T-cell activation via CD26 and caveolin-1 in rheumatoid synovium

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Abstract

CD26 is a T-cell costimulatory molecule with dipeptidyl peptidase IV (DPPIV) activity in its extracellular region. We previously reported that recombinant soluble CD26 enhances peripheral blood T-cell proliferation induced by the recall antigen tetanus toxoid (TT). Recently, we demonstrated that CD26 binds caveolin-1 on antigen-presenting cell (APC), and that residues 201–211 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. In addition, following CD26–caveolin-1 interaction on TT-loaded monocytes, caveolin-1 is phosphorylated, with linkage to NF-κB activation, followed by upregulation of CD86. Finally, reduced caveolin-1 expression on APC inhibits CD26-mediated CD86 upregulation and abrogates CD26 effect on TT-induced T-cell proliferation, and immunohistochemical studies revealed an infiltration of CD26+ T cells in the sublining region of rheumatoid synovium and high expression of caveolin-1 in the increased vasculature and synoviocytes of the rheumatoid synovium. Taken together, these results strongly suggest that CD26–caveolin-1 interaction plays a role in the upregulation of CD86 on TT-loaded APC and subsequent engagement with CD28 on T cells, leading to antigen-specific T-cell activation such as the T-cell-mediated antigenspecific response in rheumatoid arthritis.

Key words Caveolin-1 · CD26 · Memory T cell · Rheumatoid arthritis (RA) · Synovial cell

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by the progressive destruction of cartilage and bone in the synovial joints, which is associated with proliferation of synovial cells and infiltration of activated memory T cells, antigen-presenting cells (APCs) and plasma cells. Proposed etiologies for RA include genetic predisposition, dysregulation of self-tolerance, immune dysregulation triggered by environmental agents, and subsequent transformation of synovial cells. Macrophages and/or T cells are important mediators of RA pathogenesis, with cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) being proven therapeutic targets. In fact, antagonists against such cytokines have been used recently as effective RA therapy, decreasing joint damage and slowing radiographic progression of disease in patients of RA with inadequate response to methotrexate. However, as many patients do not experience effective relief even with the use of these newer biological agents, additional novel therapeutic approaches are still needed.

Major-histocompatibility-complex (MHC) class II phenotype such as HLA-DR1, DR-4 and DR-14 confers susceptibility to RA. MHC class II molecules present antigens to CD4+ T cells, suggesting an important role for T cells in the pathogenesis of RA. Moreover, the rheumatoid synovium contains activated T cells, providing further rationale for the proposal that T cells have an important role in RA. Antigen-presenting cells such as monocytes, macrophages, and dendritic cells are also present in the rheumatoid synovium, being activated and expressing both MHC class II and costimulatory molecules such as CD86 and CD80. These findings strongly suggest that the interaction between synovial T cells and APCs have a direct role in the progression of synovitis. Moreover, careful analysis of infiltrating synovial T cells has revealed a bias towards the Th1 phenotype. In particular, patients with autoimmune diseases such as multiple sclerosis, Graves’ disease, and RA have been found to have increased numbers of CD4+...
CD26+ T cells in inflamed tissues as well as in their peripheral blood, with enhancement of CD26 expression in these autoimmune diseases correlating with disease severity. In addition, we previously demonstrated that T cells migrating through endothelial cell monolayers in vitro express high levels of CD26. These findings imply that CD26+ T cells play an important role in the inflammation process and subsequent tissue damage in such diseases.

It is well established that T cells require at least two signals to be fully activated. The first signal is antigen-specific and is delivered by engagement of the T-cell receptor (TCR) complex with an MHC-peptide complex on APC. The second signal is exerted by the binding of a costimulatory receptor on T cells to a ligand on the APCs. A key costimulatory signal is provided by the interaction of CD28 on T cells with CD86 or CD80 on APCs. We showed previously that CD26 on T cells have a very strong costimulatory effect on CD4+ T-cell activation in response to memory antigen such as tetanus toxoid (TT). However, the molecular mechanism involved in the process of antigen-specific T-cell activation via CD26 has not been clearly elucidated. We recently demonstrated that caveolin-1, a binding partner of CD26 and that signaling downstream of caveolin-1 in APCs is a binding partner of the mouse thymocyte activation molecule (THAM), which is approximately 85% homology with the rat DPPIV enzyme. The amino acid sequence of human CD26 illustrates approximately 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 (dipeptidyl peptidase IV, DPPIV). The amino acid sequence of 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 (dipeptidyl peptidase IV, DPPIV). The amino acid sequence of 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 (dipeptidyl peptidase IV, DPPIV). The amino acid sequence of 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 (dipeptidyl peptidase IV, DPPIV).

CD26 is a membrane-associated ectopeptidase with DPPIV activity, and possible substrates of CD26/DPPIV include several critical cytokines and chemokines. 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. 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. 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). 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. 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 reduction in the number of mobilized HPC. Other exciting development regarding DPPIV involves its role in glucose metabolism, since 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. Selective small molecule inhibitors of DPPIV are currently being investigated in clinical trials for the treatment of impaired glucose tolerance and type 2 diabetes.

Besides its ability to regulate the effect of biological factors through DPPIV enzyme activity, CD26 has an essential role in human T-cell physiology, especially in response to memory antigens (Fig. 1B). 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. Besides being a marker of T-cell activation, CD26 is also associated with T-cell signal transduction processes as a costimulatory molecule. In addition, CD26 serves as a functional collagen receptor with a role in T-cell activation, as well as having a potential role in thymic ontogeny (Fig. 2). 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 consis-
tently demonstrate greater activation than parental CD26 negative Jurkat or cells transfected with CD26 mutated at the DPPIV enzymatic site.48 Furthermore, CD26 interacts with several molecules playing important roles in T-cell function. CD26 physically binds with adenosine deaminase (ADA), an enzyme that plays a key role in the development and function of lymphoid tissues.49–51 Adenosine deaminase is essential for purine metabolism, with the loss of ADA leading to a clinical syndrome characterized by severe immunodeficiency.52 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.53 This finding is consistent with the fact that there is a physical association between CD26 and ADA on the surface of T lymphocytes. 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 (Fig. 2).54,55 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 lipid rafts on 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 (Fig. 2).56

Since the 1970s, DPPIV-like activity has been reported in human serum. After identification of the ADA-binding
protein of plasma as CD26, soluble form of CD26 protein was characterized in the serum and seminal fluids. In the previous report, we have shown that exogenous recombinant soluble CD26 (rsCD26) enhances the proliferative response of peripheral blood lymphocytes (PBLs) to stimulation with the soluble antigen tetanus toxoid (TT). More recently, we demonstrated that the target cells of rsCD26 are the CD14+ monocytes in the peripheral blood, and that rsCD26 upregulates CD86 expression, but not CD80 or HLA-DR antigen levels on monocytes. Significantly, mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGF-IIR) was identified as a platform molecule for CD26 interaction with APC. However, while both DPPIV-positive and DPPIV-negative rsCD26 are taken up by monocytes via M6P/IGF-IIR, only DPPIV-positive rsCD26 molecules affect CD86 upregulation on monocytes, thus suggesting that additional key factors may interact with CD26 in this process. We subsequently identified caveolin-1 on APC as a binding protein for CD26, and demonstrated that CD26 stimulation upregulates surface expression of CD86 on APC by means of caveolin-1 and enhances TT-mediated T-cell proliferation. In the next section of this review, we will focus on caveolin-1 as the binding protein of CD26 in the context of antigen-driven T-cell activation.

**Structure and function of caveolin-1**

Caveolin-1 was the first family member discovered, and demonstrated as a structural component and marker for caveolae and trans-Golgi derived transport vesicles. Caveolae were described as structures resembling “little caves” due to their appearance as 50- to 100-nm vesicular invaginations of the plasma membrane. Caveolin-1 is expressed in a wide variety of cell types, especially terminally differentiated cells such as endothelial cells, adipocytes, alveolar type I pneumocytes, macrophages, synoviocytes, and smooth muscle cells. Presently, caveolin-related proteins have been identified as caveolin-1, -2, and -3, all of which serve as protein markers for caveolae. The majority of caveolae in cells and tissues require only caveolin-1 expression for their proper formation, whereas caveolin-2 is not absolutely required, although the expression of caveolin-2 is tightly associated with the expression of caveolin-1. On the other hand, caveolin-3 is found in skeletal muscle tissue and cardiac myocytes. The three human genes encoding members of the caveolin family share significant homology. The caveolin-2 protein is approximately 38% identical and 58% similar to caveolin-1, while caveolin-3 is more closely related to caveolin-1, with 65% identity and 85% similarity. All three caveolins possess an invariant “FEDVIAEP” stretch within their hydrophilic N-terminal domains which...
Caveolin-1 is composed of the N-terminal hydrophilic domain (residues 1–101), the oligomerization domain (residues 61–101), the scaffolding domain (SCD) (residues 82–101), the membrane spanning domain (residues 102–134), and the C-terminal lipid raft-anchoring domain (residues 135–178). As in trans-Golgi transport, caveolin-1 plays an important role in signal transduction via its SCD, which compartmentalizes a multitude of signaling molecules. These include G proteins, epidermal growth factor receptor, insulin receptor, endothelial nitric oxide synthase (eNOS), nonreceptor tyrosine kinase (Src, Fyn, Yes), flotillins, Ser/Thr kinases (PKA, Raf, MAPK, PI3K, Grb2), and catenins. Other cellular functions of caveolin-1 are related to the lipid metabolism, especially to cholesterol scavenging in macrophages. However, it is unknown whether caveolin-1 also plays a role in signal transduction in APCs. Although CD26 was present in caveolae of fibroblast-like synoviocytes, direct CD26–caveolin-1 interaction and associated signaling events have not been demonstrated in immune cells. Interestingly, caveolin-1 knockout mice show defects in the angiogenic response to exogenous stimuli, such as Matrigel plugs containing angiogenic growth factors (bFGF) or tumors. In this context, angiogenic vessels density and penetration was significantly reduced in caveolin-1 null mice. Moreover, electron microscopic examination revealed incomplete de novo capillary formation in tumors implanted within caveolin-1 null mice. Thus, it appears that caveolin-1 null mice have a defect in endothelial cell differentiation. This is consistent with in vitro observations demonstrating that overexpression of caveolin-1 enhances endothelial capillary-tube formation, while downregulation of caveolin-1 using an anti-sense approach blocks endothelial tube formation. With regards to inflammation and caveolin-1, a series of elegant experiments showed that caveolin-1 has a role in inflammation with association of eNOS. Using a cell permeable peptides link to the caveolin-1 scaffolding domain in aortic explants, the potent eNOS inhibiting activity of caveolin-1 was demonstrated. In vivo delivery of this peptide resulted in significant decreases in acute inflammation and edema resulting from vascular permeability. Taken together, these findings demonstrate an important relationship between caveolin-1 and vascularization, with implication for capillary formation in inflammatory processes.

**Caveolin-1: CD26 binding protein in APC**

Since CD26 on human T cells was identified as an activation antigen and costimulatory molecule of the TCR complex, several binding proteins to CD26 have been described. As described above, multiple chemokines interact with CD26/DPPIV as its substrates, and other proteins such as ADA, fibronectin, thromboxane A2 receptor, and CXCR4 are shown to be associated with CD26. However, the precise mechanism involved in T-cell activation in response to memory antigen such as TT remains to be clearly characterized. Recently, we demonstrated that CD26 binds to...
caveolin-1 on APC, and that residues 201–211 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 (Fig. 3A).31 This region in CD26 contains a caveolin-binding domain (CBD) (ΦXΦXXXΦXXΦ; Φ and X depict aromatic residue and any amino acid, respectively), specifically WYVEEVESAY in CD26.48,69 These observations strongly support the notion that DPPIV enzyme activity is necessary to exert TCR-costimulatory activation via CD26.48 In addition, following CD26–caveolin-1 interaction on TT-loaded monocytes, caveolin-1 is phosphorylated, with linkage to NF-κB activation, followed by upregulation of CD86. Finally, reduced caveolin-1 expression on monocytes inhibits CD26-mediated CD86 upregulation and abrogates CD26 effect on TT-induced T-cell proliferation (Fig. 3B). Taken together, these results strongly suggest that CD26–caveolin-1 interaction plays a role in the upregulation of CD86 on TT-loaded monocytes and subsequent engagement with CD28 on T cells, leading to antigen-specific T-cell activation.

Caveolin-1 has been reported to be an integral membrane protein with a cytoplasmic N-terminal domain and a cytoplasmic C-terminal domain.65 Our data showed that the N-terminal domain of caveolin-1 was expressed on the cell surface of monocytes 12–24 h after tetanus toxoid was loaded (Fig. 4A). Since tetanus toxoid was trafficked in cells through caveolae,79,80 caveolin-1 may be transported along with the peptide-MHC complex in APC, and is then expressed on cell surface by the antigen-processing machinery for T-cell contact.80–82 The data shown in Fig. 4B indicated that CD26 on activated memory T cells directly faces caveolin-1 on TT-loaded monocytes in the contact area, which is the immunological synapse for T cell-APC interaction. 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.

**CD26 and caveolin-1 in synovitis**

Rheumatoid arthritis is a classical example of an immune-mediated disease with chronically smoldering injury of the synovial joints resulting from infiltration of inflammatory cells, and synovitis of diarthrodial joints is its most visible manifestation. Although the observed architectures of rheumatoid synovitis vary in different individuals with RA as well as at various disease stages, the most frequent type of rheumatoid synovitis is a diffuse inflammatory infiltrate in which T cells, B cells, and macrophages are scattered around increased vasculature and synoviocytes. Meanwhile, in the remaining 40–50% of patients with RA, infiltrating inflammatory cells organize themselves into follicular structures.8 It is known that the inflammatory activation events in rheumatoid synovitis are dependent upon cell–cell contact among T cells, fibroblast-like synoviocytes, APCs, and regional tissues such as type II collagen and proteoglycan.83 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.20,22,23 These findings suggest that T cells with high levels of CD26 antigen may preferentially migrate into the rheumatoid synovium to induce inflammation and tissue destruction. To test this hypothesis, we examined the expression of CD26 and caveolin-1 in the rheumatoid synovium through immunohistochemistry. As shown in Fig. 5A, CD26+ lymphoid cells are clearly observed in diffuse synovitis. In follicular synovitis examined with the sequential sections, CD26+ lymphoid cells are infiltrated in the sublining area of caveolin-1-positive synovial cells (arrow in panel b of Fig. 5B), and are adjacent to caveolin-1-positive inflammatory cells (arrowheads in panel c of Fig. 5B). Moreover, the intimal lining layer is hyperplastic with multiple layers, and the synoviocytes in these layers highly express caveolin-1 (arrow in Fig. 5C). In addition, CD86 and caveolin-1 are coexpressed in the intimal lining synoviocytes and the sublining fibroblast-like synovial cells (black arrowhead in Fig. 5C). Furthermore, increased vas-
entry of antigens via caveolae into APC leads to presentation of antigen peptides on MHC class II molecules and exposure of caveolin-1 (inside box in phase 1 of Fig. 6). APC thus induces the activation of memory T cells through the TCR and costimulatory molecules such as CD86/CD80–CD28, leading to the formation of mature immunological synapses. Following the association between caveolin-1 on APC and CD26 on memory T cells, CD86 is upregulated on APC surface, and memory T cells are subsequently activated via the costimulatory effect of CD26 on TCR activation. By enhancing TCR activation via CD26–cavolin-1 interaction, proliferation of the immunological synapse may be maintained. CD86 upregulation therefore results in potent T cell–APC interaction, leading to the development of activated memory T cells locally and activation of the immune response, as well as the subsequent development of rheumatoid synovitis. After triggering inflammation of the synovium, memory T-cell activation leads to progression of inflammation in rheumatoid synovium, i.e., infiltration of inflammatory cells, increase of vascular vessels, formation of follicular germinal centers, and proliferation of synoviocytes (phase 2 in Fig. 6). Destructive inflammation then progresses to cartilage and bone injury by pannus (phase 3 in Fig. 6). As a result, progressive inflammation leads to synovial membrane invasion of bone, loss of cartilage and bone destruction in joints.

**Molecular-targeted therapeutic strategies in RA**

Although the specific antigens responsible for the pathogenesis of RA have not been identified, T-cell activation via interaction with APCs plays a pivotal role in disease development. In this regard, therapeutic strategies have targeted cellular pathways in RA. In addition to anti-cytokine reagents, impressive therapeutic effect has been recently reported following the blocking of CD28-mediated costimulation by the use of cytotoxic T-lymphocyte-associated antigen 4-IgG1 (CTLA4Ig). Expressed on T cells within hours to days after activation, CTLA4 is the high-avidity receptor for both CD80 and CD86, and inhibits T-cell proliferation and IL-2 production. A fusion protein, CTLA4Ig binds both CD80 and CD86 on APCs, thereby preventing these molecules from engaging CD28 on T cells. By blocking the engagement of CD28, CTLA4Ig prevents the delivery of the second costimulatory signal that is required for optimal activation of T cells. The successful usage of this agent therefore demonstrates that blocking costimulatory signal to inhibit T-cell activation is a novel and promising therapeutic concept for selected autoimmune diseases. In this regard, since we showed that CD26–cavolin-1 interaction may play a pivotal role in rheumatoid synovitis, reagents capable of blocking CD26–cavolin-1 interaction in synovitis may be potentially useful in the treatment of patients with RA.

Figure 5A–C. Architecture and immunohistochemistry of rheumatoid synovitis with inflammatory cells intermingled with fibroblast-like synoviocytes (×100). Panel a shows H&E-stained histology of diffuse synovitis of panel a, which was stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD26 antibody (×100). Panel b shows immunohistochemistry of the sequential section of panel a, which was stained with anti-caveolin-1 (Texas red). This reveals that CD26-positive lymphoid cells are scattered around the follicles (arrow) (×100). Panel c shows immunohistochemistry of the sequential section of panel b, which was stained with anti-caveolin-1 (Texas red). This reveals that the intimal lining synoviocytes and the sublining fibroblast-like synoviocytes adjacent to CD26+ lymphoid cells (arrow head) express caveolin-1 (×100). Panel a shows synovial histology of rheumatoid arthritis with H&E staining (×100). Panels b and c show immunohistochemistry of the sequential section of panel a which was stained with caveolin-1 (Texas red) and CD86 (FITC), simultaneously. Panel d shows the merged view of panels b and c. Arrow shows the intimal lining synoviocytes, and black arrowhead shows the sublining fibroblast-like synoviocytes. White arrowheads show the increased vascularization in synovitis.
Fig. 6. Schematic diagram of inflammatory progress in rheumatoid synovitis. See text for details.
Conclusions

Our results may thus provide a new approach to the treatment of autoimmune diseases or other immune-mediated disorders by directly intervening with the interaction between activated T-cell and APC. Targeting the binding of the pocket structure of CD26 and the scaffolding domain of caveolin-1 may lead to novel therapeutic approaches, including the utilization of antagonists that regulate antigen-specific immune response in immune-mediated disorders such as RA.

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