Regulation of Lymphocyte Function by Adenosine

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Abstract—Adenosine regulates the interaction between lymphocytes and the vasculature, and is important for controlling lymphocyte trafficking in response to tissue injury or infection. Adenosine can blunt the effects of T cell receptor activation primarily by activating adenosine A2A receptors and signaling via cyclic AMP and protein kinase A. Protein kinase A reduces proximal T cell receptor signaling by phosphorylation of C-terminal Src kinase, nuclear factor of activated T cells and cyclic AMP response element-binding protein. Protein kinase A activation can either enhance or inhibit the survival of T cells depending on the strength and duration of signaling. Inducible enzymes such as CD73 and CD39 regulate adenosine formation and degradation in vivo. The extravasation of lymphocytes through blood vessels is influenced by A2A receptors-mediated suppression of intercellular adhesion molecule 1 expression on lymphocytes and diminished production of interferon γ and interferon γ-inducible chemokines that are chemotactic to activated lymphocytes. Adenosine also decreases the barrier function of vascular endothelium by activating A2BRs. In sum, adenosine signaling is influenced by tissue inflammation and injury through induction of receptors and enzymes and has generally inhibitory effects on lymphocyte migration into inflamed tissues due to protein kinase A-mediated effects on adhesion molecules, interferon γ production, and endothelial barrier function. (Arterioscler Thromb Vasc Biol. 2012;32:2097-2103.)

Key Words: adenosine ■ lymphocytes ■ T cells

In addition to playing a central role in biochemical processes, adenosine is an anti-inflammatory signaling molecule that is produced by all cells in proportion to metabolic activity, injury, and hypoxia. Adenosine signaling is mediated by 4 G-protein–coupled adenosine receptors (AR): A1, A2A, A2B, and A3. These receptors are antagonized by naturally occurring and widely consumed methylxanthines, caffeine, and theophylline, as well as by more potent synthetic antagonists. Adenosine produced as a byproduct of metabolic activity readily crosses most cell membranes on nucleoside transporters. Extracellular adenosine is produced from the degradation of adenosine nucleotides by exonucleases. ATP and ADP are converted to AMP and adenosine after nucleotide release to the extracellular space through membrane channels, necrotic cell death, or as granular components of platelets, mast cells, and neuronal synaptic granules.

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The A2A receptor (A2AR) is the predominant AR subtype found on lymphocytes. Stimulation of A2ARs on activated T cells acutely inhibits proinflammatory cytokine production and effector functions. In addition, both A2A and A3B receptors are found on antigen-presenting cells (APCs) and strongly influence T cell activation. This review focuses on recent advances in our understanding of lymphocyte activation, and the interaction of lymphocytes with the vasculature by enzymes that regulate adenosine metabolism, ARs, and cyclic AMP (cAMP) signaling.

Lymphocyte Activation

T cells can be activated as a result of antigen presentation by APCs such as dendritic cells (DCs) or macrophages. Antigenic molecules are displayed on the surface of APCs by major histocompatibility proteins (MHC) and activate T cell receptors (TCRs) on lymphocytes (Figure). After TCR stimulation, lymphocyte activation can result in T cell differentiation, cytokine production, or cytotoxic activity. Antigens are presented by APCs to the ligand-binding portion (αβ subunits) of TCRs (Figure). TCR activation, known as signal 1, is transduced through γ, δ, ε, and ζ chains of the CD3 portion of the TCR. After stimulation, TCR signal transduction is initiated by lymphocyte-specific protein tyrosine kinase phosphorylation of tyrosines on immunoreceptor tyrosine-based activation motifs present in the tails of CD3 components. These phosphorylated residues provide docking sites for the SH2 domains of zeta-chain–associated protein kinase–70, which dock and phosphorylate tyrosines on linker of activated T cells. In the case of a small subset of lymphocytes known as invariant natural killer T (iNKT) cells, lipid antigens replace peptide antigens on APCs, and MHC is replaced by CD1 antigen-presenting molecules. Below we discuss how T cell activation influences lymphocyte adhesion to the endothelium and the production of chemotactic chemokines.

Adenosine A2AR Activation Selectively Inhibits Cytokine Production by T Cell Subsets

Depending on the environment during antigen encounter, CD4+ T cells can differentiate into T helper 1 cells (Th1) that secrete...
primarily type 1 cytokines including interleukin (IL) 2, interferon γ (IFN-γ), and tumor necrosis factor α; Th2 that secrete primarily IL-4, IL-5, and IL-10; or Th17 cells that secrete IL-17A, IL-17F, and IL-21. Type 1/type 2 cytokine polarization exists for both MHC class 2–restricted CD4+ T cells (Th1/Th2 subsets) and for MHC class 1–restricted cytotoxic CD8+ T cells (Tc1/Tc2 subsets). Agonist binding to A2AR activates the heterotrimeric G protein, Gs, to catalyze cAMP production. The protein kinase A (PKA) pathway negatively regulates the production of type 1 cytokines, with lessor effects on type 2 cytokines.10 A2AR activation reduces production of IL-2, tumor necrosis factor α, and IFNγ secretion from Th1 and Th2 cells, but does not affect IL-4 or IL-5 secretion.11 A2AR activation also strongly inhibits the production of IFNγ by iNKT cells.12 A2AR activation has not been reported to directly influence cytokine release from purified Th-17 cells. When given in vivo or in mixed cell T cell development assays with APCs, A2AR agonists inhibit production of IL-6 and enhance production of IL-10. This results in indirect inhibition of Th1, Th2, and Th17 effector cell development.11,14 In sum, the strongest direct effects of A2AR stimulation on lymphocytes is on type 1 cytokine production by Th1, Th1, and iNKT cells.4,11,12

**How cAMP and PKA Mediate A2AR Signaling in T Cells**

The activation of Gs after agonist binding to A2ARs stimulates adenylyl cyclase to produce cAMP. Two downstream effectors, PKA and exchange protein directly activated by cAMP, are the principal mediators of cAMP action in T cells. The cAMP mimetic 8-(4-chlorophenylthio) adenosine-3’,5’-cyclic monophosphate is a useful tool for distinguishing between these 2 pathways because it activates PKA but fails to activate exchange protein directly activated by cAMP. Experiments using 8-(4-chlorophenylthio) adenosine-3’,5’-cyclic monophosphate indicate that most transcriptional effects of cAMP in T cells are mediated by PKA. In addition to adenosine, several other Gs-coupled receptors are found on T cells. These include β2-adrenergic,15 prostaglandin E2, dopamine D1,16 and vasoactive intestinal peptide.17 Adenosine is particularly important for limiting lymphocyte activation because A2A Rs are induced on activation of T cells6,18 and iNKT cells.19 cAMP is degraded in T cells primarily by phosphodiesterase 4, and phosphodiesterase 4 inhibitors facilitate the actions of adenosine and other Gs-coupled receptor agonists. cAMP regulates T cell cytokine secretion and proliferation by directly phosphorylating the transcription factors cAMP response element-binding protein and nuclear factor of activated T cells (NF-AT).9 Suppression of proximal T cell signaling pathways indirectly inhibits activation of another transcription factor, nuclear factor κB. The most abundant isoform of PKA found in T cells, PKA-1, activates C-terminal Src kinase, which inhibits the Src family tyrosine kinases lymphocyte-specific protein tyrosine kinase and Fyn and thus functions to check T cell activation (Figure). PKA-1 is targeted to the TCR-CD3 complex during T cell activation via an A-kinase-anchoring protein that serves as a scaffold for the cAMP-PKA/C-terminal

**Figure.** Cyclic AMP (cAMP) signaling inhibits TCR and CD28 signaling in lymphocytes. cAMP accumulates in T cells in the region of lipid rafts in response to TCR activation, and more globally in response to strong Gs-coupled A2AR activation. cAMP inhibits proximal TCR signaling through a pathway involving activation of protein kinase A-1 (PKA-1) and C-terminal Src kinase (Csk) to inhibit lymphocyte-specific protein kinase (Lck) and to reduce recruitment to CD3 of zeta-chain–associated protein kinase–70 (ZAP-70). PKA-1 also phosphorylates (indicated by red dots) and inhibits NF-AT. NF-AT inhibition is reversed by the Ca2+-calmodulin-dependent phosphatase, calcineurin. TCR-induced accumulation of cAMP near lipid rafts is reduced on CD28 stimulation due to the activation of phosphatidylinositol-3-kinase (PI3-K) to produce PIP3. This results in translocation from the cytosol to the lipid raft of a complex consisting of AKT, PDE4 and (β-arr) by binding of the plextrin homology (PH) domain of AKT to PIP3, PDE4 degrades cAMP to relieve inhibition of TCR signaling. A2AR indicates adenosine A2A receptor; Ado, adenosine; Ino, inosine; ADA, adenosine deaminase; AC, adenylyl cyclase; PKC, protein kinase C; CaM, calmodulin; NF-AT, nuclear factor of activated T cells; CREB, cAMP response element-binding protein; AKAP, A kinase anchor protein; TCR, T cell receptor; LAT, linker for activation of T cells; PLC, phospholipase C; (3,4,5)-trisphosphate; PDE4, type 4 phosphodiesterase; MHC, major histocompatibility complex; Ag, antigen; RhoH, Ras Homolog, a small GTP hydrolyzing protein; AKAP, A kinase anchor protein; TCR, T cell receptor; LAT, linker for activation of T cells; PLC, phospholipase C; PKC, protein kinase C; CaM, calmodulin; NF-AT, nuclear factor of activated T cells; CREB, cAMP response element-binding protein.
Signal 2 and cAMP

Signal 1 activation of TCRs alone produces limited T cell activation because TCR engagement locally enhances cAMP production and activates C-terminal Src kinase in the region of the immunologic synapse. Activation of TCRs is amplified by signal 2, that is, costimulation of CD28 by ligands expressed on the surface of APCs, B7.1, and B7.2 (CD80 and CD86). On TCR/CD28 costimulation phosphatidylinositol-3-kinase activation leads to phosphatidylinositol-(3,4,5)-trisphosphate production. This stimulates recruitment of an AKT/β-arrestin/phosphodiesterase 4 complex to the plasma membrane via the AKT (a serine/threonine-specific protein kinase, also known as protein kinase B) pleckstrin homology domain, resulting in the degradation of cAMP located near lipid rafts. It is not entirely clear how stimulation of the TCR results in elevated cAMP levels, but recruitment of Gs to lipid rafts may be involved. It is also possible that cell activation due to TCR signaling stimulates production of adenosine that exits the cell to act on autocrine or paracrine A<sub>2a</sub>Rs.

Adenosine Signaling Increases T cell Tolerance and Treg Development

Unlike the localized production of cAMP that occurs as a result of signal 1, strong activation of A<sub>2a</sub>Rs or otherGs-coupled receptors can produce whole cell increases in cAMP that are not limited just to the region of lipid rafts. Thus, extracellular adenosine reduces the activation of T cells by APCs and modifies T cell differentiation, cytokine production, and proliferation by preventing rapid tyrosine phosphorylation of zeta-chain–associated protein kinase–70 and downstream signaling such as activation of AKT and extracellular signal-regulated kinases (ERK1/2). cAMP elevation in naïve T cells also favors development of a regulatory phenotype (Treg) characterized by high expression of CD25, cytotoxic-T-lymphocyte–associated protein 4 (CTLA4), and Forkhead box protein 3 (FoxP3). CTLA4 is involved in suppressive activities by Tregs. Unlike T effector cells, Tregs also express ecto-enzymes CD39 and CD73 (Table) that metabolize adenine nucleotides in the extracellular space to adenosine that locally inhibits the activation of effector T cells and APCs.

Paradoxical Effects of PKA on T Cells Survival Inhibition of Apoptosis

Activation-induced cell death describes an apoptotic program initiated by restimulation of previously activated peripheral T cells. A<sub>2a</sub>R activation reduces activation-induced cell death in mouse CD4<sup>+</sup> hybridomas and human Jurkat cells. A<sub>2a</sub>R activation reduces activation-induced cell death by interfering with the production of factors that stimulate T cell activation, IL-2, and the downstream expression of the costimulatory molecules CD2 and CD28.

Enhancement of Apoptosis

In contrast to the antiapoptotic effect of transient cAMP elevation, prolonged elevation of cAMP triggers T cell apoptosis. This property of persistent cAMP elevation to kill T cells has been used to select T cell lines that have mutations in the cAMP signaling pathway. Recent studies have identified the mechanism by which cAMP triggers an apoptotic program in T cells. Treatment
of wild-type S49 T cells with the PKA-activating cAMP analog, 8-(4-chlorophenylthio) adenosine-3',5'-cyclic monophosphate, increases the expression of cytotoxic T lymphocyte antigen-2α (CTLA-2α), a cathepsin L-like cysteine protease inhibitor that triggers apoptosis.35 Treatment of kinase-S49 cells T cells that lack functional PKA with 8-(4-chlorophenylthio) adenosine-3',5'-cyclic monophosphate fails to stimulate CTLA-2α expression and apoptosis indicating that the increase in CTLA-2α in wild-type S49 cells is PKA dependent.34,35

Effects of Adenosine Deaminase Deficiency on T Cell Survival

Several investigators have sought to determine whether PKA-mediated killing of T cells contributes to severe combined immunodeficiency that occurs in individuals lacking adenosine deaminase (ADA). Since ADA deficiency causes adenosine concentrations to increase in the thymus and other tissues, it is reasonable to suspect that the resulting increase in A2AR signaling via adenosine, Gs, and PKA in thymocytes might evoke T cell killing by PKA-induced apoptosis. Apasov et al36 concluded that a portion of thymocyte apoptosis that occurs in response to ADA deficiency can be attributed to A2AR activation. However, the primary cause of toxicity in developing human thymocytes is the accumulation of deoxy-ATP which triggers mitochondrial-dependent apoptosis.37

CD26 Dampens Adenosine Signaling in T Cells

In human cells a soluble form of ADA can bind to cell surface CD26 that is expressed by lymphocytes, epithelial cells, and capillary endothelial cells. CD26 expression is strongly upregulated after T cell activation.38 ADA binding to CD26 on human T cells results in ADA accumulation on the T cell surface and is associated with T cell activation.39

Effects of Adenosine Metabolism and Transport on Lymphocyte Activation

The concentration of adenosine in the extracellular space is regulated by adenosine transport as well as adenosine formation and degradation (Table). Nucleoside transporters are divided into 2 families; the Na+-dependent solute carrier family 28 and the equilibrative solute carrier family 29. Solute carrier family 28 family transporters (CNT1–3) display subtype-selective expression patterns; CNT1 is localized primarily to epithelial tissues whereas CNT2 and CNT3 have more widespread distributions. Solute carrier family 29 family transporters (ectonucleoside triphosphate 1–4) are glycosylated proteins localized to the plasma and mitochondrial membranes. They are expressed in the heart, brain, mammary gland, erythrocytes, and placenta, and also in fetal liver and spleen, and mediate nucleoside influx and efflux. Insulin and glucose induce changes in expression levels of nucleoside transporters in T lymphocytes.40

Lymphocyte CD39 and CD73 and Immune Regulation

Tregs comprise a subset of T cells that inhibit the activation of effector T cells. CD39 (ectonucleoside triphosphatase 1) and CD73 (5NTD) are coexpressed on the surface of murine T regulatory but not effector cells, and together generate extracellular adenosine from ATP, ADP, and AMP. Murine T regulatory cells are usually defined based on expression of CD4, CD25, and the transcription factor, FoxP3. However, these markers are not sufficient to uniquely define T cell subsets in humans. Liu et al41 found that the IL-7 receptor (CD127) is low on a subset of CD4+ T cells in peripheral human blood. CD39, independently of CD73, is expressed on human CD4+ CD25+ CD127+ Tregs, also characterized by high expression of Foxp3. A distinct population of human CD4+ CD39+ T lymphocytes does not express CD25 and Foxp3. The latter cells secrete proinflammatory cytokines such as IFNγ and IL-17. These cells are increased, with a concomitant decrease in Tregs, in the peripheral blood of patients exhibiting transplant rejection. Hence, CD39 may be a useful marker for the success of organ transplantation.42 Immunodeficiency from HIV is associated with a significant increase in CD39 expression on human Tregs.43 Treg inhibitory effects are enhanced by CD39 upregulation, and are replicated by activation of A2ARs on HIV patient T cells. A2ARs are expressed at higher than normal levels on the T cells of HIV patients. The expansion of CD39+ Treg cells correlates with the level of immune activation and low CD4+ T cell counts in HIV. A genetic association study identified a CD39 gene polymorphism that is associated with downregulation of CD39 expression and slower progression to AIDS.43

Adenosine Regulation of APCs

Independent of the effects of adenosine on T cells, DCs and macrophages are highly susceptible to adenosine-mediated regulation. DCs and macrophages activated by lipopolysaccharide in the presence of adenosine have a reduced capacity to induce Th1 polarization of naïve CD4+ T lymphocytes, diminished release of tumor necrosis factor α and IL-12, and enhanced release of anti-inflammatory IL-10.44–48 Inhibition of AR signaling is needed to observe optimal activation of DCs and T cell activation by pathogen-associated molecular patterns.49–55 DCs express all 4 types of ARs, but their expression level varies depending on subtype, maturation status, or progenitors from which they differentiate.53–55 Immature DCs express A1 and A3 ARs that are thought to mediate chemotaxis.53 In lipopolysaccharide-matured DCs A1 and A3 receptors are downregulated, whereas A2a and A3 receptors are upregulated.51–55 A2aR expression can be further induced by hypoxia51 or tumor necrosis factor α.56 A2aR activation also inhibits DC-mediated T cell activation because A2aR stimulation reduces lipopolysaccharide-induced surface expression of MHCIId and CD86 which results in decreased IL-2 expression by T cells.35 However, activation of the A2aR can be proinflammatory. In the absence of toll-like receptor signaling A2aR stimulation increases proinflammatory IL-6, which together with transforming growth factor β can deviate naïve CD4+ T cells to a Th17 phenotype that favors chronic inflammation.58

Interface Between Lymphocytes and the Vasculature

Adenosine and Ischemia

Vascular diseases, infections, or tissue injury can lead to vaso-occlusion and tissue hypoxia that strongly influences adenosine signaling to immune cells. Part of this effect is due
to inhibition of adenosine kinase in hypoxic cells, resulting in an increase in the cellular accumulation of adenosine. In addition, hypoxia inducible factor drives induction of immunosuppressive A$_{2B}$ receptors on APCs and CD73 on epithelial and endothelial cells. Elevated levels of CD73 produce elevated tissue and blood levels of adenosine due to enhanced conversion of AMP to adenosine.

**Adenosine and Intercellular Adhesion Molecule-1**

Tissue damage due to trauma or infection produces an inflammatory cascade resulting in chemotaxis into the inflamed tissue of lymphocytes and other leukocytes. Adenosine can inhibit this process. Part of the effect of adenosine has been attributed to inhibition of expression of intercellular adhesion molecule-1. Intercellular adhesion molecule-1 is expressed by T cells and can bind to macrophage adhesion ligand-1 (Mac-1), leukocyte function associated antigen-1, and fibrinogen, all of which are expressed on endothelial cells and other leukocytes.

**Adenosine and IFN-$\gamma$-Inducible Chemokines**

Chemokines contribute to lymphocyte extravasation into inflamed tissues. Tissue damage results in the activation of iNKT cells that rapidly produce large amounts of IFN-$\gamma$ on activation. Widespread tissue damage and iNKT cell activation is produced in sickle cell anemia by rigid red cells that cause widespread microvascular occlusion and ischemia. NY1DD mice with sickle cell anemia have increased activation of iNKT cells, and high tissue levels of IFN-$\gamma$ and IFN-$\gamma$-inducible chemokines (CXCL9, CXCL10, and CXCL11) that are chemotactic to activated lymphocytes that express CXCR3. Treating NY1DD mice with anti-CD1d antibody to inhibit CD1d-restricted iNKT cell activation reverses pulmonary dysfunction and blocks the accumulation of activated leukocytes. Neutralization of CXCR3 receptors also ameliorates pulmonary dysfunction. Infusion of an A$_{2A}$R agonist into NY1DD mice blocks iNKT IFN-$\gamma$ production, lung inflammation, and lung injury. CXCR3 also regulates NK- and T cell trafficking during sepsis, and blockade of CXCR3 attenuates the pathogenesis of septic shock. A$_{2A}$R activation also can reduce inflammation and improve survival of mice with sepsis.

**Effects of CD73 on Lymphocyte Migration into Lymph Nodes**

CD73 is expressed on the cell surface of endothelial cells. After an inflammatory stimulus, lymphocyte migration into draining lymph nodes increases dramatically to facilitate the encounter of naive T cells with antigen-loaded DCs. CD73$^{-/-}$ mice have 2.5-fold increased rates of L-selectin–dependent lymphocyte migration from the blood through high endothelial venules compared with wild-type mice after lipopolysaccharide administration. The endothelial A$_{2A}$R is a likely target of CD73-generated adenosine and inhibits the adhesion of lymphocytes and other leukocytes.

**Effects of CD73 on Allograft Survival**

In a heterotopic cardiac allotransplantation model, CD73 deficiency in either donor or recipient mice results in decreased graft survival and development of cardiac allograft vasculopathy, suggesting a contribution of CD73 on both graft-resident and circulating cells in preventing vasculopathy. Lack of CD73 results in loss of cardiac graft barrier function and diminished graft expression of A$_{2B}$ R mRNA, with a concordant exacerbation of acute inflammatory and immune responses. Antagonism of the A$_{2B}$R causes a significant increase in vascular leakage, and activation of A$_{2B}$Rs results in prolongation of graft survival and suppression of cardiac allograft vasculopathy. In another model, implantation of tracheal allografts from wild-type mice into CD73$^{-/-}$ recipients caused a large increase in airway luminal obliteration that was associated with an increase in CD3$^+$ lymphocytic infiltration. The protective effect of CD73 was attributed to generation of adenosine and stimulation of the A$_{2A}$R. Treatment of wild-type recipients with an A$_{2A}$R agonist significantly reduced CD3$^+$ lymphocyte infiltration and airway luminal obliteration; similar treatment of CD73$^{-/-}$ recipients rescued them from rejection. These data implicate CD73 acting through adenosine generation and its stimulation of A$_{2A}$ and A$_{2B}$ receptors as inhibitors of lymphocyte recruitment into allografts. In allo-mismatched in vitro coculture experiments either genetic deletion or pharmacological blockade of CD73 increased transendothelial lymphocyte migration. These data suggest that CD73 on graft-resident or circulating cells diminishes transendothelial leukocyte trafficking and mitigates inflammation and rejection.

**Summary**

Adenosine generally inhibits the activation and extravasation of lymphocytes into damaged or infected tissues. This is due to a combination of effects on T cells, APCs, and endothelial cells. A$_{2A}$ and A$_{2B}$ receptors and enzymes that control adenosine metabolism can be rapidly induced in response to inflammation or hypoxia. Adenosine signaling functions to limit inflammation and tissue injury without producing excessive immunosuppression.

**Disclosures**

J.L. owns shares of Lewis and Clark Pharmaceuticals, LLC, a drug company targeting adenosine receptors.

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