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Role of CD26/dipeptidyl peptidase IV in human T cell activation and function

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1. ABSTRACT

CD26 is a 110 kDa surface glycoprotein with intrinsic dipeptidyl peptidase IV (DPPIV, EC 3.4.14.5) activity that is expressed on numerous cell types and has a multitude of biological functions. CD26 role in immune regulation has been extensively characterized, with recent findings elucidating its linkage with signaling pathways and structures involved in T-lymphocyte activation as well as antigen presenting cell (APC)-T-cell interaction. In this paper, we will review emerging data on CD26-mediated T-cell costimulation, suggesting that CD26 may be an appropriate therapeutic target for the treatment of immune disorders. However, the identity of its putative natural ligand had not yet been clearly elucidated. Recently, using protein engineering and proteomic approach, we have recently characterized the putative costimulatory ligand for CD26 in T-cells and the proximal signaling events directly associated with the cytoplasmic region of CD26 in CD26-associated T-cell costimulation, processes that are independent of the CD28 costimulatory pathway. Our work therefore presents novel findings that contribute to the area of T-cell costimulation and signal transduction.

2. INTRODUCTION

CD26 is a 110-kDa cell surface glycoprotein with known dipeptidyl peptidase IV (DPPIV, EC3.4.14.5) activity in its extracellular domain (1-3), capable of cleaving amino-terminal dipeptides with either L-proline or L-alanine at the penultimate position (3). CD26 activity is dependent on cell type and the microenvironment, which influence its multiple biological roles (4-7). CD26 plays an important role in immunology, autoimmunity, diabetes and cancer. Interacting directly with various other cell surface and intracellular molecules, CD26 can regulate receptor specificity and the function of various chemokines and cytokines via its DPPIV activity.

In this review, we summarize current knowledge of the molecular mechanisms of CD26-mediated T-cell costimulation, focusing particularly on CD26 role in cellular pathways and programs associate with human immune regulation. In addition, we describe our recent work that identified a costimulatory ligand for CD26, which had been called "a phantom molecule" since its putative costimulatory ligand had not been previously

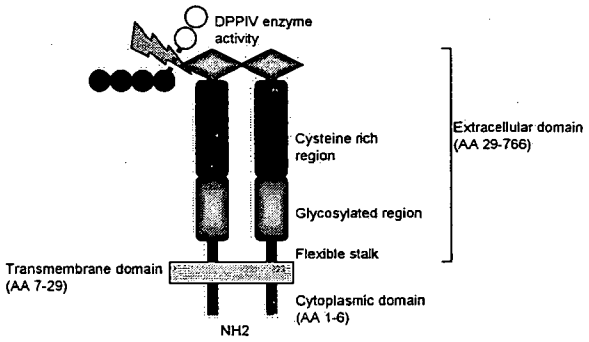

Figure 1. Schematic diagram of human CD26 structure. See text for detail.

characterized. We also discuss our data elucidating the proximal signaling events associated with CD26-mediated T-cell costimulation. Finally, we discuss CD26 involvement in various pathophysiologic states, and its suitability as a potential therapeutic target in selected immune diseases.

3. BASIC ASPECT OF CD26/DPPIV

3.1. Distribution

CD26 was originally described in 1966 as an enzyme with intrinsic DPPIV activity (8). DPPIV was later found to be the same as CD26, which is a 110 kDa extracellular membrane-bound glycoprotein that is expressed on many tissues. The amino acid sequence of human CD26 shares approximately 85% homology with the rat DPPIV enzyme and the mouse thymocyte activation molecule (THAM), the mouse homologue of human CD26 (3, 9). CD26 knockout (CD26-KO) mice with C57BL/6 background display an apparently normal phenotype (10). However, the percentage of CD4+T-cells is lower in the spleen lymphocyte population of CD26-KO mice than CD26-positive wild-type mice. After immunization of mice with pokeweed mitogen (PWM) *in vivo*, serum levels of total IgG, IgG1, IgG2a and IgE were markedly decreased in CD26-KO mice than those in wild-type mice. Moreover, IL-4 and IL-2 level in sera of CD26-KO mice were decreased and production of interferon-gamma (IFN-γ) was delayed in response to PWM immunization. These results indicate that CD26 helps to regulate the development, maturation and migration of CD4+T-cells, cytokine secretion, T-cell-dependent antibody production and immunoglobulin isotype switching of B cells. On the other hand, Abbott *et al.*, found two transcripts of human CD26 (4.2 and 2.8 kbp), both of which were expressed at high levels in the lung and liver (11). Other organs expressing CD26 include: brain, endothelium, heart, intestine, kidney, liver, lung, skeletal muscle, pancreas, placenta, and lymphocytes (11-15). Originally characterized as a T cell differentiation antigen, CD26 is preferentially expressed on a specific population of T lymphocytes, the subset of CD4+CD45RO+ memory T cells, and is upregulated following T cell activation (15). Besides being a marker of T cell activation, CD26 is also associated with T cell signal transduction processes as a costimulatory molecule (5-7). Since the 1970s, DPPIV-like activity has been also reported in human serum. Following the identification of the ADA-binding protein in plasma as CD26, soluble form of CD26 protein was characterized in the serum and seminal fluids (16, 17).

3.2. Structure

CD26 is a 110 kDa cell-surface glycoprotein that belongs to the serine protease family, which is characterized by the Ser-Asp-His catalytic triad in the C-terminal region. CD26/DPPIV is a member of a complex gene family, many of which also cleave structurally related peptides (18). These include: proly endopeptidase (PEP), acylaminoacyl peptidase (ACPH), CD26/DPPIV, fibroblast activation protein (FAP), DPP8, DPP9, DPP10, and DPPX (18, 19). The N-terminus contains the consensus sequence (DW(V/L)YEEE), with the first two Glu (E) amino acids in this sequence being necessary for enzyme activity, while the C-terminus is also highly conserved (an α/β-hydrolase domain) (20, 21). As shown in Figure 1, human CD26 is composed of 766 amino acids, including a short cytoplasmic domain of 6 amino acids, a transmembrane region of 24 amino acids, and an extracellular domain with dipeptidyl peptidase activity which selectively removes the N-terminal dipeptide from peptides with proline or alanine at the penultimate position (3). The amino acid sequence of human CD26 shares approximately 85% homology with the rat DPPIV enzyme and the mouse thymocyte activation molecule (THAM), the mouse homologue of human CD26 (22). The crystal structure of recombinant human CD26 was determined in the dimeric state at 2.5Å resolution with the DPPIV inhibitor Valine-pyrrolidide (23). The catalytic site (Ser630-Asp708-His740) is located in a large cavity (also called a central tunnel), formed between the α/β-hydrolase domain and eight-bladed beta -propeller domain, which contains the consensus sequence (DW(V/L)YEE), that is conserved in S9B protease (20, 23). Single amino acid point mutation in the β-propeller motif identified Glu205 and Glu206 as essential for DPPIV enzyme activity (20), and the central tunnel and alpha/beta -hydrolase domains both participate in DPPIV inhibitor binding (24, 25). The amino acids lining the opening to the catalytic site pocket control substrate specificity (26). Single amino acid point mutation at His750 residue is responsible for dimerization (27). As described below, a central tunnel, DPPIV enzyme pocket and dimerization site are all necessary for caveolin-1 binding, which is found to be the costimulatory ligand for human CD26 (28, 29).

3.3. Characterization of DPPIV enzyme

DPPIV is a serine protease that cleaves dipeptides from the N-terminus of peptides (3). The enzymatic activity of CD26 appears to be very important in enhancing cellular responses to external stimuli. For example, Jurkat cells transfected with wild type CD26 consistently demonstrate greater activation than parental CD26 negative Jurkat or cells transfected with CD26 mutated at the DPPIV enzymatic site (30). Moreover, we have shown that exogenous recombinant soluble CD26 (rsCD26) with DPPIV enzyme activity enhances the proliferative response of peripheral blood lymphocytes (PBLs) to stimulation with the soluble antigen tetanus toxoid (TT) (31). This enhancing effect of rsCD26 was not

observed with mutant rsCD26 lacking DPPIV enzyme activity (31). Although the precise mechanism underlying the enhancing effect of DPPIV enzyme activity has not yet been elucidated, one possible explanation for the role of DPPIV enzyme activity in T-cell activation is its binding activity to the putative ligand caveolin-1 (28, 29).

Possible substrates of CD26/DPPIV include several critical cytokines and chemokines (32). Activity of RANTES (regulated on activation, normal T cell expressed and secreted; CCL5) is altered by the enzymatic cleavage of DPPIV, as CD26/DPPIV-processed RANTES affects important activities such as those implicated in monocyte chemotaxis and HIV-1 infection (33, 34). Other important chemokines that appear to be substrates of DPPIV enzymatic activity include eotaxin (CCL11), macrophage-derived chemokine (MDC) (CCL22), interferon inducible chemokines (CXCL10), and other chemokines involved in the inhibition of HIV infection (34). In addition, recent work showed that CD26 plays an important role in the mobilization of hematopoietic stem cell (HSC) and hematopoietic progenitor cells (HPC) induced by granulocyte colony-stimulating factor (G-CSF) (35). One of the substrates of CD26/DPPIV is CXCL12 (SDF-1α, stromal cell-derived factor 1 alpha), an important chemokine that serves as a chemoattractant for HSC/HPC (36, 37). It has been shown that CXCL12 can be selectively truncated *in vitro* by CD26/DPPIV, and the truncated molecule lacks the ability to induce migration of hematopoietic cells isolated from mouse bone marrow. Furthermore, treatment of mice with CD26/DPPIV inhibitors during the process of G-CSF mobilization results in a significant increase in the number of mobilized HPC (35, 36). An exciting development regarding DPPIV involves its role in glucose metabolism, as inhibition of endogenous glucagon-like peptide 1 (GLP-1) degradation by reducing DPPIV activity is an alternative strategy for improving the incretin action of GLP-1 *in vivo* and regulating glucose levels (reviewed in (19)). The first oral selective DPPIV inhibitor was approved by the Food and Drug Administration (FDA) in October 2006, and additional DPPIV inhibitors are under review by FDA for clinical use (19, 38). More selective small molecule inhibitors of DPPIV are currently being investigated in clinical trials for the treatment of impaired glucose tolerance and type 2 diabetes (19, 39).

4. BIOLOGICAL ASPECT OF CD26/DPPIV AND T-CELL REGULATION

4.1. CD26 and T-cell function

While CD26 expression is enhanced following activation of resting T-cells, CD4+CD26high T-cells respond maximally to recall antigens such as tetanus toxoid (TT) (15). Crosslinking of CD26 and CD3 with solid-phase immobilized monoclonal antibodies (mAbs) can induce T-cell costimulation and IL-2 production by CD26+T-cells (3, 15). In addition, anti-CD26 antibody treatment of T-cells enhances tyrosine phosphorylation of signaling molecules such as CD3-ζ and $p56^{lck}$ (40, 41). High CD26 cell surface expression is correlated with the production of T_H1-type cytokines such as IFN-γ, and CD26 expression is induced by stimuli that favor the development of the T_H1 response (42-44). CD26+T-helper cells stimulate antibody synthesis in B-cells and activate MHC-restricted cytotoxic T-cells (15, 45). Moreover, previous reports showed that CD26+ T cells exhibit strong migratory ability through endothelial cells, and are present at high levels in the rheumatoid synovium and the synovial fluids (46-49). CD26 may have therefore an important role in T-cell biology and overall immune function. Moreover, DPPIV activity is required for CD26-mediated T-cell costimulation (30). Jurkat T-cell line (derived from a human T-cell leukemia) transfected with CD26 show greater activation than CD26-negative Jurkat cells or Jurkat cells transfected with CD26 mutated at the DPPIV enzymatic site (serine residue at 630 to alanine) (30). When stimulated by anti-CD3 and anti-CD26 antibodies or the combination of anti-CD3 and phorbol esters, the wild type CD26 (DPPIV containing) transfected Jurkat cells showed more IL-2 production than the mutant CD26 (DPPIV deficient) or vector-only control transfected cells. In addition, DPPIV activity can change T-cell response to a variety of external stimuli through CD26 and/or the CD3/TCR complex to regulate IL-2 production. The DPPIV activity of CD26 is therefore important in enhancing cell activation in response to external stimuli (50). We recently identified a costimulatory ligand for CD26, with its binding to CD26 being dependent on the DPPIV enzyme pocket (29, 51). This topic will be discussed in more detail in the following section.

4.2. CD26 and other immune cells

It has been reported that 0-5% of freshly isolated CD20+B cells do express the CD26 antigen (52). Following stimulation with PMA or Streptococcus aureus protein, the fraction of CD26-positive cells increased to 51%. Interestingly, induction of CD26 expression on B cells from patients with combined variable immunodeficiency occurs in a manner similar to the B-cells from healthy donors. Meanwhile, CD26 is not expressed or is found only at low levels on monocytes of healthy adult (53). Flow cytometric analysis of dendritic antigen-presenting cells (DC) generated from peripheral blood of normal donors in the presence of granulocyte/macrophage colony-stimulating factor and IL-4 revealed intermediate levels of CD26 during a 2-week culture period (54). While these findings suggest that CD26 may also be an activation marker for B-cells and APC, its precise role in these cells remains to be elucidated.

Meanwhile, only a small fraction of peripheral NK cells was found to express CD26. Using K562 as target cells, Madueno *et al.* analyzed the lytic capacity of NK cells stimulated with different anti-CD26 mAbs or following separation into CD26+ and CD26- subsets, and observed no detectable difference in the chromium released by the target cells (55). These investigators also evaluated redirected lysis through CD16 by arming both CD26+ and CD26- effector cells with an anti-CD16 antibody, and demonstrated that CD26- cells exhibited significantly less CD16-dependent lysis than CD26+ cells. These results indicate that CD26 is related to CD16-dependent lysis but not to NK cytolysis, which may be mediated through

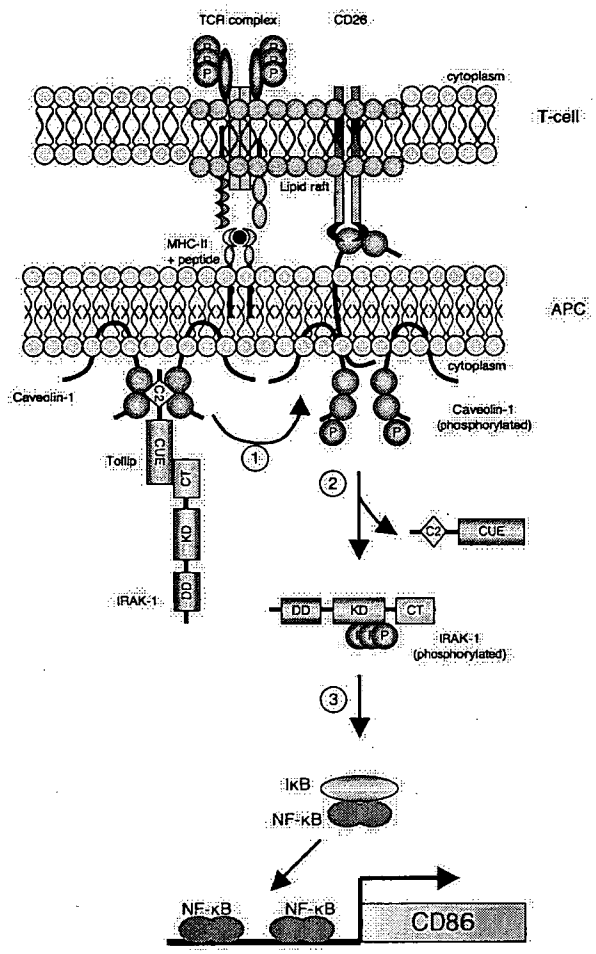

Figure 2. Model for CD26-caveolin-1 interaction leading to upregulation of CD86. (1) Caveolin-1 in monocytes (APC) resides at the inner membrane in the presence or absence of Tollip and IRAK-1. After uptake of tetanus toxoid into APC via caveolae, some population of caveolin-1 is exposed on the outer cell surface of TT-loaded APC (MHC-II + peptide). Migration of CD26+ antigen-specific memory T-cells to areas of antigen-loaded APC results in contact with TT antigen-presenting APC, leading to the association of CD26 and caveolin-1. Aggregation of caveolin-1 in the contact area occurs, presumably by homo-oligomerization (via its residues 61-101), followed by its phosphorylation. (2) Phosphorylated caveolin-1 dissociates complexed Tollip and IRAK-1, presumably due to conformational changes, and IRAK-1 is then phosphorylated in the cytosol. (3) After IRAK-1 is phosphorylated, NF-κB is activated to lead to upregulation of CD86. C2, Protein kinase C conserved region 2; CUE, Coupling of ubiquitin-conjugation to endoplasmic reticulum degradation domain; DD, death domain; KD, kinase domain, and CT, C-terminal domain.

protein tyrosine phosphorylation. On the other hand, Shingu *et al.* found that NK cell cytotoxicity against breast adenocarcinoma cells was decreased in CD26 mutant rats (DPPIV-deficient and reduced CD26 surface expression), suggesting that DPPIV activity was associated with NK cytotoxicity (56).

We recently identified caveolin-1 in APC as a binding protein for CD26 and demonstrated that CD26 on activated memory T-cells directly faces caveolin-1 on TT-loaded monocytes in the contact area, which was revealed as the immunological synapse for T-cell-APC interaction (29). Moreover, we showed that residues 201 to 211 of CD26 along with the serine catalytic site at residue 630, which constitute a pocket structure of CD26/DPPIV, contribute to binding to caveolin-1 scaffolding domain (29). More recently, we demonstrated that caveolin-1 binds to Tollip (Toll-interacting protein) and IRAK-1 (interleukin-1 receptor associated serine/threonine kinase 1) in the membrane of tetanus toxoid-loaded monocytes and that following exogenous CD26 stimulation, Tollip and IRAK-1 disengage from caveolin-1, with IRAK-1 being subsequently phosphorylated to upregulate CD86 expression (51). It is conceivable that the interaction of CD26 with caveolin-1 on antigen-loaded monocytes results in CD86 upregulation, therefore enhancing the subsequent interaction of CD86 and CD28 on T-cells to induce antigen-specific T-cell proliferation and activation (Figure 2).

5. MOLECULAR ASPECT OF CD26/DPPIV AND T-CELL REGULATION

5.1. Interacting proteins and cell structure

Many reports have hitherto showed that CD26 interacts with several molecules playing important roles in T-cell function (57). CD26 physically binds with adenosine deaminase (ADA), an enzyme that plays a key role in the development and function of lymphoid tissues (58-60). ADA is essential for purine metabolism, with the loss of ADA leading to a clinical syndrome characterized by severe immunodeficiency (61). When the ADA inhibitor pentostatin was used in the treatment of recurrent T cell lymphomas, a significant reduction in circulating CD26+ T-cells was observed in treated patients (62). This finding is consistent with the fact that there is a physical association between CD26 and ADA on the surface of T lymphocytes.

Mannnose 6-phosphate/insulin-like growth factor II receptor (M6P/IGF-IIR) binds CD26 via M6P residues in the carbohydrate moiety of CD26 (63), an interaction that is important for CD26-mediated T-cell activation and T-cell migration (63, 64). Furthermore, CD26 internalization in T-cell can be mediated by M6P/IGF-IIR (63), with CD26 internalization being associated with increasing levels of CD26 mannose 6-phosphorylation following T cell activation.

CD26 also interacts with CD45RO, a tyrosine phosphatase with a critical role in T cell signal transduction, at lipid rafts in peripheral blood T lymphocytes to modify cellular signaling events (65, 66). A lipid raft is a cholesterol-rich microdomain in cell membrane, which plays an important role in signal transduction in T-cell regulation (67, 68). CD26 interaction with lipid rafts in peripheral blood T-cells influences key cellular signaling events (65, 66). Non-activated peripheral blood T-cells treated with the anti-CD26 mAb 1F7

increased CD26 recruitment to lipid rafts, resulting in increased tyrosine phosphorylation of c-Cbl, Zap70, Erk1/2, $p56^{lck}$, and TCR-ζ (65). Interestingly, CD26 is associated with CD45 RA outside of lipid rafts in cord blood T cells, and the strong physical linkage of CD26 and CD45 RA may be responsible for the attenuation of cord blood T-cell activation signaling through CD26, which may in turn result in immature immune response and the relatively low incidence of severe graft-versus-host disease (GVHD) in cord blood transplantation (69).

5.2. T-cell costimulatory signaling via CD26

We demonstrated that CD26 binds to caveolin-1 on APC, and that residues 201 to 211 of CD26 along with the serine catalytic site at residue 630, which constitute a pocket structure of CD26/DPPIV, contribute to binding to the caveolin-1 scaffolding domain (29). This region in CD26 contains a caveolin-binding domain (CBD) (ΦXΦXXXXΦXXΦ; Φ and X depict aromatic residue and any amino acid, respectively), specifically WVYEEEVFSAY in CD26 (3, 70). These observations strongly support the notion that DPPIV enzyme activity is necessary to exert T-cell costimulatory activation via CD26 as demonstrated in our previous report using CD26 specific mAbs (30). To examine the binding of caveolin-1 to CD26 in T-cells, we made soluble Fc fusion proteins containing the N-terminal domain of caveolin-1 (NT-Fc), and found that NT-Fc binds specifically to CD26 to induce T-cell proliferation in the presence of TCR/CD3 costimulation (28). Moreover, the binding affinity between caveolin-1 and CD26 (Kd ~$2x10^{-5}$ M) as determined by the BIAcore system is comparable to that of other costimulatory molecules with important roles in immune responses and their associated ligands , such as CD2-CD5 (Kd ~10^{-6} M), CD80-CD28 (Kd ~10^{-7} M) and CD86-CD28 (Kd ~10^{-6} M) (71-73). Until now, CD26-mediated T-cell costimulation was performed using anti-CD26 mAbs, resulting in various CD26-related functions (4, 6, 7, 74). Assuming that the affinity between antigen and antibody is higher (Kd ~10^{-9} M) than that of a ligand-receptor system, and that ligand-specific conformations are capable of differentially activating distinct signaling partners (75), ligand-dependent pathways may be predicted to have different signals associated with the antigen-antibody system and ligand-receptor system.

The precise proximal signaling pathway of CD26 has not yet been identified, particularly in view of the fact that its cytoplasmic tail of CD26 contains only 6 amino acid residues without a common signaling motif structure. Moreover, it has been unclear whether the short cytoplasmic tail is responsible for signal transduction associated with CD26-mediated costimulation. Using recombinant CD26-CD10 chimeric receptor, we showed that the cytoplasmic tail of CD26 is indeed responsible for T-cell costimulation induced by anti-CD3 plus caveolin-1 (28). Furthermore, to explore the proximal signaling molecules interacting with the cytoplasmic tail of dimeric CD26, we used proteomic analyses with Fc fusion proteins containing the cytoplasmic amino acid residues of CD26 to identify that CARMA1 binds to the cytoplasmic tail of dimeric CD26 (28). We further demonstrated that a PDZ domain in CARMA1 is necessary for binding to CD26. The importance of CARMA1 in CD26-mediated costimulation is also shown by rescue experiments using the CARMA1-deficient Jurkat T-cell line JPM50.6 (28). CARMA1, containing CARD and MAGUK domains, plays an essential role in the NF-κB activation and IL-2 expression induced by CD3-CD28 or CD28-PMA stimulation (76, 77). Following its phosphorylation, CARMA1 functions as a signaling intermediate downstream of PKCθ and upstream of IKK in the TCR signaling transduction pathway leading to NF-κB activation (78, 79). We also showed that dimeric CD26, but not monomeric CD26, binds to CARMA1 (28). CD26/DDPIV is reported to exist as homodimers, a structural organization which allows access of substrates to DPPIV catalytic activity (23, 27). Although DPPIV activity is crucial for CD26-mediated T-cell costimulation (30, 31), the exact role played by DPPIV in this process is unclear. Our recent study showed that the enzymatic pocket structure of the DPPIV catalytic site is necessary for binding of CD26 to caveolin-1, leading to the upregulation of CD86 expression on APC (29, 51). More recently, we found that monomeric CD26 H750E, which has a 300-fold decrease in catalytic activity (27), does not bind to CARMA1, resulting in the loss of CD26-mediated T-cell costimulation by anti-CD3 plus caveolin-1 (28). Therefore, dimerization of CD26 is not only necessary for binding to caveolin-1, but also serves as a scaffolding structure for the cytoplasmic signaling molecule CARMA1. The precise binding position of CARMA1 in the cytoplasmic domain of CD26 remains to be elucidated in future studies, since PDZ domains bind primarily to specific C-terminal motifs (X-S/T-X-V/L; X depicts any amino acids) or internal target motifs as well as other PDZ domains (80).

Based upon the above studies, we propose the following model to explain the sequence of events leading from CD26-CD3 costimulation to NF-κB activation (Figure 3). In CD3-CD26 costimulation, TCR engagement by peptide-loaded MHC class II presented on APC activates PI3K via phosphorylation of ITAMs (immunoreceptor tyrosine-based activation motifs) in TCR, leading the recruitment of PKCθ and IKK complex in lipid rafts (77, 81-83). Concomitantly, CD26 ligation by caveolin-1 on APC recruits CD26-interacting CARMA1 to lipid rafts, resulting in the formation of a CARMA1-Bcl10-MALT1-IKK complex, and this membrane-associated Bcl10 complex then activates IKK through ubiquitination of NEMO (76, 77, 81-84).

6. CLINICAL ASPECT OF CD26 AND IMMUNE DISORDERS

6.1. Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disease that primarily affects the joints, but also has systemic symptoms. CD26/DPPIV is overexpressed on T-cells in the peripheral blood and synovial fluid of patients with RA. CD26+ T-cells induce the inflammation and tissue destruction characteristic of RA by migrating to and being active in the rheumatoid synovium (48). Cordera *et al.* studied IL-12, IL-15, soluble

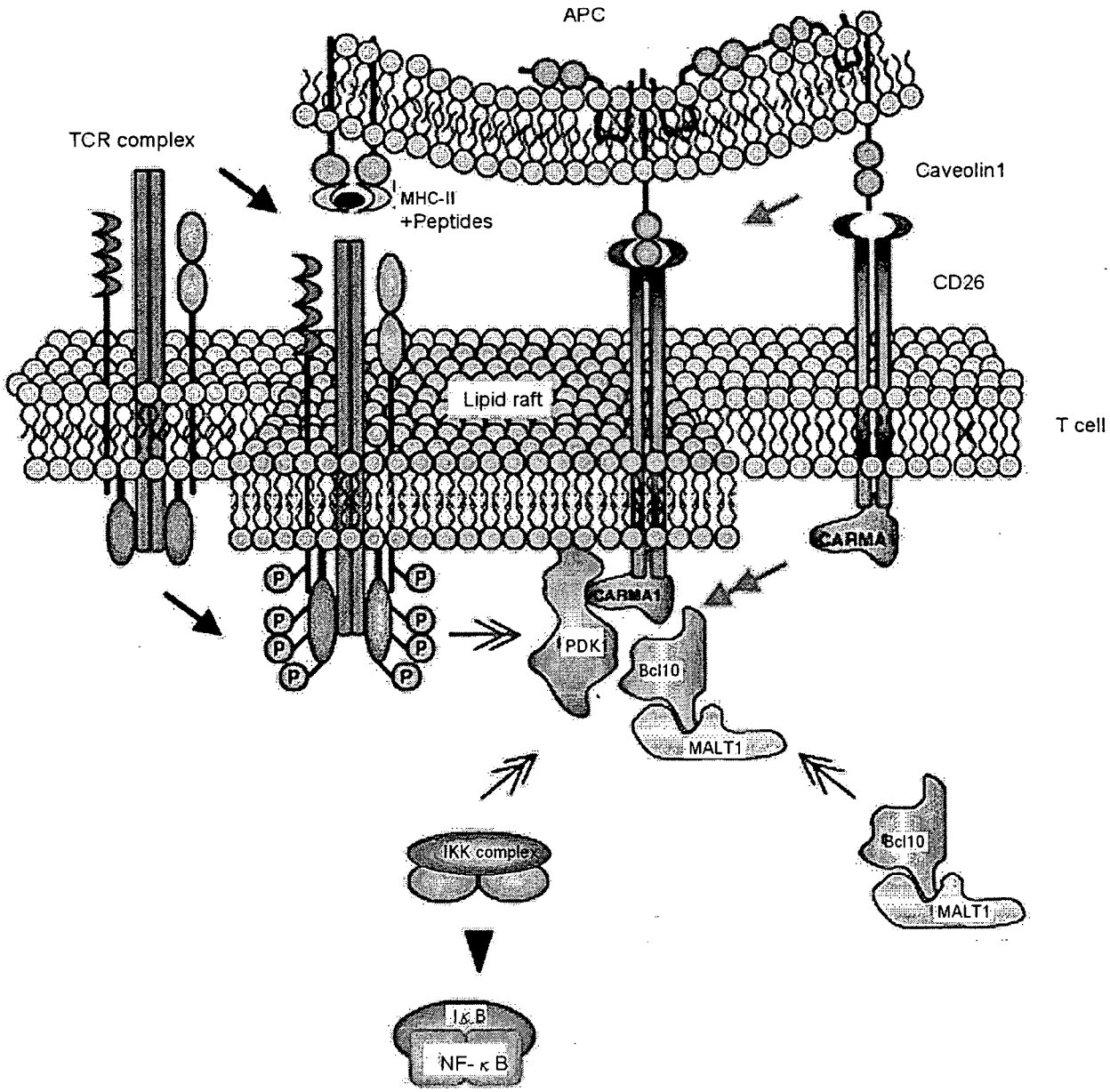

Figure 3. A model for signaling by TCR and CD26 costimulation. Stimulation of cells through TCR complexes leads to phosphorylation of cytoplasmic ITAMs by ligation of peptide-loaded MHC class II (bold arrows) and recruitment and activation of PDK1 with IKKs (gray arrow head), through activation of PI3K and PKCθ. Meanwhile, caveolin-1, the N-terminal extracellular region of which is present on antigen-loaded APC, ligates CD26 which exists as dimers on the cell surface, leading to the recruitment of lipid rafts (gray arrow) and interaction with CARMA1 (gray double-headed arrow). The recruitment of CARMA1 along with CD26 to lipid rafts also engages the CARMA1-Bcl10-PDK1-IKKs complex (black double-headed arrows), leading to activation of the IKK complex (black double-headed arrows), and finally, activation of NF-κB.

CD26, and ADA serum levels from 35 patients with active and inactive RA as well as those of controls (85). Patients' sera had higher IL-12 and IL-15 levels, and the level of soluble CD26 was inversely correlated with the number of swollen joints. These findings suggest that these cytokines and CD26 are associated with the inflammation and immune activity in RA. Mavropoulos *et al.* found that anti-tumor necrosis factor-alpha therapy increases DPPIV activity and decreases autoantibodies to the chaperone protein Bip (GRP78) and phosphoglucose isomerase in 15 patients with RA (86). DPPIV inhibitors inhibit a rat model of rheumatoid arthritis in a dose-dependent manner (87). We described CD26+ T-cells infiltrating the rheumatoid synovium using immunohistochemical studies (88). We also noted high expression of caveolin-1 in the rheumatoid synovium vasculature and synoviocytes. These data suggest that the CD26-caveolin-1 upregulation of CD86 on activated monocytes leads to antigen-specific T-

cell activation in rheumatoid arthritis. DPPIV inhibitors may be useful for suppressing the immune system in rheumatoid arthritis and other autoimmune diseases.

6.2. Autoimmune and other immune-mediated disorders

Previous studies have shown that CD26 expression is tightly regulated on human T-cells and that it has a key role in T-cell function (1, 15). CD26 expression on T-cell surface is higher in multiple sclerosis and Graves' disease (89, 90). CD26/DPPIV appears to play a critical role in the effector functions of CD4+ T-cells in experimental autoimmune encephalomyelitis systems (91). CD26+ T-cells are more sensitive to TCR-mediated activation (92), and CD26 is expressed preferentially on the subset of CD4+ helper cells in response to recall antigens (15).

CD26/DPPIV levels in human serum (soluble CD26) have been examined in patients with various medical conditions, such as autoimmune diseases, infectious diseases, and psychiatric disorders (93). To determine the role of soluble CD26 in the pathophysiology of patients with systemic lupus erythematosus (SLE), we measured levels of soluble CD26 and its specific DPPIV activity in serum (94). Serum levels of soluble CD26 and its specific DPPIV activity were significantly decreased in patients with SLE, and were inversely correlated with SLE disease activity index score, but not with clinical variables or clinical subsets of SLE (94). In patients with inflammatory bowel diseases (IBD) such as Crohn's disease or ulcerative colitis, CD26+T-cells and DPPIV activity in serum were examined (95, 96). In these studies, the DPPIV activity was reduced in patients with IBD, and the number of CD25+CD26+ T-cells in the peripheral blood was increased in patients with IBD. Taken together, these data indicate that CD26 may be potentially important for the pathophysiology of SLE and IBD, and appears to be useful as a new marker for disease activity in SLE or IBD.

CD4+ T-cells in patients with AIDS develop an intrinsic defect in their ability to recognize and respond to recall antigens prior to a detectable reduction in the total number of CD4+ T-cells (97, 98). The response to recall antigens is clearly a property of CD4+CD26+ T-cells, since this is the only helper population known to proliferate in response to soluble antigens, and to induce both MHC-restricted cytotoxic T-cells capable of killing virus-infected target cells and immoglobulin-secreting B-cells (15). In this regard, a selective decrease in CD26+ T-cells has been reported in HIV-1-infected individuals prior to a general decrease in CD4+ T-cells (99, 100). Moreover, Tat, a regulatory protein encoded by the HIV-1 genome which has been shown to suppress the response of human peripheral T-cells to soluble antigens (101, 102), can bind to CD26 and partially inhibit DPPIV enzyme activity (103). We have shown that the DPPIV enzyme activity of plasma soluble CD26 was low in HIV-1-infected individuals, and was inversely correlated with HIV-1 RNA, and that the *in vitro* addition of recombinant soluble CD26 could enhance purified protein derivative-induced lymphocyte proliferation (104). These results suggest that the specific DPPIV enzyme activity of plasma soluble CD26 in HIV-1-infected individuals contributes to the immunopathogenesis of HIV infection. Taken together, the above findings suggest that CD26/DPPIV plays an important role in the pathophysiology of autoimmune diseases and other immune-mediated disorders. Moreover, *in vivo* recombinant soluble CD26 supplementation in immunodeficient patients with decreased soluble CD26/DPPIV may be of use in restoring functional immune response.

6.3. Graft-versus-host disease (GVHD)

GVHD remains a major cause of morbidity and mortality in allogeneic stem cell transplantation (alloSCT). In GVHD, mature donor T-cells that accompany the stem cell graft attack recipient tissues, especially the skin, liver, gastrointestinal tract, and lung. Therefore, all patients undergoing alloSCT receive GVHD prophylaxis to impair T-cell function; however, treatment to prevent GVHD can be deleterious since mature donor T-cells play a critical role in mediating reconstitution of the adaptive immune system, especially in adults with diminished thymic function (105). Recipients of alloSCT are thus at great risk for infections, particularly when prolonged immunosuppression is required for treatment of GVHD. Some approaches (e.g., B7-blocking, OX40-blocking, and T-cell depletion by specific antibodies) to promote the positive effects of alloSCT without GVHD have been developed recently in the clinical settings (reviewed in (106)). Although the role of CD26/DPPIV in GVHD needs to be studied in more detail, treatment with a murine antibody against human CD26 was reported to have an effect in patients with steroid-resistant acute GVHD following alloSCT (107, 108). Moreover, we have previously examined the expression of CD26 as well as the reconstitution of CD26-mediated T-cell costimulation via the CD3 and CD2 pathways at various times in patients with CD6-positive T-cell depleted allogeneic bone marrow transplantation (alloBMT) (109). In this study, we found that the percentage of CD26- and CD3-positive cells, as well as the levels of expression of both antigens, was lower than in normal controls during the first 4 months after CD6-depleted alloBMT. Subsequently, the amount of lymphocytes expressing CD3 and CD26 and the quantitative surface expression of CD3 and CD26 were not significantly different in patients and normal controls. Functional studies showed that CD26-mediated T-cell proliferation via the CD3 pathway was considerably improved and almost reached normal levels by 1 year, whereas recovery of CD26-mediated T-cell proliferation via the CD2 pathway was delayed for at least 2 years after CD6-depleted alloBMT. As CD26 involvement in the regulation of human thymocyte activation is restricted preferentially to the CD3 pathway, unlike its involvement with both CD3 and CD2 pathways of peripheral T cells, our results suggest that the different effects of CD26-mediated costimulation via the CD3 and CD2 pathways after CD6-depleted allo-BMT may be a reflection of peripheral T-cell immaturity in those individuals. Taken together, it may be possible that the full therapeutic potential of alloSCT will be realized by approaches that aim to minimize GVHD by targeting CD26-mediated T-cell regulation.

7. SUMMARY AND PERSPECTIVES

Understanding of the molecular mechanisms involved in CD26-mediated T-cell costimulation has been hindered by the lack of specific costimulatory ligand for CD26 and proximal signaling molecules that can be assessed in comparison with those observed in experiments using anti-CD26 mAbs (6). The results presented here provide a starting point to investigate other mechanisms that have been observed in CD26-mediated cellular functions. For example, downstream targets of CARMA1 may emerge as important mediators of CD26-mediated cellular events as well as T-cell costimulation. Moreover, influencing CD26-mediated T-cell costimulation by using caveolin-1-Fc fusion proteins or by inhibiting dimerization with selected small compounds may lead to new therapeutic approaches to treat T_H1-mediated autoimmune diseases such as rheumatoid arthritis, Grave's disease and multiple sclerosis, and alloreaction following transplantation or atherosclerosis, and to induce adjuvant reaction for T_H1-mediated cancer immunotherapy. Of significance is our recent work demonstrating that CD26 on T-cell surface binds to caveolin-1, hence identifying the first endogenously expressed CD26 costimulatory ligand in the immune system. Moreover, the caveolin-1-CD26 interaction results in strong T-cell costimulation as a result of the recruitment of a molecular complex consisting of CARMA1-Bcl10-MALT1-IKK in lipid rafts. Our findings will therefore serve as a foundation for future insights into the regulation of T-cell costimulation via the CD26 molecule.

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Abbreviations: ADA: adenosine deaminase; APC: antigen-presenting cells; alloSCT: allogeneic stem cell transplantation; BMT: bone marrow transplantation; CBD: caveolin-binding domain; DPPIV: dipeptidyl peptidase IV; GLP-1: glucagon-like peptide 1; GVHD: graft-versu-host disease; IRAK-1: interleukin-1 receptor associated serine/threonine kinase 1; M6P/IGF-IIR: mannose 6-phosphate/insulin-like growth factor II receptor; NT-Fc: soluble Fc fusion proteins containing the N-terminal domain of caveolin-1; PMA: pokeweed mitogen; RA: rheumatoid arthritis; rsCD26: recombinant soluble CD26; TT: tetanus toxoid; mAb: monoclonal antibody; Tollip: Toll-interacting protein; TCR: T-cell receptor

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The role of CD26/dipeptidyl peptidase IV in cancer

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1. ABSTRACT

CD26/DPPIV is a multifunctional cell surface protein that is widely expressed in most cell types including T lymphocytes, on which it is a marker of activation. It is also present in serum and other body fluids in a truncated form (sCD26/DPPIV). It preferentially cleaves N-terminal dipeptides from polypeptides with proline or alanine in the penultimate position, and in doing so, regulates the activities of a number of cytokines and chemokines. Due in part to this ability to regulate the activity of biopeptides, it can act as a tumor suppressor or activator. It can associate with several proteins, among them fibroblast activating protein-alpha (FAP-alpha), plasminogen, adenosine deaminase (ADA), the tyrosine phosphatase CD45, and the chemokine receptor CXCR4. It can also bind to the extracellular matrix (ECM) and depending on the presence of other ligands, this process can either lead to increased or decreased invasive activity of the cells on which it is expressed. As a result of these characteristics, CD26/DPPIV plays an important role in tumor biology, and is useful as a marker for various cancers, with its levels either on the cell surface or in the serum being increased in some neoplasms and decreased in others. Our group has shown that CD26/DPPIV can be manipulated by such agents as CD26 cDNA-carrying plasmids, siRNA and monoclonal antibodies, resulting in both *in vitro* and *in vivo* inhibition of cell growth, enhanced sensitivity to selected chemotherapeutic agents, and enhanced survival of mouse xenograft models. These studies have demonstrated the utility of these tools as potential targeted therapies for specific cancers expressing CD26/DPPIV.

2. INTRODUCTION

CD26 (DPPIV) is a multifunctional membrane-bound glycoprotein present on the surface of most cell types. It is a type II cell surface protein, as the bulk of the protein including the carboxy terminus faces the extracellular space. As a dipeptidyl peptidase, it preferentially cleaves N-terminal dipeptides from polypeptides with proline or alanine in the penultimate position. This activity places it in the prolyl peptidase family and is responsible for its best known functions—chemokine regulation and glucose homeostasis (1). Its enzymatic activity, which is responsible for many but not all of its activities, resides in the carboxy terminal extracellular domain and is involved in the activation/inactivation of a number of chemokines and cytokines. CD26/DPPIV has 3 domains: an extracellular domain, a transmembrane region, and a short cytoplasmic tail of 6 amino acids. Enzymatically active CD26/DPPIV is a homodimer, each subunit containing an alpha/beta hydrolase domain and a beta-propeller domain. A large cavity formed by the alpha/beta hydrolase and the eight-bladed beta-propeller domain acts as the substrate binding site (2). CD26/DPPIV was initially considered to cleave only after a proline or alanine residue, but its substrates now include hydroxyproline, serine, glycine, valine, threonine, and leucine. The discrimination between proline and alanine in the penultimate position is much greater for the dipeptide chromogenic and fluorogenic substrates than for its natural substrates (3). In addition to its exopeptidase activity, there is some evidence that it also has endopeptidase activity, which could play a role in extracellular matrix degradation and hence invasion (4). However, it is possible that this activity is mediated through CD26/DPPIV association with FAP-alpha (5).

A soluble form of the protein (sCD26/DPPIV) is present in the serum and other body fluids, presumably as a result of shedding or secretion from different cell types. The soluble form lacks the transmembrane region and cytoplasmic residues, as it begins at amino acid 39 (6). This form is also a dimer and has been detected in seminal fluid as a larger oligomer (>900 kDa) (7).

3. PROTEINS ASSOCIATED WITH CD26/DPPIV

CD26/DPPIV also exhibits biological functions that are unrelated to its dipeptidase activity. With its beta-propeller domain containing binding sites for several proteins, in addition to forming heterodimers with FAP-alpha, CD26/DPPIV has also been reported to associate with plasminogen 2, ADA, CD45, CXCR4, and mannose 6-phosphate/insulin-like growth factor II receptor (mannose-6-P/IGFIIR). Furthermore, it can bind to the ECM proteins fibronectin and collagen.

3.1. FAP-alpha (Seprase)

FAP-alpha cDNA also codes for a type II integral membrane protein with a large extracellular domain, a transmembrane segment, and a short cytoplasmic tail with 48% amino acid sequence identity with CD26/DPPIV. Although the active form of CD26/DPPIV is a homodimer, expression of both proteins in COS-1 cells resulted in the formation of heterodimeric complexes (8). The genes for both proteins are located in the 2q23 region, suggesting that they may have arisen by gene duplication. However, their expression patterns are different, with CD26/DPPIV being constitutively expressed in most tissues (except in the case of T and B cells, where its expression is regulated by activation), and FAP-alpha expression being more restricted. FAP-alpha has been found to be localized at the invasion front during invasion into the extracellular matrix by human melanoma (9), breast carcinoma (10), and endothelial cells (5). Formation of the CD26/DPPIV-FAP-alpha complex at invadopodia of migrating fibroblasts was required for cell invasion on a collagenous matrix. In addition, antibodies to the gelatin-binding domain of CD26/DPPIV reduced cell migration and degradation of collagen (11). Interestingly, CD26/DPPIV transfectants have been shown to induce FAP-alpha (12, 13).

3.2. Plasminogen 2

Plasminogen 2 (Pg 2) binding to CD26/DPPIV was first demonstrated in rheumatoid arthritis synovial fibroblasts (14). Pg 2 bound to CD26/DPPIV residues 313-319 (15) and was dependent on the sialic acid content of the plasminogen isoform. Pg 2-gamma, Pg 2-delta, and Pg 2-epsilon bound to CD26/DPPIV, whereas Pg 2-alpha and Pg2-beta did not bind. However, in the prostate tumor cell line 1-LN, only Pg 2-epsilon induced expression and secretion of metalloproteinase 9 (MMP-9) (16). Hence, the ability of CD26/DPPIV to associate with plasminogen may be a factor in the invasiveness of certain cancers.

3.3. ADA

ADA catalyzes the deamination of adenosine and deoxyadenosine to inosine and 2'-deoxyinosine, respectively. ADA is located in both the cytosol and on the surface of lymphocytes, where it is associated with CD26/DPPIV (17) and dependent on CD26/DPP IV expression. The function of ADA located on the cell surface is to regulate extracellular adenosine and deoxyadenosine, which are toxic to lymphocytes (18). Adenosine, which accumulates in the extracellular fluid of solid tumors, caused down-regulation of CD26/DPPIV in HT29 colorectal carcinoma cells, which resulted in the depletion of ADA on the cell surface and consequently, a further increase in adenosine. Since the presence of excess adenosine suppresses the immune system, this process may facilitate tumor survival (19). Meanwhile, a different response was reported in endothelial cells. In this case, although hypoxia led to an increase in adenosine level, ADA and CD26/DPPIV mRNA and protein expression were likewise upregulated. The differential effect of adenosine on ADA and CD26/DPPIV in these distinct experimental conditions may be partially due to differences in adenosine levels and also tissue types (20).

The crystal structure of the ADA-CD26/DPPIV complex has revealed binding of one ADA molecule to each beta-propeller domain of CD26/DPPIV (21). The crystal structure also indicates that tetramerization of CD26/DPPIV is a key mechanism for the regulation of its interaction with other components (22). It has been suggested that binding of ADA could regulate

CD26/DPPIV-mediated adhesion between cells by inhibiting tetramer formation between dimers. This is consistent with an earlier study which showed that addition of exogenous ADA inhibited adhesion between lymphocytes and epithelial cells promoted by CD26/DPPIV (23).

3.4. CD45

CD45 is a membrane tyrosine phosphatase that regulates Src-family kinases. It is highly expressed in hematopoietic cells where it chiefly regulates Lck activity. CD45 can regulate Lck activity (important for TCR signaling events) by dephosphorylation of Tyr 505, the negative regulatory site, or down-regulate Lck by dephosphorylation of Tyr 394 in the kinase domain (24). In lymphocytes, CD26/DPPIV and CD45 have been shown to be associated through the binding of CD26/DPPIV to the cytoplasmic domain of CD45 (25, 26). Furthermore, cross-linking of CD26 by a CD26-specific antibody leads to increased phosphorylation of several cell signaling proteins, including $p56^{lck}$, $p59^{fyn}$, ZAP70, and MAP kinase, most likely due to its association with CD45 (27). CD45 has also been implicated in the negative regulation of CD44-mediated cell spreading (28).

3.5. CXCR4

The consequences of the association between the chemokine receptor CXCR4 and CD26/DPPIV are less well understood. Stromal cell-derived factor-1-alpha (SDF-1-alpha)/CXCL12 and its receptor, CXCR4, have recently been shown to play a critical role in tumorigenesis in addition to their well-recognized role in mediating migration and activation of leukocytes during immune and inflammatory responses (29). CXCR4 is upregulated in hypoxic tissues (30) and is the main receptor for SDF-1-alpha, a chemokine which attracts cells expressing CXCR4 and which is cleaved by CD26/DPPIV. Binding of SDF-1-alpha triggers internalization of both CXCR4 and CD26/DPPIV in the T-cell line, Jurkat J32, the B-cell line, SKW6.4, and peripheral blood lymphocytes (31), suggesting that these two proteins may function together.

3.6. Mannose-6-P/IGFIIR

CD26/DPPIV binds to mannose-6-P/IGFIIR via mannose-6-phosphate residues in the carbohydrate moiety of CD26/DPPIV (32). This interaction is critical for CD26/DPPIV-mediated T cell activation and migration (32, 33). Endothelial cells expressing mannose-6-phosphate/IGFIIR on their surface bind to mannose-6-P on sCD26, indicating that sCD26/DPPIV is involved in T-cell migration via its interaction with mannose-6-P/IGFIIR. Moreover, enhanced migration was dependent on DPPIV enzyme activity. Although CD26/DPPIV has no known motif for endocytosis, upon T cell activation, mannose-6-phosphorylation increases, leading to increased binding to the mannose-6-P/IGFIIR and resulting in CD26/DPPIV internalization. Internalization of CD26/DPPIV can also occur following SDF-1-alpha binding to CXCR4, which causes cointernalization of CXCR4 and CD26/DPPIV (31). Of note is that internalization of both CXCR4 and CD26/DPPIV require phosphorylation; for CD26/DPPIV phosphorylation of sugar residues (32), and for CXCR4, phosphorylation of serine residues (34).

3.7. Collagen and Fibronectin

Both collagen and fibronectin are components of the extracellular matrix. The interaction between collagen and CD26/DPPIV is mediated by residues in the cysteine-rich region of CD26/DPPIV and not the catalytic domain (35). CD26/DPPIV also binds to fibronectin (36-38). A detailed study of the binding of CD26/DPPIV to fibronectin was carried out using fibronectin fragments, which found this process to be mediated by the consensus motif T (I/L)TGLX (P/R)G (T/V)X (37). CD26/DPPIV binding to fibronectin is important for adhesion to specific cell types. It is thought that cancer cells initially arrest in the microvasculature of the first organ they encounter with only a few cells forming metastases at a particular secondary site. Breast carcinomas most frequently metastasized to the lungs, for example, and were shown to bind to lung endothelia expressing CD26/DPPIV mediated by the fibronectin assembled on their surface (36).

4. DIFFERENCES BETWEEN CD26/DPPIV AND OTHER FAMILY MEMBERS

4.1. FAP-alpha (Seprase)

FAP-alpha was first identified in the malignant melanoma cell line LOX on the basis of its presence in invasive cell lines. It was also found in membrane vesicles obtained from conditioned media from this cell line. However, it was not detected in a control melanoma cell line or in 32 other tumor cell lines that were unable to degrade extracellular gelatin (39). Similar to CD26/DPPIV, FAP-alpha is a type II membrane protein and a member of the prolyl peptidase family, but unlike CD26/DPPIV, it is inhibited by cysteine protease inhibitors, such as N-ethylmaleimide (39). Also, dipeptides that are CD26/DPPIV substrates are cleaved with a substantially lower catalytic efficiency (100-fold) by FAP-alpha, due to the presence of different amino acids in its active site. In contrast to CD26/DPPIV, FAP-alpha has both gelatinase and collagenase activities (40). In addition, it is generally not expressed in normal tissue, but is present in epithelial cancers and reactive stromal fibroblasts of bladder, breast, colorectal, lung and ovarian carcinomas (41).

Although FAP-alpha is not expressed in normal adult tissues, it can be induced in fibroblasts in response to wounding and also in the reactive stroma of epithelial cancers and some sarcomas (41). Its expression in some malignant cells of epithelial origin, e.g. invasive ductal carcinoma cells from breast cancers, gastric carcinoma, and melanoma, has been demonstrated in several recent studies (10, 39, 42).

Human embryonic kidney cells (HEK293) transfected with murine FAP-alpha and injected into mice formed tumors that grew more rapidly than tumors from control vector-transfectants. Rapid growth depended on protease activity, and was negated by antibodies that inhibited dipeptide cleavage (43). Human breast cancer

cells transfected with FAP-alpha cDNA also formed tumors that grew more rapidly than tumors from transfectants not expressing FAP-alpha (44). In addition, the fast growing tumors exhibited a higher microvessel density. However, there was no difference in *in vitro* proliferation between parental and FAP-alpha-expressing cells. Data from this particular study would support the conclusion that FAP-alpha drives angiogenesis. Additional support for this idea was provided by another study showing that FAP-alpha mRNA upregulation by endothelial cells was involved in capillary morphogenesis (45). Interestingly, non-small-cell lung carcinoma (NSCLC) cells transfected with either a mutant lacking DPPIV activity (S630A) or wild-type CD26/DPPIV induced expression of FAP-alpha (13).

4.2. DPP8 and DPP9

It is likely that DPP8 and DPP9 contribute to biological functions previously attributed to CD26/DPPIV. Both DPP8 and DPP9 have been expressed in baculovirus and shown to function as dimers with similar kinetic and substrate profiles (46). When transfected into 293T cells, a cell line that does not express FAPα and expresses CD26/DPPIV intracellularly at low levels, both DPP8 and DPP9 localized to the cytoplasm. Furthermore, cell migration and monolayer wound healing were impaired by overexpression of either DPP8 or DPP9 (47). Using selective inhibitors, it has been shown recently that DPPIV activity attributable to DPP8/9 is present in human peripheral blood mononuclear cells (PBMC). Most of the DPP8/9 activity was localized to the cytosol, whereas CD26/DPPIV activity was concentrated in the membrane (48).

4.3. DPP10 (DPL2)

Although this protein shares homology with CD26/DPPIV (32%), the active site serine residue is replaced by glycine, resulting in loss of enzyme activity. In contrast to CD26/DPPIV, it is not widely expressed but is present chiefly in brain and pancreas (49, 50).

Based on experiments with CD26/DPPIV enzyme inhibitors, it has become clear that CD26/DPPIV-related family members are targets of drugs initially thought to be specific for CD26/DPPIV. For example, the dipeptidyl peptidase inhibitor, val-boro-pro triggered tumor regression and rejection in WEHI 164 fibrosarcoma and EL4 and A20/2J lymphoma models. Furthermore, treatment with this inhibitor induced upregulation of cytokine and chemokine expression in the tumor and draining lymph nodes. Antitumor activity and stimulation of cytokine and chemokine production was unchanged in $CD26^{-/-}$ mice (51). Therefore, it is currently thought that this DPPIV inhibitor targets FAP-alpha in the tumor stroma as well as cytoplasmic DPP8 and DPP9, inducing a cytokine-mediated immunological response.

5. CD26/DPPIV EXPRESSION IN HUMAN CANCERS

The exact role CD26/DPPIV plays in various cancers remains to be elucidated, partly due to its variable expression on these tumors. In general, it is strongly expressed on some cancers, while being absent or present at low levels in others. Furthermore, given the plethora of its biological functions, including its ability to associate with several key proteins and its cleavage of a number of soluble factors to regulate their function, it is likely that the CD26/DPPIV effect on tumor biology is at least partly mediated by the effect of these biological functions on specific tumor types.

5.1. Cancers associated with high CD26/DPPIV expression

5.1.1. Mesothelioma

CD26/DPPIV was shown to have the highest activity among cell surface aminopeptidases in human mesothelial cells (52). Since mesothelial cells are in constant contact with bodily fluids such as ascites, the influence of ascites on DPPIV activity was measured. Mesothelial cells cultured in the presence of malignant ascites from ovarian carcinoma patients exhibited an increase in DPPIV activity of up to 200% over the control, whereas no significant increase was observed for benign ascites. Fractionation of the ascites revealed that the activity was present in the fraction containing small peptides (<3 kD) and was responsible for elevating both CD26/DPPIV mRNA and protein expression on the cell surface (52). More recent studies confirm that CD26/DPPIV is expressed at a high level on the surface of malignant mesothelioma cells, but not on cells derived from a benign mesothelioma (53). Malignant mesothelioma is an aggressive cancer involving the mesothelioma cells lining the pleura and is resistant to conventional treatments. Depletion of CD26/DPPIV by siRNA resulted in loss of binding to ECM proteins, fibronectin and collagen. Similarly, our recent work showed that incubation with the murine monoclonal antibody 14D10 or a humanized antibody against CD26/DPPIV caused loss of binding to ECM proteins and upregulation of $p27^{kip1}$. Antibody treatment of mice inoculated with human malignant mesothelioma cells inhibited tumor growth and enhanced survival when either 14D10 or the humanized CD26 antibody was injected, whereas the isotype matched control had no effect. In addition, both CD26/DPPIV-specific antibodies reduced formation of metastases (53).

5.1.2. Renal

CD26 has also been shown to be expressed on renal carcinoma cells (54, 55), including the cell lines Caki-1, Caki-2, ACHN, and VMRC-RCW (56). We recently treated Caki-2 cells, which strongly express CD26/DPPIV, with the murine monoclonal anti-CD26 antibody 14D10. Following treatment, cells arrested in G1/S, accompanied by an induction of $p27^{kip1}$, down-regulation of cyclin-dependent kinase 2, and dephosphorylation of retinoblastoma protein. When mice inoculated with human renal carcinoma cells were injected with the CD26-specific antibody, tumor growth was inhibited and survival was significantly enhanced (56).

5.2 . Cancers associated with variable CD26/DPPIV expression

5.2.1. Colon cancer

CD26/DPPIV level was found to be correlated with a differentiated phenotype in both HT-29 and Caco-2 colon cancer cells (57). Neither protein stability nor glycosylation was affected by the state of differentiation. Instead, expression appeared to be controlled at the transcriptional level, since CD26/DPPIV mRNA level was low in undifferentiated cells, but increased as differentiation progressed. Although CD26/DPPIV is not expressed in the adult colon, it has been shown in some cases to be re-expressed in some colon cancers and cell lines.

CD26/DPPIV was down-regulated by adenosine in HT-29 colorectal carcinoma cells, a process mediated by an increase in tyrosine phosphatase activity leading to decreased tyrosine phosphorylation of MAP kinase ERK (1/2) (58). Another study showed that while CD26/DPPIV expression was lower in the colon carcinoma cell lines tested, FAP-alpha was expressed at higher levels in cancer cells and adjacent stromal cells compared to normal colorectal tissue. In addition, a correlation was found between FAP-alpha expression and lymph node metastasis, with high FAP-alpha expression in colorectal cancer tissue being associated with lymph node metastasis (59, 60).

Conflicting reports have been published regarding the presence of soluble CD26/DPPIV molecules in the serum of patients with colorectal carcinoma. One study indicated that serum levels in healthy donors were significantly higher than those in colorectal carcinoma cancer patients (61). However, in another report, a higher level of soluble CD26/DPPIV was detected in patients diagnosed with colorectal cancer, being highest in those with metastatic disease (62). While the reason for the observed difference is unclear, one possibility may be due to the detection methods used-an ELISA assay in the former, and an assay for enzyme activity in the latter.

Meanwhile, tetraspanins are integral membrane proteins that play a role in organizing multimolecular complexes in the plasma membrane. Several studies have demonstrated a link between the expression of these proteins and metastasis. In a recent study utilizing a colon cancer model consisting of cell lines derived from the primary tumor and two metastases, CD26/DPPIV and the tetraspanin Co-029 (identified by mass spectrometry) were present only on the metastatic cancer cells (63).

5.2.2. Glioma

In a recent study using glioma cell lines, no simple correlation could be demonstrated between CD26/DPPIV expression and the degree of malignancy. The lack of an unambiguous result was due to the contribution of DPPIV-like enzymatic activity contributed by dipeptidyl peptidase IV activity and/or its structural homologs (DASH) (64). In an earlier report by the same group of investigators, a positive correlation had been made between the degree of transformation and DPPIV activity, but at the time the contribution of other DPPIV-like proteins was not fully appreciated (65).

5.2.3. Hematological malignancies

Immunofluorescence analysis revealed expression of CD26/DPPIV on peripheral blood lymphocytes of patients with B chronic lymphocytic leukemia (B-CLL), but not on peripheral B cells from normal donors. CD26/CPPIV could also be induced in normal B cells following treatment with interleukin-4, with RT-PCR analysis indicating that expression was regulated at the level of transcription (66). In contrast, the expression of CD26/DPPIV was decreased in the PBMC of patients with adult T cell leukemia/lymphoma (ATLL) compared with cells from normal donors. Again, expression was regulated at the level of transcription (67). Down-regulation appears to result from methylation of CpG islands in the promoter region (68).

CD26/DPPIV has also been shown to be a marker for aggressive T-large granular lymphocyte lymphoproliferative disorder (T-LGL LPD). Our work indicated that patients with CD26-positive disease were more likely to require therapies for cytopenia and infections associated with the disease than those with CD26-negative T-LGL. Furthermore, CD26-related signaling may be aberrant in T-LGL as compared to T-lymphocytes from normal donors (69). Disease aggressiveness is also correlated with CD26/DPPIV expression in other subsets of T-cell malignancies including T-lymphoblastic lymphoma/T-acute lymphoblastic leukemia (LBL/ALL), as those with CD26-positive T-LBLALL had a worse clinical outcome compared to patients with CD26- negative tumors (70, 71).

Two CD26/DPPIV inhibitors were used to elucidate the function of CD26/DPPIV in clones of the human histiocytic lymphoma cell line U937 expressing different levels of CD26/DPPIV. The presence of these inhibitors suppressed DNA synthesis and cytokine production in high expressors while having no effect in low expressors, and these findings support the notion that CD26/DPPIV plays a role in the proliferation and cytokine production of transformed cells (72). Meanwhile, we showed that treatment with anti-CD26 monoclonal antibody inhibited adhesion of the human CD30+ anaplastic large cell T-cell lymphoma cell line Karpas 299 to fibronectin. Furthermore, depletion of CD26 in Karpas 299 cells by siRNA decreased tumorigenesis and increased survival of SCID mice inoculated with these cells (38). We also showed that treatment with anti-CD26 monoclonal antibody inhibited the growth of T -leukemia cell line Jurkat transfected with CD26 through G1/S cell cycle arrest, associated with concurrent activation of the ERK signaling pathway and increased p21 expression (73). Likewise, we demonstrated that anti-CD26 monoclonal antibody treatment of the CD26-positive T-lymphoma line Karpas 299 resulted in *in vitro* and *in vivo* antitumor activity, with associated enhanced survival of SCID mice inoculated with Karpas 299 cells (74).

On the other hand, CD26/DPPIV was not detectable on tumor samples from most patients with the T-cell lymphoma subtype mycosis fungoides/Sezary syndrome, with low expression in the remaining patients in one published study (75). These findings have been corroborated by other investigators (76, 77). Therefore, the absence of CD26/DPPIV expression can be used in the clinical setting as a marker for the diagnosis of mycosis fungoides/Sezary syndrome, as suggested by our work (75). Meanwhile, a recent study suggested that the skin-homing characteristic of Sezary syndrome tumor cells is mediated by the interaction between the chemokine receptor CXCR4 and its ligand SDF-1-alpha, and is influenced by the fact that Sezary syndrome cells do not express CD26/DPPIV. The presence of exogenously added soluble CD26/DPPIV led to cleavage of SDF-1-alpha, interference with SDF-1alpha-CXCR4 interaction, and decreased SDF-1-alpha-