Internalization of CD26 by mannose 6-phosphate/insulin-like growth factor II receptor contributes to T cell activation

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CD26 is a T cell activation antigen known to bind adenosine deaminase and have dipeptidyl peptidase IV activity. Cross-linking of CD26 and CD3 with immobilized mAbs can deliver a costimulatory signal that contributes to T cell activation. Our earlier studies revealed that cross-linking of CD26 induces its internalization, the phosphorylation of a number of proteins involved in the signaling pathway, and subsequent T cell proliferation. Although these findings suggest the importance of internalization in the function of CD26, CD26 has only 6 aa residues in its cytoplasmic region with no known motif for endocytosis. In the present study, we have identified the mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGFIIR) as a binding protein for CD26 and that mannose 6-phosphate (M6P) residues in the carbohydrate moiety of CD26 are critical for this binding. Activation of peripheral blood T cells results in the mannose 6 phosphorylation of CD26. In addition, the cross-linking of CD26 with an anti-CD26 antibody induces not only capping and internalization of CD26 but also colocalization of CD26 with M6P/IGFIIR. Finally, both internalization of CD26 and the T cell proliferative response induced by CD26-mediated costimulation were inhibited by the addition of M6P, but not by glucose 6-phosphate or mannose 1-phosphate. These results indicate that internalization of CD26 after cross-linking is mediated in part by M6P/IGFIIR and that the interaction between mannose 6-phosphorylated CD26 and M6P/IGFIIR may play an important role in CD26-mediated T cell costimulatory signaling.

T cell activation antigen CD26 is a multifunctional, 110-kDa cell surface glycoprotein (1, 2). Although constitutively expressed in the liver, intestine, and kidney, the CD26 expression level is tightly regulated on T cells and its density is markedly enhanced after T cell activation. In the resting state, CD26 is expressed on a subset of $CD4^+$ memory T cells, and this $CD4^+$ $CD26^{high}$ T cell population has been shown to respond maximally to recall antigens (1, 2).

CD26 has a dipeptidyl peptidase IV (DPPIV) activity in its extracellular domain that can cleave amino-terminal dipeptides with either proline or alanine in the penultimate position (3, 4). Recently, it has been reported that an amino-terminal truncation of RANTES (regulated on activation, normal T cell expressed and secreted) by CD26/DPPIV provides a mechanism for regulation of its activity and target cell specificity (5–7). On the other hand, CD26 interacts, presumably via its extracellular domain, with CD45, a protein tyrosine-phosphatase (8). In addition, the extracellular domain of CD26 on T cells forms a complex with adenosine deaminase, which reduces the immunosuppressive activity of local adenosine by its catalytic removal (9–12). The most striking evidence for the importance of adenosine deaminase for immune function is that a defect in adenosine deaminase activity results in severe combined immunodeficiency disease in humans (13, 14).

CD26 is not only highly expressed on activated T cells, but also is involved in the signal-transducing process. Cross-linking of CD26 and CD3 with immobilized mAbs can induce T cell activation and IL-2 production (15, 16). Moreover, anti-CD26 antibody treatment of T cells leads to a decrease in the surface expression of CD26 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 $p56^{lck}$ (3, 17). From these observations, it is suggested that internalization of the CD26 molecule plays an important role in T cell activation. CD26, however, has only 6 aa residues in its cytoplasmic region with no known motif for endocytosis (16). Thus, the molecular mechanisms involved in the internalization of CD26 and the subsequent activation of T cells are unclear.

In this study, we demonstrate that CD26 binds the mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGFIIR) via M6P residues in the CD26 carbohydrate moiety of CD26. T cell activation resulted in enhanced mannose 6 phosphorylation of CD26 whereas cross-linking of CD26 with antibody induced not only internalization of CD26 but also the colocalization of CD26 with the M6P/IGFIIR. More importantly, internalization of CD26 by cross-linking and T cell proliferation by CD26-mediated T cell costimulation were inhibited by the addition of M6P. Taken together, these findings indicate that internalization of CD26 after cross-linking is mediated in part by M6P/IGFIIR and the interaction of CD26 and M6P/IGFIIR plays an important role in CD26-mediated T cell costimulation.

Materials and Methods

Cells and Reagents. K562 human erythroleukemia cell line was obtained from American Type Culture Collection. Peripheral T cells were purified as described in ref. 1. Anti-CD26 mAbs (1F7,

Abbreviations: DPPIV, dipeptidyl peptidase IV; M6P, mannose 6-phosphate; M6P/IGFIIR, M6P/insulin-like growth factor II receptor; sCD26, soluble CD26; PMA, phorbol 12-myristate 13-acetate; G6P, glucose 6-phosphate.

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5F8, 2F9, and Ta1) were described previously (1, 18). Rabbit anti-M6P/IGFIIR was kindly provided by S. Kornfeld (Washington University). Antibody-conjugated beads were prepared by using CNBr-activated Sepharose 4B (Pharmacia) or Affiprep 10 (Bio-Rad) according to the manufacturer's instructions. Soluble CD26 (sCD26) was produced by Chinese hamster ovary cells as described in ref. 19. Soluble M6P/IGFIIR was purified from FCS by affinity purification by using bovine β-galactosidase (Sigma)-conjugated Affiprep-10 (Bio-Rad). SDS/PAGE followed by silver staining confirmed a major band of soluble M6P/IGFIIR.

Flowcytometric Analysis. The sCD26 was biotinylated with Sulfo-NHS-LC-biotin (Pierce) according to the manufacturer's protocol. Cells suspended in PBS were incubated with 1 μg/ml biotinylated sCD26 for 30 min at 4°C and washed with PBS. Phycoerythrin-conjugated streptavidin (Life Technologies, Gaithersburg, MD) was used as a secondary reagent. Cells were analyzed on a FACScan flow cytometer (Becton Dickinson).

Immunoprecipitation and Protein Cross-Linking. Cell surface proteins were biotinylated as described in ref. 20. The cells were incubated with 1 μg/ml sCD26 and chemically cross-linked with 1 mM cleavable cross-linker, DTSSP (Pierce). Immunoprecipitation was done as described (20). For competitive inhibition experiments, cells were incubated with 1 μg/ml biotinylated sCD26 in the presence or absence of 100 μg/ml nonbiotinylated sCD26 followed by cross-linking with 1 mM noncleavable cross-linker, BS^3 (Pierce). sCD26 and its cross-linked complex were immunoprecipitated and analyzed as described above.

Purification of p300. About 8×10^{10} K562 cells were incubated with 1 μg/ml sCD26 and cross-linked with 1 mM DTSSP. Crude membrane fraction was prepared as described before (21) and solubilized in 1% Nonidet P-40 lysis buffer as described (20). p300 was immunoprecipitated with anti-CD26 antibody (1F7 or 9C11)-conjugated Affiprep-10 beads (Bio-Rad) for 16 h at 4°C. The precipitated proteins were separated by SDS/PAGE followed by Coomassie blue staining. Protein sequencing was carried out by the Harvard Microchemistry Facility.

Far Western Blotting Analysis of Enzyme-Treated and Native sCD26. For glycosidase or phosphatase treatment, sCD26 was denatured by boiling with SDS and incubated for 12 h at 37°C in incubation buffer [20 mM Tris-malate (pH 7.0 or pH 8.0)/1% Nonidet P-40] containing 10 microunits/μl *N*-glycosidase-F and 25 microunits/μl *O*-glycosidase (Boehringer Mannheim) (pH 7.0) or 50 milliunits/μl *Escherichia coli* alkaline phosphatase (Sigma) (pH 8.0). Both enzyme-treated and native sCD26 were electrophoresed and transferred to a polyvinylidene difluoride membrane. After blocking, the membrane was incubated with 0.5 μg/ml partially purified M6P/IGFIIR, followed by detection with anti-M6P/IGFIIR antibody and horseradish peroxidase-conjugated anti-rabbit IgG antibody (Amersham).

Modulation Study and Immunofluorescence Analysis. For antibody-induced modulation studies, cells were incubated for 1 h at 37°C in medium containing 2 μg/ml anti-CD26 antibody (1F7). The surface expression of CD26 after modulation was analyzed by flow cytometry (17). For immunofluorescence microscopy analysis, cells were fixed with 4% paraformaldehyde, permeablized with 0.1% Triton X-100, and double-stained with anti-CD26 (2F9) and anti-M6P/IGFIIR antibodies. Confocal microscopy was performed on model IX70 confocal microscopes (Olympus, Tokyo).

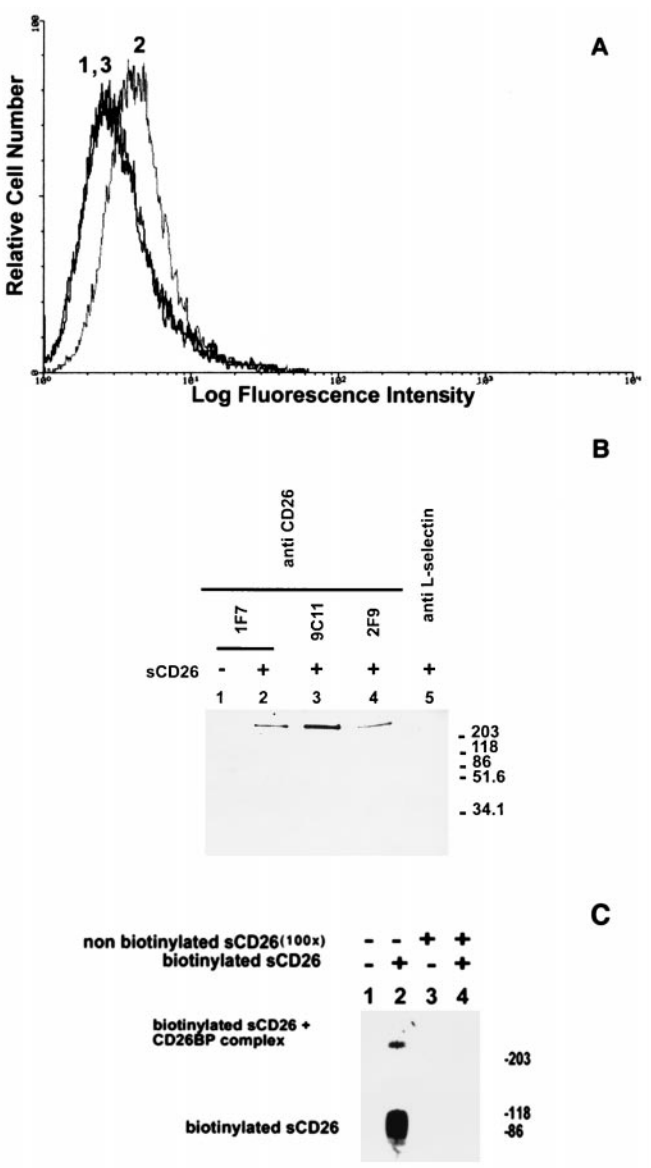

Fig. 1. Binding of sCD26 to cell surface protein p300. (*A*) Specific binding of sCD26 to cell surface of K562 cells. Cells were stained with phycoerythrin-streptavidin alone (peak 1) or with biotinylated sCD26 and phycoerythrin-streptavidin (peak 2), followed by flow cytometry analysis. Binding of biotinylated sCD26 was inhibited by a 100-fold excess of nonbiotinylated sCD26 (peak 3). (*B*) Immunoprecipitation of cell surface CD26-binding protein p300. Surface-biotinylated K562 cells were incubated with sCD26 (lanes 2–5) or PBS alone (lane 1) followed by cross-linking with cleavable cross-linker DTSSP (20). sCD26 and its cross-linked complex were immunoprecipitated with three different anti-CD26 antibodies (lanes 1–4) or isotype-matched control antibody (TQ1; anti-L-selectin, lane 5). Immunoprecipitated materials were reduced (to cut cleavable chemical cross-linker) and then analyzed by SDS/PAGE and detected with streptavidin (20). (*C*) The binding of labeled sCD26 to p300 was inhibited by an excess amount of nonlabeled sCD26. K562 cells were incubated with biotinylated sCD26 (lanes 2 and 4) or PBS alone (lanes 1 and 3) in the presence (lanes 3 and 4) or absence (lanes 1 and 2) of a 100-fold excess of nonbiotinylated sCD26. After cross-linking with noncleavable cross-linker BS^3, sCD26 and its cross-linked complex were immunoprecipitated by anti-CD26 antibody (1F7)-conjugated beads. Immunoprecipitated materials were electrophoresed and detected with streptavidin (20).

Proliferation Assay. Peripheral blood T cells were costimulated with immobilized anti-CD3 (0.05 μg/ml) with anti-CD26 (1 μg/ml), or phorbol 12-myristate 13-acetate (PMA; 5 ng/ml) as

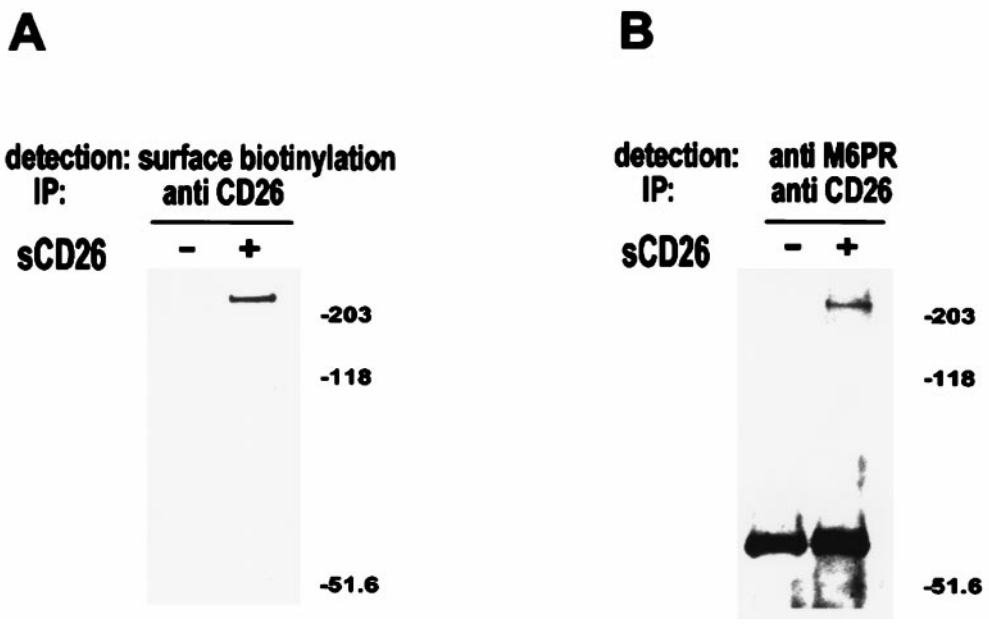

Fig. 2. CD26-binding protein p300 is M6P/IGFIIR. (*A* and *B*) CD26-binding protein p300 was immunoprecipitated from surface-biotinylated K562 cells as described in Fig. 1*B*. After SDS/PAGE under reducing conditions and transfer to a membrane, p300 was detected with streptavidin (*A*). After stripping, the membrane was probed with rabbit anti-M6P/IGFIIR polyclonal antibody (*B*).

described (1). For inhibition analysis, M6P, glucose 6-phosphate (G6P), or mannose 1-phosphate was added to each well. Proliferation assay by [^{3}H]thymidine incorporation was as described in ref. 1.

Results

Expression of CD26-Binding Protein on the Surface of K562 Cells. In an effort to define proteins that interact with CD26, we prepared a biotinylated recombinant sCD26 to screen cells by flow cytometry for those expressing a CD26-binding protein (19). The K562 cell line was one such line that bound sCD26 (Fig. 1*A*). The binding of biotinylated sCD26 to K562 cells was inhibited completely by a 100-fold excess of nonbiotinylated sCD26, suggesting the binding specificity. As shown in Fig. 1*B*, an immunoprecipitation analysis using sCD26 and anti-CD26 antibody revealed a 300-kDa CD26-binding protein (p300). The binding appears specific, because it was obtained with sCD26 and three epitope-distinct anti-CD26 mAbs (1F7, 9C11, 2F9) but not in the absence of sCD26 or in the presence of an isotype-matched control antibody (Fig. 1*B*). The binding specificity was supported further by a competitive inhibition experiment that demonstrated that the binding of biotinylated sCD26 to p300 was abolished completely in the presence of a 100-fold excess of nonlabeled sCD26 (Fig. 1*C*). These results supported the notion that CD26 binds p300. In addition, immunoprecipitation using a mutant form of sCD26 lacking DPPIV enzyme activity also precipitated p300, indicating that the DPPIV activity of CD26 was not required for binding (data not shown).

Identification of p300 as M6P/IGFIIR. To identify p300, we purified p300 and analyzed its amino acid sequences. The sequences of the four peptides that we obtained after digestion proved to be identical to residues 241–249, 530–542, 1373–1386, and 2215–2229 of the human M6P/IGFIIR (22–24). M6P/IGFIIR is a multifunctional transmembrane glycoprotein that consists of a single, 300-kDa polypeptide. A major function of this receptor is to bind and transport M6P-bearing glycoproteins (e.g., lysosomal enzymes) from the trans-Golgi network or the cell surface to lysosomes (22–24). The cell surface M6P/IGFIIR also binds and internalizes IGF-II, resulting in the lysosomal degradation of this ligand. To determine whether p300 was identical to M6P/IGFIIR, we used a rabbit polyclonal anti-M6P/IGFIIR antibody in Western blot analysis. The CD26-binding protein p300, which is shown in Fig. 2*A*, was reblotted with an anti-M6P/IGFIIR antibody and shown to be reactive (Fig. 2*B*). Additionally, the transient expression of M6P/IGFIIR cDNA in COS1 cells resulted in the strong binding of labeled sCD26 to cells, whereas mock (vector) transfected COS1 cells bound sCD26 considerably less (data not shown). These results provide additional support for the view that M6P/IGFIIR is a CD26-binding protein.

M6P/IGFIIR Binds CD26 via M6P Residues in Carbohydrate Moiety in CD26. We next tested the competing effects of free M6P on the binding of CD26 to M6P/IGFIIR to define whether M6P residues on CD26 were required for this interaction. As shown in Fig. 3*A*, sCD26 binding to M6P/IGFIIR was inhibited in a dose-dependent manner by M6P, but not by the same concentration of mannose or glucose 6-phosphate (Fig. 3*B*). These data indicate that the binding of CD26 to M6P/IGFIIR is M6P-dependent. To test whether glycosylated or phosphorylated sites on CD26 contributed to the binding, we treated sCD26 with a glycosidase or phosphatase and tested the treated material for binding to the M6P/IGFIIR by far Western analysis. As shown in Fig. 3*C*, the binding capacity of nontreated sCD26 to bind M6P/IGFIIR was demonstrated by far Western blotting by using purified M6P/IGFIIR as a probe, indicating that the binding between CD26 and M6P/IGFIIR was direct. As shown in lanes 2 and 3, either glycosidase or phosphatase treatment of sCD26 completely abolished this binding, demonstrating that both glycosylation and phosphorylation of CD26 were required. These data as well as the blotting data all suggest that binding of CD26 to the M6P/IGFIIR is mediated by M6P residues on the CD26 carbohydrate moiety.

Mannose 6 Phosphorylation of CD26 Expressed on Peripheral Blood T Cells. To address the possible physiological role of mannose 6 phosphorylation of CD26, we tried to determine whether CD26 expressed by peripheral T cells is mannose 6-phosphorylated. As

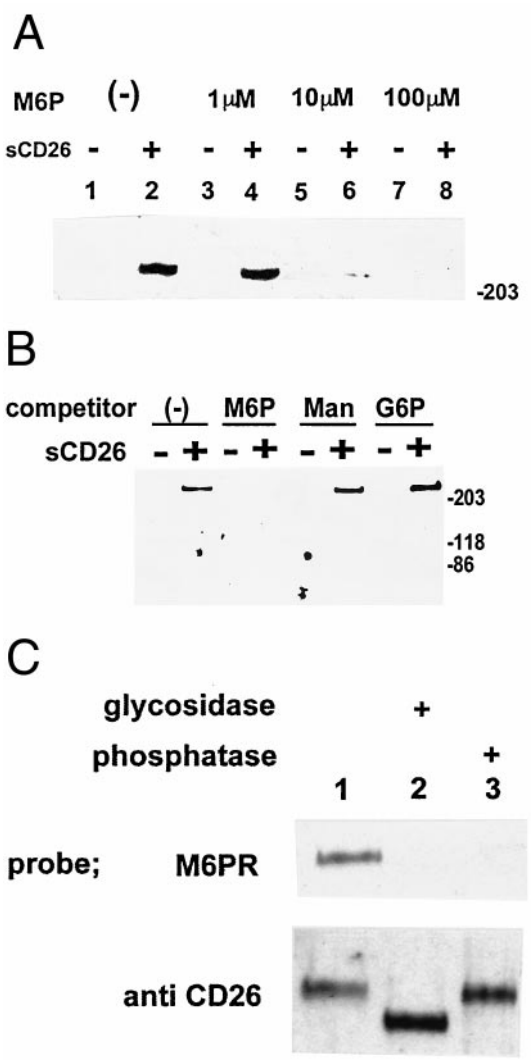

Fig. 3. Binding of sCD26 and M6P/IGFIIR is mediated by M6P residues in the carbohydrate moiety of sCD26. (*A* and *B*) Binding of sCD26 and M6P/IGFIIR was inhibited by M6P. Surface-biotinylated K562 cells were incubated with sCD26 as in Fig. 1*B* in the presence of varying concentrations of M6P (*A*) or 100 μM M6P, mannose (Man), or G6P (*B*). M6P/IGFIIR was coprecipitated with sCD26 by using anti-CD26 antibody. (*C*) Requirement of glycosylation and phosphorylation of sCD26 for M6P/IGFIIR binding. sCD26 was incubated with buffer alone (lane 1), *N*- and *O*-glycosidases (lane 2), or alkaline phosphatase (lane 3) followed by SDS/PAGE and transfer to a membrane. Binding of enzyme-treated sCD26 and M6P/IGFIIR was analyzed by far Western blotting by using soluble M6P/IGFIIR as probe (upper blot). After stripping the membrane, sCD26 also was detected with Western blotting with anti-CD26 antibody (5F8) (lower blot).

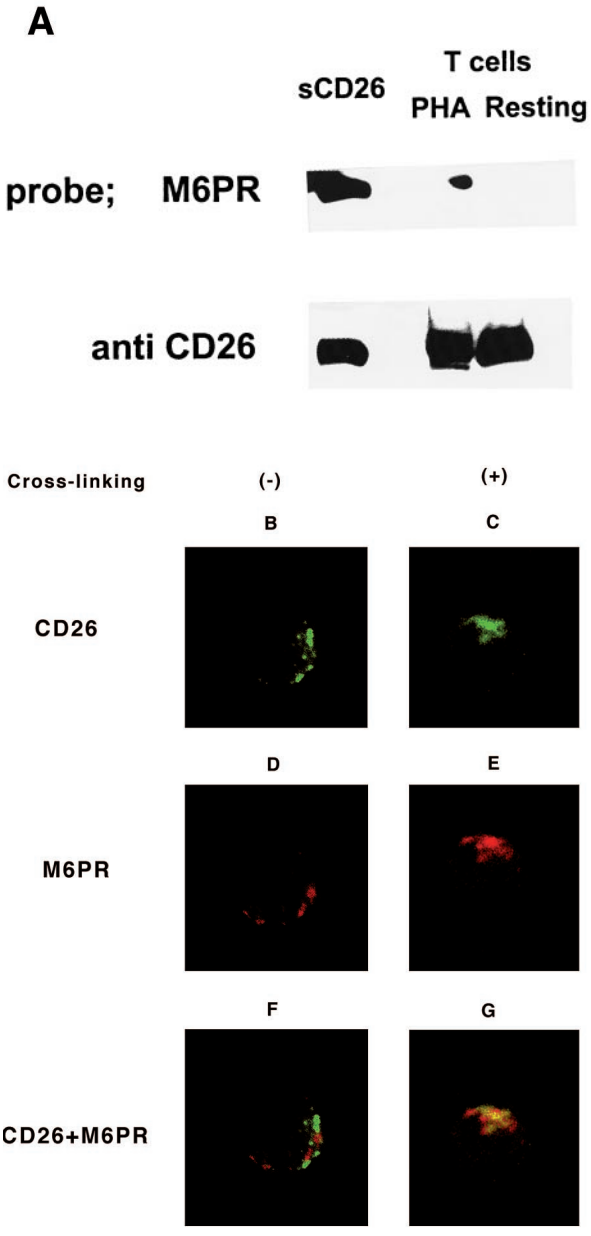

Fig. 4. (*A*) Phytohemagglutinin activation of T cells increased the level of mannose 6 phosphorylation of intracellular CD26. CD26 was immunoprecipitated from the whole-cell lysate of resting or phytohemagglutinin-activated peripheral T cells. Mannose 6 phosphorylation of CD26 was analyzed by far Western blotting with M6P/IGFIIR as probe. Equal loading of CD26 was confirmed by Western blotting probed with anti-CD26 antibody (1F7). (*B*– *G*) Immunocytochemical analysis of subcellular localization of CD26 and M6P/IGFIIR. Peripheral blood lymphocytes in resting state (*B*, *D*, and *F*) or stimulated by cross-linking of CD26 with anti-CD26 (*C*, *E*, and *G*) were stained with Oregon green 488 (green)-labeled anti-CD26 antibody (2F9) (*B* and *C*) and rabbit anti-M6P/IGFIIR antibody followed by rhodamine (red)-labeled anti-rabbit Ig antibody (16) (*D* and *E*). The stained cells were analyzed by confocal microscopy. Areas of coincidence of red and green fluorescence (giving yellow fluorescence) indicate overlapping distribution of CD26 and M6P/IGFIIR (*F* and *H*).

shown in Fig. 4*A*, mannose 6 phosphorylation was not detectable in CD26 immunoprecipitated from whole-cell lysate of resting T cells, whereas the CD26 immunoprecipitated from phytohemagglutinin-activated T cells clearly expressed mannose 6-phosphate. These results suggest that the activation of T cells induced CD26 mannose 6 phosphorylation.

Colocalization of CD26 and M6P/IGFIIR in Activated T Cells. That activated T cells express mannose 6-phosphorylated CD26 suggested a possible interaction of CD26 with M6P/IGFIIR in T cells. To test this, we first determined the localization of CD26 and M6P/IGFIIR by immunohistochemical analysis. As shown in Fig. 4 *B* and *C*, CD26 formed a dense cap after cross-linking of the cell surface of CD26 as described previously (17). Similarly, the majority of M6P/IGFIIR also was found in the dense cap of CD26 after cross-linking of CD26 (Fig. 4 *D* and *E*). These data indicated that cross-linking of CD26 resulted in the colo-

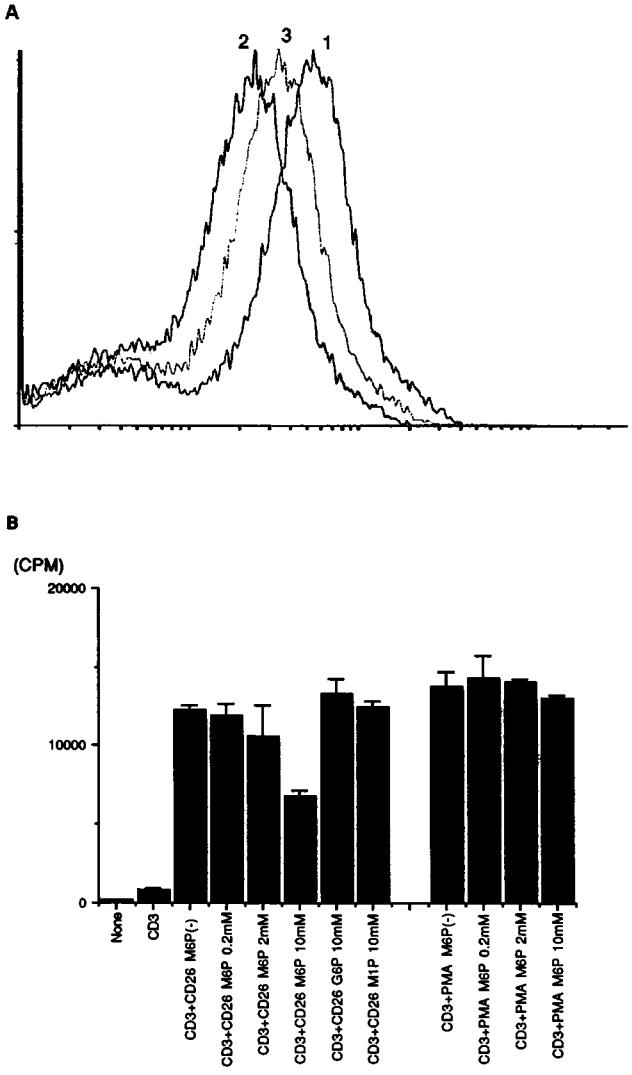

Fig. 5. (*A*) The inhibitory effect of M6P on the modulation of CD26 by anti-CD26 antibody. Purified peripheral blood T cells were stained with phycoerythrin-conjugated anti-CD26 antibody (Ta1) before (line 1) or after (lines 2 and 3) cross-linking with anti-CD26 antibody (1F7) in the presence (line 3) or absence (line 2) of 2 mM M6P and analyzed by flow cytometry. Comparable results were obtained in five independent experiments. (*B*) The effect of M6P on T cell proliferation induced by CD3 and CD26 costimulation. Purified peripheral T cells were stimulated with anti-CD3 and anti-CD26 antibodies or anti-CD3 antibody with PMA in the presence or absence of M6P. In control experiments, G6P or mannose 1-phosphate was substituted for M6P. Proliferation was assessed by incorporation of [^{3}H]thymidine into cells.

calization of CD26 and M6P/IGFIIR at the site of the dense cap (Fig. 4 *F* and *G*). Taken together, these observations strongly suggest that the internalization of CD26 after cross-linking is mediated by interaction with M6P/IGFIIR.

Inhibitory Effect of M6P on the Modulation of CD26 by an Anti-CD26 Antibody. To investigate the role of CD26 bound to M6P/IGFIIR after surface modulation of CD26, we examined whether M6P could modify the CD26 modulation induced by cross-linking with an anti-CD26 antibody. As shown in Fig. 5*A*, modulation of a significant portion of CD26 was virtually blocked by 2 mM M6P. On the other hand, G6P and mannose 1-phosphate did not inhibit CD26 modulation (data not shown). These results indicate that M6P could partially inhibit the modulation of CD26 after cross-linking with an anti-CD26 antibody.

Inhibitory Effect of M6P on T Cell Proliferation Induced by CD26 Costimulation. Next, we determined whether the inhibition of CD26 modulation by M6P also inhibited CD26-mediated T cell costimulation induced by immobilized anti-CD3 and anti-CD26 mAbs. As shown in Fig. 5*B*, T cell proliferation induced by anti-CD26 costimulation was inhibited with M6P, but not with G6P or mannose 1-phosphate. Moreover, the inhibitory effect of M6P on T cell proliferation was not observed with cells stimulated with anti-CD3 and PMA (Fig. 5*B*). These results clearly indicated that the interaction between CD26 and M6P/IGFIIR plays a role in the costimulatory signaling via the CD26 molecule.

Discussion

One of the objectives of this investigation was to identify cell surface structures that interact with CD26. In this report we have identified a 300-kDa cell surface protein present on K562, a erythroleukemia cell line, that specifically binds sCD26. Purification of the 300-kDa material from a crude membrane and its analysis by amino acid sequencing provided strong evidence that the CD26-binding protein was the M6P/IGFIIR. Support of this observation was the finding that the purified material was reactive with a M6P/IGFIIR-specific antibody in a direct binding assay and indirectly by far Western analysis. In keeping with this observation, others have shown that the M6P/IGFIIR is a multifunctional transmembrane glycoprotein composed of a 300-kDa single polypeptide chain (22–24). The M6P/IGFIIR is located mainly in the intracellular compartments, particularly in the trans-Golgi network and endosomes, with a small portion of the receptor present on the cell surface. The primary function of M6P/IGFIIR is to sort and transport M6P-containing glycoproteins (mainly lysosomal enzymes) from the trans-Golgi network or the cell surface to endosomes/lysosomes (24, 25). The receptor also binds to a mannose 6-phosphorylated precursor form of transforming growth factor β (latent transforming growth factor β) (26, 27), proliferin (28), and leukemia inhibitory factor (29, 30) and has been shown to play an important role in regulating the activation or degradation of these factors.

Although the receptor binds to M6P-bearing glycoproteins via M6P carbohydrate residues, it also has been shown to bind IGFII, a nonglycosylated peptide at a site distinct and independent of M6P. In addition, it has been reported recently that the urokinase-type plasminogen activator receptor also binds to the M6P/IGFIIR in a M6P-independent manner (25). The work reported herein indicates that the binding of CD26 to the M6P/IGFII could be inhibited by M6P and that both glycosylation and phosphorylation of CD26 were required for this binding, suggesting the importance of M6P residues in the carbohydrate moiety of CD26.

CD26 is a cell surface molecule that provides a costimulatory signal for T cell activation. Recent reports demonstrated that the internalization of cell surface CTLA-4, a negative regulator of T cell activation, contributes to regulation of T cell function (16, 31–36). CTLA-4 has a tyrosine-based motif for endocytosis in its cytoplasmic tail, which regulates the cell surface expression depending on its phosphorylation status. Our previous work demonstrated that cross-linking of CD26 with an anti-CD26 antibody leads to a decrease in the surface expression of CD26 through internalization, and such modulation resulted in an enhanced proliferative response to CD3 or CD2 (17, 18). CD26, however, has no known motif for endocytosis, suggesting the existence of another molecule that mediates endocytosis (16). The present findings strongly suggest that M6P/IGFIIR mediates internalization of CD26 and that modulation may be a consequence of enhanced mannose 6 phosphorylation of CD26. We are not certain that the mannose 6 phosphorylation of a leukocyte cell surface antigen is a general phenomenon modulating cell surface antigen expression. Immunohistochemical analysis indicated that the cross-linking of CD26 induces not only

capping and internalization of the molecule, but also its colocalization with M6P/IGFIIR. Colocalization occurred in cytoplasmic vesicles adjacent to the cell surface clustering of CD26 formed by cross-linking. Moreover, the addition of M6P inhibited not only the internalization of CD26 induced by anti-CD26 antibody cross-linking but also T cell proliferation induced by CD3 and CD26 costimulation. On the other hand, T cell proliferation induced by anti-CD3 and PMA was not inhibited by M6P. These results suggest that M6P/IGFIIR mediates internalization of CD26 via M6P residues and that the interaction between CD26 and M6P/IGFIIR may play an important role in CD26-mediated T cell costimulation.

Our findings revealed the role of M6P/IGFIIR in the regulation of T cell activation via the binding and trafficking of the CD26 molecule. Recent studies demonstrated that upon the direct binding of retinoic acid (RA) to the M6P/IGFIIR, RA functions as a regulator of endocytosis and the intracellular distribution of the M6P/IGFIIR and M6P-bearing ligands, suggesting that M6P/IGFIIR is a mediator of an RA response pathway (37, 38). In addition, the receptor also binds and modulates a subcellular distribution of the cell surface protein urokinase-type plasminogen activator receptor (25). Taken together, it is suggested that the trafficking of M6P/IGFIIR might be altered by a number of stimuli and may serve as a unique mechanism for the regulation of cellular responses. In the case of CD26/DPPIV on T cells, the interaction of CD26 with the M6P/IGFIIR may play a role in CD26-mediated T cell costimulation.

A detailed study regarding the redistribution of CD26 during T cell activation and subsequent interactions with other molecules may provide not only a clue for further understanding of the function of CD26 but also a novel mechanism in which costimulatory molecules are involved in the T cell signaling process. *In vivo* studies revealed that a large number of $CD26^+$ T cells was found in inflamed tissues of patients with autoimmune diseases (39, 40). Therefore, further study regarding the mannose 6 phosphorylation status of CD26 and the interaction with M6P/IGFIIR in chronic inflammatory sites may provide a clue for the pathogenic role of CD26 in autoimmune diseases and may lead to the development of novel therapeutic approaches.

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