

# A<sub>2B</sub> adenosine receptors in immunity and inflammation

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**A<sub>2B</sub> adenosine receptors are increasingly recognized as important orchestrators of inflammation. A<sub>2B</sub> receptor activation promotes the inflammatory response of mast cells, epithelial cells, smooth muscle cells and fibroblasts, thereby contributing to the pathophysiology of asthma and colitis. A<sub>2B</sub> receptor stimulation limits endothelial cell inflammatory responses and permeability and suppresses macrophage activation thereby preventing tissue injury after episodes of hypoxia and ischemia. A<sub>2B</sub> receptor stimulation also promotes the production of angiogenic cytokines by endothelial cells, mast cells and dendritic cells, aiding granuloma tissue formation and inflammatory resolution, but can also contribute to tumor growth. A<sub>2B</sub> receptors are, thus, potentially important pharmacological targets in treating immune system dysfunction and inflammation.**

## Introduction

Adenosine is a primordial regulator of multiple physiological processes and its modulatory actions have been demonstrated in organisms as diverse as *Drosophila* [1], the common mussel *Mytilus edulis* [2], the protozoan *Leishmania mexicana* [3] and mammals such as *Mus musculus* [4,5] and humans [4,5]. Adenosine is an extracellular purine nucleoside signaling molecule, which governs cell and tissue function both in health and disease. Adenosine is formed after the degradation of its precursor, adenosine 5' triphosphate (ATP), a process which can take place both extra- and intracellularly. ATP, a predominantly intracellular molecule, is released from the cell after stressful and injurious events, and is degraded to adenosine via a cascade of ectonucleotidases, including CD39 (nucleoside triphosphate diphosphorylase [NTPDase]) and CD73 (5'-ectonucleotidase [Ecto5'NTase]) [6]. Intracellular ATP metabolized to adenosine is exported from the cell through nucleoside transporters [7]. Cells of the immune system including neutrophils, mast cells, endothelial cells, regulatory T cells and platelets have been appreciated as the most prodigious sources of extracellular adenosine [8]. In addition to serving as a source for adenosine release, immune cells are also among the most widely studied cell types targeted by the regulatory influences of adenosine

[9]. Adenosine imparts its immune regulatory actions by binding to and activating four G protein-coupled cell surface receptors, A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> [7,9]. Although A<sub>1</sub>, A<sub>2A</sub> and A<sub>3</sub> receptors are high-affinity receptors that are activated by physiological extracellular adenosine concentrations in the submicromolar range, A<sub>2B</sub> receptors are activated by micromolar levels of adenosine, concentrations that are achieved in tissues that experience ischemia, trauma, inflammation or other types of stressful insults. This low affinity of A<sub>2B</sub> receptors for the endogenous ligand adenosine and other selective agonists had, until a few years ago, impeded exploration of the role of this receptor subtype in regulating immune function. The recent availability of sophisticated pharmacological and molecular tools has however dramatically invigorated research on the role of A<sub>2B</sub> receptors in regulating immunity. Here, we review recent advances in understanding the role of A<sub>2B</sub> receptors in modulating inflammation and immunity, which might provide a basis for the utilization of these receptors as pharmacological targets in treating immune system dysfunction and inflammation.

## Distribution of A<sub>2B</sub> receptors

A new mouse model incorporating targeted deletion of A<sub>2B</sub> receptors and replacement of exon 1 with a reporter gene has allowed *in vivo* determination of which cell types and tissues express A<sub>2B</sub> receptors in the mouse [10]. A<sub>2B</sub> receptors have been found in all tested organs, including spleen, lung, colon and kidney, and in all of these organs the primary site of expression was the vasculature. Smooth muscle cells, endothelial cells and macrophages exhibit a high level of expression [10], and A<sub>2B</sub> receptors have also been detected on colonic epithelial cells [11]. Earlier studies employing isolated primary cells or cell lines, however, clearly indicated that other cell types, such as mast cells [12,13], lymphocytes [14,15] dendritic cells [16,17] and neutrophils [15] all express A<sub>2B</sub> receptors.

## Regulation of A<sub>2B</sub> receptor expression

The signaling capacity of A<sub>2B</sub> receptors depends on several factors, which include A<sub>2B</sub> receptor density on the cell surface and A<sub>2B</sub> receptor coupling to downstream intracellular signaling pathways. A<sub>2B</sub> receptor expression on

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the cell surface is a highly regulated and dynamic process, which is influenced by metabolic, inflammatory and hormonal clues from the environment and by adenosine itself. Hypoxia is an important stimulus for the upregulation of  $A_{2B}$  receptor expression and it has been shown to induce  $A_{2B}$  receptor transcription via the oxygen-regulated transcription factor HIF-1 $\alpha$  (hypoxia inducible factor) in endothelial cells [18]. In addition, hypoxia has been documented to increase  $A_{2B}$  receptor expression on dendritic cells [19], smooth muscle cells [20] and fibroblasts [21]. Factors present in an inflammatory environment, such as the bacterial product lipopolysaccharide (LPS) [22], the pro-inflammatory cytokines TNF- $\alpha$  [23–25], IL-1 $\beta$  [23], IFN- $\gamma$  [23,26], free radicals [25] and the endogenous agonist adenosine [27] have all been shown to increase the expression of  $A_{2B}$  receptors. This occurs by varying mechanisms; for example, TNF- $\alpha$  [24,25] and IFN- $\gamma$  [26] upregulate receptor expression by increasing its transcription, whereas adenosine induces  $A_{2B}$  receptor expression on the cell membrane by rapidly recruiting it from its intracellular depot in intestinal epithelial cells via a mechanism involving the docking proteins vesicle-associated membrane protein (VAMP)-2, soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP-23) and syntaxin-3 [28]. Once in the membrane, the  $A_{2B}$  receptor associates with the Na<sup>+</sup>/H<sup>+</sup> exchanger-3 (NHE-3) kinase regulatory protein E3KARP and ezrin, which not only tether the receptor to the membrane but also serve to stabilize it in a signaling complex, facilitating activation of downstream signaling targets, most importantly protein kinase A (PKA) [27].

### Signaling pathways of $A_{2B}$ receptors

$A_{2B}$  receptors couple to a variety of intracellular signaling pathways, the best studied of which are Gs-mediated signaling to PKA, resulting in increased cAMP levels and Gq-mediated activation of phospholipase C (PLC) leading to increased protein kinase C (PKC) activation and elevations in intracellular Ca<sup>2+</sup> levels [29]. These two pathways are often activated simultaneously, which enables additional levels of fine-tuning of cellular processes. In addition to these two most important signaling pathways,  $A_{2B}$  receptor has been shown to activate mitogen activated protein (MAP) kinase signaling, in addition to membrane ion channels, the mechanisms of which are poorly understood and sometimes involve the  $\beta\gamma$  subunit of G proteins [29]. Because the intracellular pathways used by adenosine receptors are largely cell type specific, we detail each particular pathway when discussing specific immune cell types.

### The pharmacology of $A_{2B}$ receptors

The pharmacological characterization of  $A_{2B}$  receptors has lagged behind that of  $A_1$ ,  $A_{2A}$  and  $A_3$  receptors because until recently, potent and selective  $A_{2B}$  receptor ligands had not been available. NECA (5'-N-ethylcarboxamido-adenosine) is the most widely used  $A_{2B}$  receptor agonist; however, it is neither selective nor potent. Nevertheless, in those rare cellular systems in which  $A_{2B}$  receptors are the only adenosine receptors expressed, a role for  $A_{2B}$  receptors in regulating a particular response can be deduced based on the efficacy of NECA and inefficacy of selective  $A_1$ ,  $A_{2A}$

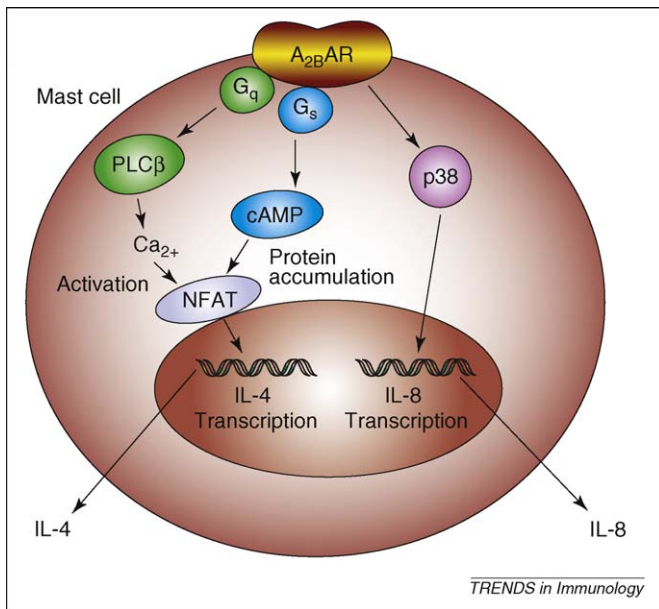
and  $A_3$  receptor agonists. When  $A_{2B}$  receptors are co-expressed with other adenosine receptors,  $A_{2B}$  receptor-mediated responses can be distinguished based on the efficacy of NECA in a manner antagonizable with selective  $A_{2B}$  receptor antagonists. MRS 1754 (N-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1, 3-dipropyl-1H-purin-8-yl)phenoxy]-acetamide) and CVT-6883 (3-ethyl-1-propyl-8-[1-(3-trifluoromethylbenzyl)-1H-pyrazol-4-yl]-3,7-dihydro-purine-2,6-dione) are recently developed, selective and potent  $A_{2B}$  receptor antagonists that are very useful in characterizing  $A_{2B}$  receptors. Enprofylline, IPDX (3-isobutyl-8-pyrrolidinoxanthine) and alloxazine are relatively selective albeit not particularly potent  $A_{2B}$  receptor antagonists. Theophylline also blocks  $A_{2B}$  receptors; however, it is not selective and can antagonize all four types of adenosine receptor. Molecular approaches such as the use of  $A_{2B}$  receptor knockout (KO) mice or siRNA-mediated knock-down of  $A_{2B}$  receptors in conjunction with pharmacological studies, are able to paint a more accurate picture of the role of  $A_{2B}$  receptors in physiological processes.

### Regulation of cell function by $A_{2B}$ receptors

#### *Mast cells*

Mast cell activation has long been recognized as a crucial factor in the pathophysiology of asthma and other allergic diseases [30]. Mast cells are also increasingly appreciated as a crucial cell type in initiating and maintaining inflammatory and immune responses, owing to their ability to rapidly release biogenic amines, proinflammatory lipid molecules and cytokines [31]. Adenosine receptors on mast cells have become of considerable interest as therapeutic targets for asthma, based on the observation that inhaled adenosine or its precursor AMP provokes bronchoconstriction in individuals suffering from asthma, furthermore this bronchoconstrictive response can be prevented using mast cell membrane stabilizers or histamine receptor antagonists [32]. The demonstration that enprofylline and theophylline, two clinically used anti-asthmatic agents, block adenosine-induced IL-8 production by the human mast cell line HMC-1 via targeting of  $A_{2B}$  receptors [33] has aroused interest in studying the role of  $A_{2B}$  receptors on mast cell function (Figure 1).  $A_{2B}$  receptor activation evokes the release of this proinflammatory chemokine by a mechanism that involves the MAP kinase p38 [34], PKC [34] and the third intracellular loop of the receptor and not the G $\alpha$  or G $\beta$  proteins [35].

Mast cells are among the earliest producers of IL-4 during a Th2 type immune response; however, until recently the stimuli eliciting IL-4 release by mast cells were unknown. It has now been demonstrated [36] that  $A_{2B}$  receptor stimulation using NECA induces IL-4 production by HMC-1 cells, indicating that adenosine might provide an early signal resulting in commitment to a Th2 type immune response. The IL-4 response of mast cells stimulated via  $A_{2B}$  receptors requires nuclear factor of activated T cells (NFAT)-mediated transcriptional activation of the IL-4 gene via PLC $\beta$  and elevations in intracellular Ca<sup>2+</sup> levels [37] (Figure 1). Although Gq proteins seem indispensable for the stimulatory effect of adenosine on NFAT transcriptional activity, simultaneous activation of Gs proteins potentiates the IL-4 response to adenosine

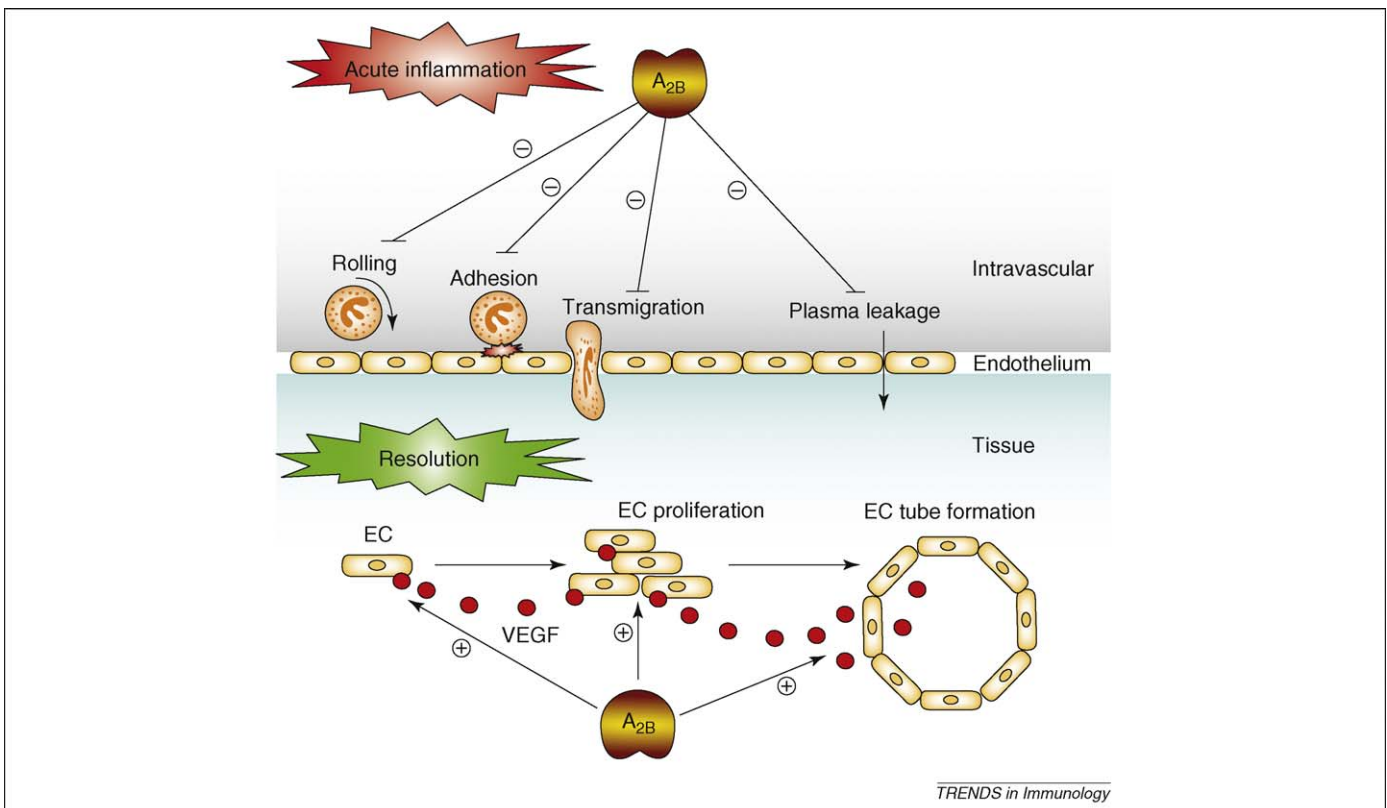


**Figure 1.**  $A_{2B}$  receptor activation on mast cells triggers the release of IL-4 and IL-8. Coupling of  $A_{2B}$  receptors to Gq proteins activates PLC $\beta$  leading to elevations in intracellular  $Ca^{2+}$  levels and activation of the transcription factor NFAT, which in turn transactivates the IL-4 promoter and results in the release of IL-4. Coupling of  $A_{2B}$  receptors to Gs proteins and subsequent accumulation of cAMP upregulates NFAT protein levels, amplifying the Gq-mediated IL-4 response. Gq and Gs protein-independent activation of p38 through the third intracellular loop of the  $A_{2B}$  receptor leads to increased IL-8 transcription and release. Abbreviations:  $A_{2B}AR$ ,  $A_{2B}$  adenosine receptor; cAMP, cyclic adenosine monophosphate; NFAT, nuclear factor of activated T cells; PLC $\beta$ , phospholipase C  $\beta$ .

by leading to increased intracellular cAMP concentrations and up-regulated NFAT protein levels (Figure 1) [36,37]. In addition, co-culturing B lymphocytes with  $A_{2B}$  receptor-stimulated mast cells triggers the former's IgE production, an effect proposed to be secondary to increased secretion of both IL-4 and another Th2 cytokine (IL-13) by the mast cells [36]. The proinflammatory effect of  $A_{2B}$  receptor activation that had first been observed in HMC-1 cells was recently also confirmed using murine bone-marrow derived mast cells [13]. Mast cells isolated from wild type (WT) but not  $A_{2B}$  receptor KO mice released IL-13 and VEGF (vascular endothelial growth factor) in response to extracellular adenosine. These studies also demonstrated that  $A_{2B}$  triggering does not directly affect antigen-induced degranulation of mast cells because adenosine was able to potentiate antigen-induced histamine release by both WT and KO cells. Taken together,  $A_{2B}$  receptor activation increases proinflammatory cytokine production by mast cells (Figure 1), which suggests that this mechanism contributes to the increased inflammatory response that is seen in asthmatic patients exposed to inhaled adenosine.

### Endothelial cells

Both innate and adaptive immune responses are dependent on the migration of leukocytes across endothelial cells [38]. Endothelial cells orchestrate leukocyte transmigration by producing inflammatory chemokines and cytokines, and upregulating adhesion molecules on their surface. In the resolution phase of inflammation, endothelial cells



**Figure 2.** Regulation of endothelial cell function by  $A_{2B}$  receptors. During acute inflammation,  $A_{2B}$  receptors suppress the expression of adhesion molecules on endothelial cells (EC), which leads to decreased leukocyte rolling, adhesion and transmigration.  $A_{2B}$  receptors also prevent plasma leakage from the intravascular compartment into the tissue. During inflammatory resolution,  $A_{2B}$  receptors facilitate endothelial cell proliferation and migration and promote the formation of blood vessels, effects that are mediated by the release of VEGF (vascular endothelial growth factor).

proliferate to form new blood vessels, a phase governed by angiogenic molecules (Figure 2).

Endothelial cells actively metabolize adenosine and have a large capacity to both take up and release adenosine. A<sub>2B</sub> receptors are expressed on the surface of endothelial cells [39] and regulate every aspect of endothelial inflammatory processes. *In vivo* studies utilizing A<sub>2B</sub> KO mice have shown that A<sub>2B</sub> receptors inhibit the expression of the adhesion molecules ICAM-1 and E-selectin, which results in decreased leukocyte rolling and adhesion [10]. A<sub>2B</sub> receptor KO mice exposed to hypoxia exhibit increased neutrophil infiltration into tissues revealing an inhibitory role for A<sub>2B</sub> receptors in neutrophil transmigration *in vivo* [15]. Similarly, pharmacologic studies indicate that neutrophil A<sub>2B</sub> receptors contribute to the decreased adhesion and transmigration of these cells to endothelial cells [40].

Leukocyte transendothelial migration has the potential to disturb vascular barrier function, resulting in fluid loss and edema. Adenosine-mediated activation of A<sub>2B</sub> receptors decreases endothelial paracellular permeability during leukocyte transmigration thereby resealing the endothelium and preventing edema formation and fluid loss [15,41] (Figure 2). This protective effect of adenosine is potentiated in a hypoxic environment, which is due to both the increased availability of extracellular adenosine and upregulation of A<sub>2B</sub> receptors [18], processes that are coordinated by the oxygen-sensitive transcription factor HIF-1 $\alpha$  [42,43]. In addition to the promoter of the A<sub>2B</sub> receptor, HIF-1 $\alpha$  transactivates the promoter of CD73 [44] leading to further generation of extracellular adenosine. Moreover, HIF-1 $\alpha$  represses the promoter of the equilibrative nucleoside transporter (ENT), which causes increased adenosine availability by limiting the cellular uptake of adenosine [45], and together creates a positive feedback loop of A<sub>2B</sub> signaling.

Once the acute inflammation has subsided, in-growth of vascular tissue containing capillary loops ensues [46]. The development of this granulation tissue is driven predominantly by the angiogenic factors produced, in a large part, by the endothelial cells themselves. A<sub>2B</sub> receptors have a key role in promoting this angiogenic response because activation of A<sub>2B</sub> receptors by adenosine increases endothelial cell proliferation, chemotaxis and capillary tube formation [47,48]. In this context, A<sub>2B</sub> receptor activation has been shown to stimulate the release of VEGF, basic fibroblast growth factor and insulin-like growth factor-I by human microvascular endothelial cells, which primarily express A<sub>2B</sub> receptors [39]. A<sub>2B</sub> receptors signal through Gq proteins to induce transcriptional VEGF upregulation; however, this process does not require HIF-1 $\alpha$ . Resting human umbilical vein endothelial cells, however, do not respond to NECA with increased VEGF release because they do not express A<sub>2B</sub> receptors [20]. Exposure of human umbilical vein endothelial cells to hypoxia increases their A<sub>2B</sub> receptor expression, which upon stimulation promotes the release of VEGF by the cells [20].

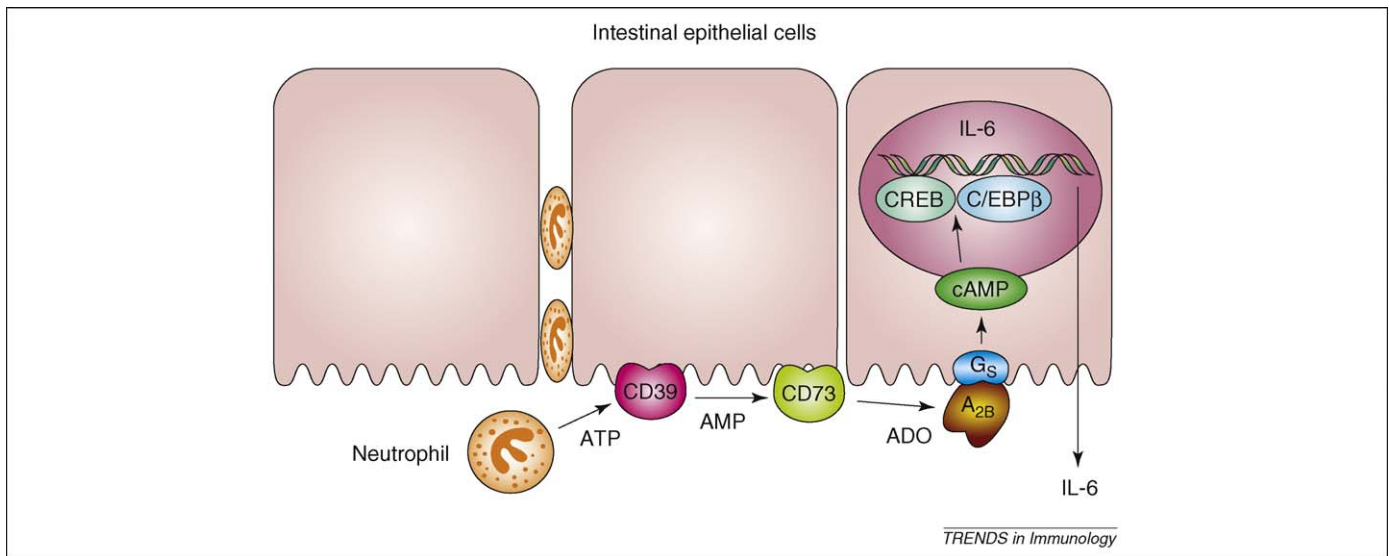
Taken together, endothelial A<sub>2B</sub> receptors seem to prevent endothelial cell-mediated inflammatory events and increase endothelial barrier function at the early stages of tissue injury, and at later phases, A<sub>2B</sub> receptor activation promotes angiogenesis (Figure 2).

### Antigen-presenting cells: macrophages and dendritic cells

Adenosine receptors have been recognized as important regulators of macrophage function; however, A<sub>2B</sub> receptors have been overshadowed by A<sub>2A</sub> receptors as the primary adenosine receptors shaping macrophage function [49,50]. In this context, the inhibitory effect of adenosine on TNF- $\alpha$ , IL-12 and MIP-1 $\alpha$  release by Toll-like receptor (TLR)-activated macrophages is mediated primarily by A<sub>2A</sub> receptors [51,52]. Because the A<sub>2B</sub> receptor antagonist MRS 1754 reverses the adenosine-induced suppression of TNF- $\alpha$  production only in the absence and not in the presence of functional A<sub>2A</sub> receptors, it was [53] proposed that A<sub>2B</sub> receptors become operational only when their effect is not masked by A<sub>2A</sub> receptors. In addition, stimulation of A<sub>2B</sub> but not A<sub>2A</sub> receptors augments TLR-induced IL-10 production by RAW264.7 cells, a macrophage-like cell line that predominantly expresses A<sub>2B</sub> receptors [54]. In spite of the most important role of A<sub>2A</sub> receptors in regulating TLR-induced cytokine production, a recent study utilizing macrophages isolated from A<sub>2B</sub> KO mice showed that adenosine elicits IL-6 production from resting macrophages through A<sub>2B</sub> receptors [55].

In contrast to cytokine production, A<sub>2B</sub> receptors down-regulate IFN- $\gamma$ -induced MHC class II expression and inducible nitric oxide synthase production (iNOS) [26]. IFN- $\gamma$  upregulates A<sub>2B</sub> receptor expression on macrophages resulting in an increased responsiveness of macrophages to the stimulatory effects of NECA, but not other agonists, as measured by intracellular cAMP [26]. This increased cAMP in turn down-regulates both MHC class II and iNOS expression at both the mRNA and protein levels. Similarly, A<sub>2B</sub> receptor activation inhibits monocyte colony stimulating factor (M-CSF)-induced macrophage proliferation by upregulating the cyclin-dependent kinase inhibitor p27<sup>Kip-1</sup> [56]. This occurs in a PKA-dependent manner and results in the growth arrest of cells at the G<sub>1</sub> phase of the cell cycle [56].

Recent studies employing A<sub>2B</sub> KO mice and selective A<sub>2B</sub> receptor agonists and antagonists have defined a novel role for A<sub>2B</sub> receptors in regulating dendritic cell function. Human monocytes differentiated towards dendritic cells using IL-4 and GM-CSF in the presence of adenosine develop into a cell type which resembles dendritic cells morphologically but is different phenotypically [16]. These 'adenosine-differentiated' cells represent an intermediate phase between monocytes and dendritic cells and are impaired in their ability to induce T-cell proliferation and IFN- $\gamma$  production [16]. However, these intermediate cells are highly angiogenic and secrete VEGF and IL-8, and are also anti-inflammatory because of their robust production of IL-10 and TGF- $\beta$ . Furthermore, because these cells also highly express the tolerance-inducing enzyme indoleamine 2,3-dioxygenase (IDO), they can render T cells anergic. Finally, A<sub>2B</sub> KO mice demonstrated that adenosine triggering of this receptor inhibits IL-12 p70 production by TLR-activated bone-marrow-derived dendritic cells [17]. In conclusion, A<sub>2B</sub> receptor activation reshapes the function of antigen-presenting cells in a way that produces an anti-inflammatory and tolerance-inducing phenotype.



**Figure 3.**  $A_{2B}$  receptors trigger the release of IL-6 by intestinal epithelial cells. Transmigrating neutrophils release ATP into the luminal compartment, where it is sequentially degraded to adenosine by CD39 and CD73 which are present on the brush border (apical side) of intestinal epithelial cells. Adenosine (ADO) released from transmigrated neutrophils activates luminal  $A_{2B}$  receptors, which results in intracellular cAMP accumulation, and subsequently, increased transcription of IL-6 via activation of the transcription factors CREB and C/EBP $\beta$ .

### Intestinal epithelial cells

Intestinal epithelial cells, which were once considered a simple physical barrier to the luminal environment, are now known to be integral to both the discrimination of pathogenic and commensal bacteria and the subsequent regulation of immune responses in the intestinal micro-environment [57].  $A_{2B}$  receptors have long been known to regulate  $Cl^-$  secretion from intestinal epithelial cells into the lumen, a key process in the development of diarrhea. Several recent studies have highlighted a novel role of  $A_{2B}$  receptors in regulating the immune response of intestinal epithelial cells (Figure 3).  $A_{2B}$  receptors are the dominant adenosine receptors that are expressed on intestinal epithelial cells, and they are found both on the apical and basolateral surface of these cells [58]. Stimulation of  $A_{2B}$  receptors on either surface triggers the release of IL-6 into the apical compartment [59].  $A_{2B}$  receptor stimulation of epithelial cells increases intracellular cAMP levels, which in turn leads to IL-6 transcription via activation of the ATF and CREB and C/EBP $\beta$  (NF-IL6) transcription factor systems [59]. The physiological relevance of this response is that it provides an amplification mechanism for intestinal inflammation because neutrophils transmigrating through the epithelial cell layer release adenosine, which in turn induces the production of the neutrophil-activating IL-6 [59]. This amplification loop is further enhanced by a rapid increase in the surface expression of  $A_{2B}$  receptors after stimulation of the cells with adenosine, which is made possible by prompt recruitment of preformed  $A_{2B}$  receptors from intracellular stores [27].

Fibronectins are multifunctional glycoproteins found primarily in the extracellular matrix and can bind a wide spectrum of molecules including heparin, DNA, collagen and the surface of bacteria [60]. Stimulation of  $A_{2B}$  receptors on intestinal epithelial cells induces the transcription and subsequent secretion of fibronectin from these cells into the apical compartment [60]. This secreted fibronectin has been shown to aid the adherence and invasion of

*Salmonella typhimurium* into intestinal epithelial cells [60]; however, the clinical relevance of this phenomenon remains to be defined.

In summary,  $A_{2B}$  receptors possess proinflammatory effects in intestinal epithelial cells (Figure 3), which is consistent with the observation that  $A_{2B}$  receptor activation contributes to the pathophysiology of inflammatory bowel disease (IBD) (see later).

### Role of $A_{2B}$ receptors in regulating inflammation and immunity in health and disease

#### *Asthma and chronic obstructive pulmonary disease*

There is an increasing body of evidence documenting the proinflammatory effects of  $A_{2B}$  receptor activation in both rodent and human asthma and chronic obstructive pulmonary disease (COPD), which indicates that  $A_{2B}$  receptor antagonists might be of therapeutic value in preventing disease progression in asthma and COPD. This is borne out by recent *in vivo* evidence [61] showing that selective antagonism of adenosine  $A_{2B}$  receptors leads to inhibition of airway inflammation and airway reactivity induced by allergen or AMP in a murine asthma model. Although mast cells mediate most of the proinflammatory effects of adenosine in the lung [32], proinflammatory effects of  $A_{2B}$  stimulation have also been observed with human bronchial smooth-muscle cells [62], human bronchial epithelial cells [63] and human lung fibroblasts [21], which produce increased levels of IL-6 [21,62] and IL-19 [63] in response to  $A_{2B}$  receptor activation.

Adenosine deaminase (ADA)-deficient mice spontaneously develop severe pulmonary inflammation, airway remodeling, fibrosis and enlargement of airspaces in association with increases in adenosine levels in lung tissue [64]. Thus, increases in endogenous adenosine levels can, at least in part, promote the pathophysiological features observed in chronic lung diseases. Treatment of ADA-deficient mice with a selective  $A_{2B}$  antagonist was able to markedly limit pulmonary inflammation, fibrosis

and airspace enlargement [65]. These findings indicate that A<sub>2B</sub> receptor signaling is an important contributor to lung injury in environments where endogenous adenosine levels are increased. Collectively, the results of pre-clinical models together with evidence that enprofylline and theophylline are A<sub>2B</sub> receptor antagonists, indicate that selective antagonism of A<sub>2B</sub> receptors is an appealing therapeutic approach for managing patients with asthma and COPD.

**Vascular injury, hypoxia, preconditioning and ischemia**  
By generating A<sub>2B</sub> KO mice, Ravid and coworkers [10,66] showed that A<sub>2B</sub> receptors are important in moderating vascular injury to various insults, such as systemic endotoxin challenge and guidewire-induced endothelial denudation of the femoral artery, a model of human restenosis after angioplasty. A<sub>2B</sub> receptor KO mice exhibited increased expression of vascular adhesion molecules, augmented leukocyte rolling and adhesion and elevated levels of pro-inflammatory cytokines [10,66]. Aortic smooth muscle cells derived from A<sub>2B</sub> receptor KO mice displayed greater proliferation in comparison with controls, which contributed to the increased deleterious neointima formation in the injured femoral artery [66]. Studies enlisting bone marrow chimeric mice showed that it was the loss of A<sub>2B</sub> receptors on bone-marrow-derived cells that was responsible for the increased inflammation seen in A<sub>2B</sub> receptor KO mice [10,66].

Extracellular adenosine has long been implicated in the adaptation to hypoxia [67,68] and recent studies have provided evidence that A<sub>2B</sub> receptors have a key role in dampening hypoxia-induced vascular leak *in vivo*. A<sub>2B</sub> receptor KO mice exposed to ambient hypoxia had increased vascular permeability in the majority of organs tested, and studies with bone marrow chimeric mice indicated that it was the lack of parenchymal (vascular) and not hematopoietic A<sub>2B</sub> receptors that caused the increased vascular leak [69]. A<sub>2B</sub> receptor KO mice suffering from ventilator-induced lung injury demonstrate increased pulmonary vascular leak and edema, which is due to lack of A<sub>2B</sub> receptors on pulmonary and not hematopoietic cells [69].

Ischemic or hypoxic preconditioning is defined as a rapid, adaptive response to brief, repeated periods of ischemia or hypoxia to render the tissue resistant to the deleterious effects of subsequent severe and long-lasting ischemia or hypoxia, respectively. It is widely accepted that adenosine has a key role in mediating preconditioning, and recent results incriminate A<sub>2B</sub> receptors as important components of the preconditioning response. Ischemic preconditioning fails to protect the heart [70] and kidney [71] in A<sub>2B</sub> receptor KO mice, and this lack of preconditioning is associated with increased NF- $\kappa$ B activation and inflammation [70–72].

#### *Inflammatory bowel disease*

Crohn's disease and ulcerative colitis are chronic, relapsing inflammatory bowel diseases (IBDs) that are characterized by a dysregulation of the inflammatory functions of intestinal epithelial cells, in addition to poorly controlled activation of both the innate and adaptive immune systems

leading to damage of the intestinal mucosa. Although the etiology of IBD remains to be defined, recent studies indicate that the disease results from a dysregulated immune response to luminal antigens and gut microflora in a genetically susceptible host [73]. Consistent with the proinflammatory role of A<sub>2B</sub> receptor activation in driving intestinal epithelial cell inflammatory responses, A<sub>2B</sub> receptor blockade [74] or KO [11] suppresses intestinal inflammation and attenuates the course of disease in murine colitis. The protection caused by A<sub>2B</sub> receptor inactivation is correlated with decreased production of IL-6 and keratinocyte-derived chemokine, in addition to limited neutrophil accumulation in gut tissue [11,74]. Because A<sub>2B</sub> receptors are upregulated in gut tissue during both human and murine colitis [24], they are components of a vicious circle that propagates inflammation in the gut.

#### *Cancer*

Inflammation in the tumor microenvironment aids in the proliferation and survival of malignant cells, promotes angiogenesis and metastasis and subverts adaptive immune responses. Concentrations of extracellular adenosine are increased in tumor microenvironments [75] because both inflammation and hypoxia, conditions which are present in most tumors, can increase the accumulation of adenosine [76–79]. Recent studies have revealed that A<sub>2B</sub> receptors can propagate the growth of Lewis lung carcinoma in mice by reprogramming host dendritic cells to produce copious amounts of VEGF, thereby supporting angiogenesis in the tumors [16,80]. It is, however, conceivable that A<sub>2B</sub> receptors directly affect the growth of other types of tumors because several tumors have been shown to overexpress A<sub>2B</sub> receptors [81,82]. Further studies will be required to determine whether A<sub>2B</sub> receptors have tumor growth-regulating roles in other models of cancer development.

#### **Conclusions and perspectives**

Research into the role of A<sub>2B</sub> receptors in regulating immunity and inflammation has gained momentum with the recent availability of KO mouse models and selective receptor ligands. Based on the new information acquired through this research it has become clear that A<sub>2B</sub> receptors represent potential targets for manipulation in the treatment of immune-mediated and inflammatory diseases. A<sub>2B</sub> receptors can be advantageous targets for pharmacological intervention because their expression is normally low and is selectively increased in tissues undergoing inflammation. Local increases in A<sub>2B</sub> receptors in inflamed tissue might thus present an opportunity for selectively targeting tissues with ongoing inflammation. An additional advantage in manipulating the adenosine-A<sub>2B</sub> receptor system lies in the fact that extracellular adenosine levels are elevated in inflammatory foci and boosting or decreasing these high levels of adenosine can alter signaling through A<sub>2B</sub> receptors in the local environment without the risk of affecting non-involved tissues.

Before the potential of targeting A<sub>2B</sub> receptors for selective pharmacological intervention can be realized there are several issues that need to be clarified. One of the issues that could complicate the translation of results from animal

models to humans is adenosine-independent signaling by A<sub>2B</sub> receptors in murine systems. This is illustrated by the observations that mast cells isolated from A<sub>2B</sub> receptor KO mice exhibit increased degranulation in response to antigen [12,13] and that macrophages obtained from A<sub>2B</sub> KO mice produce increased levels of TNF- $\alpha$  in the absence of extracellular adenosine [55]. In addition, resting A<sub>2B</sub> KO animals display increased expression of adhesion molecules and cytokines [10] and enhanced vascular permeability [15], which demonstrates that A<sub>2B</sub> receptor KO animals have a widely inflammatory phenotype. One potential explanation is that A<sub>2B</sub> receptors are constitutively active, similar to other G-protein-coupled receptors, such as  $\beta_2$ -adrenoceptors or  $\delta$  opioid receptors [83]. Because inverse agonists can block constitutive signaling, use of antagonists that are also inverse agonists can have off-target effects in tissues that are not inflamed and express constitutively active receptors. Another complicating factor is that non-traditional ligands can also activate A<sub>2B</sub> receptors. For example, the protein netrin is such a novel ligand because it was recently discovered that it can activate A<sub>2B</sub> receptors and have anti-inflammatory effects [84].

Enprofylline and theophylline, two agents with A<sub>2B</sub> receptor blocking properties, have been successfully utilized to treat patients suffering from asthma. It is hoped that a better understanding of the role of A<sub>2B</sub> receptors in regulating immunity will pave the way for additional use of selective modulators of the adenosine-A<sub>2B</sub> receptor system in the fight to improve human health.

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