Adenosine Receptors and Asthma

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Abstract The pathophysiological processes underlying respiratory diseases like asthma are complex, resulting in an overwhelming choice of potential targets for the novel treatment of this disease. Despite this complexity, asthmatic subjects are uniquely sensitive to a range of substances like adenosine, thought to act indirectly to evoke changes in respiratory mechanics and in the underlying pathology, and thereby to offer novel insights into the pathophysiology of this disease. Adenosine is of particular interest because this substance is produced endogenously by many cells during hypoxia, stress, allergic stimulation, and exercise. Extracellular adenosine can be measured in significant concentrations within the airways; can be shown to activate adenosine receptor (AR) subtypes on lung resident cells and migrating inflammatory cells, thereby altering their function, and could therefore play a significant role in this disease. Many preclinical in vitro and in vivo studies have documented the roles of the various AR subtypes in regulating cell function and

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how they might have a beneficial impact in disease models. Agonists and antagonists of some of these receptor subtypes have been developed and have progressed to clinical studies in order to evaluate their potential as novel antiasthma drugs. In this chapter, we will highlight the roles of adenosine and AR subtypes in many of the characteristic features of asthma: airway obstruction, inflammation, bronchial hyperresponsiveness and remodeling. We will also discuss the merit of targeting each receptor subtype in the development of novel antiasthma drugs.

Keywords Adenosine · Adenosine receptors · Asthma · Bronchial hyperresponsiveness · Airway smooth muscle · Airway remodeling · Airway inflammation

Abbreviations

AC	Adenylate cyclase
ADA	Adenosine deaminase
ADP	Adenosine diphosphate
AK	Adenosine kinase
AMP	Adenosine monophosphate
AR	Adenosine receptor
ATP	Adenosine triphosphate
BAL	Bronchoalveolar lavage
BHR	Bronchial hyperresponsiveness
BMMC	Bone marrow-derived mast cell
CFTR	Cystic fibrosis transmembrane conductance regulator
CPA	Cyclopentyadenosine
CXCR4	Chemokine receptor 4
cyto-5′-NT	Cytosolic form of nucleotidase
DPCPX	1,3-Dipropyl-8-cyclopentylxanthine
EAR	Early asthmatic response
ecto-5′-NT	Ecto-5′-nucleotidase
FEV1	Forced expiratory volume in 1 s
fMLP	Formyl-Met–Leu–Phe
HBEC	Human bronchial epithelial cell
HPRT	Hypoxanthine phosphoribosyltransferase
ICS	Inhaled corticosteroids
IgE	Immunoglobulin E
IL	Interleukin
IMP	Inosine monophosphate
iNOS	Inducible nitric oxide synthase
LABA	Long-acting beta-adrenoceptor agonist
LAR	Late asthmatic response
LPS	Lipopolysaccharide
MCP-1	Monocyte chemotactic protein-1

NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa B
NTPDase	Nucleoside triphosphate diphosphohydrolase
PC	Provocative concentration
PEFR	Peak expiratory flow rate
PDE	Phosphodiesterase
PG	Prostaglandin
PLC	Phospholipase C
PNC	Purine nucleotide cycle
PNP	Purine nucleoside phosphorylase
POC	Proof of concept
RASON	Respiratory antisense oligonucleotide
RT-PCR	Reverse transcriptase polymerase chain reaction
SAHH	*S*-Adenosylhomocysteine hydrolase
TNF	Tumor necrosis factor
VEGF	Vascular endothelium growth factor

1 Adenosine: An Important Signaling Molecule in Asthma

Asthma is a lung disease characterized by airway hyperresponsiveness and inflammation. The pathogenesis of asthma involves the release of a broad array of mediators such as cysteinyl leukotrienes, histamine and cytokines from various cell types, leading to bronchoconstriction, proinflammatory effects, chemoattraction of leukocytes, and airway remodeling (Busse and Lemanske 2001). A number of clinical features distinguish asthmatic subjects from other respiratory diseases and may be considered characteristic of this phenotype (Avital et al. 1995). These include an exacerbation of disease following exposure to beta-adrenoceptor antagonists (Bond et al. 2007), an impairment in the ability to bronchodilate following deep inspiration (Slats et al. 2007), and their bronchoconstrictor sensitivity to a wide range of innocuous stimuli (Cockcroft and Davis 2006; Van Schoor et al. 2002). Various mechanisms have been proposed to account for this bronchial hyperresponsiveness (BHR) phenomenon, and these include increased airway smooth muscle function (An et al. 2007; Gil and Lauzon 2007), altered airway epithelial cell function (Holgate 2007), and the recruitment and activation of numerous inflammatory cells, including dendritic cells, T lymphocytes and eosinophils (Beier et al. 2007; Hammad and Lambrecht 2007; Jacobsen et al. 2007; Kallinich et al. 2007; Lloyd and Robinson 2007; Rosenberg et al. 2007), whose cell-derived products trigger a cascade of events within the lung that lead to airway epithelial cell damage, increased bronchial smooth muscle contractility and airway remodeling.

Asthmatic subjects bronchoconstrict in response to a number of physiological stimuli, such as exercise, distilled water, cold air and hypertonic saline, to which healthy subjects are refractory. Similarly, acidification, pollutants like sulfur dioxide, and chemical substances including adenosine, bradykinin and neuropeptides

evoke bronchoconstriction in asthmatics but have little if any effect in nondiseased individuals. These agents are commonly referred to as indirect-acting stimuli, since they do not appear to mediate bronchoconstriction by the direct activation of airway smooth muscle. They are thought to elicit bronchospasm by activating a number of different cell types, including mast cells, vascular smooth muscle cells, vascular endothelial cells, and/or airway nerves (Spina and Page 1996, 2002; Van Schoor et al. 2000). It is therefore of interest that asthmatic subjects are sensitive to such stimuli whilst healthy subjects are invariably unresponsive to these agents (Van Schoor et al. 2000). This suggests that the mechanisms by which these stimuli provoke bronchoconstriction are upregulated in asthma and are characteristic of this phenotype.

Furthermore, airway inflammation appears to be correlated better with BHR to indirect stimuli like adenosine (van den Berge et al. 2001), bradykinin (Polosa et al. 1998; Roisman et al. 1996) and hypertonic saline (Sont et al. 1993) than it is to more direct-acting stimuli like methacholine. Similarly, during an exacerbation of BHR following the deliberate exposure of an asthmatic subject to an environmental allergen (e.g., house dust mite), there is a preferential increase in BHR to an indirect-acting stimulus like bradykinin in contrast to methacholine (Berman et al. 1995). On the other hand, a number of pharmacological drugs used to treat asthma, including nedocromil sodium and ipratropium bromide, suppress airway responsiveness to these indirect-acting stimuli, suggesting the likely involvement of neural reflexes (Van Schoor et al. 2000). Furthermore, it is now recognized that glucocorticosteroids preferentially suppress BHR to adenosine (Ketchell et al. 2002; van den Berge et al. 2001) and bradykinin (Reynolds et al. 2002) compared with methacholine.

It is common for clinicians to use stimuli like methacholine and histamine as provocative inhalation challenge agents to induce bronchoconstriction because these agents are relatively convenient to use. However, whilst there is a separation in airway responsiveness to these agents between asthmatic subjects and healthy individuals, there is also a considerable degree of overlap, and it has been suggested that airway responses to these agents may not be sensitive indicators of the asthma phenotype (Avital et al. 1995; O'Connor et al. 1999). In contrast, asthmatic subjects invariably bronchoconstrict in response to the indirect-acting stimuli described earlier, which provoke little if any response in otherwise healthy individuals or in subjects with other respiratory diseases (Avital et al. 1995; Van Schoor et al. 2000).

A growing body of evidence has emerged in support of the purine nucleoside adenosine in the pathogenic mechanisms of asthma (Spicuzza et al. 2006). This body of evidence is supported by the following reported findings. (a) In asthmatics adenosine levels are elevated in bronchoalveolar lavage (BAL) fluid (Driver et al. 1993), in the circulation following allergen inhalation (Mann et al. 1986a), and in exhaled breath condensate in patients with asthma (Csoma et al. 2005). (b) Adenosine given by inhalation causes a dose-dependent bronchoconstriction in subjects with asthma (Cushley et al. 1983; Polosa 2002; Rorke and Holgate 2002). (c) Inhalational challenge with adenosine monophosphate (AMP), which is metabolized locally by the ectonucleotidase 5′-nucleotidase to adenosine, increases the release of leukotrienes and other bronchoconstrictive mediators in asthmatics (Bucchioni et al. 2004). (d) Adenosine enhances mast-cell allergen-dependent activation (Polosa et al. 1995);

(e) Treatment with dipyridamole, a blocker of adenosine reuptake, significantly enhances the bronchoconstrictor response to inhaled adenosine in subjects with asthma (Crimi et al. 1988). (f) The sensitivity of airways to adenosine and AMP more closely reflects an inflammatory process and the phenotype for allergic asthma than the sensitivity of airways to other known inhalational bronchoprovocative agents, such as methacholine and histamine (de Meer et al. 2002; Holgate 2002; Spicuzza et al. 2003; van den Berge et al. 2001).

1.1 Adenosine Metabolism

The physiological effects of adenosine in asthma via its stimulation of cell-surface adenosine receptors (ARs) and subsequent downstream signaling pathways are a function of the local concentration of adenosine. Adenosine concentrations in unstressed cells and tissue are below 1 μM (estimates 10–100 nM); however, in metabolically stressed inflamed or ischemic tissues, adenosine levels may rise to 100 μM (Fredholm 2007; Hasko and Cronstein 2004). Lower concentrations of adenosine (10–100 nM) activate the high-affinity A_1, A_{2A}, and A_3 ARs and high adenosine concentrations (10 μM) stimulate low-affinity A_{2B} ARs (Fredholm 2007). Factors that determine the net effect of adenosine on specific cell and tissue function are AR expression and coupling to intracellular signaling pathways, all of which are tightly regulated in different tissues and cells.

The local adenosine concentration at its receptor subtypes is determined by several processes, which include extracellular and intracellular adenosine generation, adenosine release from cells, cellular reuptake and metabolism (Fig. 1). These processes are closely intertwined and strictly regulated. For, example, under the hypoxic and inflammatory conditions encountered in asthmatic airways, the increased intracellular dephosphorylation of adenosine 5′-triphosphate (ATP) to adenosine by the cytosolic metabolic enzyme 5′-nucleotidase may be accompanied by a suppression of the activity of the salvage enzyme adenosine kinase, which prevents the rephosphorylation of adenosine to AMP (Deussen 2000). These processes lead to high adenosine concentrations inside the cell and the release of adenosine from the dephosphorylation of AMP into the extracellular space through nucleoside transporters (Hyde et al. 2001; Pastor-Anglada et al. 2001).

The other major pathway that contributes to high extracellular adenosine concentrations during metabolic stress is release of adenine nucleotides (ATP, adenosine diphosphate (ADP), and AMP) from inflammatory and injured cells. This is followed by extracellular degradation to adenosine by a cascade of ectonucleotidases, which include CD39 (nucleoside triphosphate diphosphohydrolase (NTPDase)) and CD73 (5′-ectonucleotidase) (Eltzschig et al. 2004; Kaczmarek et al. 1996; Resta et al. 1998; Thompson et al. 2004; Zimmermann 1999). Adenosine accumulation is limited by its catabolism to inosine by adenosine deaminase. Inosine is finally degraded to the stable end-product uric acid (Hasko et al. 2000, 2004). Mechanisms of nucleotide release and metabolism, or adenosine release and metabolism, as well

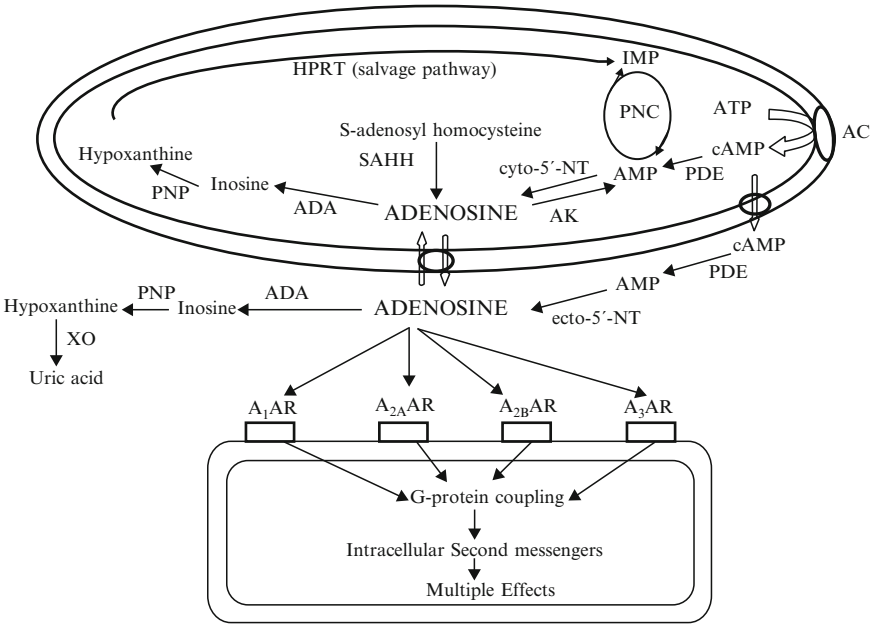

Fig. 1 Metabolism of adenosine. Adenosine is generated mainly by two enzymatic systems: intra/extracellularly localized nucleotidases and cytoplasmic *S*-adenosylhomocysteine hydrolase (SAHH). In response to hypoxia/cellular damage or other stressful/inflammatory stimuli, ATP is rapidly dephosphorylated by combined effects of adenylate cyclase (AC), phosphodiesterases (PDE) and nucleotidases to form intra/extracellular adenosine. Ecto-5′-nucleotidase (ecto-5′-NT) is one such enzyme that plays an important role in regulating local adenosine production for receptor signaling. Extracellular adenosine can interact with adenosine receptors (AR) that are coupled to heterotrimeric G proteins, which, in turn, couple AR activation to various effector molecules that can regulate second-messenger systems to influence cell and tissue function. Adenosine can also be deaminated to inosine by adenosine deaminase (ADA) that can exist intra- or extracellularly, or it can be transported into and out of the cells via membrane-associated nucleoside transporters. Intracellular adenosine is generated from the dephosphorylation of AMP by a cytosolic form of nucleotidase (cyto-5′-NT) or the hydrolysis of *S*-adenosylhomocysteine by SAHH. Adenosine can also be phosphorylated back to AMP by adenosine kinase (AK). AMP can also be directly deaminated to inosine monophosphate (IMP) by AMP deaminase. The reaction of phosphorylation predominates when adenosine occurs at a low physiological concentration ($<1\,\mu M$), whereas ADA is activated at higher concentrations of the substrate ($>10\,\mu M$). Hypoxanthine is formed after the removal of ribose from inosine by the actions of purine nucleoside phosphorylase (PNP) PNP has only negligible activity towards adenosine and degrades mainly inosine. Hypoxanthine can be salvaged back to IMP by hypoxanthine phosphoribosyltransferase (HPRT), which is again converted to AMP through the purine nucleotide cycle (PNC). Hypoxanthine can also enter the xanthine oxidase (XO) pathway to form xanthine and uric acid sequentially as byproducts

as transport mechanisms that account for the increased adenosine levels in exhaled breath condensate after exercise (Csoma et al. 2005), in the circulation following allergen inhalation (Mann et al. 1986a), and in BAL fluid (BAL adenosine concentration of $2.55 \pm 0.50\,\mu M$ in asthmatics versus $0.72 \pm 0.16\,\mu M$ in normals) (Driver et al. 1993) in human asthmatics, are yet to be determined.

There are several important cell types that are sources of extracellular adenosine. Neutrophils and endothelial cells release large amounts of adenosine at sites of metabolic distress, inflammation and infection (Cronstein et al. 1983; Gunther and Herring 1991; Madara et al. 1993; Rounds et al. 1994). Activated leukocytes are a major source of extracellular adenosine (Mann et al. 1986b). ADP released by platelets can be a significant source of adenosine after dephosphorylation (Marcus et al. 1995). Under conditions of stress including infection, activated macrophages can also serve as a major source of extracellular adenosine via ATP metabolism. Bacterial lipopolysaccharide (LPS) augments the release of ATP from macrophages (Sperlagh et al. 1998). Moreover, T-helper lymphocytes may be an important source of extracellular ATP. The presence of ecto-ATPase and antigen-triggered accumulation of extracellular ATP from T-helper cells has been reported (Apasov et al. 1995). In addition to inflammatory cells, airway epithelial cells and other structural cells in the lung may be important sources of high levels of adenosine in the airways of human asthmatics (Cohn et al. 2004).

1.2 Adenosine-Induced Bronchoconstriction, Airway Inflammation, and Airway Remodeling

In asthmatics, adenosine produces bronchoconstriction, inflammation, and airway plasma exudation, which lead to airway obstruction. Moreover, by acting on ARs, adenosine induces the release of inflammatory mediators that are important in the pathogenesis of airway remodeling in asthmatics. In both humans and animals, adenosine induces increases in BHR in asthmatics but not normal subjects, both in vivo following inhalation (Ali et al. 1994a; Cushley et al. 1983; Dahlen et al. 1983) and in vitro in small airways (Ali et al. 1994b; Bjorck et al. 1992). Adenosine produces bronchoconstriction in airways by directly acting on ARs in bronchial smooth muscle cells or indirectly by inducing the release of preformed and newly formed mediators from mast cells, and by acting on ARs on airway afferent sensory nerve endings (Hua et al. 2007a; Keir et al. 2006; Livingston et al. 2004; Polosa 2002). Multiple mechanisms may be involved in adenosine-induced bronchoconstriction; for example, the effects of adenosine in asthmatic subjects are sensitive to muscarinic receptor antagonists, suggesting that adenosine mediates obstruction indirectly (Crimi et al. 1992; Mann et al. 1985; Polosa et al. 1991), which would be consistent with the preclinical evidence that adenosine can activate afferent nerves in vivo (Hua et al. 2007a; Keir et al. 2006). However, since muscarinic antagonists do not completely abolish bronchoconstriction in response to adenosine, it is plausible to conclude that the "atropine-resistant" component of this response is mediated by direct activation of airway smooth muscle (Brown et al. 2008; Ethier and Madison 2006) and/or indirectly via mediators released from other cell types expressing these receptors.

Adenosine exposure through inhalation increases enhanced pause (Penh), a measure of airway resistance, in allergen-sensitized and -challenged mice (Fan

and Mustafa 2002). This increase in enhanced pause due to adenosine was reversed by theophylline with methacholine-mediated enhanced pause being unaffected, suggesting the involvement of ARs (Fan and Mustafa 2002). This finding that adenosine-induced bronchoconstriction is mediated by ARs is supported by an earlier study in a rabbit model of allergic asthma, where adenosine-induced bronchoconstriction was blocked by theophylline (Ali et al. 1992). Following inhalation and its local metabolism to adenosine in the airway, AMP induced bronchoconstriction is attenuated by potent cyclooxygenase inhibitors, H_1 receptor and leukotriene receptor antagonists, suggesting that adenosine induces the release of prostaglandins, histamine and leukotrienes in the airways of asthmatics (Phillips and Holgate 1989; Rorke et al. 2002; Rutgers et al. 1999). Another study has shown that inhalation challenge with adenosine, but not methacholine, produces mild airway plasma exudation (Belda et al. 2005). Collectively, these effects of adenosine on airway nerves, contraction of bronchial smooth muscle, release of mast cell mediators, and airway edema produce airflow obstruction.

Adenosine produces inflammation in airways in allergic animals and humans. Animals with increased adenosine concentrations in the lung (adenosine deaminase (ADA)-deficient mice) develop severe pulmonary inflammation, with airway accumulation of eosinophils and activated macrophages, mast cell degranulation, and mucus metaplasia in the airways—features similar to that found in asthmatic bronchi (Blackburn et al. 2000; Chunn et al. 2001). Treatment of these mice with exogenous ADA to reduce adenosine concentrations results in the reversal of these asthmatic features (Chunn et al. 2001). In a mouse model of allergic asthma, inhalation of adenosine has also been shown to cause airway inflammation, as evidenced by an increased release of proinflammatory mediators from eosinophils and mast cells (Fan and Mustafa 2002, 2006; Oldenburg and Mustafa 2005; Tilley et al. 2003). Moreover, in human asthmatics, an inhalational challenge with AMP produced an increase in eosinophils and neutrophils in the sputum (Manrique et al. 2008; van den Berge et al. 2004).

Adenosine-mediated inflammation is not limited to the lung; it also reaches the systemic circulation. In a recent report in a mouse model of asthma activities of eosinophilic peroxidase, myeloperoxidase and beta-hexosaminidase were increased not only in the lung but also in the systemic circulation of allergic mice exposed to adenosine aerosol (Fan and Mustafa 2006). In human asthmatics, adenosine aerosol increases the release of neutrophil chemotactic factor in serum (Driver et al. 1991). Moreover, in a recent study it was demonstrated that adenosine-induced effects on urinary 9α, 11β-prostaglandin (PG) F_2 levels (a sensitive biomarker of mast cell degranulation) were enhanced during repeated low-dose allergen challenge in allergic asthmatics (Ihre et al. 2006). These earlier findings in asthmatics were confirmed by a recent study showing an increase in plasma 9α, 11β-PGF_2 levels after adenosine challenge in asthmatics (Bochenek et al. 2008). These studies suggest that following inhalation, adenosine enhances the release of systemic inflammatory mediators from sensitized inflammatory cells. Thus, following inhalation, adenosine not only produces inflammation in the airways of asthmatics but it also induces a systemic inflammatory response that would, in turn, amplify the inflammation locally in the airways of asthmatics.

Adenosine in the lung may also be involved in the airway remodeling process (Cohn et al. 2004). Pathogenic hallmarks of airway remodeling are mucous gland hyperplasia, subepithelial fibrosis, hypertrophy of bronchial smooth muscle, and angiogenesis (Cohn et al. 2004; Jarjour and Kelly 2002). In a recent report, substantial angiogenesis in the tracheas of ADA-deficient mice were seen in association with high levels of adenosine (Mohsenin et al. 2007). ADA replacement enzyme therapy in these mice resulted in a lowering of adenosine levels and reversal of tracheal angiogenesis. Moreover, in lung alveolar epithelial cells and lung fibroblasts, adenosine caused an induction of fibronectin (a matrix glycoprotein highly expressed in injured tissues that has been implicated in wound healing) mRNA and protein expression in a dose- and time-dependent manner (Roman et al. 2006). Furthermore, there appears to be a connection of IL-13 levels to high adenosine levels, ADA activity and airway remodeling (Blackburn et al. 2003). Studies in CC10 IL-13 Tg mice showed that IL-13 induced high levels of adenosine, inflammation, lung collagen content and subepithelial airway fibrosis and reduced ADA activity in the lung. ADA therapy administered to these mice decreased adenosine levels, inflammation, and subepithelial airway fibrosis (Blackburn et al. 2003). Moreover, in ADA-deficient mice, IL-13 was strongly induced. These findings suggest that Il-13 and adenosine stimulate one another to amplify the pathway that contributes to airway inflammation, fibrosis, and remodeling. Similar findings were also seen in the lungs of mice overexpressing the Th2 cytokine IL-4 (Ma et al. 2006).

2 Adenosine Receptors in Asthma

Collectively, the studies presented above suggest a strong role for adenosine not only in the bronchoconstriction of allergic airways but also in the progression and amplification of airway inflammation and airway remodeling. The effects of adenosine as an important signaling molecule in asthma may depend not only on the bioavailability of the nucleoside but also on the expression, density, and affinity of ARs, which are known to be finely modulated by physiological and/or pathological conditions, signaling mechanisms, the local metabolism of adenosine, and the predominant inflammatory cell types in the asthma model, which may be species specific (Chunn et al. 2001; Fan et al. 2003; Sun et al. 2005; Zhong et al. 2006).

Adenosine produces its effects in asthmatics by acting on membrane-bound extracellular ARs on target cells. Four subtypes of ARs (namely A_1, A_{2A}, A_{2B}, and A_3) have been cloned in humans, are expressed in the lung, and are all targets for drug development for human asthma (Polosa 2002; Rorke and Holgate 2002). These receptors are heptaspanning-transmembrane G-protein-coupled receptors. Three of the AR subtypes (A_1, A_{2A}, and A_{2B}) demonstrate 80–95% sequence homology across a wide evolutionary range of species (Fredholm et al. 2001). In contrast, the A_3ARs demonstrate significant species variation. Signal transduction by the ARs varies; not only among the subtypes but also for a particular subtype between different cell sources (Fredholm et al. 2001). A_1ARs were originally characterized

Table 1 Characteristics and pharmacology of adenosine receptors

	A_1AR	$A_{2A}AR$	$A_{2B}AR$	A_3AR
Agonists	CPA, CCPA, CHA, S-ENBA	CGS 21680, ATL146e, CV-1808, CVT-3146, MRE0740; MRE0094	BAY 60-6583	IB-MECA (CF 101), 2-Cl–IB–MECA (CF102), MRS3558 (CF502)
Antagonists	DPCPX, FSCPX, N-0861, BG-9719, BG-9928, WRC-0571; KW 3902, L-97-1, SLV320, EPI-2010	ZM 241385, KW 6002, SCH 58261, SCH 442416, CSC	IPDX, MRS1754, MRS1706, CVT-6883, CVT-5440	MRS1220, MRS1191, MRS1523, VUF 5574
Transduction mechanisms	$G_{i/o}$, ↓ cAMP; ↑K^+, ↓Ca^{2+} channels, PLA_2; $G_{\alpha 16}$ NF-κB, PLC, PKC, ↑IP3/DAG	$G_{s/olf}$, ↑cAMP	G_s/↑cAMP; G_q/PLC, ↑IP3/DAG	G_i/↓cAMP; ↑Ca^{2+}, ERK1/2, G_q/PLC, ↑IP3/DAG; PLD

References: Beukers et al. (2006); Baraldi et al. (2000); Fredholm (2007); Fredholm et al. (2001); Gao and Jacobson (2007); Gessi et al. (2008); Hess (2001); Liu and Wong (2004); Moro et al. (2006); Yuzlenko and Kiec-Kononowicz (2006)

as being coupled to pertussis-toxin-sensitive G_i-coupled signal transduction pathways, but in some cells they are directly associated with, and act through, ion channels. The A_2AR subtypes (A_{2A} and A_{2B}) are typically coupled to G_s-linked signal transduction pathways. In some cells, A_1AR receptor-mediated inhibition and $A_{2A}AR$-mediated stimulation of adenylate cyclase may coexist and their functions may be counterregulatory (Fredholm et al. 2001). A summary of the AR subtypes, their signal transduction mechanisms, and selective agonists and antagonists is presented in Table 1.

Adenosine receptors have been described on a number of different cell types that are important in the pathophysiology of asthma, including dendritic, antigen-presenting cells, human airway epithelial and bronchial smooth muscle cells, lymphocytes, mast cells, eosinophils, neutrophils, macrophages, fibroblasts and endothelial cells (Thiel et al. 2003; Young et al. 2006; Wilson 2008). Activation of ARs on these different cell types is responsible for inducing the release of mediators and cytokines, leading to BHR, inflammation, edema, and airway remodeling. Activation of ARs on afferent sensory airway nerves contributes to BHR in asthma (Hua et al. 2007a). The contributions of the different AR subtypes to the pathophysiology of asthma will be discussed in the following sections and are presented in Fig. 2. In this review, the pathophysiological role of each AR and its signaling in asthma is discussed. Furthermore, the targeting of ARs with selective agonists or antagonists as therapeutic strategies in the treatment of asthma is also discussed and is presented in Table 2.

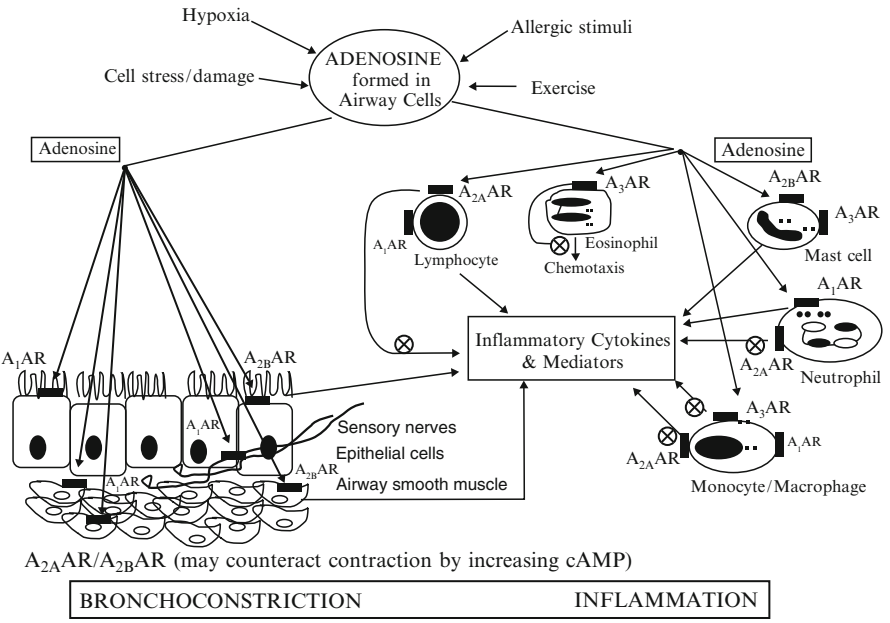

Fig. 2 Adenosine receptors and pathophysiology of asthma. By acting on adenosine receptors (ARs), A_1, A_{2A}, A_{2B}, and A_3 ARs, adenosine released under conditions of cellular stress as seen in asthmatic airways produces bronchoconstriction and inflammation. The net effect of adenosine on ARs will depend on the relative expression of these receptors on different cell types in asthmatic airways, and is concentration-dependent, as adenosine frequently exhibits opposing effects through the activation of AR subtypes expressed on the same cells coupled to different G proteins and signaling pathways. By acting on A_1ARs on bronchial smooth muscle cells and afferent sensory airway nerves, adenosine produces bronchoconstriction. By acting on A_1ARs on inflammatory leukocytes such as neutrophils, monocytes, macrophages, and lymphocytes, adenosine produces proinflammatory effects. Activation of A_{2A}ARs on the inflammatory cells suppresses the release of proinflammatory cytokines and mediators. Activation of A_{2A}ARs coupled to G_s and adenylate cyclase may also lead to bronchial smooth muscle relaxation via the cAMP–PKA (cyclic adenosine monophosphate–protein kinase A) pathway. Activation of A_{2B}ARs coupled to G_s and adenylate cyclase induce cytokine release from human bronchial epithelial and smooth muscle cells. Activation of A_{2B}ARs on murine bone marrow-derived mast cells (BMMCs) regulates the release of cytokines. The effect of adenosine on A_3ARs is species dependent. In mice, rats, and guinea pigs, activation of A_3ARs by adenosine produces bronchoconstriction, airway inflammation, mast cell degranulation, and mucus hyperplasia. In humans, activation of A_3ARs by adenosine produces anti-inflammatory effects, inhibition of chemotaxis and degranulation of eosinophils and cytokine release from monocytes. *Circled times* denote inhibition

2.1 A_1 Adenosine Receptors and Asthma

Until relatively recent times, the A_1AR received little attention as an important target in human asthma. However, a number of reports have demonstrated that expression of the A_1AR is upregulated in the airways of both animal models of allergic airway inflammation and human asthmatic subjects. Moreover, it is now appreciated

Table 2 Comparison of different potential therapeutic approaches targeting adenosine receptors in asthma

	A_1AR antagonists	A_{2A}AR agonists	A_{2B}AR antagonists	A_3AR agonists	A_3AR antagonists
Potential effects	Inhibition of bronchoconstriction, mucus hypersecretion, and inflammation	Bronchodilation and inhibition of inflammation	Inhibition of bronchoconstriction, inflammation, and airway remodeling	Inhibition of inflammation	Inhibit bronchoconstriction, inflammation, mucus hyperplasia
Disadvantages	No safety concerns reported to date for A_1AR antagonists in humans	CV side effects; tachyphylaxis; immune suppression	Reduce airway hydration; bronchoconstriction; inflammation	Tachyphylaxis; immune suppression	Inflammation
Latest developments in asthma	L-97–1 (Preclinical); EPI-2010 (Phase II; discontinued, no additional effect with ICSs)	GW328267X (Phase II; discontinued due to CV side effects)	CVT 6883 (Phase I); QAF 805 (Phase Ib)		QAF 805 (Phase Ib)
Pharmaceutical company involved in AR drug discovery	Epigenesis Pharmaceuticals; Endacea, Inc.; Biogen Idec; Merck; Solvay Pharmaceuticals; OSI Pharmaceuticals, Inc.	Glaxo Group Ltd; Pfizer; Novartis	CV Therapeutics; Novartis	Can-Fite Biopharma	Novartis

AR, Adenosine receptor; *CV*, cardiovascular; *ICSs*, inhaled corticosteroids
Adapted with permission from Wilson (2008)

that various functions relevant to asthma have also been associated with activation of the A_1AR, including bronchoconstriction, leukocyte activation and inflammation, BHR, and mucus secretion.

A pivotal study generated convincing evidence that A_1ARs could well play a significant role in the pathophysiology of asthma. The authors demonstrated that airway obstruction in response to aerosol administration of adenosine and allergen was inhibited in a rabbit model of allergic airway inflammation following treatment with antisense oligonucleotides as well as antagonist to this receptor (Ali et al. 1994a, b; Nyce and Metzger 1997). These data suggested that the A_1AR not only directly mediates bronchoconstriction following administration of exogenous adenosine, but that endogenous adenosine is an important component of the allergic response.

Although important species differences have been observed with regards to the expression and function(s) of the four AR subtypes, there is evidence that supports similar observations of an increased expression of the A_1AR and of A_1AR-induced bronchoconstriction in human asthmatic subjects. Firstly, it was demonstrated that adenosine-induced contraction of isolated bronchial tissue in vitro was greater in tissues obtained from asthmatic subjects than healthy subjects, and that this contraction could be significantly inhibited following preincubation with a selective A_1AR antagonist (Bjorck et al. 1992). Furthermore, it has been very recently demonstrated for the first time that expression of the A_1AR is increased in bronchial biopsies obtained from steroid-naïve mildly asthmatic subjects when compared with healthy subjects (Brown et al. 2008). This increased expression of the A_1AR appeared to be predominantly located in the airway epithelium and smooth muscle regions of the tissue, the latter observation thus correlating with the preclinical findings in the rabbit model of allergic asthma. In support of this, it has been demonstrated that activation of the A_1AR on human airway smooth muscle cells in vitro results in an increase in intracellular calcium mobilization, which could potentially mediate airway smooth muscle contraction (Ethier and Madison 2006). The finding of increased expression of A_1ARs in the airways and increased sensitivity of the airways to adenosine could well be of clinical significance. In asthmatics, the level of adenosine in plasma and exhaled breath condensate is increased following allergen or exercise challenge (Csoma et al. 2005; Mann et al. 1986a; Vizi et al. 2002) and therefore could lead to the activation of A_1ARs, thereby contributing toward airway obstruction during an acute exacerbation of asthma.

The report that the expression of A_1ARs is increased in bronchial biopsies of asthmatics is confirmed by the findings from another laboratory. In a preliminary study of a small number of human subjects, gene expression for A_1ARs is increased approximately 200% in bronchial tissue from small airways obtained from asthmatics ($n = 3$) versus normal subjects ($n = 3$) (Nadeem and Mustafa, unpublished data, West Virginia University). In these studies, expression of A_{2A}ARs is decreased while there is little to no change in the expression of A_{2B}ARs and A_3ARs in bronchial tissue from small airways in asthmatics versus normal subjects. The results of these studies were determined with the use of RT-PCR and confirmed with the use of western blots, with the exception of the A_{2B}AR, which was not tested in western blot studies.

A number of other studies using experimental animals have implicated a role for A_1ARs in mediating airway obstruction to adenosine. For example, the A_1AR agonist cyclopentyladenosine (CPA) selectively induces airway obstruction only in sensitized guinea pigs (Keir et al. 2006) and allergic rabbits (Ali et al. 1994a; el-Hashim et al. 1996). Further studies with the allergic rabbit model demonstrated that CPA also induced bronchoconstriction and stimulated IP_3 generation in airway smooth muscle (Abebe and Mustafa 1998). Allergic rabbits treated with the selective A_1AR antagonist L-97-1 ([3-(2-(4-aminophenyl)-ethyl]-8-benzyl-7-(2-ethyl-(2-hydroxy-ethyl)-amino]-ethyl)-1-propyl-3,7-dihydro-purine-2,6-dione]) provided bronchoprotection against inhaled adenosine (Obiefuna et al. 2005). However, atypical (Hannon et al. 2002) and adenosine A_1, A_{2B} and A_3 ARs (Fan et al. 2003; Hua et al. 2007a) have been suggested to mediate airway obstruction in response to adenosine in the brown Norway rat and mouse, respectively, underlying important species and strain differences.

Expression of the A_1AR has also been identified on a number of inflammatory cells. In general, these effects appear to be proinflammatory in nature. Activation of the A_1AR on human eosinophils, for example, promotes superoxide release (Ezeamuzie and Philips 1999). Furthermore, the A_1AR also mediates the respiratory burst in neutrophils (Salmon and Cronstein 1990), in addition to chemotaxis (Cronstein et al. 1990) and their adherence to endothelial cells (Cronstein et al. 1992). Furthermore, adenosine has been shown to promote monocyte phagocytosis (Salmon et al. 1993) and chemotaxis of immature dendritic cells (Panther et al. 2001), in addition to increasing the release of cytotoxic substances from endothelial cells that increase endothelial cell permeability (Wilson and Batra 2002) via the A_1AR.

The effects of adenosine upon inflammatory cells have been determined largely from in vitro experiments, and it should be noted that these effects are concentration dependent, as adenosine frequently exhibits opposing effects through the activation of other AR subtypes expressed on the same cells, since they are coupled to different G proteins. Thus, the relative expression of these receptors on inflammatory cells resident in asthmatic airways and the overall cellular effect of adenosine at the concentration present remain to be determined. It is likely, however, that the pattern of cellular expression for ARs changes following exposure to adenosine, since experimental evidence shows that an increased extracellular level of adenosine somewhat unusually appears to promote AR signaling. This was unequivocally demonstrated in mice partially deficient in ADA that consequently have high levels of adenosine in the lung (Chunn et al. 2001). Besides the severe pulmonary inflammation typical of this phenotype, these mice exhibited an increased transcript level for the A_1, A_{2B} and A_3 ARs.

In light of the many studies demonstrating the proinflammatory action attributed to activation of the A_1ARs, it is perhaps surprising that a preclinical study has purported to document an anti-inflammatory effect of A_1AR signaling (Sun et al. 2005). Adenosine deaminase is a ubiquitous enzyme responsible for the inactivation of adenosine, and mice deficient in this protein demonstrate profound

pulmonary injury, the presence of elevated levels of macrophages, and increased mucus production. These indices of tissue damage were exacerbated in ADA double-knockout mice also deficient in the expression of A_1AR, thereby implicating the loss of an anti-inflammatory pathway mediated by this receptor (Sun et al. 2005). However, the relevance of this model to human asthma or chronic obstructive pulmonary disease is debatable, since two of the principal cell types observed in these diseases, namely eosinophils and neutrophils, respectively, are present in such small numbers (<1.7%). In contrast to these findings, the A_1AR antagonist L-97-1 inhibited the recruitment of eosinophils and neutrophils to the airways of allergic rabbits challenged with house dust mite antigen (Nadeem et al. 2006).

Very few studies have specifically addressed the question of whether activation of A_1ARs is important in the development of BHR. Animal models of allergic inflammation are characterized by increased sensitivity to inhaled histamine, and interference in A_1AR signaling following either treatment with an antisense against this receptor (Nyce and Metzger 1997) or the use of a selective antagonist (Nadeem et al. 2006; Obiefuna et al. 2005) provided some degree of protection against the development of BHR. One can only speculate as to the mechanism by which adenosine, released within the inflammatory milieu of the airways, causes BHR via an A_1AR-dependent mechanism. Activation of these receptors on inflammatory cells including mast cells, eosinophils, dendritic cells, and lymphocytes could stimulate the release of other inflammatory mediators that, in turn, increase the sensitivity of the airways. Alternatively, adenosine might stimulate C fibers, thereby lowering the threshold for the activation of afferent input into the nucleus tractus solitarius, and thus facilitating reflex activation of parasympathetic nerves (Chuaychoo et al. 2006; Hong et al. 1998).

The mechanism(s) by which adenosine mediates airway obstruction in vivo in animal models may constitute indirect components. For example, adenosine activates pulmonary C fibers in the rat (Hong et al. 1998) and in the guinea pig (Chuaychoo et al. 2006; Lee et al. 2004), and cholinergic neural pathways in conscious mice (Hua et al. 2007a) via an A_1AR-dependent mechanism. Moreover, the effect of activation of A_1ARs by a selective A_1AR agonist, CPA, was specific for nodose but not jugular ganglion-derived C fibers (Chuaychoo et al. 2006). The consequence of activating these nerves following the endogenous release of adenosine during an inflammatory response may be airway obstruction, a phenomenon that was abolished in guinea pigs chronically treated with capsaicin in order to chemically inactivate C fibers (Keir et al. 2006). Reflex activation of parasympathetic nerves was further implicated, since vagotomy or treatment with the muscarinic antagonist atropine attenuated bronchospasm induced by CPA (Keir et al. 2006). Moreover, in mice, an adenosine-induced increase in airway resistance was abolished in A_1AR knockout mice and following vagotomy in wild type mice, but not in A_{2A}, A_{2B}, or A_3 AR knockout mice (Hua et al. 2007a). In conscious mice, the adenosine-induced increase in airway resistance was significantly reduced by the selective A_1AR antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) as well as atropine and bupivacaine, suggesting that the adenosine-induced bronchoconstriction was via the activation of A_1ARs on the cholinergic neural pathway. Similarly,

the cholinergic-dependent reflex activation of tracheal smooth muscle in situ in response to CPA was mediated by the activation of A_1AR (Reynolds et al. 2008).

Finally, in addition to its effects on bronchoconstriction, leukocyte activation and inflammation, and BHR, the A_1AR may play an important role in mucus secretion and airway remodeling of human asthma. It has been shown that adenosine is able to induce mucus secretion via activation of the A_1AR in the canine trachea in vivo (Johnson and McNee 1985), which has now been confirmed in human bronchial epithelial cells in vitro, where activation of the A_1AR was shown to increase the expression of the *MUC2* mucin gene (McNamara et al. 2004). Thus, it could be speculated that the reported increased expression of A_1AR on asthmatic bronchial epithelium (Brown et al. 2008) promotes adenosine-induced mucin secretion, although the extent to which adenosine contributes to the overall mucus hypersecretion in asthma clearly remains to be determined. Further studies will hopefully precisely define the functional effects of the A_1AR expressed in human asthmatic epithelium. With respect to a potential role of A_1ARs in airway remodeling, recent reports, albeit not pertaining to the lung per se, suggest that activation of A_1ARs may play an important role in angiogenesis and fibrosis, cardinal features of airway remodeling in human asthma (Clark et al. 2007; Cohn et al. 2004; Kalk et al. 2007). For example, activation of A_1ARs on human monocytes induces the release of vascular endothelial growth factor (VEGF) (Clark et al. 2007), and an A_1AR antagonist with high affinity and high selectivity for the human A_1AR, SLV320, significantly reduced levels of collagen I and III in an animal model of myocardial fibrosis (Kalk et al. 2007).

Validation of the A_1AR as an important target for human asthma is supported by positive proof of concept (POC) results in patients with asthma for EPI-2010, an antisense ("knockout") compound that is a respiratory antisense oligonucleotide (RASON) for the human A_1AR, in a small clinical trial conducted by EpiGenesis Pharmaceuticals (Cranbury, NJ, USA). EpiGenesis reported that a single dose of EPI-2010 reduced the need for bronchodilator drugs to control asthma symptoms concomitant with a reduction in symptom scores, an effect that was statistically and clinically significant and lasted for one week following a single dose (Ball et al. 2003). However, disappointing results in a Phase II clinical trial with EPI-2010 administered to patients who were taking inhaled corticosteroids (ICSs) were reported (Langley et al. 2005). In this Phase II clinical trial, 146 patients with persistent airway obstruction (forced expiratory volume in 1 S (FEV_1) 74.5% predicted, $\geqslant$12% reversibility) and currently receiving ICSs were administered EPI 2010 (1, 3, or 9 mg) via nebulizer once or twice weekly for 29 days. In this clinical study there was no significant change in the FEV_1 after 29 days of treatment compared to baseline. It was concluded that EPI-2010 showed no additional therapeutic effect in patients currently receiving ICSs. Patients with a stable FEV_1 of 74.5% predicted have mild/moderate asthma, depending on the frequency of symptoms and magnitude of variability in the peak expiratory flow rate (PEFR). In patients with mild/moderate asthma treated with ICSs, the FEV_1 may be 90–100% of the predicted value when measured between exacerbations and without provocation. Thus, the FEV_1 is not

a sensitive measure of asthma severity per se, vis-à-vis acute changes in airway function reflected by PEFR variability in ICS-treated patients with mild/moderate asthma. The lack of efficacy for EPI-2010 in this Phase II clinical trial (i.e., that EPI-2010 showed no additional therapeutic effect in patients taking ICSs) was not surprising.

Because of these effects of activation of A_1ARs on different cell types to produce bronchoconstriction, inflammation, mucous gland hyperplasia, angiogenesis, and fibrosis, all of which are important in the pathophysiology of human asthma, an A_1AR antagonist, L-97-1 (Endacea, Inc.), is in development as a once-daily, oral treatment for human asthma. L-97-1 is a water-soluble, small-molecule A_1AR antagonist with high affinity and high selectivity for the human A_1AR (Obiefuna et al. 2005). In an animal model of allergic asthma, L-97-1 blocks allergic airway responses, BHR to histamine, and airway inflammation (Nadeem et al. 2006; Obiefuna et al. 2005). A number of A_1AR antagonists have been or currently are in clinical trials for a number of different medical indications and, as a class, appear to be safe and well tolerated in humans (Barrett 1996; Bertolet et al. 1996; Dittrich et al. 2007; Doggrell 2005; Gaspardone et al. 1993; Givertz et al. 2007; Gottlieb et al. 2002; Greenberg et al. 2007).

2.2 A_{2A} Adenosine Receptors and Asthma

A_{2A}AR signaling in the pathophysiology of asthma may be critical considering the fact that A_{2A}ARs are present on most of the inflammatory cells (including neutrophils, mast cells, macrophages, eosinophils, platelets, and T cells; Lappas et al. 2005; Thiel et al. 2003). Activation of A_{2A}AR on these cell types is almost universally inhibitory, and therefore could modulate inflammatory events in the airways. The anti-inflammatory effects of activation of A_{2A}AR on these cell types include inhibition of chemotaxis, elastase release, phagocytosis, oxidative stress, adherence of neutrophils to endothelial cells, mast cell degranulation, and the release of proinflammatory cytokines (Lappas et al. 2005; Nadeem et al. 2007).

There are a multitude of mechanisms by which an agonist, acting through A_{2A}ARs, could suppress inflammation in asthmatic airways. In human neutrophils, stimulation of A_{2A}AR reduces neutrophil adherence to the endothelium, inhibits formyl-Met–Leu–Phe (fMLP)-induced oxidative burst, and inhibits superoxide anion generation (Visser et al. 2000). In monocytes and macrophages, activation of A_{2A}ARs inhibits LPS-induced tumor necrosis factor (TNF)-α expression (Bshesh et al. 2002). A_{2A}AR-deficient allergic mice have increased oxidative stress in the lung as well as the airway smooth muscle after ragweed/ovalbumin allergen challenge as compared to their wild type. This oxidative stress is caused by activation of inducible nitric oxide synthase (iNOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase signaling due to A_{2A}AR deficiency (Nadeem et al. 2007, 2008). Moreover, genetic removal of the A_{2A}AR from ADA-deficient mice results in enhanced inflammation (composed largely of macrophages and neutrophils, mucin

production in the bronchial airways, and angiogenesis) relative to that seen in the lungs of ADA-deficient mice with the A_{2A}AR, suggesting a protective role of this receptor in pulmonary inflammation when adenosine levels are high (Mohsenin et al. 2007). A_{2A}AR-mediated suppression of inflammation is mainly thought to be mediated by activation of protein kinase A and cyclic AMP response element-binding protein (Allen-Gipson et al. 2005; Bshesh et al. 2002), and inhibition of nuclear factor kappa B (NF-κB) signaling (Bshesh et al. 2002; Lukashev et al. 2004; Nadeem et al. 2007).

Strong anti-inflammatory properties for A_{2A}AR have been shown in an inflammatory disease model using A_{2A}AR gene-deficient mice (Lukashev et al. 2004; Nadeem et al. 2007). Consistent with this, in rat and mouse animal models of allergic asthma, the selective A_{2A}AR agonist CGS 21680 (2-*p*-(2-carboxyethyl) phenethylamino-5′-*N*-ethylcarboxoamido adenosine) significantly reduced the number of inflammatory cells in the BAL fluid during allergen-induced airway inflammation (Bonneau et al. 2006; Fozard et al. 2002). However, in these rodent animal models of allergic asthma, this selective A_{2A}AR agonist reduced airway inflammation but not BHR. Moreover, in an A_{2A}AR-deficient allergic mouse model, not only was airway inflammation enhanced but BHR was too (Nadeem et al. 2007). The discrepancy in A_{2A}AR-deficient allergic mice on airway reactivity versus the earlier report wherein airway reactivity was not reduced with the A_{2A}AR agonist, CGS 21680, in allergic mice is not apparent and may be due to differences in strains of mice.

Recently, the effects of a new A_{2A}AR agonist, GW328267X, in human asthmatics was reported (Luijk et al. 2008). In this study, treatment with GW328267X delivered as an inhalational treatment did not protect against the late asthmatic response (LAR), expressed as the decline in FEV_1 after allergen challenge, or the accompanying increase in airway inflammation (Luijk et al. 2008). However, in an earlier study, GW328267X partially inhibited the early asthmatic response (EAR) and LAR after nasal allergen challenge in patients with allergic rhinitis (Rimmer et al. 2007). There may be several possible explanations for the observed discrepancies between these two human studies. First, this A_{2A}AR agonist is not entirely selective for the A_{2A}AR; it also exhibits some inhibitory effect on A_3AR (Luijk et al. 2008). It is possible that inhibition of the A_3AR by GW328267X blocked the anti-inflammatory effects of A_3AR activation by adenosine, since it is reported that activation of the A_3AR in humans produces anti-inflammatory effects, including inhibition of migration of human eosinophils and inhibition of oxidative burst, degranulation and release of inflammatory cytokines in human neutrophils, monocytes, and macrophages (Fishman and Bar-Yehuda 2003). Thus, the inhibitory effect of GW328267X on A_3ARs may have counteracted possible beneficial effects of A_{2A}AR activation. Secondly, it is possible that the dose of the GW328267X (inhaled dose, 25 μg twice daily) was subtherapeutic. It was previously determined that higher doses of GW328267X caused cardiovascular side effects (reduction in blood pressure and increase in heart rate) following inhalational delivery (Luijk et al. 2008).

As mentioned above, even following inhalational delivery in small doses, the cardiovascular side effects of A_{2A}AR agonists may limit their clinical development. Moreover, tachyphylaxis and immune suppression may limit the clinical efficacy and safety of A_{2A}AR agonists as antiasthma drugs. For example, with the chronic administration of A_{2A}AR agonists, tachyphylaxis to the bronchodilator and anti-inflammatory effects may occur via the desensitization of G_s-coupled intracellular signaling pathways (Sullivan 2003). This potential effect of A_{2A}AR agonists was evident with the chronic administration of CGS-21680 over a two-week period wherein tachyphylaxis to the blood pressure lowering effect was reported and prevented the development of this A_{2A}AR agonist as an antihypertensive agent (Webb et al. 1993). Furthermore, because A_{2A}AR agonists act via G_s to stimulate adenylate cyclase, they may be associated with an increased risk of sudden death in asthmatics in a similar fashion to that of long-acting β2-agonists (LABAs) (Salpeter et al. 2004). Moreover, activation of A_{2A}ARs produces neovascularization (angiogenesis) (Cronstein 2006; Montesinos et al. 1997; Montesinos et al. 2006). Because of this effect of A_{2A}ARs on neovascularization/angiogenesis, an A_{2A}AR agonist, MRE-0094, is in Phase II clinical trials as a treatment for wound healing in diabetic foot ulcers (Aderis Pharmaceuticals). However, angiogenesis is a cardinal feature of airway remodeling of human asthma, and despite the report that activation of A_{2A}ARs promotes wound healing in bronchial epithelial cells (Allen-Gipson et al. 2005), the effect of A_{2A}AR agonists on angiogenesis may limit their development as antiasthma drugs.

Further to these clinical considerations for the development of A_{2A}AR agonists as antiasthma drugs, others include their potential to produce antitumor effects and immune suppression (Ohta et al. 2006; Sullivan 2003). Because A_{2A}AR agonists block oxidative and nonoxidative activity of neutrophils, cause functional repression and/or apoptosis of lymphocytes, and inhibit the release of (interleukin) IL-12, which promotes bacterial clearance in infection, these agents may cause immune suppression and predispose to infection (Sullivan 2003). Moreover, in an adenosine-rich tumor microenvironment, activation of A_{2A}ARs produces inhibition of antitumor T cells (Ohta et al. 2006). In mice, genetic deletion of the A_{2A}AR or the use of A_{2A}AR antagonists improved inhibition of tumor growth, destruction of metastasis and prevention of neovascularization by antitumor T cells. Despite what should be advantageous effects from the activation of A_{2A}ARs (i.e., bronchodilation and anti-inflammatory effects), the potential side effects of hypotension and tachycardia may limit the use of A_{2A}AR agonists as acute rescue antiasthma drugs, and the potential side effects of tachyphylaxis and immune suppression as well as the angiogenesis and antitumor effects produced by A_{2A}AR agonists may limit the use of these molecules as chronic maintenance antiasthma drugs.

2.3 A_{2B} Adenosine Receptors and Asthma

Studies in animals both in vitro and in vivo and human cell lines in vitro have suggested that A_{2B}ARs may play an important role in mediating airway reactivity,

inflammation, and remodeling in asthma. A_{2B}ARs are coupled via both G_s and G_q proteins to intracellular signaling pathways, which results in the release of cytokines and other mediators that are important in the pathophysiology of human asthma (Feoktistov et al. 1999; Zhong et al. 2004). In a human mast cell line (HMC-1), by coupling primarily to G_q, activation of A_{2B}ARs by adenosine induces the release of inflammatory cytokines such as IL-4, IL-8 and IL-13 which, in turn, can induce immunoglobulin E (IgE) synthesis by B lymphocytes (Feoktistov et al. 1999; Ryzhov et al. 2004). Moreover, in these HMC-1 cells, the selective A_{2B}AR antagonists IPDX (3-isobutyl-8-pyrrolidinoxanthine) and MRS 1754 ([*N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-phenoxy]acetamide]) inhibited activation of HMC-1 cells induced by NECA (5′-*N*-ethylcarboxoamido adenosine), a stable analog of adenosine (Feoktistov et al. 2001). Since HMC-1 cells are derived from a highly malignant, undifferentiated human mastocytoma cancer, the relevance of these findings in this human mast cell line to that in IgE immunologically sensitized human mast cells in allergic asthma is unknown. In the allergic response, antigens bind and crosslink IgE molecules bound to the functional high-affinity receptor for IgE, FcεRI, on mast cells to induce degranulation and the release of a broad spectrum of proinflammatory mediators (Nilsson et al. 1994; Xiang et al. 2001). HMC-1 cells do not express FcεRI (Nilsson et al. 1994). For this reason the reference to HMC-1 cells as human mast cells in allergic conditions, including human asthma, is misleading.

The presence of A_{2B}ARs on IgE immunologically sensitized human mast cells has not been reported. However, bone marrow-derived mast cells (BMMCs) from mice do express FcεRI (Hua et al. 2007b). Moreover, as opposed to the HMC-1 cells, A_{2B}ARs on murine BMMCs (Hua et al. 2007b), as well as human bronchial epithelial and smooth muscle cells and fibroblasts (Zhong et al. 2003, 2004, 2005), are coupled to G_s and adenylate cyclase, as compared to HMC-1 cells, where they are primarily coupled to G_q and phospholipase C (PLC) (Feoktistov and Biaggioni 1995). Furthermore, as opposed to the HMC-1 cell line, mast cell activation is enhanced in mice deficient for the A_{2B}AR (Hua et al. 2007b). The authors of this study suggested that in mice lacking this G_s-coupled receptor, BMMCs expressing FcεRI have reduced levels of cyclic AMP and an excess of intracellular calcium via store-operated calcium channels following antigen activation, thereby increasing their sensitivity to antigen-mediated degranulation. In addition, these A_{2B}AR-deficient mice display an increased sensitivity to IgE-mediated tachyphylaxis. In a recent study, genetic ablation of the A_{2B}AR had no effect on A_3AR-dependent potentiation of antigen-induced degranulation in mouse BMMCs, but abrogated A_{2B}AR-induced release of IL-13 and VEGF. The authors of this study suggest that in the mouse the A_3AR regulates mast cell degranulation, whereas the A_{2B}AR regulates mediator release, e.g., IL-13 and VEGF (Ryzhov et al. 2008a)

As mentioned above, as opposed to that seen in HMC-1 cells, by coupling to G_s and adenylate cyclase, adenosine activation of A_{2B}AR increases the release of inflammatory cytokines from human bronchial epithelial cells (HBECs) (Zhong et al. 2006), human bronchial smooth muscle cells (Zhong et al. 2004) and human fibroblasts (Zhong et al. 2005). In human bronchial smooth muscle cells, activation

of A_{2B}ARs induces the release of IL-6 and the chemokine monocyte chemotactic protein 1 (MCP-1) (Zhong et al. 2004). In HBECs, activation of A_{2B}ARs induces the release of IL-19, which in turn induces the release of TNF-α from monocytes, which in turn upregulates the expression of A_{2B}ARs on HBECs (Zhong et al. 2006). In human lung fibroblasts, activation of A_{2B}ARs induces the release of IL-6, which, in the presence of hypoxia, synergistically induced the differentiation of lung fibroblasts into myofibroblasts (Zhong et al. 2005). These effects of activation of A_{2B}ARs by NECA, a stable analog of adenosine, in human bronchial smooth muscle cells (Zhong et al. 2004) and HBECs (Zhong et al. 2006) are blocked by selective antagonists of the A_{2B}AR. Furthermore, in a recent study, genetic ablation of A_{2B}AR abrogated NECA-induced increases in IL-6 release from mouse peritoneal macrophages ex vivo and dramatically reduced the ability of NECA to increase IL-6 plasma levels in vivo (Ryzhov et al. 2008b). Moreover, stimulation of the A_{2B}AR on isolated mouse BMMCs can directly promote the production and secretion of IL-13 and VEGF (Ryzhov et al. 2008a). Taken together, these studies indicate that stimulation of A_{2B}AR is coupled to the release of proinflammatory cytokines, and may play an important role in airway remodeling of asthma.

Although the importance of these in vitro studies to support the role of the A_{2B}AR in vivo in humans with asthma remains to be determined, studies in animal models of allergic asthma support the role of this AR in asthma. In ragweed-sensitized allergic mice, airway challenge with adenosine increased bronchoconstrictor responses and amplified the pulmonary inflammatory response to an allergen challenge (Fan and Mustafa 2002, 2006). This increase in bronchoconstrictor responses and airway inflammation to adenosine was blocked by theophylline and attenuated by a specific antagonist of the A_{2B}AR, which suggests, in part, a role for the A_{2B}AR (Fan and Mustafa 2002; Fan et al. 2003; Mustafa et al. 2007). Moreover, in this allergic mouse model of asthma, adenosine-induced increases in β-hexosaminidase activity (a mast cell marker) were decreased by pretreatment with theophylline (Fan and Mustafa 2006). Furthermore, in another study involving the use of this allergic mouse model of asthma from this same group, aerosolized NECA- and AMP-elicited concentration-dependent increases in Penh were significantly attenuated by CVT-6883, an A_{2B}AR antagonist (Mustafa et al. 2007). In this study, an allergen challenge-induced increase in LAR was inhibited by CVT-6883, and the increase in the number of inflammatory cells in BAL fluid was also inhibited by CVT-6883 or theophylline.

These findings, that the A_{2B}AR antagonist CVT-6883 reduces inflammation in the lung in an animal model of allergic asthma, were demonstrated in another animal model of lung inflammation with a phenotype similar to allergic asthma, albeit not an allergic asthma animal model, ADA-deficient mice (Sun et al. 2006). As previously stated, ADA-deficient mice develop pulmonary inflammation, fibrosis, and enlargement of alveolar airspaces. In CVT-6883-treated ADA-deficient mice there was less pulmonary inflammation, fibrosis, and alveolar airspace enlargement (Sun et al. 2006). Moreover, in ADA-deficient mice, A_{2B}AR antagonism with CVT-6883 significantly reduced elevations in proinflammatory cytokines and chemokines as well as mediators of fibrosis and airway destruction (Sun et al. 2006). These findings

in these animal models suggest that A_{2B}AR signaling influences pathways critical for airway reactivity and inflammation.

As opposed to these reports suggesting that activation of A_{2B}ARs play an important role in bronchoconstriction and airway inflammation in allergic asthma, recent reports suggest that activation of A_{2B}ARs may produce bronchorelaxant and anti-inflammatory effects. In a recent study in guinea pigs, NECA evoked relaxing responses of isolated tracheal preparations precontracted with histamine in normal and sensitized animals, and this effect was reversed by the A_{2B}AR antagonist MRS 1706 (Breschi et al. 2007). Moreover, in vitro desensitization with 100 μM NECA markedly reduced the relaxing effect of NECA, raising the possibility that higher adenosine levels in the lung might desensitize this receptor to cause bronchorelaxation (Breschi et al. 2007). Furthermore, activation of A_{2B}ARs may produce anti-inflammatory effects. In A_{2B}AR knockout/reporter gene-knockin mice, there was low-grade baseline inflammation, augmented release of proinflammatory cytokines (including TNF-α and IL-6), as well as leukocyte adhesion to the vasculature (Yang et al. 2006). This finding that TNF-α levels are increased in A_{2B}AR knockout mice was confirmed by a more recent report by the same group (Yang et al. 2008). In a femoral artery injury model that resembles restenosis following angioplasty, A_{2B}AR knockout mice had higher levels of TNF-α, an upregulator of chemokine receptor 4 (CXCR4), and proliferation of vascular smooth muscle cells (Yang et al. 2008).

It is possible that the bronchorelaxant and anti-inflammatory effects of A_{2B}ARs described above may be due to an increase in intracellular cyclic AMP levels following activation of A_{2B}ARs. It is well known that an increase in intracellular cyclic AMP produces relaxation of bronchial smooth muscle and bronchodilation, suppresses inflammation, and prevents changes in endothelial cells that lead to an increase in endothelial permeability. Given these effects of intracellular cyclic AMP, it is unclear why an approach to the treatment of asthma would be to block these salutary effects of a receptor coupled via G_s to adenylate cyclase (i.e., the A_{2B}AR). It is now reported that the use of A_{2B}AR antagonists may increase endothelial permeability (Lennon et al. 1998). Moreover, in human airway epithelial cells via coupling to G_s and adenylate cyclase, A_{2B}ARs play an important role in control of the cystic fibrosis transmembrane conductance regulator (CFTR)-operated Cl^- channel (Clancy et al. 1999; Huang et al. 2001). Because of the importance of this Cl^- channel in airway hydration, the use of A_{2B}AR antagonists may induce a cystic fibrosis-like phenotype associated with an increased viscosity of mucus in humans, and may therefore limit their development as antiasthma drugs. Thus, although it appears that A_{2B}ARs may play an important role in airway remodeling of human asthma, because of their effect on the CFTR-operated Cl^- channel in human airway epithelial cells and airway hydration, the safety of A_{2B}AR antagonists in human asthmatics remains to be determined. Moreover, the efficacy of A_{2B}AR antagonists may depend on the relative contribution of this G_s-coupled receptor to adenylate cyclase and increases in intracellular cyclic AMP to produce bronchodilation and anti-inflammatory effects.

With respect to the therapeutic approach to the A_{2B}AR as a target in human asthma, based on the reports that activation of A_{2B}ARs in HBECs (Zhong et al. 2006), human bronchial smooth muscle cells (Zhong et al. 2004) and human lung fibroblasts (Zhong et al. 2005) induces the release of mediators important in the pathophysiology of airway remodeling of human asthma, as well as the efficacy of the A_{2B}AR antagonist CVT 6883 in an acceptable animal model of allergic asthma (Mustafa et al. 2007), CVT 6883 has entered Phase I clinical trials as an antiasthma drug (CV Therapeutics, Inc.). Moreover, a combined A_{2B}/A_3 AR antagonist, QAF 805 (Novartis), has been tested in humans as an antiasthma drug. This mixed A_{2B}/A_3 AR antagonist failed to increase the provocative concentration $(PC)_{20}$ for AMP (concentration of AMP required to reduce the FEV_1 by 20%) versus placebo in 24 AMP-sensitive asthmatics in a placebo-controlled, double-blind, randomized, two-way crossover Phase Ib clinical trial (Pascoe et al. 2007). The results of the clinical trials with CVT 6883 and other selective A_{2B}AR antagonists should more clearly define the role of A_{2B}ARs in human asthma, and are eagerly awaited.

2.4 A_3 Adenosine Receptors and Asthma

The functional relevance of the A_3AR in the pathogenesis of asthma is a matter of debate, primarily due to species differences. In humans, A_3ARs have been identified on eosinophils, neutrophils, and monocytes; however, they have not been identified on mast cells (Gessi et al. 2008; Walker et al. 1997). In rats and mice, A_3ARs play an important role in adenosine-induced mast cell degranulation, bronchoconstriction, eosinophilia, and mucus production; however, they exhibit poor sensitivity to methylxanthines (Fan et al. 2003; Ramkumar et al. 1993; Tilley et al. 2003; Young et al. 2006). In an A_3AR knockout mouse model, a selective A_3AR agonist, IB–MECA (N^6-(3-iodobenzyl)-adenosine-5′-*N*-methylcarboxamide) delivered via a nebulizer had no effect on lung mast cell degranulation compared to wild-type mice (Zhong et al. 2003). In murine primary lung mast cells, activation of A_3ARs induced mast cell histamine release in association with increases in intracellular calcium mediated through G_i and phosphoinositide 3-kinase signaling pathways (Zhong et al. 2003). Furthermore, in ADA-deficient mice, A_3ARs appear to be important in endogenous adenosine-induced lung mast cell degranulation in the absence of antigen stimulation (Zhong et al. 2003). Moreover, in ADA-deficient mice, an increase in eosinophils and mucus production were reversed by a selective A_3AR antagonist, suggesting an important role for A_3ARs in mediating the lung eosinophilia and mucus hyperplasia in this animal model (Young et al. 2004). Further to these studies, in a murine model of allergic asthma it appears that A_3ARs play an important role in adenosine-induced bronchoconstriction (Fan et al. 2003). Finally, in allergen-sensitized guinea pigs, an A_3AR antagonist, MRS-1220, significantly inhibited 5′-AMP-induced migration of eosinophils and macrophages into the airways (Spruntulis and Broadley 2001). Taken together, these studies suggest

that A_3ARs play an important role in adenosine-induced mast cell degranulation as well as eosinophilia, mucus hyperplasia, and bronchoconstriction in mice and guinea pigs, and would support the approach to asthma with an A_3AR antagonist.

In humans, expression of A_3ARs is elevated in lung biopsies of patients with asthma, and is mostly localized on eosinophils where activation by adenosine via this receptor inhibits chemotaxis (Walker et al. 1997). This initial report describing this anti-inflammatory effect of activation of A_3ARs on human eosinophils was reproduced, and the studies were expanded by the same group to show that the activation of A_3ARs produced a dose-dependent inhibition in the chemotaxis of human eosinophils to platelet-activating factor, RANTES, and leukotriene B4, and this effect was completely reversed by selective A_3AR antagonists (Knight et al. 1997). Moreover, following these reports, another group reported that A_3ARs on human eosinophils mediate inhibition of both degranulation and superoxide anion release, and that therapeutic concentrations of theophylline inhibit the human eosinophil partly by acting as an A_3AR agonist, thus contributing to the mechanism of the anti-inflammatory action of this drug in vivo (Ezeamuzie 2001; Ezeamuzie and Philips 1999). However, another group studied IB–MECA-induced effects on free radical generation in eosinophils of asthmatics and reported that stimulation of A_3ARs does not appear to be a prime mechanism for free radical generation by human peripheral blood eosinophils (Reeves et al. 2000). Taken together, these studies in humans suggest that an A_3AR agonist should be considered as a therapeutic option for the treatment of human asthma, as opposed to the studies in mice, rats, and guinea pigs that suggest that the A_3AR target to treat asthma should be approached with an A_3AR antagonist.

Based on the reports in animals that activation of A_3ARs produce mast cell degranulation, bronchoconstriction, eosinophilia and mucus hyperplasia, and that activation of A_{2B}ARs on human mast cells may play an important role in human asthma, a combined A_{2B}/A_3 AR antagonist QAF 805 (Novartis) is under development as an antiasthma drug (Press et al. 2005; Pascoe et al. 2007). However, as mentioned above, this mixed A_{2B}/A_3 AR antagonist has now entered human clinical trials and it failed to increase the PC_{20} for AMP versus placebo in 24 AMP-sensitive asthmatics in a placebo-controlled, double-blind, randomized, two-way crossover study Phase Ib clinical trial (Pascoe et al. 2007). With respect to the use of A_3AR agonists as antiasthma drugs, the use of this new class of drugs for this therapeutic indication may be limited by hypotension, tolerance/tachyphylaxis, and immune suppression (Gessi et al. 2008). Because of the anti-inflammatory and specifically the anti-TNF-α effects of the activation of A_3ARs on human monocytes, an A_3AR agonist, CF-101, is in Phase IIb clinical trials for the treatment of rheumatoid arthritis (Can-Fite Biopharma). It is reported that CF-101 has an acceptable safety and tolerability profile in humans (van Troostenburg et al. 2004). In this report, bronchospasm was not reported as a side effect of CF-101; however, the patients in this study were taking another anti-inflammatory immune suppressant, methotrexate, and CF-101 has not been tested in humans with asthma. Thus, the safety and efficacy of A_3AR agonists as antiasthma drugs are yet to be determined in humans.

3 Conclusions and Future Directions

It is now well accepted that adenosine is an important signaling molecule in the pathogenesis of human asthma, and all AR subtypes are important targets for anti-asthma drug development for humans. A number of AR molecules with good safety profiles and selectivity are now available for testing in humans, in order to determine the role of ARs in human asthma and the therapeutic approach to these AR targets that will produce safe and effective antiasthma therapeutics. Because many patients with asthma are either not controlled or are noncompliant with current antiasthma therapies, a shift in focus towards new mechanisms and novel targets (e.g., adenosine signaling and AR targets) is necessary to discover new classes of drugs that are safe and effective that will not only control the symptoms of asthma, but interrupt the disease of airway remodeling and the progressive loss of lung function, and thus improve not only the quality of life, but the outcome for the patient with asthma.

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