A2A receptors in inflammation and injury: lessons learned from transgenic animals

György Haskó*†1 and Pál Pacher‡

*Department of Surgery, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, New Jersey, USA; †Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary; and §Section on Oxidative Stress and Tissue Injury, Laboratory of Physiological Studies, National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland, USA

Abstract: Adenosine regulates the function of the innate and adaptive immune systems through targeting virtually every cell type that is involved in orchestrating an immune/inflammatory response. Of the four adenosine receptors (A1, A2A, A2B, A3), A2A receptors have taken center stage as the primary anti-inflammatory effectors of extracellular adenosine. This broad, anti-inflammatory effect of A2A receptor activation is a result of the predominant expression of A2A receptors on monocytes/macrophages, dendritic cells, mast cells, neutrophils, endothelial cells, eosinophils, epithelial cells, as well as lymphocytes, NK cells, and NKT cells. A2A receptor activation inhibits early and late events occurring during an immune response, which include antigen presentation, costimulation, immune cell trafficking, immune cell proliferation, proinflammatory cytokine production, and cytotoxicity. In addition to limiting inflammation, A2A receptors participate in tissue remodeling and repairation. Consistent with their multifaceted, immunoregulatory action on immune cells, A2A receptors have been shown to impact the course of a wide spectrum of ischemic, autoimmune, infectious, and allergic diseases. Here, we review the regulatory roles of A2A receptors in immune/inflammatory diseases of various organs, including heart, lung, gut, liver, kidney, joints, and brain, as well as the role of A2A receptors in regulating multiple organ failure and sepsis. J. Leukoc. Biol. 83: 447–455; 2008.

Key Words: macrophage · lymphocyte · neutrophil · cytokine · autoimmune · infection

INTRODUCTION

Adenosine and its receptors

Adenosine receptors or receptor-mediated effects have been demonstrated in virtually every tissue or organ examined [1, 2]. Adenosine, a purine nucleoside, is produced in response to metabolic stress and cell damage, and elevations in extracellular adenosine are found in conditions of ischemia, hypoxia, inflammation, and trauma (reviewed in ref. [3]). The dominant pathway leading to high extracellular adenosine levels during metabolic stress is release of precursor adenine nucleotides (mostly ATP) from the cell, followed by extracellular catabolism to adenosine by a cascade of ectonucleotidases, including CD39 (nucleoside triphosphate diphosphohydrolase) and CD73 (5’-ectonucleotidase [4–12]). Another significant source of extracellular adenosine is intracellular adenosine, which is released through nucleoside transporters when intracellular adenosine levels rise. This occurs mostly as a result of degradation of intracellular ATP in ischemic conditions. Adenosine bioavailability is limited by its catabolism to inosine by adenosine deaminase or by salvage following cellular uptake via adenosine kinase.

Adenosine produces a wide range of physiological responses by binding to and activating four cell surface adenosine receptors, designated as A1, A2A, A2B, and A3. The adenosine receptors contain seven transmembrane domains and couple to intracellular GTP-binding proteins (G proteins). Adenosine, the endogenous agonist, can activate A1, A2A, and A3 receptors with EC50 in the 0.2- to 0.7-μM range, whereas the potency of adenosine at A2B receptors is lower (EC50: 24 μM) [13]. As physiological adenosine concentrations rarely exceed 1 μM, physiological levels of adenosine can activate A1, A2A, and A3 receptors, whereas A2B receptor activation requires pathophysiological conditions. In general, A1 and A3 receptors couple to pertussis toxin-inhibited Gi/Go proteins, the activation of which results in decreases in intracellular cAMP levels. A2A and A2B receptors couple to Gi proteins and stimulate adenylyl cyclase and cAMP accumulation. A2B receptors can also couple to Gq, resulting in phospholipase C activation and stimulation of the inositoltrisphosphate and diacylglycerol pathways. Adenosine receptors couple to these various pathways in a manner that is highly cell type-dependent and temporally regulated [1, 2]. This receptor complexity reflects the multifaceted role that adenosine has in health and disease.

1 Correspondence: Department of Surgery, UMDNJ-New Jersey Medical School, 185 South Orange Avenue, University Heights, Newark, NJ 07103, USA. E-mail: haskoge@umdnj.edu

Received June 8, 2007; revised September 21, 2007; accepted November 29, 2007.
doi: 10.1189/jlb.0607359
Regulation of A2A receptor expression and function

A2A receptors are found ubiquitously in the body, and their expression is highest in the immune system and the striatopallidal system in the brain [1, 2]. The A2A receptor gene consists of multiple exons, which encode alternative transcripts, whose expression is driven by at least four independent promoters. The regulation of these promoters is now under intense investigation, and it is becoming increasingly clear that A2A receptor gene expression is highly responsive to alterations in the extracellular environment. In this regard, A2A receptor expression is exquisitely sensitive to changes in the concentrations of exogenous and endogenous factors involved in inflammation. Exposure of macrophages to LPS induced a dramatic increase in the expression of A2A receptor mRNA and protein, effects that were brought about through activation of the transcription factor NF-κB [14]. Two endogenous inducers of the NF-κB system, TNF-α and IL-1α, potently up-regulated A2A receptor expression on monocytes as well, and this increase in receptor expression was reflected in an increased responsiveness to agonists in initiating downstream signaling events [15]. However, the fact that the increase in receptor functionality following exposure to TNF-α was disproportionately greater when compared with the increase in receptor expression suggested additional regulatory levels. In fact, subsequently, it was found that TNF-α can preemt A2A receptor desensitization by preventing translocation of G protein-coupled receptor kinase 2 and β-arrestin to the plasma membrane, thereby further augmenting receptor function [16]. In addition to bacterial products and cytokines, a recent study demonstrated that A2A receptor expression was induced by heme oxygenase-1-derived carbon monoxide in macrophages, augmenting the sensitivity of macrophages toward the anti-inflammatory effect of adenosine [17].

Signaling pathways activated by A2A Receptors

A2A receptors, similar to other G protein-coupled receptors, signal through activation of adenylyl cyclase, generation of intracellular cAMP, and activation of protein kinase A (PKA), which can then phosphorylate and thereby activate the transcription factor CREB [18], directly affecting gene expression by binding to gene promoters or indirectly, by competing with NF-κB for an important cofactor, CREB-binding protein [19]. In addition, we have recently demonstrated that another transcription factor, CCAAT enhancer-binding protein β, is responsible for the stimulatory effect of A2A receptor agonists on IL-10 production [20].

Alternative to the canonical cAMP-PKA pathway, A2A receptors can also mediate activation of MAPKs and PKC [19]. Importantly, this PKC pathway was recently implicated as a crucial mechanism that is required for the induction of hypoxia-inducible factor 1 in macrophages [21].

Pharmacology of A2A Receptors

Transfection of Chinese hamster ovary, COS-7, or human embryonic kidney 293 cells with recombinant adenosine receptors had been widely used as a useful model in defining the selectivity of various adenosine receptor ligands [22–24]. Based on this approach, CGS21680 [2-[p-(2-carboxyethyl)phenylethylamino]-5’-N-ethyl-carboxamidoadenosine] was identified first as a relatively selective agonist of A2A receptors [22]. However, as these cell types express low levels of G proteins endogenously when compared with primary cells, studies subsequently used cotransfection of G proteins to refine the pharmacology of A2A receptors [25]. Studies using this technique by Linden and coworkers [25] have revealed that ATL146e [4-[3-(6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydrofuran-2-yl)-9H-purin-2-yl)-prop-2-ynyl]-cyclohexancarbonylic acid methyl ester] is a markedly more selective agonist of A2A receptors than CGS21680, and it has an ~400-fold and ~220-fold selectivity for A2A over A1 receptors and A3 receptors, respectively. Selective antagonists for the A2A receptor are also available, and they include ZM241385 (4-[2-[7-amino-2-(2-furyl)-1, 2, 4]triazolo[3,4-a](1, 3, 5)triazin-5-yl-aminophenyl]-phenyl]-2-(2-furyl)-pyrazolo[4,3-ε]-1,2,4-triazolo[1,5-c]pyrimidine [20].

Adenosine governs immunity through A2A receptor activation on immune cells

Recent studies indicate that adenosine is emerging as a key regulatory molecule, mostly protective but in certain scenarios injurious, in the pathophysiology of inflammatory diseases. In fact, adenosine receptors are expressed densely on virtually all cell types that are involved in orchestrating an inflammatory/immune response, and these cell types include monocytes/macrophages [15, 27], dendritic cells [28, 29], mast cells [30–33], neutrophils [34–36], platelets [37], endothelial cells [38, 39], eosinophils [40, 41], epithelial cells [42, 43], and fibroblasts [44], as well as lymphocytes [45–47], NK cells [48–50], and NKT cells [51]. Through the multiple adenosine receptors on the various immune cells, adenosine can protect or damage tissues and organs depending on which receptor is stimulated. In addition to the regulation of the inflammatory/immune response, recent studies have emphasized the role of adenosine in tissue restitution and wound healing. Roles for adenosine receptors in regulating inflammation had for long been deduced from studies with adenosine receptor ligands. Using adenosine receptor ligands alone, however, is a method fraught with the possibility of erroneous conclusions, owing to the fact that no ligand is fully selective for a particular adenosine receptor. The recent availability of mouse models engineered to harbor a deletion or overexpression of a specific adenosine receptor has proven critical in elucidating the function of different adenosine receptors. On the basis of these genetic studies, it is becoming increasingly recognized that A2A receptors represent the major immunoregulatory arm of the adenosine-adenosine receptor system, and there is also general agreement that A2A receptors serve to down-regulate inflammation and immunity. Numerous excellent reviews have summarized the role that A2A receptors play in regulating the functions of the various cell types that participate in inflammation and immunity [5, 52–55]. In the current review, we will attempt to focus on a different aspect of A2A receptor function in inflammation and immunity, namely on how A2A receptors govern the function of organs that are undergoing an inflammatory challenge caused by infection, ischemia, or trauma.
A2A receptors are abundantly expressed in the heart, and cardiac myocytes [56, 57], fibroblasts [58], and endothelial cells [59] as well as infiltrating hematopoietic cells [60, 61] are the major cell types on which A2A receptor expression has been detected. Reperfusion following an acute ischemic episode induces profound oxidative/nitrosative stress associated with a vigorous inflammatory response and dramatic increase in neutrophil adherence to reperfused endothelium, which leads to capillary plugging, edema, and reduction in coronary vascular flow [62] (Fig. 1), and eventually, transmigration of neutrophils and T lymphocytes, effectors of ischemic injury, into the parenchyma. Multiple lines of evidence have indicated that A2A receptors are critical for adenosine-mediated protection against ischemia-reperfusion injury in the heart [63] (Fig. 1), most compelling of which is the observation that treatment with the selective A2A receptor agonist ATL146e immediately after reperfusion of the coronary artery reduces infarct size in A2A receptor wild-type (WT) but not knockout (KO) mice [60]. There is emerging evidence that A2A receptor-mediated protection is achieved by prevention of the injurious, proinflammatory/immune response, and CD4+ T lymphocytes appear to be the major targets of this protective effect. This notion of A2A receptor activation protecting the heart from ischemia-reperfusion injury is supported by the following observations. The first evidence implicating bone marrow-derived cells and thus T cells came following the observation that the infarct-sparing effect of ATL146e disappeared in chimeric A2A receptor WT mice in which bone marrow had been replaced using bone marrow from A2A receptor KO mice [60]. In addition, administration of ATL146e provided no reduction in infarct size in Rag-1-KO mice that lack T cells, and adoptive transfer of CD4+ T cells from A2A receptor WT but not KO mice into Rag-1-KO mice re-established the protective effect of ATL146e [61]. These studies also revealed that A2A receptor stimulation attenuates the trafficking of neutrophils into the infarcted area, an effect that is secondary to decreased IFN-γ production by infiltrating T cells (Fig. 1).

**LUNG**

A2A receptors are widely expressed in the lung [64], where they exert a plethora of physiological regulatory effects, most of which are anti-inflammatory and reparative in nature. Attenuation of the inflammatory response and facilitation of subsequent wound healing by A2A receptors in the lung can be targeted to numerous sites, which include resident macrophages [65], bronchial epithelial cells [66–68], mast cells [69], eosinophils [67], neutrophils [67, 68], and lymphocytes [67, 68]. Consistent with the ability of A2A receptor activation to inhibit the proinflammatory function of most of these cell types [3, 52, 70–74], Fozard and coworkers [75] reported for the first time that A2A receptor activation using a relatively selective agonist, CGS21680, moderated allergic airway inflammation induced by sensitization to OVA in a rat model of asthma. The salutary effect of A2A receptor activation was also confirmed in OVA-sensitized mice, where exogenously administered CGS21680 prevented inflammatory cell influx into the airways [67]. Using A2A receptor-deficient mice, Jamal Mustafa and associates [68] identified the adenosine A2A receptor-cAMP axis as a potent, endogenous, anti-inflammatory signaling mechanism that mitigates airway reactivity and inflammatory cell infiltration following sensitization with ragweed. These studies offered mechanistic insight into the action of A2A receptors by demonstrating that A2A receptor deficiency produces increased activation of the proinflammatory transcription factor NF-κB and augmented expression of inducible NO synthase.

In addition to exhibiting potent, anti-inflammatory functions in animal models of asthma and other chronic obstructive pulmonary diseases (COPDs), A2A receptor activation has recently been appreciated for its ability in protecting the lung against acute lung failure and acute respiratory distress syndrome. In this regard, A2A receptor activation using CGS21680 was shown to ameliorate lung injury and neutrophil sequestration caused by an episode of trauma in combination with hemorrhagic shock [76]. The lung-protective effect of endogenous adenosine acting through A2A receptors was also reproduced in a model of LPS-induced acute respiratory distress syndrome [77]. Specifically, intratracheal injection of LPS into A2A receptor KO mice led to a markedly increased inflammatory response when compared with WT mice, as indicated by

![Fig. 1. A2A receptor activation protects the heart from ischemia-reperfusion injury by broadly inactivating the ischemia-reperfusion-induced inflammatory response. A2A receptor stimulation reduces ischemia-reperfusion-induced rolling, adhesion, and transmigration of various inflammatory cells, including lymphocytes and neutrophils. A2A receptor stimulation also limits inflammatory chemokine production, superoxide release, and IFN-γ secretion by activated lymphocytes and neutrophils, thereby preventing myocardial damage.](image-url)
augmented recovery of neutrophils from the bronchoalveolar lavage fluid (BALF), as well as enhanced BALF protein levels. These data, coupled with the observation that A2A receptor KO mice had a decrease in overall lung function, which manifested as a decrease in arterial blood oxygen tension, established A2A receptors as critical factors in limiting inflammatory lung injury and acute lung failure.

GUT

The density of A2A receptors in the intact gut is substantially lower than that in the lung [1], partly as intestinal epithelial cells express mostly A2B receptors [78]. Nevertheless, during a chronic inflammatory response, such as that which occurs during inflammatory bowel disease (IBD), a substantial number of immune cells are recruited to the inflamed tissue. In this regard, activation of A2A receptors was recently found to prevent trafficking of immune cells into the intestinal mucosa of rabbits, in which colitis was induced by formalin-immune complex or SAMP1/Yetc mice, which develop IBD spontaneously [79]. The decrease in leukocyte infiltration following A2A receptor stimulation in both models ameliorated the course of disease, as indicated by preserved mucosal architecture and villi. A2A receptor activation also attenuated colitis in SCID mice that were transferred adoptively with disease-inducing CD45RBhigh CD4+ T cells [80]. Interestingly, cotransfer of CD25+ CD4+ T regulatory cells (Tregs) or CD45RBhigh CD4+ T cells obtained from A2A receptor WT mice was able to prevent disease induction in this model, whereas coadministration of CD25+ CD4+ Tregs or CD45RBhigh CD4+ T cells isolated from A2A receptor KO mice in conjunction with disease-inducing CD45RBhigh CD4+ T cells was inefficient in blocking the development of disease [80]. These results illustrate that A2A receptors are required for regulatory cell function in colitis; however, the exact mechanisms remain poorly defined.

LIVER

In liver, studies have demonstrated A2A receptors on Kupffer cells [81], hepatocytes [82], and hepatic stellate cells [83]. The pioneering study of Ohba and Sitkovsky [84], describing the hepatoprotective effects of A2A receptors by using A2A receptor KO mice, ushered in an era of intense investigation of A2A receptors in regulating not only liver function but also inflammation in general. The major discovery of this work was that endogenous adenosine by engaging A2A receptors has a non-redundant role in the attenuation of inflammatory liver damage induced by Con A. Mice deficient in A2A receptors displayed elevated and prolonged production of proinflammatory cytokines, including TNF-α and IFN-γ, which was associated with increased biochemical and histological signs of liver injury. Liver-protecting properties of A2A receptors were also confirmed in carbon tetrachloride-induced, acute hepatotoxicity [84]. Ischemia-reperfusion injury of the liver is a clinically important manifestation of various surgical interventions, including liver transplantation, trauma repair, or partial hepatic resection. Inflammatory events that take place during reperfusion lead to disruption of the vascular endothelium, platelet aggregation, activation of immune cells, and cytokine and chemokine secretion. Pharmacological activation of A2A receptors with ATL146e during this reperfusion phase strongly depressed liver inflammation, as demonstrated by reduced neutrophil infiltration, as well as decreased up-regulation of cytokine and chemokine gene expression [85]. These anti-inflammatory effects could be correlated with improved liver function, as indicated by decreased serum alanine aminotransferase levels in drug-treated mice. The protective effect of ATL146e was abolished by genetic and pharmacological inactivation of A2A receptors, confirming involvement of A2A receptors [85]. In addition, A2A receptor KO mice display heightened indices of injury, indicating that endogenous adenosine also imparts protection from injury. Subsequent studies using a bone marrow chimera approach revealed that protection could be attributed to A2A receptors on bone marrow-derived cells [86]. The reason for this proposition was that no protection was noted when A2A agonists were administered to chimera mice that do not express A2A receptors on bone marrow cells, and substantial protection was observed in chimeras that expressed A2A receptors only on bone marrow cells. In addition, as depletion of NKT cells as well as blockade of the major NKT cell-activating molecule CD1d prevented the protective effect of ATL146e against liver reperfusion injury, a CD1d-mediated, NKT cell-dependent protection was considered as a cardinal mechanism in preventing liver injury by A2A receptor stimulation [51]. This notion of a central role for NKT cells was further supported by studies showing that ATL146e afforded no reduction in liver injury in Rag-1-KO mice, but adoptive transfer of NKT cells from A2A WT but not A2A KO mice into Rag-1 KO recipients reinstated the protective action of ATL146e.

KIDNEY

A2A receptors in kidney have been detected in the renal microvasculature [87], as well as on mesangial [88] and tubular epithelial cells [89]. A2A receptor activation by exogenous agonists or endogenous adenosine produced a dramatic reduction in renal injury following ischemia-reperfusion in rats [90, 91] and mice [92, 93], as evidenced by improved morphology and renal function in agonist-treated animals. The protective effect of A2A agonists was correlated with decreased endothelial cell adhesion molecule expression and neutrophil sequestration in the kidney parenchyma [91]. As the renal microcirculation responds to A2A receptor stimulation by vasodilation, this increased blood flow in the renal microcirculation may potentially also contribute to renal protection by A2A agonists. To discriminate between potential protective effects mediated by bone marrow-derived, inflammatory cells and vascular and other cells, Okusa and coworkers [93] generated A2A receptor bone marrow chimera mice. Studies using these mice identified bone marrow-derived cells as primary cellular targets through which A2A receptor activation affords protection from renal ischemia-reperfusion injury [93]. Although the particular cell
type in the bone marrow has yet to be determined, A2A receptor agonist-mediated protection is independent of activation of macrophage A2A receptors, as the absence of an A2A agonist-mediated, protective effect in A2A KO mice was not restored by reconstitution with WT macrophages [94], which, however, do appear to play a role in the attenuating effect of A2A receptor activation on the course of diabetic nephropathy [95]. The reason for this proposition is that tissue infiltration of macrophages, which are key players in driving kidney fibrosis, leading to renal dysfunction in diabetes, is strongly decreased by A2A receptor agonist treatment in streptozotocin-induced diabetes in mice [95]. Additionally, A2A receptor agonists reduce levels of the macrophage-derived cytokines MCP-1 and TNF-α, both of which are important in affecting tissue fibrosis and glomerulosclerosis in diabetic mice [95]. Finally, it is interesting to note that diabetic nephropathy is more severe in A2A receptor KO mice than WT mice, which suggests that endogenous adenosine might contribute to kidney protection from diabetes in a similar manner as it does in kidney ischemia-reperfusion injury [95].

**JOINTS**

Low-dose methotrexate has been demonstrated to be an effective therapy for rheumatoid arthritis and is the most commonly used disease-modifying, antirheumatic drug in the treatment of this disease [96]. Several lines of evidence now indicate that the immunosuppressive and anti-inflammatory effects of methotrexate are a result of its capacity to increase extracellular adenosine levels [97, 98]. Moreover, recent results support the view that adenosine acting at A2A receptors mediates some of the antiarthritic effects of methotrexate, as methotrexate was less effective in suppressing leukocyte accumulation in A2A receptor KO than WT mice [99]. Potential cellular targets of A2A receptor-mediated, anti-inflammatory effects in joints involve infiltrating leukocytes [99], as well as chondrocytes [100] and synovial cells [101].

**CNS**

A2A receptors are expressed at high levels on neurons, inflammatory cells, and glial cells [102]. A2A receptors on bone marrow-derived cells were recently incriminated as cardinal contributors to ischemic brain injury. Selective inactivation of A2A receptors on bone marrow cells in chimeric mice protects against ischemic brain injury following middle cerebral arterial occlusion [103]. This protection is accompanied by a reduced expression of levels of macrophage-derived, proinflammatory mediators, such as IL-1, IL-6, and IL-12 in the brain, demonstrating a proinflammatory effect of A2A receptor stimulation in this model. This injurious effect of A2A receptor activation on bone marrow cells in the ischemic brain is unique, as A2A receptor activation on bone marrow-derived cells is protective in the spinal cord [104].

**MULTIORGAN DYSFUNCTION AND SEPSIS**

Similar to data with ischemia-reperfusion in peripheral organs, pharmacologic studies using exogenous A2A receptor agonists in A2A KO and WT mice show that the activation of A2A receptors decreases organ injury and mortality, which is secondary to the overwhelming inflammation triggered by endotoxin [105, 106]. The recent demonstration of the tissue protection by endogenous adenosine acting at A2A receptors in this hyperinflammatory model of sepsis [84, 107] further suggests the relevance of A2A receptors to protection from hyperacute, systemic inflammation. The mechanistic link between A2A receptor stimulation and protection from inflammatory organ injury is suggested by studies showing that A2A receptor KO mice injected with LPS have increased plasma levels of proinflammatory TNF-α and activation of NF-κB compared with similarly treated WT controls [84, 107].

In contrast, in the clinically relevant cecal ligation and puncture (CLP) model of sepsis, which is characterized by an immune-suppressed state and insufficient bacterial clearance, A2A receptor activation proved to be harmful, as stimulation of these receptors by endogenous adenosine decreased immune responsiveness, bacterial clearance, and survival [108, 109]. The decreased survival of mice caused by A2A receptor stimulation was tightly associated with a capacity of A2A receptor stimulation to increase bacterial burden, to augment immune cell apoptosis, and to increase production of the immunosuppressive cytokine IL-10. Recent in vitro studies have verified the essential role of A2A receptor stimulation in eliciting IL-10 release in conjunction with bacterial infection, as A2A receptor KO macrophages were unable to produce IL-10 following challenge with *Escherichia coli*, whereas the ability of WT cells to release IL-10 was intact [20].

Taken together, our data that A2A receptor activation increases mortality during CLP-induced sepsis [108] might seem contradictory to observations that A2A receptor stimulation is beneficial in acute inflammation as a result of endotoxins. However, we believe the data seen as a whole suggest that differences in outcome in the two models are a result of immunosuppression being beneficial in acute inflammation/ischemia but detrimental in more clinically relevant models of infection-induced sepsis, where mortality depends more on the loss of control of bacterial growth.

**FUTURE OF A2A RECEPTOR LIGANDS IN TREATING HUMAN DISEASE**

A2A receptor activation is now considered as one of the most potent, endogenous, anti-inflammatory signals in the body. The adenosine-A2A anti-inflammatory axis is used as an endogenous means to control ischemia and inflammation-induced tissue injury, and attempts are being made to translate this anti-inflammatory mechanism to the clinical scenario. A2A receptor agonists thus have high potential in treating ischemia and immune/inflammation-induced tissue injury in a wide variety of diseases, ranging from acute myocardial infarction to Crohn’s disease (Table 1). In fact, an A2A receptor agonist, MRE-0094, is in clinical trials for chronic, neuropathic, and diabetic foot ulcers, owing to the anti-inflammatory and wound-
healing effects of A2A receptor stimulation [110]. Clinical trials with ATL146e as an anti-inflammatory compound have also been initiated [111].

There are several potential toxic effects that should be considered when testing A2A receptor agonists as a therapeutic option to treat inflammatory and ischemic disease. For example, A2A receptor agonists have been shown to adversely influence blood pressure, which is especially true for the widely used agonist CGS21680 [76]. Given the relatively moderate selectivity of CGS21680 toward A2A when compared with A1 receptors [22], it is possible that A1 receptors contribute to the adverse cardiovascular effect of this agent by directly impairing heart function [1, 2]. In addition, CGS21680 can cause vasodilation in some vascular beds in a NO- and KATP-channel-dependent manner, and this vasodilation may also contribute to alterations in blood pressure [112]. Recently developed and more selective A2A agonists, such as ATL146e, however, have the advantage of not decreasing blood pressure in rodents and larger mammals at doses that are fully effective inhibitors of inflammation [5]. A further potential issue with A2A agonists stems from the fact that certain G protein-coupled receptors are down-regulated following prolonged administration of the agonist, preventing long-term efficacy [1, 2]. Such effects, however, have not been observed so far with in vivo administration of A2A agonists, as exemplified by the effectiveness of A2A agonists in chronic models of inflammation (Table 1).

### TABLE 1. Effects of A2A Receptor Ligands in Animal Models of Disease

<table>
<thead>
<tr>
<th>Disease target</th>
<th>Animal model</th>
<th>Species</th>
<th>Compound</th>
<th>Dose</th>
<th>Route of administration</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction</td>
<td>Left anterior descending artery occlusion/reperfusion</td>
<td>Mouse</td>
<td>ATL146e</td>
<td>5 and 10 μg/kg</td>
<td>Intraperitoneal</td>
<td>Decreased infarct size</td>
<td>[60, 61]</td>
</tr>
<tr>
<td>Asthma</td>
<td>OVA-sensitization</td>
<td>Rat</td>
<td>CGS21680</td>
<td>10 and 100 μg/kg twice</td>
<td>Intratracheal</td>
<td>Decreased inflammatory cell influx</td>
<td>[75]</td>
</tr>
<tr>
<td>Asthma</td>
<td>OVA-sensitization</td>
<td>Mouse</td>
<td>CGS21680</td>
<td>10 and 100 μg/kg twice daily</td>
<td>Intranasal</td>
<td>Decreased inflammatory cell influx</td>
<td>[67]</td>
</tr>
<tr>
<td>COPD</td>
<td>LPS challenge</td>
<td>Mouse</td>
<td>CGS21680</td>
<td>10 and 100 μg/kg twice</td>
<td>Intranasal</td>
<td>Decreased elastase release</td>
<td>[67]</td>
</tr>
<tr>
<td>Trauma and hemorrhagic shock</td>
<td>Laparotomy and blood withdrawal</td>
<td>Rat</td>
<td>CGS21680</td>
<td>500 μg/kg</td>
<td>Intravenous</td>
<td>Decreased lung injury</td>
<td>[76]</td>
</tr>
<tr>
<td>IBD</td>
<td>Relapsing formalin-immune complex-induced colitis</td>
<td>Rabbit</td>
<td>ATL146e</td>
<td>0.1 μg/kg/min</td>
<td>Alzet pump</td>
<td>Decreased mucosal injury and inflammation</td>
<td>[79]</td>
</tr>
<tr>
<td>IBD</td>
<td>SAMP1/YitFc mouse spontaneous colitis</td>
<td>Mouse</td>
<td>ATL146e</td>
<td>0.1 μg/kg/min</td>
<td>Alzet pump</td>
<td>Decreased mucosal injury and inflammation</td>
<td>[79]</td>
</tr>
<tr>
<td>IBD</td>
<td>CD45RB&lt;sup&gt;kab&lt;/sup&gt; cell transfer into SCID mice</td>
<td>Mouse</td>
<td>ATL313</td>
<td>1.875 mg/kg in chow</td>
<td>Per os</td>
<td>Decreased mucosal damage</td>
<td>[80]</td>
</tr>
<tr>
<td>Viral/autoimmune hepatitis</td>
<td>Con A injection</td>
<td>Mouse</td>
<td>CGS21680</td>
<td>2 mg/kg</td>
<td>Intraperitoneal</td>
<td>Decreased liver damage</td>
<td>[84]</td>
</tr>
<tr>
<td>Liver ischemia/ reperfusion injury</td>
<td>Clamping of hepatic triad</td>
<td>Mouse</td>
<td>ATL146e</td>
<td>0.1 and 10 ng/kg/min</td>
<td>Alzet pump</td>
<td>Decreased liver damage</td>
<td>[51, 85]</td>
</tr>
<tr>
<td>Liver ischemia/ reperfusion injury</td>
<td>Clamping of hepatic triad</td>
<td>Mouse</td>
<td>ATL313</td>
<td>3 μg/kg</td>
<td>Intraperitoneal</td>
<td>Decreased liver damage</td>
<td>[86]</td>
</tr>
<tr>
<td>Ischemic acute renal failure</td>
<td>Clamping of renal artery and vein</td>
<td>Rat</td>
<td>ATL146e</td>
<td>4 ng/kg/min</td>
<td>Alzet pump</td>
<td>Improved renal function</td>
<td>[90]</td>
</tr>
<tr>
<td>Ischemic acute renal failure</td>
<td>Clamping of renal artery and vein</td>
<td>Mouse</td>
<td>ATL146e</td>
<td>10 ng/kg/min</td>
<td>Alzet pump</td>
<td>Improved renal function</td>
<td>[91–93]</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>Streptozotocin-induced diabetes</td>
<td>Rat, mouse</td>
<td>ATL146e</td>
<td>1 and 10 ng/kg/min</td>
<td>Alzet pump</td>
<td>Improved renal function</td>
<td>[95]</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>Spinal cord compression</td>
<td>Mouse</td>
<td>ATL313</td>
<td>1–10 μg/kg/twice daily</td>
<td>Intraperitoneal</td>
<td>Improved locomotor activity</td>
<td>[104]</td>
</tr>
<tr>
<td>Sepsis</td>
<td>Intrapерitoneal LPS or E. coli injection</td>
<td>Mouse</td>
<td>ATL146e</td>
<td>0.05–50 μg/kg</td>
<td>Intraperitoneal</td>
<td>Improved survival</td>
<td>[105]</td>
</tr>
<tr>
<td>Sepsis</td>
<td>CLP</td>
<td>Mouse</td>
<td>ZM241385a</td>
<td>15 mg/kg/twice daily</td>
<td>Subcutaneously</td>
<td>Improved survival</td>
<td>[108]</td>
</tr>
</tbody>
</table>
Alternatively, when there is need to provoke immune/inflammatory responses to rid the body of infections, as for example, in the immune-suppressed phase of sepsis, A2A antagonists might be useful in enhancing the immune system’s ability to fight and defeat invading pathogens (Table 1). In addition, the A2A antagonists might be useful in treating ischemic events in the brain, based on the observation that activation of A2A receptors on immune cells is responsible for mediating the deleterious effects of A2A agonism in stroke [103]. Potential side-effects with the A2A receptor antagonists might include increased blood pressure and inflammation; however, based on the results of recent trials with A2A antagonists to treat patients with Parkinson’s disease, A2A antagonists seem to be well-tolerated and devoid of side-effects [113, 114].

Taken together, despite some challenges that remain, optimism is currently high that A2A receptor ligands will have a place in the treatment of many inflammatory diseases.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health (NIH) grant R01 GM66189 and the Intramural Research Program of NIH, National Institute on Alcohol Abuse and Alcoholism, as well as Hungarian Research Fund OTKA (T 049537) and Hungarian National R&D Program 1A/036/2004.

REFERENCES


