P1 receptors and cytokine secretion

Maria P. Abbracchio · Stefania Ceruti

Received: 20 March 2006 / Accepted: 10 April 2006 / Published online: 30 January 2007
© Springer Science + Business Media B.V. 2007

Abstract Evidence has accumulated in the last three decades to suggest tissue protection and regeneration by adenosine in multiple different cell types. Adenosine produced in hypoxic or inflamed environments reduces tissue injury and promotes repair by receptor-mediated mechanisms. Among other actions, regulation of cytokine production and secretion by immune cells, astrocytes and microglia (the brain immunocytes) has emerged as a main mechanism at the basis of adenosine effects in diseases characterized by a marked inflammatory component. Many recent studies have highlighted that signalling through A1 and A2A adenosine receptors can powerfully prevent the release of pro-inflammatory cytokines, thus inhibiting inflammation and reperfusion injury. However, the activation of adenosine receptors is not invariably protective of tissues, as signalling through the A2B adenosine receptor has been linked to pro-inflammatory actions which are, at least in part, mediated by increased release of pro-inflammatory cytokines from epithelial cells, astrocytes and fibroblasts. Here, we discuss the multiple actions of P1 receptors on cytokine secretion, by analyzing, in particular, the role of the various adenosine receptor subtypes, the complex reciprocal interplay between the adenosine and the cytokine systems, their pathophysiological significance and the potential of adenosine receptor ligands as new anti-inflammatory agents.

Key words adenosine · asthma · central nervous system · chronic heart failure · cytokines · immune cells · inflammation

Abbreviations ACTH adrenocorticotropic hormone
ADA adenosine deaminase
CGS15493 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine
CHF chronic heart failure
COPD chronic obstructive pulmonary disease
COX-2 cyclooxygenase-2
GH growth hormone
IFNg interferon gamma
IL interleukin
Ins(1,4,5)P3 inositol(1,4,5)-trisphosphate
IPDX 3-isobutyl-8-pyrrolidinoxanthine
LPS lipopolysaccharide
MCP-1 monocyte chemotactic protein-1
NOS nitric oxide synthase
PBMC peripheral blood mononuclear cells
PG prostaglandin
PRL prolactin
SCH58261 5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine
Th1/2 type 1/2 helper lymphocytes
TNFα tumour necrosis factor alpha
VEGF vascular endothelial growth factor
ZM241385 4-(2-[7-amino-2-(2-furyl)(1,2,4)triazolo[1,5-c][2,3-a](1,3,5)triazin-5-y]amino)ethyl)phenol

Introduction

Adenosine acts as a local mediator with generally cytoprotective functions in mammalian organisms. These actions are mediated by the activation of four distinct adenosine re-
Adenosine modulation of cytokine secretion in immune cells: is adenosine the danger sensor that stops the immune response?

The first demonstrations of the immunosuppressive effects mediated by intracellular cyclic adenosine monophosphate (cAMP) and extracellular adenosine date back to the 1970s [3, 4]. Since then, detailed studies on the pharmacological effects of adenosine [5, 6] and on the involvement of specific adenosine receptor subtypes ([7, 8]; see also below) have expanded those original descriptions to cells of both the innate and adaptive immune systems, highlighting adenosine as a critical player in the physiological mechanisms that downregulate activated immune cells and protect tissues from inflammatory damage (for seminal and comprehensive reviews, see Sitkovsky et al. [9] and Sitkovsky and Ohta [10]).

It is known that immune cell-mediated destruction of pathogens may result in excessive collateral damage to normal tissue and that the failure to control activated immune cells and to downregulate acute inflammatory responses may cause immunopathologies and chronic inflammatory diseases. It is believed that regulation of the immune system requires at least two ‘danger’ signals (elegantly reviewed in Sitkovsky and Ohta [10]). The first danger signal indicates the presence of pathogens, injury or mutations resulting in the death or scavenging of cells (reviewed by Matzinger [11]). As a result of this danger signal, immune cells are activated to initiate immune responses: they kill pathogens by cytokines, reactive oxygen and nitrogen species and cellular cytotoxicity, and expand inflammation by attracting and activating many other effector cells through the release of pro-inflammatory cytokines and chemokines. However, uncontrolled expansion of inflammatory responses might cause tissue damage and loss of function: this is at the basis of the critical dichotomy of inflammation, which is often described as a ‘double-edged sword’ [12]. Inflammation and immune reactions are indeed believed to start as time- and site-specific defense mechanisms. Failure to resolve an acute beneficial response could later lead to a vicious and anachronistic state of chronic activation, which causes healthy tissue damage. To avoid excessive collateral tissue damage, therefore, the tissue may release a second danger signal that can evoke anti-inflammatory responses. This second danger signal indicates the danger from overactive immune cells and would trigger the downregulation of the pro-inflammatory activities of the immune system to prevent destruction of healthy tissues. As underlined by Sitkovsky and Ohta [10], the final outcome will be determined by a balance between the first pro-inflammatory danger signal and the second anti-inflammatory signal. The mechanisms that are triggered by the second danger signal are related to the extent of change to tissue microenvironment and are timed with a high level of precision, so that immune cells keep the ability to destroy remaining pathogens but with much less damage to healthy cells. Sitkovsky and Ohta [10] observed that the least tolerated damage during an immune response is damage to the structures of the microcirculation, which would cause an interruption of the local blood supply. On this basis, they proposed local tissue hypoxia as the primary event indicating the need to stop overactive immune cells and adenosine as a key “OFF” signal mediating downregulation of immune cell activity. Even short periods of hypoxia are indeed known to strongly elevate adenosine levels as a result, on the one side, of rapid breakdown of ATP, and, on the other side, of hypoxia-induced inhibition of adenosine kinase, which, under normal conditions, resphosphorylates the nucleoside to AMP. Hypoxia, such as that associated with brain ischemia, also results in upregulation of adenine nucleotide metabolizing ectoenzymes, which may lead to an even more enhanced production of adenosine. Interestingly, such an upregulation has been demonstrated to occur in vivo upon induction of middle cerebral artery occlusion in rodents as a result of brain ischaemia [13].
Among known adenosine receptors, A2 receptors have properties that make them particularly well suited to serve as sensors of pro-inflammatory activities and to act as stop signals of overstimulated immune cells. First of all, both A2A and A2B receptors are coupled to Gs proteins and to increases of intracellular cAMP, which is in line with the prime importance of cAMP elevation in inhibition of immune cell activity (in this respect, see also Raskovalova et al. [14]) (Fig. 1). Interestingly, the A2A receptor has high affinity and the A2B receptor has low affinity for adenosine, suggesting that they could be recruited sequentially, depending upon the extent of adenosine accumulation at the site of inflammation, and, thus, upon the degree of hypoxia. This would allow a graded escalation of the

![Fig. 1](image-url)
Adenosine modulation of cytokine release in the lung: a key role in asthma

Inflammation plays a major role in both the development and progression of lung diseases, such as asthma and chronic obstructive pulmonary disease (COPD). Following a chemical, environmental, mechanical or antigenic injury to the lung tissue, the release of various cytokines and chemokines is initially aimed at sustaining the repair process, but its deregulation and persistence later contribute to tissue destruction and remodelling [18].

Multiple cell types are recruited at the site of lung inflammation, including eosinophils, neutrophils, lymphocytes, macrophages and mast cells, and they represent the major sources of inflammatory mediators. In allergic asthma, a prominent role is played by Th2 lymphocytes which release various cytokines (e.g. IL-4, IL-5, IL-13 and IL-9; [19]) contributing to eosinophilia, IgE production, airway hyperreactivity and mucus hypersecretion. In all asthmatic conditions, pulmonary mast cells represent major players of the inflammatory reaction, since, following acute exposure to allergens, pollutants or other chemical stimuli, they degranulate releasing a huge variety of autacoids, including prostaglandin D2, cytokines, cysteinyl leukotrienes, enzymes and histamine [20]. These chemical entities contribute to the development of the acute asthmatic attack, modulating bronchoconstriction, but also play an important role in the chronic development of the disease. In fact, mast cells have been implicated in airway remodelling [20] and are currently believed to be primarily responsible for the development of airway hyperreactivity (a characteristic feature of chronic asthma in which airways react in an exaggerated way to exposure to mild insults; [21]). During the chronic phase of asthma, lung fibroblasts, epithelial cells and smooth muscle cells further contribute both to the evolution of the disease and to airway remodelling [18].

We have previously mentioned that adenosine concentrations rapidly increase in hypoxic and inflamed tissues, due to ATP breakdown. Thus, it was anticipated that high adenosine concentrations could be found also in the lung of asthmatic patients and that the nucleoside might contribute to the disease. The first indication of a possible role for adenosine in mediating bronchoconstriction dates back to the 1980s when Cusheley and co-workers demonstrated that inhaled adenosine was ineffective on normal, but was a potent bronchoconstrictor of asthmatic Airways [22]. These results were confirmed a few years later also in patients with chronic obstructive pulmonary disease, by utilizing
AMP (which is rapidly broken down to adenosine) as a bronchoconstrictant [23].

From the very beginning, it was hypothesized that adenosine was not acting directly on bronchial smooth muscle cells, but exerted its bronchoconstrictant activity through an indirect mechanism, involving mast cell degranulation. This hypothesis was later confirmed by the ability of histamine H1 and cysteiny1 leukotriene receptor 1-selective antagonists as well as cyclooxygenase 1 and 2 inhibitors and mast cell stabilizing drugs (i.e. sodium cromoglicate) to effectively counteract the effects of inhaled AMP [24]. Indeed, high histamine plasma concentrations were detected in asthmatic patients after AMP inhalation, due to mast cell degranulation accompanied by a rapid airway narrowing [25]. The selective effect of adenosine in asthmatic subjects has recently opened up the opportunity to develop a diagnostic test based on adenosine or AMP inhalation challenge [26] which could effectively discriminate between asthmatic and patients affected by COPD. In fact, adenosine-mediated bronchoconstriction is highly enhanced when the allergic component is relevant, like in asthma, but is lower in the case of COPD [27]. AMP inhalation might also be used to monitor the efficacy of the corticosteroid therapy [27]. Anyway, despite the importance of such a diagnostic test, its use should be carefully evaluated due to the development of an inflammatory response after AMP inhalation that could have problematic outcomes [28]. The important role played by adenosine in modulating allergic response has been very recently further highlighted in ragweed-sensitized mice where adenosine inhalation increased infiltration of inflammatory cells and the appearance of markers of inflammation (such as eotaxin) in the bronchoalveolar lavage [29].

Several studies have demonstrated that adenosine effects are mediated by activation of its surface receptors, but important differences among species have been observed. In fact, with the discovery and cloning of the A1 adenosine receptor subtype, its prominent role in modulating mast cell degranulation was described in rodents. The situation appears to be different in human mast cells, where the A2B adenosine receptor is the likely candidate for mediation of the pro-inflammatory and bronchoconstrictant effects of the nucleoside in asthmatic patients [18]. For instance, data from HMC-1 cells (a human mast cell line) have shown that activation of A2B receptors leads to IL-8 generation, which can be attenuated by rather selective antagonists [30], and to enhanced IL-4 and IL-13 secretion when cells are cocultured with human B cells [31]. Both the A3 and the A2B subtypes are low-affinity receptors for adenosine and can therefore be activated only when high levels of the nucleoside are present, such as in chronic inflammatory situations. The involvement of the low-affinity A2B (coupled to both adenylyl cyclase and phospholipase C) in the pro-inflammatory actions of adenosine could also explain its selective action on asthmatic subjects. In fact, under physiological conditions, low adenosine concentrations activate the high-affinity A2A receptor subtype localized on mast cells and coupled to increases in intracellular cAMP concentrations which, in turn, inhibit histamine release [27] (Fig. 2). Conversely, upon chronic inflammation, high adenosine levels are reached leading to activation of the A2B subtype which may, in turn, promote histamine release by raising inositol(1,4,5)-trisphosphate [Ins(1,4,5)P3] concentrations [27] (Fig. 2).

The discovery of the role of the A2B adenosine receptor in promoting lung inflammation has also helped to clarify the mechanism of action of a well-known anti-asthmatic agent, such as theophylline, which has been used in therapy for over 50 years [32]. Theophylline is known as both a phosphodiesterase inhibitor and an antagonist at adenosine receptors, but its anti-asthmatic actions were mainly ascribed to the former activity based on the fact that enprofylline, another xanthine derivative, is as effective as theophylline in treating asthma but bears a much lower affinity for adenosine receptors [33]. The discovery that the affinity of these two molecules at the A2B receptor is similar and lies below the range of plasma concentrations that are observed in therapy has further clarified the important role played by this receptor subtype in both the onset and development of lung inflammatory diseases [32].

Activation of the A2B adenosine receptor has been also demonstrated to play an important role in the development of airway hyperresponsiveness, contributing to the chronic evolution of the disease, by modulating cytokine secretion from various cell types. As previously mentioned, stimulation of the A2B receptor on human mast cells results in the engagement of Th2 lymphocytes which in turn release IL-4 and IL-13 [31]. IL-13 is involved in IgE synthesis from B cells, leading to chronic inflammation, alveolar remodeling, pulmonary fibrosis and mucus production [19]. Indeed, A2B stimulation on human lung fibroblasts induces IL-6 release which autocrinally promotes their pathological differentiation into myofibroblasts [34]. Upon adenosine stimulation also bronchial smooth muscle cells release IL-6 and monocyte chemotactic protein-1 (MCP-1, also known as CCL-2; [35]), another important mediator of disease progression. Finally, it has been also demonstrated that adenosine can upregulate mucin gene expression in human airway epithelial cells [36]. Based on these observations, selective A2B antagonists (such as IPDX, CGS15493 and CVT 6883; [37, 38]) have been proposed as powerful and effective anti-asthmatic drugs; some of them are currently in clinical trials for the long-term treatment of lung diseases [32].

In very recent years, elegant studies from Blackburn and co-workers have further highlighted the importance of high adenosine concentrations in the development of lung
diseases by utilizing adenosine deaminase (ADA)-deficient mice [39]. The most relevant phenomenon observed in these animals is the development of an “asthmatic” inflammatory phenotype, accompanied by all the classic symptoms and cellular changes observed in patients [40]. Indeed, the genetic removal of the A1 adenosine receptor subtype further strengthened the asthmatic phenotype, highlighting a protective or modulatory role for this receptor subtype against the development of lung inflammation in ADA-deficient mice which might have a functional counterpart also in humans [41].

Finally, a protective role has been also demonstrated for the A2A subtype (see also above). Its activation, in fact, suppresses activation and degranulation of neutrophils, mast cells, monocytes and T lymphocytes [27, 32, 42], thus envisaging the possible use of selective A2A agonists as therapeutic agents.

Adenosine modulation of cytokine release in the brain: beyond its role as a retaliatory neuroprotective metabolite

More than 20 years ago, a seminal paper from Newby introduced the concept of “retaliatory metabolite” to recapitulate the protective adenosine functions in brain and heart ischaemic tissues [43]. An increasing amount of details on adenosine functions have subsequently come from the work of several groups in this field, and very recent observations suggest the situation not to be so well-defined as it seemed at the beginning.

It was already known that following ischaemic and/or traumatic injury or under inflammatory situations extracellular adenosine concentrations increase several fold over basal levels due to the rapid breakdown of ATP [44].
is massively co-released from synaptic terminals together with excitatory neurotransmitters [45], and adenine nucleotides derive from damaged or dying cells undergoing nucleic acid degradation. Ectonucleotides rapidly degrade nucleotides to adenosine, whose concentration rises from the nanomolar range under basal condition to 10-50 μM following ischaemia [46]. The neuroprotective actions of adenosine have been known for several years and have been mainly associated with activation of the presynaptic A1 receptor subtype leading to decreases of neuronal firing and of excitatory neurotransmitter release [47]. Acting on postsynaptic A1 receptors, adenosine is also able to hyperpolarize plasma membranes, thus reducing the propagation of excitatory stimuli. On the contrary, the role of the A2A adenosine receptor subtype is controversial. Under some experimental paradigms, activation of A2A receptors leads to neuroprotection; however, induction of neuronal death has also been proposed, since A2A receptor antagonists are neuroprotective in several experimental models of neurodegeneration [48].

Not only neurons, but also glial cells (i.e. astrocytes and microglia) express all the four cloned adenosine receptor subtypes, with the exception of the A2B receptors that have not been found in microglia. In recent years, the discovery of the important role of glial cells in controlling brain response to traumatic injury has opened up the possibility that some of the effects mediated by adenosine in the brain may also depend on its ability to modulate glial cell functions.

Astrocytes and microglia react rapidly to noxious stimuli by increasing their proliferation rate, leading to the formation of an astrocytic scar isolating damaged neurons (the so-called reactive astrogliosis) and to the recruitment of microglial cells at the site of injury [49]. Adenosine exerts a double action on the proliferation of glial cells, with the A1 and the A2A receptor subtype reducing and enhancing astrocyte proliferation, respectively [46]. The effect on microglial cell proliferation is less clear and depends upon the cellular environment and the receptor subtypes that are expressed in any given experimental model [50, 51].

In the long term, glial cells contribute to brain remodeling and to the final outcome of the traumatic event through their ability to release both pro- and anti-inflammatory cytokines [46]. Adenosine receptors have been demonstrated to modulate cytokine release from glial cells in different ways. In astrocytes, the overall result of adenosine receptor engagement is the development of an anti-inflammatory phenotype. In fact, A1 receptor activation induced nerve growth factor (NGF) release from astrocytes [52], whereas A2A and the A3 receptor subtypes mediated increases of the cytokines IL-6 and CCL2, respectively [53, 54]. Moreover, A2A receptors play an important role in downregulating nitric oxide synthase (NOS) following pro-inflammatory stimuli, such lipopolysaccharide (LPS), IFNγ or TNFα plus IL-1β [55].

The role of adenosine in controlling cytokine release from microglial cells appears to be more contradictory. So far, no role for A1 receptors has been demonstrated, whereas activation of the A2A receptor subtype seems to drive the appearance of a pro-inflammatory phenotype, with an upregulation of cyclooxygenase-2 (COX-2) expression, followed by increased prostaglandin E (PGE)2 synthesis [56], together with an augmented NO production [57]. Nevertheless, an anti-inflammatory role for some COX-2 products, such as PGD2 and PGJ2 has been recently demonstrated [46]. Indeed, the recruitment of the microglial A2A receptor has been also associated with an increase in NGF production [58], thus suggesting that the final outcome of adenosine receptor activation is probably influenced by the cellular environment, as hypothesized for microglial cell proliferation (see above). Studies on human microglial cells have suggested that the A2A receptor subtype is preferentially expressed by activated microglia [59]. If confirmed, these observations might contribute to depicting a scenario where the A2A receptor subtype plays a neurodegenerative role upon pathologic conditions. Finally, the role of the A3 adenosine receptor in controlling microglial functions was unclear until very recently, when its activation was demonstrated to promote ERK1/2 phosphorylation [60] and to reduce LPS-induced TNFα production through inhibition of the PI3-kinase/AKT pathway [61]. The apparent contradiction in adenosine modulation of microglial cell functions, with the development of either a pro- or an anti-inflammatory phenotype, is in agreement with the double-edged sword role exerted by the inflammatory process, where failure to resolve an initial beneficial inflammatory reaction leads to a delayed and chronic detrimental situation ([12]; see also above). Thus, different receptor subtypes might be recruited at different times after the initial traumatic/inflammatory trigger, also depending upon changes in adenosine concentrations over time, contributing to the plasticity of brain response to traumatic and ischaemic events.

An important contribution to the production of cytokines and chemokines in the brain during ischaemia comes from infiltrating blood immune cells. A specific section of this review is dedicated to a detailed analysis of the role of adenosine in modulating immune cell function. Concerning the role of immune cell adenosine receptors in brain pathologies, a potent protective role for the A1 receptor subtype has been demonstrated in experimental allergic encephalomyelitis, an animal model of multiple sclerosis [62]. In fact, A1 adenosine receptor null mice develop a severe form of the disease with respect to wild-type litter mates, characterized by demyelination and oligodendrocyte cytotoxicity evoked by pro-inflammatory molecules (mainly IL-1β and matrix metalloproteinase-12) produced by macrophages [62]. This scenario recapitulates findings in
multiple sclerosis patients, thus suggesting that modulation of the A₁ adenosine receptor subtype might represent a novel pharmacological approach to currently incurable demyelinating diseases.

Again, the role of the A₂A receptor subtype expressed by infiltrating cells in modulating brain damage appears contradictory. In fact, inactivation of the A₂A receptor of bone marrow-derived cells has been demonstrated to protect brain tissue from middle cerebral arterial occlusion injury; this effect was accompanied by a parallel reduction in the production of pro-inflammatory cytokines from infiltrating macrophages [46], suggesting a detrimental role for this receptor subtype during ischaemic brain damage (see also above). On the contrary, the A₂A receptor plays a
neuroprotective and anti-inflammatory role in a rat model of endotoxin-induced meningitis [46] and has been recently demonstrated to prevent human immunodeficiency virus (HIV)-1 Tat-induced production of TNFα by macrophages [63]. Taken together, these observations further confirm an anti-inflammatory and neuroprotective role played by the A1 adenosine receptor subtype, whilst the role of the A2A receptor in neurodegeneration might depend upon the timing of the disease and the peculiar mechanisms at the basis of its aetiopathology.

A key role for adenosine A1 and A3 receptor subtypes has been also described in ischaemic preconditioning, where a mild and transient ischaemic attack reduces the susceptibility of brain tissue to a subsequent and prolonged ischaemic episode. A role for adenosine in protection against release of cytokines and cytotoxic molecules from residential macrophages in ischaemic preconditioning of the heart has been clearly demonstrated (for review, see Picano and Abbracchio [64] and references therein). Given the recent observation of an altered pattern of main cytokine expression (in particular IL-1 and IL-6) in ischaemic animals previously subjected to preconditioning with respect to non-conditioned animals [65], a possible role for adenosine receptors in modulating cytokine release during the induction of cerebral ischaemic preconditioning can also be foreseen.

Not only can adenosine influence cytokine expression and release, but a tight cross-talk between cytokine and growth factor networks and adenosine receptors can be envisaged based on recent observations. In fact, exposure of rat cortical astrocytes to IL-6 upregulates adenosine A1 receptor expression [66], and inhibition of the A2A receptor subtype by the selective antagonist SCH58261 completely prevented basic fibroblast growth factor (bFGF)-induced reactive astrogliosis [67]. Moreover, TNFα increased A2B adenosine receptor functional response and G-protein coupling in astrocytes in vitro, without any changes in receptor levels but by inhibiting receptor phosphorylation and downregulation. The functional outcome was that activation of this receptor subtype in human astrocytoma cells induced reactive astrogliosis only in the presence of the pro-inflammatory cytokine ([68]; Fig. 3). Taken together, these results suggest that a highly complex interconnection among adenosine signalling pathways, cytokines and growth factors is involved in the generation of the final outcome in response to ischaemic, traumatic and inflammatory events. The in-depth knowledge of the various regulatory pathways might open up new therapeutic strategies to both acute and chronic neurodegenerative disorders.

Finally, increases in cytokine concentrations (in particular IL-6) are known to be potent signals for hormone release by the pituitary gland (i.e. adrenocorticotropic hormone (ACTH), growth hormone (GH), prolactin (PRL) and gonadotrophin) [69]. Adenosine A2B receptors are highly expressed by the folliculostellate cells of the anterior pituitary, and their activation stimulates IL-6 and vascular endothelial growth factor (VEGF) release [69]. Since folliculostellate cells provide a cellular network integrating both locally generated and systemic signals to modulate hormone secretion, activation of the adenosine A2B receptor may represent an important regulatory pathway in the neuroimmune system [69]. A2B-mediated IL-6 and VEGF production might also play an important role during the development and growth of pituitary tumours, since it can be envisaged that high adenosine concentrations are achieved in the hypoxic core of the tumoural mass due to ATP breakdown [69].

Adenosine modulation of cytokine production in the heart: an efficient, although incomplete, mechanism of protection against heart failure progression

Since the sentinel description by Levine and colleagues of inflammatory cytokines in patients with heart failure in 1990 [70], there has been a growing interest in understanding the role of these molecules in regulating cardiovascular function under both physiological and pathological conditions. In particular, data have been accumulated to suggest that many aspects of heart failure can be explained by the known biological effects of pro-inflammatory cytokines such as TNFα, IL-1 and IL-6 [71]. When expressed at sufficiently high concentrations, such as those found in patients with chronic heart failure (CHF), cytokines are sufficient to mimic several aspects of the so-called heart failure phenotype, including progressive left ventricular dysfunction, pulmonary oedema, left ventricular remodelling, foetal gene expression and cardiomyopathy [72, 73, 74]. In CHF patients, both cardiac myocytes, heart residential macrophages and peripheral blood mononuclear cells (PBMC) are able to produce great amounts of TNFα and IL-6. Of these, TNFα represents a serious candidate as a mediator of the myocardial dysfunction progression and remodelling that are part of the natural history of CHF, since it induces hypotension, decreases myocardial contractility and ejection fraction and also exerts a direct cytotoxic effect on cardiac myocytes [75, 76, 77]. All this evidence supports the “cytokine hypothesis” [78] that heart failure progresses, at least in part, as a result of the toxic effects exerted on the heart and the peripheral circulation by endogenous cytokine cascades. Of course, this does not imply that cytokines cause heart failure “per se”, but rather that the overexpression of cytokine cascades or, alternatively, a dysfunction of the mechanisms and factors regulating their secretion (see also below), contributes to disease progression.
The possibility of exploiting endogenously generated factors that are capable of attenuating cytokine cascade in CHF is beginning to be explored. In this respect, an ideal system is represented by adenosine. This nucleoside has well-known homeostatic activities in regulating myocardial blood flow, release of catecholamines and, as more recently demonstrated, of cytokines from inflammatory cells and has been shown to reduce myocardial injury resulting from periods of ischaemia (for review, see Villarreal et al. [79]).

The A2A receptor seems to be particularly important in mediating these beneficial effects. Activation of this receptor subtype on immune cells (e.g. monocytes and lymphocytes) has long been known to mediate anti-inflammatory responses, including inhibition of TNFα release (see also above; for review see Sitkovsky et al. [9]), which may have interesting implications for the development of heart disease. In line with this hypothesis, an increase of A2A adenosine receptor expression, density and activity has been reported in the PBMC of end-stage CHF patients compared to control subjects, in parallel with significant increases of the plasma levels of TNFα and soluble TNF receptors [80]. In these patients, upregulation of A2A adenosine receptors in circulating cells progressively normalized after cardiac transplantation, in parallel with the normalization of haemodynamic conditions and with the reduction of plasma TNFα and soluble TNF receptors towards normal values (ibidem; Fig. 4). These data indicate a correlation between A2A receptors and cytokine production in CHF and suggest that A2A receptors are upregulated in an attempt to potentiate adenosine-mediated cytokine inhibition. This hypothesis is consistent with the demonstration that both adenosine and adenosine-interfering agents (i.e. dipyridamole and iodotubercidin, which inhibit adenosine uptake and intracellular phosphorylation, respectively) potently inhibit LPS-induced TNFα production and release [81]. These effects could be blocked by A2 but not by A1 or A3 receptor antagonists (ibidem). The specific involvement of the A2A receptor in these actions was confirmed in a later study. When stimulated ex vivo with LPS, the PBMC from CHF patients produce greater

![Diagram](image-url)
amounts of TNFα in comparison with cells from healthy subjects [82]. However, despite increased TNFα production, activation of A2A receptors (which are upregulated in CHF, see above) by the selective A2A receptor agonist CGS21680 induced a comparable inhibition of TNFα release in both control and CHF patients. This effect was blocked by ZM241385, a specific A2A antagonist. These results suggest that upregulated A2A receptors in CHF patients are efficiently coupled to their transduction system. The inhibitory effect of A2A adenosine receptors on LPS-induced TNFα production in monocytes could be attributed to the increase of intracellular cAMP, which has been shown to attenuate nuclear factor (NF)-κB-mediated transcriptional activity (see also below). In line with previous data suggesting the existence of a regulatory cross-talk between TNFα and A2A adenosine receptor levels [83, 84], the ex vivo incubation of PBMC from control subjects with TNFα increased the expression of this adenosine receptor subtype [82]. In contrast, under the same experimental condition, TNFα did not further increase the expression of the A2A receptor in the PBMC from CHF subjects, suggesting that, in these patients, maximal induction had already occurred in vivo.

On this basis, the following pathophysiological loop acting in chronic heart failure may be suggested: in CHF patients a high plasma level of endotoxin primes inflammatory cells to produce great amounts of cytokines; at the same time, high concentrations of TNFα may induce upregulation of the A2A adenosine receptor, in an attempt to potentiate adenosine-mediated cytokine inhibition. Upregulation of the A2A adenosine receptor in inflammatory cells from CHF patients may thus represent an efficient, although incomplete, mechanism of protection against inappropriate cytokine production in the diseased heart. These findings also suggest the A2A adenosine receptor as a pharmacological target for novel therapeutic interventions aimed at slowing down heart failure progression even after activation of inflammatory cells has occurred.

Concluding remarks

The evidence reviewed above supports a crucial role for specific adenosine receptors (mainly the A1 and A2A receptor subtypes) in inhibition of pro-inflammatory cytokine release, which has obvious important implications for human pathophysiology. In principle, adenosine signalling through these receptors is aimed at protecting tissues against excessive inflammatory damage. Compelling evidence points to a crucial role for the A2A adenosine receptor in limitation and termination of inflammation. As reviewed above and in Sitkovski et al. [9], no other factor could compensate fully for the loss of A2A receptors on immune cells, suggesting that this mechanism is non-redundant and may have important implications in human diseases characterized by excessive inflammation and/or overactivation of immune cells. In line with this hypothesis, in the PBMC of patients with chronic heart failure, A2A receptors were upregulated [80, 82], likely in an attempt to potentiate adenosine inhibition of cytokine secretion. However, in the end, this could not prevent heart disease progression, suggesting that this mechanism is not able to fully protect the heart against inappropriate cytokine production. Nevertheless, these data globally highlight the A2A receptor as an interesting target for the development of new pharmacological strategies aimed at potentiating adenosine cytoprotection. In contrast, activation of the A2B receptor seems to be responsible for pro-inflammatory actions, likely through activation of Gq proteins, calcium mobilization and stimulation of phospholipase C and mitogen-activated protein kinase (reviewed in Linden [2]). As an example of the deleterious consequences of this cascade, activation of A2B receptors contributes to exacerbating histamine release and disease progression in asthma and COPD (see above). In line with this evidence, A2B blockers have been hypothesized to be useful as anti-inflammatory agents.

The evidence summarized above also points to the existence of a highly complex interplay among adenosine receptors and cytokines. Not only can adenosine influence cytokine expression and release, but cytokines can themselves modify adenosine receptor function by either influencing their expression (e.g. [82]) or by inhibiting adenosine receptor phosphorylation and downregulation [68]. In some cases (for example in reactive astrogliosis) this may contribute to developing cytotoxic mechanisms in the first stages of the disease, but, when inflammation becomes chronic, a dysregulation of these same mechanisms may contribute to disease exacerbation. On this basis, it is clear that a more in-depth knowledge of the various regulatory pathways between adenosine and cytokines might open up new therapeutic strategies to fully exploit adenosine cytoprotection in acute and chronic degenerative diseases.

Acknowledgements The authors are grateful to Prof. Dr. Michail V. Sitkovsky, New England Inflammation and Tissue Protection Institute, Northeastern University, Boston, MA, USA, for useful discussion.

References

1. Jacobson KA, Gao ZG (2006) Adenosine receptors as therapeutic targets. Nat Rev Drug Discov 5:247–264
2. Linden J (2005) Adenosine in tissue protection and tissue regeneration. Mol Pharmacol 67:1406–1413

Springer
3. Hirschhorn R, Grossman J, Weissmann G (1970) Effect of cyclic 3′-5′-adenosine monophosphate and theophylline on lymphocyte transformation. Proc Soc Exp Biol Med 133:1361–1365
4. Henney CS, Lichtenstein LM (1971) The role of cyclic AMP in the cytolytic activity of lymphocytes. J Immunol 107:610–612
5. Cronstein BN, Kramer SB, Rosenstein ED et al (1985) Adenosine modulates the generation of superoxide anion by stimulated human neutrophils via interaction with a specific cell surface receptor. Ann NY Acad Sci 451:291–301
6. Firestein GS (1996) Anti-inflammatory effects of adenosine kinase inhibitors in acute and chronic inflammation. Drug Dev Res 39:371–376
7. Koshiha M, Kojima H, Huang S et al (1997) Memory of extracellular adenosine/A2A purinergic receptor-mediated signaling in murine T cells. J Biol Chem 272:25881–25889
8. Yaar R, Jones MR, Chen JF, Ravid K (2005) Animal models for the study of adenosine receptor function. J Cell Physiol 202:9–20
9. Sitkovsky MV, Lukashev D, Apasov S et al (2004) Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. Annu Rev Immunol 22:657–682
10. Sitkovsky MV, Ohta A (2005) The “danger” sensors that STOP the immune response: the A2 adenosine receptors? Trends Immunol 26:299–304
11. Matzinger P (2002) The danger model: a renewed sense of self. Science 296:301–305
12. Marchetti B, Abbracchio MP (2005) To be or not to be (inflamed) – is that the question in anti-inflammatory drug therapy of neurodegenerative disorders? Trends Pharmacol Sci 26:517–525
13. Braun N, Lenz C, Gillardon F et al (1997) Focal cerebral ischemia enhances glial expression of ecto-5′-nucleotidase. Brain Res 766:213–226
14. Raskovalova T, Huang X, Sitkovsky MV et al (2005) Gs protein-coupled adenosine receptor signaling and lytic function of activated NK cells. J Immunol 175:4383–4391
15. Panther E, Corinti S, Idzko M et al (2003) Adenosine affects expression of membrane molecules, cytokine and chemokine release, and the T-cell stimulatory capacity of human dendritic cells. Blood 101:3985–3990
16. Sitkovsky MV (2003) Use of the A(2A) adenosine receptor as a physiological immunosuppressor and to engineer inflammation in vivo. Biochem Pharmacol 65:493–501
17. Lukashev D, Ohta A, Apasov S et al (2004) Cutting edge: physiologic attenuation of proinflammatory transcription by the A2B adenosine receptor. J Immunol 172:7726–7733
18. Yaar R, Jones MR, Chen JF, Ravid K (2005) Animal models for the study of adenosine receptor function. J Cell Physiol 202:9–20
19. Sitkovsky MV, Lukashev D, Apasov S et al (2004) Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. Annu Rev Immunol 22:657–682
20. Sitkovsky MV, Ohta A (2005) The “danger” sensors that STOP the immune response: the A2 adenosine receptors? Trends Immunol 26:299–304
21. Matzinger P (2002) The danger model: a renewed sense of self. Science 296:301–305
22. Marchetti B, Abbracchio MP (2005) To be or not to be (inflamed) – is that the question in anti-inflammatory drug therapy of neurodegenerative disorders? Trends Pharmacol Sci 26:517–525
23. Braun N, Lenz C, Gillardon F et al (1997) Focal cerebral ischemia enhances glial expression of ecto-5′-nucleotidase. Brain Res 766:213–226
24. Raskovalova T, Huang X, Sitkovsky MV et al (2005) Gs protein-coupled adenosine receptor signaling and lytic function of activated NK cells. J Immunol 175:4383–4391
25. Phillips GD, Ng WH, Church MK, Holgate ST (1990) The response of plasma histamine to bronchoprovocation with methacholine, adenosine 5′-monophosphate, and allergen in atopic nonasthmatic subjects. Am Rev Respir Dis 141:9–13
26. van de Berge M, Polosa R, Kerstjens HA, Postma DS (2004) The role of endogenous and exogenous AMP in asthma and chronic obstructive pulmonary disease. J Allergy Clin Immunol 114:737–746
27. Spicuzza L, Bonfiglio C, Polosa R (2003) Research applications and implications of adenosine in diseased airways. Trends Pharmacol Sci 24:409–413
28. van den Berge M, Kerstjens HA, de Reus DM et al (2004) Provocation with adenosine 5′-monophosphate, but not methacholine, induces sputum eosinophilia. Clin Exp Allergy 34:71–76
29. Fan M, Mustafa J (2006) Role of adenosine in airway inflammation in an allergic mouse model of asthma. Int Immunopharmacol 6:36–45
30. Meade CJ, Worall L, Hayes D, Protin U (2002) Induction of interleukin 8 release from the HMC-1 mast cell line: synergy between stem cell factor and activators of the adenosine A2b receptor. Biochem Pharmacol 64:1163–1173
31. Ryzhov S, Goldstein AE, Matafonov A et al (2004) Adenosine-activated mast cells induce IgE synthesis by B lymphocytes: an A2B-mediated process involving Th2 cytokines IL-4 and IL-13 with implications for asthma. J Immunol 172:7726–7733
32. Fozard JR (2003) The case for a role for adenosine in asthma: almost convincing? Curr Opin Pharmacol 3:264–269
33. Dent G, Rabe KF (2000) Theophylline. Lung Biol Health Dis 145:77–124
34. Zhong H, Belardinelli L, Maa T, Zeng D (2005) Synergy between A2B adenosine receptors and hypoxia in activating human lung fibroblasts. Am J Respir Cell Mol Biol 32:2–8
35. Zhong H, Belardinelli L, Maa T et al (2004) A2B adenosine receptors increase cytokine release by bronchial smooth muscle cells. Am J Respir Cell Mol Biol 30:118–125
36. McNamara N, Gallup M, Khong A et al (2004) Adenosine up-regulation of the mucin gene, MUC2, in asthma. FASEB J 18:1770–1772
37. Feoktistov I, Garland EM, Goldstein AE et al (2001) Inhibition of human mast cell activation with the novel selective adenosine A1 receptor antagonist 3-isobutyl-8-pyridilomoxantin (IPDX) (2). Biochem Pharmacol 62:1163–1173
38. Fozard JR, Baur F, Wolber C (2003) Antagonist pharmacology of adenosine A2B receptors from rat, guinea pig and dog. Eur J Pharmacol 475:79–84
39. Blackburn MR, Datta SK, Kellemes RE (1998) Adenosine deaminase-deficient mice generated using a two-stage genetic engineering strategy exhibit a combined immunodeficiency. J Biol Chem 273:5093–5100
40. Chunn JL, Young HW, Banerjee SK et al (2005) Adenosinedependent airway inflammation and hyperresponsiveness in partially adenosine deaminase-deficient mice. J Immunol 167:4676–4685
41. Sun C-X, Young HW, Molina JG et al (2005) A protective role for adenosine in airway inflammation in chronic obstructive pulmonary disease. J Allergy Clin Immunol 114:737–746
42. Rorke S, Holgate ST (2002) Targeting adenosine receptors: novel therapeutic targets in asthma and chronic obstructive pulmonary disease. Am J Respir Med 1:99–105
43. Newby AC (1984) Adenosine and the concept of “retaliatory metabolites”. Trends Biochem Sci 9:42–44
44. Newby AC, Worku Y, Holmquist CA (1985) Adenosine formation. Evidence for a direct biochemical link with energy metabolism. Adv Myocardiol 6:273–284
45. Melani A, Turchi D, Vannuccii MG, Cipriani S, Gianfriddo M, Pedata F (2005) ATP extracellular concentrations are increased in the rat striatum during in vivo ischemia. Neurochem Int 47:442–448
46. Haskó G, Pacher P, Vizi ES, Illes P (2005) Adenosine receptor signaling in the brain immune system. Trends Pharmacol Sci 26:511–516
47. Dunwiddie TV, Masino SA (2001) The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 24:31–55
48. Stone TW (2002) Purines and neuroprotection. Adv Exp Med Biol 513:249–280
49. Panickar KS, Noreen MG (2005) Astrocytes in cerebral ischemic injury: morphological and general considerations. Glia 50:287–298
50. Gebicke-Haerter PJ, Christoffel F, Timmer J et al (1996) Both adenosine A1- and A2-receptors are required to stimulate microglial proliferation. Neurochem Int 29:37–42
51. Ciccarelli R, Di Iorio P, Bruno V et al (1999) Activation of A(1) adenosine or mGlu3 metabotropic glutamate receptors enhances the release of nerve growth factor and S-100b protein from cultured astrocytes. Glia 27:275–281
52. Schwanger M, Neher M, Viegas E et al (1997) Stimulation of interleukin-6 secretion and gene transcription in primary astrocytes by adenosine. J Neurochem 69:1145–1150
53. Brodie C, Blumberg CM, Jacobson KA (1998) Activation of the A2A adenosine receptor inhibits nitric oxide production in glial cells. FEBS Lett 429:139–142
54. Seta Y, Shan K, Bozkurt B et al (1996) Basic mechanisms in heart failure: the cytokine hypothesis. J Card Fail 2:243–249
55. Mann DL (2002) Inflammatory mediators and the failing heart. Past, present, and the foreseeable future. Circ Res 91:988–998
56. Khoa ND, Montesinos MC, Reiss AB et al (2001) Inflammatory cytokines regulate function and expression of adenosine A(2A) receptors in human monocyte THP-1 cells. J Immunol 167:4026–4032
57. Xaus J, Mirabet M, Lloberas J et al (1999) IFN-gamma up-regulates the A2A adenosine receptor expression by TNFalpha in PBMC of A2A adenosine receptor expression by TNFalpha in PBMC of