Soluble CD26/Dipeptidyl Peptidase IV Induces T Cell Proliferation Through CD86 Up-Regulation on APCs

Kei Ohnuma, Yasuhiko Munakata, Tomonori Ishii, Satoshi Iwata, Seiji Kobayashi, Osamu Hosono, Hiroshi Kawasaki, Nam H. Dang and Chikao Morimoto

http://www.jimmunol.org/content/167/12/6745

**References**
This article cites 51 articles, 24 of which you can access for free at:
http://www.jimmunol.org/content/167/12/6745.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Soluble CD26/Dipeptidyl Peptidase IV Induces T Cell Proliferation Through CD86 Up-Regulation on APCs

Kei Ohnuma,* Yasuhiko Munakata,† Tomonori Ishii,‡ Satoshi Iwata,∗ Seiji Kobayashi,∗ Osamu Hosono,∗ Hiroshi Kawasaki,∗ Nam H. Dang,§ and Chikao Morimoto∗∗

CD26 is a T cell costimulatory molecule with dipeptidyl peptidase IV enzyme activity in its extracellular region. We have previously reported that the addition of soluble CD26 (sCD26) resulted in enhanced proliferation of peripheral blood T lymphocytes induced by the recall Ag, tetanus toxoid (TT). However, the mechanism involved in this immune enhancement has not yet been elucidated. In this paper, we demonstrate that the enhancing effect of sCD26 on TT-induced T cell proliferation occurred in the early stages of immune response. The cells directly affected by exogenously added sCD26 are the CD14-positive monocytes in the peripheral blood. Mannose-6-phosphate interfered with the uptake of sCD26 into monocytes, suggesting that mannose-6-phosphate/insulin-like growth factor II receptor plays a role in the transportation of sCD26 into monocytes. When sCD26 was added after Ag presentation had taken place, enhancement in TT-induced T cell proliferation was not observed. In addition, enhancement of TT-mediated T cell proliferation by sCD26 does not result from trimming of the MHC-bound peptide on the surface of monocytes. Importantly, we also showed that exogenously added sCD26 up-regulated the expression of the costimulatory molecule CD86 on monocytes through its dipeptidyl peptidase IV activity, and that this increased expression of CD86 was observed at both protein and mRNA level. Therefore, our findings suggest that sCD26 enhances T cell immune response to recall Ag via its direct effect on APCs. The Journal of Immunology, 2001, 167:6745–6755.

The widely distributed 110-kDa cell surface glycoprotein CD26 has known dipeptidyl peptidase IV (DPPIV,3 EC 3.4.14.5; Ref. 3) activity in its extracellular domain (1, 2). This enzyme is capable of cleaving amino-terminal dipeptides with either L-proline or L-alanine at the penultimate position. CD26/DPPIV has two physiological forms in human (3). One is the membrane-bound form preferentially expressed on the CD4+ helper/ memory T cell population, whereas the other is the soluble form found in serum. Accumulating evidence suggests that DPPIV enzyme activity plays an essential role in the immune response (4). Cross-linking of CD26 and CD3 with solid-phase immobilized mAbs can induce T cell costimulation and IL-2 production by either human CD4+ T cells or Jurkat T cell lines transfected with CD26 cDNA (5, 6). In addition, anti-CD26 Ab treatment of T cells leads to a decrease in the surface expression of CD86 via its internalization, and such modulation results in an enhanced proliferative response to anti-CD3 or anti-CD2 stimulation, as well as enhanced tyrosine phosphorylation of signaling molecules such as CD3ζ and p56CΔ (7). Moreover, we have shown that DPPIV enzyme activity is required for the CD26-mediated T cell costimulation (8). More recently, we have shown that internalization of CD26 after cross-linking is mediated in part by the mannose-6-phosphate (M6P)/insulin-like growth factor II receptor (IGF-IIR), and that the interaction of CD26 and M6P/IGF-IIR plays a role in CD26-induced T cell costimulation (9).

Maximal T cell activation requires both an Ag-specific stimulus provided by an MHC peptide complex and a costimulatory signal (10). Engagement of CD28 on the surface of T cells by B7-1 (CD80) or B7-2 (CD86) expressed on APCs provides a potent costimulatory signal (10–14). CD28-B7 interactions lead to T cell proliferation, differentiation, and cytokine secretion (14, 15). In contrast, engagement of CTLA-4 on activated T cells by B7-1 or B7-2 results in an inhibition of T cell responses (16–18). However, only CD28 is constitutively expressed and, hence, it has an important role in the generation of an immune response (19–24).

In our previous report, we have shown that recombinant soluble CD26 (sCD26) enhanced proliferative responses of PBLs to stimulation with the soluble Ag, tetanus toxoid (TT) (25). In addition, this enhancing effect required DPPIV enzyme activity (8, 25). However, the precise mechanism for enhancement of TT-induced T cell proliferation by sCD26 remains unresolved.

In the present study, we demonstrated that the target cells of sCD26 were the CD14-positive monocytes in the peripheral blood and that M6P/IGF-IIR played a role in transporting sCD26 into the monocytes. Importantly, we found that sCD26 could up-regulate the expression of the CD86 but not CD80 or HLA-DR Ag on monocytes. These results suggest that sCD26 can be a useful agent in potentiating immune response in selected clinical settings.
Materials and Methods
Isolation and activation of human lymphocyte populations

Human PBMC, collected from healthy adult volunteers who were immunized with TT within two years before donation, were isolated by centrifugation on Ficoll/Paque (Amersham Pharmacia Biotech, Piscataway, NJ). PBMC were directly used for the time-course studies. For the reconstitution experiments, PBMC were further purified into T cell fractions and APC fractions. To obtain a highly purified T cell population, PBMC were separated into an E rosette-positive (E+) population and were used as resting T cells as determined by flow cytometric analysis (FACS Calibur; Nikon BD Biosciences, Tokyo, Japan) using an FITC-labeled anti-CD3 mAb (BD Pharmingen, San Diego, CA) with purity being >95%. To obtain APC, an E rosette-negative (E−) population was adhered to plastic plates for 4 h at 37°C, and adherent cells were used as APC. Monocytes were either purified by a flow cytometer on the basis of PE-labeled anti-CD14 mAb (BD Pharmingen)-oriented parameter, or by negative selection through the use of immunomagnetic beads coated with an anti-CD3, CD7, CD19, CD45RA, CD56, and IgE mAb (Miltenyi Biotec, Auburn, CA) with purity being >95%. PBMC were cultured in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies) and TT (Calbiochem, La Jolla, CA) at different concentrations for 16 h, followed by sCD26 pulses at various time intervals. T cells were also cultured with the standard medium (10% FCS-RPMI 1640 with penicillin and streptomycin) in the presence or absence of sCD26. Monocytes were incubated with TT at different concentrations for 16 h in the standard medium, followed by the addition of sCD26 in the culture medium at different times. To avoid interference by nonspecific activation of monocytes due to contamination, polymerized B subtilis (20 μg/ml; Sigma-Aldrich, St. Louis, MO) was added to all media and reagents used for APC/monocytes experiments.

All cells were preincubated in the standard medium for 24 h to minimize the risk of potential interference from sCD26 present in human serum (8). In these experiments, cells were incubated in 96-well plates (200 μl/well; Falcon, Franklin Lakes, NJ). For the time-course study, 1.0 × 10^6 PBMC were incubated with 0.5 μg/ml TT for 16 h. Then, 0.5 μg/ml sCD26 was added to the well and was incubated for 0, 6, 12, 24, 48, 72, and 96 h. The day when the first sCD26 was added to the culture well was defined as day 0 of culture. All assays in proliferation experiments were performed on day 7 of culture after cells were incubated at 37°C in a 5% CO_2 humidified atmosphere. For the reconstitution study, 0.5 × 10^6 of monocytes were preincubated with TT at a concentration of 0.5 μg/ml with different concentrations of sCD26 for 24 h. Monocytes were washed with PBS, and then 1 × 10^6/well of preincubated monocytes were subjected to the assay with 1 × 10^5/well of purified T cells from the same donor as the prepared monocytes. For fixation study, as prefixed stimulation study, after being treated with 0.05% glutaraldehyde for 30 s at room temperature, followed by being washed three times with PBS, 0.5 × 10^6 of monocytes were incubated with TT at a concentration of 0.5 μg/ml and with 0.5 μg/ml sCD26. After prefixed stimulation study, 1.0 × 10^6 of monocytes were initially incubated with TT at a concentration of 0.5 μg/ml and with 0.5 μg/ml sCD26. Subsequently, the preincubated monocytes were treated with 0.05% glutaraldehyde for 30 s at room temperature, followed by being washed three times with PBS. Monocytes (1.0 × 10^5/well) were then subjected to the assay with 1.0 × 10^5/well of purified T cells, which originated from the same donor as the prepared monocytes.

Preparation of sCD26

sCD26 with DPPIV (sCD26/DPPIV*) was produced according to the method described previously (24). Briefly, the expression vector RecSRe-26-3 days 3-9, which contains a deletion of the coding sequence for amino acids 3-9 of CD26, was transfected into a dihydrolfructose reducetase-deficit Chinese hamster ovary (CHO) cell line, DBX-11 by electroporation, together with pMT-2 providing the dihydrolfructose reductase gene. Mutant sCD26 without DPPIV (sCD26/DPPIV−) was produced in the same method except that RecSRe-26d-3 was further modified to yield RecSRe-26d-3/S630A, which contains a point mutation at the active site of the DPPIV enzyme (Ser^10 was replaced by Ala) by site-directed mutagenesis using the oligonucleotide. The transfected CHO cells, which produce either sCD26 or mutant sCD26, were cultured in serum-free CHO-S-SFM II medium (Life Technologies) supplemented with 1 μM methotrexate (Sigma-Aldrich). The culture supernatant was collected and subjected to affinity chromatography on adenosine deaminase-Sepharose according to the methods described previously (9).

mAbs and reagents

The source and working concentration of the mAbs used as primary Abs for flow cytometry are as follows: PE-conjugated anti-CD3 (UCHT1, mouse IgG1, 10 μg/ml; BD Pharmingen), anti-CD14 (Mo-2, mouse IgM; 10 μg/ml; Beckman Coulter, Miami, FL), anti-CD19 (HB919, mouse IgG1; 10 μg/ml; BD Pharmingen), and anti-CD56 (NKH1, mouse IgG1; 10 μg/ml; Beckman Coulter); FITC-labeled anti-CD80 (BB1, mouse IgM; 10 μg/ml; BD Pharmingen), anti-CD86 (IT2.2, mouse IgG2; 10 μg/ml; BD Pharmingen), and anti-HLA-DR (L243, mouse IgG2; 10 μg/ml; BD Pharmingen). Oregon green-conjugated sCD26 (sCD26-OG) was made with FluorReporter Oregon green protein labeling kit (Molecular Probes, Eugene, OR) according to the manufacturer’s instructions. Oregon green-conjugated proteins were used at a concentration of 1 μg/ml. Biotinylated anti-CD14 (Mo-2; IgM; 10 μg/ml) was purchased from Beckman Coulter. mAbs for blocking assays were obtained as follows: CD80 (BB1), CD86 (IT2.2), and HLA-DR (L243) from were BD Pharmingen; the chimeric protein of human CTLA-4 and murine Ig (CTLA-4 Ig) and relevant control murine Ig were purchased from Ancell (Bayport, MN); and mouse anti-human IFN-γ mAb was kindly provided by Dr. V. Horcsics (Academy of Science of the Czech Republic, Praha, Czech Republic). Texas red-conjugated anti-human M6P/IGF-IIR mAb (M6P/IGF-IIR-red) was made with FluorReporter Texas red protein labeling kit (Molecular Probes) according to the manufacturer’s instructions. In all experiments, relevant control mAbs of the same Ig isotype were included (IgG1 (MOPC-21), IgG2 (G155−176), and IgM (G155−228) were purchased from BD Pharmingen). Egg white lysozyme was purchased from Wako Pure Chemical (Osaka, Japan) and was conjugated with Oregon green.

![FIGURE 1. Proliferative effect on PBMC by sCD26. PBMC (1 × 10^5) were incubated in culture medium with or without TT or sCD26. The freshly isolated PBMC was cultured in standard culture medium for 24 h, then TT (0.5 μg/ml) was added to the medium to incubate for an additional 16 h. CD26 (0.5 μg/ml) was then added to the wells and was incubated for various time periods as shown in the figure. Proliferation of cells was monitored in all instances by measuring [3H]Tdr incorporation on day 7 of culture after the first sCD26 was added. Degree of proliferation is indicated as cpm in the ordinate. The experiments represent mean values ± SE calculated from three independently performed experiments.](http://www.jimmunol.org/)

Downloaded from http://www.jimmunol.org/ by guest on September 25, 2017
Table I.  sCD26-OG incorporation according to lymphocyte subpopulations

<table>
<thead>
<tr>
<th>Gated PE-Ab</th>
<th>CD3</th>
<th>CD14</th>
<th>CD19</th>
<th>CD56</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Cells with DPPIV&lt;sup&gt;+&lt;/sup&gt; sCD26-OG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT&lt;sup&gt;−&lt;/sup&gt; %</td>
<td>14.4 ± 0.5</td>
<td>80.3 ± 5.1</td>
<td>15.4 ± 1.8</td>
<td>5.6 ± 1.0</td>
</tr>
<tr>
<td>MFI</td>
<td>47.9 ± 4.8</td>
<td>51.5 ± 2.7</td>
<td>49.2 ± 4.1</td>
<td>48.8 ± 4.8</td>
</tr>
<tr>
<td>TT&lt;sup&gt;+&lt;/sup&gt; %</td>
<td>13.8 ± 1.6</td>
<td>85.8 ± 5.6</td>
<td>16.1 ± 1.9</td>
<td>4.9 ± 1.1</td>
</tr>
<tr>
<td>MFI</td>
<td>44.5 ± 2.9</td>
<td>48.5 ± 3.6</td>
<td>47.4 ± 4.8</td>
<td>40.3 ± 5.2</td>
</tr>
<tr>
<td>% Cells with DPPIV&lt;sup&gt;−&lt;/sup&gt; sCD26-OG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT&lt;sup&gt;−&lt;/sup&gt; %</td>
<td>15.8 ± 1.5</td>
<td>83.1 ± 4.9</td>
<td>14.9 ± 2.8</td>
<td>6.6 ± 1.3</td>
</tr>
<tr>
<td>MFI</td>
<td>50.4 ± 4.1</td>
<td>50.6 ± 4.3</td>
<td>49.8 ± 3.2</td>
<td>42.7 ± 5.1</td>
</tr>
<tr>
<td>TT&lt;sup&gt;+&lt;/sup&gt; %</td>
<td>13.2 ± 1.4</td>
<td>82.4 ± 4.3</td>
<td>17.3 ± 2.9</td>
<td>3.7 ± 1.4</td>
</tr>
<tr>
<td>MFI</td>
<td>49.4 ± 3.5</td>
<td>44.1 ± 6.2</td>
<td>47.8 ± 3.7</td>
<td>46.8 ± 6.6</td>
</tr>
</tbody>
</table>

*Freshly isolated PBMC (1 × 10<sup>6</sup>/well) were incubated with or without TT for 16 h after 24-h incubation and then sCD26/DPPIV<sup>−</sup>-OG was added to the culture wells. After incubation with sCD26-OG for 24 h, cells were washed in ice-cold PBS, and in acidic buffer (pH 3 PBS) for stripping the sCD26-OG on the cell surfaces. Then, cells were incubated with various PE-conjugated Abs (CD3, CD14, CD19, and CD56) for 30 min. Analysis was performed by FACS Calibur. The experiments represent mean ± SE calculated from three independently performed experiments. %, Percent of sCD26-OG-positive cell number in the various PE-positive cells. Similar experiments were performed using mutant sCD26 of defective DPPIV activity (sCD26/DPPIV<sup>−</sup>-OG). MFI, mean fluorescence intensity.
Enhancement of T cell proliferation by sCD26/DPPIV

To investigate the mechanism involved in the enhancement of TT-induced T cell proliferation by sCD26, we first performed a time-course analysis by adding sCD26 to the PBMC system stimulated by TT in vitro. In the early stage of the immune response to foreign Ags, direct interaction between APC and T cells is indispensable (21–23). However, in the latter stages, direct APC-T cell interaction is not always necessary, whereas secreted cytokines such as IL-2 are essential in maintaining the reaction (14, 21). As shown in Fig. 1, the addition of sCD26 within first 48 h resulted in an enhancement in T cell proliferation to TT. In contrast, the addition of sCD26 after 48 h did not enhance TT-mediated activation, and an enhancing effect of TT-induced T cell proliferation was not observed in the experiments with the DPPIV-deficient mutant sCD26. These results indicate that sCD26 affected the early stages of immune reaction to TT but did not have an appreciable effect on the latter stages. Moreover, these data are consistent with previous studies showing that the enhancement of TT-induced T cell proliferation requires DPPIV enzyme activity (8).

Incubation with sCD26 leads to uptake by monocytes

Because sCD26 affected the early stages of immune response to TT, we next attempted to determine the target cells of sCD26. For this purpose, we incubated PBMC with sCD26-OG for 24 h in the presence or absence of TT. As shown in Table I, leukocyte phenotypes such as CD3, CD14, CD19, and CD56 were not affected by presence of TT. In addition, sCD26 was taken up mainly by CD14-positive monocytes (Table II and Fig. 2A). In contrast, flow monocytes were washed in PBS, and once in acidic buffer (pH 3 PBS) to strip any sCD26-OG on the cell surface. The cells were then incubated with the biotinylated anti-CD14 Ab and incubated with streptavidin-Texas red. After incubation with sCD26-OG, sCD26-OG was incorporated in the monocytes (B and D, sCD26/DPPIV<sup>−</sup>-OG; C and E, sCD26/DPPIV<sup>−</sup>-OG). Although sCD26-OG was detected intracellularly following 24 h of incubation (B and C), sCD26-OG was not detectable after 36 h of incubation (D and E). Bars indicate 10 μm.

Studies in mice with antibodies to DPPIV show that there is a decrease in immune responses to TT. This suggests that DPPIV has a role in the enhancement of immune responses to TT. The current study shows that sCD26 affects the early stages of immune response to TT and that sCD26 is taken up mainly by monocytes. This suggests that sCD26 may play a role in the enhancement of immune responses to TT through the interaction with DPPIV. The mechanism of how sCD26 affects the early stages of immune response to TT remains to be elucidated.

Results

sCD26 enhances T cell proliferation in the early stage of immune response to recall Ag

To investigate the mechanism involved in the enhancement of TT-induced T cell proliferation by sCD26, we first performed a time-course analysis by adding sCD26 to the PBMC system stimulated by TT in vitro. In the early stage of the immune response to foreign Ags, direct interaction between APC and T cells is indispensable (21–23). However, in the latter stages, direct APC-T cell interaction is not always necessary, whereas secreted cytokines such as IL-2 are essential in maintaining the reaction (14, 21). As shown in Fig. 1, the addition of sCD26 within first 48 h resulted in an enhancement in T cell proliferation to TT. In contrast, the addition of sCD26 after 48 h did not enhance TT-mediated activation, and an enhancing effect of TT-induced T cell proliferation was not observed in the experiments with the DPPIV-deficient mutant sCD26. These results indicate that sCD26 affected the early stages of immune reaction to TT but did not have an appreciable effect on the latter stages. Moreover, these data are consistent with previous studies showing that the enhancement of TT-induced T cell proliferation requires DPPIV enzyme activity (8).

Incubation with sCD26 leads to uptake by monocytes

Because sCD26 affected the early stages of immune response to TT, we next attempted to determine the target cells of sCD26. For this purpose, we incubated PBMC with sCD26-OG for 24 h in the presence or absence of TT. As shown in Table I, leukocyte phenotypes such as CD3, CD14, CD19, and CD56 were not affected by presence of TT. In addition, sCD26 was taken up mainly by CD14-positive monocytes (Table II and Fig. 2A). In contrast, flow monocytes were washed in PBS, and once in acidic buffer (pH 3 PBS) to strip any sCD26-OG on the cell surface. The cells were then incubated with the biotinylated anti-CD14 Ab and incubated with streptavidin-Texas red. After incubation with sCD26-OG, sCD26-OG was incorporated in the monocytes (B and D, sCD26/DPPIV<sup>−</sup>-OG; C and E, sCD26/DPPIV<sup>−</sup>-OG). Although sCD26-OG was detected intracellularly following 24 h of incubation (B and C), sCD26-OG was not detectable after 36 h of incubation (D and E). Bars indicate 10 μm.
cytometric analyses showed that T cells (CD3+), B cells (CD19+), and NK cells (CD56+) displayed relatively low levels of sCD26. It should be noted that the above findings were observed both in the presence and absence of TT, and that DPPIV-deficient mutant sCD26 (sCD26/DPPIV−) was also preferentially taken up by CD14-positive monocytes. Additional evidence that sCD26 was taken up by monocytes was seen in studies involving confocal microscopy. Concordant data shown in Table II and Fig. 2, B and C, show that sCD26/DPPIV−, as well as sCD26/DPPIV+, was clearly taken up by monocytes. However, as shown in Fig. 2, D (sCD26/DPPIV−) and E (sCD26/DPPIV+), sCD26 was no longer detectable intracellularly 36 h after incubation of monocytes with sCD26. The disappearance of sCD26 molecules following uptake by monocytes was also observed by flow cytometric analysis (data not shown). It should be noted that this uptake of sCD26 by monocytes was not affected by the presence of TT (data not shown).

**The target cells of sCD26 are monocytes**

Because the main target cells among PBMC were monocytes, as shown in Table II and Fig. 2, A–C, we next attempted to confirm the enhancement of TT-induced T cell proliferation by monocytes that take up sCD26. For this purpose, we performed a reconstitution study by separating T cells and monocytes at the time of incubation with sCD26. As shown in Fig. 3, the enhancing effect of TT-induced T cell proliferation was observed only when monocytes were preincubated with TT and sCD26, but not T cells (Fig. 3, A and B). Importantly, these studies again confirmed that sCD26-mediated enhancement of TT-induced T cell proliferation required DPPIV enzyme activity. To further confirm that sCD26 uptake by TT-primed monocytes leads to enhancement of T cell proliferation, we performed a reconstitution study at different doses of sCD26. As shown in Fig. 3A, the degree of TT-induced T cell proliferation was dependent upon the concentration of the exogenously added sCD26. Therefore, these results indicate that the principal target cells of sCD26 are APCs, including monocytes.

**Uptake of sCD26 into monocytes occurs via its binding to M6P/IGF-IIR**

We recently showed that M6P/IGF-IIR was the binding protein for CD26 and that it played a role in internalizing CD26 molecule into T cells after ligation of CD26 (9). To examine whether M6P/IGF-IIR is involved in monocyte uptake of sCD26, we used fluorescent confocal microscopy to initially evaluate monocyte expression of sCD26-OG and M6P/IGF-IIR intracellularly and on the cell surface. For this purpose, fluorescent mouse anti-human M6P/IGF-IIR mAb was conjugated with M6P/IGF-IIR-red. Fig. 4A shows that sCD26-OG and M6P/IGF-IIR-red colocalized on the monocyte cell surface. Following incubation at 37°C for 24 h in the presence of sCD26-OG and M6P/IGF-IIR-red, intracellular colocalization of these proteins was observed (Fig. 4, B and C). Colocalization of lysozyme and M6P/IGF-IIR was similarly observed (Fig. 4D). In contrast, colocalization was not observed after incubation of sCD26-OG and Texas red-conjugated mouse IgG1 to exclude the possibility of nonspecific binding to FcγR (data not shown). To further confirm that sCD26 uptake is dependent on its binding to M6P/IGF-IIR, we have performed the inhibition assay to evaluate uptake of sCD26 by monocytes in the presence of excess amounts of M6P. As shown in Fig. 4, E and F, FACS analyses showed that the addition of M6P into the culture system (0, 0.1, 1.0, and 10 μM) inhibited the uptake of sCD26 into monocytes. The degree of inhibition of sCD26 uptake was dependent upon the concentration of the exogenously added M6P (Fig. 4E). These findings were also observed even in the absence of TT (Fig. 4F). Thus, these results indicated that uptake of sCD26 into monocytes was due to the interaction of sCD26 with its binding protein M6P/IGF-IIR. It should be noted that this inhibitory effect of M6P on uptake of sCD26 was similarly seen in experiments performed with sCD26/DPPIV− (data not shown).

**Enhancement of TT-induced T cell proliferation by sCD26 is seen when monocytes are incubated with sCD26 before fixation**

Another explanation for the sCD26-induced enhancement of TT-mediated T cell proliferation following monocyte uptake may be the trimming of the MHC class II-bound peptide, hence altering cellular responsiveness to the Ag. Such a phenomenon has been
described previously with CD13 aminopeptidase N (25). CD13 contributes to Ag processing by trimming the MHC class II-bound peptide on the APC surface. Because CD26, like CD13, is also an ectopeptidase, it may have the effect of trimming MHC class II-bound peptide on the surface of APC. To evaluate this issue, we fixed TT-pulsed monocytes before and after incubation with sCD26, and subsequently examined whether the enhancing effect of sCD26 on T cell activation was observed. As shown in Fig. 5, A and B, an enhanced T cell proliferation was seen only when the monocytes were incubated with sCD26/DPPIV™ before fixation. In contrast, monocytes incubated with sCD26 after fixation did not alter the immune response occurring after the Ag peptide bound to MHC molecule. Therefore, these results suggested that enhancement of TT-induced T cell proliferation by sCD26 does not result from trimming of the MHC class II-bound peptide on the surface of APC. Rather, it is likely that internalization of sCD26 into monocytes affects the interaction of monocytes and T cells.

Expression of the costimulatory molecule CD86 is increased on monocytes following exposure to sCD26

We next analyzed the expression of several surface molecules on monocytes that have been previously described to play a role in T cell/monocyte interaction (10–15, 21–24). For this purpose, freshly isolated monocytes were incubated with sCD26 in the presence or absence of TT, and the expression of CD80, CD86, and HLA-DR on monocytes was examined using flow cytometric analysis. As shown in Table III and Fig. 6, CD86 molecule expression on monocytes was increased within the first 48 h after TT and sCD26 stimulation. However, the increase in CD86 expression was no longer observed after 48 h of sCD26 incubation. In contrast, expression of CD80 and HLA-DR was not affected by stimulation of TT and sCD26 (Table II). Therefore, these results showed that uptake of sCD26 into monocytes resulted in an increase in CD86 expression when monocytes were pulsed with TT and sCD26. Furthermore, these findings suggested that the enhanced CD86 expression on sCD26-treated monocytes contributed to trigger TT-induced T cell proliferation in the early stages of the
immune response to recall Ag. It should be noted that sCD26/DPPIV$^-$ mutant did not affect CD86 expression on monocytes, consistent with data showing that only sCD26/DPPIV$^+$, but not sCD26/DPPIV$^-$ mutant, enhanced TT-induced T cell proliferation (8, 24).

Enhanced expression of CD86 on monocytes triggered by TT and sCD26 is dependent on increased synthesis of mRNA

To determine whether the enhancement in CD86 expression following incubation of monocytes with sCD26 in the presence of TT is dependent on increased protein synthesis, levels of mRNA encoding for CD86 were quantified by RT-PCR. Freshly isolated monocytes were incubated with or without TT for 16 h after a 24-h incubation in the standard medium alone, and then sCD26 (0.5 µg/ml) was added to the culture wells. After incubation with sCD26 for 24 h, cells were processed for RNA isolation as described in Materials and Methods. As demonstrated in Fig. 7, the increase of surface CD86 expression seen when monocytes were incubated with TT and sCD26/DPPIV$^+$ is also associated with increased mRNA levels, suggesting that enhanced protein synthesis is one potential mechanism for enhanced CD86 surface expression. Of importance is the fact that monocytes incubated with TT and sCD26/DPPIV$^-$ did not demonstrate enhanced CD86 mRNA levels, again indicating the importance of DPPIV activity in this interaction.

Inhibitory effects of mAbs and CTLA-4 Ig on T cell proliferation by monocytes triggered by TT and sCD26

To further define the role of various surface molecules on T cell proliferation induced by TT-treated monocytes, monocytes were first treated with TT and/or sCD26, and then they were incubated with mAbs against CD80, CD86, HLA-DR, or CTLA-4 Ig at a final concentration of 5 µg/ml for 15 min at 4°C before onset of culture. Cells were then incubated at 37°C for the entire culture period. As shown in Fig. 8A, although mAb against HLA-DR efficiently inhibited T cell proliferation, sCD26 did not have an effect in the HLA-DR-mediated pathway. Similarly, no effect was observed in the TT-induced T cell proliferation after treatment of monocytes with anti-CD80 in the presence or absence of sCD26. In contrast, as shown in the lower panel of Fig. 8A, whereas T cell proliferation was inhibited by the presence of mAb against CD86 molecule and CTLA-4 Ig, this inhibition was significantly stronger in the culture with TT/CD26 (sCD26/DPPIV$^+$)-treated monocytes. The blocking effect of anti-CD86 Ab and CTLA-4 Ig was not exerted through the inhibition of DPPIV activity of sCD26, as examined in liquid phase by ELISA (data not shown). To confirm whether the above costimulatory effect was observed via the induction of CD86, CD86 mAb and CTLA-4 Ig were added at different concentrations to TT and/or sCD26 stimulation cultures. As shown in Fig. 8B, TT-mediated T cell proliferation enhanced by sCD26/DPPIV$^+$ was strongly inhibited by CD86 mAb and CTLA-4 Ig in a dose-dependent manner. It should be noted that CTLA-4 Ig always exerted greater inhibitory effect on TT-mediated T cell proliferation than CD86 mAb. As previously noted in Fig. 6, TT/CD26 (sCD26/DPPIV$^+$) monocytes expressed higher levels of CD86 than monocytes incubated with sCD26/DPPIV$^+$ molecules in the presence or absence of TT. These data strongly suggested that the increased surface expression of CD86 on monocytes treated with TT and sCD26 (DPPIV$^+$) is essential for the enhancing effect of sCD26 on TT-induced T cell proliferation.

Discussion

In this paper, we report that sCD26 has an enhancing effect on T cell proliferation in the presence of the recall Ags, TT. Importantly, this enhancement resulted in an increase in the surface expression of the costimulatory molecule CD86 on monocytes following uptake of sCD26. This immune enhancing effect of sCD26 was observed within the initial 48 h of treatment with sCD26 in the presence of TT.

In the process of T cell proliferative response against a recall Ag, several major factors have been shown to contribute to the maintenance of the biological reaction, including APC, Th cells, and selected cytokines (10–15, 21–24). Initially, an APC-Th cell interaction plays a key role in triggering the T cell response, leading eventually to expression of the T cell biological program (10, 14, 15). Our studies showed that the enhancing effect of sCD26 on
Increased CD86 expression on monocytes after incubation with sCD26/DPPIV

<table>
<thead>
<tr>
<th>TT alone</th>
<th>sCD26 alone</th>
<th>0 h</th>
<th>6 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Increase of CD86 expression on monocytes following incubation with sCD26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCD26/DPPIV+</td>
<td>1.2 ± 0.2</td>
<td>1.5 ± 0.6</td>
<td>4.2 ± 0.8</td>
<td>7.4 ± 1.3</td>
<td>10.5 ± 2.4</td>
<td>12.1 ± 2.1</td>
<td>4.3 ± 1.2</td>
</tr>
<tr>
<td>sCD26/DPPIV−</td>
<td>1.8 ± 1.3</td>
<td>2.4 ± 1.9</td>
<td>12.1 ± 1.1</td>
<td>0.8 ± 1.4</td>
<td>1.6 ± 1.1</td>
<td>1.3 ± 0.8</td>
<td>1.1 ± 0.7</td>
</tr>
<tr>
<td>% Increase of CD80 expression on monocytes following incubation with sCD26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCD26/DPPIV+</td>
<td>2.0 ± 0.4</td>
<td>1.7 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>0.7 ± 0.3</td>
<td>1.6 ± 0.4</td>
<td>0.6 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>sCD26/DPPIV−</td>
<td>1.0 ± 0.4</td>
<td>0.3 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>% Increase of HLA-DR expression on monocytes following incubation with sCD26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCD26/DPPIV+</td>
<td>0.3 ± 0.2</td>
<td>1.5 ± 0.6</td>
<td>0.2 ± 0.8</td>
<td>1.4 ± 1.5</td>
<td>3.5 ± 2.6</td>
<td>2.1 ± 1.1</td>
<td>0.4 ± 1.2</td>
</tr>
<tr>
<td>sCD26/DPPIV−</td>
<td>2.8 ± 1.3</td>
<td>1.4 ± 0.7</td>
<td>0.2 ± 1.1</td>
<td>1.4 ± 0.7</td>
<td>0.8 ± 1.2</td>
<td>0.7 ± 0.8</td>
<td>2.3 ± 1.7</td>
</tr>
</tbody>
</table>

The freshly isolated monocytes (0.5 × 10^6/well) were incubated with or without TT for 16 h after 24-h incubation, and then sCD26 (0.5 μg/ml) was added to the culture wells. After incubation for different time intervals (0, 6, 24, 48, 72, and 96 h), cells were washed in ice-cold PBS, and incubated with FITC-conjugated CD86, CD80, or HLA-DR on ice for 30 min. After washing with PBS, cells were analyzed by FACSCalibur. The experiments represent mean values ± SE calculated from three independently performed experiments. %, Percent increase of FITC intensity compared to FITC intensity of non-sCD26-treated monocytes.
an important role in regulating the activation or degradation of these factors (37–41). The present findings indicate that M6P/IGF-IIR mediates the internalization of sCD26 and that this interaction induces the up-regulation of CD86 on monocytes, resulting in the enhancement of TT-induced T cell proliferation.

Accumulating evidence suggests that DPPIV enzyme activity plays an essential role in CD26-mediated T cell costimulation, as well as in T cell immune reactions (1). Previous studies suggested that several potential scenarios may explain the observed effects of DPPIV on immune activation (3, 42–47). It is possible that CD26/DPPIV exerts its effect via the membrane-bound form, particularly in T cells. It is also possible that the CD26/DPPIV exerts its effect with the soluble form, in view of the fact that CD26/DPPIV is actually present in human serum (25). Previous studies with CD26-transfected Jurkat cells showing that DPPIV activity on CD26 augmented cellular responses of CD26-transfected Jurkat cells to external stimuli would support the former possibility (25, 48). In contrast, data demonstrating that recombinant sCD26 with DPPIV enzyme activity enhanced proliferative responses of PBMC to external stimuli would support the latter scenario. Our results showed that sCD26/DPPIV− had an enhancing effect on TT-induced T cell proliferation associated with an increase in CD86 expression on monocytes. Importantly, incubation with sCD26/DPPIV+ did not enhance TT-induced T cell proliferation and had no effect on CD86 surface expression on monocytes, despite the uptake of sCD26/DPPIV− by the same cell population. Previous reports showed that CD26/DPPIV regulated immune responses by cleaving selected chemokines at the N terminus to modify their biological functions (3, 42). Chemokines processed by DPPIV enzyme exhibited lower chemotactic potency, impaired signaling effects, and altered receptor specificity (3, 42). In view of its ability to cleave selected biological factors as a serine protease, it is possible that CD26/DPPIV exerts its effect by cleavage of certain factors that may in turn affect the up-regulation and/or oligomerization of CD86 on monocytes. Further studies are required for the isolation and characterization of CD26/DPPIV-associated factors responsible for regulating the expression of CD86.

Cytokines are critical in maintaining the latter stages of immune reaction (10, 14, 22). In the initial stages of Ag presentation, the capacity of Ag presentation can be modulated by several nonexclusive mechanisms, including the efficiency of Ag capturing and loading, MHC molecule density and occupancy, or altered co-stimulatory molecule expression (32, 33). Various proteins have been demonstrated to modulate the steps described above to alter T cell responsiveness against specific Ags. For example, opsonins augment the ability of Ag capture of APCs, IL-10 increases macrophagocytosis and endocytosis by APC, and IFN-γ up-regulates the expression of MHC class II molecules on APC (49–51). Moreover, recent studies revealed that aminopeptidase N (CD13), one
of the ectopeptidases on the cell surface, may trim MHC class II-bound peptides on the surface of the APC to modify T cell responsiveness (26). However, our findings clearly show that the enhancement of TT-induced T cell proliferation by sCD26 did not result from trimming of the MHC class II-bound peptide on the APC surface, in contrast to the findings related to CD13 aminopeptidase.

It should be noted that although uptake of the sCD26 molecules by monocytes resulted in an increase in CD86 monocyte surface expression, there is the still possibility that DPPIV enzyme activity of sCD26 facilitates Ag processing in monocytes, resulting in enhanced T cell/monocyte interaction and leading to increased T cell proliferation.

In summary, we have shown that sCD26 is taken up by monocytes and exerts its enhancing effect on T cell proliferation by altering the Ag presenting function of monocytes through the up-regulation of CD86 expression. Our paper also demonstrates that sCD26 plays an important role in the immune surveillance process against recall Ags, further elucidating the key role of CD26 in immune regulation.

References


11. Chambers, C. A. 2001. The expanding world of co-stimulation: the two-signal responsiveness (26). However, our


