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Role of adenosine A_{2B} receptors in inflammation

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Abstract

Recent progress in our understanding of the unique role of A_{2B} receptors in the regulation of inflammation, immunity and tissue repair was considerably facilitated with the introduction of new pharmacological and genetic tools. However, it also led to seemingly conflicting conclusions on the role of A_{2B} adenosine receptors in inflammation with some publications indicating pro-inflammatory effects and others suggesting the opposite. This chapter reviews the functions of A_{2B} receptors in various cell types related to inflammation and integrated effects of A_{2B} receptor modulation in several animal models of inflammation. It is argued that translation of current findings into novel therapies would require a better understanding of A_{2B} receptors functions in diverse types of inflammatory responses in various tissues and at different points of their progression.

Keywords

Adenosine; Inflammation; Neovascularization; Receptors Purinergic P1; Tissue Remodeling

I. Introduction

The extracellular accumulation of adenosine contributes to the regulation of inflammation, immunity and tissue repair. Adenosine exerts its action by interacting with four adenosine receptor subtypes, A₁, A_{2A}, A_{2B} and A₃ that belong to the family of seven transmembrane G protein-coupled receptors (Fredholm et al., 2001a).

Among adenosine receptor subtypes the A_{2B} receptor has the lowest affinity to adenosine requiring micromolar concentrations to become functional, whereas the affinities of other adenosine receptor subtypes are significantly higher, rendering them active well below micromolar concentrations of adenosine (Fredholm et al., 2001b). Therefore, it is likely that A_{2B} receptors remain silent under the resting conditions when extracellular adenosine concentrations are low, estimated between ten and few hundred nM range (Fredholm, 2007), but their role becomes more important in pathophysiological conditions when adenosine concentrations are the highest.

Because both A_{2A} and A_{2B} receptor subtypes stimulate adenylate cyclase, A_{2B} receptors were often viewed as being a redundant low affinity version of A_{2A} receptors. However, there is also strong evidence that A_{2B} receptors play a non-redundant role distinct from, and often opposite to that of A_{2A} receptors.

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II. Tools to study A_{2B} adenosine receptor function

Recent progress in our understanding of the unique role of A_{2B} receptors was considerably facilitated with the introduction of new pharmacological and genetic tools. Results from using these complementary approaches, however, have not always agreed, with some publications indicating pro-inflammatory effects and others suggesting the opposite. We will address these issues later in this chapter, but first will discuss potential pitfalls in using genetic and pharmacological tools that might have contributed to inadvertent misinterpretation of results.

Generation of mice deficient in A_{2B} adenosine receptor provided a powerful tool for investigating its function in vivo and in vitro. Several studies have found significant differences between A_{2B}KO and WT mouse phenotypes at rest. These differences include increases in basal TNF- α secretion, leukocyte adhesion and vascular permeability as well as changes in expression of E-selectin, P-selectin, ICAM-1, I κ B and P2Y₁ receptors (Eckle et al., 2008a; Yang et al., 2010; Yang et al., 2006). This is surprising because basal levels of adenosine are thought to be too low to activate A_{2B} receptors. As with many gene knockouts there is always concern that potential compensatory changes in the expression of other proteins may confound the specific role of A_{2B} receptors in any given process. Therefore, it is important to keep in mind limitations of gene knockouts so that appropriate controls can be included and correct conclusions can be made.

A number of potent and selective A_{2B} receptor antagonists have been synthesized over the last decade (for review see (Kalla and Zablocki, 2009)). They were used together with antagonists of other adenosine receptor subtypes to elucidate the role of A_{2B} receptors in inflammatory models. When interpreting these studies, however, attention should be paid to the use of antagonists at concentrations that remain selective for the receptor to be targeted. For example, DPCPX and ZM241385 are often used as selective A₁ and A_{2A} antagonists, respectively, but the fact that they can also bind to human and mouse A_{2B} receptors at low nanomolar concentrations (Kreckler et al., 2006; Linden et al., 1999) is often overlooked. Table in this chapter summarizes binding affinities of adenosine antagonists at human and mouse adenosine receptor subtypes commonly used to study the role of A_{2B} receptors.

The goal of attaining selectivity for A_{2B} agonists has been more elusive than for antagonists but several potent A_{2B} agonists have been developed recently (for review see (Baraldi et al., 2009)). BAY 60-6583 has been described as a high-affinity (EC₅₀ of 3 nM) and specific A_{2B} agonist based on its effects on the activity of reporters co-expressed together with human adenosine receptors (Eckle et al., 2007). However, the reported affinity of BAY 60-6583 to A_{2B} receptors determined by radioligand binding were approximately two order magnitude lower (K_i of 330 – 750 nM) (Auchampach et al., 2009). It is likely, therefore, that the affinity of BAY 60-6583 to A_{2B} receptors was overestimated in a functional reporter assay, possibly due to the significant receptor reserve in cells overexpressing A_{2B} receptors (Linden et al., 1999). Comparative binding studies at all four adenosine receptor subtypes would be necessary to validate the selectivity of this compound toward A_{2B} receptors.

In summary, the combination of novel genetic and pharmacological approaches provides powerful tools to dissect the role of adenosine signaling through A_{2B} receptors in physiological and pathological processes. As important as these advances are, we wanted to alert the reader to the importance of taking into account the pitfalls and limitations of these approaches for the correct interpretation of results of studies on the role of A_{2B} receptors in adenosine-dependent regulation of inflammatory responses.

III. A_{2B} receptors on immune cells

A. Neutrophils, lymphocytes, platelets

A_{2B} receptors are ubiquitously expressed and, therefore, it is not surprising that they are present on various cells of hematopoietic origin. In most cases, A_{2B} receptors are co-expressed with A_{2A} receptors. A_{2B} receptor transcripts are found in neutrophils (Fredholm et al., 1996) lymphocytes (Gessi et al., 2005) and even platelets (Amisten et al., 2008). Little is known about their specific functions in these cells and A_{2A} receptors appear to predominate (Csoka et al., 2008; Fredholm et al., 1996; Yang et al., 2010).

Recently, the neuronal guidance molecule netrin-1 was proposed to inhibit neutrophil migration through activation of A_{2B} receptors located on these cells (Rosenberger et al., 2009). Although intriguing, the issue of interactions between netrin-1 and A_{2B} receptors remains controversial. It has been previously hypothesized that A_{2B} receptors can also serve as receptors for netrin-1 (Corset et al., 2000). However, there is evidence against a direct effect of netrin-1 on A_{2B} receptors. First, binding assays in COS cells overexpressing A_{2B} receptors failed to demonstrate netrin-1 binding to these receptors (McKenna et al., 2008). Second, the lack of effects of 100 μ M DPCPX and ZM241385, known to inhibit human A_{2B} receptors (see Table), on the netrin-1-dependent inhibition of neutrophil migration (Rosenberger et al., 2009) argues against this hypothesis.

B. Mast cells

1. Role of A_{2B} receptors in mast cell degranulation—Adenosine has distinct effects on mast cell degranulation of pre-formed mediators and on release of newly generated cytokine/growth factors. Adenosine is known to potentiate antigen-induced degranulation of mast cells. This effect of adenosine is mediated by A₃ adenosine receptors in rodent (Ramkumar et al., 1993; Salvatore et al., 2000), but perhaps not in human (Walker et al., 1997) or canine (Auchampach et al., 1997) mast cells.

The role of A_{2B} receptors in mast cell degranulation is less clear. A_{2B}, but not A₃ receptors both stimulated directly and potentiated the effects of the calcium ionophore A23187 on degranulation of canine BR mastocytoma cells (Auchampach et al., 1997). Conversely, A_{2B} receptors were proposed to play an inhibitory role in degranulation of mouse bone marrow derived mast cells (BMMCs), based on the finding that A_{2B}KO mice show an exaggerated antigen-induced mast cell degranulation (Hua et al., 2007). Acknowledging the implausibility of explaining this phenomenon by tonic stimulation of the low-affinity A_{2B} receptor by endogenous adenosine, Hua et al proposed the alternative explanation that A_{2B} receptors may be constitutively active in wild type BMMCs even in the absence of an agonist. However, we determined that A_{2B} receptors expressed in wild type BMMCs display no constitutive activity. Furthermore, our work demonstrated that A_{2B} receptors do not inhibit the A₃ receptor-mediated potentiation of antigen-induced degranulation of BMMCs (Ryzhov et al., 2008d). It is likely therefore that exaggerated antigen-induced degranulation in BMMCs deficient of A_{2B} receptors is unrelated to the loss of adenosine signaling function of A_{2B} receptors. Compensatory developmental changes in mice or rearrangement of proteins normally coupled to the A_{2B} receptor as a result of the A_{2B} knockout may have contributed in this phenomenon.

In contrast, pharmacological studies suggested that A_{2A} but not A_{2B} receptors can attenuate antigen-induced degranulation in human cord blood-derived mast cells (Suzuki et al., 1998) and primary human lung mast cells (Duffy et al., 2007). This effect was attributed to the ability of A_{2A} receptors to close K⁺ channel KCa3.1 by a cAMP-independent mechanism (Duffy et al., 2007).

2. Role of A_{2B} receptors in cytokine/growth factor secretion from mast cells—

The role of A_{2B} receptors in regulation of cytokine/growth factor secretion from mast cells is better understood than its effects on degranulation. Studies in HMC-1 cells showed that only A_{2B}, but not A_{2A} or A₃ receptors stimulate secretion of angiogenic factors IL-8 and VEGF, and the Th2 cytokines IL-13 and IL-4 (Feoktistov and Biaggioni, 1995; Feoktistov et al., 2003; Ryzhov et al., 2006; Ryzhov et al., 2004). Conditioned media from A_{2B} receptor-activated mast cells stimulated human umbilical vein endothelial cell (HUVEC) proliferation and migration, and induced capillary tube formation. These pro-angiogenic effects of A_{2B} receptor-stimulated mast cells were attributed primarily to VEGF release, because they were blocked by anti-VEGF antibody (Feoktistov et al., 2003). Co-culturing B lymphocytes with A_{2B} receptor-stimulated mast cells induced IgE production by B lymphocytes, an effect that appeared to be secondary to increased secretion of Th2 cytokines IL-4 and IL-13 by the mast cells (Ryzhov et al., 2004).

Like human HMC-1 cells, mouse BMMCs express A₃, A_{2A} and A_{2B}, but not A₁ receptors (Feoktistov et al., 2003; Meade et al., 2002; Ryzhov et al., 2008d). Activation of adenosine receptors stimulated IL-13 and VEGF secretion only in wild type (WT) but not in A_{2B}KO BMMCs. Contrary to adenosine action on degranulation, which is only apparent in antigen-stimulated BMMCs, these effects do not require activation of FcεRI receptors, since they are evident even in the absence of antigen (Ryzhov et al., 2008d).

The notion that only A_{2B}, but not A_{2A} receptors co-expressed in the same cells, were able to stimulate cytokine/growth factor secretion could seemed paradoxical because both receptor subtypes were thought to act by stimulation of adenylate cyclase through Gs proteins. However, studies in HMC-1 revealed that in contrast to A_{2A} receptors, A_{2B} receptors are also coupled to phospholipase C (PLC), as evidenced by increase in inositol phosphate production with consequent mobilization of intracellular calcium. These A_{2B} receptor-dependent pathways are stimulated through a cholera toxin- and pertussis toxin-insensitive G-protein, presumably of the Gq family (Feoktistov and Biaggioni, 1995). In addition, stimulation of A_{2B} receptors activates the small GTP-binding protein p21ras. This event triggers ERK signaling pathway with sequential stimulation of Raf, MEK1/2 and ERK1/2 protein kinase activities (Feoktistov et al., 1999). We have also demonstrated the coupling of adenosine receptors to JNK and p38 MAPK signaling pathways (Feoktistov et al., 1999). The fact that A_{2B} receptors are coupled to multiple intracellular signaling pathways in mast cells, explains their ability to regulate the generation and secretion of diverse cytokines and growth factors.

A_{2B} receptors stimulate release of VEGF, IL-13 and IL-8 from mast cells by mechanisms that involve activation of ERK and p38 MAPK (Ryzhov et al., 2006; Ryzhov et al., 2008d). Stimulation of the receptor tyrosine kinase c-kit with stem cell factor (Meade et al., 2002) or the receptor complex ST2/IL1RAP with IL-33 (Silver et al., 2010) synergized with A_{2B} receptors in the upregulation of IL-8 from HMC-1 cells. Functional analysis of cells transfected with full-length and truncated receptor constructs revealed that the A_{2B} receptor C-terminus is important for coupling to Gs and Gq proteins. However, the A_{2B} receptor C-terminus is not essential for upregulation of IL-8. Instead, integrity of the third intracellular loop of the A_{2B} receptor was crucial for IL-8 stimulation (Ryzhov et al., 2009).

Whereas A_{2B} receptor-mediated stimulation of IL-8 and IL-13 is cAMP-independent, stimulation of adenylate cyclase was required (but not sufficient) for upregulation of VEGF and IL-4 (Ryzhov et al., 2006; Ryzhov et al., 2008d). The dual coupling of A_{2B} receptors to Gs/Gq proteins with concurrent stimulation of diverse intracellular pathways is necessary for adenosine-dependent regulation of IL-4 production in HMC-1. A_{2B} adenosine receptors induce IL-4 generation via Gq-mediated stimulation of PLCβ, inositol trisphosphate-

mediated mobilization of intracellular Ca^{2+} and activation of NFAT by calcineurin. This process is potentiated via Gs-mediated stimulation of adenylate cyclase and activation of protein kinase A (PKA), and may involve the increase in protein levels of NFATc1. Thus, the existence of cross-talk between Gq- $\text{PLC}\beta$ and Gs-adenylate cyclase signaling pathways in regulation of IL-4 secretion enables $\text{A}_{2\text{B}}$ receptors, coupled to both Gq and Gs, to effectively stimulate IL-4 production in mast cells (Ryzhov et al., 2006).

C. Dendritic cells

1. Dendritic cell functions—Dendritic cells play an important role in bridging innate and adaptive immunity. It is generally accepted that conventional dendritic cells arise from bone-marrow hematopoietic progenitors or peripheral blood monocytes that migrate into peripheral tissues and differentiate into immature dendritic cells. Immature dendritic cells in tissues are constantly sampling their microenvironment for the presence of antigens. Upon activation by pathogens and other inflammatory stimuli, dendritic cells undergo phenotypical maturation and migrate toward the secondary lymphoid organs. On reaching these organs, dendritic cells develop into mature cells capable to present antigens to naïve T lymphocytes, thus initiating the development of adaptive immune responses (Dominguez and Ardavin, 2010). The presence of $\text{A}_{2\text{B}}$ receptors on monocytes and dendritic cells (Novitskiy et al., 2008) suggests that their activation may influence both differentiation and maturation of dendritic cells.

2. Role of $\text{A}_{2\text{B}}$ receptors in dendritic cell differentiation—Using a combination of genetic and pharmacological approaches, we have recently shown that stimulation of $\text{A}_{2\text{B}}$ receptors in vitro and in vivo induces generation of a phenotypically and functionally distinct subset of dendritic cells. These “adenosine-differentiated” cells are impaired in their ability to induce T-cell proliferation and $\text{IFN-}\gamma$ production. These cells also produce high levels of immunomodulatory cytokines IL-6, IL-10 and $\text{TGF-}\beta$. It is possible that by up-regulating IL-10 and $\text{TGF-}\beta$, adenosine-differentiated dendritic cells could affect Th1-mediated immune reactions, induce the generation of regulatory T cells, and polarize the immune response toward a Th2 type. Because these cells also express high levels of the tolerance-inducing enzymes indoleamine 2,3-dioxygenase (IDO) and arginase, they can impair T-cell signal transduction and function. Adenosine-differentiated dendritic cells also secrete high levels of angiogenic factors VEGF and IL-8. Both immunosuppressive and pro-angiogenic properties of these cells could be beneficial for tumor growth. Indeed, our studies in vivo demonstrated that the presence of “adenosine-differentiated” dendritic cells significantly promoted tumor growth in a mouse Lewis lung carcinoma model (Novitskiy et al., 2008).

3. Role of $\text{A}_{2\text{B}}$ receptors in dendritic cell maturation—In addition to modulation of cell differentiation, $\text{A}_{2\text{B}}$ receptors were shown also to affect maturation of dendritic cells. $\text{A}_{2\text{B}}$ receptors inhibited Th1 immune response-promoting cytokines IL-12 p70 and IL-23 but enhanced IL-10 secretion by TLR-activated bone-marrow-derived dendritic cells. Moreover, stimulation of $\text{A}_{2\text{B}}$ receptors during dendritic cell maturation increased expression and enzymatic activity of IDO and arginase in LPS-activated dendritic cells (Ben et al., 2008). $\text{A}_{2\text{B}}$ receptors were shown to be responsible for formation of a dendritic cell fraction expressing low levels of MHC-II and co-stimulatory molecule CD86. These cells were characterized by an increased expression of $\text{A}_{2\text{B}}$ receptor transcripts compared to the rest of dendritic cell population. Only this subset of “adenosine-matured” dendritic cells expressed lower levels of IL-12p40 but higher levels of IL-10 and had poor capacity to stimulate CD4^{+} T cells, compared to cells matured in the absence of adenosine stimulation (Wilson et al., 2009). Thus, stimulation of $\text{A}_{2\text{B}}$ receptors, not only during dendritic cell differentiation but also during maturation, may lead to the formation of a cell population capable of impairing

Th1 differentiation of CD4⁺ T cells and promoting immune tolerance. The role of A_{2B} receptors in adenosine actions on both differentiation and maturation of dendritic cells appears to be non-redundant because specific stimulation of other adenosine receptor subtypes did not produce similar effects. Furthermore, these effects were observed only in WT but not A_{2B}KO cells and were inhibited by selective A_{2B} antagonists (Ben et al., 2008; Novitskiy et al., 2008; Wilson et al., 2009).

D. Monocytes/Macrophages

Adenosine has been recognized as an important regulator of monocyte/macrophage functions. Some responses to adenosine are mediated by cAMP-dependent mechanisms. These responses include the inhibition of LPS-induced TNF- α production (Kreckler et al., 2009), the potentiation of IL-10 production (Nemeth et al., 2005), and the inhibition of proliferation induced by monocyte colony stimulating factor (M-CSF) (Xaus et al., 1999b). The role of A_{2B} receptors in adenosine-dependent regulation of these monocyte/macrophage functions is often masked by A_{2A} receptors co-expressed in the same cells. A_{2B} receptors can control cAMP-dependent functions only in those cell models where the expression of dominant A_{2A} receptors is negligible or absent. For example, macrophages generated from mouse bone marrow in vitro expressed predominantly A_{2B} adenosine receptors and only negligible levels of A_{2A} receptors. Stimulation of A_{2B}, but not A_{2A} receptors increased cAMP levels in this cell preparation and inhibited M-CSF-induced macrophage proliferation by upregulating the cyclin-dependent kinase inhibitor p27Kip-1 via activation of cAMP-PKA pathway (Xaus et al., 1999b). IFN- γ further upregulated the expression of A_{2B} receptors on bone marrow-derived macrophages resulting in an increased cAMP production in response to stimulation with NECA, which in turn down-regulated both MHC-II and iNOS expression (Xaus et al., 1999a). Due to negligible expression of A_{2A} receptors on RAW264.7 cells, A_{2B} receptors were capable of inhibiting TNF- α production and augmenting IL-10 production in this macrophage-like cell line following activation with LPS (Nemeth et al., 2005).

Similarly, A_{2B} receptors inhibited LPS-induced TNF- α release in peritoneal macrophages isolated from mice lacking A_{2A} receptor. However, selective inhibition of A_{2B} receptors had no effect on the adenosine-dependent inhibition of LPS-induced TNF- α secretion from WT mouse peritoneal macrophages due to the dominant role of A_{2A} receptors in regulation of this cAMP-dependent event (Kreckler et al., 2006). Indeed, NECA-induced cAMP accumulation was similar in peritoneal macrophages obtained from WT and A_{2B}KO animals, indicating the dominant role of A_{2A} receptors in this process. Furthermore, the absence of A_{2B} adenosine receptors did not affect adenosine receptor-dependent suppression of LPS-activated TNF- α release from peritoneal macrophages (Ryzhov et al., 2008c). Comprehensive pharmacological analysis of adenosine-dependent inhibition of LPS-induced TNF- α release from human primary monocytes (Zhang et al., 2005) and alveolar macrophages (Buenestado et al., 2010) revealed that this effect was exclusively mediated by A_{2A} receptors, corroborating the findings in mouse peritoneal macrophages.

However, A_{2B} receptors may have distinctive functions in macrophages even in the presence of otherwise dominant A_{2A} receptors. Pre-treatment of mouse primary alveolar macrophages with the selective A_{2B} antagonist MRS 1706 or genetic ablation of A_{2B} receptors resulted in a loss of NECA-stimulated increases in osteopontin expression (Schneider et al., 2010). Likewise, pharmacological inhibition with selective A_{2B} antagonists or genetic ablation of A_{2B} receptors completely abrogated NECA-induced increase in IL-6 release from peritoneal macrophages (Ryzhov et al., 2008c). Of interest, elevation of cAMP in murine macrophages attenuates LPS-induced TNF- α secretion (Kreckler et al., 2009), but has no effect on basal IL-6 release (Tang et al., 1998). It is possible that the differential regulation of TNF- α and IL-6 secretion by A_{2A} and A_{2B} receptors in mouse peritoneal macrophages can be explained

by coupling of these receptors to distinct intracellular pathways. Further studies are needed to delineate the signaling pathways linking activation of A_{2B} receptors to cytokine production in macrophages.

IV. A_{2B} receptors on endothelial cells

A. Endothelial cells in inflammation

Vascular endothelium lines all vessels in the body and serves as a dynamic and selective barrier regulating the flow of nutrients, biologically active molecules and cells across blood vessel walls. Endothelial cells, in close cooperation with other cell types, play an important role in inflammation and subsequent tissue remodeling. Activation of endothelial cells by inflammatory stimuli increases vascular permeability and the expression of adhesion molecules on the endothelial surface, which in turn promote edema, and leukocyte attachment and extravasation leading to initiation of an inflammatory cascade. Later in inflammation, endothelial cells play a central role in the expansion, regression and remodeling of pre-existing blood vessels, a process commonly known as angiogenesis. Adenosine has been implicated in modulation of all these events (Sands and Palmer, 2005).

Differential expression of cell surface adenosine receptors is part of the phenotypic heterogeneity of endothelial cells, and endothelial responses to adenosine can differ depending on the relative expression of adenosine receptor subtypes. For example, HUVECs express predominantly A_{2A} adenosine receptors, whereas the human microvascular endothelial cells HMEC-1 express predominantly A_{2B} receptors (Feoktistov et al., 2002). Predominant expression of A_{2B} receptors has been demonstrated also in cardiac microvascular endothelial cells (Ryzhov et al., 2008b) and endothelial cells of high endothelial venules (Takedachi et al., 2008). Furthermore, there are multiple factors that can modify an endothelial phenotype, including mechanical forces, biologically active compounds, the composition of extracellular matrix, and contact with circulating and tissue-based cells. Conditions present in inflammatory processes provide powerful stimuli for such phenotypic changes. The Th1 cytokines IL-1 and TNF- α increase expression of both A_{2A} and A_{2B} adenosine receptors in human dermal microvascular endothelial cells. IFN- γ treatment increases the expression of A_{2B} receptors, but decreases the expression of A_{2A} receptors (Khoa et al., 2003). Hypoxia, a condition often present in inflamed tissues, also selectively increases A_{2B} expression in endothelial cells (Eltzschig et al., 2003; Feoktistov et al., 2004) by a mechanism that involves the oxygen-sensitive HIF-1 α -dependent transactivation of the A_{2B} receptor promoter (Kong et al., 2006). An increase in the A_{2B} receptor expression may not only increase the effects of adenosine but also affect their outcomes. For example, HUVECs express predominantly A_{2A} adenosine receptors, and do not produce VEGF in response to adenosine (Feoktistov et al., 2002). Hypoxia decreased A_{2A} and increased A_{2B} receptor expression in these cells. Consistent with these changes in receptor expression, adenosine stimulated VEGF release under hypoxic but not normoxic conditions, indicating that hypoxia increased the expression of A_{2B} receptors that were functionally coupled to upregulation of VEGF (Feoktistov et al., 2004).

B. Role of A_{2B} receptors in the expression of endothelial adhesion molecules

Adenosine has been shown to inhibit the expression of adhesion molecules and leukocyte recruitment by activated endothelial cells. Whereas A_{2A} receptors have been implicated in these effects both in vitro and in vivo (Palmer and Trevethick, 2008), the role of A_{2B} receptors is less understood.

Genetic ablation of A_{2B} receptor in mice produced a phenotype that was interpreted as supporting an inhibitory role of A_{2B} receptors in the expression of endothelial adhesion molecules and leukocyte recruitment. Intravital examination of mesenteric venules revealed

an increased number of leukocytes rolling or adhered to the vascular wall of A_{2B}KO mice as compared to WT mice. Based on these findings and also on the increased levels of the adhesion molecules ICAM-1, P-selectin and E-selectin in protein extracts isolated from mesenteric arteries of A_{2B}KO mice, it has been suggested that A_{2B} receptors tonically downregulate the expression of endothelial adhesion molecules and leukocyte recruitment (Yang et al., 2006). However, these effects of A_{2B} receptor ablation in vivo are likely to be secondary to the increased basal plasma levels of TNF- α , a potent activator of endothelial adhesion molecules (Mackay et al., 1993). In contrast, our studies showed that stimulation of A_{2B} receptors in cardiac microvascular endothelial cells induced rapid cell surface expression of P-selectin by a mechanism likely involving exocytosis of the content of Weibel-Palade bodies (Ryzhov et al., 2008b). Further studies are needed to elucidate the role of endothelial A_{2B} receptor activation on the expression of adhesion molecules involved in the recruitment of inflammatory cells by activated endothelium.

C. Role of A_{2B} receptors in regulation of endothelial barrier function

Numerous studies in vitro have shown that adenosine acting on A_{2A} or/and A_{2B} receptors decreases endothelial permeability (for review see (Biaggioni and Feoktistov, 2005)). In vivo, mice lacking either apyrase (CD39) or ecto-5'-nucleotidase (CD73), enzymes involved in the generation of extracellular adenosine, had a higher leakage of albumin through endothelium in various tissues, as measured by the Evans Blue technique (Eltzschig et al., 2003; Thompson et al., 2004). Remarkably, a similar phenotype was found in mice lacking A_{2B} receptors but not other adenosine receptor subtypes (Eckle et al., 2008a). The increased basal vascular permeability was even further increased in A_{2B}KO mice subjected to ambient hypoxia (Eckle et al., 2008a) supporting the previous evidence obtained in vitro on the role of A_{2B} receptors in regulation of endothelial barrier function. Studies in bone marrow chimeric mice suggested a predominant role of vascular A_{2B} receptors but not those located on bone marrow-derived cells in this response. Surprisingly, A_{2A}KO mice did not demonstrate loss of barrier function in vivo (Eckle et al., 2008a) as it would be expected from in vitro studies that implicated A_{2A} receptors in adenosine-dependent regulation of permeability of several endothelial cell types (Umapathy et al., 2010; Wang and Huxley, 2006). Although some of the differences in vascular permeability between A_{2B}KO and A_{2A}KO mice used in these studies may be attributed to their different genetic background (C57Bl6 and CD1, respectively), these results can be explained by the predominant role of A_{2B} receptors in hypoxic endothelial cells due to hypoxia-inducible factor-1 α (HIF-1 α)-dependent increase in A_{2B} receptor expression (Kong et al., 2006). HIF-1 α was also shown to transactivate the promoter of CD73 (Synnestvedt et al., 2002) and repress the promoter of the equilibrative nucleoside transporter (Eltzschig et al., 2005). Taken together, these effects of hypoxia would upregulate A_{2B} receptors and produce high levels of adenosine at the endothelial surface, thus promoting A_{2B} receptor signaling. However the reason for the augmented vascular permeability in A_{2B}KO mice at rest, when extracellular adenosine levels are low, is less clear. Explanation of this phenomenon will require further investigation.

Reduction of endothelial permeability by adenosine seems to be mediated by the cAMP-PKA-dependent pathway, because this effect was mimicked by reagents elevating cAMP or stimulating PKA. These effects were associated with a rearrangement of the F-actin component of the cytoskeleton, enhanced cell-surface expression of cell-cell junctional protein VE-cadherin and an involvement of myosin-light-chain phosphatase (Umapathy et al., 2010). However, the exact mechanism of adenosine-induced endothelial barrier enhancement remains largely unknown. It has been proposed that it can be explained in part by relaxation of actin cytoskeletal tension, as a result of phosphorylation by PKA of actin-associated, vasodilator-stimulated phosphoprotein (Comerford et al., 2002). Inhibition of

RhoA-dependent pathway has been implicated in adenosine- and cAMP-dependent regulation of endothelial barrier function (Harrington et al., 2004; Waschke et al., 2004). In addition, stimulation of ERK via A_{2B} receptors was also proposed to promote barrier function through dephosphorylation of the myosin II regulatory light chains (Srinivas et al., 2004).

D. Role of A_{2B} receptors in regulation of endothelial cell proliferation

Depending on the endothelial cell studied, either A_{2A} or A_{2B} receptors have been implicated in stimulation of endothelial cell proliferation. Adenosine A_{2B} receptors have been shown to mediate the proliferative actions of adenosine in human retinal microvascular endothelial cells (Afzal et al., 2003; Grant et al., 2001; Grant et al., 1999; Mino et al., 2001), in porcine coronary artery and rat aortic endothelial cells (Dubey et al., 2002). The proliferative effects of adenosine on endothelial cells are mediated at least partly by stimulating the production of growth factors that facilitate new blood vessel formation. Adenosine increased VEGF production in pig cerebral microvascular endothelial cells (Fischer et al., 1995), but the adenosine receptor subtype involved in VEGF upregulation in these cells remains uncertain because of non-specific concentrations of antagonists used in that study. Takagi and associates showed that adenosine upregulates VEGF mRNA in bovine retinal microvascular cells via A_{2A} receptors (Takagi et al., 1996).

However, adenosine upregulated VEGF mRNA expression and protein secretion via A_{2B} receptors in human retinal endothelial cells (Grant et al., 1999). VEGF is not the only angiogenic factor modulated by adenosine in endothelial cells. In human retinal microvascular endothelial cells, A_{2B} receptor activation upregulated also basic fibroblast growth factor (bFGF) and insulin-like factor-1 (Grant et al., 1999). In immortalized human dermal microvascular endothelial cells HMEC-1, stimulation of A_{2B} adenosine receptors upregulated bFGF and IL-8 in addition to VEGF (Feoktistov et al., 2002). The intracellular mechanisms mediating these effects are not clear. Although some data suggest that cAMP may play a role in the effects of adenosine on VEGF secretion (Takagi et al., 1996), other studies found that cAMP-PKA-independent stimulation of MAPK pathways was primarily responsible for pro-angiogenic effects of A_{2B} receptors on microvascular endothelial cells (Feoktistov et al., 2002; Grant et al., 2001; Grant et al., 1999).

It is commonly accepted that VEGF production is regulated by HIF-1 α . However, A_{2B} receptors can upregulate VEGF production in microvascular endothelial cells by a HIF-1 α -independent mechanism (Feoktistov et al., 2004). Because adenosine levels are increased in hypoxia, stimulation of A_{2B} receptors on endothelial cells can complement HIF-1 α -dependent actions of hypoxia in the regulation of angiogenesis. Stimulation of A_{2B} receptors results in secretion of additional angiogenic factors (IL-8) not induced by hypoxia per se (when adenosine is scavenged by adenosine deaminase) and in greater VEGF production from endothelial cells (Ryzhov et al., 2007). Thus, A_{2B} receptor-dependent release of angiogenic factors can contribute to the overall effect of hypoxia and provide an autocrine pathway regulating endothelial cell growth during chronic inflammation or in the resolution phase of acute inflammation.

V. A_{2B} receptors on epithelial cells

A. Intestinal epithelial cells

Epithelial cells participate in inflammatory processes by maintaining mucosal integrity, producing biologically active mediators and modulating local immune responses. Functional adenosine A_{2B} receptors are expressed on intestinal epithelial cells. Epithelial A_{2B} receptors were shown to upregulate chloride secretion through the activation of apical cystic fibrosis conductance regulator (CFTR) (Strohmeier et al., 1995). The effect of A_{2B} receptors on

epithelial secretion has received particular attention because of its potential relevance to intestinal inflammation. As part of the pathophysiology of these disorders, neutrophils are recruited into intestinal crypts, where they release AMP, which is then converted to adenosine at the epithelial cell surface by CD73. It is adenosine that then acts on epithelial A_{2B} receptors to stimulate chloride secretion (Madara et al., 1993). This pathway normally serves to hydrate the mucosal surface, thereby protecting the intestine by preventing the translocation of bacteria, bacterial products and antigens to lamina propria. When stimulated during inflammation, chloride secretion facilitates fluid movement to lumen in order to flush pathogens from mucosal surface, thus contributing to inflammation-associated diarrhea (Kolachala et al., 2008b).

Epithelial A_{2B} receptors were also shown to modulate the release of proteins involved in intestinal inflammation. Stimulation of A_{2B} receptors on colonic epithelial cells induced an increase in IL-6 secretion into the luminal compartment at levels sufficient to activate neutrophils (Sitaraman et al., 2001). Stimulation of A_{2B} receptors also induced fibronectin synthesis and secretion from the apical surface of intestinal epithelial cells (Walia et al., 2004). Apical fibronectin significantly enhanced the adherence and invasion of *Salmonella typhimurium* to cultured epithelial cells as well as consequent IL-8 secretion (Walia et al., 2004). Intracellular mechanisms mediating these effects were suggested to involve stimulation of IL-6 and fibronectin transcription via cAMP/PKA-mediated activation of nuclear cAMP-responsive element-binding protein (CREB) (Sitaraman et al., 2001; Walia et al., 2004).

A_{2B} receptor functions on intestinal epithelium can be modulated by INF- γ and TNF- α , factors elevated at various stages of inflammation. TNF- α reportedly increase A_{2B} receptor expression in colonic epithelial cells by a post-transcriptional mechanism possibly involving downregulation of A_{2B} receptor-specific micro RNA miR27b and miR128a (Kolachala et al., 2010), whereas INF- γ affects A_{2B} receptor signaling through the direct inhibition of adenylate cyclase expression (Kolachala et al., 2005).

B. Pulmonary epithelial cells

A_{2B} receptors have been suggested to play an important role in regulation of ion and water transport in airway epithelium. Airborne particles, including pathogens, are absorbed by the mucus layer lining the airways, where they are inactivated by the innate mucosal defense system and removed via mucociliary and cough clearance. This process largely depends on active transepithelial salt transport involving the CFTR and amiloride-sensitive epithelial Na⁺ channel (ENaC). The role of A_{2B} receptors in the regulation of CFTR was demonstrated in the human airway epithelial Calu-3 cell line and primary bronchial epithelial cells (Huang et al., 2001; Lazarowski et al., 2004). Like in intestinal epithelium, A_{2B} receptors stimulate CFTR by a mechanism involving cAMP/PKA-dependent pathway (Lazarowski et al., 2004). In bronchial ciliated epithelium, CFTR can inhibit ENaC, and activation of CFTR in the presence of ENaC inhibition generates Cl⁻ secretion and liquid transport to maintain airway surface liquid volume (Donaldson and Boucher, 2007). In distal airspaces of the lung, sodium movement through ENaC and chloride movement through CFTR may be coupled (Reddy et al., 1999), and activation of CFTR-mediated chloride transport can result in fluid absorption (Fang et al., 2006). Therefore, adenosine-dependent, cAMP-mediated pathways could promote alveolar ion absorption and fluid clearance. Indeed, adenosine receptors were shown to regulate alveolar liquid clearance in rat type II pneumocytes (Factor et al., 2007), and high levels of A_{2B} receptor expression were described in murine type II alveolar epithelial cells (Cagnina et al., 2009).

In addition to stimulation of cAMP-dependent pathway, apical adenosine A_{2B} receptors in Calu-3 cells can regulate anion secretion through stimulation of basolateral K_{Ca} channels via

PLC/Ca²⁺ signaling. This pathway synergizes with cAMP-dependent modulation of apical CFTR channels for transepithelial anion secretion and a mixed secretion of chloride and bicarbonate (Wang et al., 2008). The mechanism of A_{2B} receptor-mediated transepithelial liquid transport appears even more complicated in view of the recent report that A_{2B} receptor signaling regulates gap junctional intercellular communication between epithelial cells by the release of PGE₂ and subsequent activation of basolateral EP₄ receptors. It has been suggested that this mechanism may contribute to the spread of ions, second messenger and/or co-factor exchange between cells to fully activate CFTR and ensure efficient Cl⁻ secretion (Scheckenbach et al., 2010).

Physical stimulation of airway surfaces was suggested to evoke liquid secretion by producing ATP that then locally converted to adenosine, and sensed by A_{2B} adenosine receptors (Huang et al., 2001). In nasal epithelial cells A_{2B} receptor agonists elicited sustained responses in ciliary beat frequencies (Morse et al., 2001). Thus, A_{2B} receptors were proposed to stimulate mucociliary clearance in response to injurious stimuli to remove them from airway surfaces. Whether tonic stimulation of A_{2B} receptors is required at rest in order to maintain the mucus clearance is less clear given the low affinity of this receptor subtype. A recent report by Rollins et al appeared to support this hypothesis by demonstrating that the selective A_{2B} antagonist ATL801 inhibited autoregulation of airway surface liquid height in human bronchial epithelial cells (Rollins et al., 2008). However, the lack of effects of micromolar concentrations of DPCPX and ZM241385 (Rollins et al., 2008) known to inhibit human A_{2B} receptors (see Table) adds uncertainty to the authors' conclusion.

A_{2B} receptors can also regulate cytokine secretion from pulmonary epithelium. Stimulation of A_{2B} receptors on human primary bronchial epithelial cells (HBECs) upregulated the expression of several cytokines including CXCL2, CXCL3 and IL-19 (Zhong et al., 2005). A_{2B} receptor-stimulated IL-19 released from HBECs was able to activate the monocytic cell line THP-1 and induce TNF- α secretion. In turn, TNF- α released from THP-1 upregulated the expression of A_{2B} receptors in HBECs, thus providing a positive feedback loop to facilitate the effects of adenosine (Zhong et al., 2005).

VI. Role of A_{2B} receptors on fibroblasts

The human fibroblasts cell line VA13 was the first cell type where a low affinity adenosine receptor was originally described back in 1980 (Bruns, 1980), and later designated as A_{2B} (Bruns et al., 1986). Fibroblasts represent a heterogeneous population of cells, which may differ in phenotype and function not only between anatomical sites but even within a single tissue where immature cells (often called mesenchymal fibroblasts) exist with fibroblasts of various degree of differentiation. Fibroblasts play an important role in the progression of inflammation by secreting various factors that define the tissue microenvironment and modulate immune cell functions. They also contribute to tissue remodeling by increased proliferation, differentiation and generation of various components of the extracellular matrix (for review see (Flavell et al., 2008)).

Stimulation of A_{2B} receptors on mouse cardiac fibroblasts was shown to promote IL-6 release. This effect was independent of the Gs-cAMP-PKA pathway, but required protein kinase C δ and p38 MAPK activation (Feng et al., 2010). In contrast, A_{2B} receptor-mediated increase of IL-6 release from human gingival fibroblasts was attributed, at least in part, to the activation of Gs-cAMP-PKA pathway (Murakami et al., 2000). Stimulation of these cells with high concentrations (10–50 μ M) of adenosine agonists potentiated IL-6 and IL-8 release induced by IL-1 β and upregulated the expression of hyaluronate synthase mRNA (Murakami et al., 2001). In contrast, stimulation of A_{2B} receptors on synovial fibroblasts

counteracted the effects of IL-1 β by decreasing MMP1 mRNA expression (Boyle et al., 1996).

The role of A_{2B} receptors in regulation of IL-6 secretion under normoxic and hypoxic conditions was studied in human pulmonary fibroblasts. Stimulation of A_{2B} receptors under normoxic conditions increased the release of IL-6 and promoted the differentiation of human lung fibroblasts to myofibroblasts. Hypoxia amplified these effects by upregulating the expression of A_{2B} adenosine receptors. The findings that adenosine increases the release of IL-6, and this cytokine in turn induces differentiation of fibroblasts into myofibroblasts suggest a mechanism whereby adenosine could participate in the remodeling process of chronic inflammatory diseases (Zhong et al., 2005).

Studies in corpus cavernosal fibroblast cells isolated from WT and A_{2B}KO mice suggested that A_{2B} receptors upregulate the expression of TGF- β 1 and promote fibrosis. Pharmacological analysis in WT cells showed that NECA increased TGF- β 1 mRNA expression and procollagen I mRNA levels, which was completely abolished by the A_{2B} receptor specific antagonist, MRS1754. Furthermore, this effect was not seen in the A_{2B}KO cells (Wen et al., 2010).

In contrast, A_{2B} receptors diminished collagen production and proliferation of rat cardiac fibroblasts (Dubey et al., 2001; Epperson et al., 2009). The diverse effects of A_{2B} receptors on fibroblasts of different origins can be attributed to the known phenotypical and functional heterogeneity of these cells.

VII. Role of A_{2B} receptors in animal models of inflammation

The recent generation of A_{2B}KO mice and the development of selective A_{2B} antagonists have made it possible for the testing of an integrated role of A_{2B} receptors in complex animal models of acute and chronic inflammation. Results have been interpreted as evidence for either “pro-inflammatory” or “anti-inflammatory” role of A_{2B} receptors, and results from genetic and pharmacological approach have not always been in agreement.

A_{2B}KO mice appear to have a pro-inflammatory phenotype compared to WT controls, characterized by elevated TNF- α plasma concentrations (Yang et al., 2006) and increased vascular permeability for albumin in the colon, kidney and lung (Eckle et al., 2008a). This is surprising because this is seen even at rest, when the low extracellular adenosine levels are not expected to stimulate A_{2B} receptors. Short-term exposure of mice to ambient hypoxia induced significantly higher vascular leak in all organs of A_{2B}KO mice compared to WT control (Eckle et al., 2008a). A_{2B}KO mice had also increased pulmonary albumin leakage after acute lung injury produced by either mechanical ventilation (Eckle et al., 2008b) or LPS inhalation (Eckle et al., 2008b; Schingnitz et al., 2010), compared to WT controls. In parallel to pulmonary edema, an increase in neutrophil infiltration and tissue levels of IL-1 β , IL-6 and TNF- α was also higher in A_{2B}KO mice. Studies using bone marrow chimeric mice showed that A_{2B} receptors located on stromal cells rather than those on bone marrow-derived cells contribute to these differences in lung injury between A_{2B}KO and WT animals (Eckle et al., 2008b; Schingnitz et al., 2010).

Furthermore, it has been reported that genetic deficiency of A_{2B} receptors increased the mortality of mice suffering from cecal ligation and puncture-induced sepsis. The increased mortality of A_{2B} knockout mice was associated with increased inflammatory indices measured in the spleen, heart, and plasma in comparison with WT animals. Again, experiments using bone-marrow chimeras revealed that it is the lack of A_{2B} receptors on nonhematopoietic cells that is primarily responsible for the increased inflammation of septic A_{2B} receptor-deficient mice (Csoka et al., 2010). Another study employing bone-marrow

chimeras suggested that vascular A_{2B} receptors also play an important role in protective effects of ischemic preconditioning in a mouse model of acute renal failure from ischemia (Grenz et al., 2008). Thus, it is possible that an increase in vascular permeability in mice lacking A_{2B} receptors may explain, at least in part, the observed exacerbation of acute tissue injury seen in these different disease models.

This evidence obtained in models of acute tissue injury seems to support an “anti-inflammatory” role of A_{2B} receptors. However, even similar models of acute injury may produce opposite results in different tissues. For example, A_{2B} receptors were proposed to play a protective role in gastro-intestinal model of ischemia-reperfusion injury based on enhanced intestinal injury observed in A_{2B}KO mice (Hart et al., 2009). In contrast, the lungs of A_{2B}KO mice were significantly protected in a pulmonary ischemia-reperfusion model, as evidenced by reduced pulmonary artery pressure, increased lung compliance, decreased myeloperoxidase, and reduced levels TNF- α , IL-6, CXCL-1, CCL2 and CCL5 (Anvari et al., 2010). These results would suggest a “pro-inflammatory” role of A_{2B} receptors in the lung, in contrast to their “anti-inflammatory” actions in the intestine. Experiments using bone-marrow chimeras also suggested that these effects were due to A_{2B} receptor activation primarily on resident pulmonary cells and not bone marrow-derived cells (Anvari et al., 2010).

It becomes increasingly clear that the final outcome of genetic ablation of A_{2B} receptors may be dependent not only on the tissue but also on the model of inflammation studied. Although genetic ablation of adenosine A_{2B} receptors in mice has been shown to facilitate acute inflammatory responses to antigen challenges in passively sensitized mice (Hua et al., 2007), this is not the case in chronic inflammation, a process dependent on the complex interplay between multiple cells and inflammatory factors. We studied the effects of A_{2B} receptor gene ablation in the context of ovalbumin-induced chronic pulmonary inflammation. We found that repetitive airway allergen challenge induced a significant increase in adenosine levels in fluid recovered by bronchoalveolar lavage (BAL). Genetic ablation of A_{2B} receptors significantly attenuated allergen-induced chronic pulmonary inflammation as evidenced by a reduction in the number of BAL eosinophils and in peribronchial eosinophilic infiltration. The most striking difference in the pulmonary inflammation induced in A_{2B}KO and WT mice was the lack of allergen-induced IL-4 release in the airways of A_{2B}KO animals, in line with a significant reduction in IL-4 protein and mRNA levels in lung tissue. In addition, attenuation of TGF- β 1 release in airways of A_{2B}KO mice correlated with reduced airway smooth muscle and goblet cell hyperplasia/hypertrophy. It was concluded, therefore, that genetic removal of A_{2B} adenosine receptors in mice leads to inhibition of allergen-induced chronic pulmonary inflammation and airway remodeling (Zaynagetdinov et al., 2010). This conclusion was also supported by pharmacological evidence; antagonism of adenosine A_{2B} receptors by the selective A_{2B} antagonist CVT-6883 resulted in inhibition of airway inflammation induced by chronic exposure to allergen (Mustafa et al., 2007).

Inhibition of adenosine A_{2B} receptors by the selective A_{2B} antagonist CVT-6883 also reduced pulmonary inflammation and airway remodeling in adenosine deaminase (ADA)-deficient mice (Sun et al., 2006). These mice are characterized by elevated lung tissue levels of adenosine and exhibit a lung phenotype with features of lung inflammation, bronchial hyperresponsiveness, enhanced mucus secretion, increased IgE synthesis, and elevated levels of pro-inflammatory cytokines and angiogenic factors that could be reversed by lowering adenosine levels with exogenous ADA (Blackburn et al., 2000). Paradoxically, genetic removal of the A_{2B} receptors from ADA-deficient mice led to enhanced pulmonary inflammation and airway destruction. The authors suggested that loss of pulmonary barrier function in A_{2B}KO mice and excessive airway neutrophilia contributed to the enhanced

tissue damage observed in this model (Zhou et al., 2009). In addition, TNF- α levels, known to be elevated in A_{2B}KO mice at rest (Ryzhov et al., 2008c; Yang et al., 2006), were also markedly increased in the lungs of ADA/A_{2B} double-knockout mice, which could activate pathways that influence the trafficking of neutrophils in the lung (Zhou et al., 2009). To explain the opposite effects of A_{2B} receptor antagonism and genetic ablation, the authors emphasized that pharmacological inhibition of A_{2B} receptors was introduced later in the disease process, 10 days after triggering pulmonary disease by withdrawing mice from ADA replacement therapy (Zhou et al., 2009). Thus, the differential effects of pharmacological antagonism and genetic deletion of A_{2B} receptors in ADA-deficient mice may indicate the importance of timing when elimination of A_{2B} receptor signaling could either promote or attenuate the development of pulmonary disease in ADA-deficient mouse model. The tonic increase in TNF α in A_{2B}KO but not in WT mice may also contribute to this phenomenon.

The use of A_{2B}KO mice has also resulted in different outcomes of bleomycin-induced pulmonary fibrosis depending on the research model used. Bleomycin instilled directly into the respiratory tract produced the acute damage resulting in extensive apoptosis of airway epithelial cells, followed by infiltration of granulocytes early after the challenge. These mice developed severe fibrosis due to failed wound healing. In contrast, in the intraperitoneal bleomycin model, lung injury and inflammation are not induced acutely, but mice do develop chronic pulmonary fibrosis. The fibrotic process was significantly reduced in A_{2B}KO mice in the intraperitoneal bleomycin model, but not in the intratracheal model, compared to corresponding WT controls (Zhou, 2010). In contrast, pharmacological antagonism of A_{2B} receptors with CVT-6883 attenuated pulmonary inflammation and fibrosis in WT mice subjected to lung injury induced by intratracheal instillation of bleomycin (Sun et al., 2006). These differences in the results obtained from genetic and pharmacological targeting of A_{2B} receptors could be explained by the loss of pulmonary vascular barrier seen in A_{2B}KO mice even at rest (Eckle et al., 2008a). This phenotype would be expected to amplify any model of acute pulmonary injury that involves an acute phase of inflammation with extensive edema and neutrophil infiltration.

The role of A_{2B} receptors in promoting intestinal inflammation was demonstrated in both acute and chronic mouse models of colitis. Genetic removal or pharmacological antagonism of A_{2B} receptors with ATL-801 attenuated clinical and histological features of intestinal inflammation in parallel with reduction of IL-6 and CXCL-1 secretion in several mouse models of experimental colitis (Kolachala et al., 2008a; Kolachala et al., 2008c). In contrast, both genetic ablation and pharmacological antagonism of A_{2B} with PSB1115 promoted intestinal inflammation in a mouse model of acute colitis as reflected by increased weight loss, colonic shortening, and disease activity indices (Frick et al., 2009). The reason for disparate results of these studies is unclear but may reflect the complexity of events orchestrated in these models when small differences in protocols or in composition of intestinal flora present in animals may greatly affect outcomes.

Adding more to controversy on the role of A_{2B} receptors in inflammatory responses, A_{2B} receptors were proposed to play a role in protective effects of ischemic preconditioning in models of myocardial ischemia-reperfusion injury (Eckle et al., 2007; Kuno et al., 2007). Interestingly, in situ ischemic preconditioning conferred cardioprotection in A₁KO, A_{2A}KO, or A₃KO mice but not in A_{2B}KO mice (Eckle et al., 2007). On the other hand, a recent comprehensive study also employing A_{2B}KO mice, and using the selective A_{2B} antagonist ATL-801 both in mouse and rat models of myocardial ischemia-reperfusion injury argued against contribution of A_{2B} receptors at least in the acute phase of ischemic preconditioning (Maas et al., 2010).

Stimulation of angiogenesis and modulation of immune response by A_{2B} receptors may play an important role in promotion of cancer growth, which can be considered in broad terms to share characteristics with chronic inflammatory processes. Indeed, Lewis lung carcinoma tumors grown in host animals lacking A_{2B} adenosine receptors contained significantly lower levels of VEGF and displayed lower intra-tumor vascular density compared to tumors grown in WT animals. This difference in neovascularization and tumor tissue VEGF levels was due to A_{2B} receptor-dependent VEGF production by host tumor-associated cells. Furthermore, analysis of host immune cells in tumors suggested that A_{2B} receptor signaling may favor the expansion of myeloid-derived suppressor cells. These observations raise the interesting possibility that host A_{2B} receptors on immune cells not only stimulate tumor angiogenesis, but also suppress immune surveillance, thus engaging two distinct mechanisms to promote tumor survival and growth (Ryzhov et al., 2008a). It is also possible that not only tumors, but also infectious agents may exploit host A_{2B} receptors for their advantage. Stimulation of host A_{2B} receptors was implicated in persistence of chlamydial infection (Pettengill et al., 2009) and establishing of leishmania infection (de Almeida Marques-da-Silva et al., 2008). However, the role of other adenosine receptor subtypes in these infections is not known and the mechanisms responsible for these effects need to be defined in detail.

VIII. Conclusion

To be beneficial, the inflammatory reaction must be acute, destroying an injurious agent within a short period of time and in a localized area, while inducing an immune response. This is achieved through a complex series of events involving multiple cell types and secreted factors. Given the actions of adenosine to return the host to a homeostatic state, it is not surprising that A_{2B} receptors may operate to control the extent of acute inflammatory responses. On the other hand, premature suppression of acute inflammation by adenosine may promote its progression to a chronic status to the detriment of the host.

We believe that labeling A_{2B} receptors as “anti-inflammatory” or “pro-inflammatory” may be overly simplistic and misleading because it fails to appreciate just how complex each of the different types of inflammation is and conveys little about precise mechanisms. For example, the inflammation in a model of ventilation-induced lung injury is clearly different from the inflammation in a model of allergen-induced chronic lung disease. Therefore, it is not surprising that inhibition of A_{2B} receptors produced opposite effects in these models. As we illustrated in this chapter, A_{2B} receptors may play different roles even in similar types of inflammation but occurring in different tissues. Furthermore, A_{2B} receptors can play different roles at different points in the progression of inflammation. In this respect, A_{2B} receptor functions may be reflective of pleiotropic effects of the secreted factors. As an example, IL-6 was shown to promote inflammation in models of chronic inflammatory diseases, whereas in models of acute inflammation it can suppress local and systemic acute inflammatory responses (Gabay, 2006). Moreover, various combinations of cytokines released from A_{2B} receptor-activated cells can produce different outcomes. TGF- β 1 produced by “adenosine-differentiated” dendritic cells (Novitskiy et al., 2008) favors the emergence of adaptive Tregs, but in combination with IL-4 or IL-6, it may stimulate Th9 and Th17 cells, respectively (Ernst et al., 2010).

Targeting the low affinity A_{2B} receptor, as opposed to other adenosine receptor subtypes, in the development of novel therapeutic approaches for treatment of inflammatory diseases is especially appealing because these receptors are likely activated only in the pathophysiological environment, while remaining silent in normal tissues. This characteristic could provide specificity in therapy of certain immune-related disorders, while decreasing likelihood of side effects. However, translation of current findings into novel

therapies would require a better understanding of A_{2B} receptors functions in diverse types of inflammatory responses in various tissues and at different points of their progression.

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Abbreviations

ADA	adenosine deaminase
BAL	bronchoalveolar lavage
bFGF	basic fibroblast growth factor
BMMC	bone marrow-derived mast cell
CFTR	cystic fibrosis transmembrane conductance regulator
ENaC	epithelial sodium channel
ERK	extracellular signal-regulated kinase
HIF-1α	hypoxia-inducible factor 1-alpha
HBEC	human bronchial epithelial cell
HMC-1	human mast cell-1
HMEC-1	human microvascular endothelial cell-1
HUVEC	human umbilical vein endothelial cell
ICAM	inter-cellular adhesion molecule
IDO	indoleamine 2,3-dioxygenase
IL1RAP	interleukin-1 receptor accessory protein
INF-γ	interferon-gamma
JNK	c-Jun N-terminal kinase
KO	knockout
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
M-CSF	macrophage colony-stimulating factor
MHC-II	major histocompatibility complex class II
MMP-1	matrix metalloproteinase-1
NFAT	nuclear factor of activated T-cells, PKA, protein kinase A
PLC	phospholipase C
TGF-β1	transforming growth factor beta one
TLR	toll-like receptors
TNF-α	tumor necrosis factor alpha
VEGF	vascular endothelial growth factor
WT	wild type

References

- Afzal A, Shaw LC, Caballero S, Spoerri PE, Lewin AS, Zeng D, et al. Reduction in preretinal neovascularization by ribozymes that cleave the A_{2B} adenosine receptor mRNA. *Circ Res*. 2003; 93:500–506. [PubMed: 12919950]
- Amisten S, Braun OO, Bengtsson A, Erlinge D. Gene expression profiling for the identification of G-protein coupled receptors in human platelets. *Thromb Res*. 2008; 122:47–57. [PubMed: 17920662]
- Anvari F, Sharma AK, Fernandez LG, Hranjec T, Ravid K, Kron IL, et al. Tissue-derived proinflammatory effect of adenosine A_{2B} receptor in lung ischemia-reperfusion injury. *J Thorac Cardiovasc Surg*. 2010; 140:871–877. [PubMed: 20659747]
- Auchampach JA, Jin X, Wan TC, Caughey GH, Linden J. Canine mast cell adenosine receptors: Cloning and expression of the A₃ receptors and evidence that degranulation is mediated by the A_{2B} receptor. *Mol Pharmacol*. 1997; 52:846–860. [PubMed: 9351976]
- Auchampach JA, Kreckler LM, Wan TC, Maas JE, van der Hoeven D, Gizewski E, et al. Characterization of the A_{2B} adenosine receptor from mouse, rabbit, and dog. *J Pharmacol Exp Ther*. 2009; 329:2–13. [PubMed: 19141710]
- Baraldi PG, Tabrizi MA, Fruttarolo F, Romagnoli R, Preti D. Recent improvements in the development of A_{2B} adenosine receptor agonists. *Purinergic Signal*. 2009; 5:3–19. [PubMed: 19184536]
- Ben AA, Lefort A, Hua X, Libert F, Communi D, Ledent C, et al. Modulation of murine dendritic cell function by adenine nucleotides and adenosine: involvement of the A_{2B} receptor. *Eur J Immunol*. 2008; 38:1610–1620. [PubMed: 18465770]
- Biaggioni, I.; Feoktistov, I. Adenosine and endothelial cell function. In: Hasko, G.; Cronstein, BN.; Szabo, C., editors. *Adenosine Receptors: Therapeutic Aspects for Inflammatory and Immune Diseases*. CRC Press; UK, USA: 2005. p. 109-131.
- Blackburn MR, Volmer JB, Thrasher JL, Zhong H, Crosby JR, Lee JJ, et al. Metabolic consequences of adenosine deaminase deficiency in mice are associated with defects in alveogenesis, pulmonary inflammation, and airway obstruction. *J Exp Med*. 2000; 192:159–170. [PubMed: 10899903]
- Boyle DL, Sajjadi FG, Firestein GS. Inhibition of synoviocyte collagenase gene expression by adenosine receptor stimulation. *Arthr Rheum*. 1996; 39:923–930. [PubMed: 8651985]
- Bruns RF. Adenosine receptor activation in human fibroblasts: nucleoside agonists and antagonists. *Can J Physiol Pharmacol*. 1980; 58:673–691. [PubMed: 6253037]
- Bruns RF, Lu GH, Pugsley TA. Characterization of the A₂ adenosine receptor labeled by [³H]NECA in rat striatal membranes. *Mol Pharmacol*. 1986; 29:331–346. [PubMed: 3010074]
- Buenestado A, Grassin DS, Arnould I, Besnard F, Naline E, Blouquit-Laye S, et al. The role of adenosine receptors in regulating production of tumour necrosis factor- α and chemokines by human lung macrophages. *Br J Pharmacol*. 2010; 159:1304–1311. [PubMed: 20136829]
- Cagnina RE, Ramos SI, Marshall MA, Wang G, Frazier CR, Linden J. Adenosine A_{2B} receptors are highly expressed on murine type II alveolar epithelial cells. *Am J Physiol*. 2009; 297:L467–L474.
- Comerford KM, Lawrence DW, Synnestvedt K, Levi BP, Colgan SP. Role of vasodilator-stimulated phosphoprotein in PKA-induced changes in endothelial junctional permeability. *FASEB J*. 2002; 16:583–585. [PubMed: 11919161]
- Corset V, Nguyen-Ba-Charvet KT, Forcet C, Moyse E, dotal A, Mehlen P. Netrin-1-mediated axon outgrowth and cAMP production requires interaction with adenosine A_{2b} receptor. *Nature*. 2000; 407:747–750. [PubMed: 11048721]
- Csoka B, Himer L, Selmeczy Z, Vizi ES, Pacher P, Ledent C, et al. Adenosine A_{2A} receptor activation inhibits T helper 1 and T helper 2 cell development and effector function. *FASEB J*. 2008; 22:3491–3499. [PubMed: 18625677]
- Csoka B, Nemeth ZH, Rosenberger P, Eltzschig HK, Spolarics Z, Pacher P, et al. A_{2B} adenosine receptors protect against sepsis-induced mortality by dampening excessive inflammation. *J Immunol*. 2010; 185:542–550. [PubMed: 20505145]
- de Almeida Marques-da-Silva, de Oliveira JC, Figueiredo AB, de Souza Lima JD, Carneiro CM, Rangel Fietto JL, et al. Extracellular nucleotide metabolism in *Leishmania*: influence of adenosine in the establishment of infection. *Microbes Infect*. 2008; 10:850–857. [PubMed: 18656412]

- Dominguez PM, Ardavin C. Differentiation and function of mouse monocyte-derived dendritic cells in steady state and inflammation. *Immunol Rev.* 2010; 234:90–104. [PubMed: 20193014]
- Donaldson SH, Boucher RC. Sodium channels and cystic fibrosis. *Chest.* 2007; 132:1631–1636. [PubMed: 17998363]
- Dubey RK, Gillespie DG, Jackson EK. A_{2B} adenosine receptors stimulate growth of porcine and rat arterial endothelial cells. *Hypertension.* 2002; 39:530–535. [PubMed: 11882603]
- Dubey RK, Gillespie DG, Zacharia LC, Mi Z, Jackson EK. A_{2b} receptors mediate the antimitogenic effects of adenosine in cardiac fibroblasts. *Hypertension.* 2001; 37:716–721. [PubMed: 11230362]
- Duffy SM, Cruse G, Brightling CE, Bradding P. Adenosine closes the K⁺ channel KCa3.1 in human lung mast cells and inhibits their migration via the adenosine A_{2A} receptor. *Eur J Immunol.* 2007; 37:1653–1662. [PubMed: 17474152]
- Eckle T, Faigle M, Grenz A, Laucher S, Thompson LF, Eltzschig HK. A_{2B} adenosine receptor dampens hypoxia-induced vascular leak. *Blood.* 2008a; 111:2024–2035. [PubMed: 18056839]
- Eckle T, Grenz A, Laucher S, Eltzschig HK. A_{2B} adenosine receptor signaling attenuates acute lung injury by enhancing alveolar fluid clearance in mice. *J Clin Invest.* 2008b; 118:3301–3315. [PubMed: 18787641]
- Eckle T, Krahn T, Grenz A, Kohler D, Mittelbronn M, Ledent C, et al. Cardioprotection by ecto-5'-nucleotidase (CD73) and A_{2B} adenosine receptors. *Circulation.* 2007; 115:1581–1590. [PubMed: 17353435]
- Eltzschig HK, Abdulla P, Hoffman E, Hamilton KE, Daniels D, Schonfeld C, et al. HIF-1-dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia. *J Exp Med.* 2005; 202:1493–1505. [PubMed: 16330813]
- Eltzschig HK, Ibla JC, Furuta GT, Leonard MO, Jacobson KA, Enjyoji K, et al. Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidases and adenosine A_{2B} receptors. *J Exp Med.* 2003; 198:783–796. [PubMed: 12939345]
- Epperson SA, Brunton LL, Ramirez-Sanchez I, Villarreal F. Adenosine receptors and second messenger signaling pathways in rat cardiac fibroblasts. *Am J Physiol.* 2009; 296:C1171–C1177.
- Ernst PB, Garrison JC, Thompson LF. Much ado about adenosine: adenosine synthesis and function in regulatory T cell biology. *J Immunol.* 2010; 185:1993–1998. [PubMed: 20686167]
- Factor P, Mutlu GM, Chen L, Mohameed J, Akhmedov AT, Meng FJ, et al. Adenosine regulation of alveolar fluid clearance. *Proc Natl Acad Sci U S A.* 2007; 104:4083–4088. [PubMed: 17360481]
- Fang X, Song Y, Hirsch J, Galletta LJ, Pedemonte N, Zemans RL, et al. Contribution of CFTR to apical-basolateral fluid transport in cultured human alveolar epithelial type II cells. *Am J Physiol.* 2006; 290:L242–L249.
- Feng W, Song Y, Chen C, Lu ZZ, Zhang Y. Stimulation of adenosine A_{2B} receptors induces interleukin-6 secretion in cardiac fibroblasts via the PKC-delta-P38 signalling pathway. *Br J Pharmacol.* 2010; 159:1598–1607. [PubMed: 20050850]
- Feoktistov I, Biaggioni I. Adenosine A_{2b} receptors evoke interleukin-8 secretion in human mast cells. An enprofylline-sensitive mechanism with implications for asthma. *J Clin Invest.* 1995; 96:1979–1986. [PubMed: 7560091]
- Feoktistov I, Goldstein AE, Biaggioni I. Role of p38 mitogen-activated protein kinase and extracellular signal-regulated protein kinase in adenosine A_{2B} receptor-mediated interleukin-8 production in human mast cells. *Mol Pharmacol.* 1999; 55:726–735. [PubMed: 10101031]
- Feoktistov I, Goldstein AE, Ryzhov S, Zeng D, Belardinelli L, Voyno-Yasenetskaya T, et al. Differential expression of adenosine receptors in human endothelial cells: role of A_{2B} receptors in angiogenic factor regulation. *Circ Res.* 2002; 90:531–538. [PubMed: 11909816]
- Feoktistov I, Ryzhov S, Goldstein AE, Biaggioni I. Mast cell-mediated stimulation of angiogenesis: cooperative interaction between A_{2B} and A₃ adenosine receptors. *Circ Res.* 2003; 92:485–492. [PubMed: 12600879]
- Feoktistov I, Ryzhov S, Zhong H, Goldstein AE, Matafonov A, Zeng D, et al. Hypoxia modulates adenosine receptors in human endothelial and smooth muscle cells toward an A_{2B} angiogenic phenotype. *Hypertension.* 2004; 44:649–654. [PubMed: 15452028]

- Fischer S, Sharma HS, Karliczek GF, Schaper W. Expression of vascular permeability factor/vascular endothelial growth factor in pig cerebral microvascular endothelial cells and its upregulation by adenosine. *Brain Res.* 1995; 28:141–148.
- Flavell SJ, Hou TZ, Lax S, Filer AD, Salmon M, Buckley CD. Fibroblasts as novel therapeutic targets in chronic inflammation. *Br J Pharmacol.* 2008; 153:S241–S246. [PubMed: 17965753]
- Fredholm BB. Adenosine, an endogenous distress signal, modulates tissue damage and repair. *Cell Death Differ.* 2007; 14:1315–1323. [PubMed: 17396131]
- Fredholm BB, Ijzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV Nomenclature and classification of adenosine receptors. *Pharmacol Rev.* 2001a; 53:527–552. [PubMed: 11734617]
- Fredholm BB, Irenius E, Kull B, Schulte G. Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells. *Biochem Pharmacol.* 2001b; 61:443–448. [PubMed: 11226378]
- Fredholm BB, Zhang Y, van der Ploeg I. Adenosine A_{2a} receptors mediate the inhibitory effect of adenosine on formyl-met-leu-phe-stimulated respiratory burst in neutrophil leucocytes. *Naunyn-Schmiedeberg's Arch Pharmacol.* 1996; 354:262–267. [PubMed: 8878055]
- Frick JS, Macmanus CF, Scully M, Glover LE, Eltzschig HK, Colgan SP. Contribution of adenosine A_{2B} receptors to inflammatory parameters of experimental colitis. *J Immunol.* 2009; 182:4957–4964. [PubMed: 19342675]
- Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Res Ther.* 2006; 8:S3. [PubMed: 16899107]
- Gessi S, Varani K, Merighi S, Cattabriga E, Pancaldi C, Szabadkai Y, et al. Expression, pharmacological profile, and functional coupling of A_{2B} receptors in a recombinant system and in peripheral blood cells using a novel selective antagonist radioligand, [³H]MRE 2029-F20. *Mol Pharmacol.* 2005; 67:2137–2147. [PubMed: 15788741]
- Grant MB, Davis MI, Caballero S, Feoktistov I, Biaggioni I, Belardinelli L. Proliferation, migration, and ERK activation in human retinal endothelial cells through A_{2B} adenosine receptor stimulation. *Invest Ophthalmol Visual Sci.* 2001; 42:2068–2073. [PubMed: 11481274]
- Grant MB, Tarnuzzer RW, Caballero S, Ozeck MJ, Davis MI, Spoerri PE, et al. Adenosine receptor activation induces vascular endothelial growth factor in human retinal endothelial cells. *Circ Res.* 1999; 85:699–706. [PubMed: 10521243]
- Grenz A, Osswald H, Eckle T, Yang D, Zhang H, Tran ZV, et al. The reno-vascular A_{2B} adenosine receptor protects the kidney from ischemia. *PLoS Med.* 2008; 5:e137. [PubMed: 18578565]
- Harrington EO, Newton J, Morin N, Rounds S. Barrier dysfunction and RhoA activation are blunted by homocysteine and adenosine in pulmonary endothelium. *Am J Physiol.* 2004; 287:L1091–L1097.
- Hart ML, Jacobi B, Schittenhelm J, Henn M, Eltzschig HK. Cutting Edge: A_{2B} Adenosine receptor signaling provides potent protection during intestinal ischemia/reperfusion injury. *J Immunol.* 2009; 182:3965–3968. [PubMed: 19299692]
- Hua X, Kovarova M, Chason KD, Nguyen M, Koller BH, Tilley SL. Enhanced mast cell activation in mice deficient in the A_{2b} adenosine receptor. *J Exp Med.* 2007; 204:117–128. [PubMed: 17200408]
- Huang P, Lazarowski ER, Tarran R, Milgram SL, Boucher RC, Stutts MJ. Compartmentalized autocrine signaling to cystic fibrosis transmembrane conductance regulator at the apical membrane of airway epithelial cells. *Proc Natl Acad Sci U S A.* 2001; 98:14120–14125. [PubMed: 11707576]
- Kalla RV, Zablocki J. Progress in the discovery of selective, high affinity A_{2B} adenosine receptor antagonists as clinical candidates. *Purinergic Signal.* 2009; 5:21–29. [PubMed: 18568423]
- Khoa ND, Montesinos MC, Williams AJ, Kelly M, Cronstein BN. Th1 cytokines regulate adenosine receptors and their downstream signaling elements in human microvascular endothelial cells. *J Immunol.* 2003; 171:3991–3998. [PubMed: 14530318]
- Kim YC, Ji X, Melman N, Linden J, Jacobson KA. Anilide derivatives of an 8-phenylxanthine carboxylic congener are highly potent and selective antagonists at human A_{2B} adenosine receptors. *J Med Chem.* 2000; 43:1165–1172. [PubMed: 10737749]

- Klotz KN, Hessling J, Hegler J, Owman C, Kull B, Fredholm BB, et al. Comparative pharmacology of human adenosine receptor subtypes - characterization of stably transfected receptors in CHO cells. *Naunyn-Schmiedeberg's Arch Pharmacol*. 1998; 357:1–9. [PubMed: 9459566]
- Kolachala V, Asamoah V, Wang L, Srinivasan S, Merlin D, Sitaraman SV. Interferon-gamma down-regulates adenosine 2b receptor-mediated signaling and short circuit current in the intestinal epithelia by inhibiting the expression of adenylate cyclase. *J Biol Chem*. 2005; 280:4048–4057. [PubMed: 15550390]
- Kolachala V, Ruble B, Vijay-Kumar M, Wang L, Mwangi S, Figler H, et al. Blockade of adenosine A_{2B} receptors ameliorates murine colitis. *Br J Pharmacol*. 2008a; 155:127–137. [PubMed: 18536750]
- Kolachala VL, Bajaj R, Chalasani M, Sitaraman SV. Purinergic receptors in gastrointestinal inflammation. *Am J Physiol*. 2008b; 294:G401–G410.
- Kolachala VL, Vijay-Kumar M, Dalmaso G, Yang D, Linden J, Wang L, et al. A_{2B} adenosine receptor gene deletion attenuates murine colitis. *Gastroenterology*. 2008c; 135:861–870. [PubMed: 18601927]
- Kolachala VL, Wang L, Obertone TS, Prasad M, Yan Y, Dalmaso G, et al. Adenosine 2B receptor expression is post-transcriptionally regulated by microRNA. *J Biol Chem*. 2010; 285:18184–18190. [PubMed: 20388705]
- Kong T, Westerman KA, Faigle M, Eltzschig HK, Colgan SP. HIF-dependent induction of adenosine A_{2B} receptor in hypoxia. *FASEB J*. 2006; 20:2242–2250. [PubMed: 17077301]
- Kreckler LM, Gizewski E, Wan TC, Auchampach JA. Adenosine suppresses lipopolysaccharide-induced tumor necrosis factor- α production by murine macrophages through a protein kinase A- and exchange protein activated by cAMP-independent signaling pathway. *J Pharmacol Exp Ther*. 2009; 331:1051–1061. [PubMed: 19749080]
- Kreckler LM, Wan TC, Ge ZD, Auchampach JA. Adenosine inhibits tumor necrosis factor- α release from mouse peritoneal macrophages via A_{2A} and A_{2B} but not the A₃ adenosine receptor. *J Pharmacol Exp Ther*. 2006; 317:172–180. [PubMed: 16339914]
- Kuno A, Critz SD, Cui L, Solodushko V, Yang XM, Krahn T, et al. Protein kinase C protects preconditioned rabbit hearts by increasing sensitivity of adenosine A_{2b}-dependent signaling during early reperfusion. *J Mol Cell Cardiol*. 2007; 43:262–271. [PubMed: 17632123]
- Lazarowski ER, Tarran R, Grubb BR, van Heusden CA, Okada S, Boucher RC. Nucleotide release provides a mechanism for airway surface liquid homeostasis. *J Biol Chem*. 2004; 279:36855–36864. [PubMed: 15210701]
- Linden J, Thai T, Figler H, Jin X, Robeva AS. Characterization of human A_{2B} adenosine receptors: radioligand binding, western blotting, and coupling to G_q in human embryonic kidney 293 cells and HMC-1 mast cells. *Mol Pharmacol*. 1999; 56:705–713. [PubMed: 10496952]
- Maas JE, Wan TC, Figler RA, Gross GJ, Auchampach JA. Evidence that the acute phase of ischemic preconditioning does not require signaling by the A_{2B} adenosine receptor. *J Mol Cell Cardiol*. 2010; 49:886–893. [PubMed: 20797398]
- Mackay F, Loetscher H, Stueber D, Gehr G, Lesslauer W. Tumor necrosis factor α (TNF- α)-induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55. *J Exp Med*. 1993; 177:1277–1286. [PubMed: 8386742]
- Madara JL, Patapoff TW, Gillece-Castro B, Colgan SP, Parkos CA, Delp C, et al. 5'-adenosine monophosphate is the neutrophil-derived paracrine factor that elicits chloride secretion from T84 intestinal epithelial cell monolayers. *J Clin Invest*. 1993; 91:2320–2325. [PubMed: 8486793]
- McKenna WL, Wong-Staal C, Kim GC, Macias H, Hinck L, Bartoe JL. Netrin-1-independent adenosine A_{2b} receptor activation regulates the response of axons to netrin-1 by controlling cell surface levels of UNC5A receptors. *J Neurochem*. 2008; 104:1081–1090. [PubMed: 17995930]
- Meade CJ, Worrall L, Hayes D, Protin U. Induction of interleukin 8 release from the HMC-1 mast cell line: synergy between stem cell factor and activators of the adenosine A_{2b} receptor. *Biochem Pharmacol*. 2002; 64:317–325. [PubMed: 12123753]
- Mino RP, Spoerri PE, Caballero S, Player D, Belardinelli L, Biaggioni I, et al. Adenosine receptor antagonists and retinal neovascularization in vivo. *Invest Ophthalmol Visual Sci*. 2001; 42:3320–3324. [PubMed: 11726639]

- Morse DM, Smullen JL, Davis CW. Differential effects of UTP, ATP, and adenosine on ciliary activity of human nasal epithelial cells. *Am J Physiol.* 2001; 280:C1485–C1497.
- Murakami S, Hashikawa T, Saho T, Takedachi M, Nozaki T, Shimabukuro Y, et al. Adenosine regulates the IL-1 beta-induced cellular functions of human gingival fibroblasts. *Int Immunol.* 2001; 13:1533–1540. [PubMed: 11717194]
- Murakami S, Terakura M, Kamatani T, Hashikawa T, Saho T, Shimabukuro Y, et al. Adenosine regulates the production of interleukin-6 by human gingival fibroblasts via cyclic AMP/protein kinase A pathway. *J Periodontal Res.* 2000; 35:93–101. [PubMed: 10863963]
- Mustafa SJ, Nadeem A, Fan M, Zhong H, Belardinelli L, Zeng D. Effect of a specific and selective A_{2B} adenosine receptor antagonist on adenosine agonist AMP and allergen-induced airway responsiveness and cellular influx in a mouse model of asthma. *J Pharmacol Exp Ther.* 2007; 320:1246–1251. [PubMed: 17159162]
- Nemeth ZH, Lutz CS, Csoka B, Deitch EA, Leibovich SJ, Gause WC, et al. Adenosine augments IL-10 production by macrophages through an A_{2B} receptor-mediated posttranscriptional mechanism. *J Immunol.* 2005; 175:8260–8270. [PubMed: 16339566]
- Novitskiy SV, Ryzhov S, Zaynagetdinov R, Goldstein AE, Huang Y, Tikhomirov OY, et al. Adenosine receptors in regulation of dendritic cell differentiation and function. *Blood.* 2008; 112:1822–1831. [PubMed: 18559975]
- Ongini E, Dionisotti S, Gessi S, Irenius E, Fredholm BB. Comparison of CGS 15943, ZM 241385 and SCH 58261 as antagonists at human adenosine receptors. *Naunyn-Schmiedeberg Arch Pharmacol.* 1999; 359:7–10. [PubMed: 9933143]
- Palmer TM, Trevethick MA. Suppression of inflammatory and immune responses by the A_{2A} adenosine receptor: an introduction. *Br J Pharmacol.* 2008; 153:S27–S34. [PubMed: 18026131]
- Pettengill MA, Lam VW, Ojcius DM. The danger signal adenosine induces persistence of chlamydial infection through stimulation of A_{2b} receptors. *PLoS One.* 2009; 4:e8299. [PubMed: 20011598]
- Ramkumar V, Stiles GL, Beaven MA, Ali H. The A₃ adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J Biol Chem.* 1993; 268:16887–16890. [PubMed: 8349579]
- Reddy MM, Light MJ, Quinton PM. Activation of the epithelial Na⁺ channel (ENaC) requires CFTR Cl⁻ channel function. *Nature.* 1999; 402:301–304. [PubMed: 10580502]
- Rollins BM, Burn M, Coakley RD, Chambers LA, Hirsh AJ, Clunes MT, et al. A_{2B} adenosine receptors regulate the mucus clearance component of the lung's innate defense system. *Am J Respir Cell Mol Biol.* 2008; 39:190–197. [PubMed: 18367727]
- Rosenberger P, Schwab JM, Mirakaj V, Masekowsky E, Mager A, Morote-Garcia JC, et al. Hypoxia-inducible factor-dependent induction of netrin-1 dampens inflammation caused by hypoxia. *Nat Immunol.* 2009; 10:195–202. [PubMed: 19122655]
- Ryzhov S, Goldstein AE, Biaggioni I, Feoktistov I. Cross-talk between G_s- and G_q-coupled pathways in regulation of interleukin-4 by A_{2B} adenosine receptors in human mast cells. *Mol Pharmacol.* 2006; 70:727–735. [PubMed: 16707627]
- Ryzhov S, Goldstein AE, Matafonov A, Zeng D, Biaggioni I, Feoktistov I. Adenosine-activated mast cells induce IgE synthesis by B lymphocytes: an A_{2B}-mediated process involving Th2 cytokines IL-4 and IL-13 with implications for asthma. *J Immunol.* 2004; 172:7726–7733. [PubMed: 15187156]
- Ryzhov S, McCaleb JL, Goldstein AE, Biaggioni I, Feoktistov I. Role of adenosine receptors in the regulation of angiogenic factors and neovascularization in hypoxia. *J Pharmacol Exp Ther.* 2007; 322:565–572. [PubMed: 17132813]
- Ryzhov S, Novitskiy SV, Zaynagetdinov R, Goldstein AE, Carbone DP, Biaggioni I, et al. Host A_{2B} adenosine receptors promote carcinoma growth. *Neoplasia (New York).* 2008a; 10:987–995.
- Ryzhov S, Solenkova NV, Goldstein AE, Lamparter M, Fleenor T, Young PP, et al. Adenosine receptor-mediated adhesion of endothelial progenitors to cardiac microvascular endothelial cells. *Circ Res.* 2008b; 102:356–363. [PubMed: 18032734]
- Ryzhov S, Zaynagetdinov R, Goldstein AE, Matafonov A, Biaggioni I, Feoktistov I. Differential role of the carboxy-terminus of the A_{2B} adenosine receptor in stimulation of adenylate cyclase, phospholipase C β , and interleukin-8. *Purinergic Signal.* 2009; 5:289–298. [PubMed: 19125355]

- Ryzhov S, Zaynagetdinov R, Goldstein AE, Novitskiy SV, Blackburn MR, Biaggioni I, et al. Effect of A_{2B} adenosine receptor gene ablation on adenosine-dependent regulation of proinflammatory cytokines. *J Pharmacol Exp Ther*. 2008c; 324:694–700. [PubMed: 17965229]
- Ryzhov S, Zaynagetdinov R, Goldstein AE, Novitskiy SV, Dikov MM, Blackburn MR, et al. Effect of A_{2B} adenosine receptor gene ablation on proinflammatory adenosine signaling in mast cells. *J Immunol*. 2008d; 180:7212–7220. [PubMed: 18490720]
- Salvatore CA, Tilley SL, Latour AM, Fletcher DS, Koller BH, Jacobson MA. Disruption of the A₃ adenosine receptor gene in mice and its effect on stimulated inflammatory cells. *J Biol Chem*. 2000; 275:4429–4434. [PubMed: 10660615]
- Sands WA, Palmer TM. Adenosine receptors and the control of endothelial cell function in inflammatory disease. *Immunol Lett*. 2005; 101:1–11. [PubMed: 15878624]
- Scheckenbach KL, Losa D, Dudez T, Bacchetta M, O'Grady S, Crespin S, et al. PGE₂ regulation of CFTR activity and air surface liquid volume requires gap junctional communication. *Am J Respir Cell Mol Biol*. 2010
- Schingnitz U, Hartmann K, Macmanus CF, Eckle T, Zug S, Colgan SP, et al. Signaling through the A_{2B} adenosine receptor dampens endotoxin-induced acute lung injury. *J Immunol*. 2010; 184:5271–5279. [PubMed: 20348420]
- Schneider DJ, Lindsay JC, Zhou Y, Molina JG, Blackburn MR. Adenosine and osteopontin contribute to the development of chronic obstructive pulmonary disease. *FASEB J*. 2010; 24:70–80. [PubMed: 19720619]
- Silver MR, Margulis A, Wood N, Goldman SJ, Kasaian M, Chaudhary D. IL-33 synergizes with IgE-dependent and IgE-independent agents to promote mast cell and basophil activation. *Inflamm Res*. 2010; 59:207–218. [PubMed: 19763788]
- Sitaraman SV, Merlin D, Wang L, Wong M, Gewirtz AT, Si-Tahar M, et al. Neutrophil-epithelial crosstalk at the intestinal luminal surface mediated by reciprocal secretion of adenosine and IL-6. *J Clin Invest*. 2001; 107:861–869. [PubMed: 11285305]
- Srinivas SP, Satpathy M, Gallagher P, Lariviere E, Van Driessche W. Adenosine induces dephosphorylation of myosin II regulatory light chain in cultured bovine corneal endothelial cells. *Exp Eye Res*. 2004; 79:543–551. [PubMed: 15381038]
- Strohmeier GR, Reppert SM, Lencer WI, Madara JL. The A_{2b} adenosine receptor mediates cAMP responses to adenosine receptor agonists in human intestinal epithelia. *J Biol Chem*. 1995; 270:2387–2394. [PubMed: 7836474]
- Sun CX, Zhong H, Mohsenin A, Morschl E, Chunn JL, Molina JG, et al. Role of A_{2B} adenosine receptor signaling in adenosine-dependent pulmonary inflammation and injury. *J Clin Invest*. 2006; 116:2173–2182. [PubMed: 16841096]
- Suzuki H, Takei M, Nakahata T, Fukamachi H. Inhibitory effect of adenosine on degranulation of human cultured mast cells upon cross-linking of Fc epsilon RI. *Biochem Biophys Res Commun*. 1998; 242:697–702. [PubMed: 9464280]
- Synnestvedt K, Furuta GT, Comerford KM, Louis N, Karhausen J, Eltzschig HK, et al. Ecto-5'-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. *J Clin Invest*. 2002; 110:993–1002. [PubMed: 12370277]
- Takagi H, King GL, Ferrara N, Aiello LP. Hypoxia regulates vascular endothelial growth factor receptor KDR/Flk gene expression through adenosine A₂ receptors in retinal capillary endothelial cells. *Invest Ophthalmol Visual Sci*. 1996; 37:1311–1321. [PubMed: 8641834]
- Takedachi M, Qu D, Ebisuno Y, Oohara H, Joachims ML, McGee ST, et al. CD73-generated adenosine restricts lymphocyte migration into draining lymph nodes. *J Immunol*. 2008; 180:6288–6296. [PubMed: 18424752]
- Tang Y, Feng Y, Wang X. Calcitonin gene-related peptide potentiates LPS-induced IL-6 release from mouse peritoneal macrophages. *J Neuroimmunol*. 1998; 84:207–212. [PubMed: 9628464]
- Thompson LF, Eltzschig HK, Ibla JC, Van De Wiele CJ, Resta R, Morote-Garcia JC, et al. Crucial role for ecto-5'-nucleotidase (CD73) in vascular leakage during hypoxia. *J Exp Med*. 2004; 200:1395–1405. [PubMed: 15583013]

- Umapathy NS, Fan Z, Zemskov EA, Alieva IB, Black SM, Verin AD. Molecular mechanisms involved in adenosine-induced endothelial cell barrier enhancement. *Vascul Pharmacol*. 2010; 52:199–206. [PubMed: 20045081]
- Walia B, Castaneda FE, Wang L, Kolachala VL, Bajaj R, Roman J, et al. Polarized fibronectin secretion induced by adenosine regulates bacterial-epithelial interaction in human intestinal epithelial cells. *Biochem J*. 2004; 382:589–596. [PubMed: 15193149]
- Walker BA, Jacobson MA, Knight DA, Salvatore CA, Weir T, Zhou D, et al. Adenosine A₃ receptor expression and function in eosinophils. *Am J Resp Cell Mol Biol*. 1997; 16:531–537.
- Wang D, Sun Y, Zhang W, Huang P. Apical adenosine regulates basolateral Ca²⁺-activated potassium channels in human airway Calu-3 epithelial cells. *Am J Physiol*. 2008; 294:C1443–C1453.
- Wang J, Huxley VH. Adenosine A_{2A} receptor modulation of juvenile female rat skeletal muscle microvessel permeability. *Am J Physiol Heart Circ Physiol*. 2006; 291:H3094–H3105. [PubMed: 16815983]
- Waschke J, Drenckhahn D, Adamson RH, Barth H, Curry FE. cAMP protects endothelial barrier functions by preventing Rac-1 inhibition. *Am J Physiol*. 2004; 287:H2427–H2433.
- Wen J, Jiang X, Dai Y, Zhang Y, Tang Y, Sun H, et al. Increased adenosine contributes to penile fibrosis, a dangerous feature of priapism, via A_{2B} adenosine receptor signaling. *FASEB J*. 2010; 24:740–749. [PubMed: 19858092]
- Wilson JM, Ross WG, Agbai ON, Frazier R, Figler RA, Rieger J, et al. The A_{2B} adenosine receptor impairs the maturation and immunogenicity of dendritic cells. *J Immunol*. 2009; 182:4616–4623. [PubMed: 19342636]
- Xaus J, Mirabet M, Lloberas J, Soler C, Lluís C, Franco R, et al. IFN-gamma up-regulates the A_{2B} adenosine receptor expression in macrophages: a mechanism of macrophage deactivation. *J Immunol*. 1999a; 162:3607–3614. [PubMed: 10092821]
- Xaus J, Valledor AF, Cardo M, Marques L, Beleta J, Palacios JM, et al. Adenosine inhibits macrophage colony-stimulating factor-dependent proliferation of macrophages through the induction of p27kip-1 expression. *J Immunol*. 1999b; 163:4140–4149. [PubMed: 10510349]
- Yang D, Chen H, Koupenova M, Carroll SH, Eliades A, Freedman JE, et al. A new role for the A_{2B} adenosine receptor in regulating platelet function. *J Thromb Haemost*. 2010; 8:817–827. [PubMed: 20102488]
- Yang D, Zhang Y, Nguyen HG, Koupenova M, Chauhan AK, Makitalo M, et al. The A_{2B} adenosine receptor protects against inflammation and excessive vascular adhesion. *J Clin Invest*. 2006; 116:1913–1923. [PubMed: 16823489]
- Zaynagetdinov R, Ryzhov S, Goldstein AE, Yin H, Novitskiy SV, Goleniewska K, et al. Attenuation of chronic pulmonary inflammation in A_{2B} adenosine receptor knockout mice. *Am J Respir Cell Mol Biol*. 2010; 42:564–571. [PubMed: 19556606]
- Zhang JG, Hepburn L, Cruz G, Borman RA, Clark KL. The role of adenosine A_{2A} and A_{2B} receptors in the regulation of TNF-alpha production by human monocytes. *Biochem Pharmacol*. 2005; 69:883–889. [PubMed: 15748700]
- Zhong H, Belardinelli L, Maa T, Zeng D. Synergy between A_{2B} adenosine receptors and hypoxia in activating human lung fibroblasts. *Am J Resp Cell Mol Biol*. 2005; 32:2–8.
- Zhou, Y. The role of A_{2B} adenosine receptor signaling in adenosine dependent lung disease; UT Graduate School of Biomedical Sciences at Houston: Dissertations and Theses. 2010. p. Paper 13 http://digitalcommons.library.tmc.edu/utgsbs_dissertations/13
- Zhou Y, Mohsenin A, Morschl E, Young HW, Molina JG, Ma W, et al. Enhanced airway inflammation and remodeling in adenosine deaminase-deficient mice lacking the A_{2B} adenosine receptor. *J Immunol*. 2009; 182:8037–8046. [PubMed: 19494329]

Table

Affinity of commonly used antagonists to human (h) and mouse (m) adenosine receptor subtypes determined in radioligand binding assays (inhibition constants in μM).

Compounds	Subtypes			
	A ₁	A _{2A}	A _{2B}	A ₃
ALT-801	h 5.0 ^a	h 0.7 ^a	h 0.02 ^a	h 6.3 ^a
	m 5.2 ^a	m 3.5 ^a	m 0.2 ^a	m >10 ^a
CVT 6883	h 1.9 ^b	h 3.3 ^b	h 0.02 ^b	h 1.0 ^b
MRS 1706	h 0.2 ^c	h 0.1 ^c	h 0.001 ^c	h 0.2 ^c
MRS 1754	h 0.4 ^c	h 0.5 ^c	h 0.002 ^c	h 0.6 ^c
	m 0.009 ^d	m $\times 10^d$	m 0.003 ^d	m > 10 ^d
PSB1115	h > 10 ^b	h > 10 ^b	h 0.05 ^b	h > 10 ^b
DPCPX	h 0.004 ^e	h 0.1 ^e	h 0.05 ^f	h 4.0 ^e
	m 0.002 ^d	m 0.6 ^d	m 0.09 ^d	m > 10 ^d
ZM 241385	h 0.3 ^g	h 0.0008 ^g	h 0.03 ^f	h > 10 ^g
	m 0.2 ^d	m 0.0007 ^d	m 0.03 ^d	m > 100 ^d

^a(Kolachala et al., 2008a),

^b(Kalla and Zablocki, 2009),

^c(Kim et al., 2000)

^d(Kreckler et al., 2006),

^e(Klotz et al., 1998),

^f(Linden et al., 1999),

^g(Ongini et al., 1999)