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$CD4^+$-T-Cell Counts, Spontaneous Apoptosis, and Fas Expression in Peripheral Blood Mononuclear Cells Obtained from Human Immunodeficiency Virus Type 1-Infected Subjects

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We examined the relationships among $CD4^+$-T-cell counts, spontaneous apoptosis, and Fas expression among peripheral blood mononuclear cells obtained from human immunodeficiency virus type 1 (HIV-1)-infected patients. After 2 days of incubation, propidium iodide DNA staining and flow cytometry revealed that peripheral blood mononuclear cells from subjects with the lowest $CD4^+$-cell numbers (0 to 99/μl; $n = 20$) showed the highest frequency of apoptosis: 22.4% ± 2.7% (mean ± standard error) versus 13.8% ± 1.2% and 12.7% ± 1.4% among peripheral blood mononuclear cells obtained from patients with 100 to 499 $CD4^+$ cells/μl ($n = 19$) and >500 $CD4^+$ cells/μl ($n = 17$), respectively. Each of these means differed significantly from the mean frequency of apoptosis (6.3% ± 0.7%) of peripheral blood mononuclear cells obtained from HIV-1-seronegative controls ($P < 0.001$, Student's t test). After incubation, the percentage of peripheral blood mononuclear cells expressing Fas antigen was increased for the HIV-1-infected subjects, and this was most evident for patients with more advanced disease. Among patients with fewer than 100 $CD4^+$ cells/μl, 64.4% ± 5.4% of peripheral blood mononuclear cells were Fas^+, as opposed to 25.8% ± 3.0% and 14.5% ± 1.7% Fas^+ cells among patients with more than 100 $CD4^+$ cells/μl and healthy controls, respectively ($P < 0.05$ for each group comparison). Interestingly, in all populations, most apoptotic cells did not express Fas. Thus, apoptosis and Fas expression are increased in incubated peripheral blood mononuclear cells obtained from HIV-1-infected patients and these phenomena are enhanced as disease progresses.

Human immunodeficiency virus type 1 (HIV-1) infection is characterized by a progressive decline in circulating-lymphocyte counts. Although productive HIV-1 infection induces lysis of infected cells, the precise mechanism of cell loss in HIV-1 disease is not well understood. Peripheral blood lymphocytes from HIV-1-infected persons have been shown to undergo spontaneous apoptosis after in vitro culture (2, 3, 23), and it has been postulated that this enhanced tendency to undergo programmed cell death may contribute to the cell loss that characterizes HIV-1 infection and AIDS (5, 9, 15, 24, 34). Fas antigen (CD95), a member of a protein superfamily that includes nerve growth factor receptor and tumor necrosis factor receptor, has been shown to mediate apoptosis following interaction with the Fas ligand (FasL) (1, 5, 8, 11, 17).

The present studies examine the relationships of spontaneous apoptosis and Fas expression in unstimulated, incubated peripheral blood mononuclear cells (PBMC) to the stage of HIV-1 disease. We found an enhanced tendency to undergo apoptosis in PBMC obtained from patients with advanced HIV-1 disease. We also found that the percentage of Fas-expressing PBMC was increased in HIV-1-infected subjects, particularly among those with $CD4^+$-T-cell counts less than 100/μl. Interestingly, Fas expression was demonstrated in only a minority of apoptotic cells.

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MATERIALS AND METHODS

Cell preparation and culture conditions. Blood was obtained from HIV-1-infected and HIV-1-seronegative healthy controls; informed consent was obtained from all study participants. PBMC were isolated by Ficoll-Hypaque (Pharmacia LKB Biotechnology, Uppsala, Sweden) density sedimentation. One million PBMC were incubated in duplicate in 1 ml of complete medium, consisting of RPMI 1640 (BioWhittaker, Walkersville, Md.) supplemented with 10% heat-inactivated pooled human serum (Gemini Bioproducts, Calabasas, Calif.), 2 mM L-glutamine, 10 mM HEPES buffer, 100 U of penicillin per ml, and 100 μg of streptomycin per ml, at 37°C in a humidified 5% CO_2-enriched atmosphere.

Measurement of apoptosis and Fas expression. Cells from duplicate wells were harvested after 1, 2, or 3 days of culture, were methanol fixed, and were processed for the measurement of apoptosis by propidium iodide (PI) staining and flow cytometry as described earlier (20, 26, 28–31). In short, after fixation, cells were washed twice with phosphate-buffered saline (PBS, pH 7.0) and resuspended in 250 μl of PBS. Ten microliters of DNase-free RNase A solution (250 μg/ml [Boehringer Mannheim, Indianapolis, Ind.] prepared in PBS containing 10 mM EDTA) was added, and the cells were incubated at 37°C for 30 min. An equal volume of PI stain solution (100 μg of PI [Sigma, St. Louis, Mo.] per ml, 0.1% Nonidet P-40, 0.1% sodium azide in PBS) was added, and the cells were incubated at 4°C for a minimum of 1 h. Cells were analyzed with an EPICS Elite flow cytometer (Coulter Electronics, Inc., Hialeah, Fla.). A 15-mW argon laser was used (excitation wavelength of 488 nm) and red fluorescence was collected above 640 nm. Cells were gated by using side versus forward scatter to eliminate cell debris. The discriminator was set at 50 on the PI fluorescence scale to eliminate debris, mitochondria, and subcellular bodies. The reduced DNA content of apoptotic nuclei resulted in hypodiploid DNA staining in the red fluorescence channels. In these studies, apoptotic cells were gated three standard deviations below the modelled G_0/G_1 peak. Thus, assuming a normal distribution, fewer than 1% of G_0/G_1 cells were misidentified as apoptotic and criteria for the definition of apoptosis were standardized across experiments. This method clearly distinguishes between apoptotic and necrotic cells.

For simultaneous measurement of apoptosis and Fas antigen expression, cells were first stained with fluorescein isothiocyanate-conjugated anti-Fas monoclonal antibody (clone UB2; Immunotech Inc., Westbrook, Maine) and then fixed with PBS containing 1% paraformaldehyde at 4°C for 20 min. Cells were washed twice with PBS and then permeabilized with methanol. Cells then were stained

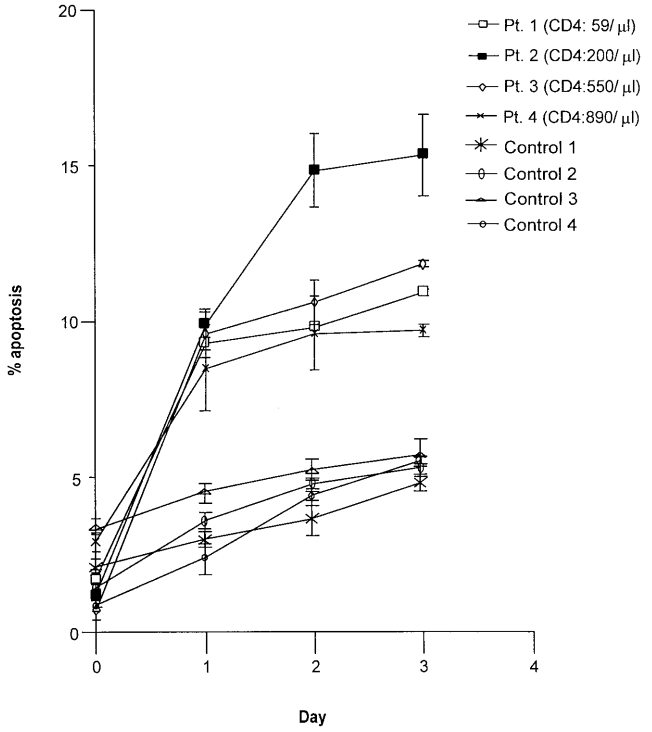

FIG. 1. Measurement of spontaneous apoptosis in PBMC obtained from HIV-1-infected and uninfected subjects. Duplicate PBMC samples either were examined immediately or were incubated in complete medium for 1, 2, and 3 days. Cells were methanol fixed and apoptosis was measured by flow cytometry after PI staining. Data points represent means, and error bars indicate standard deviations. Pt, patient.

with PI as described above. By using a 15-mW argon laser with an excitation wavelength of 488 nm, PI-stained cells were detected by fluorescence above 640 nm and cells reactive with anti-Fas monoclonal antibody were detected by fluorescence at 525 nm. Isotypic fluorescein isothiocyanate-conjugated immunoglobulin G monoclonal antibody (clone 679.1MC7; Immunotech Inc.) was used to eliminate the background green fluorescence.

RESULTS

Optimal conditions for the measurement of spontaneous apoptosis. PBMC from four HIV-1-infected patients with $CD4^+$-cell counts ranging from 59 to 890/μl and four HIV-1-seronegative healthy control subjects were examined in duplicate for apoptosis immediately after preparation and after incubation for 1, 2, and 3 days (Fig. 1). Freshly prepared cells from HIV-1-infected or uninfected subjects were infrequently apoptotic (0.7 to 3.2%). After incubation for 1 day, 8.5 to 9.9% of cells obtained from HIV-1-infected subjects were apoptotic as determined by this assay, whereas only 2.5 to 4.5% cells obtained from healthy controls showed programmed cell death. The percentage of PBMC that were obtained from HIV-1-seronegative subjects that were undergoing spontaneous apoptosis after incubation was significantly lower than the percentage of apoptotic PBMC among HIV-1-infected subjects at all times examined ($P < 0.05$, Student's t test). Maximum apoptosis of PBMC obtained from HIV-1-infected and uninfected subjects was achieved after incubation for 2 to 3 days. A 2-day incubation was therefore utilized for all subsequent experiments. As indicated by the standard deviations, the frequency of apoptosis was highly reproducible in replicate experiments.

To ascertain the effect of cell storage on measurement of spontaneous apoptosis, PBMC from three subjects were incubated for 2 days, harvested, methanol fixed, and stored at −20°C. Apoptosis was measured immediately after fixation (day 0) and then on days 7, 14, and 21 after storage in methanol at −20°C (Table 1). Storage of methanol-fixed cells for up to 3 weeks had no appreciable effect on flow cytometric measurement of apoptosis after PI staining.

To evaluate intrasubject variability over time, apoptosis in PBMC obtained on two to four occasions from 15 HIV-1-infected subjects over a 1- to 3-week period was measured (Table 2). The frequency of spontaneous apoptosis ranged from 4.9 to 30.1%. Coefficients of variation ranged from 6.4 to 42.3% and averaged 20.6%. In the next series of experiments, blood was obtained from three HIV-1-infected subjects on two to three consecutive days and incubated PBMC were assayed for spontaneous apoptosis (Table 3). In these experiments, the frequency of apoptosis was fairly consistent over this shorter period, with coefficients of variation ranging from 3.9 to 7.4%.

Relationship between $CD4^+$-T-lymphocyte count and spontaneous apoptosis. Spontaneous apoptosis was examined in incubated PBMC obtained from 56 HIV-1-infected patients and 12 healthy controls. Patients were stratified into three groups according to their circulating-$CD4^+$-T-cell counts. Among the patients, the frequency of apoptotic cells averaged 16.7% and ranged from 4.3 to 42.3% (Fig. 2). Among controls the frequency of apoptotic cells averaged 6.3% and ranged from 2.9 to 9.6%. In each of the three patient groups (with 0 to 99, 100 to 499, and >500 $CD4^+$ cells/μl) the frequency of apoptotic cells was greater than among the healthy controls ($P < 0.005$, Student's t test). Although there was substantial overlap among the groups, patients with lower $CD4^+$-T-cell counts tended to have more apoptotic cells, with the mean frequencies (± the standard errors) of apoptotic cells averaging 22.4% ± 2.7, 13.8% ± 1.2, and 12.7% ± 1.4% for the patients with 0 to 99, 100 to 499, and >500 $CD4^+$ cells/μl, respectively.

Spontaneous apoptosis of and Fas expression in incubated PBMC. Since activation-induced apoptosis of PBMC in HIV-1 infection may be associated with Fas expression (18, 27, 36), we examined Fas antigen expression and apoptosis among freshly isolated PBMC obtained from 11 HIV-1-infected subjects and 4 healthy controls. The frequencies of apoptotic cells among

TABLE 1. Measurement of spontaneous apoptosis in methanol-fixed PBMC stored at −20°C[a]

Subject	$CD4^+$-lymphocyte count/μl	% Apoptosis after no. of days of storage				Coefficient of variation
		0	7	14	21	
5	10	26.9 ± 3.1	25.8 ± 1.2	27.3 ± 2.1	26.1 ± 1.9	2.6
6	340	8.8 ± 0.2	10.8 ± 0.7	10.2 ± 1	10.1 ± 0.1	8.4
7	490	13.8 ± 0.7	14.2 ± 0.6	14.4 ± 0.4	15.1 ± 0.9	3.8

[a] PBMC obtained from three HIV-1-infected subjects were incubated for 2 days and then were fixed in methanol. One set of samples was stained, and rest were frozen at −20°C; the frequency of apoptosis in duplicate samples was measured immediately and after 7, 14, and 21 days of storage. Values are means ± standard deviations.

TABLE 2. Variability of spontaneous apoptosis of PBMC obtained from HIV-1-infected subjects over a 1- to 3-week period

Subject	$CD4^+$-lymphocyte count	% Apoptosis[a]				Coefficient of variation
		Wk 0	Wk 1	Wk 2	Wk 3	
8	10	20.5 ± 2.1	26.9 ± 3.1			19.1
9	90	15.4	8.3			42.3
10	180	29.5 ± 0.3	20.7 ± 1.2			24.8
11	210	19.1 ± 1.9	24 ± 3.2			16.1
12	340	7.1 ± 0.1	8.8 ± 0.2			15.1
13	390	29.4 ± 3.0	20.7 ± 1			24.5
14	420	18.8 ± 0.8	20.7 ± 3.3			6.8
15	510	12.7 ± 1.5	11.6 ± 1.2			6.4
16	550	13.4 ± 1.3	11.8 ± 0.1	16.4 ± 0.5	14.9 ± 2.8	13.9
17	590	4.9 ± 1.4	8.3 ± 3.4			36.4
18	710	20.1 ± 1.6	30.1 ± 1.3	20.1 ± 1.9	23.8 ± 2.0	20.1
19	750	9.7 ± 2.9	12.2 ± 0.4			16.1

[a] Data are means ± standard deviations.

freshly obtained PBMC (day 0) were comparable for patients and controls, averaging 1.8, 1.0, 1.0% among patients with fewer than 100 $CD4^+$ cells/μl, patients with more than 100 $CD4^+$ cells/μl, and healthy controls, respectively (Table 4). After 2 days' incubation, apoptosis was more frequent in cells from the two HIV-1-infected groups, averaging 22.4% ± 4.9% and 16.0% ± 6.8%, than in cells from the controls (2.2% ± 0.5%). Fas expression by freshly prepared PBMC tended to be higher in the infected groups, averaging 10.2% ± 2.8% and 8.3% ± 3.7%, than in controls (5.2% ± 1.5% Fas^+ cells). After 2 days' incubation, apoptosis and the frequency of Fas^+ cells increased in all three populations, but these phenomena were most prominent among patients, particularly those with fewer than 100 $CD4^+$ cells/μl. In these experiments we also analyzed the relationship between Fas expression and apoptosis on a single-cell level. Representative flow cytograms for one control and one HIV-1-infected subject are shown in Fig. 3. The control subject had 6% Fas^+ cells and 1% apoptotic cells on day 0. Following 2 days' incubation, 14% of cells were Fas^+, 2% were apoptotic, and 1% were both apoptotic and Fas^+. The HIV-1-infected subject had 10% Fas^+ cells and 1% apoptotic cells at day 0. These frequencies increased significantly to 68% Fas^+ cells and 17% apoptotic cells following 2 days' incubation, and at 2 days, 10% of cells were both apoptotic and Fas^+. Of interest, after 2 days' incubation, a small percentage of cells from the HIV-1-infected patient progressed through the cell cycle. These cells most likely represent a subset of activated PBMC recognized to be expanded among persons with HIV-1 infection (14). Moreover, these activated cells all were Fas^+, suggesting that the activated, proliferating cells in persons with HIV disease are prepared to undergo apoptosis.

Summary data on concurrent expression of Fas and apoptosis are shown in Table 5. Among HIV-1-infected persons with $CD4^+$-cell counts of <100/μl, 22.4% ± 4.9% of cells were apoptotic after 2 days of incubation. Among patients with $CD4^+$-cell counts of >100/μl, 16% ± 6.8% of cells were apoptotic, compared to 2.2% ± 0.5% of cells obtained from healthy controls. Although among the same populations, 64.4% ± 5.4%, 25.8% ± 3.0%, and 14.5% ± 1.7% of cells were Fas^+, respectively, only a minority of cells (9.7, 6.2, and 0.6%, respectively) were both Fas^+ and apoptotic. Thus, the majority of apoptotic cells in each subject group did not express detectable Fas, yet among patients with $CD4^+$-cell counts of <100/μl, the majority of all PBMC (64.4%) were Fas^+.

TABLE 3. Variability of spontaneous apoptosis of PBMC obtained from HIV-1-infected subjects over 2 to 3 consecutive days

Subject	$CD4^+$-lymphocyte count	% Apoptosis[a]			Coefficient of variation
		Day 1	Day 2	Day 3	
20	150	38.6 ± 2.1	41.6 ± 0.1	41.3 ± 1.8	3.9
21	220	26.6 ± 0.5	27.7 ± 0.3	29.2 ± 0	4.3
22	390	9.9 ± 0.9	8.8 ± 0.5		7.4

[a] Data are means ± standard deviations.

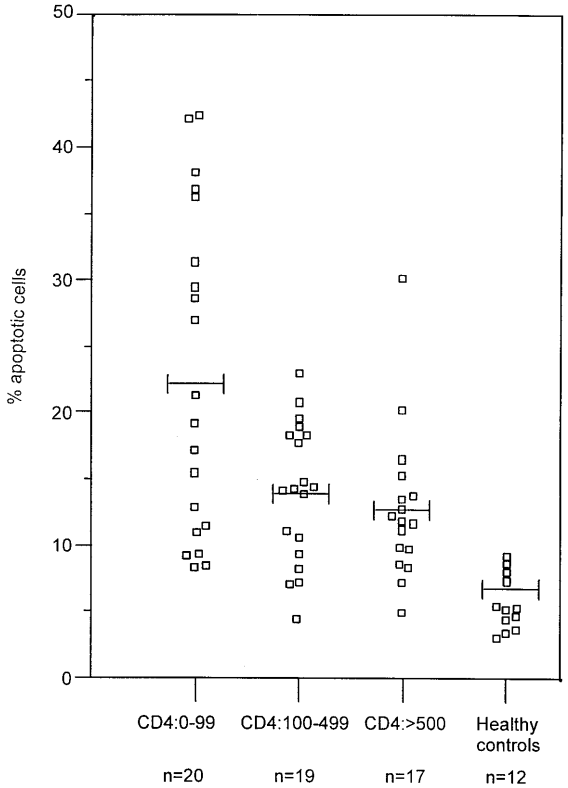

FIG. 2. PBMC obtained from 56 HIV-1-infected subjects and 12 healthy controls were examined for spontaneous apoptosis after 2 days of incubation. Bars represent means. The means of groups 1, 2, and 3 each differ significantly from the mean of the healthy control group ($P < 0.005$, Student's t test). A significant difference between groups 1 and 3 ($P < 0.01$, Student's t test) was seen.

TABLE 4. Relationships among $CD4^+$-cell count, age, sex, antiretroviral therapy, Fas expression, and apoptosis in freshly isolated and 2-day-incubated PBMC obtained from HIV-1-infected subjects and healthy controls

Subjects	$CD4^+$-lymphocyte count	Age (yr)	Sex[a]	Antiretroviral therapy	Fas expression		% Apoptosis	
					Day 0	Day 2	Day 0	Day 2
HIV-1 infected								
57	0	35	M	AZT, 3TC	13	58	1	20
58	10	53	M	AZT, ddC	6	59	1	24
59	10	35	F	AZT	9	68	1	17
60	20	35	M	AZT, 3TC	12	68	5	21
61	30	29	M	AZT	11	69	1	30
Mean ± SD					10.2 ± 2.8	64.4 ± 5.4	1.8 ± 1.7	22.4 ± 4.9
62	150	45	F	AZT	13	25	1	9
63	160	35	M	AZT, 3TC	6	26	1	8
64	200	31	M	ddC	3	27	1	15
65	210	35	M	AZT, ddC	7	31	1	26
66	490	45	F	Nil	12	24	1	18
67	880	32	F	Nil	9	22	1	20
Mean ± SD					8.3 ± 3.7	25.8 ± 3.0	1.0 ± 0	16.0 ± 6.8
Uninfected healthy control								
13	Not done	32	M	Nil	3	17	1	2
14	Not done	33	M	Nil	6	14	1	2
15	Not done	34	M	Nil	6	14	1	3
16	Not done	33	M	Nil	6	13	1	2
Mean ± SD					5.2 ± 1.5	14.5 ± 1.7	1.0 ± 0	2.2 ± 0.5

[a] M, male; F, female.
[b] AZT, zidovudine; 3TC, lamivudine; ddC, didanosine.

In these experiments, the subjects' ages were not associated with either the frequency of Fas^+ cells or the frequency of apoptosis. This contrasts with observations made for non-HIV-1-infected subjects, in which Fas antigen expression increased with aging (32).

DISCUSSION

Utilizing a standard method for the measurement of apoptosis, we have optimized the conditions for and determined the variability of spontaneous apoptosis of incubated PBMC obtained from HIV-1-infected subjects. We found that 2 or 3 days' incubation results in comparable levels of induction of apoptosis. We also have shown that the intra-assay variability is minimal and that methanol-fixed cells can be stored at −20°C for as long as 3 weeks before flow cytometric assay. Although there is substantial intrasubject variation in spontaneous apoptosis as measured over a 1- to 3-week interval (Table 2), daily repeated measurements of apoptosis revealed only minimal variability (Table 3). This suggests that the variability in spontaneous apoptosis over time may actually reflect a true temporal variation in programming for cell death in HIV-1-infected patients.

As others have, we found that the frequency of spontaneous apoptosis is enhanced in PBMC obtained from HIV-1-infected persons (13, 14, 19, 22, 23, 25). Meyaard et al., however, found no relationship between the stage of HIV-1 disease and the frequency of apoptosis (23). In contrast, we found that PBMC obtained from patients with more advanced HIV-1 disease tended to have more apoptotic cells after brief incubation. Recently, Guogeon et al. also found an inverse relationship between $CD4^+$-T-cell count and the frequency of spontaneous apoptosis (14). This group also demonstrated that the majority of apoptotic cells were activated as determined by expression of HLA-DR and CD38 (14). HIV-1 infection of T cells and monocytes increases surface expression of Fas (10, 21), and HIV-1 infection of monocytes induces de novo expression of FasL. Our studies have shown increased Fas expression by freshly prepared PBMC. McCloskey et al. (21) and Debatin et al. (10) have shown increased Fas expression in freshly isolated PBMC from HIV-1-infected patients with Centers for Disease Control and Prevention category 3 HIV-1 disease and among HIV-1-infected children, respectively. Apoptosis mediated through Fas-FasL interaction may contribute to the lymphocyte depletion seen in HIV-1-infected persons (3). We have demonstrated that incubated PBMC obtained from HIV-1-infected patients show heightened Fas expression compared to incubated PBMC of controls. We have also shown that the frequency of Fas^+ cells increases with more advanced disease. In additional experiments we found that both $CD4^+$ and $CD8^+$ cells from HIV-1-infected subjects were apoptotic after 2 days' incubation, and in both cell populations apoptosis was observed among Fas^+ and Fas^- cells (data not shown). This was also demonstrated by Gougeon et al. (14). The mechanism whereby Fas expression increases after incubation is not clear. The observation that Fas expression increases in PBMC of HIV^+ patients and HIV^- controls is of some interest and suggests that in HIV disease, physiologic mechanisms that regulate cell survival may be excessively activated. Additional studies will be needed to clarify this. The rapid induction of Fas upon phytohemagglutinin activation (33) and the observation

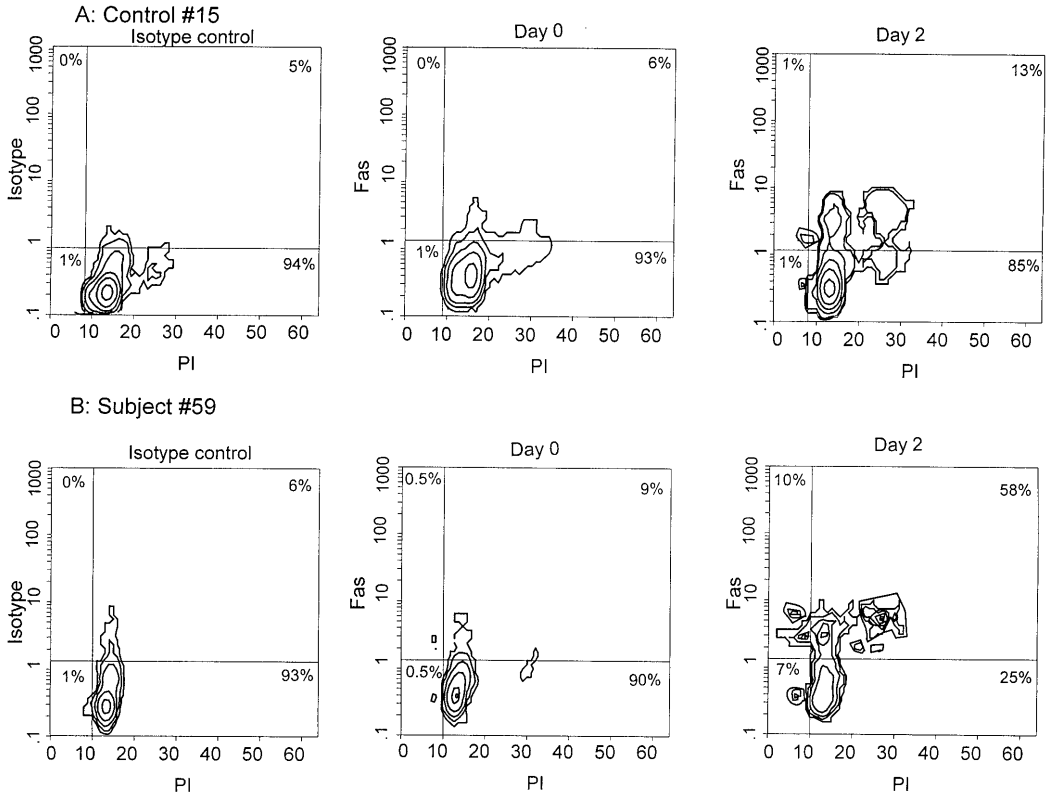

FIG. 3. Flow histograms showing Fas antigen expression on apoptotic and nonapoptotic freshly isolated and 2-day-incubated PBMC from an HIV-1-infected subject and an uninfected control. Cells were prepared for flow cytometric analyses as described in Materials and Methods.

that spontaneous apoptosis is seen primarily among cells with an activated phenotype suggest that HIV-1 infection results in cellular activation and Fas expression that render cells programmed for death in vitro and possibly in vivo (18, 25). Interestingly, despite increased apoptosis among incubated PBMC from patients with less advanced HIV-1 disease ($CD4^+$-T-cell counts of $>100/\mu l$), the frequency of Fas^+ nonapoptotic PBMC was comparable to that observed among PBMC obtained from controls (16 and 14%, respectively), whereas among patients with advanced disease ($CD4^+$-T-cell counts of $<100/\mu l$), more than half of nonapoptotic PBMC expressed Fas. The significance of this observation is unclear. On the one hand, these assays provide only a snapshot of events. With additional cultivation more Fas^+ cells may become apoptotic. Moreover, apoptotic cells may disintegrate in culture and the half-life of apoptotic cells in this system is unknown. High-frequency Fas expression by incubated PBMC from patients with advanced disease may indicate more frequent activation of programmed cell death in these patients and is compatible with the concept of rapid cell turnover in advanced HIV-1 infection (16, 35). On the other hand, the relatively normal frequency of Fas^+ nonapoptotic cells in the presence of increased numbers of apoptotic PBMC in HIV-1-seropositive patients with higher $CD4^+$-T-cell counts may indicate that Fas-independent mechanisms of programmed cell death are important in earlier stages of HIV-1 infection. In this regard, in both early and later stages of disease, the majority of apoptotic cells did not express detectable Fas antigen. Whether this represents loss of Fas expression with cell death or is reflective of Fas-independent mechanisms for cell death is not clear.

Other mechanisms have been implicated in induction of cellular apoptosis in HIV-1 infection. Recently, Boudet et al. have shown that the down regulation of Bcl-2 protein in $CD8^+$ T cells is correlated with increased apoptosis and HIV-1 disease progression (4). More recently, Estaquier et al. found that

TABLE 5. Fas expression on apoptotic and nonapoptotic PBMC from HIV-1-infected subjects and HIV-1-seronegative controls[a]

Group	% Cells of indicated type					
	Apoptotic	Fas^+	Apoptotic and Fas^+	Apoptotic and Fas^-	Nonapoptotic and Fas^+	Nonapoptotic and Fas^-
Subjects with $CD4^+$-cell counts of:						
$<100/\mu l$ ($n = 5$)	22.5 ± 4.9*	64.4 ± 5.4**	9.7 ± 2.2*	12.5 ± 3.7*	54.6 ± 6.2**	22.5 ± 2.4**
$>100/\mu l$ ($n = 6$)	16.0 ± 6.8	25.8 ± 3.0*	6.2 ± 4.2	9.7 ± 2.8*	15.8 ± 3.0	68.2 ± 7.0
Healthy controls ($n = 4$)	2.2 ± 0.5	14.5 ± 1.7	0.6 ± 0.1	1.5 ± 0.3	14.0 ± 1.7	83.8 ± 1.6

[a] PBMC were incubated for 2 days. Data are means ± standard deviations. *, $P < 0.05$ (Student's t test) compared with value for healthy controls; **, $P < 0.05$ (Student's t test) compared with values for subjects with >100 $CD4^+$ cells/μl and for controls.

T-cell-receptor-induced apoptosis of $CD4^+$ lymphocytes obtained from HIV-1-infected patients could be blocked by an antagonistic anti-Fas antibody whereas apoptosis of $CD8^+$ lymphocytes was unaffected (12). Oxidative stress may be increased in persons with HIV-1 disease (6), and oxidant-mediated apoptosis has been posited as a mechanism of cell loss in persons with AIDS (7). Thus, Fas-mediated and Fas-independent mechanisms of programmed cell death may contribute to the progressive loss of circulating cells in HIV-1 disease, including the loss of cells that are not ordinarily susceptible to HIV-1 infection, such as $CD8^+$ T cells.

In conclusion, incubated PBMC from HIV-1-infected subjects show an increased frequency of apoptosis. Fas expression is increased in freshly isolated PBMC from HIV-1-infected subjects, and this increases substantially after incubation. Both apoptosis and Fas expression increase in more advanced stages of HIV-1 disease. This notwithstanding, most apoptotic cells do not express Fas, suggesting the possibility that both Fas-dependent and Fas-independent mechanisms may contribute to the occurrence of spontaneous apoptosis among incubated PBMC from HIV-1-infected persons.

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