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REVIEW

Suppression of inflammatory and immune responses by the A_{2A} adenosine receptor: an introduction

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The purine nucleoside adenosine has been described as a 'retaliatory metabolite' by virtue of its ability to function in an autocrine manner to modify the activity of a range of cell types following its extracellular accumulation during cell stress or injury. These effects are largely protective and are triggered by the binding of adenosine to any of four G-protein-coupled adenosine receptors. Most of the anti-inflammatory effects of adenosine have been assigned to the adenosine A_{2A} receptor subtype, which is expressed in many immune and inflammatory cells. In this brief article, we will outline the growing evidence to support the hypothesis that the development of agonists selective for the A_{2A} receptor is an effective strategy for suppressing the exaggerated inflammatory responses associated with many diseases by virtue of the receptor's ability to inhibit multiple pro-inflammatory signalling cascades.
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Abbreviations: ADA, adenosine deaminase; cAMP, cyclic AMP; CRM1, chromosomal region maintenance 1; Epac, exchange protein directly activated by cAMP; ERK, extracellular signal-regulated kinase; HEK, human embryonic kidney; IκB, inhibitor of NF-κB; IFN, interferon; IKK, IκB kinase; IL, interleukin; IR, ischaemia–reperfusion; JAK, Janus kinase; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; NK, natural killer; PDE, phosphodiesterase; PKA, cAMP-dependent protein kinase; SOCS, suppressor of cytokine signalling; STAT, signal transducer and activator of transcription; TLR, Toll-like receptor; TNFα, tumour necrosis factor-α; TNFR, TNFα receptor

Introduction

Adenosine is an endogenous purine nucleoside that mediates a wide variety of physiological functions by interaction with four cell-surface receptors (A_1, A_{2A}, A_{2B} and A_3; Fredholm *et al*., 2000, 2001). Numerous studies have highlighted the anti-inflammatory role of the A_{2A} receptor from studies of receptor distribution on inflammatory cells (Gessi *et al*., 2000) as well as from observations of inhibitory effects of A_{2A} receptor-selective agonists *in vitro* and *in vivo* and enhanced inflammatory responses *in vivo* in A_{2A} receptor-deficient mice (Sitkovsky, 2003; Hasko and Cronstein, 2004).

This article is written as an introduction to the symposium on *New Insights into the Anti-inflammatory Effects of A_{2A}AR Agonists* presented at the Life Sciences Meeting in Glasgow on July 11, 2007. Its purpose is to introduce the reader to some key concepts and serve as a basis for other chapters in this journal from the symposium discussing the following: (1) A_{2A} adenosine receptors in tissue protection from reperfusion injury, by Professor Joel Linden (University of Virginia, USA); (2) Adenosine receptors and asthma, by Professor Clive Page (King's College, London); (3) Moving out and turning tail: restricted collision of the A_{2A}-adenosine receptor revisited, by Professor Michael Freissmuth (University of Vienna, Austria); and (4) Hypoxia-adenosinergic regulation of immune response and tissue damage by Professor Misha Sitkovsky (Northeastern University, Massachusetts, USA).

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Generation of extracellular adenosine

Before considering what is currently known about A_{2A} receptor activation and its intracellular effects on inflammatory and immune signalling, it is important to consider how the endogenous agonist is generated *in vivo*. Adenosine is released from all cells upon the degradation of ATP.

Consequently, conditions of stress, hypoxia and increased tissue energy expenditure can lead to a rapid >200-fold elevation in tissue adenosine over basal levels (typically <50 nM) (Rivkees *et al*., 2001). Specifically, ATP is converted to adenosine at sites of inflammation and injury by the action of CD39, an ecto-apyrase, and the subsequent conversion of AMP to adenosine by the ecto-5′-nucleotidase CD73. These enzymes are abundantly expressed on endothelial cells as well as on leucocytes and regulatory T cells (Lennon *et al*., 1998; Eltzschig *et al*., 2004; Deaglio *et al*., 2007). The protective role of this pathway has been elegantly demonstrated by gene-targeting studies in mice, which have shown that the presence of functional CD39 and CD73 is necessary to maintain endothelial barrier function and thus prevent excessive vascular leakage following hypoxia (Eltzschig *et al*., 2003; Thompson *et al*., 2004). However, the accumulation of extracellular adenosine is transient due to its conversion to inosine by ecto-adenosine deaminase (ADA) or uptake into endothelial cells via specific transporters and conversion either to inosine by intracellular ADA or to AMP by adenosine kinase. Because adenosine exerts profound auto-/paracrine effects on critical aspects of the immune and inflammatory responses, it is perhaps not surprising that the expression of enzymes involved in its accumulation is subject to tight regulation. For example, both CD39 and CD73 are each strongly induced in vascular endothelial cells upon the onset of hypoxia, and CD73 expression can be potentiated also by interferon (IFN)-α and adenosine itself via receptor-mediated elevation of cyclic AMP (cAMP) (Narravula *et al*., 2000; Niemela *et al*., 2004). Thus, control of adenosine accumulation and initiation of protective signalling clearly represents an important adaptive mechanism by which hypoxia-mediated damage to the endothelium can be minimized.

Activation of signalling pathways by the A_{2A} receptor: *in vitro* studies

The A_{2A} receptor was originally identified by virtue of its ability to elevate intracellular levels of cAMP via receptor interaction with the heterotrimeric G-protein G_s and subsequent activation of adenylyl cyclase (Linden, 2001). Changes in cAMP levels are translated into pleiotropic intracellular effects by a panel of cAMP-binding effector proteins, which include cyclic nucleotide-gated ion channels, cAMP-dependent protein kinase (PKA) and exchange proteins directly activated by cAMP (Epacs) (Beavo and Brunton, 2002). Signal termination is achieved by hydrolysis of cAMP to 5′-AMP catalysed by the large superfamily of cyclic nucleotide phosphodiesterases (PDEs). A key aspect of cAMP's effects is the generation of intracellular cAMP gradients arising from the opposing effects of adenylyl cyclases and PDEs (Lynch *et al*., 2006). The ability of distinct regions within the cell to sample these gradients is dictated in part by specific A-kinase anchoring protein scaffolds that localize PDEs, RI and RII regulatory cAMP-binding subunits of PKA and Epacs to defined intracellular compartments. In the case of PKA, binding of cAMP to R subunits releases catalytic C subunits from the PKA holoenzyme and allows phosphorylation of nearby substrates (Baillie *et al*., 2005).

However, similar to the other three receptors, the A_{2A} receptor can also activate the extracellular signal-regulated kinase (ERK) pathway; this has been demonstrated in Chinese hamster ovary cells as well as in human embryonic kidney (HEK)-293, PC12 and vascular endothelial cells (reviewed by Schulte and Fredholm, 2003). Intriguingly, multiple mechanisms are responsible that appear to differ between cell types. For example, whereas A_{2A} receptor activation of ERK in CHO cells occurs via an Src-mediated process that can be blocked by H-89 and mimicked by treatment with the cell-permeable cAMP analogue 8-bromo-cAMP, stimulation of ERK by either endogenous A_{2A} receptors in human umbilical vein endothelial cells or recombinant A_{2A} receptors expressed in HEK293 cells appears to occur via a Ras- and Src kinase-dependent mechanism independent of cAMP elevation (Seidel *et al*., 1999). Identification of the Arf6 guanine nucleotide exchange factor as an A_{2A} receptor-binding protein revealed that its interaction with the receptor's unique cytoplasmic C-terminal domain was critical for the sustained cAMP-independent ERK activation observed upon receptor expression in HEK293 cells, although not affecting the initial spike of ERK phosphorylation detectable at 5 min (Gsandtner *et al*., 2005).

Suppression of immune and inflammatory events by the A_{2A} receptor: *in vitro* studies

Functional and immunological approaches have shown that A_{2A} receptors are expressed in specific haematopoietic cell populations. These include $CD4^+$ and $CD8^+$ T cells and natural killer cells as well as monocytes, macrophages and neutrophils. In contrast, the receptor is largely absent from B cells (Koshiba *et al*., 1999; Sullivan *et al*., 2001; Pinhal-Enfield *et al*., 2003; Zhang *et al*., 2005; Raskovalova *et al*., 2006). The majority of the A_{2A} receptor's inhibitory effects on immune and inflammatory processes in these cell types have been proposed to occur via cAMP-/PKA-dependent pathways, which are known to have wide-ranging effects on immune cell function (Tasken and Stokka, 2006). For example, selective activation of A_{2A} receptors expressed on lymphokine-activated killer cells using CGS21680 has been shown to suppress perforin- and FasL-mediated cytotoxicity via an RI PKA-dependent pathway (Raskovalova *et al*., 2006). Also, inhibition of both the oxidative burst response (Sullivan *et al*., 2001) and VLA-4 induction (Sullivan *et al*., 2004) in human neutrophils by A_{2A} receptor-selective agonists can be reversed by the PKA-selective inhibitor H-89 and potentiated by inclusion of rolipram, an inhibitor of PDE-4. Similarly, the inhibitory effect of A_{2A} receptor agonists on anti-CD3ε-mediated tyrosine phosphorylation of ZAP70, a critical step in T-cell activation, could also be reversed by H-89, indicative of a cAMP-/PKA-dependent mechanism (Sevigny *et al*., 2007). However, an alternative cAMP-activated pathway has recently emerged by which the A_{2A} receptor can suppress JAK (Janus kinase)–STAT (signal transducer and activator of transcription) pathway activation by cytokines

that utilize the transmembrane protein gp130 to trigger downstream signalling. In vascular endothelial cells and embryonic fibroblasts, cAMP-mediated activation of Epac1 results in the induction of the gene encoding 'suppressor of cytokine signalling-3' (SOCS-3) (Sands *et al.*, 2006). SOCS-3 is one of the eight SOCS family members (cytokine-inducible SH2 domain protein, SOCS-1 to -7) defined by the presence of a distinct N-terminal region linked to a central SH2 and C-terminal SOCS box domains (Yoshimura *et al.*, 2007). Upon induction, the SH2 domain binds to specific phosphor-Tyr residues present on target cytokine receptors, such as gp130 and the leptin receptor Ob-R (Heinrich *et al.*, 2003) (Figure 1). Once bound, a so-called 'kinase inhibitory region' within the N-terminal domain of SOCS-3 binds and inhibits the kinase activity of receptor-associated JAKs, thereby inhibiting STAT phosphorylation and activation (Yoshimura *et al.*, 2007) (Figure 1). The exact mechanism by which Epac1 activation triggers SOCS-3 gene transcription remains to be elucidated, but it may provide a common mechanism by which Epac-mediated, PKA-independent effects of cAMP on the induction of genes such as those for AQP-2 and pro-glucagon can be rationalized (Lotfi *et al.*, 2006; Umenishi *et al.*, 2006).

A key molecular mechanism that has emerged as being critical for the inhibitory effects of the A_{2A} receptor on inflammatory and immune responses is suppression of the nuclear factor-κB (NF-κB) pathway activated by cytokines such as tumour necrosis factor (TNF)-α, IL-1β as well as pathogen-derived Toll-like receptor (TLR) agonists such as lipopolysaccharide (LPS). Interestingly, the molecular basis for this inhibition appears to be cell type-specific. Thus, A_{2A} receptor expression in rat glioma cells prevents the accumulation of transcriptionally active NF-κB dimers in the nucleus in response to either LPS or TNFα by specifically blocking the phosphorylation and degradation of inhibitor of NF-κB (IκB) proteins, resulting in an abolition of inducible nitric oxide synthase (iNOS) induction in response to LPS or TNFα in combination with IFNγ (Sands *et al.*, 2004). This would suggest that the receptor inhibits a common step in TNFα receptor/TLR signalling at or above the level of IκB kinases (IKKs). Similarly, deletion of the A_{2A} receptor in macrophages increases the rate and extent to which TNFα triggers the degradation of IκBα, resulting in an enhanced accumulation of multiple κB-regulated transcripts (Lukashev *et al.*, 2004). In contrast, overexpression of the A_{2A} receptor in vascular endothelial cells blocks the TNFα-stimulated nuclear accumulation of p50/RelA heterodimers, thereby reducing κB-regulated adhesion molecule expression, without altering degradation of IκB proteins (Sands *et al.*, 2004). Thus, presumably A_{2A} receptor expression is either blocking the nuclear import of NF-κB dimers or accelerating chromosomal region maintenance 1 (CRM1)-dependent export back to the cytoplasm, although experiments utilizing CRM1 inhibitor leptomycin B suggest the former mechanism (EW Strong and TM Palmer, unpublished observations). Interestingly, recent work describing how the adenosine generated during hypoxic preconditioning suppresses cytokine activation of NF-κB has suggested that activation of adenosine receptors inactivates the Skp-cullin-F box-1 E3 ligase complex required for poly-ubiquitylation and degradation of IκBα by promoting the removal of the ubiquitin-like protein Nedd8 from the Cul1 component of the complex, although the identity of the adenosine receptor subtype involved and the relative

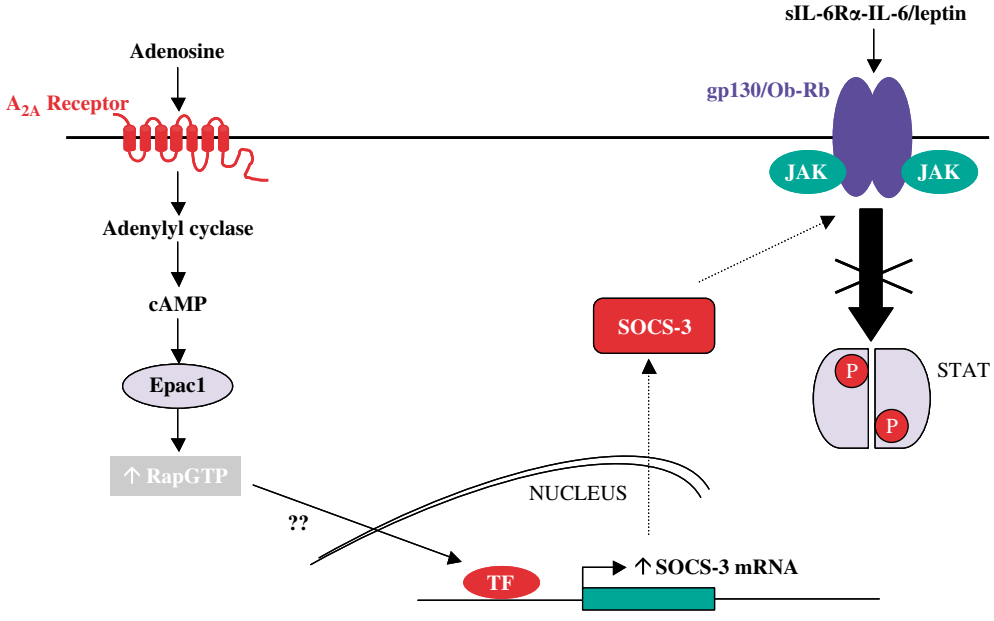

Figure 1 A_{2A} receptor-mediated inhibition of cytokine receptor activation of the JAK-STAT pathway. Binding of adenosine or synthetic agonists to the A_{2A} receptor triggers the activation of the stimulatory heterotrimeric G-protein G_s, which in turn activates adenylyl cyclase. The resulting elevation of cAMP levels is sensed by multiple intracellular cAMP-binding effectors, including Epac1. Epac1 functions as a cAMP-activated guanine nucleotide exchange factor for the Rap family of Ras-related small G-proteins, promoting the formation of GTP-bound Rap1a. This initiates transcription of the SOCS-3 gene via a transcription factor (TF) that has yet to be identified. In vascular endothelial cells, SOCS-3 inhibits JAK-mediated tyrosine phosphorylation of STATs after binding to tyrosine-phosphorylated Ob-Rb and gp130 following their activation by leptin and soluble IL-6 receptor α/IL-6 (sIL-6Rα/IL-6) trans-signalling complexes, respectively.

contributions of cAMP or other signalling pathways have not been determined (Khoury *et al.*, 2007). Nevertheless, such a mechanism could potentially account for the enhanced IκBα degradation observed in macrophages from A_{2A} receptor-deficient mice, as one would predict that the absence of the A_{2A} receptor should increase the proportion of active Cul1, thereby potentiating IκBα degradation (Lukashev *et al.*, 2004). Finally, it is important to note that the expression of the A_{2A} receptor is positively regulated by the pro-inflammatory stimuli whose responses it inhibits. For example, IL-1β, LPS and TNFα each increase A_{2A} receptor mRNA and protein levels in microvascular endothelial cells, macrophages and THP-1 monocytic cells (Nguyen *et al.*, 2003; Khoa *et al.*, 2004; Murphree *et al.*, 2005). The effect is inhibited, although not abolished, by pre-incubation with an IKK inhibitor (Murphree *et al.*, 2005), indicative of a role for NF-κB in up-regulating A_{2A} receptor gene transcription. This potentiates the ability of receptor activation to inhibit LPS-stimulated TNFα production in macrophages and also reduces TNFα-mediated up-regulation of vascular endothelial growth factor mRNA in endothelial cells (Nguyen *et al.*, 2003; Murphree *et al.*, 2005). Potentiation of A_{2A} receptor expression therefore constitutes an additional negative feedback mechanism by which inflammatory responses may be limited *in vivo*.

Anti-inflammatory potential of the A_{2A} receptor: *in vivo* evidence

The evidence that activation of the A_{2A} receptor *in vitro* leads to suppression of inflammatory responses is overwhelming as reviewed above. Three lines of experimental evidence targeting the A_{2A} receptor directly in animal models of inflammation–tissue damage should support the *in vitro* evidence if: (1) administration of an A_{2A} receptor-selective antagonist enhances inflammation following induction of an inflammatory response, (2) inflammation is exaggerated in animals with targeted deletion of the A_{2A} receptor and (3) application of selective agonists reduces inflammation and tissue damage.

Exacerbation of tissue damage and inflammation with A_{2A} receptor antagonists has been shown in a variety of inflammatory models. For instance, treatment of mice with the selective A_{2A} receptor antagonist ZM241385 enhanced liver injury and inflammation in response to concanavalin A, *Pseudomonas aeruginosa* and carbon tetrachloride (Ohta and Sitkovsky, 2001; Chan *et al.*, 2006). Similarly, ZM241385 prevented both the anti-inflammatory effects and the increased survival rates induced by low-dose ketamine administration, which promotes adenosine accumulation, in mice where sepsis was induced by LPS or *Escherichia coli* (Mazar *et al.*, 2005). In the mouse lung, treatment with ZM241385 enhanced lung neutrophilia in response to intratracheal administration of LPS (Thiel *et al.*, 2005). However, in other models, ZM241385 did not enhance inflammation/tissue damage in response to an inflammatory stimulus (see, for example, Peirce *et al.*, 2001; Fozard *et al.*, 2002). This may reflect either varying amounts of endogenous adenosine in the different models or, alternatively, administration of a near-maximal dose of inflammatory stimulus, which would make it difficult to observe any further enhancement with an A_{2A} receptor-selective antagonist.

Studies in animals with targeted deletion of the A_{2A} receptor largely support the conclusions from experiments using ZM241385. For instance, mice deficient in the A_{2A} receptor displayed an exaggerated inflammatory response and cytokine release in response to a wide variety of inflammatory insults, such as concanavalin A- and carbon tetrachloride-induced liver damage, and LPS-induced inflammation in an air pouch model (Ohta and Sitkovsky, 2001; Chan *et al.*, 2006). Mice lacking the A_{2A} receptor were recently reported to have exaggerated lung inflammation in response to sensitization and inhalation with ragweed antigen (Nadeem *et al.*, 2007). In addition, adoptive transfer studies employing inflammatory cells from mice lacking the A_{2A} receptor have also supported the concept that this receptor functions as a physiological anti-inflammatory mechanism (Yang *et al.*, 2006).

The above-mentioned two groups of studies, in which signalling via the A_{2A} receptor has been inhibited, support the concept that in an inflammatory environment the A_{2A} receptor functions as a 'physiological brake' on inflammatory processes. A number of reviews have been written, highlighting the concept that the A_{2A} receptor is involved in inflammation (e.g., Sitkovsky, 2003; Sitkovsky *et al.*, 2004; Hasko and Cronstein, 2004). From a therapeutic perspective, these data also suggest that administration of selective A_{2A} receptor agonists should inhibit the excessive inflammation and tissue damage associated with disease. There are many publications supporting the view that agonists of the A_{2A} receptor can inhibit inflammation in a wide variety of *in vivo* models. In addition, CGS21680, the first reported selective agonist at the A_{2A} receptor (Hutchinson *et al.*, 1989), and the more recently developed ATL-146e (Sullivan *et al.*, 2001) have also shown a broad spectrum of anti-inflammatory and tissue-protective effects in many different animal models of inflammation (Akkari *et al.*, 2006; and see below).

Investigation of the anti-inflammatory mechanisms *in vivo* have supported many of the observations made *in vitro* and have provided evidence for the anti-inflammatory effects of A_{2A} receptor agonists in a variety of target tissues, as discussed below.

Liver

Concanavalin A induces polyclonal T-cell activation and subsequently liver cell death (Tiegs *et al.*, 1992). The suggested mechanism of injury involves $CD4^+$ T cells with secretion of cytokines such as TNFα, IFNγ and IL-6 (Gantner *et al.*, 1995; Ohta and Sitkovsky, 2001). TNFα and IL-6 are both known to be important for induction of liver cell damage, apoptosis and necrosis. CGS21680 and ATL-146e inhibit both liver damage and the associated elevation in serum cytokine levels induced by concanavalin A administration, suggesting that inhibition of cytokine production by T cells contributed to the protective effect observed (Odashima *et al.*, 2006). This conclusion was supported by the observations of Ohta and Sitkovsky (2001), who demon-

strated that concanavalin A-induced liver damage was enhanced in mice lacking the A_{2A} receptor.

Kidney

A tissue protection mechanism via an inhibitory action on $CD4^+$ T cells has also been supported by *in vivo* studies examining the protective effects of ATL-146e on ischaemia–reperfusion (IR) damage in the mouse kidney (Day *et al*., 2003, 2006). In these studies, the protective effect of ATL-146e was inhibited in mice whose bone marrow had been ablated and reconstituted with bone marrow from A_{2A} receptor-deficient animals. Using adoptive transfer techniques, the mechanism of protection of ATL-146e was shown to be due to an action of A_{2A} receptor agonists on $CD4^+$ T cells and involved an inhibition of IFNγ release.

Lungs

In rats and mice, intratracheal or intranasal administration of CGS21680 has been shown to suppress lung inflammation in response to pro-inflammatory stimuli such as antigen (ovalbumin) and involved inhibition of neutrophil, eosinophil, macrophage and lymphocyte infiltration (Fozard *et al*., 2002; Bonneau *et al*., 2006). In the study by Fozard *et al*. (2002), the effects of CGS21680 were inhibited by the selective A_{2A} receptor antagonist ZM241385, although this agent did not appear to exaggerate inflammation induced by antigen itself. In contrast, CGS21680 was much less effective in inhibiting lung inflammation in response to LPS–*N*-formyl-methionine-leucine-phenylalanine administration in mice, but it did appear to inhibit neutrophil activation, as assessed by elastase release (Bonneau *et al*., 2006). Thiel *et al*. (2005) recently reported that the deleterious effect of oxygen therapy on lung inflammation and survival in mice was prevented by treatment with CGS21680, and several publications have also documented the inhibitory effects of A_{2A} receptor agonists on lung injury and inflammation in response to IR (Hasko *et al*., 2006).

Heart

Agonists at the A_{2A} receptor reduce infarct size and inflammation in studies of IR in canine and mouse models of myocardial infarction. Initial studies in canine models demonstrated that inhibition of P-selectin induction, neutrophil infiltration and other indices of inflammation accompanied the reduction in infarct size (Glover *et al*., 2005, 2007). Subsequent studies in mouse models of cardiac IR have highlighted the potential central role of $CD4^+$ T cells in orchestrating several aspects of the inflammatory process, such as neutrophil infiltration, in response to IR. For instance, infarct size in response to IR is smaller in Rag1 knockout mice, which lack mature lymphocytes, and is increased by adoptive transfer of $CD4^+$ T cells from control animals and those lacking the A_{2A} receptor but not in those T cells lacking the ability to secrete IFNγ. Importantly, activation of the A_{2A} receptor reduced both infarct size and inflammation in Rag1 knockout mice reconstituted with mature T cells but not in those reconstituted with $CD4^+$ T cells lacking the A_{2A} receptor (Yang *et al*., 2006).

Overwhelmingly, therefore, the anti-inflammatory/tissue-protective effects of the A_{2A} receptor have been repeatedly demonstrated in several organs in a wide variety of *in vivo* model systems. Indeed, some anti-inflammatory medications have been proposed to derive some of their clinical benefit by promoting adenosine release (Cronstein *et al*., 1991; Gadangi *et al*., 1996; Hwang *et al*., 2001), and data from animal models suggest that this triggers an essential activation of the A_{2A} receptor (Cronstein *et al*., 1993; Montesinos *et al*., 2007).

The biochemical mechanism(s) of protection *in vivo* have also supported the *in vitro* observations. For instance, mice lacking the A_{2A} receptor display an enhanced release of TNFα and other cytokines, which is associated with increased gene transcription in response to injection of LPS. NF-κB is the major transcription factor responsible for production of pro-inflammatory cytokines in immune cells and, as described previously, studies in A_{2A} receptor-deficient mice have demonstrated an enhanced NF-κB activation associated with elevated IKK-mediated phosphorylation and proteasomal degradation of IκBα (Lukashev *et al*., 2004). In wild-type mice, administration of CGS21680 and ZM241385 were able to inhibit and enhance, respectively, CpG-mediated increases in inflammatory gene transcripts, presumably by modulating activation of the NF-κB pathway (Lukashev *et al*., 2004). Influences on the mitogen-activated protein kinase activation pathways and, in particular, activation of ERK1/2 have also been investigated, as these have been suggested to play a role in cell protection. For example, studies in lung have demonstrated that administration of A_{2A} receptor agonists induce a robust activation of the ERK pathway that is associated with reduced levels of pro-apoptotic markers (Rivo *et al*., 2007).

Clinical potential of A_{2A} receptor agonists

Despite all the overwhelming pre-clinical evidence that A_{2A} receptor-selective agonists can reduce inflammation and tissue damage, the most advanced agents (Regadenson from CV Therapeutics and Apadenoson from Adenosine Therapeutics) are actually in late-phase development as imaging agents/cardiac stress agents for coronary artery disease. This is based on the principle that activation of the A_{2A} receptor in the coronary circulation produces a profound vasodilation. Thus, infusion with a compound with a longer half-life (in the order of a few minutes) and better selectivity for the A_{2A} receptor than adenosine, which is currently utilized for such applications, would be expected to have a better and safer clinical profile in these conditions (Cerqueira, 2004).

Why then have the anti-inflammatory and tissue-protective properties of A_{2A} receptor agonists not yet been adequately tested in clinical trials? The A_{2A} receptor has a widespread tissue/cell-type distribution, and therefore activating this receptor might lead to unwanted side effects. For instance, the A_{2A} receptor mediates inhibition of platelet aggregation, hypotension and a variety of effects within the CNS, some of which involve interactions with dopamine D_2

receptors (see Ledent *et al.*, 1997; Schwarzschild *et al.*, 2006). Thus, chronic treatment of inflammatory conditions with an A_{2A} receptor-selective agonist might have an unacceptable side-effect liability. In support of this concept, Fozard *et al.* (2002) demonstrated that, following intratracheal administration, inhibition of lung inflammation by CGS21680 was accompanied by falls in blood pressure, and these authors speculated that strategies would have to be developed to target the action of adenosine A_{2A} receptor agonists within the lung to avoid the observed systemic side effects. Not withstanding the above consideration, at least two companies (Adenosine Therapeutics and Pfizer) are reported to be testing the anti-inflammatory potential of A_{2A} receptor-selective agonists in a variety of clinical situations.

Concluding remarks

Many studies have now established the ability of selective A_{2A} receptor activation to repress the exaggerated immune and inflammatory responses associated with many diseases. Although several high-affinity agonists with strong selectivity for the A_{2A} receptor have now been developed and tested in several animal models, a major aim in future will be to assess whether these will have a sufficiently low side-effect liability to be suitable for systemic administration in patients. The identification of adenosine as a critical factor that accumulates in tumours in response to hypoxia, and which prevents their destruction by inhibiting anti-tumour T-cell function via A_{2A} receptor activation (Lukashev *et al.*, 2007), would also suggest that selective antagonists would be beneficial in many types of cancers. Finally, although the vast majority of the A_{2A} receptor's effects appear to be mediated via elevation of cAMP, unravelling the detailed molecular mechanisms by which it suppresses specific pro-inflammatory signalling pathways will be essential if we are to (1) understand why A_{2A} receptor activation is such a powerful strategy for turning off immune and inflammatory responses and (2) devise ways to minimize unwanted side effects of receptor activation arising from initiation of cAMP-independent signalling pathways.

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Conflict of interest

The authors state no conflict of interest.

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