CD26/dipeptidyl peptidase IV differentially regulates the chemotaxis of T cells and monocytes toward RANTES: possible mechanism for the switch from innate to acquired immune response

Satoshi Iwata1, Noriko Yamaguchi1, Yasuhiko Munakata1, Hideto Ikushima1, James F. Lee2, Osamu Hosono3, Stuart F. Schlossman1 and Chikao Morimoto1,3

1Division of Tumor Immunology and 2Molecular Biology Core Facility (MBCF), Dana-Farber Cancer Institute; and Departments of 1Medicine and 2Molecular Biology, Harvard Medical School, Boston, MA 02115, USA
3Department of Clinical Immunology and AIDS Research Center, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108, Japan

Keywords: β-chemokine, CD26, dipeptidyl peptidase IV, RANTES, transendothelial migration

Abstract
CD26, a 110 kDa cell surface glycoprotein, exhibits dipeptidyl peptidase IV (DPPIV; EC 3.4.14.5) enzyme activity and plays an important role in T cell co-stimulation. In the present study, the function of CD26/DPPIV in transendothelial migration was examined using β-chemokines as chemoattractants. When soluble recombinant CD26 (sCD26/DPPIV) was added to the transendothelial chemotaxis system, chemotactic migration of T cells toward RANTES was significantly enhanced. Addition of sCD26 to 50 ng/ml of RANTES enhanced the migratory response by a factor of two compared to RANTES alone, whereas mutant soluble CD26 (mCD26), lacking the DPPIV enzyme activity, had no enhancing effect on RANTES-induced T cell migration. In the process of analyzing the mechanisms of the enhancement of T cell migration by sCD26, we showed that RANTES was cleaved by sCD26 under physiologic conditions at the precise site characteristic of its enzyme specificity. However, synthesized RANTES which lacks two N-terminal amino acids showed a chemotactic activity equivalent to full-length RANTES on T cells. Furthermore, addition of sCD26 showed enhancement of T cell migration induced by both forms of RANTES. In contrast to T cells, the truncated RANTES is inactive in chemotaxis of purified monocytes and supplement of sCD26 but not mCD26 reduced the migratory response of monocytes to RANTES. These results suggest that CD26/DPPIV differentially regulate the chemotactic response of T cells and monocytes to RANTES.

Introduction
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 (1–4). This ectoenzyme can cleave out an N-terminal dipeptide from a polypeptide with either L-proline or L-alanine at the penultimate position, although a significant physiological substrate remains to be identified (5). On T cells, CD26 expression is preferentially restricted to the CD4+ helper/memory population (2) and...
CD26 can deliver a potent co-stimulatory signal for T cell activation (6,7). Moreover, we demonstrated that DPPIV enzyme activity is essential for CD26-mediated T cell co-stimulation (7). From its cDNA sequence, CD26 is predicted to be a type II integral membrane protein with a large extracellular domain, a transmembrane segment and a cytoplasmic tail of six amino acids. It has been suggested that besides DPPIV enzyme activity, other signal inducing molecules might be associated with CD26 (8). Recently, we showed that CD26 physically associates with CD45, a membrane-linked protein-tyrosine phosphatase (9), and with adenosine deaminase (ADA) (10) presumably in its extracellular domain. Both molecules are thought to be important in T cell activation and signal transduction. Furthermore, we, as well as others, demonstrated that CD26 is identical to the ADA binding protein (10–12). In previous studies, it was shown that β-chemokines play a key role in T cell migration and that migrating T cells were those which expressed the highest levels of CD26 (13–17). These findings were consistent with other studies indicating that a major population of T cells was CD26 positive in inflamed tissues of patients with rheumatoid arthritis (18) and multiple sclerosis (19), again suggesting that CD26 might be involved in β-chemokines-induced transendothelial migration of T cells. The β-chemokines are 8–15 kDa soluble proteins produced and released from a variety of cell types in response to inflammatory reactions (20), and are thought to be major suppressers of macrophage-tropic HIV infection (21, 22). What is more important, the β-chemokine receptor CCR5 serves as a co-receptor of HIV-1 virus entry along with CD4 (23–26).

To evaluate the role of CD26 in chemotactic migration, we utilized a transendothelial chemotaxis assay system known to be sensitive, physiologically relevant and dependent on an endothelial cell layer. To circumvent the membranous nature of CD26, we utilized a soluble form of the recombinant protein (sCD26) which lacked a transmembrane domain and was secreted from transfected CHO cells (27). In addition, a mutant soluble CD26 (mCD26) which lacked DPPIV enzyme activity was also prepared by replacing the serine protease site; specifically, serine with alanine (7).

Here, we report that RANTES (regulated on activation, normal T expressed and secreted), a key member of the β-chemokine family (28), appears to be a physiological substrate for CD26/DPPIV. RANTES was digested at a site known to be specific for the DPPIV enzyme. Moreover, we describe that the soluble form of recombinant CD26 enhances the
Differential regulation of RANTES-induced migration of T cells and monocytes by CD26/DPPIV

Production and purification of sCD26 and mCD26

sCD26 was produced according to the method described previously (27). Briefly, the expression vector named RcSRα-26Δ3-9, which has the deletion of the coding sequence for amino acids 3–9 of CD26, was transfected into dihydrofolate reductase (DHFR)-deficient CHO cell line, DXB-11, by electroporation together with pMT-2 providing the DHFR gene. mCD26 was produced in the same method except that RcSRα-26Δ3-9 was further modified to yield RcSRα-26Δ3-9 S629A, which contains a point mutation at the active site of DPPIV enzyme (Ser629 was replaced by Ala) by site-directed mutagenesis using the oligonucleotide. The transfected CHO cells, which produce either sCD26 or mCD26, were cultured in serum-free CHO-S-SFM (Life Technologies, Grand Island, NY). The culture supernatant was collected and subjected to affinity chromatography on ADA–Sepharose according to the method described elsewhere (29).

Transendothelial chemotaxis assay

Recombinant human chemokines were obtained from PharMingen (San Diego, CA) or Peprotech (Rocky Hill, NJ). Human umbilical vein endothelial cells (HUVEC) and ECV304 cells were obtained from ATCC (Rockville, MD) and cultured on 6.5 mm diameter, type I collagen-coated cell culture inserts (Becton Dickinson, Bedford, MA) or Transwell cell culture chamber inserts (Corning Costar, Cambridge, MA) with a 3.0 µm pore size. Chemokines with or without sCD26 were diluted in assay medium consisting of RPMI 1640 and 0.6% BSA, then added to culture plates in a final volume of 600 µl. Endothelial cell-covered inserts were transferred to culture plates and 10⁶ of T cells or monocytes were added to each insert in a volume of 100 µl. A chemotaxis assay was performed by 37°C incubation for 4 h in 5% CO₂/95% air. Cells migrating to the bottom chamber were harvested and counted by flow cytometry for a set time period of 30 s (17) or directly counted using hemacytometer.

Enzymatic digestion of RANTES by sCD26 or mCD26 in vitro

RANTES (3 µg) was mixed with sCD26 or mCD26 at a molecular ratio of 1:1. The mixture was incubated in PBS (pH 7.5) at 37°C for 4 h. After incubation, sCD26 was removed from the reaction mixture using a Microcon-30 concentrator (Amicon, Bedford, MA) which cut off molecules above 30 kDa. The filtrate was collected and subjected to N-terminal sequencing. Alternatively, 1 µg of RANTES was incubated with sCD26 or mCD26 at 37°C for 4 h and the reaction mixture was directly subjected to protein sequencing.

Protein sequencing

The determination of protein sequence, performed by the Molecular Biology Core Facility at the Dana-Farber Cancer Institute, was accomplished using an Applied 477 pulsed liquid sequencer (Applied Biosystems, Foster City, CA). The cleaved N-terminal amino acids were analyzed on-line with an Applied Biosystems 120A analyzer. Standard chemistry cycles were used with liquid samples. The liquid samples were applied to the glass filter which was pretreated with Biobrene.

Methods

Preparation of human T cells and monocytes

Isolation of human peripheral blood mononuclear cells and E rosette-positive (E⁺) cells from healthy volunteer donors was described previously (2). For further elimination of monocytes, E⁺ cells were cultured in plastic dishes and non-adherent cells were used as a source of T cells throughout this experiment. Flow cytometry showed that the purified T lymphocyte preparations contained >95% T lymphocytes. Stimulation of T cells by phytohemagglutinin (PHA) was performed at a concentration of 1 µg/ml for 4 days. To obtain monocytes, the E⁺ fraction was further purified by negative selection with anti-CD3 mAb (OKT3, IgG2a), anti-CD20 (B1, IgG2a) mAb and anti-CD56 (NKH-1, IgG1) mAb using magnet beads (BioMag Goat Anti-Mouse IgG) obtained from PerSeptive Biosystems (Framingham, MA). The purity of monocytes was ~90% as determined by flow cytometry.

Transendothelial migration of T cells. In contrast to T cells, sCD26 reduced RANTES-induced migration of monocytes. These findings suggest that CD26/DPPIV may differentially regulate RANTES-induced migration of T cells and monocytes partially through its DPPIV enzymatic activity.

Fig. 2. The effect of sCD26 and mCD26 on transendothelial chemotaxis of resting T cells induced by RANTES. Human recombinant RANTES was used at the concentrations indicated in ng/ml diluted with assay medium consisting of RPMI 1640, 0.6% BSA. sCD26 or mCD26 was added to RANTES at a molecular ratio of 1:1. Chemotaxis to assay medium, sCD26, and mCD26 were also measured as control. The concentration of sCD26 and mCD26 used as control was the same as that was added for 50 ng/ml RANTES. The data represents the mean cell count ± SD from three independent experiments performed in duplicate.
Differential regulation of RANTES-induced migration of T cells and monocytes by CD26/DPPIV

sCD26 + RANTES

mCD26 + RANTES
**Differential regulation of RANTES-induced migration of T cells and monocytes by CD26/DPP IV**

421

Fig. 3. N-terminal amino acid sequence of the reaction products of RANTES incubated with sCD26 or mCD26. (A) Elution profiles of N-terminal amino acid sequence analyzed by the Applied 477 pulsed liquid sequencer. RANTES (1 µg) was mixed with 14 µg of sCD26 or mCD26 in 30 µl of 0.1 M phosphate buffer (pH 7.5) and incubated at 37°C for 4 h. Both reaction mixtures were directly subjected to the sequencer. The elution profiles of the first four cycles are shown. sCD26-RANTES, the reaction mixture of sCD26 and RANTES; mCD26 + RANTES, the mixture of mCD26 and RANTES. (B) Schematic representation of the predicted N-terminal amino acid sequence. sCD26, sCD26 alone; sCD26 + RANTES, the reaction mixture of sCD26 and RANTES; mCD26 + RANTES, the mixture of mCD26 and RANTES. By comparison, amino acid sequences of RANTES, MCP-1 are also shown. The single letter code of amino acid is used. X stands for the amino acid which could not be determined. The characteristic cysteines and tyrosine are marked in boxes, and gaps are introduced for optimal alignment.

**Peptide synthesis**

The full-length (corresponds to amino acids 1–68) and truncated (amino acids 3–68) form of RANTES were synthesized on an Applied Biosystems 431 peptide synthesizer using FMOC chemistry and HBTU activation. They were purified by HPLC on a C18 column. The purity and sequence of the peptides were confirmed by mass spectrometry.

**Results**

sCD26 enhances the migratory response of T cells induced by RANTES

In our initial experiments we prepared human peripheral blood T lymphocytes and measured the transendothelial migration induced by β-chemokines (RANTES, MCP-1, MIP-1α and MIP-1β) at various concentrations. Repeated experiments with different donors indicated that the chemotactic response to RANTES was reproducible and relatively constant among four chemokines (data not shown). T cells from several different donors were prepared in order to examine the effect of sCD26 on transendothelial migration. In some case of donors, T cells were further activated with PHA to enhance CD26 expression prior to the chemotaxis assay (1,8). Figure 1 shows the chemotactic response of resting T cells (Fig. 1A) and PHA blasts (Fig. 1B) to human recombinant RANTES, MCP-1, MIP-1α and MIP-1β at a concentration of 100 ng/ml. As shown in Fig. 1(A), there is a variability in chemotactic responses of resting T cells from various donors to each of the chemokines. Among them, the migratory response to RANTES seems relatively consistent compared to the other three chemokines. In this condition, Fig. 1(A) shows that the supplement of sCD26 to the lower chamber with RANTES augmented the chemotactic responses of resting T cells significantly (1.6- to 1.77-fold), whereas no enhancement was observed in the case with MCP-1, MIP-1β and MIP-1α. As shown in Fig. 1(B), sCD26 enhanced the migration of PHA blasts together with RANTES and MCP-1 (1.7- and 1.5-fold respectively). In the case of PHA blasts, the number of migrating cells was consistently higher than resting T cells in the absence of exogenous RANTES. It may be partly due to endogenous production of RANTES by activated T cells. Based on these findings, we focused on the transendothelial migration of resting T cells induced by RANTES.

In the next set of experiments, we used RANTES at concentrations of 50 and 100 ng/ml with or without sCD26 or mCD26. Figure 2 displays the mean cell count from three independent experiments using different preparations of T cells. The addition of sCD26 to 50 ng/ml of RANTES at a molar ratio of 1:1 enhanced the migratory response dramatically. The degree of enhancement was almost twice that observed using 50 ng/ml RANTES alone and was almost the same as with 100 ng/ml of RANTES. We also examined the enhancing effect of sCD26 with 25 and 75 ng/ml of RANTES. The migratory responses enhanced by sCD26 at 25 and 75 ng/ml of RANTES showed intermediate values between 0–50 and 50–100 ng/ml respectively (data not shown). In this case, enhancement of migration by sCD26 was not observed at 100 ng/ml of RANTES, probably due to the donor variation in dose response of RANTES. The effect of sCD26 may be maximal at the concentration of RANTES which is suboptimal for migration. In contrast to the case with sCD26, mCD26 has no effect on transendothelial migration at any concentration of RANTES (Fig. 2). The above results suggest that DPP IV activity of CD26 is required for the enhancement of transendothelial migration of resting T cells. It should be noted that sCD26 or mCD26 alone has no effect on the transendothelial migration of resting T cells as shown in Figs 2 and 4(B).
sCD26, but not mCD26 cleaves out N-terminal dipeptide from RANTES

What is the mechanism of the enhancing effect of sCD26 on T cell chemotaxis for RANTES? As mentioned before, substrates of DPPIV share a sequence displaying a proline or alanine at the second position from the N-terminus. Amino acid sequences of RANTES contain a proline residue at the penultimate position (Fig. 3B). Therefore, it is a probable candidate for the substrate of DPPIV (CD26).

To evaluate this possibility, RANTES was incubated with sCD26 or mCD26 at 37°C for 4 h, the same conditions in which the transendothelial migration assay was performed. First, we eliminated sCD26 and mCD26 from the reaction mixture by a Microcon concentrator prior to protein sequencing. However, the protein recovery from the Microcon concentrator was not quantitative and was sometimes insufficient for sequencing even after pretreatment (passivation) with 5% Tween 20 to prevent the non-specific binding of protein. Thus, we had to utilize a relatively large amount of RANTES (3 µg) compared to the sensitivity of protein sequencing. Under these conditions, the sequence which appeared was S-P, which corresponds to the N-terminal first and second amino acid of RANTES. In contrast, a sample from the reaction mixture of mCD26 and RANTES did not show any detectable peptide sequence (data not shown). To determine whether this sequence (S-P) was liberated from RANTES by sCD26 or derived from the intact form of RANTES, the reaction mixtures of RANTES and sCD26 or mCD26 were directly subjected to protein sequencing. As shown in Fig. 3(A), the elution profiles to identify the amino acids from the protein sequencer were slightly complicated because of a co-existing N-terminal amino acid sequence derived from sCD26 or mCD26. However, by virtue of the protein sequences of sCD26 and mCD26 which were also confirmed by the same sequencer (N-K-G-T-), we were able to identify the peptide fragment sequences derived from RANTES. Amino acid sequence analysis from the N-terminus showed that there were two peptide sequences of S-P and Y-S-S-X-T-T derived from RANTES as well as the sequence from sCD26 (Fig. 3A and B). Thus, the above result suggests that RANTES was digested by sCD26 at the exact position according to DPPIV enzyme specificity. On the other hand, amino acid sequencing from the reaction mixture of RANTES and mCD26 showed only the intact RANTES sequence (Fig. 3A). These results obtained from protein sequencing suggest that sCD26 cleaved out dipeptides from RANTES and that RANTES is a substrate for DPPIV enzyme activity. On the contrary, cleavage of MCP-1, which also has potential consensus sequence for DPPIV substrate, by sCD26 was not observed in the same experimental condition (data not shown).

Evaluation of biological activity of synthetic RANTES on T cells

How is the chemotactic activity of RANTES regulated or altered by CD26/DPPIV-mediated cleavage? To determine the effect of N-terminal dipeptidyl cleavage on the chemotactic activity of RANTES, we next synthesized and analyzed two forms of RANTES, one is the full-length (amino acids 1–68) and the other is the truncated (amino acids 3–68) form, by transendothelial migration assay. In contrast to our speculation, the truncated form of RANTES (amino acids 3–68) showed equivalent chemotactic activity on resting T cells (Fig. 4A). However, addition of sCD26 was still able to enhance the activity of truncated RANTES (amino acids 3–68) on PHA-stimulated T cells, as well as the recombinant and the full-length synthetic form of RANTES (Fig. 4B). In the case of resting T cells, similar enhancement was observed (data not shown). It was thus suggested that the mechanism by which sCD26 enhances RANTES-induced migration of T cells is distinct from its N-terminal dipeptidyl cleavage of RANTES, and that co-existence of sCD26 and full-length and/or cleaved RANTES is required for the enhancement.
Fig. 5. Effect of the concentration gradient of sCD26 on its enhancement of migratory response toward RANTES in T cells. Effect of the concentration gradient of sCD26 was analyzed on resting T cells. RANTES was supplemented in the lower well at 50 ng/ml in the lanes designated *+. sCD26 was simultaneously administrated at a molar ratio of 0.1, 1 or 10 respectively in the upper and/or lower well. Resting T cells were subjected to transendothelial migration assay using HUVEC. The cells which migrated into the lower wells were harvested and counted by a hemacytometer. Statistical significance against the case with RANTES alone was evaluated by use of the Student’s t-test and indicated on each bar (defined as *P < 0.05 and **P < 0.01 respectively).

The concentration gradient of sCD26 is not necessary to enhance migratory response of T cells toward RANTES

To further characterize the sCD26-mediated enhancement of the migratory response, we next analyzed the effect of concentration gradient of sCD26 on its enhancing activity. As shown in Fig. 5, supplement of sCD26 in the upper well or both wells caused equivalent enhancement on RANTES-mediated migration of resting T cells. These data suggest that sCD26 elicits its enhancing activity in a gradient-independent manner. It should be noted that sCD26-mediated enhancement of the migratory response to RANTES was statistically significant in the case with the lower well alone (1 and 10 µg/ml of sCD26), both the lower and upper wells (1 µg/ml), and the upper well alone (1 and 10 µg/ml).

N-terminal truncated RANTES is inactive in transendothelial migration of monocytes

As described above, enzymatic cleavage of RANTES itself did not modulate its chemotactic activity on resting T cells. To study the biological role of the DPPIV enzyme activity, we tested its effect on resting monocytes. In the case of monocytes, a relatively high amount of RANTES was required to attract through the endothelium compared to resting T cells. To our surprise, N-terminal truncated RANTES (amino acids 3–68) was proved to be completely inactive in the range of the concentration up to 500 ng/ml, whereas full-length synthetic RANTES exhibited a significant chemotactic activity on monocytes as shown in Fig. 6(A).

Supplement of sCD26, but not mCD26, reduced the migratory response of monocytes induced by RANTES

Further, we evaluated the effect of sCD26 and mCD26 on RANTES-induced migration of monocytes and subsequently found that simultaneous incubation of full-length RANTES with sCD26 reduced the migratory response of monocytes, whereas the addition of mCD26 showed almost no effect on the migratory activity of full-length RANTES (Fig. 6B). These results suggest that enzymatic cleavage of RANTES by sCD26/DPPIV may negatively regulate the transendothelial migration of monocytes and show marked contrast to the case with resting T cells.

Discussion

The present study demonstrated that sCD26 enhances transendothelial migration of T cells induced by RANTES, whereas it reduces the migratory response of monocytes toward RANTES. DPPIV enzyme-negative mCD26 does not exhibit such effects either on T cells or monocytes. We also showed...
that RANTES was digested by sCD26 at the exact position of its specificity. Furthermore, synthetic RANTES (amino acids 3–68) lacking N-terminal 2 amino acids showed equivalent chemotactic activity to its full-length form on resting T cells. On the contrary, RANTES (amino acids 3–68) have little biological activity if any, on the monocytes. Thus, RANTES is a substrate for CD26/DPP IV, and the interaction between CD26/DPP IV and RANTES may differentially regulate the transendothelial migration of T cells and monocytes.

Reported physiological substrates for DPP IV include substance P, β-casomorphin, kentsin, somatoliberin, growth hormone releasing factor, the neuropeptides and the fibrin α-chain (30). Currently, physiological substrates related to the immunological aspects of CD26/DPP IV have not yet been identified. Recently, Oravecz et al. have also demonstrated CD26-mediated cleavage of RANTES by a different method, and the differential effect of full-length (amino acids 1–68) and truncated (amino acids 3–68) RANTES on Ca2+ mobilization in T cells and monocytes in the system without sCD26 (31). Similar to their results, we found that MCP-1 was not cleaved by sCD26/DPP IV in our system, although it shares the common sequence having l-proline at the penultimate position of the N-terminus. The mechanism for this substrate specificity remains unclear.

The modulation of the N-terminus of RANTES is of great importance for binding to its receptors and the following reactions. Extension of recombinant human RANTES by methionine (Met-RANTES) failed to induce calcium mobilization and chemotaxis of THP-1 (32). A deletion mutant of RANTES which lacks 8 amino acids from the N-terminus (amino acids 9–68) lost its receptor binding affinity from 1/4 to 1/6 and was also rendered inactive for chemotaxis. RANTES (amino acids 9–68) competed for the binding and inhibited the activities of other chemokines (33). These results indicated that N-terminal residues partly determine the receptor specificity and the binding affinity of RANTES. The structure–activity relationships have been extensively studied on MCP-1 (34).

Gong et al. reported that the analogue that presented the sequential deletion of the three N-terminal residues exhibited lower binding affinity and activity than full-length native MCP-1 (33). It has been believed that for β-chemokines, the 10 N-terminal residues are critical for the receptor activation and the receptor binding. As shown in Fig. 3(B), N-terminal 9 amino acid residues to the first cysteine of RANTES are different from those of MCP-1 and these sequences determine receptor specificity. The receptors for RANTES are CCR1, CCR3, CCR4 and CCR5, although the MCP-1 receptor is reported to be only CCR2 (35). According to the report by Oravecz et al. and by De Meester et al. which has appeared very recently, the sCD26/DPP IV-mediated loss of chemotactic activity of RANTES may probably be due to its loss of signal transducing ability through CCR1 and/or CCR3 (31,36,37).

RANTES (amino acids 9–68) is not only inactive in chemo-
taxis for mononuclear cells, but is also a potent inhibitor of interaction with macrophage-tropic HIV (38). The structural modification of chemokines yields a variant lacking chemotactic properties, but retaining the affinity with their receptors and the ability to block HIV infection. These findings supported the hypothesis that chemokine derivatives are potent antagonists, revealing their possible usefulness as anti-inflammatory reagents and potential antiviral substances in HIV-infected patients.

Our results, as well as others, suggested that one of the major modulators of RANTES in vivo is CD26. The observation that CD26 enhances the T cell chemotaxis for RANTES does not conflict with the N-terminal modification of RANTES. Nosò et al. reported that the truncated form of RANTES (amino acids 3–68) was mainly observed in the culture medium of dermal fibroblasts stimulated with tumor necrosis factor-α or IL-1. Moreover, they reported that the loss of the two N-terminal residues, serine and proline, does not affect the eosinophil chemotactic activity of RANTES (39). In concert with their study, results obtained in this study by using two forms of synthetic RANTES demonstrated that N-terminal dipeptidyl truncation does not affect the biological activity of RANTES in the case of T cell migration.

In contrast to the monocytes, the precise mechanism of sCD26-mediated modulation of the migratory response remains unclear in the case of T cells. The first possibility is that sCD26 might regulate chemokine receptor either directly or indirectly through the complex formation with RANTES. The second one is that sCD26 might interact with other components, such as endothelial cells. The possibility of the complex formation was examined by surface plasmon resonance analysis and co-precipitation assay. Subsequently, we sometimes detected possible binding of sCD26 and RANTES, however, it was difficult to neglect the non-specific binding. It is due to the extremely sticky nature of RANTES which has a high pl value (9). Under physiological pH, RANTES is supposed to be positively charged, which may cause an electrostatic interaction with any negatively charged proteins or chemical moiety. Indeed, the sticky nature of RANTES is strong enough in the presence of detergents and physiological ionic strength. For above reason, whether RANTES and sCD26 bind specifically or not is an open question. In this paper, however, there may be some clues for the explanation. According to our results, the co-existence of sCD26 and RANTES, but not enzymatic cleavage of RANTES is necessary for the enhancement of T cell migration—nonetheless enzymatic inactive mCD26 failed to do so. This might reflect that enzymatic activity of sCD26 is still required in other steps. Moreover, administration of sCD26 in the upper chamber alone or both chambers resulted in a similar extent of enhancement. This result itself does not conflict with possible complex formation between sCD26 and RANTES, it may imply the site of action. Therefore, the second possibility cannot be excluded so far. Further studies will be required to determine the precise mechanism of action in sCD26-mediated enhancement of T cell migration.

Previously, it had been reported that in the migration assay using the endothelial cell monolayer on collagen gels, lymphocyte populations that migrated into the collagen gel through the endothelial monolayer showed higher CD26 expression compared with those which did not migrate (14,15). Furthermore, in vivo studies revealed that a large number of CD26+ T cells was found in inflamed tissues of patients with multiple sclerosis and rheumatoid arthritis (18,19). Thus, the finding that sCD26 enhanced the chemotactic migration of T cell can partly explain the observation that CD26 highly positive cells have most migratory capacity.
in vitro and were the dominant phenotype at the chronic inflammatory sites in vivo. In addition, the fact that biological activity of RANTES is modulated by CD26 represents the new aspects of its contribution to the regulation of immune response and HIV-1 virus infection. Indeed, our previous study suggested that the DPPIV enzyme activity confers relative resistance to HIV-1 infection (40).

Finally, of particular interest is the difference in the response of T cells and monocytes to sCD26. In general, the primary immune response, or innate immunity, is mainly mediated by monocytes and NK cells, followed by the secondary, or acquired, immune response that is mediated by antigen-specific T cells and B cells. In view of the inhibition of RANTES-induced migration of monocytes, CD26-bearing migrating T cells, which are known to be a major population in inflamed tissue, may contribute to some extent to the shut-off of migration of monocytes and the switching from the primary immune response to the secondary immune response.

Acknowledgements
The authors thank M. Berne (Tufts Medical School, Department of Physiology) for peptide synthesis of RANTES, L. Willis and N. Asahara for excellent secretarial assistance, and D. Cho for indispensable technical support and proof-reading of the manuscript. This work was supported by National Institutes of Health grants AR33713, AI29530 and AI12069. S. I. was supported by research fellowship from the Uehara Memorial Foundation.

Abbreviations

ADA adenosine deaminase
DHFR dihydrofolate reductase
DPPIV dipeptidyl peptidase IV
FCM flow cytometry
HUVEC human umbilical vein endothelial cells
mCD26 mutant soluble CD26
PHA phytohemagglutinin
sCD26 soluble CD26

References


chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell 85:1149.


