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Regulation of Leukocyte Function by Adenosine Receptors

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Abstract

The immune system responds to cues in the microenvironment to make acute and chronic adaptations in response to inflammation and injury. Locally produced purine nucleotides and adenosine provide receptor-mediated signaling to all bone-marrow derived cells of the immune system to modulate their responses. This review summarizes recent advances in our understanding of the effects of adenosine signaling through G protein-coupled adenosine receptors on cells of the immune system. Adenosine A_{2A} receptors (A_{2A}Rs) have a generally suppressive effect on the activation of immune cells. Moreover, their transcription is strongly induced by signals that activate macrophages or dendritic cells through toll-like receptors, or T cells through T cell receptors. A_{2A}R induction is responsible for producing a gradual dissipation of inflammatory responses. A_{2A}R activation is particularly effective in limiting the activation of invariant NKT (iNKT) cells that play a central role in acute reperfusion injury. A_{2A} agonists have clinical promise for the treatment of vaso-occlusive tissue injury. Blockade of A_{2A} receptors may be useful to enhance immune-mediated killing of cancer cells. A_{2B}R expression also is transcriptionally regulated by hypoxia, cytokines, and oxygen radicals. Acute A_{2B}R activation attenuates the production of proinflammatory cytokines from macrophages, but sustained activation facilitates macrophage and dendritic cell remodeling and the production of acute phase proteins and angiogenic factors that may participate in evoking insulin resistance and tissue fibrosis. A_{2B}R activation also influences macrophage and neutrophil function by influencing expression of the anti-inflammatory netrin receptor, UNC5B. The therapeutic significance of adenosine-mediated effects on the immune system is discussed.

Keywords

Leukocytes; Lymphocytes; platelets; dendritic cells; macrophages; invariant NKT cells

I. Introduction

Both innate and adaptive immunity are strongly influenced by purinergic signaling. Innate immunity is the most ancient system that protects multicellular hosts from infections and comprises of immune cells that are activated in response to either to pathogen-associated molecular patterns (PAMPs) or sterile host tissue injury resulting in inflammation in

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response to damage-associated molecular patterns (DAMPs; Pelegrin, 2008). The adaptive immune system evolved subsequent to the innate system and utilizes antigen presenting macrophages and DCs, MHC molecules, and TCRs to recognize specific pathogenic antigens or host autoantigens. All cells of the immune system express multiple purinergic receptors, and these receptors play a major role in their regulation. The reader is directed to previous reviews for background information about adenosine signaling in the immune system (Hasko et al., 2007; Kumar & Sharma, 2009). This review focuses on recent findings that have shed new light on the role that purinergic signaling plays in regulating both innate and adaptive immune responses. Of particular interest are recent discoveries demonstrating that adenosine receptor transcripts can be rapidly upregulated in response to local cues such as activation of excitatory receptors or tissue hypoxia. It has also become evident that the extra-cellular metabolism of adenine nucleotides by ectoenzymes such as CD39 and CD73 is a major source of adenosine, based on proinflammatory responses in mice upon deletion of these enzymes.

A diagram of the suppressive effects of $A_{2A}R$ on adaptive and innate immunity is shown in Fig. 1. Conventional T cells are part of the adaptive immune system. Selective activation of highly variable T cell receptors results in the expansion of these cells and the release of cytokines such as INF-γ. A minor subset of T cells known as invariant NKT (iNKT) cells express invariant T cells receptors. In addition to responding to various pathogens, iNKT cells are activated by injury to host tissues and contribute to sterile inflammation. Since NKT cells possess T cell receptors than can be rapidly activated by innate signals either from pathogens or danger signals produced by the injured host, they bridge innate and adaptive immunity. Both systems are strongly influenced by inducible $A_{2A}R$ signaling, as well as other purinergic receptors. Suppression of the innate immune response due to adenosine signaling can be beneficial to limit tissue inflammation and injury. However, too much immunosuppression by adenosine can blunt the ability of the immune system to control infections (Hasko et al., 2008). Activation of adaptive immune responses can be beneficial, for example, by enhancing immune surveillance of tumors (Jin et al., 2010), or harmful, for example, by reducing immune sensitization to persistent viral infections (Alam et al., 2009). We discuss how recent developments may be useful to the goal of exploiting adenosine signaling for therapeutic uses such as treatment of reperfusion injury, chronic inflammatory diseases, and tumor killing.

II. Immune Responses to Adenosine Receptor Signaling

Activation of the immune system elicits immune cell-mediated killing of pathogens and the release of proinflammatory cytokines. The rapid induction of proinflammatory mediators by the immune system is accompanied by the initiation of transcriptional programs that limit inflammation. These include production of TGF-β, IL-10, vascular endothelial growth factor (VEGF), insulin-like growth factor-1, HO-1, and netrin-1. Adenosine and the A_{2A} and A_{2B} receptors are included among anti-inflammatory factors that are produced or induced during inflammation. A_1R signaling also is important in immune regulation, but it acts primarily by influencing the sympathetic nervous system. Prejunctional A_1 receptors inhibit the release of the sympathetic cotransmitters norepinephrine and ATP. All primary and secondary immune organs receive sympathetic innervations from sympathetic postganglionic neurons (Nance &

Sanders, 2007). Innate immune cells express both α- and β-adrenergic receptor subtypes, while T and B lymphocytes express anti-inflammatory β2 adrenergic receptors exclusively. The A_3 receptor has been implicated in influencing neutrophil chemotaxis (Chen et al., 2006) and mast cell degranulation (Feoktistov et al., 2003), and may contribute to inhibiting reperfusion injury (Ge et al., 2010), but in general, the role of the A_3 receptor in immune regulation remains enigmatic (Gessi et al., 2008).

A. Platelets

Platelets are activated during sterile inflammation that occurs in response to tissue trauma or ischemia reperfusion injury (IRI). Substantial platelet activation is associated with sickle cell disease that has been extensively studied as a model of simultaneous IRI in multiple tissues. Intravital microscopy analyses in mice with sickle cell disease indicate that sickle RBCs interact primarily with adherent platelets and leukocytes in postcapillary and collecting venules leading to vascular obstruction (Turhan et al., 2002). ATP and ADP released from activated or damaged cells activate platelets via two G protein-coupled ADP receptors ($P2Y_1$ and $P2Y_{12}$) and via ATP through the ligand-gated P2X1 receptor (Oury et al., 2006).

It is now appreciated that the metabolic flux of adenine nucleotides and adenosine in the extracellular space regulates platelet activation due to counterbalancing signaling through P2 and adenosine receptors (Iyu et al., 2010). Activation of A_{2A} receptors on platelets causes an increase in cyclic AMP accumulation and a decrease in platelet aggregation (Cooper et al., 1995; Table I). In A_{2A} receptor-knockout mice, platelet aggregation is increased, proving the importance of this receptor subtype in limiting platelet activation (Ledent et al., 1997). Platelet activation is not only important for regulation platelet aggregation and secretion but also because it stimulates the production of platelet heteroaggregates with other leukocytes including monocytes, eosinophils, and neutrophils (Polanowska-Grabowska et al., 2010). Blockade of P-selectin-mediated platelet–leukocyte aggregation is beneficial in the animal models of vascular injury (Merhi et al., 1999). Hence, platelet $A_{2A}R$ activation may contribute to reduced sterile inflammation by direct effects on singlet platelets and platelet–leukocyte heteroaggregates. Although it was thought that the only adenosine receptor on platelets was the $A_{2A}R$, Yang et al. (2010) recently showed that systemic inflammation induces the expression of $A_{2B}Rs$ on platelets, and activation of these receptors inhibits the expression of the $P2Y_1$ receptor and ADP-induced platelet aggregation.

B. Neutrophils

Tissue trauma or IRI results in an inflammatory cascade that ultimately results in neutrophil infiltration into tissues (Lappas et al., 2006; McDonald et al., 2010). In the absence of infection, neutrophil accumulation in tissues can be very destructive. Platelet activation is associated with increased platelet adhesion to microvascular endothelium (Brittain et al., 1993), and formation of platelet heteroaggregates with erythrocytes (Inwald et al., 2000) and leukocytes including neutrophils, monocytes, and eosinophils. Oxidative burst in activated neutrophils and elevated expression of α4/β1 integrin (VLA-4, CD49d/CD29) are decreased as a result of $A_{2A}R$ activation (Fredholm et al., 1996; Revan et al., 1996; Sullivan et al., 2001, 2004b).

Neutrophils release ATP through pannexin-1 hemichannels in response to inflammatory mediators (Chen et al., 2010). Released ATP is necessary for maintaining neutrophil activation, but metabolism of ATP to adenosine inhibits neutrophil activation and adhesion to endothelial cells by direct effects on neutrophils (Sullivan et al., 2001) as well as indirect effects that reduced cytokine-mediated expression of P-selectin and ICAM-1 on endothelial cells (Okusa et al., 2000). Neutrophils are guided to sites of tissue injury by chemokines and formal peptides released from necrotic cells (McDonald et al., 2010). Thus, purinergic signaling is one of the several mechanisms required for regulation of neutrophil trafficking during inflammation. A_{2B}Rs also indirectly influence neutrophil trafficking by effects on tissue production of cytokines that are chemotactic to neutrophils such as KC. For example, A_{2B}R activation plays a role in mediating lung inflammation after ischemia–reperfusion by stimulating neutrophil chemotaxis (Anvari et al., 2010).

C. Macrophages and DCs

Macrophages are broadly classified into inflammatory M1 (NOS2+) and angiogenic M2 (arginase+). Toll-like receptor (TLR) 2, 4, 7, and 9 agonists, together with A_{2A}R agonists, switch macrophages from an M1 to an M2-like phenotype. This switch involves induction of A_{2A}Rs by TLR agonists, diminished TNF-α and IL-12 production, and enhanced production of VEGF and IL-10 (Grinberg et al., 2009). LPS suppresses PLCβ1 and β2 expression in macrophages *in vitro* and in several tissues *in vivo*. Signaling through TLRs suppresses PLC-β2 and this switches M1 macrophages into an M2-like state (Grinberg et al., 2009). Recognition of apoptotic cells also polarizes macrophages toward the anti-inflammatory M2-like phenotype by a process involving macrophage production of sphingosine-1-phosphate and VEGF and the induction of the A_{2A}R (Weis et al., 2009). These responses are mediated in part by the transcription factor HO-1. These findings suggest that HO-1, which is induced by apoptotic cell-derived S1P, is involved in macrophage polarization toward an M2 phenotype that includes A_{2A}R induction (Weis et al., 2009).

The release of proinflammatory cytokines such as TNF-α and IL-12 can be inhibited by either A_{2A}R or A_{2B}R activation. A_{2B}R receptors are induced in response to arterial injury or by IFN-γ. Stimulation of A_{2B}Rs inhibits the IFN-γ-induced expression of MHC class II genes, nitric oxide synthase, and proinflammatory cytokines (Xaus et al., 1999).

In addition to binding adenosine, the A_{2B}R has also been reported to bind another anti-inflammatory signaling molecule, netrin-1 (Corset et al., 2000). Netrin-1 mediates its functions through stimulation of the deleted in colorectal cancer (DCC) family receptors DCC and neogenin, and the UNC5 family receptors UNC5A, UNC5B, UNC5C, and UNC5D (Barallobre et al., 2005). Netrin-1 can act as chemoattractant or chemorepellent. The DCC family of receptors mediates attraction to netrin-1, whereas the UNC5 family of receptors forms a netrin-1-dependent complex with DCC and mediates repulsion (Hong et al., 1999). In addition to its function in neuronal development, netrin-1 expressed outside the nervous system inhibits migration of leukocytes *in vitro* and *in vivo* and attenuates inflammation-mediated tissue injury. The netrin-1 receptor UNC5B is highly expressed on human monocytes, granulocytes, and lymphocytes, and netrin-1 acting through UNC5B receptor inhibits migration of monocytes (Wang et al., 2009) *in vitro*. Activation of the

$A_{2B}R$, originally proposed to contribute to netrin effects on axons, is not required for axon outgrowth or Xenopus spinal axon attraction to netrin-1. Thus, DCC plays a central role in netrin signaling of axon growth and guidance independent of $A_{2B}R$ activation (Stein et al., 2001). Administration of recombinant netrin-1 before or after renal IRI reduced kidney injury, apoptosis, monocyte and neutrophil infiltration, and cytokine and chemokine production (Tadagavadi et al., 2010). Analysis of different netrin-1 receptors on leukocytes showed very high expression of UNC5B but little or no expression of UNC5A, UNC5C, UNC5D, neogenin, or DCC. These findings suggest that the $A_{2B}R$ may in fact not be the netrin-1 receptor. Rather, $A_{2B}R$ activation may influence the expression of the netrin receptor, UNC5B, on macrophages and other leukocytes. Neutralization of UNC5B receptor reduced netrin-1-mediated protection against renal IRI, and it increased monocyte and neutrophil infiltration, as well as serum and renal cytokine and chemokine production, with increased kidney injury. These studies suggest that netrin-1 acts through UNC5B receptors that are regulated by $A_{2B}R$ signaling to reduce inflammation.

D. T Cells

Incubation of purified C57BL/6 murine CD4(+) T lymphocytes with anti-CD3 mAb serves as a model of TCR-mediated activation and results in increased IFN-γ production and cell surface expression of activation markers, CD25 and CD69. Signaling through the TCR causes a rapid fivefold increase in $A_{2A}R$ mRNA, which is correlated with a significant increase in the efficacy of $A_{2A}R$-mediated cAMP accumulation in these cells (Lappas et al., 2005). $A_{2A}R$ stimulation not only inhibits the generation of adaptive effector T cells but also promotes the induction of adaptive regulatory T cells. *In vitro*, antigen recognition in the setting of $A_{2A}R$ engagement induces T-cell anergy, even in the presence of costimulation (Zarek et al., 2008). T cells initially stimulated in the presence of an $A_{2A}R$ agonist fail to proliferate and produce IL-2 and IFN-γ when rechallenged in the absence of $A_{2A}R$ stimulation.

$A_{2A}R$ stimulation inhibits interleukin-6 expression while enhancing the production of TGF-β. TGF-β favors the production of anti-inflammatory T regulatory cells, while IL-6, in conjunction with TGF-β, favors the production of inflammatory Th17 inflammatory cells. Consequently, treating mice with $A_{2A}R$ agonists not only inhibits Th1 and Th17 effector cell generation but also promotes the generation of Foxp3(+) T regulatory cells. Overall, the effect of $A_{2A}R$ activation on T cells is to promote long-term T-cell anergy and the generation of adaptive T regulatory cells.

$A_{2A}Rs$ also regulate the function of T regulatory cells. Although the transfer of T regulatory cells (CD45RB(low)) blocks colitis induced by pathogenic CD45RB(high) Th cells, CD45RB(low) cells from $A_{2A}R$-deficient mice do not prevent colitis (Naganuma et al., 2006). $A_{2A}R$ agonists suppress the production of proinflammatory cytokines by CD45RB(high) and CD45RB(low) T cells in association with a loss of mRNA stability. In contrast, anti-inflammatory cytokines, including IL-10 and TGF-β, are minimally affected. Oral administration of the $A_{2A}R$ agonist ATL313 attenuated colitis in mice receiving CD45RB(high) Th cells. These data suggest that $A_{2A}R$ activation controls T-cell-mediated

colitis by suppressing the expression of proinflammatory cytokines while sparing anti-inflammatory activity mediated by IL-10 and TGF-β.

A_{2B}R stimulation has not been reported to have strong direct effects on T-cell function. However, activation of A_{2B}Rs may indirectly promote the development of tissue rejection by inhibiting $CD4^+/CD25^+/Foxp3^+$ regulatory T-cell infiltration (Zhao et al., 2010).

E. NKT Cells

A_{2A} agonists have also been found to reduce injury following ischemia or trauma in liver (Alchera et al., 2008; Ben-Ari et al., 2005; Cao et al., 2009; Day et al., 2004, 2005b; Harada et al., 2000), kidney (Day et al., 2003, 2005a; Okusa et al., 1999, 2001), skin (Peirce et al., 2001), lung (Gazoni et al., 2008; Rivo et al., 2007; Sharma et al., 2010), heart (Patel et al., 2009; Rork et al., 2008; Xi et al., 2009; Yang et al., 2006b), intestine (Di Paola et al., 2010), and spinal cord (Cassada et al., 2002; Li et al., 2006; Reece et al., 2008). The cellular targets of A_{2A}Rs initially were not clear. As noted above, platelets, neutrophils, and macrophages express A_{2A}Rs that, respectively, inhibit oxidative burst and adhesion molecule expression (Sullivan et al., 2004a) and cytokine production (Murphree et al., 2005). We introduced *loxp* sites flanking the first A_{2A}R gene, *adora2a*, and crossed these mice to LysMCre mice. All lines were made congenic to C57BL/6J using marker-assisted selection. The resultant LysMCre × $A_{2A}R^{f/f}$mice selectively lack A_{2A}Rs in neutrophils and macrophages. Nevertheless, A_{2A}R activation was still highly effective at reducing injury in response to liver or lung IRI (Reutershan et al., 2007). Adoptive transfer of $CD4^+$ (but not $CD8^+$ T cells) to $Rag1^{-/-}$ mice reconstituted injury from IRI (Zhai et al., 2006). The A_{2A} agonist, ATL146e, inhibited this injury if the transferred cells had A_{2A}Rs, but not if they lacked A_{2A}Rs (Yang et al., 2006b). This result is striking because $Rag1^{-/-}$ mice reconstituted with $A_{2A}R^{-/-}$ $CD4^+$ T cells have a normal complement of A_{2A}Rs in all cells except the reconstituted T cells. The results indicate that despite the widespread distribution of A_{2A}Rs on platelets and leukocytes, A_{2A} agonists reduce IRI primarily by their effects on T cells.

In 2005, Shimamura et al. found that liver reperfusion injury was associated with an expansion and activation of CD1d-restricted NKT cells (Shimamura et al. (2005)). Subsequently, we found that depletion of NKT and NK cells with PK136, an antibody that binds to NK1.1 found only on NKT and NK cells, or an anti-CD1d antibody produces protection from liver IRI that is equivalent to and not additive to protection by ATL146e (Lappas et al., 2006). These studies indicate that the adenosine-sensitive T cells that mediate IRI are iNKT cells. The putative endogenous ligands that are responsible for activating iNKT following IRI have not been identified, but recent studies suggest that tissue injury may result in the formation of one or more galactose-containing glycolipids that can activate the invariant TCR (Darmoise et al., 2010). In addition, iNKT cell activation may be facilitated by the binding of phosphatidylserine on the surface of apoptotic cells to T cell Ig-like mucin-like-1 (TIM-1) receptors on NKT cells (Lee et al., 2010). Hepatic preconditioning produced by preactivating NKT cells protects the liver from IRI via an IL-13 response and induction of A_{2A}Rs (Cao et al., 2009).

As sickle cell disease is characterized by persistent multiorgan microvascular IRI, we examined the role of iNKT cells in sickle cell disease. Deletion or blockade of iNKT cell

activation was found to greatly attenuate pulmonary vaso-occlusive pathophysiology in sickle cell mice. In addition, sickle cell patients were found to have increased numbers of activated iNKT cells in their blood (Wallace et al., 2009). These findings suggest that iNKT cells orchestrate a leukocyte inflammatory cascade that triggers vaso-occlusive episodes. $A_{2A}R$ agonists produce substantial protection to mouse lungs in sickle cell disease, primarily by targeting A_{2A} receptors that are induced on iNKT cells and NK cells (Wallace & Linden, 2010).

III. Disease Relevance of Adenosine to Immune Signaling

A. Diabetes

Inflammation in diabetes may be triggered in part by elevated concentrations of free fatty acids that increase CD11c+ macrophage accumulation and activation in adipose tissue (Nguyen et al., 2007). Insulin resistance due to a high-fat diet causes macrophage accumulation in adipose tissue and M2-like remodeling (Shaul et al., 2010). Endothelial dysfunction is also a hallmark of diabetes because inflammatory mediators activate receptors and transcription factors such as nuclear factor-κB, TLRs, c-Jun amino terminal kinase, and the receptor for advanced glycation end products, which cause systemic endothelial dysfunction (Goldberg, 2009). Signaling through the $A_{2B}R$ also contributes to insulin resistance by altering the production of IL-6 and other cytokines. IL-6 is produced primarily by macrophages and adipocytes and drives the production of CRP.

Several studies have linked adenosine receptor blockade with reversal of insulin resistance. Challis and coworkers reported that adenosine receptor antagonists (Challis et al., 1984) or degradation of adenosine with adenosine deaminase (Budohoski et al., 1984) reverse insulin resistance in skeletal muscle isolated from diabetic animals. The orally active antagonist, adenosine receptor antagonist BW-1433, was found to persistently reverse insulin resistance in obese Zucker rats (Crist et al., 1998, 2001; Xu et al., 1998). In mice rendered insulin resistant due to a high-fat diet, *ADORA2B* gene deletion was reported to reduce body fat, reduce liver glycogen, increase energy expenditure, and increase lean body mass (Treadway et al., 2006). It is notable that statins stimulate the induction of CD73 and have been shown to cause insulin resistance. Statins also enhance ischemia-mediated vasodilation in people, and this is blocked by caffeine, consistent with an effect to enhance adenosine production (Meijer et al., 2010). Enhanced adenosine production, by activating $A_{2B}Rs$, may contribute to the effect of statins to provoke insulin resistance.

Diabetes triggers induction of $A_{2B}R$ mRNA in macrophages and endothelial cells, resulting in increased IL-6 production in response to $A_{2B}R$ activation (Figler et al., 2011). Deletion of the mouse $A_{2B}R$ resulted acutely in a proinflammatory phenotype manifested as mild vascular inflammation at baseline and exacerbation of cytokine production in response to endotoxin (Yang et al., 2006a). Thus, in some settings, signaling by the $A_{2B}R$ reduces inflammation. However, persistant activation of $A_{2B}Rs$ increased IL-6 plasma levels in mice, and by several types of isolated cells (Linden, 2006), including macrophages (Ryzhov et al., 2008b) and dendritic cells (Novitskiy et al., 2008; Ryzhov et al., 2008b). IL-6 is directly involved in stimulating the production of transcription factors that enhance CRP production (Young et al., 2008). Analyses of the cloned human $A_{2B}R$ promoter identified a

functional binding site for hypoxia-inducible factor (Kong et al., 2006) and identified TNF-α and the oxidative stress-promoting enzyme NAD(P)H oxidase as additional regulators of A_{2B}R gene expression (Kolachala et al., 2005). Since elevated TNF-α and oxidative stress are associated with diabetes (Castoldi et al., 2007; Gokulakrishnan et al., 2009), it is reasonable to speculate that these factors contribute to induction of A_{2B}R mRNA in diabetics. Hence, A_{2B}R-facilitated production of IL-6 and other adipokines by macrophages that accumulate in adipose tissue of obese animals and people may contribute to insulin resistance associated with type II diabetes (Figler et al., 2011). Chronic activation of A_{2B}Rs has been implicated in other pathological processes, such as pulmonary fibrosis (Sun et al., 2006).

B. Cancer

Both agonists and antagonists of adenosine receptors have been evaluated in mouse models of cancer, and in some cases have direct effects on tumor cells that sometimes express various adenosine receptor subtypes (Fishman et al., 2009; Merighi et al., 2007). Another approach has been to target adenosine receptors in immunocompetent hosts for blockade as a means of enhancing immune killing of tumors. Most tumors are thought to produce some degree of immune activation that might be exploited to facilitate tumor rejection. For example, in bladder cancer, activation of the immune system by the immune adjuvant bacillus Calmette–Guerin (BCG) has been shown to significantly reduce tumor progression (Demkow et al., 2008). Sequential activation of NKT cells and NK cells provides effective innate immunotherapy of cancer (Smyth et al., 2005). As discussed above, signaling through $A_{2A}$and A_{2B} receptors generally has a strong negative effect on T cell responses. Activation of the A_{2A}R on T effector cells can reduce by 98% INF-γ release (Lappas et al., 2005). A_{2A}R activation on CD1d-restricted NKT cells reduces the production of INF-γ, TNF-α, and IL-2 in response to glycolipid antigens (Lappas et al., 2006). Treating mice with synthetic A_{2A} agonists inhibits Th1 and Th17 effector cell generation and promotes the generation of $Foxp3^+$ regulatory T cells (Zarek et al., 2008). Given the suppressive effects of A_{2A}Rs on T cells and other leukocytes, A_{2A}R blockade or deletion has been investigated to enhance immune killing of tumors. These studies have met with some success in immunocompetent mouse models with syngeneic tumors (Lukashev et al., 2007; Ohta and Sitkovsky, 2011; Ohta et al., 2006). Ohta et al. (2006) showed that solid tumors produce high concentrations of adenosine and demonstrated that genetic deletion of the A_{2A}R resulted in rejection of established immunogenic lung tumors in ∼60% of mice with no rejection observed in control WT mice. Caffeine, a weak nonselective adenosine receptor antagonist, also significantly increased tumor rejection.

In addition to conventional Foxp3+ T regulatory cells, adaptive regulatory T cells (Tr1) are induced in the periphery upon encountering cognate antigens. In cancer, their frequency is increased; however, Tr1-mediated suppression mechanisms have only recently begun to be studied. Both ectonucleotidases (CD39/CD73) and cyclooxygenase 2 (COX-2) are involved in Tr1-mediated suppression. The concomitant inhibition of prostaglandin E2 and adenosine receptors via their common intracellular cyclic AMP pathway has been suggested as an additional approach for improving results of immune therapies for cancer (Mandapathil et al., 2010).

In addition to their effects on the function of T cells, $A_{2A}R$ and $A_{2B}R$ blockade may have indirect effects on tumor angiogenesis. In addition to effects of A_{2B} signaling on macrophages and DCs, both A_{2B} and A_3 receptors have been shown to facilitate the release of angiogenic factors from mast cells (Feoktistov et al., 2003). $A_{2B}R$ blockade impairs production of IL-8 in a mouse melanoma model (Merighi et al., 2009). In a Lewis lung carcinoma isograft model, deletion of the host $A_{2B}R$ lowered tumor levels of VEGF and attenuated tumor growth (Ryzhov et al., 2008a). Since $A_{2A}R$ activation strongly suppresses the production of IFN-γ by both NKT and NK cells, blockade of these receptors increases the production of IFN-γ-inducible chemokines. CXC chemokines are important in controlling leukocyte trafficking, enhancing innate and adaptive immunity, and regulating angiogenesis. CXC chemokines behave as both potent promoters of Th1-dependent cell-mediated immunity and inhibitors of angiogenesis. These chemokines bind to a specific receptor known as CXCR3. This receptor has been found on Th1 T cells, B cells, NK cells, and endothelial cells. The CXCR3 ligands represent the major chemoattractants for the recruitment of Th1 cells during cell-mediated immunity. Recently, CXCR3 has been found to exist in two alternatively spliced mRNAs (CXCR3A and CXCR3B). CXCR3B is expressed on endothelial cells and mediates the angiostatic effects of CXCR3 ligands, whereas CXCR3A appears to be expressed on T cells, B cells, and NK cells (Struyf et al., 2010). IL-2 is the major agonist for triggering the expression of CXCR3A on these leukocytes. The regulation of the expression of CXCR3B on endothelial cells remains to be fully elucidated. In addition to their role in mediating Th1-mediated immunity, CXCR3 ligands are potent and efficacious cytokines for inhibiting angiogenesis induced by VEGF, bFGF, and ELR+ CXC chemokines. $A_{2A}R$ blockade enhances the production of interferon-inducible CXC chemokines to promote Th1 immunity and inhibit angiogenesis. Studies are ongoing in several laboratories to evaluate effects of $A_{2A}R$ and $A_{2B}R$ blockade on tumor progression.

IV. Conclusion

It is now clear that purineric signaling exerts major regulatory effects on the immune system. $A_{2A}R$ activation produces strong anti-inflammatory effects on multiple cell types. As A_{2A} agonists make their way toward the clinic, it may be possible to exploit their anti-inflammatory effects to inhibit tissue injury in response to acute insults such as tissue transplantation, myocardial infarction, and flares in autoimmune diseases or sickle cell anemia. $A_{2B}R$ signaling is more complex. Although $A_{2B}R$ activation seems to produce some of the acute antiinflammatory effects on macrophages as are produced by A_{2A} agonists, acute $A_{2B}R$ activation may elevate blood glucose, and prolonged $A_{2B}R$ signaling results in tissue reparative programs, such as fibrosis, angiogenesis, and IL-6 production that may be detrimental in some instances. $A_{2B}R$ antagonists are currently in clinical development for the treatment of asthma (due in part to inhibition of mast cell deregulation). It will be of interest to determine if such antagonists prove to be useful for the treatment of chronic inflammatory states such as pulmonary fibrosis, type II diabetes, and others.

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Abbreviations

DAMPs	damage-associated molecular patterns
DCC	deleted in colorectal cancer
DCs	dendritic cells
ECs	endothelial cells
HIF-1	hypoxia-inducible factor-α
HO-1	heme oxygenase-1
IL	interleukin
iNKT	invariant NKT
IRI	ischemia reperfusion injury
MHC	major histocompatibility complex
TCR	T cell receptor
TGF-β	transforming growth factor-β
TIM-1	T cell Ig-like mucin-like-1
VEGF	vascular endothelial growth factor

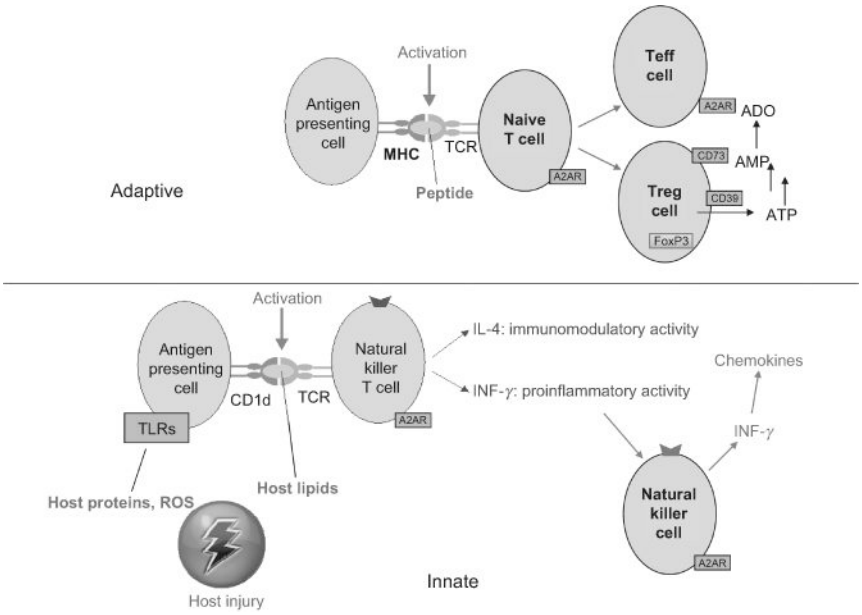

Figure 1.

Comparison of $A_{2A}R$ effects on T cells and iNKT cells. The *top panel* illustrates that the adaptive immune response to peptide antigens are processed by antigen presenting cells and presented on major histocompatibility complex (MHC) molecules to variable T cell receptors. Upon TCR activation, naive T cells expand and generate T effector (Teff) cells, T regulatory (Treg) cells, or other types of daughter T cells. $A_{2A}R$ activation on naive T cells during antigen presentation enhances the production of Treg cells and produces persistent anergy of Teff cells. Activation of $A_{2A}Rs$ on Teff cells during TCR activation suppresses their expansion and cytokine production. Among lymphocytes, only Treg cells express ectonucleotidases CD39 and CD73 that generate adenosine from the extracellular metabolism of adenosine nucleotides. The *bottom panel* illustrates the innate response of NKT cells. Glycolipid antigens can be derived from pathogens but also are thought to be derived from glycolipids derived from necrotic host cells and are presented by the MHC-like antigen presenting molecule, CD1d, to invariant TCRs on NKT cells. NKT cells usually express TCRs and NK cell markers such as NK1.1. Upon activation of their TCR, INKT cells rapidly produce large quantities of several cytokines including IFN-γ and IL-4. NK cells are transactivated by cytokines released form NKT cells and produce additional IFN-γ which stimulates the production of IFN-γ inducible chemokines that recruit additional leukocytes into the inflamed tissues. $A_{2A}Rs$ are induced upon TCR activation of NKT and NK cells, and $A_{2A}R$ signaling strongly suppresses cytokine production by these cells.

Table I

Summary of the Effects of $A_{2A}R$ and $A_{2B}R$ Signaling on Some Cells of the Immune System

	A_{2A}	***A_{2B}***	***Other***
Platelets	↑ Cyclic AMP ↓ Aggregation ↓ Secretion ↑ Leukocyte heteroaggregates	↓ $P2Y_1$ expression ↓ ADP-induced aggregation	ADP and ATP receptors
Neutrophils	↑ Cyclic AMP ↓ Oxidative burst ↓ α4/β1 integrin(VLA-4)		ATP release, pannexin channels
Macrophages	↑ M1 to M2-like switch ↓ TNF-α, IL-12 ↑ VEGF, IL-10 Induced by HO-1 Induced by endotoxin	↓ TNF, IL-12 ↑ VEGF, IL-10 ↑ IL-6 Induced by HIF Induced by IFN-γ Induced by diabetes Controls UNC5B expression	M1 inflammatory M2 angiogenic
T cells	↑ Cyclic AMP ↓ IFN-γ production ↓ CD-69 ↓ Proliferation, IL-2 ↑ Anergy Induced by TCR activation ↑ Treg production ↑ Treg function		CD73 and CD39 (Tregs only)
iNKT cells	↑ Cyclic AMP ↓ IFN-γ production ↓ TNF-α Induced by TCR activation		Activated by lipidantigens Coactivated by TIM-1 Coactivates NK cells