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Immunology

Enhancement of antigen-induced T-cell proliferation by soluble CD26/dipeptidyl peptidase IV

(cell-mediated immunity/costimulation/memory T cells)

TOSHIAKI TANAKA, JONATHAN S. DUKE-COHAN, JUNICHI KAMEOKA, ARIEH YARON, IRIS LEE, STUART F. SCHLOSSMAN, AND CHIKAO MORIMOTO

Division of Tumor Immunology, Dana–Farber Cancer Institute and Departments of Pathology and Medicine, Harvard Medical School, 44 Binney Street, Boston, MA 02115

Contributed by Stuart F. Schlossman, December 10, 1993

ABSTRACT **The addition of a soluble recombinant CD26 (sCD26) enhanced proliferation of peripheral blood lymphocytes induced by the recall antigen tetanus toxoid. sCD26 itself did not provide a mitogenic signal and did not augment the proliferative response of T cells to other mitogenic stimuli such as phytohemagglutinin and anti-CD3. Dipeptidyl peptidase IV-negative sCD26 did not have this enhancement effect, implying a requirement for enzyme activity. It was found that there exists a large variation in the levels of human plasma sCD26/dipeptidyl peptidase IV *in vivo* which may regulate T-cell activity. Peripheral blood lymphocytes from individuals whose plasma sCD26 was high and responded strongly to tetanus toxoid stimulation were insensitive to the enhancing effects of exogenously added sCD26. This suggests that plasma sCD26 had modulated the responsiveness of T cells of these individuals *in vivo* and that the endogenous plasma sCD26 regulates immune responses by allowing antigen-specific T cells to exert a maximal response to their specific antigen.**

CD26 is a 110-kDa glycoprotein present on T cells and was first described as a T-cell activation antigen defined by the monoclonal antibodies (mAbs) Ta1 and 1F7 (1, 2). The expression of CD26 is enhanced after activation of T cells (2), though it is expressed on a subset of $CD4^+$ memory T cells in a resting state (1). In addition, the $CD4^+$ $CD26^+$ subset responds maximally to recall antigens such as tetanus toxoid (TT) (1). CD26 has dipeptidyl peptidase IV (DPPIV, EC 3.4.14.5) enzyme activity in its extracellular domain (3, 4). This enzyme can cleave amino-terminal dipeptides with either L-proline or L-alanine in the penultimate position (5), but a significant physiological substrate remains to be identified.

Considerable evidence suggests the involvement of CD26 in T-cell activation and function (6–9). Crosslinking of CD26 and CD3 with solid-phase immobilized mAbs can induce T-cell costimulation and interleukin 2 (IL-2) production by human $CD4^+$ T cells in the absence of antigen-presenting cells (6). This costimulatory activity of CD26 was also recently confirmed by utilizing a CD26-transfected Jurkat human T-cell line that secretes IL-2 after stimulation (10).

The CD26 molecule is predicted to be a type II transmembrane protein with only 6 amino acids in its cytoplasmic region (10), which may be too short to be directly involved in signal transducing activity. One possible mechanism for the costimulatory activity is the association of CD26 with other molecules which have signal transducing activity, such as membrane-bound protein-tyrosine-phosphatase CD45 (11) or adenosine deaminase (12).

Our recent studies have shown that the DPPIV enzyme activity of CD26 functions to augment cellular responses of CD26-transfected Jurkat cells to external stimuli (13). These studies raise questions as to how CD26 functions in a physiological situation. CD26 may exert its costimulatory activity through binding with a putative ligand on the same or other cells, which then acts as a signal transducing molecule. Another possibility is that CD26 participates in the control of cellular functions through DPPIV processing of biologically active molecules.

Here we show that recombinant soluble CD26 (sCD26) enhanced proliferative responses of peripheral blood lymphocytes (PBLs) to stimulation with soluble antigen. This enhancing effect required DPPIV enzyme activity. However, this enhancing effect of T-cell responses to the recall antigen by sCD26 was not observed in all individuals. Individuals with high plasma sCD26 and with a pronounced response to TT were insensitive to the effects of added sCD26. This observation supports the view that plasma sCD26 may have already modulated the T-cell response of individuals *in vivo* and suggests that sCD26 may have biologically important immunomodulatory activity and be of use in immunodeficiency disorders.

MATERIALS AND METHODS

Production of sCD26. Mutagenesis of CD26 cDNA was performed with the oligodeoxynucleotide 5′-ACGCCGACGATGAAGGGACTGCTGGGTGCT-3′ (13), which directs the deletion of the coding sequence for amino acids 3–9 of CD26 (Fig. 1, line 2). The small *Xba* I–*Dra* III DNA fragment of the mutant CD26 cDNA containing the deletion was substituted for the corresponding DNA fragment of the wild-type CD26 cDNA in the expression vector RcSRα to yield RcSRα-26Δ3-9 (13). A mixture of RcSRα-26.Δ3-9 and pMT-2 (14) providing the dihydrofolate reductase (DHFR) gene was used to transfect a DHFR-deficient CHO cell line, DXB-11, by electroporation (15).

Transfectants grown in nucleoside-deficient selection medium were screened for sCD26 production by measuring DPPIV enzyme activity (13) and appearance of a 110-kDa band in the supernatant of metabolically labeled cells (16). Production of sCD26 was amplified by culturing transfected CHO cells in selection medium containing increasing concentrations of methotrexate (up to 0.5 μM).

Purification of sCD26. The mutant CD26 cDNA (Δ3–9)-transfected CHO cells were first cultured in selection medium for 1 day and then cultured in serum-free CHO-S-SFM (GIBCO/BRL). Protein from the culture supernatant was precipitated with saturated ammonium sulfate at 75% satu-

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Abbreviations: DPPIV, dipeptidyl peptidase IV; IL-2, interleukin 2; mAb, monoclonal antibody; PBL, peripheral blood lymphocyte; sCD4, soluble CD4; TT, tetanus toxoid.

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1. MKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATAD
2. MK-------GLLGAAALVTIITVPVVLLNKGTDDATAD
3.                              NKGTDDATAD
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FIG. 1. Amino-terminal amino acid sequences of predicted wild-type CD26 (line 1), a derivative of CD26 encoded by the mutant cDNA (line 2), and sCD26 secreted by transfected CHO cells (line 3). Underline shows the putative signal/anchoring (transmembrane) sequence. Dashes indicate amino acids that are lacking because of the deletion of corresponding DNA sequence.

ration, resuspended in phosphate-buffered saline (PBS), loaded on a concanavalin A-Sepharose (Pharmacia) column, and eluted with 0.2 M methyl α-D-mannopyranoside (Sigma). The sCD26-containing fractions identified by DPPIV enzyme assay were pooled and precleared by passage through a column of bovine serum albumin-conjugated Affi-Gel 10 (Bio-Rad) and then applied to an anti-CD26 mAb (1F7)-conjugated Affi-Gel-10 column. The 1F7 column was washed with PBS, and sCD26 was eluted with PBS containing 3 M sodium thiocyanate.

Biochemical Characterization of the sCD26 Protein. SDS/PAGE and staining of proteins were done as described (16). Amino-terminal amino acid sequence analysis was performed by the Harvard University Microchemistry Unit.

Purified sCD26 was ^{125}I-labeled with Iodo-Beads (Pierce) to yield a specific activity of 5–10 × 10^6 dpm/μg. Labeling of cell surface proteins with ^{125}I by lactoperoxidase, the method of immunoprecipitation, and autoradiography were performed as described (1). The anti-CD26 mAbs (1F7, Ta1, and 5F8), anti-L-selectin mAb (TQ1), and anti-CD3 mAb (Rw24B6) have been described (1, 2, 3, 17).

To inactivate DPPIV enzyme activity, sCD26 was treated with diisopropyl fluorophosphate (18).

T-Cell Proliferation Assays. Human PBLs were isolated from healthy donors as described (1–3). For T-cell proliferation ([^{3}H]thymidine incorporation) assays, sCD26 or other soluble proteins were added to each well simultaneously with the anti-CD3 or TT. Soluble recombinant CD45 (full-length transcript) was a gift from M. Streuli (Dana–Farber Cancer Institute). Soluble recombinant CD4 was obtained from Biogen. For preclearing, the medium containing sCD26 or other soluble molecule was incubated with appropriate antibody-conjugated beads for 4 hr prior to use.

For generation of T-cell clones, human PBLs isolated from a TT-sensitized donor were stimulated with TT (10 μg/ml; Connaught Laboratories) for 6 days in RPMI 1640 medium containing 10% fetal bovine serum. Cloning of activated T cells was performed by limiting dilution in the presence of recombinant IL-2 (20 units/ml) and irradiated autologous PBLs as feeder cells (19).

Measurement of sCD26 and DPPIV Activity in Human Plasma. sCD26 was assayed by incubation of samples diluted in PBS containing 0.05% Tween 20 (Sigma) on plates coated with a 10-μg/ml solution of anti-CD26 (5F8) (3) in 0.05 M carbonate/bicarbonate buffer (pH 9.6), followed by blocking with Superblock (Pierce). All incubations were for 1 hr at room temperature. After washing in PBS/Tween, the plates were then incubated with anti-CD26 (1F7)-biotin (1 μg/ml) followed by streptavidin-alkaline phosphatase (0.2 μg/ml; Fisher). *p*-Nitrophenyl phosphate (1 mg/ml) in 0.5 M diethanolamine buffer, pH 9/5 mM $MgCl_2$ was added and color development was measured at 405 nm.

DPPIV was determined by capture on anti-CD26-coated plates followed by incubation with substrate, Gly-Pro-*p*-nitroaniline *p*-tosylate (1 mg/ml in water; Bachem) and color development was measured at 405 nm, with *p*-nitroaniline (Sigma) as standard.

Deletion of sCD26 from Plasma. Plasma samples were incubated with anti-CD26 (1F7) or irrevelant isotype-matched antibody (IgG1) for 1 hr and then added to anti-mouse IgG-magnetic beads (Advanced Magnetics) and incubated for 1 hr at 4°C, after which the CD26/anti-CD26/anti-mouse IgG-magnetic bead complexes were removed with a magnet.

RESULTS

Production of sCD26. In previous *in vitro* translocation studies, Hong and Doyle (20) used rat DPPIV to show that a deletion mutation of the cytoplasmic region resulted in release of DPPIV from the membrane (20). In addition, it appears that the shorter the length of the signal/anchoring sequence of type II proteins, the more likely it is that cleavage of the anchoring sequence will occur (21).

Accordingly, human CD26 cDNA was mutated to delete the sequence coding for amino acids 3–9. The mutant CD26 lacks four of the six amino acids within the predicted cytoplasmic region and three amino acids of the putative signal/anchoring (transmembrane) domain (Fig. 1).

The mutant CD26 cDNA was introduced into CHO cells, and a transfectant which produced the largest amount of sCD26 was selected for purification of the protein.

Characterization of Purified sCD26. The purified sCD26 showed a single band of correct molecular weight by SDS/PAGE analysis (Fig. 2). Amino acid sequence analysis of the purified protein revealed that 90% of the purified sCD26 had NKGTDDATA as the amino-terminal sequence, corresponding to the sequence immediately after the putative signal/anchoring (transmembrane) sequence of wild-type CD26 (Fig. 1, lines 1 and 3).

The reactivity of sCD26 with anti-CD26 mAbs was examined. ^{125}I-labeled sCD26 was immunoprecipitated by the anti-CD26 antibodies 1F7 and 5F8 (Fig. 3, lanes 2 and 3) as well as Ta1 (data not shown), which recognize different epitopes of the CD26 antigen. The purified sCD26 was not precipitated by the isotype-matched control antibodies recognizing L-selectin and CD3 (Fig. 3, lanes 4 and 5). The purified sCD26 thus retains the original tertiary structure defined by three different anti-CD26 mAbs.

The purified sCD26 was examined for DPPIV activity and exhibited a K_m of 0.22 mM, similar to that of membrane CD26. The V_{max} of the purified protein for Gly-Pro-*p*-nitroanilide was 8.86 $mM \cdot min^{-1} \cdot mg^{-1}$.

Enhancement of T-Cell Proliferation by sCD26. To determine how CD26 functions in T-cell costimulation, the effect of addition of sCD26 on TT-induced T-cell proliferation was examined. In Fig. 4*A*, representative results from one donor reveal that the addition of sCD26 at 1 and 25 μg/ml increased TT-induced T-cell proliferation 3- and 5-fold, respectively. The addition of two other soluble T-cell surface antigens, soluble CD45 and CD4, did not have a significant enhancing effect on T-cell proliferation (Fig. 4*A*). For positive respond-

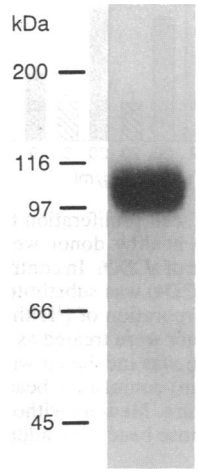

FIG. 2. SDS/PAGE of the purified sCD26. Five micrograms of sCD26 purified from culture supernatant transfected CHO cells was analyzed by SDS/6% PAGE under reducing conditions. Then the protein was visualized by staining with Coomassie brilliant blue R250.

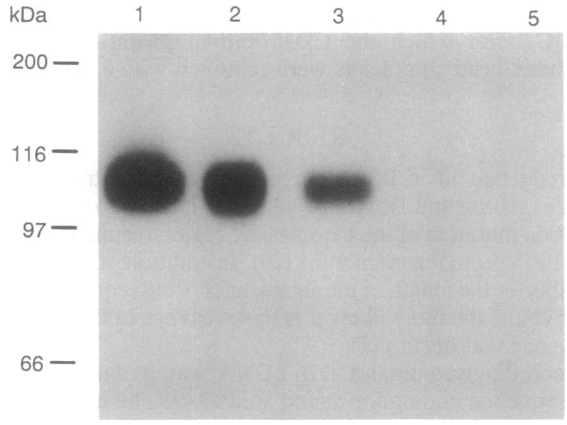

FIG. 3. Immunoprecipitation of sCD26 by anti-CD26 mAbs. Purified sCD26 was labeled by ^{125}I (lane 1) and immunoprecipitated by the mAbs 1F7 (anti-CD26) (lane 2), 5F8 (anti-CD26) (lane 3), TQ1 (anti-L-selectin) (lane 4), and Rw24B6 (anti-CD3) (lane 5). Precipitates were separated by SDS/6% PAGE under reducing conditions and detected by autoradiography.

ers to TT stimulation, the addition of sCD26 increased TT-induced T-cell proliferation 1.5–5 times in 50% of the cases. In all instances, the addition of sCD26 without any other stimulation did not enhance background proliferation of T cells (data not shown).

To confirm that this effect was specifically mediated by sCD26, sCD26-containing solution was precleared with beads conjugated with anti-CD26 (1F7) or an irrelevant (anti-L-selectin) mAb before addition to the T-cell proliferation assay mixture. Preclearing with anti-CD26 mAb removed the enhancing effect of sCD26, whereas preclearing with an irrelevant antibody had no effect (Fig. 4*B*). Similarly, preclearing with antibody, against either CD26 or irrelevant antibody, of control medium not containing CD26 had no effect upon T-cell proliferative responses (Fig. 4*B*). These results confirmed that the enhancement of TT-induced T-cell proliferation was mediated by sCD26 itself.

The same PBLs used in Fig. 4*B* were tested for the response to anti-CD3 mAb or phytohemagglutinin stimulation, which induces polyclonal T-cell proliferation. The addition of sCD26 or other soluble proteins did not affect anti-CD3 mAb- or phytohemagglutinin-stimulated T-cell proliferation for the mitogens at their optimum dose (data not shown), although the responses to TT could be enhanced by sCD26 (Fig. 4*B*).

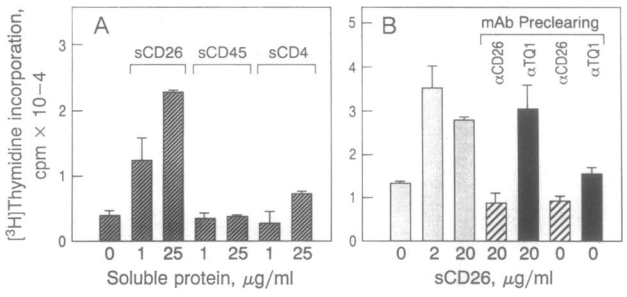

FIG. 4. Enhancement of antigen-induced T-cell proliferation by addition of sCD26. (*A*) PBLs purified from a healthy donor were stimulated with TT with or without the presence of sCD26. In control experiments, soluble CD45 (sCD45) or CD4 (sCD4) was substituted for sCD26. Proliferation was assessed by incorporation of [^{3}H]thymidine into cells. (*B*) PBLs from a different donor were treated as in *A*. As a control, the medium containing sCD26 was incubated with mAb 1F7 (anti-CD26) or TQ1 (anti-L-selectin)-conjugated beads prior to addition to the proliferation assay mixture. Medium without exogenous sCD26 was treated similarly with those beads and added to the assay mixture.

Importance of DPPIV Enzyme Activity for the Enhancing Effect of sCD26. To confirm the effect of sCD26 in a more reproducible system, a TT-specific T-cell clone was employed. Addition of sCD26 increased the response of the T-cell clone with the optimal concentration at 0.5 μg/ml (Fig. 5*A*). Addition of more sCD26 reproducibly reduced the enhancement effect, in contrast to the results with PBLs. As observed with PBLs, the addition of sCD26 without stimulation did not increase the background proliferation of this T-cell clone (Fig. 5*A*).

To test whether the DPPIV activity was important for this enhancing effect, DPPIV activity-negative sCD26 was tested in the same assay system. The DFP-treated sCD26 still retains reactivity with anti-CD26 mAbs but lacks DPPIV enzyme activity. Addition of DPPIV-negative sCD26 did not enhance T-cell proliferation (Fig. 5*B*). These results suggest that the DPPIV activity of sCD26 contributes to the enhancement effect of the molecule.

Correlation of Plasma sCD26/DPPIV Levels with the TT-Specific T-Cell Response to Added sCD26. As noted previously, not all the TT responders responded to added sCD26. The difference in responsiveness of T cells from individual donors may depend on the degree of exposure to endogenous plasma sCD26/DPPIV. Consequently, plasma sCD26/DPPIV levels and the responsiveness of T cells to exogenous sCD26 were determined. The content of sCD26 protein and DPPIV enzyme activity in the plasma were measured by using CHO cell-produced sCD26 as a standard. The results are summarized in Table 1 and representative data are shown in Fig. 6. All the DPPIV activity was attributed to sCD26, since clearing of the plasma with anti-CD26 mAb (1F7) removed the DPPIV activity completely (data not shown).

For a donor with high endogenous levels of sCD26/DPPIV and who responded strongly to TT stimulation, exogenous sCD26 had no effect or some inhibitory effect (Fig. 6*C*; Table 1, group 1). For those donors with lower levels of sCD26/DPPIV and who responded moderately to TT, exogenous sCD26 enhanced their TT-induced T-cell response (Fig. 6 *A* and *B*; Table 1, group 2). As shown previously, the addition of sCD26 had no effect if the donor PBLs did not respond to TT (Fig. 6*D*; Table 1, group 3). These results suggest that high endogenous levels of sCD26 render a cell insensitive to any further effect of exogenous sCD26. Since the results were not different when TT stimulation was performed in the presence of untreated or sCD26-depleted autologous plasma (Fig. 6), it appears that the responding cells retain the "primed state"

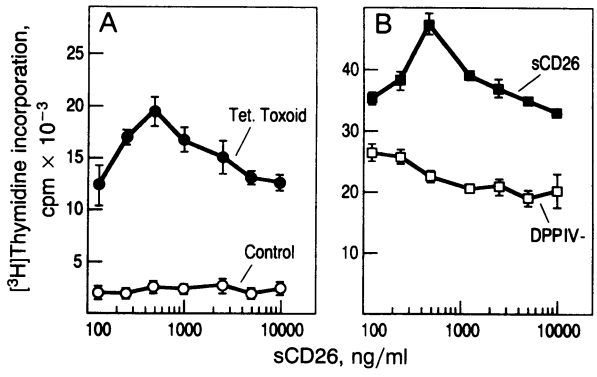

FIG. 5. Enhancement of proliferation of a TT-specific T-cell clone by sCD26/DPPIV. (*A*) The clone was stimulated with (●) or without (○) TT in the presence of autologous irradiated PBLs and various concentrations of sCD26. (*B*) The clone was stimulated as above with various concentrations of wild-type sCD26 (■) or diisopropyl fluorophosphate-treated sCD26 (□).

Table 1. Correlation between plasma sCD26/DPPIV level and the responsiveness of T-cell TT response to exogenous sCD26

Group	Donor	sCD26, μg/ml	DPPIV activity, nmol/min	[^{3}H]Thymidine incorporation, cpm	
				Response to TT*	Effect of sCD26†
1	1	10.6	1.84	21,611 (+++)	19,271 (−)
	2	14.8	2.35	125,570 (++++)	83,136 (−)
	3	12.1	1.41	82,181 (++++)	59,630 (−)
	4	11.3	2.70	32,589 (+++)	25,860 (−)
	$\bar{X}$ ± SD	12.2 ± 1.8	2.07 ± 0.57		
2	5	8.0	1.51	1,536 (+)	11,241 (+++)
	6	7.8	0.75	1,310 (+)	14,623 (+++)
	7	5.6	0.78	10,140 (++)	22,930 (++)
	8	4.9	0.36	8,188 (+)	26,470 (++)
	9	7.8	0.75	10,132 (++)	17,309 (+)
	10	6.5	0.68	27,284 (+++)	34,324 (+)
	$\bar{X}$ ± SD	6.8 ± 1.3	0.81 ± 0.38		
3	11	7.8	2.35	893 (−)	559 (−)
	12	7.8	2.30	0 (−)	0 (−)
	13	7.3	0.69	0 (−)	0 (−)
	14	5.2	0.36	0 (−)	516 (−)
	$\bar{X}$ ± SD	7.0 ± 1.2	1.43 ± 1.05		

$\bar{X}$ ± SD, mean ± 1 standard deviation. All results were determined in triplicate and the coefficient of variation was <15% of the mean.

*Ranges are delineated as follows: −, no response (<1000 cpm); +, 1000–10,000 cpm; ++, 10,000–20,000 cpm; +++, 20,000–60,000; ++++, >60,000 cpm, all as increases over background.

†Ranges are as follows: +, 1- to 2-fold increase; ++, 2- to 8-fold increase; +++, 8- to 20-fold increase. sCD26 was added at 1 μg/ml.

induced by endogenous plasma sCD26, even after transfer to the *in vitro* antigen-induced reaction mixture.

DISCUSSION

The present study shows that the addition of sCD26 enhanced T-cell proliferative responses to the recall antigen TT. Intact DPPIV enzyme activity appears to play a necessary enhancing role with sCD26, as it does in the costimulatory activity of the membrane-bound form of CD26 (18). In addition, human plasma exhibited a wide range of levels for sCD26/DPPIV which inversely correlated with the TT-specific T-cell response to added sCD26. These results suggest that CD26 is involved in T-cell activation through its DPPIV activity and that sCD26/DPPIV participates in regulation of the T-cell memory response *in vitro* as well as *in vivo*.

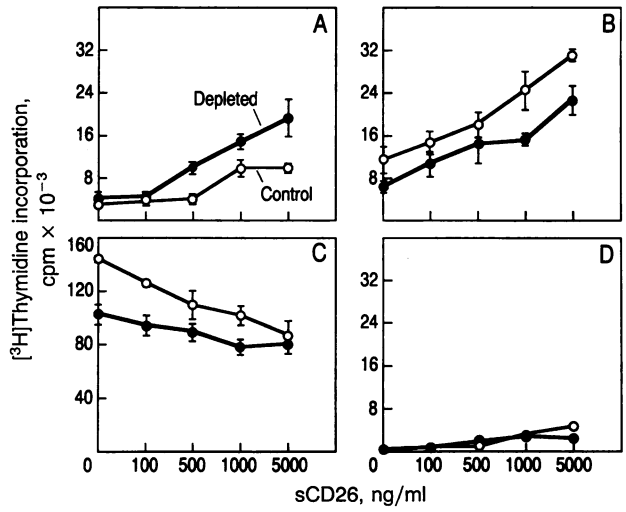

FIG. 6. Heterogeneity of TT-specific T-cell responses to added sCD26. Responses of PBLs to TT were examined with various concentrations of sCD26 in the presence of CD26-depleted (●) or control plasma (○) in the culture system. (*A* and *B*) Exogenous sCD26 enhances TT-induced T-cell responses (donors 5 and 7). (*C*) Exogenous sCD26 inhibits TT-induced T-cell responses (donor 1). (*D*) Addition of sCD26 has no effect because the donor PBLs do not respond to TT (donor 14).

Engagement of the membrane form of CD26 has been shown to mediate costimulatory activity for T-cell activation. For example, solid-phase immobilized anti-CD26 can mediate a comitogenic effect on highly purified CD4$^+$ T cells as well as CD26-transfected Jurkat T cells via the CD3 pathway in the absence of accessory cells (6, 10). Based on these observations, it is assumed that a putative ligand for CD26 may exist either on the same T cell or on other T cells. Evidence for a putative ligand for CD26 on T cells is provided by previous studies which showed that the DPPIV activity of membrane-expressed CD26 was important in the elevated IL-2 production from CD26-transfected Jurkat T cell lines, with no other immunoregulatory cells present (13). Similarly, DPPIV activity may be required for the enhancing effect of sCD26 (Fig. 5). The DPPIV activity of sCD26 may directly modulate T-cell activity through a processing of bioactive molecules (22), thus enhancing responsiveness of T cells to antigenic stimulation, although preliminary data (not shown) suggest that CD26/DPPIV has no effect upon IL-2 or any active component of T-cell growth factor over a range of sCD26 from 1 ng/ml to 10 μg/ml. Nevertheless, other cytokines of biologically active molecules may be involved in the process.

In comparison with other T-cell costimulatory molecules such as CD28 and CTLA-4 (23, 24), it was reported that soluble recombinant CTLA-4–immunoglobulin fusion protein inhibited T-cell proliferation in a mixed lymphocyte reaction, as well as T-cell-dependent B-cell IgG synthesis *in vitro* (24). Since the B7 molecule is expressed on both B cells and antigen-presenting cells, the CTLA-4 fusion protein blocks immune responses by interfering with the interaction of CD28, B7-1, and B7-2 which triggers a necessary costimulatory signal for T cells (25). With regard to other costimulatory molecules such as CD40/CD40–ligand (26), a CD40–immunoglobulin fusion protein has been shown to block the interaction of T helper cells with activated B cells (27), suggesting that this interaction is a key to T-cell-dependent B-cell activation. Consequently, the mechanism of costimu-

lation by sCD26 appears to differ from that of other costimulatory molecules such as CD28, CTLA-4, and CD40.

The binding of sCD26 to the putative ligand on T cells may trigger T-cell activation and subsequently induce T-cell proliferation. It is clear that sCD26 specifically enhances antigen-specific T-cell memory responses and is not an activator in its own right, since no enhancement was developed by PBLs which did not respond at all to TT. These cells may well have responded to other recall antigens, but without this primary stimulus, the cells did not respond to sCD26. This suggests that sCD26 affects either antigen presentation or the efficiency of signal transduction on the T cell after antigen presentation. One possibility results from the documented interaction between CD26 and CD45RO, the latter being preferentially expressed on $CD4^+$ memory T cells which respond maximally to recall antigens. We may envisage that CD26 enhances the phosphatase activity of CD45RO, subsequently regulating the activity of CD4- and CD3-associated kinases $p56^{lck}$ and $p59^{fyn}$ (28, 29). Earlier studies indicated that modulation of CD26 resulted in enhanced phosphorylation of CD3ζ tyrosine residues and increased CD4-associated $p56^{lck}$ tyrosine kinase activity (11).

Although sCD26 enhances the proliferative response of PBLs and T-cell clones, the optimal concentration for TT-specific T-cell clones was 0.5 μg/ml (Fig. 5) and for PBLs was 0.5 to >5 μg/ml, depending on the donors. It is possible that freshly isolated PBLs have been exposed already to sCD26 in plasma and are relatively refractory to further stimulation by sCD26, since the putative ligand on the T-cell surface may be saturated, thus reducing the efficacy of added sCD26. In group 2 of Table 1, an inverse correlation may be drawn between the response to TT and the efficacy of sCD26, implying that sCD26 does not increase the maximum response to recall antigen but, rather, helps the responding T cell achieve that maximum response. Accordingly, in group 2, lower levels of endogenous plasma sCD26 may leave free putative ligand to which sCD26 exogenously added may bind.

Accordingly, in this report we demonstrate that sCD26 enhances reactions by T cells toward specific recall antigens. This enhancement occurs only for cells reacting at a low to moderate level with recall antigen, implying that sCD26 does not increase the maximal response but instead aids cells in achieving this level. This has very important consequences for immunodeficient states, where administration of sCD26 may help increase antigen-specific responses.

Several important questions immediately arise. (*i*) What is the mechanism of the enhancing effect of sCD26 on T-cell memory responses? (*ii*) Does plasma sCD26 originate from T cells and have any specific function or target with reference to its DPPIV activity. (*iii*) Would the introduction of exogenous sCD26 *in vivo* enhance antigen-specific responses in immunodeficient states? Of particular importance here is the observation that the Tat product encoded by the genome of human immunodeficiency virus type 1 not only has an inhibitory effect upon the antigen responsiveness of human peripheral blood T cells but is also able to inhibit the DPPIV activity of CD26 (30). With regard to function, CD26 is a binding or carrier protein for adenosine deaminase (12), an enzyme whose deficiency is strongly associated with severe combined immunodeficiency disease (31). Further, it has been suggested that CD26 is a putative coreceptor for human immunodeficiency virus (32).

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