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Anti-HIV-1 and chemotactic activities of human stromal cell-derived factor 1α (SDF-1α) and SDF-1β are abolished by CD26/dipeptidyl peptidase IV-mediated cleavage

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ABSTRACT **CD26 is a leukocyte-activation antigen that is expressed on T lymphocytes and macrophages and possesses dipeptidyl peptidase IV (DPPIV) activity, whose natural substrates have not been identified yet. CXC chemokines, stromal cell-derived factor 1α (SDF-1α) and 1β (SDF-1β), sharing the receptor CXCR-4, are highly efficacious chemoattractants for resting lymphocytes and CD34$^+$ progenitor cells, and they efficiently block the CXCR-4-mediated entry into cells of T cell line tropic strains of HIV type 1 (HIV-1). Here we show that both the chemotactic and antiviral activities of these chemokines are abrogated by DPPIV-mediated specific removal of the N-terminal dipeptide, not only when the chemokines are produced in transformed mouse L cell line to express human CD26 but also when they were exposed to a human T cell line (H9) physiologically expressing CD26. Mutagenesis of SDF-1α confirmed the critical requirement of the N-terminal dipeptide for its chemotactic and antiviral activities. These data suggest that CD26-mediated cleavage of SDF-1α and SDF-1β likely occurs in human bodies and promotes HIV-1 replication and disease progression. They may also explain why memory function of CD4$^+$ cells is preferentially lost in HIV-1 infection. Furthermore, CD26 would modulate various other biological processes in which SDF-1α and SDF-1β are involved.**

Stromal cell-derived factor (SDF-1), also named pre-B-cell growth-stimulating factor (PBSF), is a member of the CXC chemokine family (1, 2). Chemokines constitute a large family of small chemotactic cytokines. There are two subfamilies of chemokines, CC-chemokines and CXC-chemokines, which differ in the spacing of the first two cysteine residues (3). The CC-chemokine subfamily includes macrophage inflammatory peptide 1α and 1β (MIP-1α and MIP-1β) and regulated on activation normal T cell expressed and secreted (RANTES) protein. The CXC-chemokine subfamily includes interleukin 8 (IL-8) and platelet factor 4 (PF4), in addition to SDF-1. Of these chemokines, SDF-1 appears to be most efficacious as a chemoattractant on resting T lymphocytes and monocytes (4). SDF-1 arises in two forms, SDF-1α and SDF-1β, by differential splicing from a single gene (5).

Both CC- and CXC-chemokines are now attracting growing attention, because their receptors were proved to be used as coreceptors for HIV type 1 (HIV-1) to enter susceptible CD4 (receptor)-expressing target cells by a mechanism of envelope fusion with cellular membrane (6–11) and because they competitively block this virus–cell interaction (12–16). Coreceptor usage by viruses and virus inhibition by the respective chemokines are strain specific. For instance, macrophage tropic/non-syncytium-inducing strains use CCR-5 for their entry into cells (6–10) and their infection can be blocked by the corresponding ligands, MIP-1α, MIP-1β, and RANTES (12, 14, 16). On the other hand, CXCR-4 serves as a coreceptor for T cell line tropic/syncytium-inducing strains (11), and its ligand SDF-1 can block the infection (13, 15). The emergence of the latter type of HIV-1 is closely associated with progression to AIDS in HIV-1-infected individuals, whereas the former ones predominate early in infection (17–19).

Many chemokines, including SDF-1α and SDF-1β, possess a proline residue at the second position from the N terminus. This residue conforms to the substrate specificity of dipeptidyl peptidase IV (DPPIV), because it specifically cleaves the first two amino acids from peptides carrying proline or alanine at the second position (20). DPPIV is also known as leukocyte activation marker CD26, which is a 110-kDa glycoprotein expressed on the surface of CD4$^+$ T lymphocytes (21). CD26 is capable of delivering a potent costimulatory T cell activation signal (22), and it is strongly associated with the development of immunological memory (23). The DPPIV enzyme activity of CD26 is known to play an important role in CD26-mediated T cell costimulation (24). It is also reported that CD26 interacts with CD45 protein tyrosine phosphatase and adenosine deaminase (ADA) (25, 26). Moreover, the soluble form of DPPIV activity is readily detectable in human sera (27), suggesting its ubiquitous action in the body.

In this report, we demonstrate that human CD26 inactivates both the chemotactic and antiviral capabilities of SDF-1α and SDF-1β by specifically cleaving their N-terminal dipeptide, raising the possibility of its role *in vivo* in facilitating replication of HIV-1 of T cell-line-tropic phenotype and promoting progression to AIDS.

MATERIALS AND METHODS

Viruses and Cells. HIV-1 strain NL43 (28) was grown in the MT4 T cell line. MT4 and H9 T cell lines were grown in RPMI medium 1640 supplemented with 10% fetal bovine serum

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Abbreviations: SDF-1α and -β, stromal cell-derived factor 1α and -β; MIP-1α and -1β, macrophage inflammatory peptide 1α and 1β; RANTES, regulated on activation normal T cell expressed and secreted; DPPIV, dipeptidyl peptidase IV; SeV, Sendai virus; PBL, peripheral blood lymphocytes.

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(FBS). Murine fibroblast L and monkey kidney CV1 cells were grown in minimal essential medium (MEM) supplemented with 10% FBS.

Chemokines. Recombinant human SDF-1α and SDF-1β were produced as described previously (29). Briefly, human SDF-1α or SDF-1β genes were inserted just upstream of the open reading frame of the 3′-proximal *N* gene of Sendai virus (SeV), a nonsegmented negative-strand RNA virus, according to the method described previously (30–32) to generate recombinant SeV, SeV/SDF-1α or SeV/SDF-1β. CV1 cells were infected with SeV/SDF-1α, SeV/SDF-1β, or wild-type SeV at a multiplicity of infection (moi) of 10 plaque-forming units per cell and maintained in serum-free MEM. After 72 hr at 37°C, culture media were harvested, and SeV virions were removed by centrifugation. Resultant culture supernatant, which contained approximately 3 μg/ml SDF-1α or SDF-1β, was used in this study as SDF-1α or SDF-1β preparation. Culture supernatant from CV1 cells infected with wild-type SeV was always used as a negative control.

Generation of a Recombinant SeV. The mutant SDF-1α gene lacking the N-terminal dipeptide was generated by site-directed mutagenesis by two successive PCRs using a *Not*I-tagged primer pair. After digestion with *Not*I, the amplified fragments were directly introduced to the *Not*I site of pSeV18$^+$b(+) (31), generating pSeVSDF-1αN-2(+). All PCRs were performed with ExTaq DNA polymerase (Takara, Tokyo), and the sequences of all the PCR products were verified. Recombinant SeV was recovered from pSeVSDF-1αN-2(+) essentially according to the previously described procedures (30–32). The second passages in embryonated chicken eggs were used as the stock viruses for all the experiments.

Generation of Mouse L Cells Expressing Human CD26. L cells were transfected with the CD26 expression plasmid pSRα-CD26 (24) and pHyg (33), which confers hygromycin B resistance, with *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methylsulfate (DOTAP; Boehringer Mannheim). Cells were maintained in the presence of 200 μg/ml hygromycin B, and resultant colonies were assayed for the expression of CD26 by Western blotting using a monoclonal antibody against CD26, 1F7 (21).

Fluorescence-Activated Cell Sorting Analysis. Approximately 1×10^6 MT4 or H9 cells were incubated with 2 μg/ml SDF-1α for 1 hr at 37°C, washed once with PBS, and subsequently incubated with anti-CXCR-4 monoclonal antibody IVR7 (34) at a concentration of 40 μg/ml in PBS containing 2% BSA for 1 hr at room temperature. Cells were washed three times with PBS and incubated with fluorescein-5-isothiocyanate-conjugated sheep affinity-purified F(ab′)$_2$ fragments to mouse IgG (Organon Teknika) for 1 hr. Stained cells were washed, fixed in 1% formaldehyde in PBS, and analyzed by FACScan (Becton Dickinson).

Western Blotting. Culture supernatant of infected cells were electrophoresed in SDS/15% polyacrylamide gels. The resolved proteins were electrotransferred onto poly(vinylidene difluoride) (PVDF) membranes (Millipore, Bedford) and probed with anti-SDF-1 antiserum, which was prepared by immunizing rabbits with the multiple-antigen peptide containing residues 33–45 (RFFESHVARANVK). The peptide was synthesized by Research Genetics (Huntsville, AL) (35).

Chemotaxis Assay. Lymphocyte chemotaxis assays were performed according to the method described by Bluel *et al.* (4). Briefly, human peripheral blood lymphocytes (PBL) were obtained from healthy donors by the Ficoll/Histpaque method. Monocytes were removed by a 1-hr step of plastic adherence. Cells (5×10^5) in 100 μl of RPMI medium 1640 containing 0.25% human serum albumin were added to the upper chamber of a 5-μm pore polycarbonate Transwell culture insert (Costar) and incubated with the chemokines for 3 hr. Transmigrated cells were counted with a FACScan for 20 sec at 60 μl/min. Data points are means of triplicate determinations.

Anti-HIV-1 Assay. MT4 or H9 cells (5×10^5) were incubated with indicated concentrations of chemokines for 1 hr, and then exposed to 1,000 50% tissue culture infective dose of HIV-1 for 2 hr at 37°C. The cells were washed twice with RPMI medium 1640 and maintained in the culture medium containing indicated concentrations of chemokine. Culture supernatants of the infected cells were assayed for the levels of p24 core antigen (Abbott, Wiesbaden-Delkenheim, Germany). Data points are the means of duplicate cultures.

RESULTS

Different Effects of SDF-1α on MT4 and H9 T Cell Lines. When the expression of CXCR-4, the receptor for SDF-1α and SDF-1β, on the surface of two different T cell lines, MT4 and H9, was compared by flow cytometry using anti-CXCR-4 antibody (34), both cell lines were found to express significant amounts of CXCR-4 (Fig. 1*A*). The fluorescence intensity was nearly 2-fold higher for H9 cells than for MT4 cells. Thus, these two cell lines were susceptible to infection with a T cell line-tropic HIV-1 strain, NL43, which uses CXCR-4 as the coreceptor. Consistent with its coreceptor usage, the replication of HIV-1 strain NL43 was inhibited by the anti-CXCR-4 antibody in both of these cell lines (Fig. 1*B*). However, greatly different patterns of virus inhibition by SDF-1α were found between the two cells. In MT4 cells, SDF-1α almost completely suppressed the viral replication at a concentration of 100 ng/ml (Fig. 1*B*), whereas at the same and 2-fold higher concentrations (200 ng/ml, data not shown), SDF-1α could not

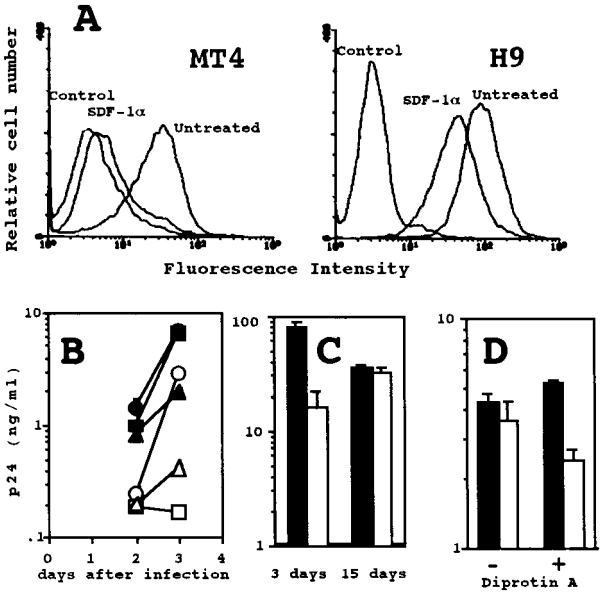

FIG. 1. Differential effects of SDF-1α on MT4 and H9 T cell lines. (*A*) MT4 and H9 cells were treated with or without 2 μg/ml SDF-1α for 1 hr at 37°C, labeled with anti-CXCR-4 antibody, and analyzed by flow cytometry. Control indicates cells stained with secondary antibodies alone. (*B*) MT4 (open symbols) and H9 (closed symbols) cells were mock-treated (circles) or treated with 100 ng/ml SDF-1α (squares) or 10 μg/ml anti-CXCR-4 antibody (triangles) and then infected with the NL43 strain of HIV-1. The culture supernatants were periodically assayed for the levels of p24 core antigen. (*C*) Three-day-old and 15-day-old PBL were mock-treated (open bars) or treated with SDF-1α (solid bars) and then infected with NL43 as described above. The levels of p24 antigen production 6 days after infection are shown. (*D*) H9 cells were mock-treated (open bars) or treated with SDF-1α (solid bars) and then infected with NL43 as described above. Diprotin A (4 mM) was always included in this experiment. The levels of p24 antigen production 4 days after infection are shown.

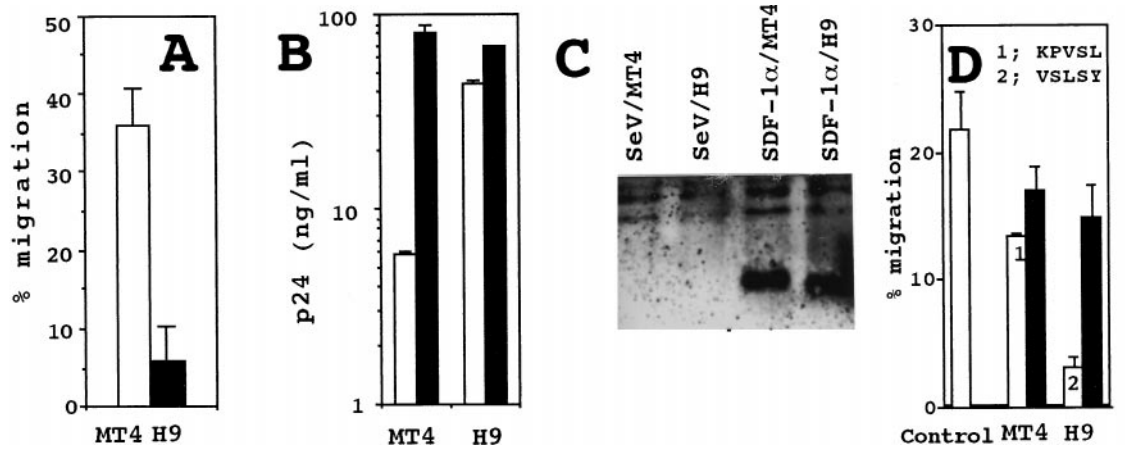

FIG. 2. Lack of chemotactic and anti-HIV-1 activities in SDF-1α produced by H9 cells or exposed to the same cells. (*A*) Culture supernatants of MT4 or H9 cells infected with the wild-type SeV or SeV/SDF-1α were diluted 4-fold and assayed for chemotactic activity. (*B*) MT4 cells were treated with 4-fold-diluted culture supernatants of H9 or MT4 cells infected with the wild-type SeV or SeV/SDF-1α and then infected with NL43. The levels of p24 antigen production 4 days after infection are shown. (*C*) Proteins in 250 μl of culture supernatant of H9 or MT4 cells infected with wild-type SeV (SeV) or SeV/SDF-1α (SDF-1α) were precipitated with ethanol and analyzed by Western blotting using anti-SDF-1 antiserum. (*D*) Two-hundred microliters of SDF-1α (2 μg/ml) was mixed with an equal volume of culture supernatant of MT4 or H9 cells (open bars) or with 1×10^6 MT4 or H9 cells in the same volume (solid bars). After incubation at 37°C for 16 hr, SDF-1α was recovered, diluted 5-fold, and assayed for chemotactic activity. The N-terminal amino acid sequences of SDF-1α incubated with MT4 (1) and H9 (2) cells are shown.

suppress the replication at all in H9 cells (Fig. 1*B*). Our previous study and studies by others have shown that CXCR-4 is endocytosed from the cell surface upon ligation with SDF-1α or SDF-1β (31, 36). This down-regulation of CXCR-4 was indeed observed by an extensive shift of fluorescence intensity after incubation of MT4 cells with SDF-1α (Fig. 1*A*). In contrast, such a clear-cut down-regulation of CXCR-4 did not occur for H9 cells, as evidenced by a high level of specific fluorescence remaining on the cell surface. These results suggested that H9 cells possess a certain mechanism to abrogate the antiviral effect of SDF-1α, even though they fully express the specific receptor CXCR-4.

Lack of Chemotactic and Anti-HIV-1 Activities in SDF-1α Produced by H9 Cells. The above-described remarkable differences in the effect of SDF-1α on MT4 and H9 cells prompted us to produce SDF-1α in these distinct cell lines and compare the biological activities. For this purpose, we used a recombinant SeV expressing human SDF-1α (SeV/SDF-1α) (29). SeV grows well in a wide variety of cells, particularly those of epithelial origin, reaching a high copy number in cells with a final titer of 10^8 plaque-forming units/ml. Cells of blood origin, including H9 and MT4, are also susceptible to SeV, although the virus yield is only 2×10^6/ml for these cells. Three days after infection with SeV/SDF-1α, the culture supernatants were assayed for chemotactic and anti-HIV-1 activities (Fig. 2 *A* and *B*). Culture supernatants of wild-type SeV-infected cells exhibited no chemotactic activity (data not shown). Very low or only marginal levels of chemotactic and anti-HIV-1 activities were detected in the culture supernatant of infected H9 cells, in contrast to that of MT4, which exhibited remarkably high levels of both activities. On the other hand, Western blot analysis with anti-SDF-1α antibody revealed the presence of comparable amounts of SDF-1α in the supernatants of MT4 and H9 cells infected with SeV/SDF-1α (Fig. 2*C*). However, we consistently noticed that the SDF-1α released from H9 cells migrated slightly faster than that from MT4 cells (Fig. 2*C*). This latter finding suggested some chemical modification of SDF-1α in H9 cells and its association with the abolished chemotactic and anti-HIV-1 activities.

Specific Cleavage of the N-Terminal Dipeptide of SDF-1α by H9 Cells. H9 cells are known to possess surface markers characteristic of activated T lymphocytes (37), whereas MT4 cells possess those characteristic of unactivated T lymphocytes. CD26, which is one of the cell-surface markers of activated T lymphocytes, was indeed found to be expressed on H9 cells but not on MT4 cells (Fig. 3 *A* and *B*). This marker is also known to possess DPPIV activity, which cleaves the first two amino acids from polypeptides carrying proline or alanine at the second position. The N-terminal amino acid sequence of SDF-1α is KPVSL, which conforms to CD26 substrate specificity. We therefore speculated that the faster migration of SDF-1α from H9 cells would be because of specific cleavage of the dipeptide from its N terminus by CD26 on the H9 cell

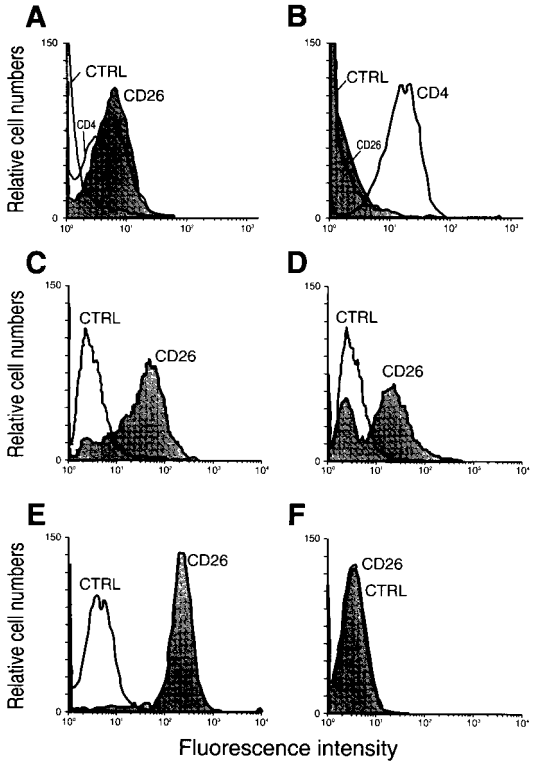

FIG. 3. Cell surface expression of human CD26. H9 (*A*), MT4 (*B*), 15-day-old PBL (*C*), 3-day-old PBL (*D*), L cell clone B5 expressing CD26 (*E*), and clone C3 (*F*) were stained with monoclonal antibody against human CD26 or CD4 (Leu3A) and analyzed by flow cytometry. CTRL indicates cells stained with secondary antibodies alone.

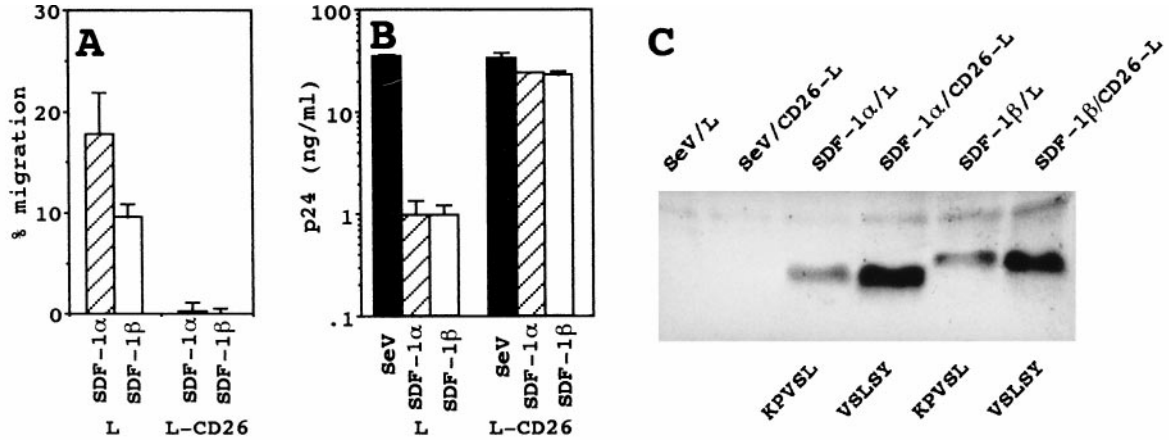

FIG. 4. Inactivation of SDF-1α by specific cleavage of CD26/DPPIV. (*A*) Tenfold-diluted culture supernatants of clone C3 (L) or B5 (L-CD26) infected with SeV/SDF-1α or SeV/SDF-1β were assayed for the level of chemotactic activity. (*B*) MT4 cells were treated with tenfold-diluted culture supernatants of clone C3 or B5 infected with the wild-type SeV (SeV), SeV/SDF-1α (SDF-1α), or SeV/SDF-1β (SDF-1β) and then infected with NL43. The levels of p24 antigen production 4 days after infection are shown. (*C*) Proteins in 250 μl of culture supernatant of clone C3 or B5 infected with the wild-type SeV, SeV/SDF-1α, or SeV/SDF-1β were precipitated with ethanol and analyzed by Western blotting using anti-SDF-1 antiserum. The N-terminal amino acid sequence of each protein band is shown.

surface. To verify this possibility, we exogenously added SDF-1α to H9 or MT4 cultures and incubated them at 37°C for 16 hr. Comparable amounts of SDF-1α could be recovered from both cultures (data not shown), and they were used for the chemotactic assay and the N-terminal amino acid sequence analysis. The results showed that the SDF-1α incubated with H9 cells possessed greatly reduced chemotactic activity and the N-terminal sequence VSLSY (Fig. 2*D*), identical to that of SDF-1α lacking the N-terminal dipeptide. In contrast, the dipeptide and chemotactic activity were retained in the SDF-1α incubated with MT4 cells (Fig. 2*D*). Incubation of SDF-1α with the culture supernatant of H9 cells did not affect the chemotactic activity (Fig. 2*D*). These results strongly suggested that CD26/DPPIV naturally expressed on the surface of H9 cells specifically cleaved the N-terminal dipeptide of SDF-1α, and that this cleavage could be associated with the loss of chemotactic activity.

Further Verification of SDF-1α Inactivation Because of Specific Cleavage by CD26/DPPIV. We established two stably transformed L cell clones, A4 and B5, which were positive for cell surface CD26 expression in more than 95% of the population (Fig. 3*E*). Both the parental line and the clone C3 obtained from mock-transfected culture were totally negative for CD26 expression (Fig. 3*F*). Western blotting of these transformed and control cell lines confirmed the specificity of CD26 expression (data not shown). Both the wild-type SeV and SeV/SDF-1α grew in these cell clones to comparable titers of approximately 10^7 plaque-forming units/ml. The culture supernatant of the CD26-negative control clone C3 infected with SeV/SDF-1α was found to contain chemotactic and anti-HIV-1 activities, whereas the supernatant from CD26-positive clone B5 totally lacked these activities (Fig. 4 *A* and *B*). Exactly as in the comparison of SDF-1α from H9 and MT4 cells, B5-derived SDF-1α migrated slightly faster in SDS/PAGE than that derived form C5, with the N-terminal sequence being VSLSY, and thus devoid of the N-terminal dipeptide (Fig. 4*C*). The dipeptide was retained in the SDF-1α produced by C5 cells (Fig. 4*C*). Exactly the same results were obtained, when clone A4, instead of clone B5, was used (data not shown).

To further substantiate that the removal of N-terminal dipeptide but not other modifications is responsible for the loss of the chemotactic and anti-HIV-1 activities of SDF-1α, we generated a new recombinant SeV expressing SDF-1α lacking the N-terminal dipeptides (SeV/SDF-1αN-2) by deleting six bases encoding the dipeptide. This mutant SDF-1α was expressed in infected cells and secreted into the culture supernatant as efficiently as the authentic SDF-1α (Fig. 5*A*). The lack of dipeptide in the mutant SDF-1α was confirmed directly by sequencing the product (Fig. 5). As expected, mutant SDF-1α exhibited neither chemotactic (Fig. 5*B*) nor anti-HIV-1 (Fig. 5*C*) activity. The same conclusion was recently reached by Crump *et al.* (38). The mutant SDF-1α was further found not to interfere with the chemotactic or anti-HIV-1 activity of intact SDF-1α even in the presence of 10-fold excess (data not shown). Taken together, these results unequivocally demonstrated that CD26/DPPIV specifically cleaved the N-terminal dipeptide from SDF-1α and thereby inactivated its chemotactic and antiviral activities.

Inverse Correlation of the Level of CD26 Expression with Sensitivity to SDF-1α. The level of CD26 expression in primary T lymphocytes increases gradually as activation progresses (27). To know whether there is a correlation between the level of CD26 and resistance to SDF-1α-mediated

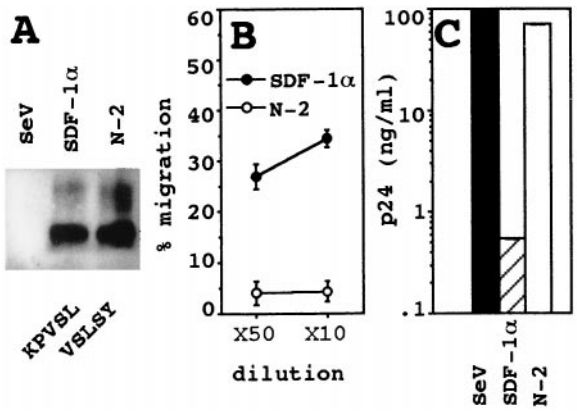

FIG. 5. SDF-1α lacking the N-terminal dipeptide does not possess chemotactic and anti-HIV-1 activities. (*A*) Proteins in 100 μl of culture supernatant of CV1 cells infected with the wild-type SeV (SeV), SeV/SDF-1α (SDF-1α), or SeV/SDF-1αN-2 (N-2) were precipitated with ethanol and analyzed by Western blotting. The N-terminal amino acid sequence of each protein band is shown. (*B*) Culture supernatants of CV1 cells infected with the SeV/SDF-1α or SeV/SDF-1αN-2 were diluted as indicated and assayed for the level of chemotactic activity. (*C*) MT4 cells were treated with 40-fold-diluted culture supernatants of CV1 cells infected with wild-type SeV, SeV/SDF-1α, or SeV/SDF-1αN-2 (N-2) and then infected with NL43. The levels of p24 antigen production 4 days after infection are shown.

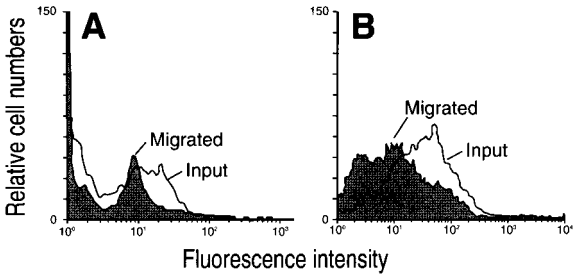

FIG. 6. PBL migrated toward SDF-1α (Migrated) and input cells (Input) were stained with monoclonal antibody against human CD26 (*A*) or CXCR-4 (*B*) and analyzed by flow cytometry.

HIV-1 suppression, we compared the anti-HIV-1 effect of SDF-1α in PBL that were in different activation stages. Three days after stimulation with phytohemagglutinin and interleukin 2, nearly 50% of PBL were still CD26 negative (Fig. 3*D*), whereas more than 90% were CD26 positive 15 days later (Fig. 3*C*). The HIV-1 growth in the former cells was moderately inhibited by the addition of 100 ng/ml SDF-1α but there was virtually no inhibition at the same concentration in the latter (Fig. 1*C*). Furthermore, addition of 4 mM diprotin A, a potent inhibitor for DPPIV (37), to infected H9 cell culture significantly increased the antiviral action of SDF-1α (Fig. 1*D*). Thus, good inverse correlation was obtained between the levels of CD26 expression or function and the antiviral action of SDF-1α.

SDF-1α Preferentially Attracts Resting Lymphocytes Not Expressing CD26. To know whether $CD26^+$ and $CD26^-$ lymphocytes differently respond to SDF-1α, we compared the proportion of $CD26^+$ cells in the population that had migrated toward 200 ng/ml of SDF-1α and in the initial input population. As shown in Fig. 6*A*, cells with intense CD26-specific fluorescence, which were present in the input population, were almost absent in the attracted cell population. These results indicated that $CD26^+$ PBL were less efficiently attracted to SDF-1α than were $CD26^-$ PBL. Consistent with the fact that CXCR-4 molecules were endocytosed from the cell surface upon ligation with SDF-1α, the attracted cell population contained lower numbers of CXCR-4-positive cells than did the original input population (Fig. 6*B*).

SDF-1β Is also a Substrate for DPPIV Activity of CD26. The human and murine SDF-1 genes give rise to two forms, SDF-1α and SDF-1β, by alternative splicing. They differ in the C-terminal four amino acid residues, which are present in SDF-1β and absent from SDF-1α (5). We investigated whether SDF-1β can also be a substrate for DPPIV activity of CD26. A recombinant SeV expressing SDF-1β (SeV/SDF-1β) (29) and the L cell clone B5 were used to express SDF-1β in the presence of CD26. Similar to SDF-1α, SDF-1β produced by B5 exhibited no chemotactic and anti-HIV-1 activities (Fig. 4 *A* and *B*), migrated slightly faster in SDS/PAGE than that from the control C3 line, and was devoid of the N-terminal dipeptide (Fig. 4*C*). These data indicated that not only SDF-1α but also SDF-1β is a substrate for DPPIV activity of CD26.

DISCUSSION

In this report, we demonstrated that CD26/DPPIV-mediated cleavage of SDF-1α and SDF-1β abolished their chemotactic and anti-HIV-1 activities. This cleavage inactivation of SDF-1 may occur upon contact with cell surfaces expressing CD26 in the human body. Because the DPPIV activity is readily detectable in human sera, the cleavage inactivation can also occur even ubiquitously in the body. Thus, CD26/DPPIV-mediated inactivation of SDF-1α and SDF-1β is likely occurring in the body. If so, CD26 is an important factor determining the levels of functional SDF-1α and SDF-1β in the body, and thereby lowering the antiviral state. This view is highly relevant to the recent discovery that the particular genotype of SDF-1 gene retards progression to AIDS, probably because of higher levels of SDF-1 accumulation (39). Such increased SDF-1 restricts spread and growth of T cell line-tropic viruses generally dominating in a late stage of HIV-1 infection. One of the feasible approaches taken then will be to differentially measure the levels of intact SDF-1 and truncated inactive SDF-1 as well as the level of CD26/DPPIV in patients' sera and correlate these levels with disease stages. It will also be tempting to convert natural SDF-1 to a CD26-resistant form in designing SDF-1-based therapeutics.

Expression of CD26 is closely associated with development of immunological memory. Previous studies have shown that $CD4^+$ lymphocytes from HIV-1-infected individuals exhibit a qualitative defect in their ability to mount memory responses to previously encountered antigens, while their responses to nonspecific mitogens remain normal (40, 41). Although many studies have addressed this issue, the precise mechanism for preferential loss of memory function in HIV-1 infection has not been fully understood (42, 43). It can now be speculated that CD26-mediated inactivation of SDF-1α and SDF-1β facilitates HIV-1 replication in memory cells rather than in naive cells, and thus contributes at least partly to the preferential loss of memory function in HIV-1 infection.

In view of the presence of a soluble form of DPPIV in human sera (27), DPPIV could help HIV-1 replication even in CD26-negative naive $CD4^+$ cells. Persistent immune activation frequently observed in HIV-1-infected individuals (44) could facilitate HIV-1 replication not only by increasing numbers of activated T lymphocytes, which are highly susceptible to HIV-1 infection, but also by up-regulating CD26, which inactivates SDF-1α and SDF-1β.

SDF-1 efficaciously attracts monocytes, resting lymphocytes (4), and $CD34^+$ hematopoietic progenitor cells (45), and it promotes the growth of B-cell progenitors (2). The mice lacking SDF-1 gene exhibit severe impairment in B-cell lymphopoiesis in both fetal liver and bone marrow, whereas myelopoiesis was impaired only in bone marrow and not in fetal liver (46). These results suggest that SDF-1 plays a critical role in B cell lymphopoiesis and migration of $CD34^+$ hematopoietic progenitor cells between fetal liver and bone marrow during development. In addition, SDF-1-deficient mice had a defect in cardiac ventricular septal formation. Thus, SDF-1 is multifunctional and hence its inactivation by CD26 could also be multiplex in its outcome. CD26 most likely modulates physiological functions of SDF-1α and SDF-1β by limiting the numbers and types of cells that respond to these chemokines. It will be interesting to analyze details of surface markers of cells migrating to SDF-1α and SDF-1β.

Not all but many CXC- and CC-chemokines possess a proline residue at the second position from the N terminus as do SDF-1α and SDF-1β, and thus they are potentially the natural substrates for CD26. Recently, Oravecz *et al.* (47) reported that removal of the N-terminal dipeptide of CC-chemokine RANTES by a soluble form of CD26 abolished its interaction with CCR1, but not with another RANTES receptor, CCR5. Because macrophage-tropic strains of HIV-1 use CCR5 but not CCR1 as the coreceptor, CD26-mediated cleavage of RANTES did not affect the anti-HIV-1 activity of RANTES. It is important to determine whether CD26-mediated cleavage alters the chemotactic and anti-HIV-1 activity of other chemokines such as MIP-1β and macrophage-derived chemokine (MDC) (48), which conform to the substrate specificity of CD26.

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