form mass spectrometry. For the TEM study, we used a JEOL JEM-4000EX transmission electron microscope with a top-entry, double-tilting sample holder (±15° tilt), a structure resolution limit of 1.7 Å, and a spherical aberration coefficient (Cs) of 1.0 mm. A 40-μm objective aperture and a 150-μm condenser aperture were used for high-resolution TEM.
24. Fourier transform mass spectra (FTMS) were obtained using an Extrel FTMS-2000 instrument. Fullerene ions were generated by either laser desorption (using an Nd:YAG pulsed laser) or by thermal desorption/electron capture. For each sample, a few micrograms of solid were loaded onto a stainless steel probe tip for examination. For the laser desorption experiments, the fourth harmonic of the YAG (266 nm) was used at 10^6 to 10^7 W/cm^2 to desorb and simultaneously ionize the fullerenes (*37*). Single laser shots of the "as is" samples generated ions that were trapped for between 3 and 20 ms at a base pressure of 8×10^{-8} Torr and subsequently detected in the FTMS ion cell. Both positive and negative ions were generated and examined in these studies. For the thermal desorption experiments, the probe tip containing the sample was heated slowly from 30° to 350°C to thermally desorb compounds from the sample. These neutral molecules were then ionized by low-energy electron capture to generate characteristic negative ions that could be monitored and compared to the laser desorption spectra.
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Programmed Death of T Cells in HIV-1 Infection

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In human immunodeficiency virus (HIV) infection, functional defects and deletion of antigen-reactive T cells are more frequent than can be explained by direct viral infection. On culturing, both CD4$^+$ and CD8$^+$ T cells from asymptomatic HIV-infected individuals died as a result of programmed cell death (apoptosis). Apoptosis was enhanced by activation with CD3 antibodies. Programmed cell death, associated with impaired T cell reactivity, may thus be responsible for the deletion of reactive T cells that contributes to HIV-induced immunodeficiency.

Early in HIV infection, abnormalities in the immune system can be demonstrated in clinically stable asymptomatic individuals. HIV infection affects such CD4$^+$ and CD8$^+$ T cell functions as interleukin-2 production and proliferation after stimulation with soluble antigens and CD3 antibodies. These changes occur before CD4$^+$ T cell numbers are decreased (*1–5*) and cannot be attributed to direct HIV infection in vivo because only a relatively small number of T cells are infected (*6, 7*). In addition to deletion of T memory cells, intrinsic nonresponsiveness occurs in both CD4$^+$ and CD8$^+$ cells in long-term infections (*3, 4, 8*). In their nonresponsiveness to antigenic stimulation and lack of interleukin-2 production despite intact cell-signaling pathways (*1–4*), T cells in HIV-infected individuals exhibit the properties of the unresponsive state known as anergy (*9*).

CD8$^+$ T cells from HIV-infected individuals have increased expression of such activation markers as CD38, HLA-DR, and CD57, which suggests that there is continuous immune activation (*10, 11*). CD8$^+$ cells expressing activation markers have severely decreased proliferative responses and clonogenic potential (*12*) and are reported to die in culture (*13*). This suggests hyporesponsiveness in CD8$^+$ cells as a result of hyperactivation.

We considered the possibility that in this apparent anergic or hyperactivated state, T cells could be programmed for death and whether the loss of antigen-reactive cells could occur as a result of apoptosis. Programmed cell death (PCD), also known as apoptosis or activation-induced cell death, is a physiological mechanism of cell deletion that differs morphologically and biochemically from necrosis (*14*). PCD is involved in a wide variety of immunological regulatory processes (*14–16*). The process is characterized by a typical cellular morphology and degradation of the chromatin into discrete fragments that are multiples of about 190 base pairs of DNA (*17*). It has been proposed that in HIV infection interaction of soluble gp120 with CD4, previously shown to lead to impaired lymphocyte function (*18*), would prime CD4$^+$ T cells for PCD (*19*). This hypothesis is supported by results obtained with mature murine lymphocytes that die from PCD after stimulation through the T cell receptor complex when CD4 was previously ligated by CD4 antibodies (*20*).

Peripheral blood mononuclear cells (PBMC) from HIV-infected persons displayed morphology characteristic of PCD after being cultured overnight in the presence of antibodies to CD3 (anti-CD3) (Fig. 1). Cells showed extensive peripheral chromatin condensation, dilation of the endoplasmatic reticulum, and preservation of mitochondrial structures (*21*). To look for DNA fragmentation, we studied PBMC from 29 asymptomatic seropositive (CDC class II or III) homosexual men. They were selected from a prospective cohort study in Amsterdam (*22*) as having normal numbers of circulating CD4$^+$ cells (mean 540 per cubic millimeter, range 320 to 880) and being seropositive for longer than 3.5 years. In all experiments, a healthy male seronegative control was tested for each seropositive individual. All except one of the HIV-infected subjects showed decreased proliferative responses compared to the seronegative controls. Proliferation in response to anti-CD3 ranged from <1 to 80% of control values, as reported (*2–4, 8, 23*).

When low molecular weight DNA fractions were isolated from lysed cells and subjected to gel electrophoresis, the DNA cleavage pattern specific for apoptosis was observed. Fragmentation could be prevented by Zn^{2+}, which inhibits endonuclease activity (*24*) (Fig. 2). DNA fragments corresponding to 1 to 7 nucleosomes were identified by gel electrophoresis, but longer fragments were also detected that formed a smear in the gel near the origin of migration. In unstimulated cultures, DNA fragmentation was observed in

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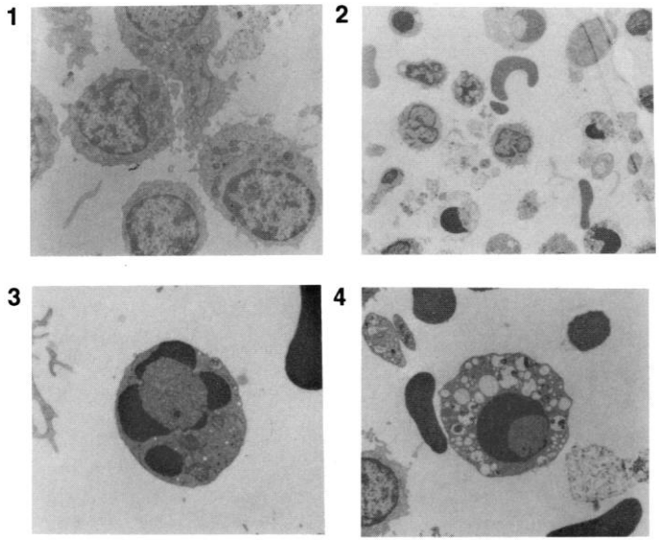

Fig. 1. Transmission electron microscopy analysis revealing the typical morphological changes in cells undergoing PCD. PBMC from a seronegative control (1, magnification ×1600) and PBMC from an HIV-infected individual (2, ×740; 3, ×2700; and 4, ×1600) cultured overnight in the presence of anti-CD3.

15 out of 29 HIV-infected men but in none of the seronegative controls. After stimulation with anti-CD3, PBMC from all HIV-infected persons showed DNA fragmentation, compared with 14 out of 37 seronegative controls. PBMC from eight homosexual seronegative controls showed the same pattern as heterosexual subjects.

Cells undergoing PCD can be labeled by in situ nick translation. Biotin-labeled nucleotides are incorporated into the DNA of cells with DNA strand breaks (*25*). This provides a specific and accurate method of determining the percentages of apoptotic cells. The results from PBMC of 34 asymptomatic HIV-infected individuals from the Amsterdam cohort are shown in Fig. 3. $CD4^+$ T cell numbers range from 70 to 950 per cubic millimeter. On culturing, the percentage of apoptotic cells from HIV-infected individuals was significantly higher than in PBMC from seronegative controls (Fig. 3). Up to 25% of the cells were apoptotic after stimulation with anti-CD3, which supports the data obtained by analysis of DNA fragmentation.

DNA fragmentation was observed in cell fractions enriched for either $CD4^+$ or $CD8^+$ cells (Fig. 4A). Quantitative analysis also revealed that in both $CD4^+$- and $CD8^+$-enriched fractions, cells were dying from apoptosis (Fig. 4B). Polyclonal activation with CD3 antibodies increased the percentage of apoptotic $CD4^+$ and $CD8^+$ cells. In these patients, selected for having normal $CD4^+$ T cell counts, the percentage of apoptotic cells in the $CD4^+$ subset was low compared to that in the $CD8^+$ subset.

It is unlikely that CD4-gp120 ligation is the only mechanism of PCD in HIV infection, because it does not explain PCD observed in $CD8^+$ cells. Up regulated release of cytokines such as tumor necrosis factor (TNF) or transforming growth factor–β (TGF-β) could program cells for death (*26*). Peripheral blood monocytes from HIV-infected persons show an increase in TNF production, which is known to induce PCD (*27*). Overproduction of

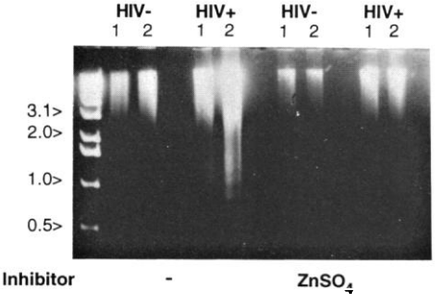

Fig. 2. Apoptosis of PCD in PBMC of HIV-infected individuals can be prevented by $ZnSO_4$. PBMC cultured in the absence (1) or presence (2) of CD3 antibodies were treated with 2 mM $ZnSO_4$. PBMC were freshly isolated by Percoll density gradient centrifugation from heparinized blood. Cells (2×10^6) were cultured in 1 ml of Iscove's modification of Dulbecco's medium supplemented with 10% fetal bovine serum in the absence or presence of CD3 monoclonal antibodies CLB-T3/4.E (*32*). Cells were collected by centrifugation at 800*g* for 15 min. The extent of DNA fragmentation was determined by a modification of the method of Sellins (*33*). Molecular size markers are shown at the left (in kilobases).

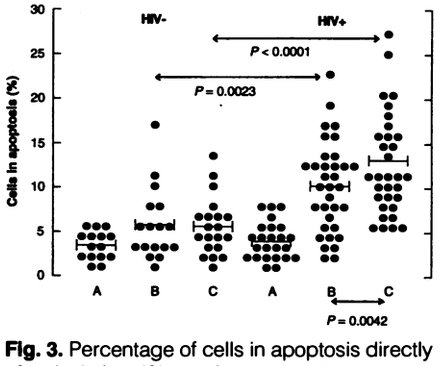

Fig. 3. Percentage of cells in apoptosis directly after isolation (**A**) or after overnight culture in the absence (**B**) or presence (**C**) of anti-CD3. PBMC from 34 HIV-1 seropositive and 17 seronegative controls were cultured as described (Fig. 2). Percentages of apoptotic cells were determined by in situ translation as described by Jonker *et al.* (*25*). Percentages of apoptotic cells in HIV-infected individuals were significantly higher than in controls, as determined by the Mann-Whitney U-test. The increase by polyclonal stimulation of apoptosis in HIV-infected individuals was significant, as determined by the Wilcoxon matched pairs signed ranks test.

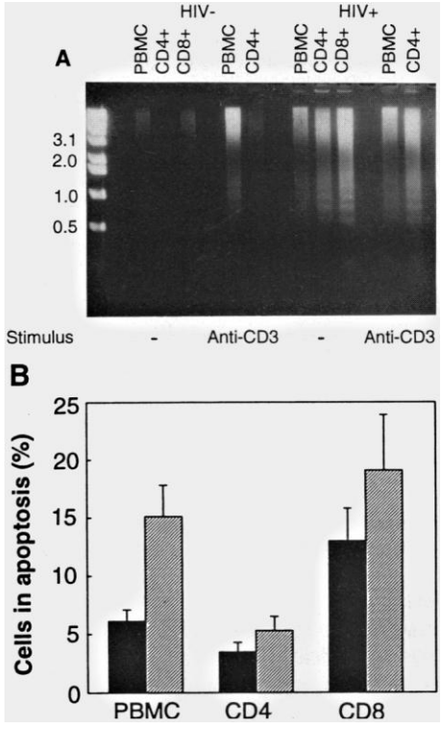

Fig. 4. (**A**) PCD of cells of HIV-1–infected individuals in both the $CD4^+$ and the $CD8^+$ subset, after overnight culture in the absence or presence of anti-CD3. PBMC were cultured and DNA fragmentation determined as described in Fig. 2. PBMC were depleted for either $CD19^+$, $CD16^+$ and $CD8^+$ or $CD19^+$, $CD16^+$ and $CD4^+$ cells by CD19 (CLB-CD19), CD16 (CLB-FcR gran1), CD8 (CLB-T8/4), or CD4 (CLB-T4/1) monoclonal antibodies and anti-mouse IgG–coated magnetic beads, as described (*34*). Molecular size markers are shown at the left (in kilobases). (**B**) Cells in apoptosis in cell fractions enriched for $CD4^+$ and $CD8^+$ cells after overnight culture. PBMC from five HIV-1 seropositive individuals were purified and cultured as described in Fig. 2. The percentage of cells in apoptosis was determined as described (*25*). The percentage of apoptotic cells in PBMC as well as $CD4^+$ and $CD8^+$ subsets from three seronegative controls were at background levels (<2.5%). Cells were cultured in the absence (solid bars) or presence (hatched bars) of anti-CD3.

TGF-β by HIV-infected PBMC has been proposed to cause T cell functional defects that may be due to PCD (*28*). In addition, functional defects in antigen-presenting cells in HIV-infected men (*2*) may result in inadequate T cell activation that induces refractoriness to stimulation and PCD on subsequent activation. Groux *et al.* (*29*), by means of vital dye exclusion, observed activation-induced cell death of $CD4^+$ cells from HIV-infected individuals, after the cells had been cultured for 48 hours. Spontaneous cell death was not observed, and cell death after stimulation involved only $CD4^+$ cells, which might have resulted from their use of stimuli that preferentially activated $CD4^+$ cells.

We demonstrate that on culturing as well as after polyclonal activation, both $CD4^+$ and $CD8^+$ cells from HIV-infected individuals die as a result of apoptosis. In HIV infection in vivo, exposure to HIV proteins or disturbance of cytokine regulatory networks may cause a continuous, PCD-mediated depletion of T cells. Gradual deletion of immune regulatory T cells by PCD, in addition to the direct effects of highly cytopathic HIV variants (*30*, *31*), may contribute to the attenuation and collapse of the immune system.

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Matrix-Mediated Synthesis of Nanocrystalline γ-Fe_2O_3: A New Optically Transparent Magnetic Material

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A new magnetic material with appreciable optical transmission in the visible region at room temperature has been isolated as a γ-Fe_2O_3/polymer nanocomposite. The synthesis is carried out in an ion-exchange resin at 60°C. Magnetization and susceptibility data demonstrate loading-dependent saturation moments as high as 46 electromagnetic units per gram and superparamagnetism for lower loadings where particle sizes are less than 100 angstroms. Optical absorption studies show that the small-particle form of γ-Fe_2O_3 is considerably more transparent to visible light than the single-crystal form. The difference in absorption ranges from nearly an order of magnitude in the "red" spectral region to a factor of 3 at 5400 angstroms. The magnetization of the nanocomposite is greater by more than an order of magnitude than those of the strongest room-temperature transparent magnets, $FeBO_3$ and FeF_3.

The design and synthesis of materials with nanometer dimensions, so called mesoscopic materials, are the subjects of intense current research. Materials with particles in the range 10 to 100 Å exhibit novel electronic, optical, magnetic, and chemical properties due to their extremely small dimensions (*1*). Although several studies have been devoted to the synthesis of nanometer-sized compound semiconductors, relatively little work exists for magnetic materials of similar dimensions (*2–8*). Potential applications for the latter exist in information storage (*9*, *10*), color imaging (*11*), bioprocessing (*12*), magnetic refrigeration (*13*), and ferrofluids (*14*). Such materials may also serve as models for small magnetic particles, which may comprise a portion of interstellar dust (*15*).

A critical obstacle in assembling and

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