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A CD26-Controlled Cell Surface Cascade for Regulation of T Cell Motility and Chemokine Signals

Zhiwen Liu,* Marta Christensson,* Anna Forslöw,† Ingrid De Meester,‡ and Karl-Gösta Sundqvist2*

Chemokines are key regulators of cell trafficking, and dipeptidyl peptidase IV/CD26 (CD26) inactivates chemokines. Here we show that the CD26-processed chemokines SDF1α/CXCL12 and RANTES/CCL5, in contrast to a control chemokine not processed by CD26, are potent inducers of cell surface expression of thrombospondin-1 (TSP-1) in T lymphocytes through a CD26-controlled mechanism and that TSP-1 stimulates expression of lipoprotein receptor related protein/CD91. Accordingly, intact TSP-1 and a peptide mimic of a sequence in TSP-1 were sufficient to stimulate CD91 expression. The chemokine-induced expression of TSP-1 and CD91 was mimicked by inhibitors of CD26 and CXCL12 and CCL5 as well as inhibitors of CD26 stimulated polarized cytoplasmic spreading and migration through TSP-1. Silencing of CD26 using small interfering RNA or Ab-induced modulation of CD26 also increased TSP-1 expression and enhanced cytoplasmic spreading and T cell migration markedly. These results indicate that CD26 is an endogenous inhibitor of T cell motility through inhibition of TSP-1 expression and that chemokines stimulate cell polarity and migration through abrogation of the CD26-dependent inhibition. This suggests that T cell motility is regulated by a cascade of interacting cell surface molecules. The Journal of Immunology, 2009, 183: 3616–3624.

The chemokines are a superfamily of small peptide molecules (8–20 kDa) produced by various cell types including leukocytes, epithelial cells, perivascular myofibroblasts (1), and tumor cells (2). Chemokines regulate the migration of inflammatory cells, stem cells, and cancer cells (3–5) to specific destinations within the organism (6) and determine the extravasation as well as the migration within target organs (7). In addition, chemokines induce release of proteinases from leukocytes, control angiogenesis (8), and regulate T cell differentiation and function as scavenger molecules (9). The chemokine superfamily includes >50 ligands and 20 receptors and is currently classified into 4 groups based on the amino acid motif that occurs within the first 2 N-terminal cysteines (10, 11). Chemokines bind to G-protein-coupled surface receptors on target cells (12), and almost all chemokine receptors recognize more than one chemokine (12, 13). Chemokines have been shown to be important orchestrators of the capacity of lymphocytes to extravasate and migrate that makes it possible for these cells to reposition themselves between a free-floating vascular state and active migration in tissues (14, 15). Although chemokines thus play a critical role for lymphocyte adhesion and migration, and hence for immune surveillance against pathogens, altered self-components, and cancer throughout the organism, the mechanisms via which chemokines regulate adhesion and migration are only partly known (16–20). Understanding of this regulation therefore requires deeper mechanistic insights and perhaps even identification of additional regulatory factors and pathways. This lack of understanding applies particularly to the formation of active cell edges formed by adhering and migrating cells that probably senses the environment and is critical for recognition, a key feature of the adaptive immune system.

Thrombospondin-1 (TSP-1) is a 420-kDa glycoprotein composed of three identical disulfide-linked polypeptide chains that display binding sites for various cell surface receptors including glycosaminoglycans; the integrins α5β1, αvβ3, αvβ5, αvβ6 and αvβ1; calreticulin (CRT); CD91; CD36; and CD47 (21–29). T lymphocytes express endogenous TSP-1 associated with CD91 and CRT that seem to function as a cell surface ligand for regulation of T cell adhesion and migration (30–32). In vivo findings further suggest that TSP-1 has a role in immune regulation. Thus, TSP-1-deficient mice show inflammatory disturbances in lungs and other organs and TSP-1 or TSP-2 as well as CD47-deficient mice show defective delayed-type hypersensitivity with prolonged inflammation (33–35). Deficiency of TSP-1 also reduces Th17 differentiation and attenuates experimental autoimmune encephalomyelitis (36). The starting point for the present investigation was a working hypothesis, based on preliminary evidence that chemokines may influence T cell TSP-1, that the chemokine receptor system may cross-talk with the cell surface receptors that bind TSP-1. We further hypothesized that such a cross-talk might be important for regulation of T cell functions such as environmental recognition by active cell edges, adhesion, and migration, and perhaps contribute to the extensive lymphocyte infiltration seen in autoimmune and allergic inflammatory diseases. It is interesting in this context that the chemokine CXCL12, which is expressed in many tissues (37), binds to the chemokine receptor CXCR4, which is associated with the membrane-bound ectopeptidase dipeptidyl

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Abbreviations used in this paper: TSP-1, thrombospondin-1; CD26, dipeptidyl peptidase IV/CD26; CRT, calreticulin; siRNA, small interfering RNA; 4N1K, KRFVY-VMWKK; Sc4N1K, KVFRWRYVMR (scrambled 4N1K).

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peptidase IV/CD26 (CD26), a cell surface protease with a wide-
spread organ distribution, composed of two identical 110-kDa sub-
units, that inactivates chemokines and other peptides by selective
removal of the N-terminal proline or alanine in the second position
(38–40). CD26 affects T cell activation, proliferation, and cyto-
kine production (41–44), and CD26 inhibitors introduced into an-
imal models have demonstrated a role for CD26 in the develop-
ment of autoimmune diseases (45). However, the severity of autoimmune diseases including rheumatoid arthritis and experi-
mental autoimmune encephalomyelitis is increased in CD26-defi-
cient mice indicating that CD26 rather exerts a protective effect
against disease development (46, 47). Our results show that
CXCL12 and CCL5, which both are processed by CD26, are po-
tent inducers of cell surface expression of TSP-1 and that TSP-1
induces expression of CD91 and CRT and stimulates cytoplasmic
spreading and migration. Small interfering RNA (siRNA) silenc-
ing of CD26, inhibitors of CD26 as well as Ab-induced modulation
of CD26 also stimulate this chemokine-induced molecular cas-
cade, thus implicating CD26 as a suppressor factor for T cell for-
mation of cytoplasmic projections and migration.

Materials and Methods

Chemicals and Abs

Human plasma fibronectin and rat tendon collagen type I were purified and prepared as described elsewhere (48, 49). Poly-y-lysine (molecular mass, 5300) was purchased from Miles-Yeda. IL-2 and IL-4 were from Genzyme Diagnostics. Brefeldin A was from Sigma-Aldrich, and ICAM-1 was from R&D Systems. Human recombinant CD26 was obtained from Alexius Bio-
chemicals. Anti-fibronectin (clone IST1, IgG1) was obtained from Sera-
Lab. Anti-CD3 (clone SK7, IgG1) and anti-CD4 (clone SK4/14B, IgG1) were obtained from Dako. Anti-CD8 (C8/144B, IgG1) were from Dako. Goat anti-mouse Ab was from Dakopatts. Anti-TSP-1 clone MBC 101.1 (also called TSP-Ab-9, IgG1), clone A6.1 (also called TSP-Ab-4, IgG1) and clone C 6.7 (also called TSP-Ab-3, IgG1), were from NEO-MARKERS. Anti-CD91 (clone A2MrO2, IgG1) was from Santa Cruz Biotechnology. Anti-CD26 (clone TA5.9) has been described previously (50). Anti-CD26 (clone BA5) was obtained from Dako. Anti-CRT (clone FMC75, IgG1) was from Biosite. Anti-CD47 (clone B6H12.2, IgG1) was from NEO-MARKERS. Biotiny-
lated peroxidase and avidine were from Vector Laboratories. The peptides KRFFYVVMWK (4N1K) and KVFRWKYVMK (scrambled (Sc) 4N1K) were synthesized with Tri pep (Novum Research Park). Protease inhibitors KRFYVVMWK (4N1K) and KVFRWKYVMK (scrambled (Sc) 4N1K) were synthesized with Tri pep (Novum Research Park). Protease inhibitors were present with the cells in adhesion experiments. A total of 10^6 cells in AIM-V medium was added followed by boiling for 4 min. Proteins were separated on 6%
agarose gels.

Quantitative immunocytochemistry

Glass chambers were coated with ICAM-1 (2 μg/ml) or fibronectin (10 μg/ml) overnight at 4°C and washed. Anti-CD3 activated cells in serum-
free AIM-V medium (15 μl of 1 × 10^6 cells/ml per position) were allowed to adhere to the coated surface for 30 min at 37°C followed by fixation with 2% paraformaldehyde at 4°C for 20 min. For detection of CD26, the cells were subsequently permeabilized using 0.1% saponin. Mouse anti-human Abs (TSP-1, CD91, CD26), conjugated with FITC or TRITC, were added, followed by detection of the primary Ab with the biotin-avidin-HRP-based Vectastain ABC Kit (Vector Laboratories) and a peroxidase substrate (3-amino-9-ethylcar-
bazole). Pictures were taken of each slide using the program NIKON
ACT-1 and a NIKON Eclipse E1000M microscope at ×600. Mean
staining intensity (arbitrary units) was determined using the Image J
picture analysis program.

Cell migration

Collagen type I was diluted in serum-free RPMI 1640 and H_2O (8:1:1), applied in plastic petri dishes (30 mm; BD Biosciences), and allowed to polymerize at room temperature. The chemokines were dis-
olved in the gel, whereas Abs and peptides were present with the cells in migration experiments. A total of 1.0 × 10^6 cells in AIM-V medium was added to each well and allowed to migrate for different times. The cells were fixed in 2.5% glutaraldehyde and washed twice with PBS. Cell mor-
phology and cell migration were routinely, unless otherwise stated, eval-
uated in nine fixed positions in each well and at 50-μm intervals through-
out the gel by the use of an inverted microscope (Nikon Eclipse TE300) and a digital depth meter (Heidenheim ND221). The results are given as maximal infiltration depth or mean number of infiltrating cells/field (×20 objective) per infiltration depth (50 μm) for the first two layers immediately beneath the gel surface and 100 μm for other layers further down. The infiltrating cells were identified in situ in the collagen gels using immuno-
cytochemistry after fixation in paraformaldehyde.

Cell adhesion

To study cell adhesion, plastic petri dishes (90 mm; Heger) were coated with ICAM-1 (2 μg/ml) or fibronectin (10 μg/ml) and extensively washed before use. The cells (10,000/position) in AIM-V medium were incubated on the substrates in a humidified CO_2 incubator at 37°C for 15 or 30 min. Cells were fixed in 2.4% cold glutaraldehyde for 10 min, and unbound cells were removed by gentle aspiration. The number of adherent cells per mi-
dere coup was determined by light microscopy. A total of 5 × 10^4 cells in AIM-V medium were added followed by boiling for 4 min. Proteins were separated on 6%
agarose gels.

Statistical analysis

Staining intensity in immunocytochemistry experiments is presented as mean ± SD. For analysis of differences between groups, the Mann-Whitney U test was used to evaluate differences between groups. For analysis of adhesion and spread-
ning, mean values ± SD are shown. For determination of differences be-
tween inhibitor-treated lymphocytes and control lymphocytes, the paired

Biocytin labeling and immunoprecipitation of cell surface proteins

Anti-CD3-activated cells were biotinylated in solution, 3 × 10^6/well, and after adhesion to tissue culture dishes (Falcon 35 3001), 5 × 10^6/well, coated overnight at 4°C with fibronectin (20 μg/ml) using reagents for biotinyltion from the Cellular Labeling and Immunoprecipitation Kit (Roche). The reaction was stopped and cells were washed and lysed with 1 ml of lysis buffer on ice for 30 min, followed by centrifugation at 12,000 × g for 10 min at 4°C. The cells were allowed to adhere for 30 min. Where stated, cells were treated with 4N1K at 50 μM or scrambled 4N1K (Sc-4N1K) at 50 μM or inhibitor of CD26, dipeptide A, at 50 μM for 30 min before biotinylation. Adherent cells were washed and biotinylated while remaining attached (0.5 ml of biotinyl buffer/well). Adherent cells were sonicated off the plate, washed, lysed, and centrifuged as above. For pre-clearing, each tube with lysate was incubated with protein G agarose at 4°C overnight. Agarose beads were discarded and lysates mixed with 1 μg of TSP-1 Ab or CD91 Ab and incubated at 4°C overnight. Protein G agarose was added to each tube followed by incubation at 4°C over-
night. Beads were washed, and 20 μl of reducing sample buffer were added followed by boiling for 4 min. Proteins were separated on 6%
SDS-PAGE gels.
Student t test was used. A two-tailed value of <0.05 was considered statistically significant.

Results
CXCL12 and CCL5 induce cell surface expression of TSP-1 and CD91

To study the possible influence of chemokines on the cell surface expression of TSP-1, we exposed anti-CD3-activated lymphocytes (generally >92% CD3 positive cells) in suspension and on a fibronectin substrate to CXCL12 and CCL5, two chemokines processed by CD26 (53, 54). The cells were cultured in serum-free medium to exclude interference of exogenous TSP-1. Anti-CD3-activated cells were used because we intended to examine cell migration and formation of pseudopodia that develop poorly in lymphocytes fresh from the blood (55). Both chemokines were potent inducers of cell surface expression of TSP-1, CD91, and CRT as determined by SDS-PAGE of immunoprecipitated biotinylated cell surface components from cells in suspension (Fig. 1A) and quantitative immunocytochemistry of adherent cells fixed in situ to prevent Ab-induced perturbations of Ag expression and turnover of Ags during the detection procedure (Fig. 1, C–G). In contrast, CXCL12 and CCL5 (not shown) did not affect the cell surface expression of CD3 and CD4 (Fig. 1H). Brefeldin A inhibited the cell surface expression of TSP-1 induced by CXCL12.
indicating that the chemokines induced TSP-1 transport to the cell surface from an intracellular source. The effect of CXCL12 on the expression of TSP-1 was concentration dependent with a peak at 50–200 ng/ml (Fig. 1D). Immunoprecipitation of surface-biotinylated cells demonstrated intact 175-kDa TSP-1 as well as 130- and 115-kDa fragments that also could be identified as TSP-1 using Western immunoblotting (Fig. 1B). It is also evident from Fig. 1B that CXCL12 increased the amount of intact TSP-1 in comparison with control cells. In contrast, these showed a prominent 115-kDa TSP-1 band that was weak in cells cultured in the presence of CXCL12. In addition, there were multiple minor bands immunoprecipitated with anti-TSP-1 Abs as revealed by the gradient gel in Fig. 1A. It is possible that some of the multiple bands seen on the gel in Fig. 1A represent components that associate with TSP-1, such as CRT or CD47. CXCL12 also markedly increased the cell surface expression of TSP-1 in a T cell clone as revealed by SDS-PAGE gels of immunoprecipitated biotinylated cell surface components (results not shown). In contrast to CXCL12 and CCL5, CXCL8, which binds to and induces functional responses in activated T cells (56) but is not processed by CD26 (57), did not affect TSP-1 expression (Fig. 1C). The results in Fig. 1 indicate that CD26-processed chemokines induce cell surface expression of TSP-1 through a brefeldin A-sensitive transport mechanism and also expression of CD91 and its coreceptor CRT, which has been shown to coprecipitate with cell surface TSP-1 in T cells (32).

**TSP-1 induces cell surface expression of CD91**

TSP-1 and a peptide mimetic of a sequence in TSP-1, 4N1K, were found to be inducers of cell surface expression of CD91 whereas they did not affect CD3 expression (Fig. 2). In contrast, a scrambled control peptide, Sc4N1K, did not induce CD91 expression. This suggests that the chemokine-induced cell surface expression of CD91 demonstrated in Fig. 1 is dependent on TSP-1.

**CD26 influences cell surface expression of TSP-1**

The results in Fig. 1 suggested that chemokines metabolized by CD26, such as CXCL12 and CCL5 (53, 54, 58), that cleave N-terminal dipeptides from various chemokines regulate the cell surface expression of CD91 and TSP-1 through CD26. Therefore, we examined the possible influence of the CD26 inhibitors diprotin A, KR 62436, and vildagliptin on the cell surface expression of TSP-1 in anti-CD3-activated lymphocytes using quantitative immunocytochemistry and SDS-PAGE of immunoprecipitated biotinylated TSP-1.

**FIGURE 2.** A TSP-1-CD47-CD91 cascade on the T cell surface. A–C, The influence of TSP-1 (5 μg/ml), the CD47-binding site in TSP-1, 4N1K, and a scrambled control peptide, Sc4N1K, on the cell surface expression of CD91 (A and B) and CD3 (C) as showed by quantitative immunocytochemistry (A and C) and immunoprecipitation of biotinylated cell surface components (B). One representative experiment of two to six independent experiments is shown.

**FIGURE 3.** CD26 inhibitors increase the cell surface expression of TSP-1. A–C, Quantitative immunocytochemistry showing that incubation for 30 min in the presence of diprotin A (50 μM), KR 62436 (50 μM), or vildagliptin (100 μM), and modulation by the anti-CD26 Ab TA5.9 (5 μg/ml) increases the cell surface expression of TSP-1 (A) and CD91 (B). C, Comparison of the influence of CXCL12 and diprotin A on TSP-1 expression in cells adherent to fibronectin. D, SDS-PAGE gel analysis (6% and 4–12% gels) showing immunoprecipitated biotinylated cell surface proteins from lymphocytes allowed to adhere to fibronectin for 30 min in the presence of diprotin A (50 μM) or GM6001 (10 μM). Diprotin A enhances expression of intact 175-kDa TSP-1, 130- and 115-kDa proteins as well as several other coprecipitated components and/or fragments of TSP-1. One representative experiment of three to four independent experiments is shown. Ac-IETD-CHO, Ile-Glu-Thr-Asp aldehyde.
Arrows, Bands of lower molecular mass. It can be seen that CD26 influences the detectability of TSP-1.

CXCL12 (50 M) coated with ICAM-1 in the presence and absence of T cells were allowed to adhere to a plastic surface. CXCL12 and diprotin A stimulate polarized cytoplasmic spreading and development of pseudopodia. 4N1K inhibited cytoplasmic spreading both with and without diprotin A or CXCL12. One representative experiment of five independent experiments is shown. PBT, Peripheral blood T cells.

FIGURE 5. CXCL12 and diprotin A stimulate polarized cytoplasmic spreading and pseudopodia formation. T cells were allowed to adhere to a plastic surface coated with ICAM-1 in the presence and absence of CXCL12 (50 μg/ml), diprotin A (50 μM), 4N1K (50 μM), or Sc4N1K (50 μM). Control cells and cells allowed to adhere in the presence of Sc4N1K show uniform circumferential formation of active cell edges, whereas cells allowed to adhere in the presence of diprotin A or CXCL12 show pronounced polarization and development of pseudopodia. 4N1K inhibits cytoplasmic spreading both with and without diprotin A or CXCL12. One representative experiment of five independent experiments is shown. PBT, Peripheral blood T cells.

CD26 inhibits T cell polarity and migration through TSP-1

In the light of the previous findings that endogenous TSP-1 participates in the regulation of T cell migration and adhesion (30, 32), together with the present evidence that chemokines and CD26 affect TSP-1 expression (Figs. 1 and 3), it seemed reasonable to assume that chemokines as well as CD26 may influence T cell adhesion and migration through TSP-1. To investigate this, we examined the influence of chemokines and diprotin A on adhesion of lymphocytes to ICAM-1 coated on a plastic surface. CXCL12 and diprotin A stimulated polarized cytoplasmic spreading with formation of pseudopodia in T cells adhering to these substrata whereas control cells exhibited a relatively uniform circumferential spreading along the cell periphery (Fig. 5). A peptide mimetic of a sequence in TSP-1 (4N1K) prevented spontaneous spreading (Fig. 5) as well as the potentiation of spreading induced by diprotin A and CXCL12 (not shown), probably through interference with the association of endogenous TSP-1, whereas a scrambled control peptide (Sc4N1K) had a negligible effect on spreading.

To analyze the influence of CD26 and chemokines on lymphocyte migration anti-CD3-activated cells were allowed to migrate into three-dimensional collagen gels, a well established test system for analysis of lymphocyte migration (30, 61–64), in the presence of CXCL12, the CD26 inhibitors vildagliptin, diprotin A, and KR 62436. In addition, the influence of modulation of CD26 by Ab to induced a 70-kDa band (Fig. 3D). This points to the possibility that a metalloprotease also influences TSP-1 expression, and further suggestive evidence for involvement of several proteases is provided by the loss of cell surface TSP-1 when GM6001 was combined with diprotin A.

CD26 may inhibit the expression of TSP-1 and CD91 if capable of binding to TSP-1 and inhibit its stimulatory effect leading to CD91 expression. To study the possible influence of CD26 on TSP-1 the two molecules were mixed at increasing enzyme:substrate (w/w) ratios and incubated at 37°C for 15 h. CD26, at a relatively low concentration, prevented reduction of TSP-1 as shown by the persistence of a 420-kDa band. In addition, CD26 induced a 320-kDa band, most probably derived from the 420-kDa band, 140-, 90-, and 70-kDa bands (Fig. 4). Moreover, a 110-kDa band appeared, that did not seem to be CD26, although it had the same molecular mass, because it was not present in the control lane with CD26 alone. The results in Fig. 4 suggest that CD26 can associate with TSP-1, but further studies are needed to elucidate whether CD26 can cleave TSP-1.
CD26 (TA5.9) on T cell migration was examined. Migration was
determined by counting the number of cells at different depths of
the collagen. It can be seen in Fig. 6A that diprotin A and
vildagliptin stimulated T cell migration into collagen. The CD26-spe-
cific Ab (TA5.9) also enhanced T cell migration significantly,
whereas an Ab to CD8 did not affect migration (Fig. 6A). A mAb
to CD47, B6H12, previously found to block responses to CD47
ligands (25, 65), inhibited diprotin A-induced as well as CXCL12-
induced stimulation of T cell migration (Fig. 6, B and C). In con-
trast, Abs to CD4 and CD8 did not affect T cell migration. This
indicates that CXCL12 induced T cell migration through TSP-1
and CD47. The stimulatory effects of CXCL12 and diprotin A
were not additive, indicating that they affect the same pathway.
Diprotin A markedly increased the tendency to cytoplasmic spreading
but did not affect the expression of CD4 (Fig. 7A). CD26 siRNA
markedly increased the tendency to cytoplasmic spreading and
development of locomotor morphology (Fig. 7B). Furthermore,
CD26 siRNA also markedly increased T cell migration into a
collagen gel, whereas control siRNA did not increase T cell
migration (Fig. 7C). This strengthens the conclusion that CD26
is a major inhibitor of the cell surface expression of TSP-1 and
of T cell motility.

**Discussion**

The present results unveil a previously unknown CD26-controlled
intraplasma membrane pathway for T cell regulation of adhesion,
particularly cytoplasmic spreading, and migration connecting che-
monokes/chemokine receptors with TSP-1, CD91, including core-
ceptor CRT, and CD47 (31, 32, 66). CD26 thus down-regulates
TSP-1 expression, cell polarization, and migration at the plasma
membrane level, where environmental interactions take place.
CXCL12 and CCL5 seem to abrogate this CD26-dependent neg-
ative regulation and hence are potent inducers of a molecular cas-
cade characterized by up-regulated cell surface expression of
TSP-1 that stimulates expression of CD91. This indicates that the
CD26-controlled chemokine-activated TSP-1-CD91 cascade is es-
cential for the motile capacity that enables T cells to carry out
immune surveillance throughout the organism. The involvement of
CD47, a TSP-1 receptor for induction of growth, apoptosis, and
differentiation of lymphocytes as well as for the regulation of T
cell inflammation (34, 67–70), suggests that this cascade may also
have bearing on other aspects of immune regulation besides T cell
adhesion and migration. Furthermore, the fact that chemokines as
well as TSP-1, CD47, CD91, and CD26 are present also in non-
lymphoid cells means that interactions in cis between these

**FIGURE 6.** CD26 inhibits T cell migration and adhesion dependent on CD47 and chemokines stimulate T cell migration through CD47. A, T cells were allowed to migrate into a three-dimensional collagen type I gel for 30 min in the presence of vildagliptin (200 μM), diprotin A (200 μM) and anti-CD26 TA5.9 (5 μg/ml). Anti-CD8 (5 μg/ml) was used as a control Ab. *, p < 0.05; §, p < 0.01; †, p < 0.001 vs control. B and C, T cells were allowed to migrate into a collagen gel for 30 min in the presence of diprotin A and mAbs to CD47, CD4, or CD8 (B). *, p < 0.05 vs control; §, p < 0.01 vs control; †, p < 0.05 vs diprotin A, or into a collagen gel containing CXCL12 (50 ng/ml) in the presence and absence of mAbs to CD47 and CD4 (C). *, p < 0.01 vs control; §, p < 0.01 vs CXCL12; †, p > 0.05 vs CXCL12. D, Cells with or without diprotin A were allowed to migrate into a collagen gel containing CXCL12 (50 ng/ml). E, Cells were allowed to migrate into a collagen gel in the presence of various protease inhibitors. D and E, *, p < 0.05 vs control; §, p < 0.01 vs control; †, p > 0.001 vs control. The number of cells at different depths of the collagen was determined using a depth meter. It is evident from the results that diprotin A markedly increased migration and that the anti-CD47 Ab inhibited migration, whereas the Abs to CD4 and CD8 did not. One representative experiment of three independent experiments is shown. Ac-IETD-CHO, Ile-Glu-Thr-Asp aldehyde; Z-VAD.fmk, Z-ValAlaAsp-fluoromethyl ketone.
components within the same plasma membrane may have implications also for other cell types.

It is well established that chemokines regulate T cell migration through G-protein-coupled receptors and that CD26 is involved in the truncation of CCL5, CCL4, and CXCL12, which is considered to inactivate or reduce their cell-stimulating capacity (53, 71, 72). However, the present results suggest that the regulatory influence of chemokines and CD26 is more complex and that chemokines stimulate T cells through abrogation of a CD26-dependent suppression. This abrogated suppression probably reflects a different handling of TSP-1 in comparison with that of cells not stimulated by chemokines. It is reasonable to assume that the CD26 control mechanism counteracts the capacity of TSP-1 to stimulate CD91 expression and as a consequence of this TSP-1 is not associated with and processed by CD91 and TSP-1 turnover maintained at a low level. CD26 may thus be postulated to have a dual role as a regulator of T cell functions being engaged either in suppression of the TSP-1-CD91 cascade or in the processing of chemokines stimulating the same cascade. Chemokines processed by CD26 may be presented to T cells associated with extracellular matrix components, on cell surfaces such as endothelial cells, or in solution, and may thus increase the locomotor capacity of T cells through TSP-1 and CD91 by acting as CD26 inhibitors.

Transient chemokine-induced inhibition of CD26 may up-regulate the motility of vascular lymphocytes as part of a commitment to extravasation. Under normal physiological conditions with respect to chemokine concentrations and endothelial presentation, CD26 probably controls the TSP-1-CD91 cascade effectively. However, inflammation-induced chemokine production provoked by microorganisms, autoantigens, or allergens may lead to persistent inhibition of CD26 and down-regulate its suppressive effect on this cascade. Thus, it is possible that persistent down-regulation of the suppressive CD26 effect on TSP-1 expression and T cell motility may account for a propensity of certain individuals to develop autoimmune and other types of T cell-dependent inflammation. Involvement in TSP-1 regulation may explain the protective effect of CD26 against autoimmunity, as demonstrated in knockout mice (46, 47). It is interesting in this context that recent studies of regulatory T cells, which are important for ensuring that the immune system does not attack self and does not overreact to external Ags, suggest that the ability of regulatory T cells to suppress bystander T cells depends on their capacity to produce TSP-1 (73). The present finding that CD26 exerts a negative control of T cell activity through TSP-1 taken together with the notion that CD26 suppresses autoimmune disease in vivo models (46, 47) suggest that TSP-1, CD91, CD47, CD26, and chemokine/chemokine receptors are components in a multimolecular network for integrated positive and negative T cell regulation of immunity.

FIGURE 7. Silencing of CD26 enhances cell surface expression of TSP-1 and CD91 as well as development of locomotor morphology and migration in T cells. A. Comparison of the expression of CD26, TSP-1, CD91, and CD4 in T cells after transfection with CD26 siRNA and negative controls (control siRNA and nontransfected cells). †, p < 0.001 vs negative control. CD26 was determined after permeabilization of the cells. B and C. CD26-silenced cells were allowed to migrate into a collagen gel for 30 min. B, morphological appearance of CD26-silenced cells in comparison with negative control cells. C Depth of migration in comparison with negative control cells. One representative experiment of two independent experiments is shown.

FIGURE 8. Model of a chemokine-activated CD26-suppressed molecular cascade for T lymphocyte regulation. In the absence of chemokine stimulation, CD26 inhibits TSP-1-induced cell surface expression of CD91. Chemokines inhibit CD26 and abrogate its suppression of T cell motility through TSP-1. TSP-1 is transported to the cell surface through a brefeldin A-sensitive process and CD91 when present on the cell surface is postulated to drive a pathway for endocytosis of TSP-1. CD26-processed chemokines stimulate T cell polarity and migration and probably also other T cell functions through CD26 inhibition and up-regulation of the TSP-1-CD91 cascade.
In conclusion, the present investigation provides evidence for the existence of a CD26-controlled chemokine-activated cell surface cascade for regulation of T lymphocyte functions through cis receptor communication, as outlined in Fig. 8. According to this model, CD26-processed chemokines inhibit the capacity of CD26 to suppress the formation of active cell edges and motility in T cells through TSP-1 and CD91. This CD26-mediated suppression seems to target a mechanism that probably plays a pivotal role for T cell recognition and interaction with the environment and that may be important for the pathogenesis of immunological diseases. An inherent poor CD26 expression or down-regulation of the CD26-dependent suppression owing to overproduction of chemokines may thus induce sustained chronic T cell stimulation that aggravates inflammatory processes. This implies that CD26 is protective against sustained T cell stimulation by Ags and chemokines. It is possible that this protective function is impaired secondary to overstimulation and sometimes even primarily defective in inflammatory disorders such as autoimmune diseases.

Disclosures
The authors have no financial conflict of interest.

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