contribute significantly to the rate of apoptosis in patients. A recent study we performed showing a high expression of tissue transglutaminase, a pre-apoptotic marker, in CD4 T-cells but not CD8 T-cells from patients’ lymph-nodes is in agreement with such a mechanism [32a]. (iii) The impaired production of Th1 cytokines, such as interleukin-2 or interleukin-12, would contribute to the excess of cell death, since these cytokines were shown to block apoptosis in patients’ CD4 and CD8 lymphocytes [7,33,34]. In other respects, apoptosis can also significantly contribute to AIDS. Since apoptosis was shown in vivo to involve activated CD4 and CD8 T-cells [4,13] and mostly non-infected lymphocytes [12], it could be responsible for the clearance of activated but healthy T-cells and consequently of the impoverishment of memory T-cells, as suggested by our recent studies [4,16]. Furthermore, the chronic expression of Fas on patients’ lymphocytes [4,16,22], the increased susceptibility of these cells to Fas-mediated apoptosis [16,22], and the influence of some viral proteins on Fas-L expression [27] suggest an important role for Fas-based apoptosis in peripheral T-cell deletion. In conclusion, the uncontrolled and chronic immune activation occurring throughout HIV deletion is probably the primary mechanism responsible for lymphocyte apoptosis, whose deleterious effects contribute to the collapse of the immune system.
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