Activation-Associated Necrosis in Human Immunodeficiency Virus Infection

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Mitogenic stimulation of lymphocytes from persons infected with human immunodeficiency virus (HIV) resulted in massive cell death. In addition to early apoptosis, a second wave of cell death occurred 48–72 h after stimulation. At that time, the cells were enlarged, leaked content, and had plasma membrane damage—all indicative of necrosis. Furthermore, DNA fragmentation as determined by TUNEL assay was virtually absent. This activation-associated necrosis could not be prevented by interfering with CD95/CD95-ligand interactions or by blocking caspase activity and was unaffected by neutralizing antibodies to tumor necrosis factor-α or interferon-γ. Necrosis was also induced by activation of normal lymphocytes in the presence of ribavirin, which inhibits the de novo pathway of nucleotide synthesis. Lymphocytes from HIV-infected persons are defective in their ability to synthesize nucleotides via this pathway, indicating one possible mechanism for the activation-associated necrosis seen in HIV infection.

Lymphocytes from persons infected with human immunodeficiency virus (HIV) are susceptible to cell death when cultured in vitro. A number of mechanisms of cell death have been described in such patients. First, the virus itself can induce death either by direct infection or through the interaction of viral gp120 with the CD4 molecule [1]. Second, spontaneous cell death in unstimulated cultures can be partially prevented by interleukin (IL)-2, indicating it may be similar to the cell death observed during acute herpes virus infections [2, 3]. Third, stimulation through the T cell receptor (TCR) induces an activation-associated death that involves the up-regulation of CD95 (Fas/APO-1) and its ligand, CD95-L [4–6]. Cell death via the CD95/CD95-L pathway is an early event and occurs within 18 h of stimulation. We previously showed that following activation, a second wave of death 48–72 h after stimulation accounts for the majority of cell loss [7]. This late-stage death occurs in both CD4 and CD8 T lymphocyte subsets in response to stimulation with anti-CD3 or phytohemagglutinin (PHA) and can be detected in asymptomatic HIV-positive persons and in subjects with AIDS [7, 8]. Early activation events seem normal in these cells as they up-regulate CD25 and begin DNA synthesis but do not increase in size and fail to divide [8, 9].

Both HIV-induced death and spontaneous and CD95-mediated cell death occur by apoptosis. However, we have observed that many necrotic cells appear later in cultures, suggesting there may be a second mechanism leading to death of lymphocytes of HIV-1–infected persons. The morphologic appearance of cells dying by apoptosis is very different from that of cells dying by necrosis [10, 11]. In apoptosis, the main target of damage is the nuclear DNA, which is cleaved into oligonucleosomal fragments of 180–200 bp through the activation of an endogenous nuclease [12]. Nuclear degradation is accompanied by a massive loss of cell volume and distortions of cell shape that may be mediated by elevations in transglutaminase activity [13]. The plasma membrane retains its integrity but becomes extensively convoluted, acquiring a characteristic blebbed appearance, and simultaneously loses specialized surface structures such as microvilli [10]. Necrosis involves damage to the mitochondria, resulting in the loss of cell ability to regulate osmotic pressure and ultimately to cell swelling and lysis [10]. The key morphologic features of necrosis are nuclear swelling, chromatin flocculation, and the formation of ghost cells due to cell lysis.

We recently showed that T lymphocytes from HIV-positive persons are deficient in their ability to synthesize ribonucleotides via the de novo pathway in response to stimulation with PHA [14]. This pathway is essential for the provision of the precursors necessary for RNA and DNA synthesis and for a range of other processes associated with cell division, including the purine and pyrimidine sugars required for membrane lipid synthesis and glycosylation [15]. The inability of HIV-infected persons to transcribe genes that activate the de novo synthetic pathway effectively results in a metabolic cell death [14].

In the present study, we examined the mechanisms of cell death in HIV-infected subjects after in vitro activation and attempted to determine whether blocking the de novo pathway
of nucleotide synthesis in lymphocytes from uninfected persons would induce a similar necrotic cell death after activation. We used both morphologic and biochemical criteria to identify apoptosis/necrosis plus cell counting to investigate total cell loss.

Methods

**Patients and controls.** The HIV-seropositive cohort in this study attended outpatient clinics at the Royal Free Hospital, London. This cohort was composed mainly of homosexual men (85%), mean age 35 years (range, 19–61). The patients were divided into asymptomatic or symptomatic groups on the basis of the presence or absence of AIDS-defining symptoms [16]. The HIV-seronegative group was made up of healthy laboratory personnel, mean age 31 years (range, 21–60).

**Lymphocyte purification and culture.** Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by density gradient centrifugation. Cells were cultured in RPMI 1640 medium containing 10% fetal calf serum (both Life Technologies, Paisley, UK) and antibiotics (Sigma, Poole, UK). Lymphocytes were activated with either anti-CD3 (OKT3; 0.5 μg/mL; American Type Culture Collection, Rockville, MD) plus IL-2 (2 ng/mL; R&D Systems, Abingdon, UK) or PHA (1 μg/mL; Murex Biotechnology, Dartford, UK) at pretitrated, optimal concentrations. In some experiments, cells were supplemented with IL-2, -7, -12, or -15 (R&D Systems) at the concentrations indicated in the text or were cultured in the presence of neutralizing antibodies to tumor necrosis factor (TNF)–α or IFN-γ (both Genzyme Diagnostics, West Malling, UK).

IL-2-dependent T cell lines were generated from HIV-negative subjects by PHA stimulation of PBMCs for 3 days followed by maintenance in IL-2 (2 ng/mL). Cell lines generated in this way remained viable for 4–6 weeks and were used after ≥1 week in IL-2. Apoptosis was induced in T cell lines by activation for 24 h with immobilized anti-CD3 (10 μg/mL).

Apoptosis was induced in lymphocytes by activating cells with anti-CD3 in the presence of okadaic acid (20 μM; Sigma), which blocks protein phosphatases and induces cell cycle arrest and apoptosis [17]. The role of CD95 in cell death after stimulation was investigated using a monoclonal antibody (MAb) (M3; 5 μg/mL; gift of Immunex, Seattle) that interferes with CD95:CD95-L interactions and thus blocks apoptosis [18]. A nonblocking antibody (M33; 5 μg/mL; Immunex) was used as a control. The broad-spectrum caspase inhibitor z-VAD-fmk (50 μM; Enzyme System Products, Dublin, CA) inhibits apoptosis and was added to anti-CD3-stimulated cells at the initiation of the cultures.

**Enumeration of viable cells and cells in apoptosis.** Viable cells were distinguished by their forward angle scatter and 90° side scatter profiles and were counted by flow cytometry [8]. The number of viable CD4 and CD8 T cells was determined by dual staining with CD4-PE (RFT4; Royal Free Hospital) and CD8–fluorescein isothiocyanate (FITC) (RFT8; Royal Free Hospital) before analysis. Cell morphology was examined in cytospin preparations stained with May-Grunwald-Giemsa (Sigma) and by transmission electron microscopy as described elsewhere [19]. DNA fragmentation was quantitated by terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling (TUNEL). In brief, DNA strand breaks were labeled with FITC-dUTP and deoxynucleotidyl transferase in situ using an apoptosis detection kit (Promega, Southampton, UK). Nuclei were identified by propidium iodide (PI) staining. A minimum of 500 cells per slide were scored by two independent investigators. The exposure of phosphatidylserine on the external surface of the plasma membrane is an indication of apoptosis [20]. This was measured by flow cytometry using FITC-labeled annexin V, a probe for phosphatidylserine, together with PI, which, when used on unpermeabilized cell preparations, distinguishes necrotic and apoptotic cells (R&D Systems).

**Blocking the de novo pathway of nucleotide synthesis.** The de novo pathway of nucleotide synthesis was blocked using either azaserine (20 μM; a glutamine antagonist; Sigma) or ribavirin (50 μM; an IMP dehydrogenase inhibitor; gift of ICN Pharmaceuticals, High Wycombe, UK) [15]. Lymphocytes were preincubated with inhibitors for 30 min before addition of PHA and then incubated for the periods indicated in the text.

Results

**Activation-associated necrosis (AAN) in HIV infection.** We previously reported that mitogenic stimulation of PBMCs from HIV-infected persons induces massive cell death that is not restricted to one particular phenotype and that covers a broad range of lymphocyte subsets. Cell death after activation has also been described in T cell lines and clones and occurs by apoptosis mediated by CD95/CD95-L interactions [21–23]. Therefore, we compared deaths induced in T cell lines with that in PBMCs of HIV-positive persons after stimulation with anti-CD3. The kinetics of the death response were quite different in the two cell types: The minimum cell recovery times of the T cell lines and of PBMCs from HIV-positive subjects occurred 24 and 48–72 h after activation, respectively. Thereafter, cell recovery increased, reflecting the proliferation of residual viable cells (table 1). In addition, most cell death in PBMCs of HIV-positive persons induced by stimulation through CD3 was morphologically very different from the apoptosis induced in T cell lines similarly stimulated. The most characteristic features of apoptosis that can be identified in May-Grunwald-Giemsa-stained cells viewed under light microscopy are cell shrinkage and nuclear condensation. Freshly isolated lymphocytes from HIV-positive patients and anti-CD3–activated cells from controls showed little evidence of cells in apoptosis, although such features were observed in T cell lines stimulated with anti-CD3.

| Table 1. The effect of mitogenic stimulation on lymphocyte recovery. |
|--------------------------|--------------------------|--------------------------|--------------------------|
|                          | 24 h                     | 48 h                     | 72 h                     |
| HIV-negative (n = 13)     | 83.7 ± 4.7               | 87.1 ± 7.0               | 107.6 ± 11.5             |
| HIV-positive             |                          |                          |                          |
| Asymptomatic (n = 10)    | 72.6 ± 6.4               | 54.7 ± 5.0               | 41.5 ± 5.8               |
| Symptomatic (n = 16)     | 59.5 ± 4.5               | 32.6 ± 4.3               | 48.8 ± 5.2               |
| T cell line (n = 5)      | 36.4 ± 5.1               | 52.4 ± 12.5              | ND                       |

**NOTE.** Peripheral blood mononuclear cells were stimulated with anti-CD3 as described in text. Data are viable cell recovery as % of initial cell input (mean ± SE). ND, not determined.
Conversely, in HIV-infected patients, anti-CD3-stimulated cultures consistently showed few apoptotic cells (<5%) but many cells with damaged plasma membranes, anucleated ghost cells, and cell debris indicative of cell lysis (figure 1D, top). An investigation of DNA fragmentation using the TUNEL assay confirmed these morphologic observations: Few TUNEL-positive cells (<5%) were observed in cultured lymphocytes from HIV-positive subjects compared with the T cells lines (>20%; figure 1C, 1D, bottom).

We then investigated the morphology of the activated cells under transmission electron microscopy. Cells with apoptotic morphologic features were evident in T cell lines after stimulation, and we observed condensation of nuclear material combined with cytosolic contraction leading to the loss of surface microvilli (figure 2B). In cells of HIV-infected persons, although some apoptotic cells were identified, a greater proportion of the lymphocytes showed morphologic changes similar to necrosis, including evidence of cell swelling, rupture of the plasma membrane, and leakage of cell content (figure 2D, 2E). While transmission electron microscopy revealed necrotic-like features, the exposure of phosphatidylserine on the plasma membrane identified by the binding of annexin V-FITC in the absence of PI staining was retained (figure 3). Thus, in HIV-infected persons, mitogenic activation induces a form of lymphocyte death that does not involve nuclear fragmentation but instead appears morphologically as necrosis.

To determine if T lymphocytes from HIV-positive subjects had a generalized defect in their ability to undergo DNA fragmentation that might account for the morphologic features described, apoptosis was induced in both T cell lines and PBMCs from HIV-positive and control subjects using okadeic acid, an inhibitor of phosphoprotein phosphatases 1 and 2A [17]. In these experiments, PHA was used as the mitogenic stimulus. Again, after activation with PHA, although viable cell recovery was severely impaired in HIV-positive subjects, the number of cells in apoptosis identified by TUNEL assay was very low (<5%; n = 4) compared with those in T cell lines similarly activated (>50%). The addition of okadeic acid, however, induced similar high levels of apoptosis in HIV-positive subjects and controls (>50%; figure 1F, top and bottom). The induction of apoptosis in HIV-positive patients by okadeic acid was confirmed morphologically by electron microscopy (figure 2C). We conclude, therefore, that the apoptotic pathway induced by okadeic acid remains intact in HIV-infected persons.

Apoptosis induced by CD95/CD95-L interactions is followed in vitro by secondary necrosis, which presumably occurs because of the absence of phagocytes to “mop up” apoptotic cells. To ensure that our observations were not similarly due to secondary necrosis, apoptosis was blocked first by interfering with CD95/CD95-L interactions using an antagonistic CD95 MAb (M3) and second by blocking the downstream events of apoptosis using the caspase inhibitor ZVAD-fmk. Activation of T cell lines with anti-CD3 in the presence of the blocking MAb M3 increased the viable cell recovery and decreased the degree of cell death measured by the binding of annexin V (figure 4). This same MAb, however, had no effect on either viable cell recovery or binding of annexin V when lymphocytes from HIV-infected subjects were similarly activated. The addition of ZVAD-fmk to the cultures decreased the proportion of apoptotic cells induced in control cells by okadeic acid (okadeic acid 45.0%, okadeic acid + ZVAD-fmk 10.5%); however, ZVAD-fmk did not influence either the number of apoptotic cells or...
lymphocyte recovery in HIV-positive patients after stimulation with anti-CD3. These data indicate that CD95/CD95-L interactions do not play a significant role in initiating most cell death seen in HIV infection and strongly suggest that the mechanism of cell death is AAN, not classical apoptosis.

The effect of cytokines on AAN. In HIV and other systems, cytokines can greatly influence the outcome of TCR ligation. IL-2 and other cytokines that utilize the common γ-chain of the IL-2 receptor, including IL-7 and IL-15, prevent apoptosis in IL-2-dependent T cell lines after growth factor withdrawal [24]. To determine the effect of these cytokines on cell death in HIV-1 infection, lymphocytes from HIV-positive and control subjects were stimulated with anti-CD3 in the presence of cytokines at varying concentrations. As shown in figure 5, lymphocytes from control subjects were unaffected by the presence of either IL-2 or IL-15; however, IL-7 augmented recovery, indicating that this cytokine enhances lymphocyte proliferation. The asymptomatic patient illustrated had a cell recovery of 77.9% after lymphocytes were activated for 3 days in the absence of cytokines. Although IL-2 and IL-15 had no effect, IL-7 again increased cell recovery. However, microscopic examination of the cultures revealed substantial cell death was also occurring, indicating that IL-7 augments proliferation in cells that retain the ability to be stimulated through CD3 but does not prevent cell death. This was more evident in the symptomatic patient, who had reduced cell recovery (39.4%) and in whom none of the cytokines had a major effect. Thus, IL-2, IL-7, and IL-15 cannot prevent the death of lymphocytes from HIV-infected persons after activation. Investigation of IL-4, another γ-chain cytokine, found it had no effect on lymphocyte recovery after activation. In addition, although IL-12 is reported to prevent cell death in HIV infection, this cytokine had no effect on cell recovery after 72 h (IL-12 at 50 ng/mL; HIV-positive Patient 1 nil 53.0%, IL-12 38.2%; Patient 2 nil 55.1%, IL-12 40.0%). The cytokines TNF-α and IFN-γ have been shown to induce cell death in other systems; however, neutralizing antibodies to these cytokines, added at a range of concentrations, did not prevent lymphocyte death after stimulation with anti-CD3 (ta-
Figure 3. Exposure of phosphatidylserine detected by annexin V–fluorescein isothiocyanate (FITC). Exposure of phosphatidylserine on plasma membrane of intact cells is a feature of cells in apoptosis. This was detected by flow cytometry using annexin V–FITC, which binds to phosphatidylserine, together with propidium iodide (PI), which is excluded from cells with intact plasma membranes. Apoptotic cells are annexin V positive, PI negative. A, PBMCs, from HIV-positive person, activated for 72 h with anti-CD3. Cell recovery at 72 h was 17.0%, and 34.2% of cells were located within the apoptotic gate (B). T cell line stimulated with anti-CD3 for 24 h. Cell recovery at 24 h was 41.6%, and 13% of cells were within the apoptotic gate.

Figure 4. Representative experiment shows that blocking CD95/CD95-L interactions does not prevent activation-induced cell death in HIV infection. Cell death was induced in T cell line (open bars) and in PBMCs from HIV-positive asymptomatic patient (closed bars) using immobilized anti-CD3. Monoclonal antibody (MAb) M3 blocks CD95/CD95-L interactions; M33 is nonblocking anti-CD95 MAb. MAbs were added at 5 μg/mL at start of cultures. Cell death was assessed by annexin V–propidium iodide staining after 48 h.

Blocking the de novo pathway of nucleotide synthesis induces necrosis. To determine whether interfering with the de novo pathway of nucleotide synthesis could induce the necrotic death observed in HIV patients, this pathway was blocked in lymphocytes from HIV-negative persons using the inhibitors azaserine and ribavirin [15]. Lymphocytes activated with PHA in the presence of these inhibitors showed a marked reduction in cell recovery (day 3: PHA 123%, + azaserine 40%, + ribavirin 23%) and increased cell death measured by annexin V/PI staining (day 3: PHA 24%, + azaserine 47.2%, + ribavirin 45.4%). Cell death induced in this way did not result in increased apoptosis as identified by TUNEL (figure 1E, bottom), but the cells showed many of the morphologic features of AAN seen in HIV-1–positive persons (figure 1E, top; figure 2F). Although these data do not conclusively show that nucleotide starvation is the cause of AAN in HIV-positive persons, it indicates that interrupting the de novo pathway can result in a form of cell death that shares many of its features.

Discussion

It seems clear that multiple mechanisms of cell death operate in HIV infection, each of which could play a key role in disease pathogenesis. These mechanisms may not immediately be apparent unless the total cell loss determined by simple cell counting is compared with that determined by quantitative biochemical assays of apoptosis, such as TUNEL, combined with an examination of lymphocyte morphology. In this study, we identified an AAN in lymphocytes from HIV-positive subjects that occurs after mitogenic stimulation of lymphocytes in vitro and that greatly contributes to the overall cell death observed in these persons.
Figure 5. Effect of interleukin (IL)-2, -7, and -15 on cell recovery after activation. Peripheral blood mononuclear cells isolated from HIV-negative control (A) and HIV-positive asymptomatic (B) and HIV-positive symptomatic (C) subjects were activated with soluble anti-CD3 in presence of IL-2 ( ), -7 ( ), or -15 ( ) at concentrations indicated. Cells were harvested after 72 h. % Cell recovery in relation to initial input is illustrated for representative subjects. Cell recovery in absence of cytokines was 97.5%, 77.9%, and 39.4% for HIV-negative, HIV-positive asymptomatic, and HIV-positive symptomatic subjects, respectively.

Table 2. The effect of neutralizing antibodies to tumor necrosis factor (TNF)-α or interferon (INF)-γ on cell recovery after mitogenic stimulation.

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NOTE: Peripheral blood mononuclear cells from HIV-positive patients (P) and HIV-negative controls (C) were stimulated with anti-CD3 in presence of antibodies to either TNF-α or IFN-γ. % Cell recovery shown after 72 h in culture. NU, neutralizing units; ND, not determined.

Morphologic examination remains one of the most reliable means of identifying apoptotic cells. The majority of activated lymphocytes from HIV-positive patients did not, however, exhibit any of the features normally associated with apoptosis. In particular, the nucleus was not condensed or fragmented, and there was no cell shrinkage or cytoplasmic vacuolation. Instead, the nucleus remained intact, and there was evidence of damage to the integrity of the plasma membrane, both features linked to necrotic injury [10].

The most dramatic and distinguishable features of apoptosis are those associated with the nucleus. The fragmentation of nuclear DNA is induced by the action of a Ca²⁺/Mg²⁺-dependent endonuclease that cleaves DNA into oligosomal fragments of 180–200 bp [12] and is detected by TUNEL assay. Although cells exhibiting necrotic morphologies can in some cases be stained with TUNEL [25, 26], this assay is a well-recognized method for quantifying DNA fragmentation [20]. The lack of TUNEL positivity in lymphocytes triggered by anti-CD3 strongly indicates that DNA fragmentation does not occur in these cells. Key morphologic features of apoptosis can occur without DNA fragmentation. Cohen et al. [27] showed that in the presence of zinc, which is thought to inhibit endonuclease activity, thymocytes treated with dexamethasone did not have fragmented DNA but showed morphologic changes associated with early apoptosis. Also, apoptosis in some leukemic cell lines occurs in the absence of DNA fragmentation and has been described in anucleate cytoplasts [28]. These studies indicate that the action of the endonuclease can be separated from other morphologic changes; however, apoptosis is always associated with characteristic changes to the cytoplasm and plasma membrane. In most T cell systems, including thymocytes [29], mature T lymphocytes [30], and T cell hybridomas [31], cell death induced by anti-CD3 results in DNA fragmentation and apoptotic morphologies. The absence of both of these strongly suggests that the cell death mechanism we have described in HIV-1–infected persons is not apoptotic.

One apoptotic feature retained by the dying cells was the binding of annexin V–FITC to the plasma membrane. In normal blood cells, phosphatidylserine is usually confined to the
inner surface of the plasma membrane; however, on entry into apoptosis, phosphatidylserine becomes exposed at the cell surface [32]. This occurs while the integrity of the membrane is retained. The anticoagulant annexin V preferentially binds to negatively charged phospholipids, such as phosphatidylserine, and when conjugated to FITC can be used to detect exposure of phosphatidylserine on dying cells [20]. In HIV infection, however, annexin V binding was the only apoptotic feature observed, and under electron microscopy, the plasma membrane did not show the smooth appearance associated with apoptosis. It may be that in this necrotic-like cell death pathway, early damage to the plasma membrane allows exposure of phosphatidylserine before the membrane is sufficiently compromised to allow entry of PI. Alternatively, this death pathway, like apoptosis, may induce activation of the molecular mechanisms that lead to exposure of phosphatidylserine at the cell surface. This has important implications in vivo, since exposure of phosphatidylserine is one means whereby phagocytes recognize and engulf dying cells, thus preventing inflammation [33].

Cell death following TCR stimulation in other T cell systems involves ligation of the death receptor CD95 by its ligand, CD95-L [21–23]. This receptor is a member of the TNF–nerve growth factor receptor superfamily, a group of molecules with structural and functional homology (reviewed in [34]). These receptors contain a homologous intracellular region called the death domain, which upon the receptors’ clustering binds other death domain–containing proteins and signals the death cascade [34]. In this study, we were unable to prevent cell death by interfering with CD95/CD95-L interactions, which indicates that this death receptor does not play a key role in activation-associated death in HIV infection. This observation is in agreement with the findings of Estaquier et al. [35], who were also unable to demonstrate a role for CD95 in the death of CD8 T cells after mitogenic stimulation, although it was important in the death of the CD4 subset. Recently, TNF-related apoptosis-inducing ligand (TRAIL), a member of the TNF–nerve growth factor family of ligands [36], was shown to be involved in mitogen-induced cell death in HIV-1 infection, as anti-TRAIL MAbs partially prevented death [37]. TRAIL binds to the death receptor DR4 and induces apoptosis [38]. It remains to be seen whether it is involved in the AAN described here.

Direct infection of cell lines and peripheral CD4 T cells with HIV inhibits the responses of these cells to further mitogenic stimuli and to induce cell death [39]. The gp120 envelope glycoprotein that is released by infected cells and that can be detected in the serum [40] produces a similar effect. This protein suppresses T cell proliferation and primes CD4 T cells for death by apoptosis after subsequent stimulation through the TCR [41]. Similarly, the HIV tat protein inhibits antigen-specific proliferation of normal T cells and accelerates CD95-mediated cell death after activation in vitro [42]. Although we were unable to detect an association between levels of HIV p24 in activated cultures and degree of AAN, it is possible that HIV or one of its proteins interferes with normal cell division. If so, one might expect other dividing cells (e.g., epithelial cells or fibroblasts) from HIV-positive persons to be similarly affected, and it would be interesting to determine whether AAN can be detected in these cell types.

TNF, as its name implies, can induce a necrotic form of cell death in some tumor cell lines through the release of reactive oxygen intermediates [43]. These free radicals can initiate reactions of lipid peroxidation that damage cellular DNA, RNA, proteins, and phospholipids and are involved in the toxicity of agents other than TNF [44, 45]. However, we were unable to demonstrate a role for this cytokine in AAN, as blocking anti-TNF antibodies did not influence cell death. Similarly, IFN-γ was involved in the death of mature T cells and thymocytes, since death could be prevented by neutralizing IFN-γ activity [46], although these antibodies had no effect on lymphocytes of HIV-infected persons.

Mitogenically activated T lymphocytes meet their nucleotide requirements through the de novo pathway of nucleotide synthesis [15]. This pathway is essential for providing new RNA and DNA and the pyrimidine sugars necessary for the massive expansion in membrane biosynthesis induced by cell division. In symptomatic HIV-infected persons, mitogenic stimulation does not induce increases in purine and pyrimidine pools or of NAD, which is required for NAD-dependent reactions such as DNA repair. The pattern of abnormalities observed in HIV infection indicates a defect in the de novo pathway, particularly in glutamine-dependent reactions involving both pyrimidine and purine synthetic enzymes and NAD synthetase [14]. These defects in the de novo pathway can be mimicked in normal cells preincubated with azaserine, a glutamine agonist, or ribavirin, an inhibitor of IMP dehydrogenase [15]. These agents also induced the death of control lymphocytes after stimulation, and these cells shared many of the necrotic features observed in HIV-infected persons.

It is unclear whether there is a direct link between the perturbation of the nucleotide synthetic pathway and necrosis in HIV infection. Further studies will be necessary to define the precise steps of the synthetic pathway that are affected in HIV-positive persons. It is possible that a number of biochemical pathways are disrupted in dying cells and that the necrosis per se induces aberrant nucleotide synthesis. Levels of ATP play an important role in determining death pathways. Death in Jurkat T cells initiated through CD95 requires ATP generation for the execution of nuclear condensation and DNA degradation. In the absence of ATP, the cells switch to a necrotic cell death in response to anti-CD95 [47]. Although the mechanism of this ATP dependence is not clear, the activation of caspase 9 by the human ced-4 homologue requires ATP and cytochrome C as cofactors. This is a key step in the initiation of the final proteolytic steps that result in DNA fragmentation. Cell death in HIV-infected persons increases with lymphocyte
activation and disease progression [48]. Our investigations of the prognostic significance of cell death in HIV infection show that high levels of cell death (low proliferation) are associated with an increased risk of developing AIDS, even among persons with similar numbers of CD4 cells (unpublished data). These studies suggest that cell death in response to mitogenic stimulation provides a clinically useful marker of disease progression. Determining the contribution of AAN to the overall cell death phenomenon could reveal important information on HIV pathogenesis and provide new avenues of HIV therapy.

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