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Adenosine and Adenosine Receptors in the Pathogenesis and Treatment of Rheumatic Diseases

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Abstract

Adenosine, a nucleoside derived primarily from the extracellular hydrolysis of adenine nucleotides, is a potent regulator of inflammation. Adenosine mediates its effects on inflammatory cells by engaging one or more cell-surface receptors. The expression and function of adenosine receptors on different cell types change during the course of rheumatic diseases, such as rheumatoid arthritis. Targeting adenosine receptors directly for the treatment of rheumatic diseases is currently under study; however, indirect targeting of adenosine receptors by enhancing adenosine levels at inflamed sites accounts for most of the anti-inflammatory effects of methotrexate, the anchor drug for the treatment of rheumatoid arthritis. In this Review, we discuss the regulation of extracellular adenosine levels and the role of adenosine in regulating the inflammatory and immune responses in rheumatic diseases such as Rheumatoid Arthritis, psoriasis and other types of inflammatory arthritis. In addition, adenosine and its receptors are involved in promoting fibrous matrix production in the skin and other organs and the role of adenosine in fibrosis and fibrosing diseases will also be discussed.

Introduction

Many different intercellular signals maintain homeostasis in tissues and organs. One of the first of these signals identified in the regulation of a large number of physiological and pathological processes was adenosine. First identified as a potent vasodilator in 1929 by Drury and Szent-Gyorgi[1], adenosine was subsequently shown to mediate its effects on cells via engagement of specific receptors (A_1, A_{2a}, A_{2b} and A_3 [2,3]). Indeed, adenosine has been described as a 'retaliatory metabolite' [4] that is released from cells in response to hypoxia, metabolic stress or injury and promotes the processes required to alleviate these noxious stimuli. Among its other functions, adenosine is an endogenous regulator of inflammation

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Author contributions

Both authors contributed to all aspects of this manuscript.

that mediates the transition from inflammation to healing. Pathological changes in, or pharmacological manipulation of, adenosine metabolism or adenosine receptor expression and/or function might have a role in both the pathogenesis and therapy of rheumatic diseases. We review the metabolic changes that regulate adenosine levels in inflamed tissue, the receptors that mediate the pharmacological and pathological effects of adenosine and their role in rheumatic diseases, as well as the potential role for therapeutic targeting of adenosine and its receptors.

Adenosine in inflammation and fibrosis

Inflammation of the joints, connective tissue, muscle and bone are the most common manifestations of the rheumatic diseases; both the innate and adaptive immune systems can contribute to the inflammation observed in diseases such as rheumatoid arthritis (RA) and psoriatic arthritis. In most inflammatory arthritides neutrophils are the most abundant cells in synovial fluids whereas T cells, B cells and macrophages predominate in the synovial tissues. At inflamed sites a large number of intercellular messengers and effector molecules secreted by the various cells are present, ranging in size from low molecular weight products (such as nitric oxide and prostaglandins among many others) to proteins (cytokines, growth factors, proteolytic enzymes and others). The cells (for example, synovial fibroblast-like cells and vascular endothelial cells) of the tissues that comprise the joint also contribute to injury and destruction of the structures of the joint; osteoclasts are critical to bone destruction, and chondrocytes secrete enzymes that destroy cartilage in inflamed joints. Adenosine, acting at its receptors, regulates the activation of all of these cell types and their secretion of intercellular messengers and effector molecules of all classes.

A common end point of inflammation is fibrosis or scarring, which can disrupt the appropriate functioning of the organ affected. Thus, joint contractures are also characteristic of inflammatory arthritis, and fibrosis characterizes organs affected in extra-articular RA, such as in the lung. The major manifestations of systemic sclerosis (SSc) are fibrosis of the skin and internal organs. Adenosine and its receptors also have a role in the pathogenesis of fibrosis in many different organs and might have a role in SSc as well.

Sources of adenosine

Intracellular ATP, the most abundant molecule in the cell, serves as the reservoir for the production of adenosine; however, most adenosine is formed in the extracellular space as a result of the sequential dephosphorylation of adenine nucleotides to adenosine (Figure 1). A number of transporters are involved in the export of ATP, including the proteins connexin-43 (also known as gap junction alpha-1 protein) [5], progressive ankylosis protein homolog (ANK) [6], pannexin-1 and pannexin-3 [7], and probably others as well, including P2×7[8]. Under resting conditions some ATP is dephosphorylated to adenosine but injury, hypoxia or other metabolic assault can trigger increased rates of intracellular conversion of ATP to adenosine or, more commonly, stimulate the release of adenine nucleotides into the extracellular space where they are dephosphorylated to adenosine by ectoenzymes at the cell surface (ecto-5'nucleotidase [CD73] and ecto-nucleoside triphosphate phosphohydrolase [CD39]) and by enzymes in blood or other extracellular fluids (for example, alkaline or acid

phosphatases, including tissue nonspecific alkaline phosphatase [TNAP]). Once formed or released into the extracellular space adenosine can be deaminated to inosine and, in humans, ultimately to uric acid or taken up directly by cells by specific nucleoside transporters (ENT1 and ENT2) [9] and re-phosphorylated to ATP (Figure 1) [6,9-16].

Inflammation in the joint has long been known to lead to hypoxia due, in part, to pressure from synovial exudates, the disordered vasculature that develops in chronically inflamed synovium and the marked influx of inflammatory cells [17-19]. Although acute hypoxia leads to increased adenosine release that helps to ameliorate tissue injury following ischemia–reperfusion (reviewed elsewhere[20]), in settings of chronic hypoxia and inflammation the ongoing cellular injury results in mitochondrial dysfunction[21]. In addition to release of inflammatory reactants from dysfunctional mitochondria, reduced ATP production probably leads to a reduction of ATP and/or adenosine levels in extracellular fluid (Corciulo and Cronstein, unpublished observations. Despite upregulation of adenosine receptors at inflamed sites (see below) the diminished adenosine level present in chronically inflamed joints probably permits further activation of inflammatory cells in the synovium [21]. Hypoxia contributes to both acute and chronic inflammation by other mechanisms as well [21].

Adenosine receptors

Four subtypes of adenosine receptor exist, named in order of discovery: A_1, A_{2a}, A_{2b} and A_3. These receptors are all members of the G-protein-coupled family of 7-transmembrane spanning receptors [3] (FIGURE 2). A_1 and A_{2a} are high affinity receptors with activity in the low to mid-nanomolar range whereas A_{2b} has a substantially lower affinity for adenosine (micromolar). Considerable species-dependent variability in A_3 exists[22]. Adenosine receptors are widespread in their expression and are important regulators of many different types of physiological and pathological processes. A_1 and A_3 signal via $G\alpha_i$ signalling proteins, which downregulate cAMP expression, whereas A_{2a} and A_{2b} are linked to $G\alpha_S$ proteins, which trigger increases in cellular cAMP content. A_{2b} also signals through Gq proteins[23].

Expression of adenosine receptors is regulated by a number of stimuli, including inflammatory stimuli. In particular, A_{2a} is upregulated by agents that stimulate activation of NFκB (a central transcriptional regulator in the inflammatory process), such as TNF, IL-1 and endotoxin, [24-28] and acts, as described below, as a feedback inhibitor of inflammation. Evidence from patients with RA confirms these observations: increased expression of A_{2a} on peripheral white blood cells in these patients is reduced by treatment with anti-TNF agents[29,30]. In addition to regulation of A_{2a} expression, TNF and other proinflammatory cytokines increase the function of these receptors by preventing receptor desensitization[31], further downregulating inflammation. By contrast, interferon-γ (IFNγ) downregulates both the expression and function of A_{2a} [32-34]. This downregulation (which occurs following the increase in both extracellular adenosine levels and A_{2a} expression and function after cellular injury or necrosis) indicates the potent role of adenosine and its receptors as feedback regulators of inflammation and innate immune responses.

Similarly, A_3 adenosine receptor expression is increased on peripheral blood white cells in patients with RA, and treatment with anti-TNF antibodies reduces adenosine receptor overexpression[29,35-37].

Adenosine receptors and innate immunity

Neutrophils

The anti-inflammatory effects of adenosine were first suggested in 1983 when the capacity of extracellular adenosine to inhibit stimulated neutrophil superoxide anion generation was demonstrated [38], effects subsequently confirmed and expanded upon by many others [39-42]. Later studies revealed that these effects were mediated by adnesoine receptor A_{2a} [43-45]. Thus, A_{2a} engagement inhibits phagocytosis.

Before neutrophils can destroy invading bacteria or clear debris from a wound they must be recruited from the vasculature into the extravascular space, a process mediated by a series of adhesive interactions among neutrophils, endothelial cells and matrix. Numerous studies revealed that engagement of adenosine receptor A_{2a} and A_{2b} inhibited adhesion of neutrophils to both endothelial cells and other surfaces [46-50] by inhibiting both selectin and integrin-mediated adhesive interactions. Interestingly, *in vitro* studies demonstrated that A_1 enhanced neutrophil adhesion to endothelial cells via $\alpha 4$ integrins [49]. This same group had reported, based on studies with pharmacological inhibitors, that stimulation of A_1 enhanced neutrophil chemotaxis; [43,51] however, later studies using knockout mice and their neutrophils demonstrated that, in fact, it was A_3 that mediated enhanced chemotaxis [52,53].

One of the mechanisms by which inflammatory cell activation is terminated or downregulated is by engulfment of apoptotic cells, such as occurs at sites of crystal-induced inflammation. A_{2a} engagement promotes engulfment-mediated neutrophil downregulation, whereas A_3 engagement diminishes engulfment-mediated downregulation of proinflammatory actions (TABLE 1) [54,55].

It has often been noted that imitation is the most sincere form of flattery and this adage is no less true for imitation of critical biological mechanisms by pathogens. Although most of the adenosine that mediates suppression of inflammation is produced endogenously some is produced by pathogens as a virulence factor. Invasive forms of *Candida albicans*, *Staphylococcus aureus* and *Streptococcus suis* have all been reported to produce adenosine as a virulence factor to protect against phagocyte-mediated injury [56-58]. The hijacking of adenosine-mediated immunosuppression by pathogens provides strong evidence for the importance of this biological mechanism.

Monocytes and macrophages

Macrophages have critical roles in initiating the innate immune response against microbes and in eliminating debris at sites of injury. In addition to destroying most microbes, macrophages produce a variety of cytokines and mediators involved in regulating inflammatory responses and present antigens to T cells in order to orient the adaptive immune response. Macrophages are antigen presenting cells that promote the selective expansion and differentiation of lymphocytes specific for invaders or cancer cells and, in the

rheumatic diseases, direct immune response to self-antigens. Two types of macrophages have been identified: M1 or classical macrophages and M2 or alternatively activated macrophages [59,60]. In diseases such as RA, M1 macrophages have an important role by releasing a variety of proinflammatory cytokines (including TNF, IFNγ and IL-12 among many others), as well as oxidants, nitric oxide and other small-molecule mediators of tissue injury. M2 macrophages have a role in terminating inflammation after ingestion of apoptotic cells and promote wound healing by producing angiogenic and profibrotic cytokines.

Adenosine, acting at A_{2a}, suppresses production of proinflammatory cytokines and stimulates expression of anti-inflammatory mediators such as IL-10 and vascular endothelial growth factor (VEGF) [61-72]. More generally, A_{2a} stimulation induces a switch from an M1 to a modified M2 phenotype [62,71]. One mechanism by which A_{2a} ligation alters macrophage function is via stimulating expression of the orphan nuclear receptor NR4A, which diminishes activation of NFκB-dependent gene expression [73,74]. A_{2b} also stimulates the switch from an M1 to an M2 phenotype [75-77]. The role of A_3 in regulation of inflammatory macrophage function is less clear; A_3 stimulation clearly suppresses cytokine expression and release by macrophages and diminishes inflammation in mouse models of arthritis and inflammatory bowel disease [70,78-86]. Indeed, the relatively poor anti-infective function of macrophages from human neonates is thought to reflect the dominance of A_3 expressed by these cells [80]. By contrast, studies have indicated that A_3 ligation diminishes the suppression of inflammation induced by engulfment of apoptotic cells [54]. In patients with RA, A_3 expression is a marker of disease severity and can predict the efficacy of treatment of symptoms with a selective adenosine receptor A_3 ligand (TABLE 1) [35].

The effect of A_{2b} activation on the overall immune response remains ambiguous. As noted above, A_{2b} promotes a phenotypical switch from M1 to M2 macrophages, [75-77] but also stimulates the differentiation of dendritic cells, which can promote type 17 T helper cells (T_H17) immune responses via production of IL-6 [87-89]. Indeed, in *in vivo* studies high concentrations of adenosine exacerbated arthritis and tissue destruction in rats via an A_{2b} mechanism [90].

Multinucleated giant cells, formed from the fusion of macrophages, are characteristic accompaniments of inflammation in response to foreign bodies or certain pathogens (most notably tuberculosis) and their presence in inflamed vessels gives Giant Cell Arteritis its name.. Adenosine receptors have a critical role in the formation of these syncytia as A_1 stimulation is essential for formation of giant cells *in vitro* [91]. Osteoclasts, a specialized form of multinucleated giant cell involved in bone remodelling and turnover, also depend on A_1 to complete differentiation by a mechanism involving the signalling molecule TRAF6 [92,93]. By contrast, A_{2a} and A_{2b} stimulation inhibits osteoclast differentiation; mice lacking these receptors have osteopaenia [94-96] due, in part, to increased osteoclast-mediated bone resorption. Owing to its effects on osteoclasts and macrophages and production of proinflammatory cytokines, A_{2a} also inhibits inflammatory bone damage induced by prosthetic wear particles[97]. Interestingly, both A_{2a} and A_{2b} stimulate bone formation *in vivo* [98] although only the direct stimulatory effects of A_{2b} on osteoblast function have been well characterized [99,100].

The adaptive immune system

Regulation of T cells by A_{2a}

Effector functions of T cells in RA synovium are triggered by the T cell receptor (TCR) recognition event followed by transmembrane signalling of the TCR–CD3 complex and by multiple interconnected intracellular biochemical pathways; therefore, it is important to explore the therapeutic opportunities of targeting T cells in both hypoxic and adenosine-rich microenvironments and normoxic and adenosine-poor tissue microenvironments of inflamed joints in RA. Studies of adenosine receptors on T cells were largely motivated by the need to understand their role in anti-pathogen and anti-tumour immunity, with a primary focus, until recently, on A_{2a} [101,102]. The high-affinity A_{2a}, but not A_1 or A_3, was identified as the predominant adenosine receptor on the surface of mouse T cells[103,104]. Expression and functional coupling of A_{2a} in mouse peripheral T cells and B cells correlated with adenosine-induced cAMP accumulation in lymphocytes and with adenosine-induced inhibition of TCR-triggered T cell activation[103,104]. The capacity of A_{2a} stimulation to inhibit effector functions of different subsets of mouse T cells was subsequently confirmed[105] and *in vivo* genetic and pharmacological evidence was provided showing that A_{2a} is critical and non-redundant in controlling the extent of T-cell-mediated immune responses, inflammation and tissue damage in different models of antipathogen immune responses and antitumour immune responses [101,106]. Non-redundance of A_{2a} in T cells and myeloid cells was demonstrated by the observation that none of the other cAMP-elevating G-protein-coupled receptors on immune cells could replace A_{2a} and compensate for the A_{2a}-mediated attenuation of T-cell-driven immune responses and inflammatory damage in models of autoimmune and viral hepatitis[101].

ADORA2A gene–dose effect

Naive T cells express low levels of A_{2a} [103,104] but following TCR-triggered activation of T cells *ADORA2A* mRNA and A_{2a}-mediated signalling rapidly increase[105]. Other studies have demonstrated a clear gene–dose effect for extracellular adenosine signalling at A_{2a} and that no receptor reserve exists on these cells [107]. The lack of ‘spare’ immunosuppressive A_{2a} on T cells points toward the importance of every individual A_{2a} molecule on these cells in maintaining the balance between immunosuppression and immunostimulation and the need for the precise regulation of numbers of A_{2a} molecules to avoid autoimmunity without tolerization of T cells. Extracellular adenosine is short-lived *in vivo* but its immunosuppressive effects on T cells are prolonged due to the intracellular biochemical ‘memory’ of exposure of T cells to adenosine[104].

In view of the direct evidence for the effect of A_{2a} on T cells and the role of T_H17 and T regulatory (T_{reg}) cells in the pathogenesis of RA it would be of significant interest to investigate the genetic differences in *ADORA2A* between individual patients with RA and to search for correlations between the number of A_{2a} molecules per immune cell and severity of the disease. *ADORA2A* gene polymorphisms, as well as the number of A_{2a} molecules per immune cell, might also predispose a patient to developing RA or, equally importantly, to respond to adenosine-dependent therapies such as methotrexate (see below). An interesting experimental approach to testing this hypothesis was presented in studies of

functional changes caused by genetic variation of *ADORA2A* that used personalized lymphoblastoid cell lines from twins as a model system[108]. This study demonstrated that there was altered potency of a partial A_{2A} agonist for A_{2A} receptor activation in cells expressing a receptor in which there was a previously described polymorphism associated with caffeine-induced sleep disorders.

A_{2a} and subsets of human T cells

Further characterization of which T cell subsets express A_{2a} and whether and how their functions are regulated is required, although early studies clearly demonstrated differential expression and function of this receptor on T cell subsets[109,110]. $CD4^+$ T helper cells express A_{2a} which, when stimulated by adenosine or an A_{2A} agonist, inhibits the proinflammatory functions of these cells[110,111]. Similar to mouse T cells, human T helper cells increased *ADORA2A* mRNA levels as soon as 4 hours after activation, and A_{2a} was the predominant subtype of adenosine receptor expressed by these cells. Activation of A_{2a} on T cells is of great consequence not only to T cell function but also to the fate of T cells, memory T cells and T-cell homeostasis as A_{2a} counteracts processes leading to activation-induced cell death and Fas–Fas-ligand-mediated cell death in CD4+ T lymphocytes[112]. (TABLE 1) These original studies were carried out on mouse CD4+ hybridomas and human Jurkat cells (a transformed T cell line and were subsequently expanded to *in vivo* studies of naive T cell development and peripheral maintenance, which is the overall outcome of extracellular signalling to T cells in different tissue microenvironments[113]. The possibility of A_{2a} involvement in T-cell selection processes was also the implication of early studies of immature T cells[114].

A_{2a} signalling in T cells

A_{2a} is a Gs-linked receptor that stimulates an increase in intracellular cAMP levels, which in turn activates protein kinase A (PKA). cAMP–PKA signalling directly inhibits all known TCR-triggered effector functions[104,106] because cAMP–PKA is the 'high fidelity' immunosuppressor that intercepts both early and late events in the TCR-triggered intracellular T cell activation pathway [115,116]. Moreover, signalling mediated by A_{2a}, cAMP, PKA, cAMP response element (CRE) and cAMP response element binding (CREB) protein can redirect proinflammatory T cell responses toward an immunosuppressive phenotype. This change in phenotype also occurs because the extracellular adenosine accumulation driven by hypoxia and hypoxia-inducible factor (HIF)-1α results in hypoxia response element (HRE) and CRE-dependent redirection of CD8 and CD4 and myeloid-cell-mediated immune responses toward production of immunosuppressive cytokines and molecules such as TGFβ, IL-10 and CTLA-4, and promotes the development of T_{reg} cells and their inhibition of effector T cells[117,118]. Taken together, any interpretation of the effects of adenosine on T cells should consider A_{2a} signalling regulating T cell development, maintenance and effector functions.

Regulation of T cells by A_{2b}

Compared with adenosine receptor A_{2a}, much less is known about the low affinity A_{2b} on T cells, but—of relevance to RA—evidence indicates that activation of A_{2b} by synthetic agonists could have T_H17-promoting effects by redirecting the differentiation of bone

marrow cells to a $CD11c^{+}GR1^{+}$ dendritic cell subset[89,119]. (TABLE 1) Moreover, A_{2b} stimulation might also promote T_H17 differentiation by stimulating IL-6 expression in a cAMP-independent manner[88,119]. Others have reported that A_{2b} promotes T_{reg}-cell differentiation and limits inflammatory injury[120]. A_{2b} signals by multiple pathways; not only does A_{2b} increase cAMP via coupling to G_s proteins but they might also signal via G_q and G_i proteins, thereby explaining their cAMP-independent effects on generation of IL-6[121].

ADORA1 and ADORA3 in T cells

The expression and function of A_1 and A_3 on T cells and their subsets is not fully established. A report indicates that A_3 is expressed on Jurkat cells but its expression and function in primary cells has not been established[122].

Other cell types in inflammation

New blood vessel formation is a characteristic of the inflamed synovium, and hypoxia is a major driving force for neovascularization. Adenosine and its receptors have direct and indirect roles in stimulating new vessel formation. Acting at both A_{2a} and A_{2b}, adenosine stimulates both angiogenesis and production of such proangiogenic mediators as VEGF[123]. Moreover, both A_{2a} and A_{2b} directly stimulate endothelial cells to form new vessels[123]. (TABLE 1)

The effects of adenosine receptor stimulation on the formation and resolution of vascular oedema seems to vary with the tissue studied. A_{2a} has been reported to promote vascular leakage in healing wounds and inflamed sites as a result of the increase in new blood vessels that form at these sites[124]. By contrast, the barrier integrity of human umbilical vein endothelial cells is enhanced by A_{2a} stimulation[125]. Interestingly, A_{2b} stimulation promotes clearance of pulmonary oedema[126]. A_{2b} stimulation might have opposing effects in other vascular beds: excess adenosine generation, acting via A_{2b}, can increase inflammation and inflammatory oedema in rat models of arthritis[127]. Thus, targeting adenosine receptors in the prevention of angiogenesis and vascular oedema in inflammation is complex.

Similar to the effects on oedema formation and angiogenesis, the effects of adenosine and adenosine receptors on collagen and matrix production by fibroblasts and fibroblast-like cells varies from tissue to tissue. Adenosine, acting at A_{2a}, directly stimulates dermal fibroblasts to produce collagen and also stimulates the production of factors, such as IL-13 and connective tissue growth factor, that are capable of further amplifying collagen production [128]. By contrast, A_{2b} stimulation inhibits collagen production by some tissue fibroblasts (cardiac fibroblasts) but stimulates collagen production by fibroblasts in other tissues [128].

Adenosine and resolution of inflammation

The inflammatory response is critical for clearance of debris and protection from invading microorganisms; inflammation sets the stage for tissue repair. As with suppression of inflammation, adenosine, acting at its receptors, promotes tissue repair and regeneration. As noted above, A_{2a} stimulation promotes the transformation of macrophages to an M2 phenotype, which is associated with lower levels of production of TNF, IL-12 and other

proinflammatory mediators than the M1 phenotype, and with increased production of IL-10, IL-13 and VEGF,[59,60] which promote angiogenesis and fibrosis. Moreover, A_{2a} and A_{2b} stimulation promotes angiogenesis and generation of matrix by directly stimulating endothelial cells, endothelial cell precursors and fibroblasts [123,129].

Adenosine receptors and fibrosis

Adenosine mediates the transition from inflammation to wound healing but an excess of wound healing might result in fibrosis, which can disrupt organ architecture and function. Fibrous contractions of the joints and fibrosis in the lungs and other tissues are common complications of RA and other rheumatic diseases, and fibrosis characterizes SSc. Results from animal models of scarring or diffuse fibrosis, a manifestation of SSc, indicate a role for adenosine and its receptors in fibrosis [130-133]. Moreover, mice lacking adenosine deaminase have high levels of adenosine in the skin and spontaneously develop diffuse dermal fibrosis [131]. Elevated adenosine levels in the tissue of mice lacking adenosine deaminase also lead to pulmonary fibrosis by engaging both A_{2a} and A_{2b} [134-141]. In the liver, extracellular adenosine production with A_{2b} stimulation mediates hepatic fibrosis in mouse models, a phenomenon that is inhibited by caffeine, a nonselective adenosine receptor antagonist[142-144]. This latter finding might account for the well-documented observation that coffee drinking and caffeine ingestion reduces deaths from liver disease [145-149]. Peritoneal fibrosis also depends on A_{2a} stimulation in animal models[150]. In the setting of priapism, in which the chronically engorged penis becomes hypoxic, adenosine release leads to both vasodilation and fibrosis via activation of A_{2b} [151,152]. Thus, although adenosine and its receptors generally have a beneficial role in terminating inflammation, these same receptors, if persistently stimulated, lead to enhanced fibrosis with resulting tissue and organ dysfunction.

Regulation of extracellular adenosine

Under some pathological conditions tissue adenosine levels are markedly increased. Perhaps the best studied condition under which adenosine levels increase is hypoxia. As noted above, adenosine was first described as a coronary vasodilator in 1929[1] and in subsequent studies it became increasingly clear that the hyperaemic response to hypoxia was attributable, in large part, to adenosine release[153]. Presumably the breakdown of ATP and release of adenine nucleotides into the extracellular space has a role in the generation of increased extracellular adenosine; HIF-1α and adenosine kinase were shown to have an important role in this pathway[154]. In addition, ENT1-mediated uptake of adenosine is downregulated by increases in intracellular cAMP[155], and increases in extracellular adenosine drive further increases in adenosine levels by diminishing ENT1 function via adenosine-receptor-mediated increases in cAMP [156]. It was recognized quite early that inflamed joints tend to be chronically hypoxic as a result of the increased cellular bulk, tissue oedema (with resulting compression of the microvasculature) and the pressure of increased synovial fluid[17-19,157,158]. Nonetheless, estimating the actual level of adenosine in synovial fluid is difficult as cells present in synovial fluid might undergo lysis, thus releasing ATP (which is converted to adenosine during processing); furthermore, adenosine is hydrolyzed by the adenosine deaminase present in synovial fluid[159-161].

Extracellular adenosine levels can also fluctuate in other settings. A number of drugs can regulate adenosine levels in the extracellular fluid. Most notable among these are dipyridamole and ticagrelor, which are agents that inhibit ENT1-mediated adenosine uptake [156,162,163]. Ethanol, which is metabolized as a lipid and requires ATP hydrolysis in order to be metabolized to acetyl-CoA, also causes adenosine release as a result of ATP breakdown[156,164-167]. More recently, it has been appreciated that extracellular adenosine levels might reach extremely high concentrations in the setting of rapid cellular breakdown and hypoxia, such as occurs in tumours, leading to consequences such as immunosuppression by tumours[101,102,168].

Therapeutic targeting of adenosine

Although no drugs have been developed to treat rheumatic diseases that directly target adenosine and its receptors, adenosine-based targeting has been reported for a number of commonly used drugs. Anti-inflammatory and antirheumatic drugs that mediate at least some of their anti-inflammatory effects via targeting adenosine metabolism include methotrexate, sulfasalazine and high-dose aspirin.

Methotrexate

Weekly, low-dose methotrexate remains the gold standard therapy for patients with rheumatic diseases. Although its mechanism of action has not been fully elucidated (Box 1), it seems that enhancing adenosine release at inflamed sites has a major role in the anti-inflammatory actions of low-dose methotrexate. The enzyme inhibited best by intracellular methotrexate polyglutamates is aminoimidazolecarboxamidoribonucleotide (AICAR) transformylase; intracellular accumulation of AICAR leads to adenosine release[169] (Figure 1). In 1991, the first results were published suggesting that methotrexate promotes adenosine release, which diminishes inflammation *in vitro*[170]; it was subsequently demonstrated that adenosine mediates the anti-inflammatory effects of methotrexate in an *in vivo* model in which increased intracellular AICAR levels at an inflamed site were documented[171]. Adenosine mediates the anti-inflammatory effects of methotrexate via occupancy of A_{2a} and A_3[169]. Moreover, adenosine receptor antagonists, including caffeine, block the anti-inflammatory effects of methotrexate treatment in an animal model of arthritis[172] and in patients with Rheumatoid Arthritis. in prospective studies[173,174]. A large retrospective study of patients taking methotrexate did not support the contention that caffeine ingestion reversed the anti-inflammatory effects of weekly low-dose methotrexate; however, given that patients in this study had been successfully treated for RA for a number of years, those who had not responded to methotrexate were excluded from the analysis[175]. Nonetheless, other studies provide further evidence for the role of adenosine in the mechanism of action of methotrexate; for example, the expression of CD39, an ectoenzyme that catalyzes the dephosphorylation of ATP and ADP to AMP (which is critical for extracellular adenosine production), is a biomarker for methotrexate efficacy in patients with RA[176]. Other mechanisms of action have been described for the anti-inflammatory actions of methotrexate in the treatment of Rheumatoid Arthritis and other rheumatic conditions that do not include a role for adenosine[169,177-179].

Sulfasalazine

Sulfasalazine was first developed as a specific therapy for RA in the 1940s and remains in use today. Sulfasalazine is broken down by gut bacteria to 5-aminosalicylate and sulfapyridine; sulfasalazine itself and 5-aminosalicylate are poorly absorbed from the gut and sulfapyridine acts as an antifolate once absorbed. Indeed, folate deficiency is a recognized side effect of sulfasalazine[180]. Among its other effects sulfasalazine promotes accumulation of intracellular AICAR and release of adenine nucleotides into the extracellular space where they are dephosphorylated to adenosine; blockade or loss of ecto-5'nucleotidase prevents adenosine release induced by sulfasalazine both *in vitro* and *in vivo* and reverses the adenosine-receptor-dependent anti-inflammatory effects of the drug in a mouse model of inflammation[181,182] (Figure 1). These results suggest that, like methotrexate, adenosine mediates at least some of the anti-inflammatory effects of sulfasalazine.

High-dose aspirin

Although not commonly used anymore for the treatment of RA and other rheumatic diseases, high-dose aspirin was a mainstay of antirheumatic therapy until the development of less-toxic NSAIDs in the 1970s. Before the recognition that aspirin and other NSAIDs mediated many of their actions by inhibiting cyclooxygenases, it was thought that one mechanism by which high-dose aspirin mediated its therapeutic and toxic effects was by uncoupling oxidative phosphorylation and loss of intracellular ATP [183,184]. Results of studies in human neutrophils indicated that some of the effects of high-dose salicylates on stimulated neutrophil function resulted from ATP loss and extracellular conversion to adenosine [185]. Subsequent studies in mice confirmed that adenosine mediates the anti-inflammatory effects of high-dose aspirin *in vivo* as well [186]. The doses of aspirin required verge on the toxic and are almost never used at present.

Antirheumatic drugs in development

On the basis of their anti-inflammatory activity in a variety of animal models [187], A_3 agonists are currently under development for the treatment of RA. Indeed, a phase II clinical trial of CF101, an A_3 agonist, reduced RA activity although the improvement did not achieve statistical significance[188]. Interestingly, an increased clinical response to CF101 was seen in patients in whom A_3 was overexpressed on peripheral blood mononuclear cells [188]. CF101 has also been administered to patients for the treatment of psoriasis and an early phase study demonstrates clear evidence of efficacy for this indication [189].

Conclusions

The role of adenosine and its receptors in regulating inflammation and immune responses has been well established. Adenosine, released by hypoxic or injured tissues or following treatment with drugs such as methotrexate, suppresses inflammation and immune responses via stimulation of its receptors on inflammatory and immune cells. Although this mechanism of action is useful for the treatment of diseases such as RA, in the setting of tumours the immunosuppression that occurs might be deleterious. The conditional benefits of modulating

inflammation and immunity dictate that targeting adenosine receptors with agonists or antagonists will require individual tailoring.

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Biography

Bruce Cronstein is the Paul R. Esserman Professor of Medicine at NYU School of Medicine where he directs the Clinical and Translational Science Institute. Trained as a physician and Rheumatologist, he first described the role of adenosine and its receptors in inflammation and demonstrated that adenosine mediates the anti-inflammatory effects of low-dose methotrexate in the treatment of rheumatic diseases. In other studies he has demonstrated the role of adenosine and its receptors in bone and liver physiology and the pathologic role of adenosine in fibrosis of the skin and liver.

Michail Sitkovsky is the E.W. Black Professor of Immunophysiology and Director of the New England Inflammation and Tissue Protection Institute at Northeastern University, Boston, Massachusetts, USA. Trained as a biophysicist and immunologist, he uncovered the non-redundant and domineering role of A_{2A} adenosine receptor and hypoxia-inducible factor 1α in pathophysiological immunosuppression and redirection of immune response. These findings led to the conceptually novel repurposing of several classes of drugs in order to weaken the immunosuppressive hypoxia–A_{2A} adenosine receptor axis and thereby enable the use of immunotherapies for cancer and infectious diseases.

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BOX 1

Methotrexate was originally developed as a folic acid antagonist to prevent the *de novo* synthesis of purines and pyrimidines in the treatment of cancer and leukaemia, which remain indications for use of this drug. Although high-dose aminopterin, an analogue of methotrexate, was reported to be useful in the treatment of rheumatoid arthritis (RA) in the early 1950s[190], methotrexate was not widely used to treat inflammatory arthritis until anecdotal reports and uncontrolled trials were carried out in the 1970s followed by placebo-controlled trials in the 1980s and approval for the treatment of RA in 1989[191]. Although initially thought to block lymphocyte proliferation via inhibition of purine and pyrimidine synthesis, the inability of folic acid supplementation to reverse the efficacy of methotrexate treatment while preventing folate-dependent toxicities did not support this effect for methotrexate used at doses up to 1/100th of the dose given to treat malignancy[192-194]. In the treatment of Rheumatoid Arthritis, psoriasis and other inflammatory conditions methotrexate is administered, in low doses (5-25mg) once per week and has a half-life that is relatively short. It is undetectable in the circulation after 18 h but is widely distributed and present in tissues as long-lived polyglutamates, which remain potent inhibitors of a number of enzymes involved in the synthesis of purines and pyrimidines. The enzyme inhibited most potently by methotrexate polyglutamates is aminoimidazole carboxamidoribonucleotide (AICAR) transferase (AICART); inhibition of this enzyme leads to intracellular AICAR accumulation (see Figure 1). Increased intracellular AICAR promotes adenosine release, and blockade or deletion of adenosine receptors reverses the anti-inflammatory effects of methotrexate in animal models and patients with Rheumatoid Arthritis [169].

Key Points

- Adenosine, generated from the extracellular hydrolysis of ATP, is a potent endogenous regulator of inflammation and immune reactions via interaction with one or more cell surface receptors.
- The principal adenosine receptor involved in regulation of adaptive T cell responses is ADORA2A.
- ADORA2A, ADORA2B and ADORA3 downregulate macrophage-mediated inflammatory actions, although ADORA2B might indirectly stimulate type 17 T helper cell immune responses via increased IL-6 production.
- Adenosine mediates the anti-inflammatory effects of low-dose methotrexate treatment as well as some of the anti-inflammatory effects of sulfasalazine.

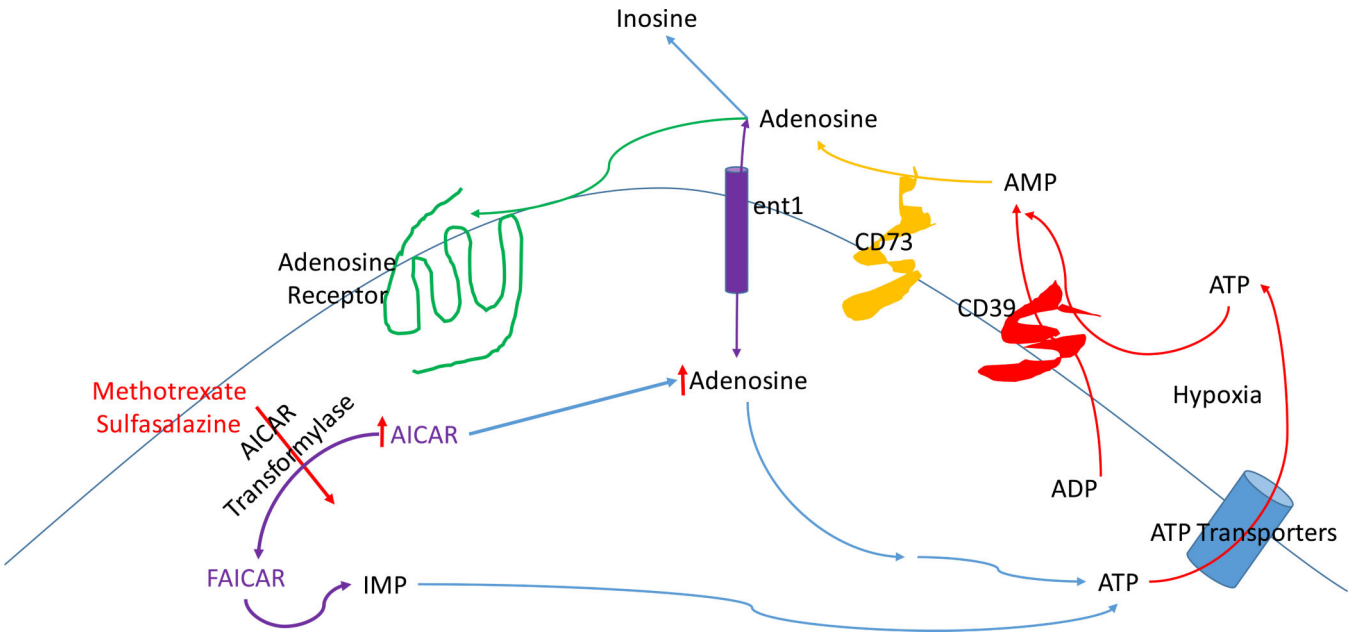

Figure 1. Cellular adenosine production in response to hypoxia and medications

Adenosine is produced extracellularly from the hydrolysis of adenine nucleotides (ATP, ADP and AMP) by the ectoenzymes nucleoside triphosphate phosphohydrolase (NTPP or CD39) and ecto- 5'nucleotidase (CD73) whereas adenosine levels in the extracellular space are reduced by cellular uptake via the extracellular nucleoside transporter (ent1) and breakdown extracellularly to inosine by adenosine deaminase. Both methotrexate and sulfasalazine diminish the activity of aminoimidazolecarboxamido ribonucleotide (AICAR) transformylase leading to accumulation of AICAR and reduction of its metabolite formyl AICAR (FAICAR). Intracellular accumulation of AICAR leads to increased ATP release into the extracellular space.

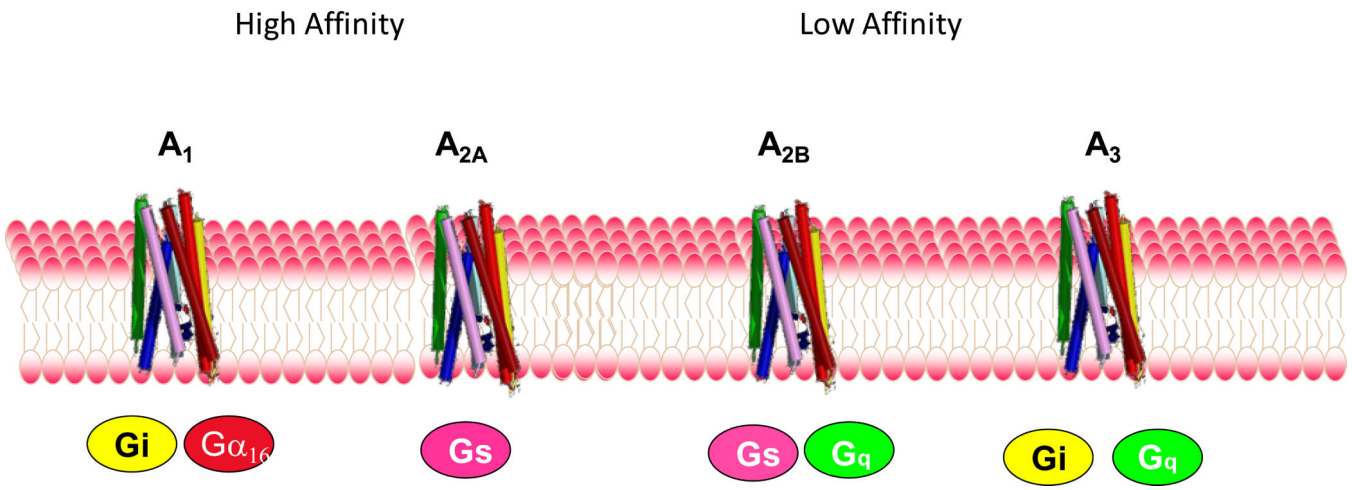

Figure 2. Adenosine receptors

Adenosine receptors are all G protein-coupled receptors whose signal transduction is mediated by activation of intracellular G proteins. ADORA1 has the highest affinity for adenosine and is activated by high picomolar to low nanomolar adenosine concentrations; ADORA2A is activated by concentrations of adenosine in the mid-nanomolar range. By contrast, ADORA2B and ADORA3 are activated by adenosine concentrations in the micromolar range.

TABLE 1

Adenosine receptors regulate the function of inflammatory cells

Inflammatory cell	Adenosine receptor			
	ADORA1	**ADORA2A**	**ADORA2B**	**ADORA3**
Neutrophil	Increases adhesion to matrix[49]	Inhibits superoxide anion generation[43-45] Inhibits adhesion and recruitment Increases engulfment-mediated downregulation of neutrophil function[46-50]	Unknown	Chemotaxis[52,53]
Macrophage	Increases giant cell formation[91] Increases osteoclast differentiation[92,93]	Promotes M1 to M2 transition[62,71] Inhibits cytokine expression[61-72] Inhibits osteoclast formation[94-96]	Inhibits osteoclast formation[94-96] Promotes M1 to M2 transition[75-77]	Inhibits cytokine expression[70,78-86]
T cell	Unknown	Inhibits TCR-triggered activation[103,104] Inhibits activation-induced cell death[112] Inhibits Fas/FasL-mediated cell death[112]	Stimulates T_H17 differentiation by increasing dendritic cell IL-6 production[87-89]	Unknown
Endothelial cell	Unknown	Increases angiogenesis[123] Increases barrier integrity (prevents oedema) [123,125]	Increases angiogenesis[123] Increases oedema formation in arthritis[90] Promotes clearance of pulmonary oedema[126]	Unknown
Fibroblast	Unknown	Stimulates fibroblast production of collagen I and III[130-133] Promotes skin, lung and hepatic fibrosis[130-144]	Stimulates collagen production[130-133]	Unknown