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# Enzymatic and Extraenzymatic Role of Adenosine Deaminase 1 in T-Cell–Dendritic Cell Contacts and in Alterations of the Immune Function

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**ABSTRACT:** Adenosine deaminase 1 (ADA1) is an enzyme of the purine metabolism whose congenital defect leads to severe combined immunodeficiency (SCID)[[AU: OK?]]. Although classically considered a cytosolic enzyme, early evidence from work in brain synaptosomes suggested that the enzyme could be an ectoenzyme. In lymphoid cells, ectoenzymatic activity of ADA1 was also found. The obvious role of this enzyme located on the cell surface of lymphocytes and monocytes was to deaminate adenosine, making it less available for uptaking and metabolism, and also for adenosine-receptor activation[[AU: OK?]]. Quite unexpectedly, ADA1 was shown to act extraenzymatically. In addition, cell surface ADA1-binding proteins have been identified. Interestingly, the interaction of ADA1 with these anchoring proteins leads to T-cell costimulation. Recent studies performed with professional antigen-presenting cells and T lymphocytes have shown that ADA1 can bridge the two cell types together by a "cross-linking" established between different anchoring molecules in each cell. Some aspects of ADA action are similar to[[AU: OK?]] growth factors. In fact, ADA1 is a member of the adenosine deaminase growth factor (ADGF) family. Some molecular mechanisms that occur in ADA-related SCID and the role ADA1 may play in acquired immunodeficiency are also reviewed here.

**KEY WORDS:** SCID, ADA1, ADA2, CD26, ADGF, GPCR, adenosine receptors, $A_1$, $A_{2B}$

## I. INTRODUCTION

Adenosine deaminase (ADA1) catalyzes deamination of adenosine or deoxyadenosine to inosine or deoxyinosine, respectively. Traditionally, ADA1 has been considered a cytosolic enzyme acting on the purine metabolism. As shown below, there is no clear indication that the enzyme is indeed exclusively cytosolic. In fact, there is the possibility that the enzyme is anchored to cell membranes, even intracellular membranes. This possibility is supported by various factors; for instance, from a structural point of view, it is not a typical globular protein because the molecule has a number of hydrophobic patches that are susceptible to interaction with membrane lipids or membrane proteins (see Fig. 3 in Franco et al.,[1]). Moreover, the topology of ADA1 activity was the same as that of the ectoenzyme 5′-nculeotidase and different from that of cytosolic enzymes such as lactate dehydrogenase (see below).

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Because its congenital deficit leads to severe combined immunodeficiency (SCID), ADA1 has been heavily studied and was one of the first proteins whose cDNA was isolated. Several mutations and alterations in the ADA1 gene have been reported in SCID patients.[2] Early after the discovery that a significant percentage of SCID patients were lacking ADA1, it was suspected that the main mechanism leading to ADA1-mediated SCID was the accumulation of adenosine and of adenine nucleotides, mainly dATP, which are toxic for lymphocytes (Fig. 1; see, also, Hershfield[3]). Later on, the discovery of cell surface ADA1 (see below) and the possibility that this ectoADA1 could act both enzymatically and extraenzymatically, even as a growth factor or intercellular adaptor, has introduced new ways of looking at the mechanism by which ADA1 participates in the regulation of immune system function.

## II. BACKGROUND

### A. Cell Surface Adenosine Deaminase

Characterization of subcellular fractions from cultured brain cells led to the finding that ADA1 activity was enriched with the same fractions as the membrane-bound enzyme 5′-nucleotidase.[4] A similar enrichment was also found in our laboratory in rat brain synaptosomes.[5] Furthermore, we found that the topology of ADA1 activity was the same as that of the ectoenzyme 5′-nucleotidase and different from cytosolic enzymes such as lactate dehydrogenase. In fact, the increase of ADA1 and 5′-nucleotidase activity by detergent disruption of synaptosomes was analogous. At the end of the 1980s and beginning of the 1990s, an extracellular orientation of ADA1 was also reported in blood cells[6–9] and coronary arterial endothelial cells.[10] In peripheral nucleated blood cells, ADA1 is found as ectoenzyme in the majority of monocytes and B cells and in some (10%–20%) resting T cells.[9] Apart from the higher percentage of B cells expressing surface ADA1, the number of ADA1 molecules on the plasma membrane of ectoADA1-expressing B cells is much higher than that of ectoADA1-expressing resting T cells. This contrasts with the total cell activity, which is much higher in T cells than in B cells.[9,11]

It should be noted that no differences in catalytic activity or molecular characteristics between intracellular ADA1 and ectoADA1 have so far been encountered. Apart from biochemical evidence, this is supported by the fact that only one gene for ADA1 has been detected that codes for a protein lacking both signal peptide and putative transmembrane domains. Therefore, it seems that intracellular and ectoADA1 are identical, at least at the level of protein sequences. Although it has not yet been possible to elucidate the mechanism by which ADA1 appears on the cell surface, it is possible to remove all intracellular



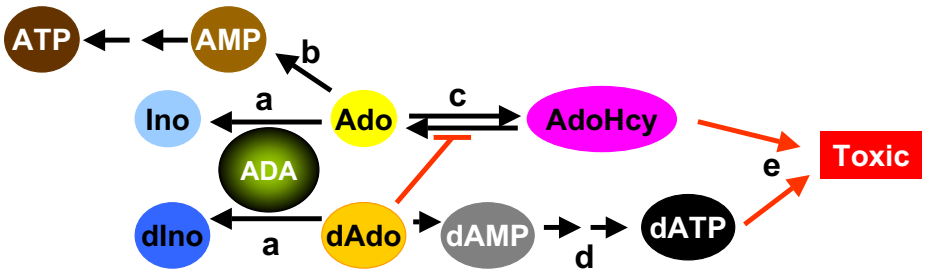


**FIGURE 1.** Intracellular metabolism of adenosine. Inside the cell, ADA catalyzes (a) deamination of adenosine (Ado) or deoxiadenosine (dAdo) to, respectively, inosine (Ino) or deoxiinosine (dIno). Alternatively, adenosine may be metabolized by adenosine kinase to AMP (b), and, subsequently, AMP can be converted into ADP and ATP, or by adenosylhomocysteinase to S-adenosylhomocyteine (AdoHcy) (c). Elevated intracellular deoxyadenosine inhibits the hydrolysis of adenosylhomocysteine. Although deoxyadenosine is a weak substrate for adenosine kinase and deoxicytidine kinase, in the absence of ADA these enzymes may phosphorylate deoxyadenoisne to dAMP, dADP, and dATP (d). Accumulation of adenosylhomocysteine becomes[[AU: OK?]] toxic because it is a potent inhibitor of all transmethylation reactions (e). On the other hand, the pool of dATP inhibits DNA synthesis and activates apoptosis (e).

ADA1 from mouse lymphocytes stabilized at low pH acetate buffer. These cells remain structurally intact and other intracellular proteins are not released.[12] We have shown that activation of T cells by using anti-CD3 antibodies leads to a steady increase of ectoenzymatic ADA1 activity (unpublished results). A portion, probably small, of ectoADA1 would also come[[AU: APPEAR?]] after release from dead cells and attach[[AU: OK?]] to the surface of neighbor living cells. It should be noted that there are important growth factors (eg, interleukin-1β) lacking signal peptides whose mechanism of secretion is not known either.

## B. CD26 and Adenosine Receptors as ADA1-Anchoring Proteins

ectoADA1 anchors to the cell surface via CD26 and adenosine receptors (see below). ectoADA1 was also found to bind plasminogen 2 in the absence of CD26 via the plasminogen kringle 4 (K4) domain.[13] Intracellular ADA1 may also interact with other proteins as the Grb2 isoform Grb3-3.[14]

Despite being known as a T-cell-activation marker molecule,[15] CD26 is found in many cell types, even in resting T cells. CD26 is also known as dipeptidylpeptidase IV because it demonstrates an enzyme activity consisting of the cleavage of dipeptides from the N-terminus of polypeptides having Pro or Ala at the penultimate position. Physiologically, this activity is important for processing, for instance, chemokines.[16] CD26 is a type II membrane sialoglycoprotein composed of two identical subunits of about 100 kDa each.[16–19] The human protein is predominantly extracellular, with a 22-amino acid hydrophobic transmembrane region and a cytoplasmic tail of only six amino acids corresponding to the N-terminus (Fig. 2). By proteolytic cleavage, CD26 can be released from the plasma membrane, and a soluble form of CD26, which retain full dipeptidylpeptidase activity, can appear in extracellular/interstitial body



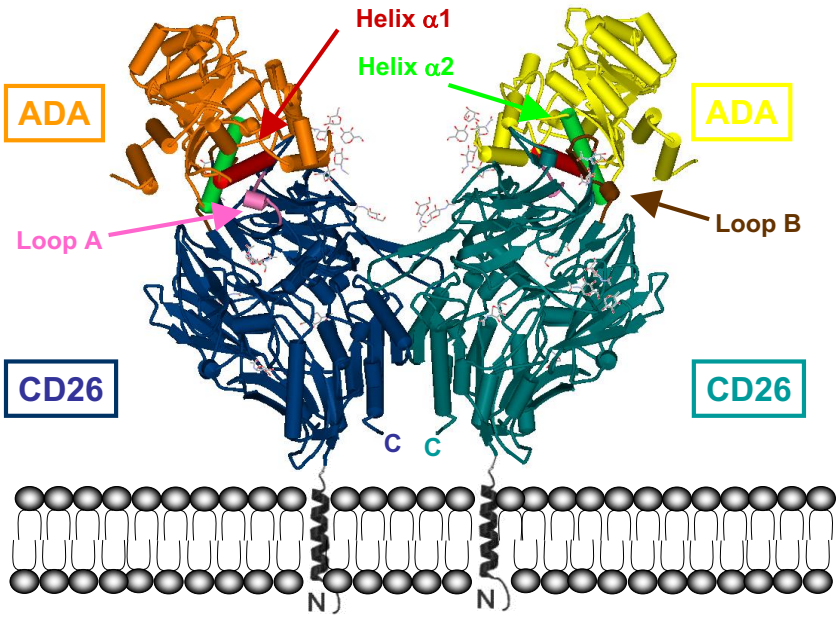


**FIGURE 2**. Structure of the $(CD26:ADA)_2$ complex at 3.03 Å resolution. Schematic representation of a CD26 dimer (a monomer as **dark blue** and the other monomer as **light blue**) interacting with two molecules of ADA (**orange and yellow**) is shown. Secondary structures such as α-helix and β-sheets are indicated. Areas of interaction between CD26 and ADA are indicated as follows: the helix α1 (Arg76-Ala91, **red**) of ADA interacting with loop A (Ile287-Asp297, pink) of CD26 and helix α2 (Pro126-Asp143, green) of ADA interacting with loop2 (Asp331-Gln344, brown) of CD26. Structure was reconstituted using data from X-ray diffraction of crystal structure published by Weihofen et al.,[84] available in protein data bank web site http://www.rcsb.org/pdb/ by using the viewerlite software. [[AU: IF YOU WANT THIS ILLUSTRATION PUBLISHED IN GREYSCALE, PLS. REVISE ALLUSIONS TO COLOR ACCORDINGLY.]]

fluids. In lymphocyes, CD26 is unable to signal by itself and requires the presence of the T-cell receptor–CD3 complex. Because of its short cytoplasmic tail, signal transduction via CD26 requires that the molecule interacts with other components of the plasma membrane in T cells. Among them, it has been suggested that CD26 interacts, directly or indirectly, with the zeta chain of the T-cell-receptor CD3 complex[20] and with the phosphotyrosine phosphatase CD45.[21]

In 1993, Kameoka et al.[22] demonstrated that CD26 was an ectoADA1-anchoring protein in Jurkat cells stably transfected with the cDNA encoding for CD26. Dong et al.,[23] have shown that neither the protease nor the deaminative activity are required for the association between CD26 and ADA1. By the use of immunoelectron microcopy, the same authors have found that CD26 and ectoADA1 colocalize in the cell surface but not inside the cells. This indicates that CD26 does not transport ADA1 to the plasma membrane of T cells. Recently, Kameoka et al.,[24] have reported that ADA is coexpressed with CD26 in vivo on the cell surface of malignant neoplasms. To further determine the binding domain of CD26 to ADA, the correlation of the epitopes defined by anti-CD26 mAbs and the functions of CD26 were determined. It was reported that the residues of L340, V341, A342, and R34.3 [[AU: EDITS OK?]] on the CD26 molecule were essential amino acids for ADA binding.[25]

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.[26,27] Importantly, in mouse ADA, which is not able to bind either human CD26 or mouse CD26, the amino acid in position 142 is Gln instead of Arg.[27] 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.[28,29] These 3D structures show that each CD26 dimer binds two ADA molecules (Fig. 2). 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 α2) of ADA (Fig. 2). CD26 binds ADA with two adjacent loops.[28,29] 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 α2 of ADA. Also, the same authors have described an additional region of ADA implicated in CD26-ADA binding, Arg76-Ala91 (helix α1). The interaction between helix α1 of ADA and loop A of CD26 completes the interface.[29]

Interestingly, murine cells transfected with human CD26, which express the human CD26 but do not express murine ADA1 on the cell surface, are able to acquire human ADA1 from human CD26$^-$ cells cocultured in vitro.[23] Since the only source of ADA1 was the intracellular ADA1 derived from the human CD26$^-$ cell lines, ADA1 may be released to the medium in a CD26-independent manner and bind to human CD26 expressed on the surface of the other cell type[[AU: WHAT OTHER CELL TYPE?]]. This would fit with the idea that intracellular ADA1 and cell surface ADA1 (ectoADA1) are indistinguishable molecules.

Adenosine, acting through specific receptors located on the cell surface of several kinds of cells, is an autocoid, which demonstrates multiple physiological activities[[AU: OK?]] in various systems.[30–33] There are four subtypes of adenosine receptors ($A_1$, $A_{2A}$, $A_{2B}$, and $A_3$), which have been cloned and pharmacologically characterized. All four subtypes share a significant sequence homology and belong to the superfamily of G-protein-coupled receptors. Whereas $A_1$ and $A_3$ are negatively coupled to the adenylate cyclase, $A_{2A}$ and $A_{2B}$ are positively coupled to this enzyme. The affinity of each receptor subtype for adenosine is relatively different, especially that of the $A_{2B}$ receptor; in fact, it may be speculated that the $A_{2B}$ receptor is not a true adenosine receptor due to the poor affinity of the adenosine/$A_{2B}$ receptor interaction[[AU: EDITS OK?]].

The effect of ADA1 on agonist binding to $A_1$ adenosine receptors has been somewhat controversial. The binding of 2-[$^3$H]-chloroadenosine to rat membranes was first studied in two laboratories. Basically depending upon the absence or presence of exogenous ADA1, one single[34,35] or two binding sites (low- and high-affinity[36,37]) were found. The appearance of a high-affinity binding

site in the presence of ADA1 was explained by the disappearance of endogenous adenosine, which acts as a competitor of $A_1$ adenosine receptor agonists, or by assuming that ADA1 had an extracatalytic high-affinity binding site for the adenosine-receptor agonist.[38] 3D resolution of the structure of the enzyme[39] has ruled out the existence of an allosteric site in ADA1. Therefore, it has been assumed, so far, that ADA1 affected agonist binding to $A_1$ adenosine receptors by deaminating adenosine present in the preparations. For many years it has been considered true that ADA1 acted by removing endogenous adenosine; therefore, ADA1 is always added in assays of ligand binding to adenosine receptors or in assays of adenosine-mediated physiological effects.

The first evidence that ADA1 could interact with cell surface $A_1R$ (Fig. 3) came from colocalization and coimmunoprecipitation experiments using a rodent cell type naturally expressing these receptors.[40] In these cells, the degree of colocalization between ADA1 and CD26 was lower than the colocalization between ADA1 and $A_1R$. Interestingly, when cells were incubated with commercial ADA1 (from calf intestine), the degree of colocalization of ADA1/$A_1R$ approached 100%. These data suggest a direct interaction between ADA1 and $A_1R$. The interaction has been confirmed in another laboratory using commercial bovine ADA1 and human embryonic HEK293 cells transfected with the cDNA coding for human $A_1R$.[41] Recently, we have proven the interaction by using a purified human enzyme preparation and brain samples from human striatum (data in preparation). When tested, the interaction leads to a significant increase in the affinity of $A_1R$ for the agonists.[40–42] This allosteric effect is not due to the enzymatic activity because it also happens when the activity is abolished by pretreatment of ADA1 with Hg ions.[40]

The expression of $A_1R$ in immune cells is relatively low, whereas other adenosine receptor subtypes are expressed in significant amounts. Quite unexpectedly, Herrera et al.,[43] demonstrated that cell surface ADA1 in ADA1$^+$/CD26$^-$ T lymphocytes anchors to adenosine receptors of the $A_{2B}$ subtype ($A_{2B}R$). The interaction between $A_{2B}R$ and cell surface ADA1 was also shown in transfected Chinese hamster ovary cells and Jurkat J32 T lymphocytes (Fig. 3). This was proven by



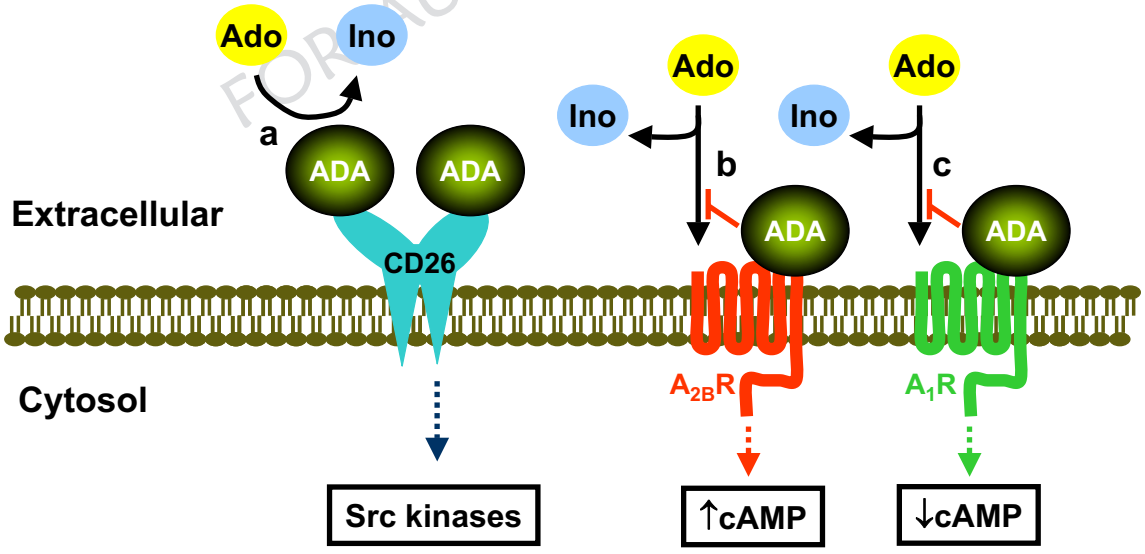


**FIGURE 3**. Enzymatic and extraenzymatic activity of ecto-ADA. Anchored on the cell surface by binding to CD26 (a) or to adenosine receptors A2BR (b) or A1R (c), ecto-ADA degrades adenosine (Ado, **yellow**) to inosine (Ino, **blue**), thus regulating adenosine availability to bind and stimulate adenosine receptors. In addition, by binding to CD26 (a), ecto-ADA may stimulate costimulatory signal for T cells. Moreover, when it binds to adenosine receptors A2BR (b) or A1R (c), by modulating affinity of the receptor-adenosine interaction, ecto-ADA regulates intracellular signaling triggered by stimulation of these receptors. [[AU: IF YOU WANT THIS ILLUSTRATION PUBLISHED IN GREYSCALE, PLS. REVISE ALLUSIONS TO COLOR ACCORDINGLY.]]

coimmunoprecipitation, binding of exogenous ADA1 to $A_{2B}R^+$ cells, and coimmunolocalization. The specificity of the interaction was demonstrated by the lack of interaction with other members of the G-protein-coupled receptor superfamily. As in the case of $A_1R$-ADA1 interaction, binding of ADA1 to $A_{2B}R$ increases the affinity of the agonist 5′-N-ethylcarboxamidoadenosine and cAMP production. This effect occurs even when ADA1 devoid of enzyme activity is used. Thus, ADA1 anchoring on the lymphocyte surface not only mediates extracellular adenosine degradation but also regulates $A_{2B}R$-mediated effects. To our knowledge, these data constitute the first evidence demonstrating molecular interactions between a degradative ectoenzyme and the receptors whose ligand is the enzyme substrate. What is known about the physiological role of such interactions is reviewed below.

## III. ROLE OF ADA1 IN T-CELL ACTIVATION

In [[AU: PLS. DEFINE "PBL".]]PBLs triggered by an anti-CD3 mAb, there was a significant increase in the percentage of expression of ADA and CD26 in the population of activated cells. In the case of activation via TCR-CD3 but in the presence of IL-2 or via phorbol esters, the increase was higher. The results of increased expression of surface ADA and CD26 were similar in whole T cells or in purer preparations such as $CD3^+$ or $CD4^+$ lymphocytes. Polyclonal Abs against ADA were not able to induce an activation response in T cells even when cross-linked by a secondary Ab. Interestingly, these Abs produced anergy in $CD4^+$ cells subjected to an anti-CD3 stimulus. In contrast, the addition of ADA produced an enzyme-independent synergism in the response through the TCR-CD3 complex.[44] In T cells, ADA and CD26 colocalized on the surface of T cells; thus, the effect of exogenous ADA seems to be mediated by CD26 molecules that are not interacting with endogenous ADA (spare CD26 molecules). The presence of spare CD26 molecules on the surface of $CD4^+$ cells was demonstrated by flow cytometry in the presence of exogenous ADA and also by confocal microscopy. The set of results strongly indicates that ADA binding to CD26 produces a costimulatory response in T-cell activation events.[44]

As indicated above, the presence of TCR-associated CD3ζ with at least one functional ITAM is required for CD26-induced costimulation.[20,45] Conflicting data exist about subsequent activation of the important T-cell receptor-associated adaptor protein LAT by ZAP-70 during CD26-induced signaling, because it becomes phosphorylated[46] but does not colocalize with CD26.[47] Nevertheless, CD26 induces activation of downstream signaling molecules, such as MAP kinases ERK1/2,[46,48,49] phospholipase C-γ (PLP-Cγ),[49] and Src kinase regulator c-Cb1.[21,48,49] Additionally, ADA[1] or antibody-mediated CD26 cross-linking[50] induces a synergic effect upon calcium mobilization triggered via the TCR/CD3 complex (Fig. 4). Therefore, the costimulatory signal triggered via CD26-ADA interaction potentiates the TCR/CD3 engagement during 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 costimulation.[46]

## IV. ADA1, DENDRITIC CELLS, T CELLS, AND THE IMMUNOSYNAPSE

### A. The Novel Costimulatory Intercellular Interaction

The physiological activation of T cells requires at least two signals. The first is provided by stimulation of the TCR/CD3 complex by a specific peptide/MHC complex. The second signal can be delivered by triggering costimulatory surface molecules that, similar to adhesion molecules, belong to the group of «accessory molecules»[[AU: WHAT DO THE DOUBLE BRACKETS MEAN? SHOULD THEY BE REPLACED BY QUOTATION MARKS?]], which are involved in a series of antigen nonspecific interactions between antigen-presenting cells and T cells in the immunosynapse. The engagement of costimulatory molecules can positively affect T-cell function, enhancing activation, proliferation, survival, and cytokine secretion. The critical role of costimulation in the activation of T cells is reflected by the fact that in the absence of any costimulatory signal, the antigenic presentation induces T cells to be-

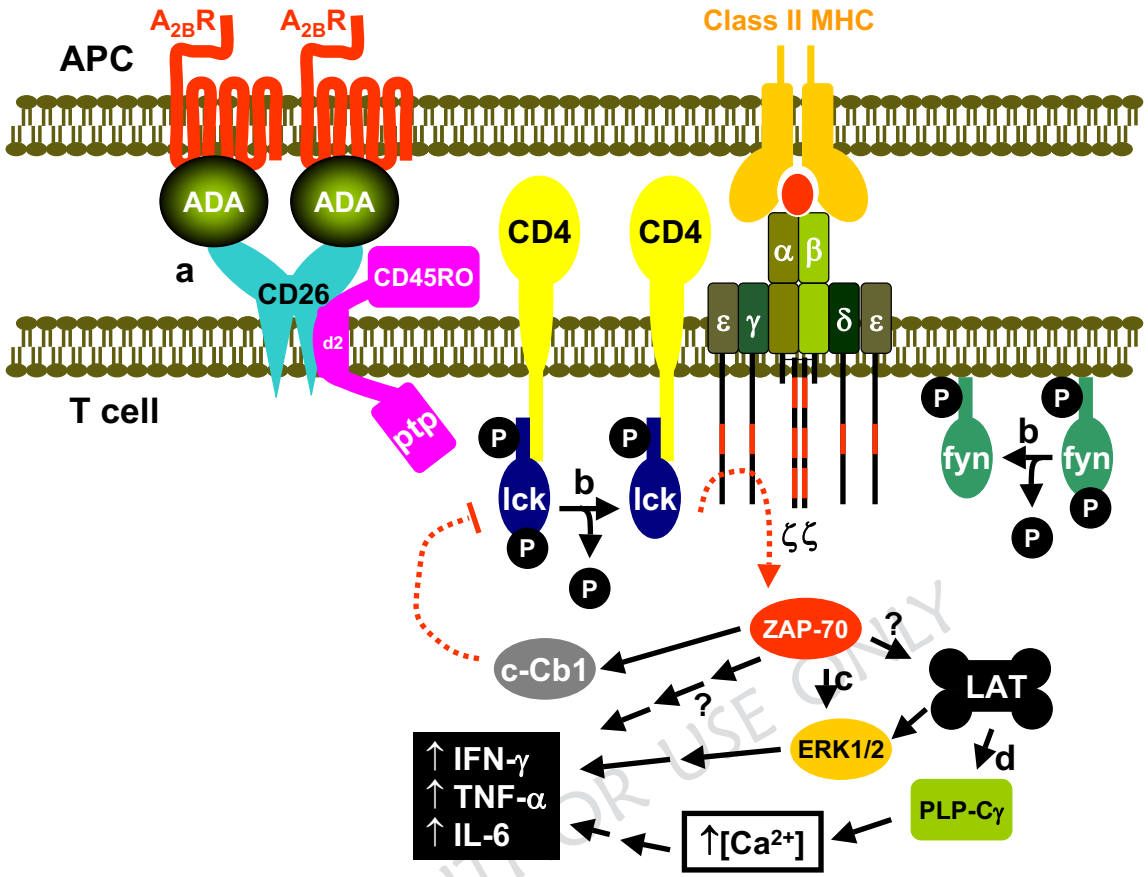


**FIGURE 4.** Proposed model of the CD26-ADA-mediated costimulation in T-cell activation. Protein Src kinases such as Lck (which constitutively binds to the cytosolic domain of coreceptors CD4 or CD8) and Fyn are activated following TCR/CD3/CD4 cross-linking on T-cell surface promoted by MHC:peptide complex formation on the antigen-presenting cell surface. These Src kinases phosphorylate immunoreceptor tyrosine-based activation motif (ITAM) of the cytosolic region of CD3γ, CD3δ, CD3ε, and CD3ζ (red lines). Phosphorylation allows recruitment of other protein tyrosine kinases, including ZAP-70, to the ITAM on CD3ζ, which is phosphorylated and activated by Lck. Activated ZAP-70, through adaptor protein phosphorylation such as LAT, promotes activation of the Ras/ERKs pathway and the PLP-Cγ activation, with subsequent rise in intracellular $Ca^{2+}$. ADA anchored to A2B adenosine-receptor homodimers on the antigen-presenting cell surface (a) promotes a cross-link of CD26 on the T-cell surface simultaneous to the TCR/CD3 engagement. Subsequently, CD45RO associated to CD26, through its d2 intracellular domain, is activated and recruited to the lipid rafts. The ptp domain of CD45RO catalyses the dephosphorylation of the regulatory domain of Src kinases including Lck and Fyn, thus inducing Src kinase activation (b). The subsequent activation of ZAP-70 induces the activation of the MAPK pathway, PLP-Cγ, and perhaps a still unknown pathway, which results in regulation of gene transcription resulting in IL-6 production and upregulation of TNF-α and IFN-γ (c). Additionally, a regulatory protein (c-Cb1) is activated, which induces a negative feedback of T-cell activation by promoting Src kinase degradation. Whether activation of MAPK pathway and PLP-Cγ depends on LAT activation (d) during CD26-induced signaling is not clear. The model shown for $CD4^+$ T cells would be similar for $CD8^+$ T cells. Abbreviations: d2, intracellular domain d2 of CD45RO; ptp, phopho-tyrosine-phosphatase domain of CD45RO; PLP-Cγ, phospholipase C-γ.



come anergic and tolerant.[51,52] So far, the costimulatory molecules described fall into three main groups, namely, Ig superfamily members, tumor necrosis factor receptor (TNFR) [[AU: OK?]] superfamily members, and cytokine receptors.[53] CD28, ICOS, and CD2 typify costimulatory 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, costimulatory 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 as OX40 (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 antigen-presenting cell surface.[54]

Recently, we have discovered that subsequent to immunosynaptic contacts,[[AU: OK?]] the costimulatory effect occurring through CD26 on the T-cell surface is promoted by ecto-ADA colocalizing with the $A_{2B}$ adenosine receptor expressed on the antigen-presenting cell surface.[55] Similar to members of the TNFR family, CD26 is weakly expressed in resting T cells, but it is strongly upregulated by reagents that engage T-cell receptor (TCR)/CD3[[AU: OK?]].[44,53] Different from members of the TNFR/TNF family, CD26-mediated costimulation has been suggested to occur by a tri-molecular interaction between CD26, ADA, and the $A_{2B}$ adenosine receptor.[55] Thus, CD26 -a type II transmembrane protein on T cells-, $A_{2B}$ adenosine receptor -G-protein-coupled receptors on dendritic cells[[AU: OK?]] (DC)-, and ADA bound simultaneously to both may constitute an example of a novel module leading to enhanced T-cell activation[[AU: WHAT IS THE SIGNIFICANCE OF THE UNDERLINED HYPHENS? OK TO DELETE OR CHANGE TO EM DASHES? THEY ARE NOT CORRECT AS SET.]] (Fig. 5). Similar to other G-protein-coupled receptors,[56] $A_{2B}$ adenosine receptors may form homodimers on the cell surface. Ecto-ADA, therefore, is likely to interact with $A_{2B}$ adenosine recep-



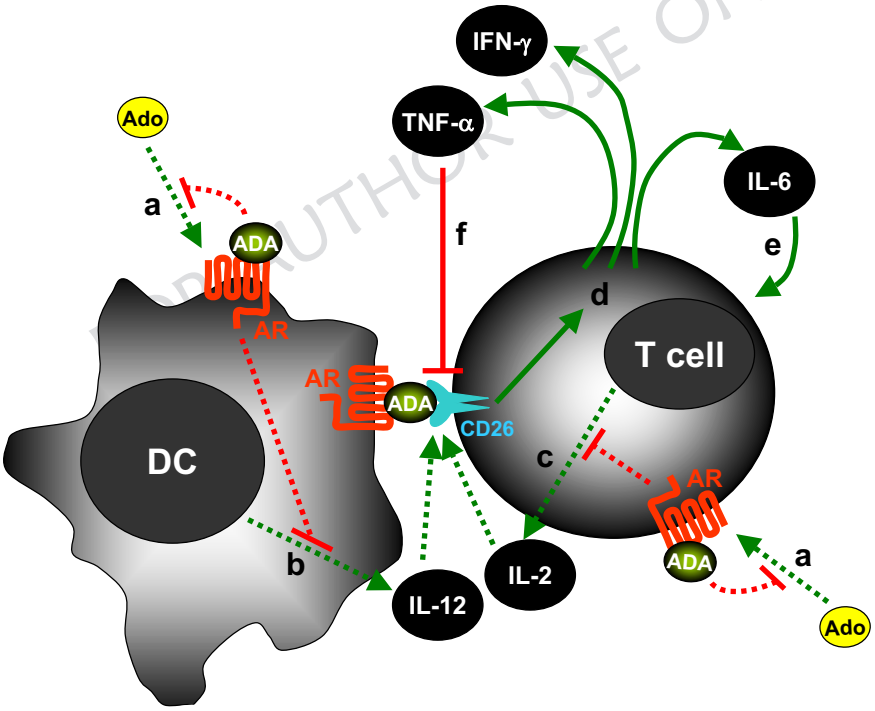


**FIGURE 5**. The CD26–ADA-mediated costimulatory signal potentiates Th1 polarization. Dotted lines indicate the enzymatic activity–mediated costimulatory effect of ADA. Solid lines indicate CD26–ADA binding–mediated costimulatory intercellular interactions. Green arrows represent stimulation/upregulation, whereas the red flat heads represent inhibition/downregulation. Subsequent to immunosynaptic contacts, ecto-ADA decreases (a) the adenosine (Ado, yellow) concentration able to activate adenosine receptors (AR, red). In these conditions, DC produce IL-12 (b) and T cells produce IL-2 (c). Both events promote upregulation of CD26 on the T-cell surface. The costimulatory signal promoted by the CD26–ADA interaction induces secretion of IL-6 and upregulation of TNF-α and IFN-γ (d). IL-6 likely promotes additional costimulation (e), perhaps inducing initial upregulation of IFN-γ. TNF-α produced by T cells (f) promotes a negative feedback inducing downregulation of CD26 on the T-cell surface. [[AU: IF YOU WANT THIS ILLUSTRATION PUBLISHED IN GREYSCALE, PLS. REVISE ALLUSIONS TO COLOR ACCORDINGLY.]]

tor homodimers on the DC surface, promoting a cross-link of CD26 on the T-cell surface during the immunosynapse, thus inducing costimulation. In addition, it has been reported that neither dipeptidyl peptidase activity of CD26[47,57] nor deaminase activity of ADA[44,55] are required to initiate a CD26–ADA-mediated costimulatory signal. CD26 cross-linking causes coaggregation of CD26 and CD45RO into lipid rafts.[48] Subsequently, through its interaction with a cytoplasmic domain of CD45RO, CD26 promotes dephosphorylation of the C-terminal regulatory domain of Src kinases such as Lck and Fyn, thus activating them [21,48,49] (see Fig. 4). 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 ITAMs. Thus, Lck activates ZAP-70.[58,59] In this regard, the presence of the TCR-associated CD3ζ with at least one functional ITAM is required for CD26-induced costimulation.[20,45] Recently, we have demonstrated that during the immunosynapse between superantigen-pulsed DC and $CD4^+$ T cells, the costimulatory effect promoted by CD26-ADA interaction induces IL-6 production and enhanced IFN-γ and TNF-α secretion.[55] In agreement with the augmented IFN-γ secretion, CD26 has been proposed to be a Th1 marker.[60–63] In this regard, superantigen-pulsed DC secreting IL-12 promote a Th1 cytokine pattern and phenotype in $CD4^+$ T cells (Fig. 5). Indeed, it has been reported that IL-12 lead to upregulation of ADA expression on the cell surface.[60]

Interestingly, indirect evidence indicates that TNF-α induces a downregulation of T-cell surface CD26 expression.[63] In this way, the increased TNF-α secretion induced via CD26-ADA costimulation could exert negative feedback by regulating CD26 expression and, therefore, the Th1 response during immunosynapse (Fig. 5). In addition, IL-6 possibly plays a role during T-cell activation, promoting additional costimulation[64,65] and/or inducing initial production of IFN-γ in T cells[66] (Fig. 5). This tri-molecular interaction involving both DC and T cells promotes enhanced TCR/CD3 signal, proliferation, and proinflammatory[[AU: PROINFLAMMATORY WHAT?]] and a Th1 cytokine pattern.[55]

## B. ADA1 As a Putative Growth Factor

As mentioned above, the ADA1 action during DC–T-cell contacts is to increase proliferation. This and other extraenzymatic features of ADA1 in lymphocytes are similar to some interleukins[[AU: OK?]]. Recent data on a new family of growth factors with adenosine deaminase activity suggest that ADA1 should be considered both an enzyme and a growth factor.

For many years it has been known that two adenosine deaminase activities occur in human plasma. One of them is displayed by the enzyme whose DNA was cloned more than 20 years ago and which is the object of the present review. This activity is classically referred to as ADA1. In contrast, the nature of the so-called ADA2 has only been recently elucidated by Zavialov and Engstrom.[67] ADA2 is a member of a new class of ADA-related growth factors, which is present in almost all organisms, from flies to humans. Six *Drosophila* adenosine deaminase-related growth factor (ADGF) genes have been identified at three different chromosomal locations, with one singleton, two in an inverted orientation, and three in a tandem arrangement. These genes show distinct patterns of expression as measured by RT-PCR and Northern blots, indicating gene-specific function.[68] The presence of six ADGF genes in the *Drosophila* genome suggests that gene duplication and divergence has been important for these growth factors in insect development. Phylogenetic analysis of the 14 extant ADGF-like gene products shows that there are at least three major groups, two of which are found in *Drosophila*.[68] The products of these genes not only act as growth factors, but it has also been reported that they stimulate cell proliferation in *Drosophila* by depleting extracellular adenosine.[69] The deaminase activity is also important for *Drosophila* larval development.[70] Interestingly, ADA1 was first described as an enzyme with deaminase activity, whereas other members of the ADGF family were first described as growth factors and were later ~~on a role for the~~ discovered to demonstrate deaminase activity ~~has been adscribed~~[[AU: OK?]]. On the basis of the proliferating effect of ADA1, it is tempting to hypothesize that ADA1 is a putative growth factor. This hypothesis is sustained by the high homology in the catalytic region between ADA1

and ADGF family members[69,71] and by the analogies found between the above-indicated release of intracellular ADA1 by lymphoid cells and the release of ADA2 from activated human peripheral blood monocytes.[72]

## V. ADA1 ROLE IN ALTERATIONS OF THE IMMUNE FUNCTION

### A. ADA1 and Severe Combined Immunodeficiency (SCID)

The congenital deficit of ADA1 leads to severe combined immunodeficiency (SCID). When only the cytosolic enzyme was described, all the alterations due to this congenital defect were ascribed to the accumulation of adenosine and a subsequent increase of adenosine nucleotides. dATP inhibits ribonucleotide reductase, and this prevents formation of the deoxynucleotides that are needed for DNA synthesis (see Fig. 1, reviewed in Ref. 1). This hypothesis could not explain why immune cell systems are more prone to ADA-related alterations than cells in other tissues/systems. After the discovery of ectoADA1 and the extranezymatic activities of ADA1, other mechanisms for ADA-related SCID are considered possible, as well[[AU: OK?]].

The most obvious role for ectoADA1 is ~~the~~ to[[AU: OK?]] deaminate the extracellular adenosine available for adenosine receptors. In 1997, Resta and Thompson[[AU: THOMSON (SEE REF.)?]][73] speculated about the possibility that overexpression of adenosine receptors by accumulation of extracellular adenosine could have a role in ADA1-related SCID. More recently, Hashikawa et al.[74] have demonstrated, by using CD26-transfected cells, that cell surface ecto-ADA1 can regulate adenosine receptor engagement by degrading extracellular adenosine to inosine. This ability is dependent upon CD26 expression, the extent of CD26 saturation with ecto-ADA, and the kinetics of the cAMP response. Thus, the cAMP response was markedly decreased when CD26-transfected cells were incubated with an exogenous source of ADA to increase ecto-ADA expression. Abolishment of the enzyme activity inhibited the ability of ectoADA to degrade adenosine and increased the cAMP response. Although CD26 expression on human thymocytes was low compared with that of CD26-transfected cells, it was saturated with ectoADA. When thymocytes incubated at high densities (to mimic the situation in tissues) were exposed to exogenous adenosine, the cAMP response was dramatically decreased by ecto-ADA. There is evidence that adenosine, acting through its $A_{2A}$ receptor, interferes with NF-kappaB activation and IL-2 function in antigen-receptor-stimulated B and T lymphocytes.[3] Hashikawa et al.[74] concluded that ecto-ADA has the potential to regulate adenosine receptor-mediated cAMP responses in vivo in tissues with $CD26^+$ cells.

### B. 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.[75–77] Furthermore, it has been described that ADA binding to CD26 is inhibited by recombinant soluble HIV-1 envelope glycoprotein gp120 and HIV-1 infectious particles.[7] [[AU: SHOULD THIS BE "78"? IF NOT, PLS. MARK CALLOUTS AND REFERENCE SECTION FOR RENUMBERING. REFERENCES MUST BE CALLED OUT IN NUMERICAL SEQUENCE FOR THE FIRST INSTANCE.]] 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.[79] Importantly, direct interaction and comodulation of CD26 and CXCR4 on the T-cell surface has been demonstrated.[80] Studies with overlapping synthetic peptides covering the entire gp120 sequence have revealed that the region of gp120 implicated in the inhibition of ADA binding to CD26 is the third constant domain gp120 (C3 region).[78] Because the C3 region of gp120 is hidden in soluble gp120,[81] 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 in gp120 occurs before binding to CD26.[80] The impairment of T-cell physiology promoted by gp120-mediated disruption of ADA binding to

CD26 is evident because preincubation of T cells with gp120 inhibits TCR/CD3-dependent activation of Fyn and Lck (~~Morio et al., 1997~~[[AU: PLEASE FILL IN REF. #]]), and blocks the IP3-sensitive calcium mobilization,[82] resulting in altered antigen-induced proliferation and IL-2 production.[83] On the other hand, HIV has also been implicated in the inhibition of CD26 enzymatic activity. The HIV-transactivating protein Tat plays a role in viral replication and also exerts immunosuppresive properties in vitro, which has been attributed to its interaction with CD26.[84] When T cells are infected by HIV-1, they release Tat into the extracellular 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,[85] thus contributing to the HIV-1-promoted impairment of T-cell-mediated immunity. Additionally, because chemokines can be substrates of CD26, the catalytic activity of CD26 has 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 [[AU: PLS. DEFINE "PBMC".]]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.[86] 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.[86]

In recent studies we have observed that HIV-1 gp120 added to cultures inhibited the CD26/ADA costimulatory signal that induces T-cell activation by anti-CD3 or by superantigen-pulsed autologous monocyte-derived dendritic cells (MDDC) in healthy subjects. This effect seems to be mediated by the displacement of ADA by gp120, as it has previously been demonstrated.[79,80] In HIV-1-infected patients, although ADA costimulation occurred, the costimulation ratio was highly variable, both in anti-CD3-induced proliferation and in cocultures of SEA-pulsed MDDC. The higher response was observed in those patients with low viral load and relatively high CD4 counts. In addition, ADA caused a robust increase of cytokine secretion (n = 15) in cocultures of MDDC pulsed with heat-inactivated HIV. The costimulation ratio in HIV patients was correlated with CD26 expression. These results suggest that gp120 disruption of CD26/ADA interaction is a novel mechanism to explain, at least in part, the altered immunological synapse function observed in HIV-1-infected patients. ADA costimulation seems to improve T-cell activation events, although mainly in patients with low viral load and high CD4 T-cell levels.

## VI. CONCLUSIONS

ADA1 is a remarkable example of a multifunctional adaptor protein with a seminal role in the development of the immune system and the function of immune cells. Both the enzymatic activity and the signaling events occurring through the interactions with ADA1-binding proteins are important to fully elucidate the physiological role of ADA1 in healthy conditions or in immune-related diseases. It is likely that its role as adaptor is redundant in different systems but not in the immune system where, in humans, ADA1 is crucial for immune system development and immune cell function. Although the consequences of the interaction with CD26 and with adenosine receptors are being elucidated, little is known about the physiological effects of the interaction with the intracellular protein Grb 3-3 or with plasminogen. It has been demonstrated that the interaction of ADA1 with partner proteins in DCs and T cells is important for T-cell activation and polarization of the effector response[[AU: MEANING AS MEANT?]]. The disruption of the interaction by HIV strongly indicates that ADA1-related interactions play a role in AIDS. Further studies are required to know whether the infusion of ADA1 could be of benefit to improve antigen presentation in HIV-infected individuals.

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