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Commentary

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A₁ antagonism in asthma: better than coffee?

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Adenosine is a ubiquitous biological mediator with the capacity to produce both pro- and anti-inflammatory effects in tissues. Proinflammatory and bronchoconstrictive actions of adenosine in the asthmatic lung are well recognized, with the latter being mediated, in part, through A₁ receptor activation on airway smooth muscle. In this issue of the *JCI*, Sun et al. report findings in adenosine deaminase-deficient mice that suggest the occurrence of anti-inflammatory actions of adenosine in the lung, mediated through A₁ adenosine receptors on macrophages (see the related article beginning on page 35). Here we discuss the history of the study of adenosine receptor ligands for asthma and how enhanced understanding of adenosine receptor biology may aid in the rational exploitation of these receptors as therapeutic targets.

A startling rise in asthma prevalence has occurred over the past 2 decades, making it one of the most common chronic diseases of industrialized countries. Most morbidity and all mortality from asthma is the result of acute exacerbations (commonly known as asthma attacks), and treatment of these exacerbations accounts for the majority of the economic burden attributable to this disease. As research efforts have been expanded to further elucidate disease pathogenesis, vast arrays of inflammatory mediators and proinflammatory pathways have been discovered, and pharmacological interruption of many of these pathways has been proposed. However, the challenge to medical scientists and the pharmaceutical industry is in determining which of a growing list of candidates warrants investment of the time and resources required for transition from the bench to the bedside.

The endogenous purine nucleoside adenosine is one proinflammatory mediator that has garnered interest as a contributor to asthma pathogenesis, particularly with regard to acute exacerbations of the disease. ATP, released by cells, is rapidly metabolized by extracellular nucleotidases to adenosine, a potent signaling molecule that can activate several cell surface receptors to produce myriad effects on both parenchymal and immune cells

throughout the body (Figure 1). As growing lines of evidence have supported a proinflammatory role for adenosine in the asthmatic lung, interest in adenosine receptor antagonists has risen.

Nonselective adenosine receptor antagonism

Evidence for efficacy of adenosine receptor antagonism may date back to the Victorian era, when physicians noted the beneficial effects of strong black coffee in patients with bronchial asthma. Coffee beans and tea leaves contain the plant alkaloid caffeine, a compound later recognized as an antagonist of adenosine receptors (1). A structurally similar methylated xanthine, theophylline, was developed into one of the first effective therapies for asthma in the 1940s and today remains the most widely prescribed drug for the treatment of airway disease worldwide (2), albeit with decreasing frequency in the US and Europe due to the advent of long-acting β agonists and inhaled steroids.

Theophylline can act as both a bronchodilator and immunomodulator, depending on the serum concentration achieved. At higher doses (serum levels of 10–20 mg/l), bronchodilation is believed to occur through phosphodiesterase (PDE) inhibition. However, at lower doses (serum levels 5–10 mg/l), which have been shown to be anti-inflammatory, PDE is not significantly inhibited. Several mechanisms have been proposed for the anti-inflammatory effects of low-dose theophylline, including the antagonism of adenosine receptors, which occurs at concentrations 20- to 100-fold lower than that required for PDE inhibition (3).

Receptors for adenosine

Adenosine acts through 4 distinct cell surface receptors, each with varying ligand affinities, tissue distributions, and signal transduction mechanisms (Table 1). Thus, both proinflammatory and anti-inflammatory signals can be transmitted to cells by adenosine, depending on which adenosine receptors are present and activated (Figure 2). Theophylline is a nonselective adenosine receptor antagonist that may block both pro- and anti-inflammatory actions of adenosine, potentially decreasing its efficacy. Selective antagonists of adenosine receptors mediating a pure proinflammatory signal – or conversely, agonists of receptors transmitting a pure anti-inflammatory signal – conceivably may be more potent than nonselective ligands such as theophylline.

The task of fully defining the effects of each adenosine receptor in an adenosine-rich environment, such as that present during an exacerbation of asthma (4), has been challenging in the past due to poor selectivity of adenosine receptor ligands, the expression of multiple receptor subtypes by both immune and parenchymal cells, and the difficulty establishing a model with chronic elevations of adenosine in the lung. Recently, mice deficient in adenosine deaminase (ADA), the primary catabolic enzyme for adenosine, have been generated. These animals have marked elevations of lung adenosine and die from respiratory failure at 18–21 days of age (5). Examination of these animals at the time of death has revealed several features similar to human asthma, including eosinophilic lung inflammation, goblet cell hyperplasia, mast cell degranulation, elevation of the Th2 cytokine IL-13, elevation of IgE, and airway hyperresponsiveness. ADA-deficient mice have been used in conjunction with more selective adenosine receptor ligands and through intercrosses with mice lacking specific adenosine receptors to begin to define the role of each individual receptor in the presence of sustained elevations in lung adenosine. Many of the inflammatory changes observed in the lungs of ADA-deficient mice were

Nonstandard abbreviations used: ADA, adenosine deaminase; MOG, myelin oligodendrocyte glycoprotein; PDE, phosphodiesterase.

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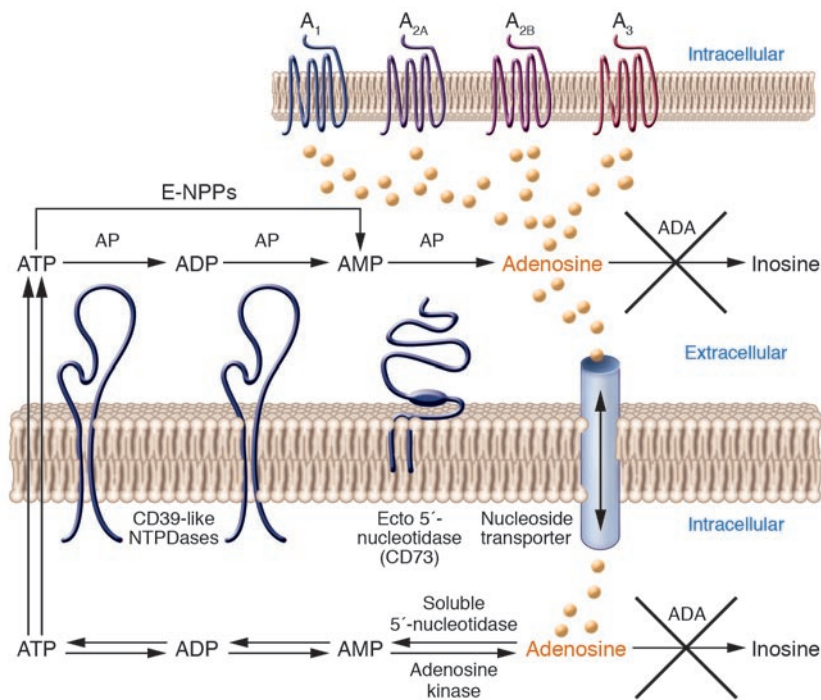


Figure 1
 Extracellular adenosine is produced predominantly by the metabolism of ATP released from cells. ATP is sequentially dephosphorylated by a series of membrane-bound and soluble ectonucleotidases to produce adenosine. Adenosine can act at 4 different 7-transmembrane, G-protein-coupled receptors present on the surfaces of both infiltrating leukocytes and resident parenchymal cells. While both proinflammatory and anti-inflammatory signals can be sent depending on the specific adenosine receptor activated, adenosine produces a net proinflammatory effect in the asthmatic airway. ADA is the primary catabolic enzyme for adenosine, and its absence in ADA-deficient mice results in marked elevations of extracellular adenosine. Elevations of extracellular adenosine are present in the asthmatic lung due to both increased release of ATP from cells and inhibition of ADA by local hypoxia. E-NPPs, ectonucleotide pyrophosphatase/phospho-diesterases; AP, alkaline phosphatase; NTPDases, ectonucleoside triphosphate-diphosphohydrolases.

attenuated when these animals were treated with a selective A₃ receptor antagonist or intercrossed with A₃-deficient mice, which suggests a proinflammatory role for the A₃ adenosine receptor under conditions of marked elevations of lung adenosine (6) (Figure 2).

Anti-inflammatory actions of A₁ adenosine receptor activation

In this issue of the *JCI*, Sun et al. report that mice deficient in both ADA and the A₁ adenosine receptor die at days 15–16 from an inflammatory lung disease that is more severe than that of age-matched littermates deficient only in ADA (7). In addition to enhanced eosinophilic lung inflammation, the authors observed exaggerated expression of Th2 cytokines and chemokines, increased mucus metaplasia, and increased expression of MMPs in animals lacking both the catabolic enzyme

and the A₁ receptor. These findings suggest an anti-inflammatory role for chronic A₁ receptor activation by high levels of adenosine in the lung, a surprising and important finding in light of the fact that A₁ receptor antagonists are being investigated as a potential treatment for asthma.

In ADA-deficient mice, A₁ expression is increased 3-fold in whole-lung RNA extracts and 50-fold in RNA isolated from bronchoalveolar lavage (BAL) cell pellets. Cell pellets from these animals contain pre-

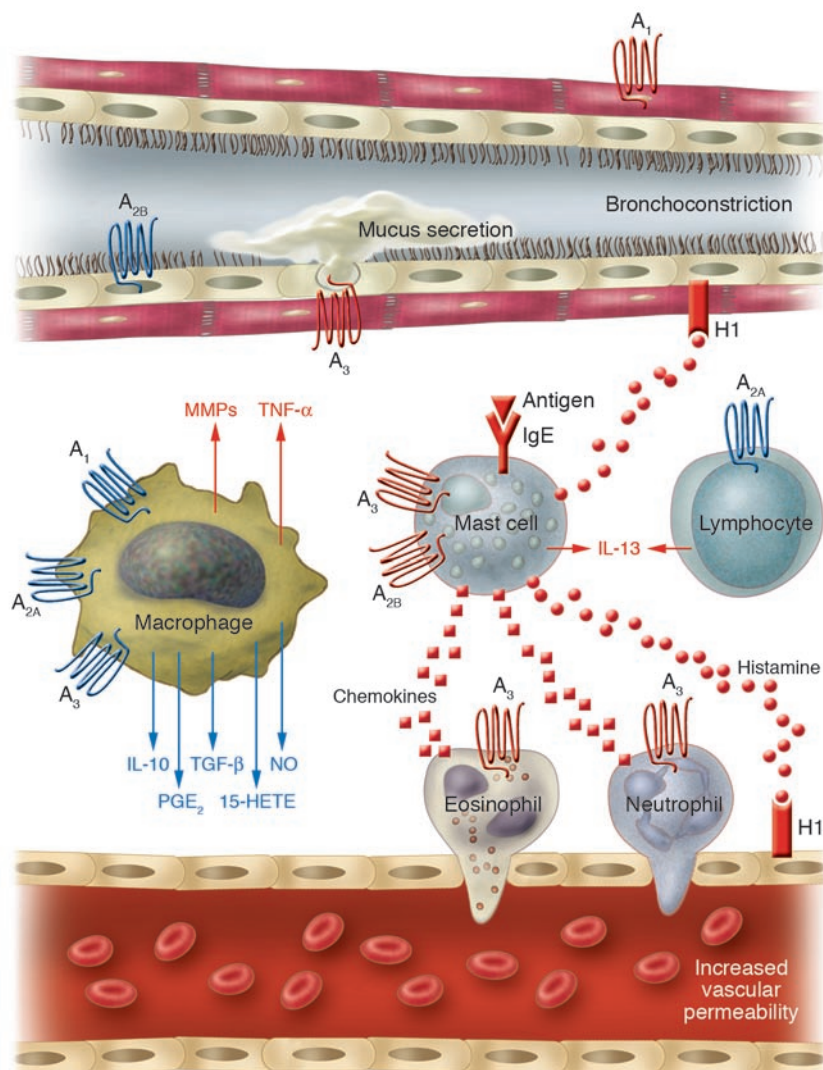
dominantly macrophages (200-fold more macrophages than all other cells combined), suggesting that this cell is the predominant cell type expressing A₁ in this model. Indeed, in situ hybridization with an A₁-specific probe localized expression to macrophages in the lungs of ADA-deficient mice. Thus, the anti-inflammatory effects of adenosine via A₁ are likely the result of activation of this receptor on macrophages.

Adenosine-mediated anti-inflammatory effects have been studied extensively in macrophages and macrophage cell lines. Adenosine inhibits the production of several proinflammatory cytokines (TNF-α, IL-6, and IL-8) by LPS-stimulated macrophages and enhances the release of the anti-inflammatory cytokine IL-10 (8–10). Despite these well-established anti-inflammatory effects of adenosine on the macrophage, the adenosine receptor(s) mediating these actions have been difficult to delineate due to incomplete selectivity of adenosine receptor ligands. While newer, increasingly selective compounds have been developed recently, there is a paucity of published data regarding their impact on macrophage function.

A similar anti-inflammatory role for the A₁ receptor on macrophages has been reported in studies with A₁-deficient mice in an experimental model of allergic encephalomyelitis (11). In this model, myelin oligodendrocyte glycoprotein-driven (MOG-driven) activation of macrophages in the CNS results in demyelination and axonal injury, producing an animal model of multiple sclerosis in WT mice. In addition to a worsened physiological and histopathological phenotype observed in A₁-deficient mice exposed to MOG, macrophages isolated from these animals showed increased expression of the proinflammatory genes *IL-1β* and *MMP-12* when compared to expression of these genes in macrophages from similarly exposed WT mice (11). Interestingly, diminished A₁ receptor expression on macrophages from patients with multiple sclerosis has been reported,

Table 1
 Signal transduction pathways of adenosine receptors

	A ₁	A _{2A}	A _{2B}	A ₃
G protein coupling	G _{i/o}	G _s	G _s G _q	G _i G _q
Effects	↓ cAMP ↑ IP ₃ ↑ K ⁺	↑ cAMP	↑ cAMP ↑ IP ₃	↓ cAMP ↑ IP ₃

**Figure 2**

Model of receptors and cell types mediating the pro- and anti-inflammatory effects of adenosine in the lung. Proinflammatory pathways are depicted in red while anti-inflammatory pathways are depicted in blue. A₃ receptor activation has been implicated in a number of proinflammatory events including mast cell-dependent increases in vasopermeability, adenosine-induced mast cell degranulation, enhancement of antigen-induced mast cell degranulation, mucus metaplasia and secretion, and recruitment of eosinophils and neutrophils to the airway. It remains unclear whether this chemotactic effect of adenosine on granulocytes is due to direct activation of A₃ receptors on these leukocytes or indirect activation through A₃-induced mediator release by other cell types, such as mast cells. A_{2B} receptors have also been implicated in mediating mast cell activation by adenosine. IL-13 and adenosine have been shown to stimulate one another in an amplification pathway that may contribute to the proinflammatory capacity of each mediator. Macrophages play an important anti-inflammatory role in asthma, and adenosine sends anti-inflammatory signals to macrophages through A₁ and A₃ receptors. These effects may occur through both the enhanced release of anti-inflammatory mediators, such as IL-10 and PGE₂, and the inhibition of release of proinflammatory mediators, including TNF- α and MMPs. Adenosine elicits bronchoconstriction in the asthmatic airway both directly from the activation of A₁ receptors on airway smooth muscle and indirectly by bronchoconstrictive substances released by mast cells. A_{2A} receptors are believed to send anti-inflammatory signals to all cell types on which they are expressed. Events depicted in the interstitium may also occur in the airway lumen.

suggesting that increased macrophage activation seen in this disease may be due, in part, to altered adenosine signaling (12). Taken together, these *in vivo* studies utilizing A₁-deficient animals in models of neurological and pulmonary disease support an anti-inflammatory role of A₁ receptor activation *in vivo*.

A₁ adenosine receptors and asthma

In contrast to patients with multiple sclerosis, those with asthma are believed to have increased expression of A₁ receptor in the lungs. Such increased expression of the A₁ receptor has previously been believed to contribute to the asthmatic phenotype largely through its capacity to facilitate adenosine-induced bronchoconstriction (Figure 2). Recently, an antisense oligonucleotide (EPI-2010) that binds the initiation codon of the

human A₁ receptor has been introduced into clinical trials for asthma (13). Pre-clinical studies in rabbits and primates focused on the effects of A₁ antisense on adenosine- and allergen-induced bronchoconstriction. However, measurements of inflammatory indices such as BAL cellularity or lung histopathology were not reported (13, 14). Thus, while A₁ antisense oligonucleotides have been shown to attenuate adenosine- and allergen-induced bronchoconstriction, their effect on the inflammatory component of asthma remains unknown.

The macrophage has been labeled as the forgotten cell in asthma, but data from animal models suggest that it may play an important anti-inflammatory role in allergic inflammation (15). ADA/A₁-deficient mice provide the first *in vivo* evidence suggesting that adenosine signal-

ing through the A₁ receptor represents a nonredundant anti-inflammatory signal to the pulmonary macrophage, which dampens the inflammatory response. If these findings can be extrapolated from mice to humans, then the potential clinical benefits of blocking A₁ receptors on airway smooth muscle, particularly in the adenosine-rich environment of an asthma attack, may be offset by increased inflammation. While we await more data, have another cup of coffee!

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Ca_v2.3 channel and PKC λ : new players in insulin secretion

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Insulin secretion is critically dependent on the proper function of a complex molecular network. Ca_v2.3-knockout (Ca_v2.3^{-/-}) and PKC λ -knockout (PKC λ ^{-/-}) mouse models now suggest that these 2 players, the Ca_v2.3 channel and PKC λ , are important constituents of this molecular network. Subsequent to glucose stimulation, insulin is released from the pancreatic β cell in a biphasic pattern, i.e., a rapid initial phase followed by a slower, more sustained phase. Interestingly, Ca²⁺ influx through the Ca_v2.3 channel regulates only the second phase of insulin secretion. PKC λ seems to enter the β cell nucleus and in turn modulates the expression of several genes critical for β cell secretory function. Studies by Hashimoto et al. and Jing et al. in this issue of the *JCI* set out to answer the question of why numerous isoforms of proteins with similar functions are present in the β cell. This is important, since it has been difficult to understand the modulatory and/or regulatory roles of different isoforms of proteins in defined subcellular compartments and at various times during the secretory process in both β cell physiology and pathophysiology (see the related articles beginning on pages 138 and 146).

The pancreatic β cell adequately and efficiently secretes insulin to maintain glucose homeostasis. Numerous players with distinct roles act in concert to precisely regulate the complex process of insulin secretion. The β cell relies on common mechanisms shared by other types of cells to execute exocytosis of insulin-containing granules, but also exhibits unique features. The β cell is exquisitely sensitive to glucose. Upon elevation

of the plasma glucose level, the β cell efficiently takes up glucose through glucose transporters. Thereafter, subsequent glucose metabolism results in the activation of a series of signal transduction events. A well-known paradigm demonstrates that an increase in the ATP/ADP ratio derived from glucose metabolism closes ATP-sensitive K⁺ (K_{ATP}) channels, resulting in depolarization of the plasma membrane. The membrane depolarization in turn opens Ca_v channels, mediating Ca²⁺ influx. The resultant increase in [Ca²⁺]_i triggers direct interactions between exocytotic proteins situated in the insulin-containing granule membrane and those localized in the plasma membrane. Eventually, the interaction between exocytotic

proteins initiates the fusion of insulin-containing granules with the plasma membrane, i.e., insulin exocytosis (1). There is no doubt that this K_{ATP} channel-dependent pathway plays a central role in the β cell stimulus-secretion coupling. However, abolition of this pathway does not entirely block glucose-stimulated insulin secretion. This observation has led to several significant discoveries of novel mechanisms of glucose-stimulated insulin secretion, which constitute a K_{ATP} channel-independent pathway (2). For example, application of high glucose together with activators of PKA and PKC significantly stimulates insulin secretion from the β cell even under conditions where there is neither Ca²⁺ influx through the plasma membrane nor Ca²⁺ mobilization from intracellular stores (3). These K_{ATP} channel-dependent and K_{ATP} channel-independent mechanisms operate in a highly cooperative manner, always guaranteeing adequate release of insulin to maintain normoglycemia (4).

Dynamics of insulin secretion

When the β cell is exposed to an abrupt and sustained increase in the concentration of glucose, it responds with a biphasic insulin secretory pattern (Figure 1, inset). This response is characterized by a rapid initial phase of insulin release, which is maintained for about 10 minutes, followed by a nadir, and subsequently, a gradually increasing second phase, which reaches a

Nonstandard abbreviations used: Ca_v2.3^{-/-}, Ca_v2.3-knockout; DAG, diacylglycerol; K_{ATP}, ATP-sensitive K⁺; PS, phosphatidylserine.

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