Role of CD26-adenosine deaminase interaction in T cell-mediated immunity

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ABSTRACT

CD26 is a 110 kDa surface-bound ectopeptidase with intrinsic dipeptidyl peptidase IV (DPP IV) activity, which binds adenosine deaminase (ADA) on the surface of T cells. ADA is an enzyme of the purine metabolism that has been the object of considerable interest mainly because the congenital defect causes severe combined immunodeficiency (SCID). This review focuses on work demonstrating that CD26-ADA interaction has a key role in T cell mediated immunity. The enzymatic and extra-enzymatic roles of ADA and CD26 in the immune context are discussed. Furthermore, the structure of the CD26-ADA complex, the signal transduction pathway triggered by the co-stimulatory ADA-CD26 interaction and the cytokine pattern induced during the immunosynapse are analyzed. In this context, the implications of the impairment of CD26-ADA interaction and its catalytic activities in the pathophysiology of AIDS are discussed.

KEY WORDS: Co-stimulation/ immunosynapse/ ecto-enzyme/ signal pathways/ CD26/ dipeptidyl peptidase IV/ ADA/ AIDS.
CD26, STRUCTURE AND FUNCTION

CD26 or dipeptidyl peptidase IV (DPPIV, EC 3.4.14.5) is a multifunctional type II transmembrane glycoprotein expressed as a homodimer on the surface of a variety of epithelial, endothelial and lymphoid cells. As an exopeptidase it cleaves N-terminal dipeptides from polypeptides with proline or alanine in the penultimate position, thereby regulating the activity of a variety of biologically important peptides (1,2). Some of them are closely related to immune function, such as RANTES (regulated on activation, normal T cell expressed and secreted) (3), MDC (monocyte-derived chemokine) (4), SDF-1α and SDF-1β (stromal derived factor) (5), eotaxin (6) and LD78α (7).

CD26 is expressed at detectable levels by some resting CD4+ or CD8+ T cells but the cell-surface expression increases 5-10 fold following stimulation with either antigen, anti-CD3 plus interleukin (IL)-2, or mitogens such as phytohemagglutinin (PHA) (2). On CD4+ T cells, CD26 expression is induced by stimuli that favour the development of Th1 responses (8). The correlation between the CD26 expression and Th1-like immune responses has been suggested to be due to an IL-12-dependent up-regulation (9). In this regard, it is hypothesized that CD26 expression on T cell is a marker for the Th1 subset (9-12). The expression of CD26 in B cells is very low, but increases with stimulation by mitogens such as pokeweed mitogen (PWM) or Staphylococcus aureus protein (2). On the other hand a soluble form of CD26 (sCD26), which lacks the intracellular tail and transmembrane regions, has been reported. It is found at high levels in seminal fluid, whereas moderate and low amounts are detected in plasma and cerebrospinal fluid, respectively (1).

The human CD26 gene encodes a protein of 766 amino acids (110 KDa), which is anchored to the lipid bilayer by a single hydrophobic helix of 23 amino acids located at the N-terminus, and has a short cytosolic tail of 6 amino acids (Fig. 1) (1). A flexible stalk of 20 amino acids links the membrane anchor to a β-propeller domain (Arg54-Asn497) which contains eight-blades with four anti-parallel strands each (13). Seven out of nine glycosylation sites are located in the β-propeller domain (14). The catalytic domain in the C-terminus, which contains two glycosylation sites, spans residues Gln508-Pro766. It adopts a typical α/β-hydrolase fold with a central eight-stranded β-sheet sandwiched by several α-helices (13,14). Only small peptides (typical length of <30 amino acids) are hydrolyzed by CD26 because there are only two possible small entrances to the active site, namely...
through the tunnel of the β-propeller and through the side opening generated by the kinked arrangement of blades I and II(13) (Fig. 1).

CD26 belongs to the s9b family of serine proteases. Other members are, fibroblast activation protein (FAP)(15), DPP6(16), DPP8(17), DPP9(18) and DPP10(19). The best studied are CD26 and FAP, sharing a sequence identity of 54%(20). Both are integral membrane proteins and require dimerization for catalytic activity(14,21). Nucleophile-acid-base (Ser-Asp-His) is the linear order of the catalytic triad in this family of peptidases, an arrangement not common for typical serine-type proteases (trypsin or chymotrypsin-like enzymes) but characteristic of the α/β-hydrolase fold(12,21). The catalytic triad of CD26 is Ser630-Asp708-His740, but other amino acids are also essential for enzymatic activity, such as Glu205, Glu206, Tyr547 and His750. The highly conserved Glu205-Glu206 motif interacts with the free amino terminus of the P1-residue, thus determining the dipeptidyl «amino» peptidase activity of the enzyme, and the point mutations Glu205Lys and / or Glu206Leu abolish enzyme activity(21,22).

The Tyr547, which may stabilize the oxyanion formed in the tetrahedral intermediates by a strong hydrogen bond, is also essential for catalytic activity(21,22). In addition, His750 of CD26 is essential to the dimerization, and its replacement by a negatively charged Glu results in nearly exclusive monomer expression with a 300-fold decrease in catalytic activity(23). An interesting feature of CD26 traffic is its ability to enter into an endocytosis/exocytosis cycle, which involves re-entry into the Golgi apparatus and results in glycosylation changes. This might explain the different forms of CD26 during T cell activation(1). However, it has been recently demonstrated that none of the nine N-linked glycosylation sites of CD26 contributes significantly to its dimerization and peptidase activity(14).

The enzymatic activity of CD26 promotes an augmented proliferation in T cell activation induced by TCR/CD3 complex engagement or other mitogenic stimuli(25). Apart from the enzymatic activity-dependent co-stimulatory effect, CD26 is able to provide a enzymatic activity-independent co-stimulatory signal to T cells with augmented responses to foreign antigen, increased proliferation and cytokine secretion, up-regulation of CD25, CD71 and CD69, and induced differentiation into effector cells(12,26). In response to anti-CD3 stimulation, the co-stimulatory signal of CD26 in T cells is initiated by an enhanced phosphorylation of c-Cbl, ZAP-70, ERK1/2, Lck, and CD3ζ(26). Because CD26 has a short conserved cytosolic region consisting in only six amino acid residues, without any common signalling motif or known binding motif, it needs association with other molecules able to transduce the signal. In this regard, Ishii et al.(27) have demonstrated that CD26 binds to the cytosolic domain of CD45, a protein tyrosine phosphatase involved in activation of Src kinases such as Lck during T cell activation. When CD26 binds to CD45RO, both are recruited to lipid rafts promoting co-stimulation(27). In contrast, when CD26 interacts with the CD45 isoform CD45RA, the two proteins are excluded from lipid rafts and the co-stimulatory signal is attenuated(28). Similar to other co-stimulatory molecules such as CD28, CD26-binding proteins have been object of considerable interest. In 1993, CD26 was identified as the binding protein for adenosine deaminase (ADA)(29), whose hereditary lack causes severe impairment of cellular and humoral immunity(30). Two years later, the interaction of CD26 with ADA was shown to be involved in the co-stimulatory properties of CD26 in T cell activation(31). Recently, the role of the CD26-ADA interaction in the immunosynapse has been demonstrated(32). This is the central aspect reviewed here and is discussed in detail later.

ADA, STRUCTURE AND FUNCTION

Adenosine deaminase (ADA, EC 3.5.4.4) is an enzyme involved in purine metabolism. It catalyzes the hydrolytic deamination of adenosine or 2-´deoxyadenosine to inosine or 2-´deoxyinosine and ammonia. The product of the human ADA gene consists of 363 amino acids and there is a high degree of amino acid sequence conservation amongst species(30). ADA is one ubiquitous, soluble, and globular enzyme with a TIM barrel-fold consisting of eight parallel β-strands forming a barrel decorated by α-helices(33) (Fig. 1).

For many years, ADA was considered to be cytosolic, but it has been found on the cell surface of many cell types and can therefore be considered an ecto-enzyme. Importantly, ecto-ADA is expressed on the T cell surface(33) and on the surface of dendritic cells (DC), the most potent antigen presenting cells (APC)(34). Because ecto-ADA is not a membrane protein, it needs association with cell surface ADA anchoring-proteins. So far, three ADA-anchoring proteins have been described: CD26(29), and adenosine receptors A₁(35) and A₂B(36). In this regard, ecto-ADA mediates a co-stimulatory signal through its interaction with CD26(28). It is not known whether ADA generates a signal when it binds to adenosine receptors. However, ADA binding to A₁ or A₂B receptors is required for high affinity binding of the agonist and for efficient signalling(34,35).

The congenital defect of ADA causes severe combined immunodeficiency (SCID), which is characterized by the absence of functional T and B lymphocytes in affected
individuals. Skeletal, neurological, and hepatic abnormalities that occur in some patients may be due to the metabolic disorder, but these are of less clinical relevance than the immunodeficiency. In contrast to the phenotype in humans, ADA knockout mice have normal lymphoid development at birth and die perinatally of hepatic and pulmonary injury. Lymphopenia develops postnatally in strains genetically engineered to express only placental ADA, but these animals die at a few weeks of age from lung injury. In genetically engineered to express only placental ADA, but these animals die at a few weeks of age from lung injury. In genetically engineered to express only placental ADA, but these animals die at a few weeks of age from lung injury.

In this context it is intriguing that, despite the good homology between murine and human ADA, murine ADA does not interact with human or murine CD26 nor does murine CD26 interact with human or murine ADA. Taking these results together, it is likely that the ADA-CD26 interaction is involved in the lymphoid related abnormalities developed in ADA-deficient SCID individuals. However, Richard et al. have demonstrated that an individual who only expresses the Arg142Gln ADA mutant, which displays a normal catalytic activity but reduced CD26 binding, has no apparent immunological impairment.

Due to its enzymatic activity, ecto-ADA regulates the concentration of available adenosine (Ado) that binds to adenosine receptors. The surface expression of adenosine receptors has been described in T cells as well as on DC. In human T cells the major adenosine receptor expressed is the A2A receptor, which promotes cAMP increases with subsequent PKA activation. PKA and cAMP induce a marked impairment in T cell activation and IL-2 production, promoting energy and tolerance in Th1 clones through inhibition of MAPK and JNK, C-terminal Src kinase (CSK) activation, NF-AT deactivation and blockade of NF-kB activation. In this regard, the enzymatic activity of ecto-ADA on the cell surface decreases the adenosine receptor engagement of T cells and thymocytes, suggesting an activatory role of the ecto-enzyme. On the other hand, Ado acts through A2A adenosine receptors on the mature DC (mDC) surface where it promotes an enhancement of intracellular cAMP levels, thus impairing the capacity to initiate and amplify a Th1 response. In immature DC (iDC), Ado acts through A1 adenosine receptors, which promote an increase of intracellular calcium levels resulting in enhanced chemotaxis, actin polymerization, macropinocytotic activity and augmented surface expression of CD80, CD86, MHC class I and HLA-DR. However, the action of Ado through A1 adenosine receptors on these cells promotes the rise of intracellular calcium levels thus decreasing the expression of CCR5 and MDR-1 and subsequently impairing the migratory activity.

In summary, ecto-ADA possesses two different complementary mechanisms to promote a correct Th1 cell-mediated immune response. First, an enzymatic activity-dependent mechanism, which by degrading extracellular Ado, makes adenosine less available to the receptors positively coupled to adenylylate cyclase, subsequently enhancing the immune response. Second, a catalytic activity-independent mechanism, which induces a co-stimulatory signal through its interaction with CD26 that, together with the TCR/CD3 triggered-signal, induces strong T cell activation.

THE CD26-ADA INTERACTION

Similarly to other T cell-activation markers, the increase of ecto-ADA expression on the T cell surface after exposure to mitogens has been reported. In 1993, ADA binding protein was identified, first by Morrison et al. as a negative marker of melanocyte transformation, and three months later by Kameoka et al. as the T cell activation marker CD26. After this, the CD26-ADA interaction has been extensively studied.

Dong et al. have demonstrated that neither the dipeptidyl peptidase nor the deaminase activities are required for the association between CD26 and ADA. By the use of immunolabeling microscopy, the same authors found that CD26 and ecto-ADA co-localize on the cell surface but not inside the cells. This indicates that CD26 does not transport ADA to the T cell plasma membrane. Also, when murine cells transfected with human CD26 are co-cultured with CD26-deficient human cells, murine cells are able to acquire human ADA on their surface. Therefore, intracellular human ADA may be released to the medium in a CD26-independent manner and may bind to human CD26 expressed on the surface of other cells. In this regard, several cytokines may regulate the translocation of ADA towards the cell surface through a mechanism not involving CD26. IL-2 and IL-12 would lead to T cell surface ADA up-regulation and IL-4 to down-regulation.

To determine the amino acid residues of CD26 implicated in ADA binding, systematic studies have been performed using CD26 constructs including deletion, human-rat swapping, point mutations and monoclonal antibodies that inhibit ADA binding. These studies have shown that region Leu140-Val142-Ala142-Arg143 and Leu249 are essential for ADA binding. Additionally, Aertgeerts et al. have described that none of the nine glycosylation sites of CD26 are required for ADA binding. On the other hand, to localize the ADA region implicated in CD26 binding, studies with human-mouse ADA hybrids and point mutants have been performed. These studies have demonstrated that the helical segment residues 126-143 of ADA are implicated in CD26 binding, specifically amino acids Arg142, Glu139,
and Asp143(39,62). Importantly, in mouse ADA, which is not able to bind neither human CD26 nor mouse CD26, the amino acid in position 142 is Gln instead of Arg(62).

The 3D structure of the CD26-ADA complex has been resolved at 3.0-Å by X-ray diffraction and by single particle cryo-EM at 22-Å resolution(63,64). These 3D structures show that each CD26 dimer binds two ADA molecules (Fig. 1). In agreement with the previous studies, these works indicate that the CD26-ADA binding involves regions Ile287-Asp297 (loop A) and Asp331-Gln344 (loop B) of CD26, and region Pro126-Asp143 (helix a2) of ADA (Fig. 1). CD26 binds ADA with two adjacent loops(63,64). Loop A connects blades IV and V, and loop B links β-strands β3 and β4 of blade V. The loops protrude from the propeller blades and form a cleft accommodating helix a2 of ADA. Also, the same authors have described an additional region of ADA implicated in CD26-ADA binding, Arg76-Ala91 (helix a1). The interaction between helix a1 of ADA and loop A of CD26 completes the interface(64).

ADA DEPENDENT CD26-MEDIATED SIGNALLING

Due to the fact that the endogenous CD26 ligand is unknown, most studies aimed at elucidating the signal pathway triggered via CD26 have been performed using monoclonal antibody-mediated cross-link of CD26. Recently, we have demonstrated that ADA co-localizing with A2B adenosine receptor on the DC surface induces a co-stimulatory effect in T cell activation via CD26(32). Also, similar to other G protein-coupled receptors(65), A2B adenosine receptors may be forming homodimers on the cell surface. Ecto-ADA, therefore, is likely to interact with A2B adenosine receptor homodimers on the DC surface, promoting a cross-link of CD26 on the T cell surface during the immunosynapse, and inducing co-stimulatory effect. In addition, it has been reported that neither dipeptidyl peptidase activity of CD26(66,67) nor deaminase activity of ADA(31,32) are required to initiate a CD26-ADA mediated co-stimulatory signal.

CD26 cross-linking causes co-aggregation of CD26 and CD45RO into lipid rafts(27). Subsequently, through its interaction with cytoplasmic d2 of CD45RO, CD26 promotes dephosphorylation of the C-terminal regulatory domain of Src kinases such as Lck and Fyn, activating them(26-48) (Fig. 2). Active Lck binds to the cytosolic domain of CD4 or CD8 and phosphorylates the immunoreceptor tyrosine-based activation motif (ITAM) of CD3ζ, allowing ZAP-70 binding to phosphorylated ITAM. Thus, Lck activates ZAP-70(69,70) (Fig. 2). In this regard, presence of the TCR associated CD3ζ with at least one functional ITAM is required for CD26-induced co-stimulation(71,72). Conflicting data exist about subsequent activation of the important T cell receptor associated adaptor protein LAT by ZAP-70 during CD26-induced signalling, because it becomes phosphorylated(28), but does not co-localize with CD26(66). Nevertheless, CD26 induces activation of downstream signalling molecules such as the MAP kinases ERK1/2(27,28,68), the phospholipase C-γ (PLP-Cγ)(69), and the Src kinases-regulator c-Cb(28,27,69).

Additionally, ADA(30) or antibody-mediated CD26 cross-linking(72) induces a synergetic effect upon calcium mobilization triggered via the TCR/CD3 complex (Fig. 2). Therefore, the co-stimulatory signal triggered via CD26-ADA interaction potentiates the TCR/CD3 engagement during the T cell activation. Importantly, the signal pathway triggered by CD26 cross-linking described above depends on which CD45 isoform is present. When CD26 is associated with CD45RA, these two proteins are removed from lipid rafts, promoting the attenuation of CD26-mediated co-stimulation(28).

THE NOVEL CO-STIMULATORY INTERCELLULAR INTERACTION

The physiological activation of T cells requires at least two signals. The first is provided by stimulation of the TCR/CD3 complex via specific peptide/MHC complex. The second signal can be delivered by triggering co-stimulatory surface molecules that, similar to adhesion molecules, belong to the group of «accessory molecules», which are involved in a series of antigen non-specific interactions between APC and T cells in the immunosynapse. The engagement of co-stimulatory molecules can positively affect T cell function, enhancing activation, proliferation, survival and cytokine secretion. The critical role of co-stimulation in the activation of T cells is reflected by the fact that in the absence of any co-stimulatory signal, the antigenic presentation induces T cells to become anergic and tolerant(26,73).

So far, the co-stimulatory molecules described fall into three main groups, namely Ig superfamily members, TNFR superfamily members, and cytokine receptors(76). CD28, ICOS and CD2 typify co-stimulatory molecules of the Ig superfamily. Cytokine receptors that can control T cell growth or survival include IL-1R, IL-2R, IL-6R, IL-7-R and IL-15R. Finally, co-stimulatory signals through a number of TNFR/TNF family members have also been shown to augment T cell responses in various settings. These latter signals include type I transmembrane proteins of the TNFR family expressed in T cell surface such asOX40(CD134), 4-1BB (CD137), CD27, CD30 and HVEM, whose ligands are type II transmembrane proteins of the TNF family (OX40L, 4-1BBL, CD27L (CD70), CD30L and LIGHT, respectively) expressed on the APC surface(77).
Recently, we have discovered that the co-stimulatory effect through CD26 on the T cell surface is promoted by ecto-ADA co-localizing with the A2B adenosine receptor expressed on the APC surface(32). Similar to members of the TNFR family, CD26 is weakly expressed in resting T cells, but it is strongly up-regulated by reagents that engage TCR/CD3(31,76). Differently to members of TNFR/TNF family, CD26-mediated co-stimulation has been suggested to occur by a tri-molecular interaction between CD26, ADA and the A2B adenosine receptor(32). Thus, CD26-a type II transmembrane protein on T cells, A2B adenosine receptors -G protein-coupled receptors on DC-, and ADA bound simultaneously to both may constitute an example of a novel module leading to enhanced antigen presentation (Fig. 3). Recently we have demonstrated that during the immunosynapse between superantigen-pulsed DC and CD4+ T cells, the co-stimulatory effect promoted by CD26-ADA interaction induces IL-6 production and enhanced INF-γ and TNF-α secretion(32). In
agreement with the augmented INF-γ secretion, CD26 has been proposed to be a Th1 marker\(^{8-12}\). In this regard, superantigen-pulsed DC secreting IL-12, promote a Th1 cytokine pattern and phenotype in CD4\(^+\) T cells. Interestingly, indirect evidence indicates that TNF-α induces a down-regulation of the CD26 surface expression\(^{111}\). In this way, the increased TNF-α secretion induced via CD26-ADA co-stimulation could exert a negative feedback, by regulating CD26 expression, and therefore, the Th response during immunosynapse (Fig. 3). In addition, IL-6 possibly plays a role during T cell activation promoting additional co-stimulation\(^{79,80}\) and/or inducing initial production of INF-γ in T cells\(^{80}\) (Fig. 3). This tri-molecular interaction involving both DC and T cells promotes enhanced TCR/CD3 signal, proliferation and pro-inflammatory and a Th1 cytokine pattern\(^{80}\).

**IMPLICATIONS OF CD26-ADA INTERACTION IN THE PATHOPHYSIOLOGY OF AIDS**

Several studies have revealed a correlation between depletion of CD4\(^+\)/CD26\(^-\) T cells, increased serum levels of ADA, and the evolution of AIDS in infected individuals\(^{81-83}\). Furthermore, it has been described that ADA binding to CD26 is inhibited by recombinant soluble HIV-1 envelope glycoprotein gp120 and by HIV-1 infectious particles\(^{84}\). This inhibition occurs through a mechanism requiring the previous interaction of gp120 with CD4 for efficient inhibition of ADA binding to CD26. In the presence of CXCR4 the interaction of gp120 with CD4 may be dispensable\(^{85}\). Importantly, direct interaction and co-modulation of CD26 and CXCR4 on the T cell surface has been demonstrated\(^{86}\). Studies with overlapping synthetic peptides covering the entire gp120 sequence have revealed that the region of gp120 implicated in the interaction of ADA binding to CD26 is the third constant domain gp120 (C3 region)\(^{84}\). Because the C3 region of gp120 is hidden in soluble gp120\(^{87}\), the previous interaction of gp120 with CD4 or CXCR4 could contribute to unmasking this hidden region and allow inhibition of ADA binding to CD26. In fact, it has indirectly been demonstrated that a conformational change of gp120 occurs before binding to CD26\(^{88}\). The impairment of T cell physiology promoted by gp120-mediated disruption of ADA binding to CD26 is evident because pre-incubation of T cells with gp120 inhibits TCR/CD3 dependent activation of Fyn and Lck\(^{89}\), and blocks the IP3-sensitive calcium mobilization\(^{89}\), resulting in altered antigen-induced proliferation and IL-2 production\(^{88}\).

On the other hand, HIV has been also implicated in the inhibition of CD26 enzymatic activity. The HIV-1 transactivating protein Tat plays a role in viral replication, and also exerts immunosuppressive properties *in vitro*, which has been attributed to its interaction with CD26\(^{90}\). When T cells are infected by HIV-1, they release Tat into the extra-cellular space where it suppresses antigen- and mitogen-induced activation of human T cells due to its inhibitory effect in the dipeptidyl peptidase activity of CD26\(^{90}\), thus contributing to the HIV-1 promoted impairment of T cell-mediated immunity. Additionally, due to the fact that chemokines can be substrates of CD26, the catalytic activity of CD26 possesses a dual role during HIV infection depending on the HIV-tropism. When intact and CD26-cleaved RANTES molecules were compared for their ability to inhibit HIV-1 infection of PBMC with M-tropic strains, truncated RANTES was found to be a much more potent HIV-1 inhibitor than intact RANTES. In contrast, intact SDF-1α is a more potent HIV-1 inhibitor than CD26-truncated SDF-1α\(^{84}\). Therefore, CD26 could be beneficial in early stages of HIV infection where M-tropic CCR5-using HIV-1 predominate, but at later stages, when T-tropic CXCR4-using HIV-1 appear, CD26 could facilitate viral dissemination\(^{91}\).
CONCLUSIONS

Both, ecto-ADA and CD26 have catalytic activity-dependent and catalytic activity-independent synergistic or co-stimulatory effects on T cell-mediated immunity. The hydrolytic activity of ecto-ADA reduces the Ado available for adenosine receptor activation, thus counteracting the adenosine receptor-mediated inhibition of DC and T cell function. In addition, the dipeptidyl peptidase activity of membrane bound or soluble CD26 increases T cell activation. Interestingly, the CD26-ADA interaction on the T cell surface induces a dipeptidyl peptidase and deaminase activity-independent co-stimulatory effect in the activation of T cells. This co-stimulatory effect occurs through activation of several signal pathways shared or converging with the TCR/CD3 complex-triggered signal pathways, resulting in enhanced secretion of Th1 cytokines and IL-6, which may promote additional co-stimulation.

A physiological CD26-ADA interaction-mediated co-stimulation is likely to occur through ADA binding to CD26 on the T cell surface and simultaneously ADA binding to A32 adenosine receptors on the DC surface inducing CD26 cross-linking on the T cell surface. This tri-molecular interaction involving APC and T cells constitutes a novel group of co-stimulatory modules.

In serious immunodeficiencies such as AIDS or SCID, deaminase activity, dipeptidyl peptidase activity and/or CD26-ADA interaction are impaired, contributing to T cell unresponsiveness. Taken together, all the evidence support a key role of the CD26-ADA interaction in CD4+ T cell-mediated immunity.

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