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Role of CD26/dipeptidyl peptidase IV in human immunodeficiency virus type 1 infection and apoptosis

(CD95)

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ABSTRACT **To examine the role of CD26/dipeptidyl peptidase IV (DPPIV; EC 3.4.14.5) in infection by human immunodeficiency virus type 1 (HIV-1), we utilized CD26 cDNA-transfected Jurkat T-cell lines. Both CD26$^-$ parental Jurkat cells and mutant CD26$^+$ (DPPIV$^-$) transfected Jurkat cells were readily infected with HIV-1, whereas wild-type CD26$^+$ (DPPIV$^+$) transfected Jurkat cells were more resistant to HIV-1 infection. Our results suggest that CD26 is not essential for HIV-1 infectivity as suggested by others but that DPPIV enzyme activity may decrease the efficiency of HIV-1 infection. Of great interest, we found that mutant CD26$^+$ (DPPIV$^-$) transfectants and CD26$^-$ parental Jurkat cells strongly expressed CD95 (Fas/Apo-1) and were more sensitive than wild-type CD26$^+$ (DPPIV$^+$) transfectants to the induction of apoptosis by anti-CD95 monoclonal antibody. These results suggest that CD26 may play a role in HIV-1-associated loss of CD4$^+$ cells through the process of programmed cell death.**

CD26 is a widely distributed 110-kDa cell surface glycoprotein with known dipeptidyl peptidase IV (DPPIV; EC 3.4.14.5) activity in its extracellular domain (1–5). This ectoenzyme is capable of cleaving amino-terminal dipeptides from polypeptides with either L-proline or L-alanine in position 2 (6). On T cells, CD26 expression is preferentially restricted to the CD4$^+$ helper/memory population (2), and CD26 can deliver a potent costimulatory T-cell activation signal (7). Crosslinking of CD26 and CD3 with solid-phase immobilized monoclonal antibodies (mAbs) can induce T-cell costimulation and interleukin 2 production by either human CD4$^+$ T cells or Jurkat T-cell lines transfected with CD26 cDNA (7, 8). The cDNA sequence of CD26 predicts a type II membrane protein with only six amino acids in its cytoplasmic region (8), suggesting that in addition to DPPIV enzyme activity other signal-inducing molecules may be associated with CD26. There is evidence that CD26 interacts, presumably in its extracellular domain, with both CD45, a protein-tyrosine-phosphatase, and adenosine deaminase, each of which is capable of functioning in a signal transduction pathway (9, 10).

CD4$^+$ lymphocytes in patients with acquired immunodeficiency syndrome (AIDS) have an intrinsic defect in their ability to recognize and respond to "recall antigens" some time before a reduction in the total number of CD4$^+$ cells occurs (11, 12). The response to recall antigens is clearly a property of CD4$^+$CD26$^+$ T cells, since this is the only helper population known to proliferate in response to soluble antigens and to induce both major histocompatibility complex-restricted cytotoxic T lymphocytes, capable of killing virally infected target cells and B cells to secrete immunoglobulins (2). Each of these properties is a key to the host's response to viral infection. In this regard, a selective decrease in CD26$^+$ T cells has been reported in human immunodeficiency virus type 1 (HIV-1)-infected individuals prior to a general decrease in CD4$^+$ T cells (13, 14). Moreover, Tat, a regulatory protein encoded by the HIV-1 genome (15), has been shown to suppress the response of human peripheral T cells to soluble antigens (16). It has been suggested that Tat can bind to and partially inhibit DPPIV (17). More recently, Hovanessian and colleagues (18) have proposed that CD26 serves as an essential cofactor for HIV-1 entry, capable of rendering CD4-expressing murine NIH 3T3 cells infectible by HIV-1. These authors found that the anti-CD26 mAb 1F7 and inhibitors of DPPIV enzyme activity could inhibit viral entry.

Previously (19), using wild-type CD26$^+$ (DPPIV$^+$)- as well as mutant CD26$^+$ (DPPIV$^-$)-transfected Jurkat T-cell lines, we demonstrated that DPPIV enzyme activity plays an important role in CD26-mediated T-cell costimulation. This system utilizing the parental Jurkat T-cell line, which is CD4$^+$CD26$^-$, and transfected cell lines that are CD4$^+$ wild-type CD26$^+$ (DPPIV$^+$) and CD4$^+$ mutant CD26$^+$ (DPPIV$^-$) provided an ideal system to determine whether CD26 and/or DPPIV enzyme activity are involved in HIV-1 infection. Both CD26$^-$ parental Jurkat cells and mutant CD26$^+$ (DPPIV$^-$)-transfected Jurkat cells were readily infected with HIV-1, whereas wild-type CD26$^+$ (DPPIV$^+$)-transfected cells appeared to be more resistant to HIV-1 infection. This observation suggests that CD26 is not absolutely necessary for HIV-1 infection and that DPPIV enzyme activity may protect against HIV-1 infection. More importantly, both parental and mutant CD26$^+$ (DPPIV$^-$) Jurkat cells strongly expressed CD95 (Fas/Apo-1) (20) and were more sensitive to the induction of apoptosis. Our results support the notion that CD26-associated cellular events may contribute to loss of CD4$^+$ cells through the process of programmed cell death in HIV-1-infected individuals.

MATERIALS AND METHODS

Establishment of DPPIV Activity-Deficient Jurkat Transfectants. The *Xba* I DNA fragment which encodes the entire CD26 protein was subcloned into the *Xba* I site of pTZ19u (Bio-Rad) so that its 5′ end was located proximal to the *Eco*RI site of the vector DNA (8). With single-stranded DNA prepared from this plasmid as a template and the oligodeoxynucleotide 5′-GGCTGGGCATATGGAG-3′ (which directs the substitution of alanine for the putative catalytic serine at position 630), as a primer, site-directed mutagenesis was performed by the method of Kunkel (21). After the sequence was verified, the *Xba* I CD26 cDNA fragment was inserted between the *Hin*dIII and *Xba* I sites of the mammalian

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Abbreviations: DPPIV, dipeptidyl peptidase IV; HIV-1, human immunodeficiency virus type 1; mAb, monoclonal antibody.

expression vector RcCMV (Invitrogen). Then, the *Mlu* I–*Xba* I cytomegalovirus promoter fragment was replaced with the *Hin*dIII–*Xba* I SRα promoter fragment derived from pSRα-26 (8). The resultant plasmid DNA allows the expression of mutant CD26 cDNA under the control of the SRα promoter on the framework of RcCMV plasmid DNA. This plasmid DNA also carries a neomycin-resistance gene. Wild-type CD26 expression plasmid DNA was constructed and used as a control. These plasmid DNAs were digested with *Sal* I before transfection. Jurkat cells were transfected by electroporation (8). Transfectants were selected in RPMI 1640 medium supplemented with 10% fetal bovine serum, 4 mM glutamine, and the neomycin analog G418 (1 mg/ml: GIBCO). G418-resistant clones were further screened for high expression of CD3, CD4, and CD26 antigens by cell surface immunofluorescent staining as described below. Transfectants were maintained in the aforementioned medium containing G418 at 0.25 mg/ml, and parent Jurkat cells were maintained in the regular RPMI 1640 medium with 10% fetal bovine serum.

Characterization of the Antigen. The following mAbs were used to characterize the antigen and to assess the inhibitory effects of HIV-1 infection: anti-CD3 (T3/RW24B6, IgG2b), anti-CD4 (19thy4D6, IgG2b), anti-CD29 (4B4, IgG1), anti-CD26 (Tal/4EL-1C7, IgG1; 1F7, IgG1; 5F8, IgG1), and anti-CD95 (7C11, IgM) (1, 2, 5, 22, 23). Procedures for staining cell surface antigens with mAbs and for flow cytometry analyses with an EPICS V cell sorter (Coulter) have been described (1, 2).

Assay for DPPIV Enzyme Activity. The indicated number of cells in 100 μl of phosphate-buffered saline were incubated for 2 hr at room temperature with 100 μl of glycylproline *p*-nitroanilide *p*-tosylate (Sigma) in phosphate-buffered saline. Release of *p*-nitroaniline was measured by absorbance at 405 nm with a microplate spectrophotometer (Micro Devices, Menlo Park, CA) and activity was calculated by comparison with a standard curve for *p*-nitroaniline.

HIV-1 Replication in CD26-Transfected Jurkat Cells. Jurkat cells, maintained in culture at a 2.5×10^5 per ml, were incubated overnight with cell-free HIV-1, washed, and placed in culture with fresh medium. Culture supernatants were harvested every 3–4 days and replaced with fresh medium (RPMI 1640 with 10% fetal bovine serum). The harvested culture supernatants were assessed for HIV-1 p24 antigen concentration with a commercial antigen-capture assay kit (Coulter).

Analysis of Apoptotic Cell Death by Flow Cytometry. Parental and transfected Jurkat cells were suspended in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin sulfate (100 μg/ml) and were induced to undergo apoptosis by incubation in medium containing 7C11, a mAb that recognizes CD95 (Fas/Apo-1) antigen (23). After treatment, 1 million cells in 2 ml of medium from each sample were stained for 10 min at 37°C with the bisbenzimidazole dye Hoechst 33342 (1 μg/ml) and centrifuged. The cell pellets were resuspended in 1 ml of cold phosphate-buffered saline containing propidium iodide (5 μg/ml) and analyzed for the percentage of apoptotic cell death by flow cytometry (24). A small number of cells positive for propidium iodide were excluded by electronic gating. Apoptotic cells were distinguished from normal cells by their higher fluorescence intensity of Hoechst 33342 and by their smaller size as judged by forward light scatter.

RESULTS

Establishment of Jurkat Transfectant Cells Expressing High Levels of CD4 and Wild-Type or Mutant CD26. In our previous study (19), to determine the involvement of DPPIV enzyme activity and its functional relationship to the costimulatory activity of CD26, we established Jurkat transfectants which expressed wild-type CD26 and DPPIV enzyme activity-deficient (DPPIV$^-$) mutant CD26 (19). We have screened transfectants for high expression of CD4 and wild-type and mutant CD26, although mutant CD26-transfected cells expressed a lower density of CD26 antigen (Fig. 1). Both wild-type CD26$^+$ (DPPIV$^+$) and mutant CD26$^+$ (DPPIV$^-$) Jurkat cells expressed not only the 1F7 epitope but also the 5F8 and Tal epitopes of the CD26 antigen. Eighty percent of the parental Jurkat cells expressed CD4 and did not express CD26 (at background level). When the cell lines were examined for DPPIV enzyme activity (Fig. 2), only wild-type CD26-transfected cells (nos. 23 and 11) contained high DPPIV enzyme activity. On the other hand, mutant CD26 (DPPIV$^-$)-transfected cells and parental Jurkat cells had no DPPIV enzyme activity over a large range of cell numbers. Thus, we confirmed that the wild-type CD26-transfected cells expressed surface DPPIV enzyme activity but that mutant CD26-transfected cells did not.

DPPIV Enzyme Activity and HIV-1 Infection. To define the role of the CD26/DPPIV molecule in HIV-1 infection, the transfected Jurkat cell lines were assessed for their relative susceptibility to infection *in vitro* by a limiting multiplicity of infection of cell-free HIV-1. Since the doubling time of each of these cell lines varied, cell numbers were adjusted every 3–4 days in each line to a density of 2.5×10^5 cells per ml.

Three representative experiments are shown in Table 1. In Exp. 1, one of the mutant CD26-transfected cell lines (D28) was readily infected by both the IIIb and MN strains of HIV-1. The other mutant CD26 transfectant, 3-13, and the CD4$^+$CD26$^-$ parental cells could be infected by HIV-1 IIIb but not HIV-1 MN. Both wild-type CD26-transfected cell lines (nos. 11 and 23) were relatively resistant to HIV-1 infection. In Exp. 2, both the parental (CD26$^-$) cells and mutant CD26 (DPPIV$^-$)-transfected Jurkat cells (3–13) were readily infected with both the IIIb and MN isolates of HIV-1. Interestingly, another mutant CD26 (DPPIV$^-$)-transfected Jurkat cell line, D28, which was the most readily infected in Exp. 1, did not survive following infection. On the other hand, both wild-type CD26-transfected cell lines, 11 and 23, were relatively resistant to HIV-1 infection. In Exp. 3, mutant CD26-transfected cells (D28) and the parental cell lines were readily infected, whereas wild-type CD26-transfected cells (line 23) were relatively resistant to infection. These results indicate that CD4$^+$CD26$^-$ parental Jurkat

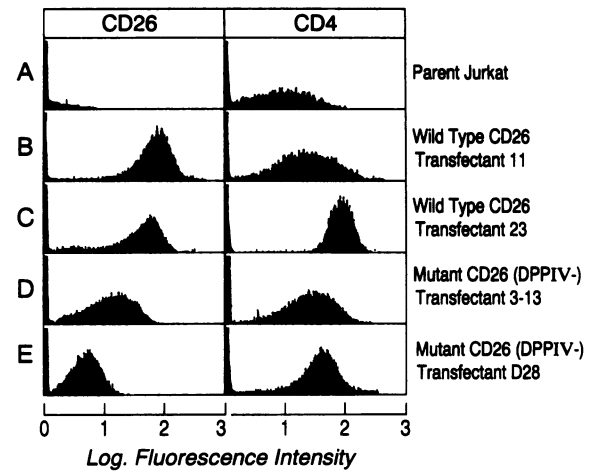

FIG. 1. Cell surface expression of CD26 and CD4 on parental Jurkat cells (*A*), wild-type CD26$^+$ (DPPIV$^+$) transfectants 11 (*B*) and 23 (*C*), mutant CD26$^+$ (DPPIV$^-$) transfectant, 3-13 (*D*), and mutant CD26$^+$ (DPPIV$^-$) transfectant D28 (*E*). Cell surface expression of CD26 and CD4 was assessed by indirect fluorescence using anti-CD26 mAb 1F7 and anti-CD4 mAb 19thy, with mouse immunoglobulin as a negative control. The *y* axis represents relative cell number.

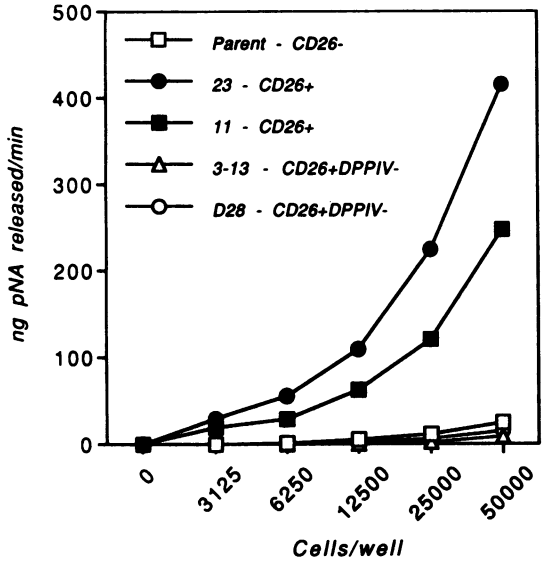

FIG. 2. Membrane-associated DPPIV activity of Jurkat transfectants. The indicated number of cells were incubated for 2 hr with glycylproline *p*-nitroanilide *p*-tosylate. Release of *p*-nitroaniline (pNA) was measured by absorbance at 405 nm with a microplate spectrophotometer (Micro Devices) and activity was calculated by comparison with a standard curve for *p*-nitroaniline.

T cells can be infected with HIV-1 and that the DPPIV enzyme activity of CD26 conferred a relative resistance to HIV-1 infection in this system.

Induction of Apoptosis in Various CD26-Transfected Jurkat Cells. It has been proposed that T cells in HIV-1-infected individuals are programmed for death and that upon activation they die due to apoptosis (25, 26). Since one of the mutant CD26 ($DPPIV^-$)-transfected Jurkat cell lines (D28) was susceptible to death following HIV-1 infection, we next examined the expression of CD95 (Fas/Apo-1) on parental Jurkat cells and on wild-type and mutant CD26-transfected cells. CD95 is a member of the nerve growth factor/tumor necrosis factor receptor family of proteins and can mediate apoptosis in certain transformed cell lines (20, 23, 27–29). CD95 was strongly expressed on all three CD26 mutant Jurkat cells tested and on parental ($CD26^-$) Jurkat cells (Fig. 3). CD95 was weakly expressed on all three wild-type $CD26^+$ ($DPPIV^+$) Jurkat cells tested. In all experiments to date, we have noted a variable expression of CD95 on mutant $CD26^+$ ($DPPIV^-$) transfectants, but it has always been higher than that found on wild-type $CD26^+$ ($DPPIV^+$) transfectants. Next we examined the occurrence of anti-CD95-induced programmed cell death in the above transfected cell lines. For this purpose, cells were treated with anti-CD95 mAb at 0.25 μg/ml and 0.5 μg/ml and percent cell death was assessed by flow cytometry. In a representative experiment (Fig. 4), both doses of anti-CD95 antibody appeared to enhance apoptosis in mutant $CD26^+$ ($DPPIV^-$) cells, whereas wild-type $CD26^+$ ($DPPIV^+$) Jurkat transfectants and parental cells were more resistant to the induction of apoptosis. These results may help explain the sensitivity to cell death of mutant $CD26^+$ Jurkat cells (D28) following HIV-1 infection.

DISCUSSION

Both $CD26^-$ parental and $CD26^+$ mutant ($DPPIV^-$) Jurkat T cells were readily infected by HIV-1 whereas wild-type $CD26^+$ ($DPPIV^+$) Jurkat T cells were more resistant to infection. In addition, mutant CD26-transfected Jurkat cells (D28) were more susceptible to programmed cell death following HIV-1 infection and expressed significantly more cell surface CD95 (Fas/Apo-1) antigen than did wild-type Jurkat cells. These findings suggest that DPPIV enzyme activity may play a role in programmed cell death and protection against HIV-1 infection.

Several reports have shown a selective decrease in the proportion of $CD26^+$ cells among the $CD4^+$ cell population in HIV-1 infected individuals prior to a general reduction in $CD4^+$ T cells (13, 14). One explanation to account for these findings is the proposal by Hovanessian and coworkers (18) that CD26 serves as an essential cofactor for HIV-1 entry. These authors showed that both CD26 and CD4 were required to render murine NIH 3T3 cells infectible by HIV-1. In addition, they demonstrated that entry of HIV-1 was inhibited by both CD4 and a specific mAb against CD26 (1F7) and by specific inhibitors of CD26/DPPIV. Although these activities seemed to parallel one another, earlier studies from our laboratory indicated that mAb 1F7 neither reacted with the catalytic site of DPPIV nor affected DPPIV enzyme activity (5, 19). In addition, we have been unable to demonstrate that 1F7 binds directly to cell surface CD4, blocks the binding of anti-CD4 mAb, or binds to either immobilized recombinant CD4 or HIV envelope glycoprotein gp120 (data not shown). In addition, we found no evidence to support the view that gp120 either binds to or is a substrate for recombinant soluble CD26, arguing strongly against the suggestion that DPPIV has endopeptidase activity capable of cleaving the V3 loop of gp120 during HIV-1 entry. Moreover, it is clear from Table 1 that parental $CD26^-$ Jurkat cells can be infected with HIV-1 despite a lack of CD26 antigen expression or measurable DPPIV enzymatic activity. Others have shown that CD26 is not required for syncytium formation and some have been unable to confirm Hovanessian and coworkers' studies (18) on viral entry (30–32). Taken together, these data suggest that CD26 is not a necessary cofactor for HIV infection.

Unexpectedly, our results suggest that DPPIV enzyme activity confers relative resistance to HIV-1 infection. These results support earlier studies demonstrating a differential susceptibility of subsets of CD4 cells to HIV-1 infection (11, 12). Although phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes that express both CD4 and CD26 can be infected with HIV-1, in our present experiments Jurkat transfectants with a comparable phenotype expressing wild-type CD26 were relatively resistant when compared with either the parental Jurkat cell lacking CD26 or the transfectants expressing mutant CD26. Further studies are needed to clarify the mechanisms responsible for this differential susceptibility to HIV-1 infection. Perhaps the distinction between wild-type $CD26^+$ transfectants and PHA blasts is that the transfectants stably express very high levels of cell surface CD26/DPPIV whereas in PHA blasts both CD4 and CD26 can be readily modulated off the surface. It is also possible that the distinctions between our findings and those of Hovanessian and colleagues (18) could be attributable to different assay systems. Possibly both the noncatalytic and the DPPIV enzymatic sites of CD26 can still promote virus entry, but subsequent virus replication within cells is negatively regulated by DPPIV enzyme activity. Finally, the known roles of CD26/DPPIV in T-cell activation, signaling, and membrane function may also be key to viral replication (8, 9, 19, 33).

In the asymptomatic phase of HIV infection, a diminished response to recall antigens and loss of the $CD29^+$ $CD45RO^+$ (memory) $CD4^+$ cells have been amply demonstrated (11, 12). The mechanism by which HIV infection inhibits selective immunologic function at this stage of the disease is still not fully resolved. One plausible explanation is the preferential infection with HIV-1 of the $CD4^+$ memory ($CD29^+$-$CD45RO^+$) T cells, which are known to be responsive to recall antigens (34, 35). More recently, Blazquez *et al.* (13) not only detected a selective loss of $CD26^+$ T cells prior to a

Table 1. *In vitro* HIV-1 infection of CD26-transfected Jurkat cell lines

Exp.	Cell line*	p24 antigen, pg/ml											
		HIV-1 MN (2 $TCID_{50}$)				HIV-1 IIIb (5 $TCID_{50}$)				HIV-1 IIIb (2 $TCID_{50}$)			
		d5	d8			d5	d8			d5	d8		
1	A	10.0	17.6			4.0	0			6.5	0		
	B	0.8	0			0.9	0			2.0	0.3		
	C	2.1	0			0.4	0			1.8	0.1		
	D	10.2	20.9			11.2	28.4			3.9	0		
	E	102	>600			217	209			17.5	130.8		
		d3	d6	d11	d14	d3	d6	d11	d14	d3	d6	d11	d14
2	A	13.2	556	584	520	0	497	576	565	3.2	76	565	564
	B	1.1	2.7	3.3	2.2	0	0.4	2.5	0	1.3	1.3	4.0	6.1
	C	1.1	2.8	58	130	0	11	28	0	0	1.3	11	12
	D	0	10.7	>600	562	0.2	2.4	>600	468	0	3.0	488	550
	E†	—	—	—	—	—	—	—	—	—	—	—	—
		d3	d6	d9									
3	A	0.4	63.2	111.8									
	B		ND										
	C	0.6	0	0									
	D		ND										
	E	24	171.2	349.5									

Jurkat transfectants maintained in culture at 2.5×10^5 cells per ml, were incubated overnight with HIV-1 IIIb [5 or 2 median tissue culture infective doses ($TCID_{50}$)] or MN (2 $TCID_{50}$), washed, and placed in culture with fresh medium (RPMI 1640 with 10% fetal bovine serum). Culture supernatants were harvested every 3–4 days and replaced with fresh medium. p24 in culture supernatant on the indicated days (d) was determined by ELISA. ND, not done.

*A, parental Jurkat; B, wild-type $CD26^+$ ($DPPIV^+$) transfectant 11; C, wild-type $CD26^+$ ($DPPIV^+$) transfectant 23; D, mutant $CD26^+$ ($DPPIV^-$) transfectant 3-13; E, mutant $CD26^+$ ($DPPIV^-$) transfectant D28.

†Cell line died following infection.

general reduction of $CD4^+$ T cells but, more importantly, also demonstrated that HIV-1 preferentially infected the $CD4^+CD26^-$ T-cell subset as measured by higher levels of HIV-1 DNA in this population (13). These authors concluded that $CD26^-$ T cells were a principal reservoir of HIV-1 *in vivo*. Since the $CD4^+CD29^+$ subset of T cells extensively overlaps with the $CD4^+CD26^+$ population, it also suggests that the $CD4^+CD29^+CD26^-$ population may be an important reservoir for HIV infection. Our findings that $CD4^+CD29^+$ wild-type $CD26^+$ ($DPPIV^+$) Jurkat cells were relatively resistant to HIV-1 infection whereas both $CD4^+CD29^+CD26^-$

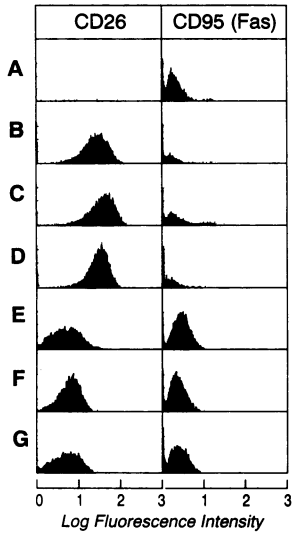

FIG. 3. Cell surface expression of CD26 and CD95 (Fas/Apo-1) on parental Jurkat cells (*A*), wild-type $CD26^+$ ($DPPIV^+$) transfectant 11 (*B*), wild-type $CD26^+$ ($DPPIV^+$) transfectants 23 (*C*) and 37 (*D*), and mutant $CD26^+$ ($DPPIV^-$) transfectants 3-13 (*E*), D11 (*F*), and D28 (*G*). Cell surface expression of CD26 on representative transfectants was assessed by indirect fluorescence using anti-CD26 mAb 1F7 and anti-CD95 mAb 7C11.

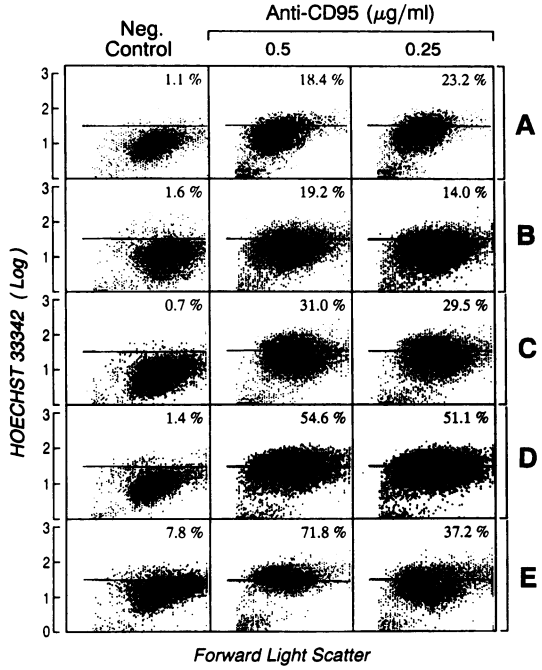

FIG. 4. Induction of apoptosis in various CD26-transfected Jurkat cells by anti-CD95 mAb. Cells were incubated overnight at 37°C in medium alone or in the presence of anti-CD95 mAb 7C11 at final concentrations of 0.5 and 0.25 μg/ml. The cells were then stained with Hoechst 33342 and propidium iodide and analyzed on a flow cytometer (EPICS Elite, Coulter). The percentages of apoptotic cells with higher levels of Hoechst 33342 fluorescence are indicated. (*A*) Parental Jurkat cells. (*B* and *C*) Wild-type $CD26^+$ ($DPPIV^+$) transfectants 23 and 11, respectively. (*D* and *E*) Mutant $CD26^+$ ($DPPIV^-$) transfectants 3-13 and D28, respectively.

and $CD4^+CD29^+$ mutant $CD26^+(DPPIV^-)$ cells were susceptible supports this point of view. Given these findings, how can one explain both the selective reduction of $CD26^+$ T cells and response to recall antigens in asymptomatic HIV-1-infected individuals? It has been proposed that viral infection or the abnormal priming of noninfected CD4 cells in AIDS patients leads to selective cell loss through apoptosis (25, 26). Indeed, *in vitro* activation of mature T cells from asymptomatic, HIV-1-infected individuals by polyclonal activators, bacterial superantigens, or antibody to the T-cell antigen receptor readily induces apoptosis (36–38). Even crosslinking of bound gp120 to normal $CD4^+$ T cells primes these cells for apoptosis through T-cell receptor signaling (39). A more recent association of apoptosis and AIDS has been made through the demonstration that CD95 (Fas/Apo-1), a marker known to trigger apoptosis, was strongly expressed on T lymphocytes from HIV-1-infected individuals (40). Although not detected on normal resting T cells, CD95 is expressed on and can induce apoptosis in activated T cells with a $CD45RO^+$ helper phenotype (41). These cells are also known to exhibit CD26 and participate in the response to recall antigens (2, 35). We suggest that the HIV Tat protein can contribute to both diminished immune responsiveness and enhanced susceptibility to the induction of apoptosis through its binding to CD26. The HIV-1 Tat protein is known to be essential for transactivation of viral genes as well as for viral replication (15) and can also be detected in the sera of HIV patients (16, 17). HIV-1 Tat has also been shown to suppress the response of human peripheral T cells to recall antigen *in vitro* (16, 17), a property of the $CD4^+CD26^+$ T cell. More importantly, HIV-1 Tat protein can bind to CD26 and partially inhibit DPPIV enzyme activity (16). As shown in Fig. 3, we found not only that the CD95 molecule was strongly expressed on mutant $CD26^+$ $(DPPIV^-)$ T-cell lines but that these $CD26^+$ $(DPPIV^-)$ enzyme-deficient lines were highly susceptible to both HIV-1 infection and the induction of apoptosis. Our *in vitro* observations suggest an important role for CD26 in HIV infectivity and provide a clinically relevant mechanism to partially explain both the selective loss of $CD4^+$ helper cells and helper cell function in asymptomatic HIV-infected patients.

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