

Adenosine: an endogenous regulator of innate immunity

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Although inflammatory and immunological reactions protect the host from invasion by microorganisms and eliminate debris at sites of tissue injury, they can also be responsible for significant tissue damage. Thus, regulatory mechanisms that limit damage from an overly exuberant immune response have evolved. It is increasingly apparent that adenosine, a purine nucleoside that is elaborated at injured and inflamed sites, has a central role in the regulation of inflammatory responses and in limiting inflammatory tissue destruction. Adenosine, called a 'retaliatory metabolite' because it is a regulatory autocoid that is generated as a result of cellular injury or stress, interacts with specific G protein-coupled receptors on inflammatory and immune cells to regulate their function. The effects of adenosine, acting at its receptors, on the functions of the cells that mediate innate immune responses, will be reviewed.

Adenosine is an endogenous purine nucleoside that, following its release from cells or after being formed extracellularly, diffuses to the cell membrane of surrounding cells where it binds specific cell-surface structures that recognize it, termed adenosine receptors [1,2]. There are four types of adenosine receptor, all of which are members of the G protein-coupled family of receptors [2] (Table 1). The genes for these receptors have been analyzed in detail and they are designated A_1 , A_{2A} , A_{2B} and A_3 . Although adenosine is constitutively present in the extracellular space at low concentrations, metabolically stressful conditions dramatically increase its extracellular levels.

The role of adenosine as an extracellular signaling molecule was first established by the seminal study of Drury and Szent-Györgyi [3], which demonstrated that adenosine (extracted from heart muscle) is both a potent negative inotropic agent and a coronary vasodilator. Because extracellular adenosine formation was later shown to occur in the hypoxic and ischemic heart, the hypothesis was proposed that adenosine served a protective function in the heart against the consequences of metabolically detrimental situations, both by decreasing the metabolic demands of the myocardium and by increasing coronary blood flow [1]. Subsequently, evidence was obtained for similar protective actions for extracellular adenosine in other cellular and organ systems, including the brain, kidney, skeletal muscle and adipose tissue. Based on this evidence, a unifying hypothesis for adenosine action was formulated by Newby [4], and the term 'retaliatory metabolite' was coined to describe the protective function of adenosine. This hypothesis states that adenosine, released in response to a wide range of stressful injurious stimuli, mediates an autoregulatory loop, the function of which is to protect organs from injury following the initiating stressful stimuli.

Adenosine exerts its protective effects by two different mechanisms. First, adenosine decreases the energy demand of the tissue by a direct inhibitory effect on parenchymal cell function, exemplified by the negative inotropic effect of adenosine on the heart muscle. Second, adenosine indirectly protects the tissue by providing a more favorable environment for parenchymal cells, the best

Table 1. Characterization of adenosine receptors^a

Receptor	A_1	A_{2a}	A_{2b}	A_3
Intracellular second messenger pathways coupled to adenosine receptor activation	↓ cAMP, ↑ Ca^{2+} , ↑ p38, ↑ p42/44	↑ cAMP, ↑ p42/44	↑ cAMP, ↑ p38	↓ cAMP, ↑ Ca^{2+}
Selective agonists	CPA, CCPA	CGS-21680	None	IB-MECA, 2CI-IB-MECA
Selective antagonists	DPCPX, PACPX	ZM241385, CSC	Alloxazine	MRS-1220
Affinity to adenosine	High	High	Low	Low
G protein-coupling	Gi/o	Gs	Gs, Gq	Gi, Gq

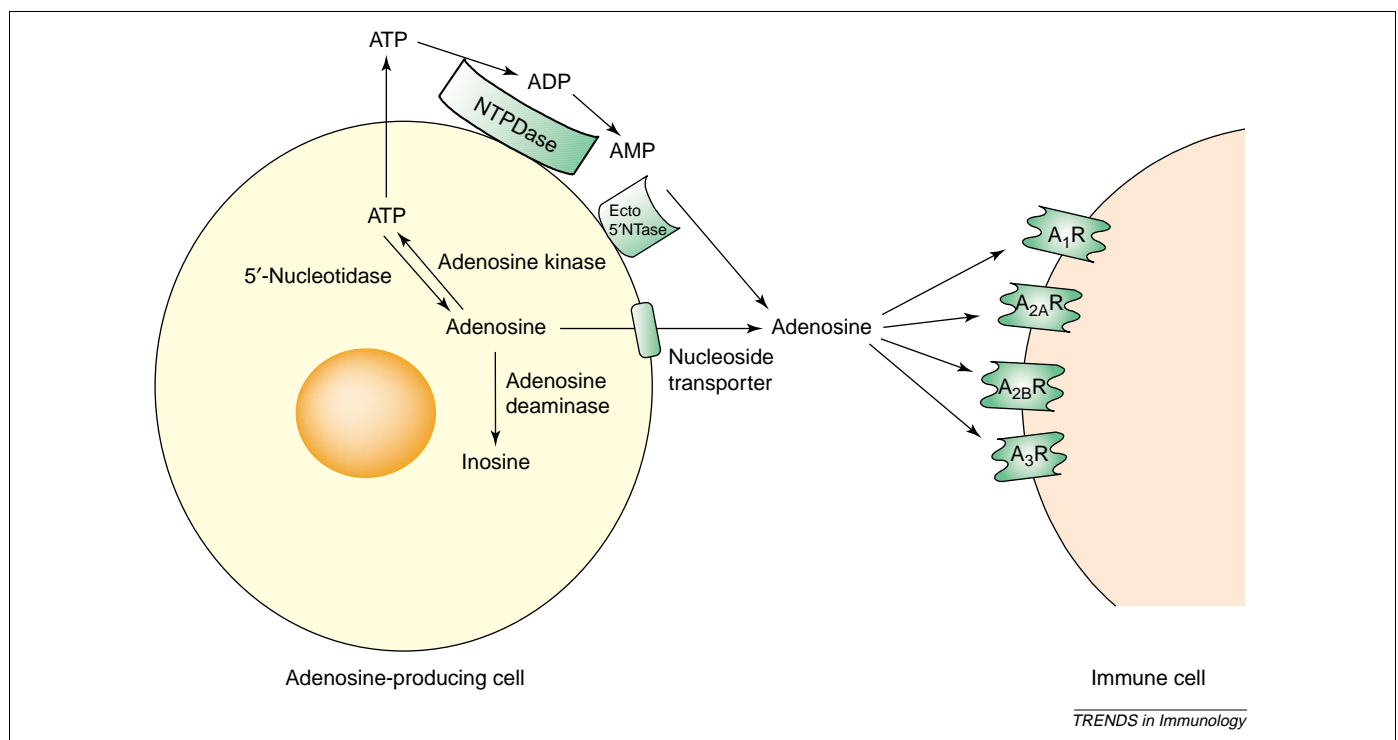
^aAbbreviations: CCPA, 2-chloro- N^6 -cyclopentyladenosine; CGS-21680, 2-p-(2-carboxyethyl) phenethylamino-5'- N -ethyl-carboxamidoadenosine, 2CI-IB-MECA, 2-chloro- N^6 -(3-iodobenzyl)-adenosine-5'- N -methyluronamide; CPA, N^6 -cyclopentyladenosine; CSC, 8-(3-chlorostyryl)caffeine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; IB-MECA, N^6 -(3-iodobenzyl)-adenosine-5'- N -methyluronamide; IE-MECA, N^6 -(3-iodobenzyl)-adenosine-5'- N -methyluronamide; MRS-1220 (9-chloro-2-(2-furyl)-5-[(phenylacetyl)amino] [1,2,4]-triazolo[1,5-c]quinazoline; PACPX, 1,3-dipropyl-8-(2-amino-4-chlorophenyl)-xanthine; ZM241385, 4-(2-[7-amino-2-(2-furyl)-(1,2,4)-triazolo(2,3-a)(1,3,5)triazin-5-ylamino]ethyl)phenol.

example of which is adenosine-mediated augmentation of nutrient availability through vasodilation. More recent evidence indicates that adenosine helps to maintain tissue integrity by another major indirect route: modulation of immune system function. Although the immune response to tissue injury has an essential role in preserving tissue homeostasis, uncontrolled inflammation or immune activation can inflict further damage on the affected tissues. It appears that the release of adenosine followed by its binding to adenosine receptors on immune cells represents a potent endogenous immunosuppressive pathway that regulates the exuberant immune response to harmful external insults.

Recent *in vitro* and *in vivo* studies clearly confirm the beneficial role of adenosine as an immune modulator. First, adenosine is released in the vicinity of immune cells in tissues subjected to various forms of injurious stimuli, including ischemia and inflammation. Second, in the majority of experimental systems, adenosine is immunosuppressive as a result of adenosine receptor occupancy on the various immune cell types. Third, removal of endogenous adenosine signaling exacerbates immune activation and consequently aggravates tissue dysfunction following acute injurious stimuli. In this Review, we discuss recent developments that have increased our understanding of the role that adenosine has in the regulation of immune function. Special emphasis is given to the question of how adenosine regulates the innate immune system because the most substantial new findings have been obtained in this area.

Adenosine bioavailability is a key determinant of adenosine action in the innate immune system

Physiological actions of adenosine almost exclusively result from its occupancy of cell surface adenosine receptors and the activation of downstream intracellular pathways. Processes related to its production, release, cellular uptake and metabolism determine the bioavailability of adenosine at receptor sites. These processes are closely interdependent and highly regulated. One good example for this interdependence is the concerted action of intracellular purinergic metabolic pathways, which results in a substantial increment of intracellular adenosine concentrations during tissue hypoxia or ischemia. Under these conditions, the increased dephosphorylation of ATP to adenosine by the metabolic enzyme 5'-nucleotidase is paralleled by a suppression of the activity of the salvage enzyme adenosine kinase, which prevents the rephosphorylation of adenosine [2]. Once adenosine reaches high concentrations inside the cell, it is then shunted into the extracellular space through the operation of specialized nucleoside transporters [5] (Figure 1). The other major, and probably dominant, pathway that contributes to high extracellular adenosine levels during metabolic stress is comprised of the release of precursor adenine nucleotides (ATP, ADP and AMP) from the cell followed by extracellular catabolism to adenosine by a cascade of ectonucleotidases, which include CD39 [nucleoside triphosphate dephosphorylase (NTPD)] and CD73 (5'-ectonucleotidase). Adenosine bioavailability is limited by its catabolism to inosine by adenosine deaminase,



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Figure 1. Major pathways involved in adenosine metabolism. Adenosine is formed from its precursor ATP in both the intracellular and extracellular spaces. Intracellular adenosine is shunted into the extracellular space through membrane nucleoside transporters. The adenosine salvage enzyme adenosine kinase rephosphorylates adenosine to ATP while adenosine deaminase deaminates adenosine to inosine. The extracellular formation of adenosine is the result of an enzymatic cascade consisting of NTPDases and ecto-5'-nucleotidase (Ecto5'NTase). Extracellular adenosine ligates adenosine receptors (AR) 1, 2a, 2b and 3, all of which are expressed on the surface of immune cells.

which is then further degraded to the stable end product uric acid.

It is not entirely clear which cell types are the most important producers of extracellular adenosine, however, endothelial cells and neutrophils have both consistently been reported to release high levels of adenosine at sites of metabolic distress, inflammation and infection [6]. In addition, Sperlagh *et al.* [7] recently documented that nerve terminals are a major source of extracellular adenosine in the spleen that has been subjected to ischemia. ADP released by platelets at injured sites can also be dephosphorylated to adenosine [1]. It is generally thought that the concentrations of extracellular adenosine are below 1 μM in unstressed tissues, whereas adenosine levels in inflamed or ischemic tissues can be as high as 100 μM . For example, a recent study has documented that systemic (plasma) adenosine levels reach 4–10 μM in patients with sepsis, a condition associated with both ischemia and inflammation, whereas adenosine concentrations in healthy individuals are < 1 μM [8]. Even higher adenosine concentrations, in the 10–100 μM range, were recently found in the synovial fluid of patients with rheumatoid arthritis [9]. Because, in general, adenosine levels < 1 μM have little influence on immune processes, ischemic and inflammatory conditions represent scenarios in which the levels of endogenous adenosine become high enough to exert immunomodulatory and especially immunosuppressive effects.

Finally, it needs to be emphasized that adenosine signaling in the immune system should be viewed in context with signaling initiated by both upstream and downstream metabolites of adenosine. It is well established that adenine nucleotides have powerful immunoregulatory effects, which are mediated by P_2 -purinoceptors [10]. Furthermore, recent evidence indicates that the adenosine breakdown products inosine and uric acid can influence many facets of the innate immune response [11,12]. Because the relative levels of these various purinergic products closely reflect the metabolic status of the tissue, it can be proposed that the purinergic apparatus could represent an ideal sensor system providing the immune system with essential information about the 'health' of the tissue.

Immune cell trafficking between tissue compartments is regulated by endothelial adenosine receptors

Endothelial cells have multiple roles in inflammation and innate immunity. Indeed, the first events recognized as inflammation are mediated solely or in large measure by endothelial cells. In response to inflammatory mediators, ranging from complement components and immune complexes to cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1), endothelial cells express adhesion molecules, which are responsible for the recruitment of leukocytes to inflamed sites. Endothelial cells also synthesize and release mediators, such as platelet activating factor, IL-8 and IL-6, which have a direct role in the inflammatory process by conducting the movement of leukocytes between tissue compartments. Expression of adenosine $\text{A}_{2\text{A}}$ and $\text{A}_{2\text{B}}$ receptors on various types of vascular endothelial cells is well documented, whereas evidence that A_1 and A_3 receptors are expressed on

endothelial cells is based only on the demonstration of mRNA for these receptors [13–16].

Endothelial cells have been recognized as a significant source of adenosine by virtue of their capacity to dephosphorylate adenine nucleotides to adenosine, and the interaction of neutrophils with endothelial cells appears to involve this pathway [15,16]. Neutrophils release AMP during transmigration through the endothelial monolayer and endothelial dephosphorylation of AMP to adenosine by ecto-5' nucleotidase promotes endothelial barrier function through $\text{A}_{2\text{B}}$ receptors [15,16]. In other studies, adenosine inhibited IL-6 and IL-8 secretion and adhesion molecule [E-selectin and vascular cell adhesion molecule-1 (VCAM-1)] expression by immunostimulated human umbilical vein endothelial cells by a mechanism probably related to adenosine $\text{A}_{2\text{A}}$ receptors [17]. Results of later studies indicated that adenosine alone stimulated IL-8 release in human microvascular cells but not in human umbilical vein endothelial cells, an effect that resulted from occupancy of $\text{A}_{2\text{B}}$ receptors [14]. It appears that activation of phospholipase C by Gq proteins, rather than activation of the classical G_s -cAMP pathway, is responsible for the increment in IL-8 production following $\text{A}_{2\text{B}}$ receptor stimulation on human microvascular cells [14]. Thus, both $\text{A}_{2\text{A}}$ and $\text{A}_{2\text{B}}$ receptors regulate endothelial cell inflammatory functions, although their relative importance varies with the source of the endothelial cells.

Neutrophils: crucial players in the early protective effect of adenosine against ischemic and inflammatory tissue injury

Neutrophils are the first inflammatory cells to be recruited to sites of injury and inflammation. These cells, the footsoldiers of the innate immune system, kill microorganisms, eliminate debris remaining after injury and release factors responsible for the recruitment of other inflammatory cells. Although these cells are crucial for preventing dissemination of infections, if unchecked they can also be responsible for significant tissue injury.

Soon after adenosine receptors were described, efforts were made to determine whether they were present on neutrophils and whether they modulated neutrophil behavior. In 1983, Cronstein and coworkers published a seminal study demonstrating that adenosine suppressed the stimulated generation of superoxide anion, an effect that occurred through cell surface receptors [18]. Subsequent studies established that adenosine, acting at A_2 (now $\text{A}_{2\text{A}}$) receptors, inhibited stimulated neutrophil adhesion to, and killing of, endothelial and other cells, bactericidal activity, apoptosis, expression and shedding of adhesion molecules, secretion of cytokines, growth factors and synthesis of leukotriene B₄ (reviewed in Refs [6,19]).

Generally speaking, the effects of $\text{A}_{2\text{A}}$ receptors are attributed to their capacity to signal through cAMP-dependent pathways. Although some laboratories have reported that the influence of adenosine $\text{A}_{2\text{A}}$ receptors on all neutrophil functions is mediated by cAMP [20,21], others have reported that adenosine receptor occupancy inhibits neutrophil superoxide anion generation by cAMP-independent mechanisms, including activation of

a membrane-associated phosphatase and desensitization of chemoattractant receptors (reviewed in Ref. [6]).

Early work had suggested that neutrophils might possess more than one type of adenosine receptor [22]. Subsequent studies demonstrated that neutrophils express A_1 receptors that, when occupied, increase neutrophil chemotaxis and phagocytosis (reviewed in Ref. [6]). Nevertheless, in general, the anti-inflammatory effects of adenosine acting at A_{2A} receptors dominate the effects of A_1 receptors (reviewed in Ref. [6]). Neutrophils appear to express A_{2B} receptors that, when occupied, inhibit the stimulated release of vascular-endothelial growth factor and the transmigration of neutrophils across endothelial monolayers [23]. The presence of A_3 receptors on human neutrophils is controversial. The first paper reporting their existence and function did not use appropriate ligands to characterize the pharmacologic effects of adenosine on neutrophils [24]. A recent study using both pharmacologic and molecular approaches reported that A_3 receptors were expressed on human neutrophils, however, the authors could not demonstrate a clear functional role for these receptors [25].

Thus, adenosine regulates neutrophil function in opposing fashion through the ligation of A_1 (immunostimulatory) and A_{2A} (immunosuppressive) receptors. This counter-regulatory effect suggests that adenosine enhances the inflammatory response at sites where adenosine is present in low concentrations, such as in the relatively acellular dermis (perhaps in an autocrine fashion due to adenosine release from the neutrophils), or at sites where net adenosine uptake and metabolism is greater than adenosine production, as can occur at sites of bacterial infection. Once neutrophils have arrived at a site where there is significant tissue injury, adenosine, generated in high concentrations by damaged tissues or cells, acts as a feedback inhibitor of inflammatory neutrophil functions.

Interaction of adenosine receptor signaling and pattern recognition receptor transduction pathways in APCs

Macrophages and dendritic cells (DCs) are specialized phagocytes that have an important role in the clearance of apoptotic host cells and injurious molecules, as well as in defense against infection. These antigen-presenting cells (APCs) are widely dispersed throughout the body, including at portals of entry to microorganisms. They participate in the initial capture and processing of antigens and then in the activation of specific lymphocyte-effector mechanisms. These activated lymphocytes in turn cooperate with macrophages to enhance destruction of pathogens. Probably the most important recent development in the field of APC immunology is the discovery of pattern recognition receptors (PRRs), which have evolved to permit recognition of conserved repetitive microbial elements [e.g. lipopolysaccharide (LPS), CpG DNA, viral RNA]. These include the Toll-like receptors (TLRs), mannose-binding lectins and scavenger receptors [26]. Recent studies have made it abundantly clear that signals initiated by adenosine receptor occupancy can interfere with the intracellular pathways activated by PRRs.

Adenosine receptor ligation on monocytes and macrophages strongly suppresses the production of IL-12 induced by LPS through TLR4 [27–30]. Because IL-12 is instrumental in directing a strong inflammatory response, the adenosine suppression of IL-12 production is probably one of the central mechanisms whereby adenosine receptor occupancy prevents inflammation-induced tissue injury. In addition to observations with IL-12, adenosine receptor stimulation has been documented to decrease the TLR4-induced release of a host of other proinflammatory mediators including TNF- α , MIP-1 α , and nitric oxide, as well as to augment the production of the anti-inflammatory IL-10 [31–34]. Remarkably, the inhibitory effect of adenosine on TNF- α production by macrophages is not confined to TLR4-mediated induction of this cytokine because adenosine downregulates TNF- α production when induced by agonists of TLR2, 3, 4, 7 and 9 [35]. Although it is probable that adenosine receptor ligation targets a common major intracellular pathway to exert such a general anti-inflammatory effect, the nature of this intracellular target is unclear at this juncture. The possibility that NF- κ B, a central transcription factor mediating most of the proinflammatory effects of TLR stimulation, could be such a target was recently dismissed because not only did adenosine fail to decrease TLR-mediated NF- κ B activation but it also had no effect on cytokine transcript levels as assessed by cDNA array analysis [35–37]. Recent studies using knockout mice for the A_{2A} and A_3 receptors have illustrated that both of these receptors contribute to the adenosine suppression of proinflammatory mediator production following TLR stimulation [28,38,39]. Furthermore, the A_{2B} receptor was recently implicated as the receptor responsible for the downregulation of both inducible NO synthase and MHC II expression in response to interferon- γ (IFN- γ) [40].

Again, as seen with neutrophils, under certain conditions adenosine can exert activating effects on APCs. In immature DCs, in the absence of TLR stimulation, adenosine promotes chemotaxis, which appears to be mediated by A_3 receptors and is associated with increased intracellular calcium levels and actin reorganization [41,42]. Although, at first glance, this stimulation of immature DCs might indicate a potentially injurious role, this is not the case. Because the early accumulation of DCs at sites of microbial invasion is crucial for the initiation of an immune response, such a chemotactic effect of adenosine can be viewed as protective by providing an early signal for the recruitment of immune cells able to fight the intruding microorganism. However, in fully mature DCs that are encountered at the site of an established inflammatory and/or immune response, adenosine strongly suppresses the TLR-mediated production of IL-12 [43], providing a stop signal that prevents potential tissue injury caused by an unchecked inflammatory and/or immune response. Consistent with this downregulation of IL-12 production by mature DCs, DCs, in the presence of adenosine, have a diminished capacity to promote differentiation of T cells to a Th1 direction [43]. Such a decrease in Th1-cell differentiation constitutes a further mechanism for the adenosine suppression of inflammation because

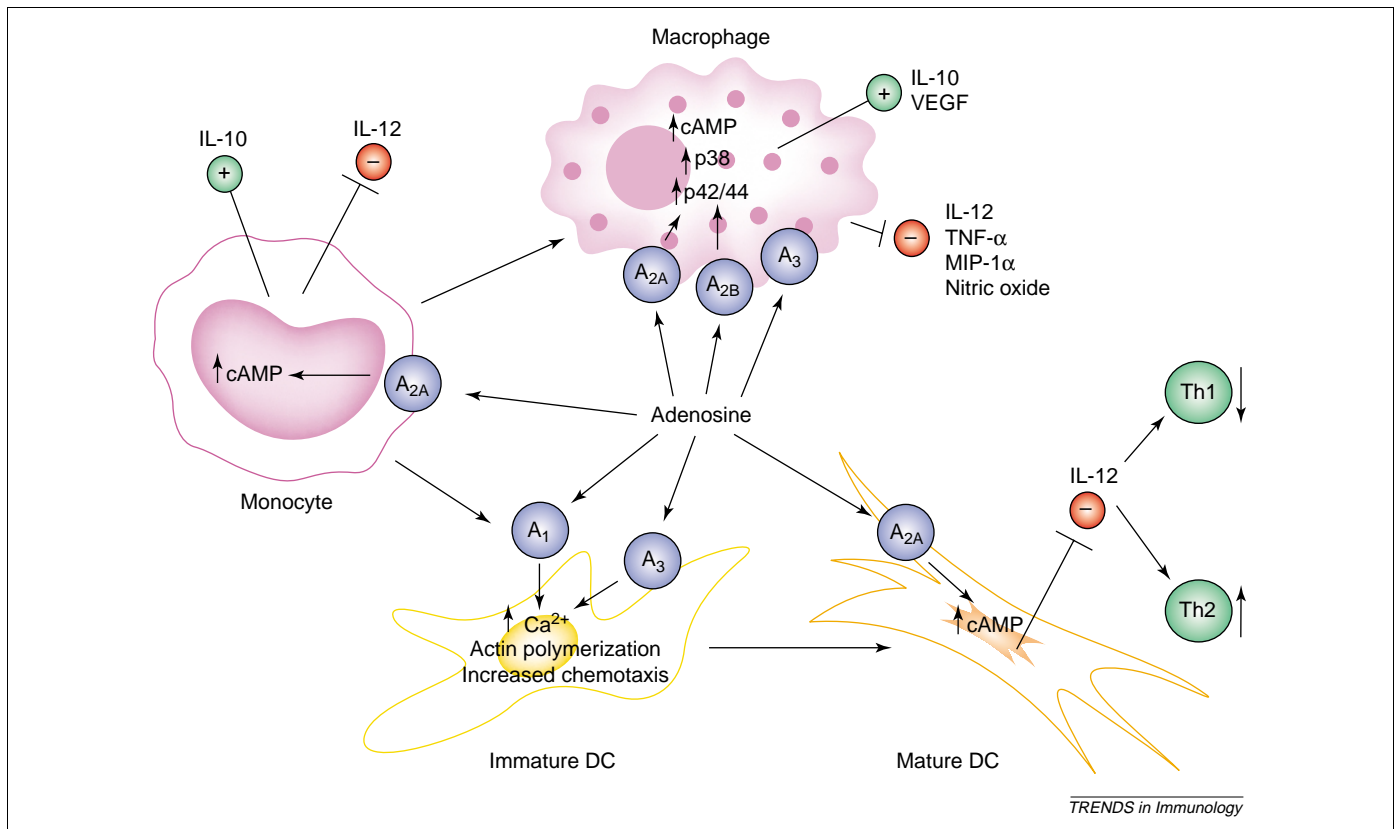


Figure 2. Antigen-presenting cells (APCs) are equipped with adenosine receptors, which on occupation regulate APC function. Adenosine receptor (AR) occupancy signals through alterations of intracellular cyclic AMP and Ca^{2+} concentrations and activation of the mitogen-activated protein kinases p38 and p42/44. AR occupancy on monocytes and macrophages diminishes production of the proinflammatory mediators interleukin-12 (IL-12), tumor necrosis factor- α (TNF- α), macrophage inflammatory protein-1 α (MIP-1 α), and nitric oxide while augmenting secretion of the anti-inflammatory cytokine IL-10 and vascular endothelial growth factor (VEGF). Adenosine receptor ligation stimulates the chemotaxis of immature dendritic cells (DCs). Adenosine acting on A_{2a} receptors suppresses IL-12 production by mature DCs leading to diminished Th1- versus Th2-cell development.

Th1 cells are strong inducers of a macrophage-mediated inflammatory response (Figure 2).

Adenosine in mast cells – a double-edged sword?

Mast cells have traditionally been associated with IgE-mediated immune responses against parasites, however, mast cells are now generally recognized as essential components of innate immune responses against bacteria and can also have a role in the pathogenesis of inflammatory arthritis [44]. Interestingly, mast cells could represent the exception to the 'rule' that adenosine is tissue protective and immunosuppressive because adenosine is a potent stimulator of mast-cell function. Adenosine, mainly through occupancy of A_{2B} and A_3 receptors, causes degranulation of mast cells, which liberates histamine, serotonin, chemokines and a host of injurious proteases [38,45–48]. There is some recent evidence that mast-cell adenosine receptor stimulation, through the release of histamine, could provide a negative feedback signal for macrophage TNF- α production, and thus inflammation, through H_2 histamine receptors expressed on macrophages [49]. However, the proinflammatory effects of mast-cell adenosine receptor stimulation, especially in situations associated with long-lasting high adenosine concentrations, seem to overshadowed the anti-inflammatory potential. Recent studies have revealed that mice, which accumulate high levels of endogenous adenosine owing to a deficiency of the adenosine catabolizing enzyme,

adenosine deaminase, develop a pulmonary phenotype with mast cell-mediated inflammation resembling the symptoms of asthma [50–52]. This evidence, together with the observation that patients suffering from asthma have elevated pulmonary adenosine levels as well as augmented expression of adenosine receptors, suggests that increased adenosine signaling could be an important feature of asthma and chronic obstructive pulmonary diseases [53].

Adenosinergic therapy of inflammatory diseases

Based on the evidence summarized, adenosine appears to promote a self-limiting, healthy immune response. Early after the injurious or infectious signal, high concentrations of extracellular adenosine favor a transition from neutrophil infiltration to DC recruitment, providing a framework in which less sophisticated defense mechanisms represented by neutrophils give way to a highly efficient specific immune response initiated by DCs. At later stages of immune or inflammatory processes, adenosine contributes to the resolution of inflammation, both by downregulating macrophage activation and by advancing Th2- versus Th1-cell development [54]. Thus, adenosine receptors on cells of the immune system present an intriguing target for immunomodulatory therapies for inflammatory, autoimmune and acute ischemic diseases. The use of exogenously administered adenosine in the therapy

of inflammatory or ischemic diseases, however, does not seem to be practical; systemically administered adenosine, owing to its non-selective nature and the expression of adenosine receptors on virtually all cell types, leads to undesirable side effects that prevent it from being an effective therapeutic modality. However, agents that increase local (at the site of injury or inflammation) ambient adenosine concentrations or stable compounds with high selectivity towards adenosine receptor types could be successfully used to treat inflammatory, autoimmune and acute ischemic diseases.

Methotrexate is probably the most widely used agent for the therapy of rheumatoid arthritis and other inflammatory states; in the therapy of early rheumatoid arthritis it is nearly as effective as newer biologic agents [55]. In 1991, Cronstein and co-workers [56] provided *in vitro* evidence to support the hypothesis that low dose methotrexate therapy induces an increase in adenosine release from injured cells by the selective inhibition of AICAR (5-aminoimidazole-4-carboxamidoribonucleotide) transformylase, an enzyme that catalyzes an intermediate reaction in *de novo* purine biosynthesis, and the adenosine so released inhibits inflammation. Subsequently, *in vivo* studies demonstrated that adenosine, acting at A₂ receptors, mediates the anti-inflammatory effects of methotrexate in mice [57], an observation confirmed more recently in adenosine A_{2A} and A₃ knockout mice [58]. Studies in the adjuvant arthritis model reveal that the adenosine receptor antagonist, caffeine, reverses the anti-inflammatory effects of methotrexate in the adjuvant arthritis model of inflammation [59]. Drinking caffeinated coffee also interferes with the anti-inflammatory actions of methotrexate in patients [60,61], an observation that suggests that avoidance of caffeine could enhance the therapeutic effects of methotrexate. Besides methotrexate, there is evidence that other therapeutic agents, such as sulfasalazine and FK-506, could exert their anti-inflammatory effects by promoting adenosine release [62,63].

Finally, it needs to be emphasized that although increasing ambient adenosine concentrations or stimulating adenosine receptors might be useful in the treatment of some forms of inflammation and acute ischemia, there appear to be a few scenarios when an inverse approach could be required. For example, in certain disease states associated with long-lasting high endogenous levels of adenosine, such as asthma and chronic obstructive pulmonary disease, as well as sepsis and septic shock, it could be necessary to downregulate adenosinergic signaling. In this way, the injurious consequences of adenosinergic mast-cell stimulation in asthma or the immunocompromised state that might be secondary to high concentrations of adenosine during the late phase of sepsis, could be counteracted by adenosine receptor blockade and/or diminishing adenosine concentrations [53,64].

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