Monocytes are required to prime peripheral blood T cells to undergo apoptosis

(programmed cell death/human immunodeficiency virus 1 gp120)

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Contributed by Stuart F. Schlossman, November 15, 1994

ABSTRACT Freshly isolated, human peripheral blood T (PBT) cells are largely resistant to the apoptotic effects of anti-CD3 monoclonal antibody, ionomycin, or phorbol 12-myristate 13-acetate (PMA). We demonstrate here, however, that PBT cells, including both CD4+ and CD8+ cell populations, can be readily induced to undergo apoptosis when cocultured with either autologous or allogeneic monocytes (Mo) in PMA-containing medium. Incubation of PBT cells with Mo at a ratio of 1:1 for 18 hr resulted in maximal levels (80%) of apoptotic cell death. The mechanism whereby Mo enable PBT cells to undergo apoptosis in PMA-containing medium appeared to depend on cell–cell contact or close proximity between Mo and PBT cells rather than solely via soluble mediators. It was demonstrated that Mo acquire the ability to prime PBT cells for apoptosis after treatment with PMA and that treated Mo maintain this ability even after fixation with formaldehyde. It was also found that once PBT cells became primed for apoptosis by incubation with PMA-pretreated Mo, the primed PBT cells were susceptible to apoptosis triggered not only by PMA but also by either ionomycin or by monoclonal antibody crosslinking of T-cell surface molecules such as CD4 and CD3. Interestingly, the degree of apoptosis of CD4+ T cells by crosslinking of CD4 molecules via a combination of gp120, anti-gp120, and goat anti-mouse IgG was significantly greater for T cells primed with PMA-pretreated Mo than for unprimed T cells. Together, these findings reveal an important role for accessory cells in priming resting PBT cells for apoptosis and suggest a possible Mo-dependent mechanism by which T cells may become primed for apoptosis in human immunodeficiency virus-infected asymptomatic individuals.

Treatment with anti-CD3 antibody, ionomycin, and/or phorbol ester can induce apoptosis in both human and mouse immature thymocytes (1, 2). However, truly resting mature T cells are resistant to the induction of apoptosis (3, 4), and it is believed that this resistance to apoptosis may be correlated with T-cell maturity. Bcl-2, a known protooncogene that can block apoptosis, is present at much higher levels in mature, peripheral T cells than in immature thymocytes (5), and, within the mouse thymus, mature CD3\(^{\text{high}}\) thymocytes are largely resistant to apoptosis induced by glucocorticoid treatment compared with immature CD3\(^{\text{low}}\) thymocytes (6). Such findings have suggested that upregulation of CD3 expression in thymocytes correlates with cell survival and selection for export into the periphery.

Peripheral blood T (PBT) cells can gradually acquire susceptibility to apoptosis induced by anti-CD3 monoclonal antibody (mAb), antigen, and mitogenic activation, or by cytoxin-induced cycling after extended culture in vitro (4). Apoptosis can be induced as well in T lymphoblasts, transformed T cells, and T-cell hybridomas by treatment with anti-CD3 antibody, and susceptibility to apoptosis has also been demonstrated with some long-lived T-cell clones (7). In these cases, it is thought that this acquired susceptibility to apoptosis by mature T cells may be linked to the Fas (CD95)-dependent death pathway (8). These findings suggest that the programmed cell death (PCD) pathway may be absent or inactivated during differentiation of T lymphocytes but may reappear after prolonged stimulation.

In contrast to PBT cells isolated from healthy individuals, a significant number of PBT cells isolated from human immunodeficiency virus (HIV)-infected individuals die through apoptosis upon overnight culture in vitro (9). This apoptosis of T cells occurs in both CD4+ and CD8+ cell populations and is enhanced by activation in vitro with anti-CD3 antibody, ionomycin, pokeweed mitogen, or staphylococcal enterotoxin B—i.e., with reagents that stimulate normal PBT cells to proliferate (10, 11). PBT cells from HIV-infected individuals do express low levels of both activation (12) and CD95 (Fas/Apo-1) antigens (13), and they are apparently nondividing cells. Nevertheless, it is still not clear why PBT cells in HIV-infected individuals are so susceptible to apoptosis triggered by T-cell stimulation. An understanding of such a question may be crucial toward understanding the mechanisms underlying the decline in the number of CD4+ lymphocytes that occurs with disease progression in AIDS patients.

To ascertain whether a PCD pathway, similar in nature to that which exists in immature thymocytes, might exist in resting, mature PBT cells, a number of in vitro culture systems were tested and we found that coculturing T cells with phorbol 12-myristate 13-acetate (PMA)-treated monocytes (Mo) can prime both CD4+ and CD8+ cells for apoptosis. In this system, the primed PBT cells undergo apoptosis upon activation-related stimulation, such as with PMA or ionomycin, or by crosslinking CD3 or CD4. In addition to providing us with the knowledge of the existence of a PCD pathway in resting PBT cells that can be activated by PMA-treated Mo, this study gives us an insight for an important role that antigen presenting cells may have in priming resting PBT cells for apoptosis.

MATERIALS AND METHODS

Antibody Reagents. The following mAb reagents were obtained from Coulter: fluorescein isothiocyanate (FITC)-, phycoerythrin- or biotin-conjugated mAbs reactive with the lymphocyte surface antigens CD2 (T11-FITC), CD3 (T3-FITC, T3-RD1), CD4 (T4-FITC, T4-biotin), and CD8 (T8-FITC); My4-FITC reactive with the Mo marker CD14; control non-immune mouse IgG (MsIgG)-FITC and MsIgG-RD1; and GAMlgG, goat anti-mouse IgG; Ho, Hoechst 33342 vital dye; Mo, monocyte(s); MsIgG, nonimmune mouse IgG; PBT cells, peripheral blood T cells; PI, propidium iodide; PMA, phorbol 12-myristate 13-acetate; mAb, monoclonal antibody; PCD, programmed cell death; HIV, human immunodeficiency virus; FITC, fluorescein isothiocyanate; E−, E rosette negative; TNF-α, type α tumor necrosis factor; r, recombinant.

Abbreviations: GAMlgG, goat anti-mouse IgG; Ho, Hoechst 33342 vital dye; Mo, monocyte(s); MsIgG, nonimmune mouse IgG; PBT cells, peripheral blood T cells; PI, propidium iodide; PMA, phorbol 12-myristate 13-acetate; mAb, monoclonal antibody; PCD, programmed cell death; HIV, human immunodeficiency virus; FITC, fluorescein isothiocyanate; E−, E rosette negative; TNF-α, type α tumor necrosis factor; r, recombinant.

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purified mAbs specific for CD3 (T3; IgG2) and CD14 (MY4). mAbs against CD4 (19th5D7), CD20 (B1), CD8 (21th2D3), CD56 (N901), and CD6 (6D3) (14, 15) were developed in this laboratory. mAbs against CD16 (3G8) and CD95 (Fas/Apo-1, 7C11) were generous gifts of P. Anderson and M. J. Robertson (Dana–Farber Cancer Institute), respectively. Mouse anti-gp120 (HIV-1) mAb was purchased from Intracell (Cambridge, MA).

**Purification of PBT Cells and Mo.** Human peripheral blood mononuclear cells were isolated by Ficoll/Hypaque (Pharma-
cia) gradient centrifugation fromuffy coats of healthy blood
donors; resuspended in culture medium consisting of RPMI
1640 medium, 10% human AB serum, 2 mM L-glutamine, 25
mM Hepes buffer (Sigma), 100 units of penicillin per ml, and
100 μg of streptomycin per ml (GIBCO); and cultured at 37°C
in tissue culture flasks. After 1 hr of incubation, nonadherent
cells were collected, washed with antibody reaction buffer [2%
human AB serum (BioWhittaker) in RPMI 1640 medium] and
then treated with a mixture of mAbs specific for CD56 (1:250
diluted ascites), CD20 (1:100 diluted ascites), and CD14 (5
μg/ml), which recognize natural killer cells, B cells, and Mo,
respectively. For CD4+ cell purification, a mAb specific for
CD8 (1:200 ascites) was also included in the mAb solution.
Cells were then purified by negative depletion as described
(15) and resuspended in culture medium at 10^6 cells per ml.
Purified PBT cells and CD4+ cells were used in all experiments
unless otherwise indicated.

For purification of Mo, E rosette-negative (E−) cells at 2 × 10^6
cells per ml in culture medium were prepared as described
(15) and plated at 5 ml per 25-cm² flask or 0.5 ml to each well
of a 24-well plate (Costar). After 1 hr of incubation at 37°C
in 5% CO2/95% air, nonadherent cells were removed by washing
flasks or plates a total of three times with washing buffer (5%
newborn calf serum in RPMI 1640 medium). Remaining adher-
ent cells, consisting of a population of ~90% Mo, as
determined by flow cytometry with FITC-anti-CD14 antibody,
were used directly as Mo in apoptosis induction assays.

**Flow Cytometric Analysis of Apoptosis.** Cells to be analyzed
were collected, washed once with fluorescence-activated cell
sorter medium (2% human AB serum in PBS), and incubated
with either FITC-anti-CD2 or FITC-anti-CD3 mAb for 20 min
on ice. After washing, cells were resuspended in Hobeck in 33342
vital dye (Ho) solution (1 μg/ml in RPMI 1640 medium),
icubated for 12 min at 37°C, and then immediately placed on
ice. Propidium iodide (PI) was added to each sample to give
a final concentration of 1 μg/ml (both Ho and PI were pur-
chased from Sigma) before flow cytometric analysis. Quanti-
fication of apoptotic cell death in the CD3+ population was
performed by flow cytometric analysis using an Epics Elite
(Coulter) (6). Cell debris and clumps were excluded from
analysis by using forward and side scatter parameters. All
fluorescent signals were recorded on a logarithmic scale and
analyzed with Epics Elite software.

**Apoptosis Induction.** Mo were incubated either in culture
medium alone or with PMA in culture medium (10 ng/ml) for
1 hr at 37°C and then washed three times. In some experiments,
formaldehyde (0.1–1% in RPMI 1640 medium) was added to
fix the cells for 15 min at room temperature before washing.
To wells containing either treated or untreated Mo, purified
PBT cells were added (0.5 ml at 10^6 cells per ml) and cocultures
were incubated for 16–18 hr at 37°C in the presence or absence
of various stimuli as indicated. PBT cells were then analyzed
for apoptosis as described for the particular experiment.

In some cases, PBT cell surface antigens were crosslinked
using saturating concentrations of specific mAbs plus goat
anti-mouse IgG (GAMlgG), where indicated (15), prior to
culture. Crosslinking CD4 molecules via recombinant HIV-1
gp120 was carried out on purified CD4+ cells that were first
incubated with gp120 (American Biotechnologies, Cambridge,
MA) at 2.5 μg per 10^6 cells in 100 μl of antibody reaction buffer
for 40 min at room temperature, washed once with antibody
reaction buffer, and then incubated with anti-gp120 mAb (10
μg/ml) for 20 min on ice. Cells were washed again and treated
with GAMlgG as described above before coculturing with Mo.

**DNA Fragmentation.** PBT cells (10^7 cells per 10 ml) were
cultured in 25-cm² flasks containing Mo that had been pre-
viously treated as indicated; PMA was included in cultures where
noted. After overnight culture at 37°C, the cells were washed
once in PBS and then pelleted by centrifugation in Eppendorf
tubes. DNA extraction and electrophoresis were done as de-
scribed (16).

**Staining of Apoptotic Cells and Fluorescence Microscopy.**
Cells to be examined microscopically were pelleted in Eppen-
dorf tubes by centrifugation for 10 sec at 13,000 rpm. The cell
pellet was resuspended, stained with 10 μl of Ho solution
containing 1 μg of PI per ml, placed on ice, and immediately
examined under a Zeiss ICM fluorescence microscope with
UV excitation and blue emission. Blue fluorescence was vis-
ualized when the sample was warmed up during the time of
examination. Photographs were taken with either Kodak Tri-X
or TMAX 400 film.

**RESULTS**

**PBT Cells Undergo Apoptosis in the Presence of Both Mo and PMA.** To define the conditions whereby resting T cells
might be induced to undergo apoptosis, a series of preliminary
experiments were performed. Our initial studies supported the
notion that PBT cells, isolated from normal individuals, are
resistant to apoptosis (3, 4). It was found, for example, that
overnight treatment of purified, freshly isolated PBT with
PMA, ionomycin, dexamethasone, and anti-CD95 (Fas/
Apo-1) mAb, or mAb crosslinking of cell surface molecules
such as CD4 and/or CD3 failed to induce apoptosis (data not
shown). In contrast, a significant degree of apoptosis in the
CD3+ population was noted when PBT cells were cultured
with autologous, E− cells in the presence of PMA as shown in
Fig. 1 (D–F). The effect of the E− cells appears to be density
dependent since at a E−/PBT cell ratio of 1:2 ~50% of the T

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**FIG. 1.** Effect of accessory cells on induction of apoptosis in PBT cells by Mo. PBT cells were incubated for 18 hr in medium alone (A and C) or in PMA-containing medium (10 ng/ml; B and D–H), stained, and analyzed for apoptotic cells on CD3+ cells by flow cytometry as described. Percentages denote the number of Ho^high and smaller cells within the CD3+ population. In some cases, accessory cells were included in cultures as follows: unfractionated, E− cells at 1:2 (D), 1:1 (E), or 5:1 (F) the number of PBT cells; equal numbers of adherent, E− cells (C and H); or equal numbers of nonadherent, E− cells (G). The final volume of all cultures was 0.5 ml except for F (1.5 ml).
cells became apoptotic and this increased to >70% when equal numbers of E− and PBT cells were cultured together. However, no further increase in the percentage of apoptotic T cells was observed when greater numbers of E− cells were included in the cultures and, indeed, when high numbers of E− cells were added, larger volumes of media were required to maintain a consistent cell density, because it was found that, at a high cell density, apoptotic cell death of T cells actually decreased from 70% to 52% due to poor metabolic activity of the cells. Without the addition of E− cells, the percentage of PBT cells undergoing apoptosis in PMA-containing medium (Fig. 1B) was only slightly higher than that of PBT cells cultured in medium alone (Fig. 1A). No apoptosis over background levels was observed for T cells cultured in the presence of an equal number of E− cells when PMA was replaced with ionomycin, dexamethasone, or anti-CD95 (Fas/Apo-I) antibody, or by mAb crosslinking of CD4 and CD3 molecules (data not shown).

Within the E− population, it was found that cells in the adherent population induced a strong degree of T-cell apoptosis in the presence of PMA (Fig. 1H), yet no apoptosis above background levels was observed when T cells were cultured with nonadherent E− cells (Fig. 1G). Furthermore, no induction of T-cell apoptosis by adherent cells occurred if PMA was not included in the culture medium (Fig. 1C). Consistent with these results, proliferation of PBT in PMA-containing medium, as measured by thymidine incorporation, was 2-fold greater in the presence of 1/10th the number of Mo as in the presence of an equal number of Mo.

In addition, an identical degree of T-cell apoptosis in PMA-containing medium was found in the presence of either allogeneic or autologous Mo (data not shown). Moreover, it was demonstrated by using purified T subpopulations that not only CD4+ but also CD8+ T cells undergo apoptosis at a high level (80%) through this Mo-dependent mechanism (data not shown).

To confirm the apoptotic nature of HoH bright PBT cells, HoH bright and HoH dim populations were sorted by flow cytometry, and low molecular weight DNA was extracted from an equivalent number of cells (10⁷) of these two populations. DNA fragmentation was clearly demonstrated in the HoH bright T cells, but little was seen in the HoH dim T cells (Fig. 2 Upper, lanes 1 and 2, respectively). Fluorescence microscopy (Fig. 2 Lower) also showed HoH bright cells; cells labeled a had the characteristic condensed nuclei of apoptotic cells compared with normal sized nuclei observed in the HoH dim cells labeled b.

Similar DNA fragmentation analysis was performed on PBT cells cultured with equal numbers of Mo in PMA-containing medium for various time periods. DNA fragmentation could be seen in the culture containing PBT cells and Mo as early as 6 hr after stimulation with PMA (Fig. 2 Upper, lane 5) and a much greater degree of fragmentation was observed in DNA isolated from cells cultured for 12 hr (lane 6). A control 12-hr culture of Mo alone in PMA-containing medium demonstrated that DNA fragmentation as a result of the death of Mo in these cultures was negligible (lane 3).

**Contact or Close Proximity Between PMA-Pretreated Mo and PBT Cells Is Required to Prime PBT for PCD.** The finding that major histocompatibility complex-mismatched Mo are capable of inducing apoptosis of PBT cells in the presence of PMA raises the possibility that the apoptosis-inducing function of Mo may be mediated by soluble factors released into the culture medium after stimulation with PMA. Such soluble factors might then bind to PBT cells, resulting in the T cells now becoming susceptible to PMA-induced apoptosis. Two approaches were taken to test such a possibility. First, cell-free culture supernatants were prepared from Mo cultured alone in PMA medium for 1 hr and added to PBT cells. Apoptosis of T cells was then assessed by PI and Ho staining after 18 hr of culture, and it was found that no apoptosis was induced by Mo conditioned medium (data not shown).

Second, a series of experiments were performed to determine whether contact or close proximity between PBT cells and Mo was required to trigger apoptosis in T cells. In one set of experiments, semipermeable membrane culture inserts were used to separate Mo and PBT cells within the same culture well. It was found that when Mo and PBT cells were cultured on the same side of the membrane, in the presence of PMA, a high degree of PBT cell apoptosis was observed (Fig. 3). In contrast, when PBT cells and Mo were separated, the percentage of apoptotic cells in the PBT cell population was not significantly greater than that observed with PBT cells cultured alone. In a second set of experiments, Mo were first cultured in PMA medium for 1 hr at 37°C, fixed using 0.5% formaldehyde, and then washed extensively; then PBT cells were added. Apoptosis of PBT was measured by DNA fragmentation analysis after 18 hr of culture and it was found that fixed, PMA-pretreated Mo maintained their ability to sensitize PBT cells for apoptosis (Fig. 4, lane 2). The degree of apoptosis was diminished by ~50% compared to that seen using nonfixed, PMA-pretreated Mo (lane 1). As expected, when Mo were fixed prior to treatment with PMA using an even lower
Fig. 3. Contact or close proximity between PBT cells and Mo is required for induction of apoptosis of PBT cells. PBT cells (T) were cultured alone or cocultured with Mo (M) in either the same chamber or different chambers separated by a semipermeable membrane in the presence of PMA. Results with cells from two different donors and percentage of apoptotic cells in the T-cell population are shown.

concentration of formaldehyde (0.1%), the fixed Mo were no longer able to prime PBT cells for PMA-induced apoptosis (lane 3). Together, these results suggest that contact between PBT cells and Mo is required to trigger apoptosis in PMA medium. However, we cannot exclude the possibility that the leakage of soluble ligands into the microenvironment of PBT cells from the PMA-pretreated Mo may contribute to the induction of apoptosis.

PMA-pretreated Mo Enable PBT to Undergo Apoptosis Triggered by Ionomycin, PMA, or Crosslinking of CD3 or CD4. To investigate whether T cells themselves also need to be stimulated in order to initiate the apoptosis process after priming by PMA-pretreated Mo, Mo were first treated with PMA for 1 hr, washed extensively, and then fixed in 0.1% formaldehyde. To the fixed, PMA-pretreated Mo, fresh PBT cells, some of which had been surface antigen crosslinked using mAb to CD3, CD4, or CD6 plus GAM IgG, were added and cultured in either culture medium alone or culture medium containing ionomycin or PMA as indicated in Fig. 5A. After 18 hr, apoptotic cells within the T-cell population increased by a factor of almost 2–3 with anti-CD4 and anti-CD3 crosslinking relative to cells that were not treated with mAb in the presence of PMA-pretreated Mo. Crosslinking of CD6 or prior treatment with control IgG caused no increase in the percentage of apoptotic cells. Culturing PBT cells with PMA-treated Mo in medium containing ionomycin or PMA increased the level of apoptosis by almost 3- and 6-fold, respectively. In contrast, when resting PBT cells were cocultured with Mo that were not pretreated with PMA prior to fixation, no significant increase in apoptotic cell death of PBT was observed regardless of the stimuli used (Fig. 5A, Mo), confirming both that Mo have no ability to prime T cells for apoptosis unless treated with PMA prior to fixation and that unprimed PBT cells are resistant to apoptosis. Importantly, the findings suggest that upon culturing with fixed, PMA-pretreated Mo, further stimulation of PBT is required for apoptosis in that no apoptosis was observed for T cells cultured in medium alone or for T cells treated with anti-CD6 mAb plus goat anti-Mo IgG. Although CD6 is present on virtually all T cells, crosslinking of CD6 with the 6D3 mAb does not result in T-cell activation (R.A.R., unpublished observation).

In addition to crosslinking by anti-CD4 mAb, it was found that if CD4 molecules on the cell surface of T cells were crosslinked by using a combination of gp120, the envelope glycoprotein of HIV-1, plus anti-gp120 mAb and GAM IgG, apoptosis of primed PBT cells increased by >2-fold when compared to unprimed PBT cells (Fig. 5B). No increase in apoptotic cell death was observed by the binding of gp120 alone without crosslinking.

**DISCUSSION**

Our data show that freshly isolated PBT cells can be readily induced to undergo apoptosis by stimulation via crosslinking of CD3 or CD4 molecules, ionomycin, or PMA through a major histocompatibility complex-nonrestricted Mo-dependent mechanism. Our findings suggest that Mo/macrophages play an important role in regulating T-cell death, which in itself may be essential for maintaining a normal T-cell repertoire.

When stimulated with PMA, Mo express and release a number of factors, among which are apoptosis-related mediators such as \( \text{H}_2\text{O}_2, \text{O}_2^- \) (17), and type \( \alpha \) tumor necrosis factor (TNF-\( \alpha \)) (18). Superficially, these factors do not appear to be involved in priming PBT cells for apoptosis since cell-free
culture supernatants prepared from PMA-treated Mo were unable to induce apoptosis of PBT cells in PMA-containing medium. In addition, apoptosis of PBT cells was not observed when PBT cells and Mo were cultured in the same chamber but separated by a semipermeable membrane. Further support for this idea was the demonstration that PMA-treated Mo, which were fixed before the addition of PBT cells, were still capable of triggering apoptosis of PBT cells. We also showed that PMA-treated Mo, which were fixed before the addition of PBT cells, were still capable of priming PBT cells for apoptosis, suggesting that this process involves a membrane-associated molecule(s) expressed by PMA-treated Mo. However, the membrane-bound form of TNF-α, which is expressed by Mo upon PMA stimulation (19), is unlikely to be involved in apoptosis of PBT cells triggered by PMA (data not shown).

It is unclear at this point whether the CD95 (Fas/Apo-1) molecule is involved in the observed Mo-mediated apoptosis of PBT cells. What is unlikely, however, is that the process is mediated solely through CD95 molecules, since no enhancement in the number of apoptotic T cells was detected when freshly isolated PBT cells were cultured with a combination of PMA and anti-CD95 (Fas/Apo-1) antibody. Indeed, no increased apoptosis of PBT cells was observed when CD95 expression on the activated PBT cell surface is reduced by treatment with CD95 antagonist mAb (50%–60%) of a CD4+/CD8+ T-cell clone but simultaneously triggered a proliferative response in the surviving fraction (50–60%) of the cloned cells.

It has been proposed that T-cell apoptosis is a key in AIDS development. However, how T cells in HIV-infected individuals are primed for apoptosis is entirely unknown. Indeed, HIV-infected chimpanzees show no increase in the degree of apoptotic T-cell death (11). These HIV-infected chimpanzees neither develop AIDS-like disease nor have significantly immunodeficient T-cell function, even though they are persistently infected with T-cell-tropic HIV variants. Interestingly, HIV does not infect Mo in chimpanzees, in contrast to human HIV infection, where Mo-tropic HIV variants can be isolated in all the stages of HIV infection (20). In support of the view that Mo play a crucial role in maintaining T-cell number in HIV-infected individuals was the observation that macrophage-tropic HIV strains but not T-cell-tropic HIV strains cause extensive CD4+ cell depletion in the hu-PBL-SCID model despite equivalent virus burden (21). Banda et al. (22) have suggested that crosslinking of CD4 molecules with a combination of gp120 and anti-gp120 antibody can prime T cells for apoptosis since crosslinking of bound gp120 on human CD4+ cells followed by engagement of T-cell receptor results in apoptosis in vitro (22). This, however, cannot explain the death of CD8+ T cells from HIV+ individuals upon in vitro culture. Our demonstration that both CD4+ and CD8+ T cells can be primed for apoptosis by PMA-treated Mo and that the primed T cells can now undergo apoptosis upon polyclonal stimulation of T cells suggests a model whereby infection of macrophages with a macrophage-tropic HIV results in activation of these cells, either by direct infection or secondarily to cytokines released as a result of infection (23). The activated macrophages would then be envisioned to facilitate induction of apoptosis on susceptible PBT cells in a manner similar to that described for PMA-treated Mo. This mechanism, applicable to other virus infections as well, might be used in lymphoid tissues where a high ratio of infected macrophages to T cells can be found (24). These T cells would be particularly susceptible to apoptosis upon further stimulation, as might occur after antigen recognition, superantigen binding, CD4 molecule crosslinking by membrane-associated gp160 on the infected macrophages, and/or CD4 crosslinked by gp120 in the presence of anti-gp120 antibody. Such a process could result in the continuous and slow depletion of CD4+ cells and even of those activated CD8+ cells that are primed to kill virus-infected targets.

S.F.S. is a member of the scientific advisory board of Apoptosis Technology Inc. The authors thank Dr. C. Morimoto and his colleagues for mAb reagents and helpful discussions and Suzan B. Lazo for flow cytometry technical assistance. This work was supported by Grants AI-12069 and CA-34183 from the National Institutes of Health (to S.F.S.).