Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1525–1529, February 1995
Immunology

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

(programmed cell death/human immunodeficiency virus 1 gp120)

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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-treated 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 $CD3^{bright}$ thymocytes are largely resistant to apoptosis induced by glucocorticoid treatment compared with immature $CD3^{-/dim}$ 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 cytokine-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 nonimmune mouse IgG (MsIgG)-FITC and MsIgG-RD1; and

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Abbreviations: GAMIgG, 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 (19thy5D7), CD20 (B1), CD8 (21thy2D3), 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 (Pharmacia) gradient centrifugation from buffy 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-cm^2 flask or 0.5 ml to each well of a 24-well plate (Costar). After 1 hr of incubation at 37°C in 5% CO_2/95% 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 adherent 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 a wash, cells were resuspended in Hoechst 33342 vital dye (Ho) solution (1 μg/ml in RPMI 1640 medium), incubated 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 purchased from Sigma) before flow cytometric analysis. Quantification 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 (GAMIgG), 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 GAMIgG as described above before coculturing with Mo.

DNA Fragmentation. PBT cells (10^7 cells per 10 ml) were cultured in 25-cm^2 flasks containing Mo that had been previously 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 described (16).

Staining of Apoptotic Cells and Fluorescence Microscopy. Cells to be examined microscopically were pelleted in Eppendorf 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 visualized 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

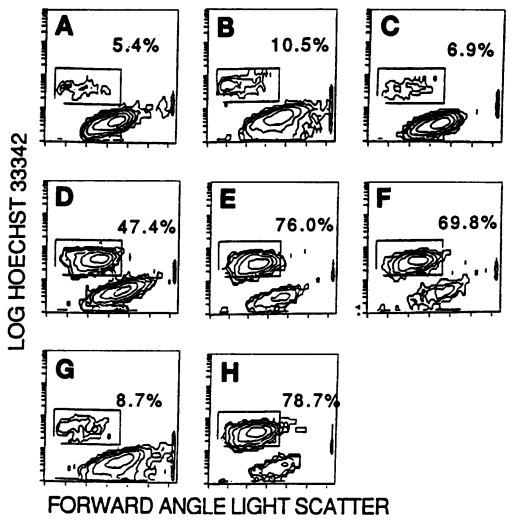

FIG. 1. Effect of accessory cells on induction of apoptosis in PBT cells by PMA. PBT cells were incubated for 18 hr in medium alone (*A* and *C*) or in PMA-containing medium (10 ng/ml; *B* and *D–H*), stained, and analyzed for apoptotic cells on $CD3^+$ cells by flow cytometry as described. Percentages denote the number of Ho^{bright} 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. 1*B*) was only slightly higher than that of PBT cells cultured in medium alone (Fig. 1*A*). 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-1) 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. 1*H*), yet no apoptosis above background levels was observed when T cells were cultured with nonadherent E^- cells (Fig. 1*G*). Furthermore, no induction of T-cell apoptosis by adherent cells occurred if PMA was not included in the culture medium (Fig. 1*C*). 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 autologous or allogeneic 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 Ho^{bright} PBT cells, Ho^{bright} and Ho^{dim} populations were sorted by flow cytometry, and low molecular weight DNA was extracted from an equivalent number of cells (10^7) of these two populations. DNA fragmentation was clearly demonstrated in the Ho^{bright} T cells, but little was seen in the Ho^{dim} T cells (Fig. 2 *Upper*, lanes 1 and 2, respectively). Fluorescence microscopy (Fig. 2 *Lower*) also showed Ho^{bright} cells; cells labeled a had the characteristic condensed nuclei of apoptotic cells compared with normal sized nuclei observed in the Ho^{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 raised 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

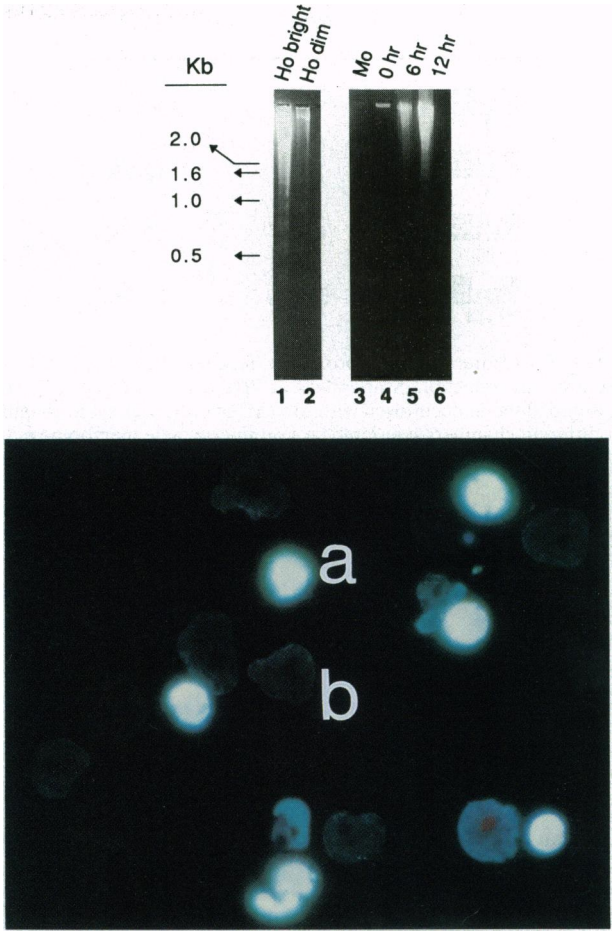

FIG. 2. Measurement of apoptosis by visualization of DNA fragmentation (*Upper*) and fluorescence microscopy of apoptotic cells (*Lower*). (*Upper*) Lanes 1 and 2, DNA was extracted from 10^7 cells of cell sorter purified Ho^{bright} (lane 1) and Ho^{dim} (lane 2) populations (as shown in Fig. 1); lanes 4–6, DNA extracted from PBT cells cultured for the indicated times with an equal number of Mo in PMA medium; lane 3, DNA extracted from Mo alone treated with PMA for 12 hr. Size of DNA (kb) is indicated on the left. (*Lower*) PBT cells were cocultured with Mo in PMA medium for 16 hr and collected for staining with Ho and PI. Ho^{bright} cells (a) show condensed nuclei compared with Ho^{dim} cells (b).

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

double chamber	exp.1	exp.2
T	6.9%	9.3%
T M	7.2%	12.0%
T M	6.5%	12.1%
T+M	56.4%	73.7%

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-treated 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 GAMIgG, were added and cultured in either culture medium alone or culture medium containing ionomycin or PMA as indicated in Fig. 5*A*. 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-treated Mo. Crosslinking of CD6 or prior treatment with control MsIgG 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

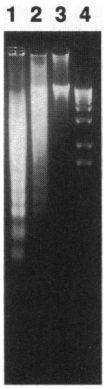

FIG. 4. Effect of fixation on the ability of PMA-pretreated Mo to induce apoptosis of PBT cells in PMA medium. Mo were incubated with PMA (lanes 1 and 2) or medium alone (lane 3) for 1 hr at 37°C and fixed by treatment with 0.5% (lane 2) or 0.1% (lane 3) formaldehyde; Mo in lane 1 remained unfixed. After washing Mo, PBT cells were cocultured with treated Mo and DNA fragmentation analysis was performed as described in Fig. 2 (*Upper*). Lane 4, 1-kb ladder DNA size markers.

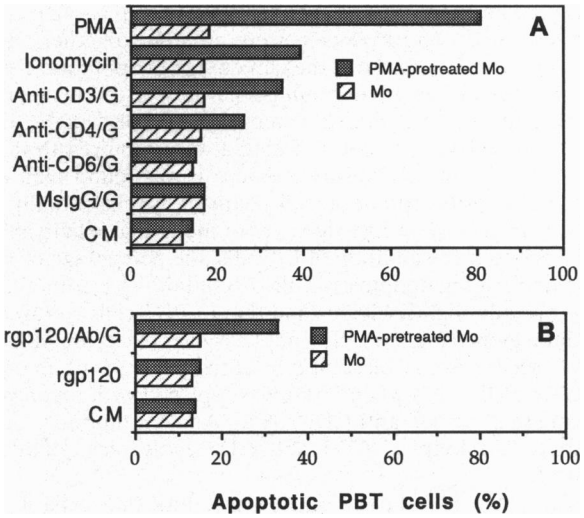

FIG. 5. T-cell stimulation induces apoptosis of PBT cells cultured with PMA-pretreated Mo. (*A*) PBT cells were treated with MsIgG or mAb specific for CD6, CD4, or CD3; bound mAbs were crosslinked with GAMIgG (G); and PBT cells were cocultured with 0.1% formaldehyde-fixed Mo for 18 hr. Prior to fixation, the Mo were incubated in either PMA-containing medium (PMA-pretreated Mo) or culture medium (Mo). For ionomycin or PMA stimulation, PBT cells were cultured directly, in the presence of fixed Mo, in culture medium (CM) or in medium containing ionomycin (1 μg/ml) or PMA (10 ng/ml). (*B*) $CD4^+$ cells were treated before culture with rgp120 or with rgp120, anti-rgp120 mAb plus GAMIgG and then cocultured with Mo treated as described in *A*. At the end of culture, cells were stained and analyzed for percentage of apoptotic $CD3^+$ cells as described in Fig. 1.

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. 5*A*, 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-MsIgG. 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 GAMIgG, apoptosis of primed PBT cells increased by >2-fold when compared to unprimed PBT cells (Fig. 5*B*). 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 H_2O_2, O_2^- (17), and type α tumor necrosis factor (TNF-α) (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 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 the case because neither anti-rTNF-α mAb nor anti-rTNF-α (r, recombinant) receptor p60 and p80 mAbs, either alone or in combination, were able to block Mo-dependent 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 alone were stimulated with a combination of PMA and anti-CD95 (Fas/Apo-1) antibody, 7C11, for 18 hr at 37°C (data not shown). Whether or not PMA treatment induces expression of CD95 ligand on the surface of Mo, which upon engagement with CD95 on the activated PBT cell surface partly contributes to apoptosis of PBT cells in our system, remains to be determined. Other experiments are necessary to determine the nature of additional molecules that may be involved in cell–cell contact in the priming of PBT cells for apoptosis.

PMA is a pleiotropic reagent and is widely used to study cell function and growth of T cells. Our data, however, showed that PMA also induces apoptotic cell death of PBT cells in the presence of Mo and suggested that the mitogenic effects of PMA are a balance between cell death and proliferation. Such opposite effects exerted by the same signal have also been seen in other systems (7). For instance, treatment with staphylococcal enterotoxins A, D, and E induced apoptotic death in a fraction of the cells (40–50%) of a $CD4^+$ 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 immunodefective 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.).

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