Adenosine, an endogenous anti-inflammatory agent

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Cronstein, Bruce N. Adenosine, an endogenous anti-inflammatory agent. J. Appl. Physiol. 76(1): 5-13, 1994.—Adenosine receptors are present on most cells and organs, yet, although the physiological effects of adenosine were first described over 60 years ago, the potential therapeutic uses of adenosine have only been recognized and realized recently. A decade ago the potent anti-inflammatory effects of adenosine were first described; adenosine, acting at specific A2 receptors, inhibits some, but not all, neutrophil functions. Adenosine inhibits phagocytosis, generation of toxic oxygen metabolites, and adhesion (to some surfaces and to endothelial cells) but does not inhibit degranulation or chemotaxis. Occupancy of adenosine A2 receptors modulates leukocyte function by a novel mechanism. Although adenosine A2 receptors are classically linked to heterotrimeric Gs signaling proteins and stimulation of adenylate cyclase, adenosine 3',5'-cyclic monophosphate does not act as the second messenger for inhibition of leukocyte function. By a mechanism that still remains obscure, occupancy of adenosine A2 receptors on neutrophils “uncouples” chemotactant receptors from their stimulus-transduction proteins. The concentrations of adenosine that inhibit inflammatory cell function are similar to those observed in vivo and suggest a role for adenosine in the modulation of inflammation in vivo. Indeed, recent studies indicate that nonmetabolized adenosine receptor agonists are potent anti-inflammatory agents, and other studies indicate that methotrexate, a commonly used anti-inflammatory agent, diminishes inflammation by increasing adenosine release at inflamed sites. The observations reviewed here suggest that adenosine and agents that act through adenosine are excellent candidates for development as anti-inflammatory agents.

leukocyte; neutrophil; methotrexate; inflammation

INFLAMMATION IS RESPONSIBLE for tissue injury in pathological conditions ranging from myocardial infarction to rheumatoid arthritis. Recent studies suggest that adenosine is a potent regulator of the inflammatory response and, as such, may be useful as a pharmacological agent in the therapy of inflammatory disease. I review the effects of adenosine on leukocyte (primarily polymorphonuclear leukocytes) function and discuss the signal transduction mechanisms responsible for adenosine’s effects on inflammatory cells and inflammation. Finally, I discuss the potential utility of adenosine and agents that act via adenosine as therapeutic modalities in controlling inflammation.

NEUTROPHILS, THE MOST ABUNDANT LEUKOCYTES, MEDIATE ACUTE INFLAMMATION

The histological determinant of acute inflammation is the presence of polymorphonuclear leukocytes (neutrophils). Neutrophils are the most abundant circulating leukocytes and are, generally, the first to respond to bacterial invasion or injury. To arrive at an inflamed site, neutrophils must first travel via the circulation, adhere to the vascular endothelium of postcapillary venules, and finally migrate between the endothelial cells lining the vasculature and into the extravascular space. A variety of adhesion molecules expressed on both the surface of neutrophils and endothelium mediate this complex interaction. L-selectin is a member of the selectin family of lectinlike adhesion molecules that effects the initial loose contact of the resting neutrophil with the endothelium (rolling). When the leukocyte is further activated by tissue factors or bacterial products, L-selectin is shed from the neutrophil surface. Concurrently, other adhesion molecules, CD11a and CD11b/CD18 (β2-integrins) on the neutrophil and intracellular adhesion molecule 1 on the endothelial cell surface, mediate other more stable adhesive interactions between neutrophils and the endothelium. After adhesion to the vascular wall, neutrophils migrate into the inflamed or infected site by following a trail of chemotactic agents, such as the surrogate bacterial chemotactant N-formyl-methionyl-leucyl-phenylalanine (FMLP), the activated complement component C5a, and the cytokine interleukin 8 (34).

Once in the extravascular space, the leukocytes phago-
cytose bacteria or debris, fuse the phagocytic vacuole with lysosomal granules (phagolysosomes), and digest or kill the ingested agents using a variety of granule enzymes (e.g., collagenase and elastase) and toxic oxygen metabolites (e.g., $O_2^-$ and $H_2O_2$). Neutrophils may also injure tissues and organs by the overzealous secretion of these granule components and oxygen metabolites into the extracellular milieu. Recognition of the capacity of neutrophils to mediate tissue injury has driven the search for agents designed to specifically diminish leukocyte accumulation or function in the setting of myocardial injury and other acute inflammatory conditions.

Tissue mast cells, tissue macrophages, monocytes, and other cells also participate in the acute inflammatory response. Indeed, collaboration of these cells is essential for stimulating neutrophil endothelial interactions and priming neutrophils so that they become more potent effector cells in the extracellular environment. On activation, mast cells release histamine, a potent vasodilator, which stimulates neighboring endothelial cells to express P-selectin. Tissue macrophages and monocytes release cytokines (e.g., interleukin 1 and tumor necrosis factor-α (TNF-α)), which provoke endothelial expression of E-selectin and intracellular adhesion molecule 1 and “prime” neutrophils, thereby permitting them to generate greater quantities of reactive oxygen metabolites in response to chemoattractants (34).

EFFECTS OF ADENOSINE ON NEUTROPHIL FUNCTION

In 1980 Marone et al. (69) observed that, unlike epinephrine, adenosine, an agent known to promote intracellular adenosine 3',5'-cyclic monophosphate (cAMP) accumulation in a variety of cell types, did not affect degranulation by stimulated neutrophils. These results were somewhat disappointing, and no further studies were performed to explore the effect of adenosine on neutrophil function for several years. In 1983 Cronstein et al. (28) reported that adenosine inhibited neutrophil $O_2^-$ generation stimulated by chemoattractants (FMLP and C5a) and the Ca$^{2+}$ ionophore A-23187 but not by phorbol myristate acetate (a direct activator of protein kinase C). This group also confirmed that adenosine was, at best, a poor inhibitor of granule release from stimulated neutrophils and did not inhibit leukocyte aggregation (neutrophil-neutrophil adhesion). Of note, adenosine mediated its effects on neutrophil function by acting extracellularly, since blockade of purine uptake did not reverse the effect of added adenosine on stimulated neutrophil function. Subsequent studies (33, 87) demonstrated that adenosine inhibited stimulated $O_2^-$ generation via occupancy of specific adenosine receptors (A$_1$). The effects of adenosine on generation of oxygen radicals ($O_2^-$ and $H_2O_2$) by activated neutrophils have now been reproduced in a number of laboratories (5, 10, 15, 35, 36, 47, 58, 74, 78, 94, 99, 115). In contrast to the original studies, some laboratories have reported that adenosine inhibits stimulated neutrophil degranulation and aggregation (86, 92, 95), whereas other laboratories could not demonstrate any great effect of adenosine on stimulated neutrophil degranulation or aggregation (11, 27, 45, 74, 110).

The effects of adenosine on chemotaxis, adhesion to endothelial cells, and phagocytosis have subsequently been explored. Rose et al. (88) observed that adenosine and its analogues promoted chemotaxis to FMLP and C5a at concentrations two to three orders of magnitude below that which inhibited $O_2^-$ generation. Cronstein et al. (30) first demonstrated that the stable adenosine analogue 2-chloroadenosine inhibited adhesion of stimulated neutrophils to endothelial cells, but subsequent studies by this same group have demonstrated that the effects of adenosine and its analogues on stimulated neutrophil adhesion to endothelial cells and other surfaces are more complex (31). As is discussed below, neutrophils, like other cell types, possess two different types of receptors for adenosine, A$_1$ and A$_2$ (23). Low concentrations of compounds specific for the adenosine A$_1$ receptor actually promote stimulated neutrophil adhesion to cultured endothelial cells and some surfaces. Higher concentrations of adenosine and adenosine A$_2$-receptor-specific analogues inhibit stimulated neutrophil adhesion to endothelium. Similarly, low concentrations of adenosine A$_2$-receptor agonists promote phagocytosis of immunoglobulin-coated red blood cells, whereas higher concentrations of adenosine or A$_2$-receptor agonists inhibit phagocytosis of immunoglobulin-coated red blood cells (91).

After exposure to a variety of agents (e.g., phorbol myristate acetate, endotoxin (lipopolysaccharide), platelet-activating factor (PAF), TNF-α), neutrophils become primed to generate greater quantities of $H_2O_2$ and $O_2^-$ after stimulation with chemoattractants. Adenosine has been reported to inhibit TNF-α-mediated priming for adherent neutrophils but not for neutrophils in suspension (36, 97). In contrast, Stewart and Harris (97) found that adenosine did inhibit PAF-mediated priming of neutrophils in suspension. When primed, neutrophils showed a diminished chemotactic response to chemoattractants and adenosine reversed the effect of TNF-α on neutrophil chemotaxis (98). The effects of adenosine on TNF-α priming of adherent neutrophils are qualitatively similar to those of adenosine and its agonists on TNF-α-mediated reduction of chemotaxis, since migrating cells must adhere to the underlying substratum (36, 97). Because priming is only poorly understood, the mechanism by which adenosine, acting on its receptor, inhibits priming remains a mystery.

One early observation that suggested a physiological role for adenosine as a regulator of inflammation was the demonstration that adenosine is released by suspensions of neutrophils or endothelial monolayers in vitro (10, 28, 30, 47, 77, 105). Removal of this endogenously released adenosine (by addition of adenosine deaminase) enhanced $O_2^-$ generation by stimulated neutrophils. This finding led to the hypothesis that adenosine is an endogenously released anti-inflammatory agent, a “retaliatory metabolite” (28, 30, 77). Cronstein et al. (30) tested this hypothesis in vitro and found that stimulated neutrophils injured endothelial cells; endothelial cell injury was greatly enhanced when extracellular adenosine was removed by the addition of adenosine deaminase. Subsequent studies have confirmed that adenosine may function as an endogenous regulator of inflammation in vivo (19, 47). Indeed, recent studies have suggested that the anti-inflammatory effects of methotrexate, a commonly
used agent in the treatment of rheumatoid arthritis, resulted from enhanced adenosine release at inflamed sites (24, 32). A similar mechanism is probably responsible for the anti-inflammatory effects of acadesine (5-aminimidazole-4-carboxamide ribonucleoside) (46), an agent currently under study for the prevention of reperfusion injury in patients undergoing cardiac surgery (see below).

In addition to neutrophils, other leukocytes also possess adenosine receptors. Adenosine inhibits generation of O$_2^-$ (18, 64), phagocytosis of immunoglobulin-coated particles (90), and secretion of the complement component C2 (63) by stimulated monocytes. Probably much more central to the potential anti-inflammatory effects of adenosine is inhibition, by adenosine, of stimulated monocyte secretion of the proinflammatory cytokine TNF-α (82), although it is not known whether this effect is mediated by an adenosine receptor. Mast cells have previously been reported to possess adenosine receptors that stimulate release of such mediators as histamine (50, 70, 71), but subsequent studies have suggested that adenosine-mediated enhancement of mediator release is not mediated by a cell surface receptor, since cellular uptake is required for adenosine’s functional effects (19, 20, 49, 66).

**ADENOSINE RECEPTORS ON LEUKOCYTES: SUBTYPE AND SIGNALING**

It was clear from even the earliest studies that adenosine interacted with a site on the cell surface to regulate neutrophil function. Metabolism of adenosine to inosine abrogated the effect of adenosine on neutrophil function, and, as noted above, adenosine uptake is not required for inhibition of neutrophil function (21, 28, 106). It had previously been discovered that other cell types possess two distinct receptors for adenosine designated A$_1$ and A$_2$ (67, 104). By using adenosine analogues specific for the individual receptors, it was demonstrated that adenosine interacted with an A$_2$ receptor to inhibit O$_2^-$ generation by stimulated neutrophils (33, 87; Table 1). Similarly, adenosine interacted with A$_1$ receptors to inhibit stimulated neutrophil adhesion and phagocytosis of immunoglobulin-coated particles (31, 91).

**Table 1. Effects of adenosine receptor occupancy on stimulated neutrophil function**

<table>
<thead>
<tr>
<th>Oxygen radical generation (H$_2$O$_2$ and O$_2^-$)</th>
<th>A$_1$ Receptor Occupancy</th>
<th>A$_2$ Receptor Occupancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulate</td>
<td>Inhibit</td>
<td>Inhibit</td>
</tr>
<tr>
<td>Chemotaxis</td>
<td>Inhibit</td>
<td>Inhibit</td>
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<tr>
<td>Phagocytosis</td>
<td>Inhibit</td>
<td>Inhibit</td>
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<tr>
<td>Adhesion</td>
<td>Inhibit</td>
<td>Inhibit</td>
</tr>
</tbody>
</table>

†, Stimulates; †, inhibits; *, no effect.

Radioligand (2-[³H]chloroadenosine and 5′-N-[³H]-ethylcarboxamidaenosine)-binding studies (using intact cells) demonstrated the presence of a single type of adenosine receptor on the surface of neutrophils with a dissociation constant of ~0.23 μM (33). In this study it was estimated that there were ~11,000 receptors per neutrophil. Although interpretation of these studies was rendered more difficult by poor ligand specificity for A$_2$ receptors and the high nonspecific binding of the ligands used, subsequent ligand-binding studies (to isolated membranes) using a more specific ligand ([³H]CGS-21680) have generally confirmed these observations (72). Because of the high nonspecific binding of all of the ligands used, no evidence of a high-affinity A$_1$ receptor has been provided by radioligand-binding studies.

It quickly became clear that interaction with an A$_2$ receptor could not explain all of the effects of adenosine on neutrophil function; extremely low concentrations of adenosine or its agonists promoted adhesion and chemotaxis, and higher concentrations of adenosine or different agonists inhibited adhesion and generation of oxygen metabolites. This pharmacological profile of adenosine effects on neutrophil function is consistent with the presence of two distinct receptors that respond to different concentrations of adenosine (23, 31, 88). Thus, occupancy of adenosine A$_1$ receptors promotes phagocytosis and chemotaxis (half-maximal effective concentration in the picomolar range), whereas occupancy of A$_2$ receptors inhibits O$_2^-$ generation and adhesion (half-maximal inhibitory concentration in the nanomolar range) (23, 31, 88). Although no radioligand-binding studies have yet demonstrated the presence of A$_1$ receptors on neutrophils, recent studies that used a monoclonal antibody directed against adenosine A$_1$ receptors confirmed the presence of adenosine A$_1$ receptors on the surface of human neutrophils (90).

**SIGNAL TRANSDUCTION AT NEUTROPHIL ADENOSINE A$_1$ RECEPTORS**

Adenosine receptors were first demonstrated and their subtypes differentiated on the basis of the effect of adenosine on cellular content of cAMP (67, 104). In cultured neural cells, A$_1$-receptor occupancy diminishes cAMP accumulation in response to other ligands (e.g., β-adrenergic agents) and occupancy of A$_2$ receptors directly stimulates the accumulation of cAMP. In a series of experiments performed in a number of laboratories, the susceptibility of adenosine A$_1$-receptor-mediated functions to inhibition by pertussis toxin [an agent that ADP-ribosylates and thereby inactivates inhibitory G (G$_i$) proteins] indicated that A$_1$ receptors were linked to G$_i$ signal transduction proteins (3, 13, 37, 40, 43, 51, 75, 83–85, 89, 100). This observation was confirmed by the cloning of an A$_1$ receptor and its characterization as a member of the seven transmembrane-spanning family of G protein-linked receptors (103). In recent experiments it was found that, under conditions in which pertussis toxin only minimally affected chemotaxis to FMLP, pertussis toxin treatment reversed the effect of adenosine receptor occupancy on neutrophil chemotaxis (88). These results confirmed that the effect of adenosine on chemotaxis was mediated by A$_1$ receptors and indicated that, in neutrophils, A$_1$ receptors are similarly coupled to G$_i$ proteins (88). In this regard it is interesting to note that the chemotactant receptors of neutrophils are similarly linked to pertussis toxin-sensitive signal transduction systems (12, 14, 41, 59–61, 79–81, 96, 107–109). One can postulate that amplification of G$_i$ stimulated signals by A$_1$ agonists may account for their enhancement of che-
motaxis and phagocytosis. Alternatively, occupancy of $A_2$ receptors may promote more rapid recycling of chemoattractant and Fe receptors to effect more rapid chemotaxis and greater phagocytosis, respectively.

**SIGNAL TRANSDUCTION AT NEUTROPHIL ADENOSINE $A_2$ RECEPTORS**

Stimulus transduction at neutrophil adenosine $A_2$ receptors has been an area of great interest since the first description of the effects of adenosine on O$_2$ generation. Adenosine $A_2$ receptors were first differentiated in neural cells on the basis of their capacity to stimulate intracellular accumulation of cAMP, presumably via stimulatory G protein (G$_s$)-linked stimulus transduction proteins (67, 104). Indeed, cloning of an adenosine $A_2$ receptor from dog thyroid confirmed that $A_2$ receptors are members of the family of seven transmembrane-spanning G protein linked receptors as well (68). Like other $G_s$-linked receptors, adenosine receptors on neutrophils mediate the intracellular accumulation of cAMP (27, 62). Moreover, occupancy of adenosine $A_2$ receptors enhances the increase in neutrophil cAMP that can be measured after stimulation with chemoattractants (27, 62). In general, accumulation of intracellular cAMP, either as a result of ligand-receptor interactions or treatment of neutrophils with cell-soluble cAMP analogues (dibutyryl cAMP), inhibits stimulated neutrophil functions, such as O$_2$ generation. It seemed likely therefore that cAMP mediated inhibition of O$_2$ generation by adenosine receptor ligand; however, there is little support for this hypothesis. Occupancy of adenosine receptors does not provoke detectable increases in neutrophil cAMP content except in the presence of a nonmethylxanthine phosphodiesterase inhibitor (Ro-20–1724), yet Ro-20–1724 does not increase the functional effect of adenosine receptor occupancy on stimulated generation of O$_2$ (27). More recent studies also cast doubt on the hypothesis that cAMP is the intracellular messenger for inhibition of O$_2$ generation by adenosine. Treatment of neutrophils with cell-soluble analogues of cAMP inhibits O$_2$ generation, and, as would be expected, the inhibition of O$_2$ generation by dibutyryl cAMP is completely reversed by inhibitors of the cAMP-dependent protein kinase (protein kinase A) (26). In contrast, the effects of adenosine $A_2$ receptor occupancy on O$_2$ generation are totally unaffected by protein kinase A inhibitors (26). Thus, although cAMP can inhibit O$_2$ production via its effects on protein kinase A, adenosine does not utilize the cAMP-protein kinase A system to inhibit O$_2$ generation. It remains possible that other neutrophil functions are affected as a result of increased intracellular cAMP concentrations.

Stimulation of neutrophils with chemoattractants provokes increased phospholipid turnover with generation of diacylglycerol and inositol 1,4,5-trisphosphate, events closely tied to activation of G$_i$ proteins. Cronstein and Haines (25) have reported that adenosine does not inhibit the early wave of diacylglycerol formation (15 s after stimulation). Similarly, Walker et al. (111) have reported that adenosine receptor occupancy does not affect the FMLP-stimulated peak in inositol 1,4,5-trisphosphate, an increase observed 10 s after FMLP stimulation. Thus, adenosine does not inhibit the early signals that follow chemoattractant receptor occupancy. In addition to the early increase in diacylglycerol release, stimulated neutrophils also undergo a late sustained increase in diacylglycerol synthesis. In contrast to the early transient increase in diacylglycerol, occupancy of adenosine receptors diminished, by ~50%, the sustained increase in diacylglycerol that followed stimulation (25).

Chemoattractant-stimulated generation of inositol 1,4,5-trisphosphate leads to mobilization of intracellular Ca$^{2+}$ stores and increments in free cytosolic Ca$^{2+}$ in the neutrophil, as in other cell types. As adenosine receptor occupancy does not affect the early events after occupancy of chemoattractant receptors, it was not surprising that the initial peak in free cytosolic Ca$^{2+}$ was unaffected by treatment with adenosine analogues (27, 112). The observation that adenosine receptor occupancy does not affect either chemoattractant stimulate alterations in phospholipids or rapid-onset fluxes in [Ca$^{2+}$], metabolism suggests that adenosine does not interfere with the early steps in cell activation. Moreover, as neutrophil degranulation is much more tightly linked to the early stimulated changes in [Ca$^{2+}$], the lack of an effect of adenosine on [Ca$^{2+}$], is consistent with previous observations that adenosine does not inhibit degranulation.

Although adenosine does not affect the early increment in free cytosolic Ca$^{2+}$ in stimulated neutrophils, Ward et al. (112) and Thiel and Bardenheuer (99) have observed that adenosine inhibits the sustained increase in free cytosolic Ca$^{2+}$ that follows stimulation. Laghi Pasini et al. (62) have observed similar changes in free cytosolic Ca$^{2+}$ and have further reported that adenosine is a Ca$^{2+}$-channel blocker, since adenosine inhibits binding of flunarizine, a Ca$^{2+}$-channel blocker, to neutrophil plasma membrane sites, a finding largely confirmed by Tsuruta et al. (101). Because the effect of adenosine on A-23187-stimulated O$_2$ formation (chemiluminescence) could be reversed by addition of excess Ca$^{2+}$ to the medium, Laghi Pasini et al. concluded that adenosine inhibits production of O$_2$ by inhibiting stimulated Ca$^{2+}$ influx or mobilization. Moreover, Thiel and Bardenheuer (99) observed that chelation of intracellular and extracellular Ca$^{2+}$ reversed the effect of adenosine on the generation of O$_2$ stimulated by insoluble particles (latex beads); the effect of adenosine on O$_2$ generation by cells in which only the intracellular Ca$^{2+}$ had been chelated was not tested. In contrast, Cronstein et al. (27) observed that adenosine inhibited chemoattractant-stimulated O$_2$ generation even in the absence of extracellular Ca$^{2+}$. Although it is difficult to reconcile all of the experimental differences, it is clear that the signals for generation of O$_2$ generation differ depending on whether the stimulus is a particle (e.g., latex particles or opsonized red blood cells), an ionophore (A-23187), or a chemoattractant (FMLP). Thus, although it is likely that adenosine, acting at its receptor, inhibits a Ca$^{2+}$-dependent step in neutrophil activation, it is by no means clear that adenosine...
inhibits neutrophil function by inhibiting mobilization or transmembrane fluxes of Ca\(^{2+}\). It is interesting to note that inhibition by adenosine of the sustained increase in [Ca\(^{2+}\)], parallels the inhibition by adenosine of the sustained increase in diacylglycerol synthesis.

Whether adenosine acts as a Ca\(^{2+}\)-channel blocker or inhibits a Ca\(^{2+}\)-dependent step in stimulus transduction, recent studies indicate that adenosine receptors inhibit a proximal step in signal transduction for chemoattractant receptors. Adenosine receptor occupancy does not affect the capacity of NaF, an agent that directly activates G proteins, to stimulate O\(_2\) generation, an observation that suggests that adenosine inhibits the interaction between occupied chemotactic receptor and G proteins (15, 23).

Moreover, adenosine A\(_2\)-receptor (and \(\beta\)-adrenergic receptor) occupancy, but not cAMP or dibutyryl cAMP, inhibits FMLP-stimulated G protein activation, determined as the FMLP-stimulated increment in guanosine-triphosphatase activity (26). This observation was recently confirmed by Burkey and Webster (15). These data indicate that adenosine receptor occupancy either inhibits or uncouples bound FMLP receptors from signal transduction mechanisms.

A separate line of evidence suggests a possible mechanism by which adenosine receptor occupancy can uncouple FMLP receptors from signal transduction mechanisms. Once occupied by ligand, FMLP receptors assume a high-affinity configuration for FMLP and associate with the cytoskeleton (16, 38, 53–56, 114). Subsequently, these bound receptors are no longer capable of stimulating neutrophil function. Occupancy of adenosine receptors promotes more rapid and complete association of bound FMLP receptors with the cytoskeleton (22, 23). Disruption of actin filaments (by cytochalasin B) diminishes the number of FMLP receptors that are associated with the cytoskeleton, disrupting the normal “turn-off” process, and thereby amplifying the FMLP-stimulated generation of O\(_2\) (55). Adenosine A\(_2\)-receptor occupancy promoted the association of bound FMLP receptors to cytoskeletal components even in the presence of cytochalasin B and appeared to do so without affecting either basal or FMLP-stimulated actin filament formation (22).

Thus adenosine restores, or augments, the regulatory mechanisms that dampen O\(_2\) generation. In contrast to these observations, Tsukuda et al. (102) have observed that increased adenosine release could be utilized to diminish inflammation. Interestingly, the existence of a potential counterregulatory effect of extracellular adenosine deaminase (released from dead cells or bacteria) was inferred from the observation that adenosine deaminase can bind to opsonized particles (zymosan) and, by metabolizing extracellular adenosine to inosine, may promote phagocytosis and O\(_2\) generation by activated leukocytes (29).

Adenosine and its analogues are potent inhibitors of inflammation in two different animal models (44, 57, 93). Green et al. (44) have reported that a single daily dose (intraperitoneal) of adenosine reduced the severity of experimental adjuvant arthritis in rats, a finding that is quite surprising considering the extremely rapid metabolism of adenosine [half time in whole blood is 2 s (76)]. Although adenosine diminishes leukocyte function via A\(_2\) receptors and, in general, promotes inflammatory functions via A\(_1\) receptors, Schrier and co-workers (55, 93) have observed that adenosine A\(_2\)-receptor agonists are better inhibitors of pleural and peritoneal inflammation than A\(_1\)-receptor agonists when studied in vivo. It is not
clear whether the apparent A$\_1$-receptor specificity is a result of individual characteristics of the agonists used, which, although possessing higher affinity for A$\_1$ than A$\_2$ receptors, are not absolutely specific. Indeed, subsequent studies using adenosine receptor antagonists that are highly specific for their subtype suggested that adenosine modulates inflammation in vivo via A$\_2$ receptors (4).

Further in vitro studies suggested that pharmacologically enhanced release of endogenous adenosine may already be utilized to diminish inflammation (24). Methotrexate is an antifolate that is commonly administered in low doses at weekly intervals to treat such inflammatory diseases as rheumatoid arthritis. Methotrexate is rapidly polyglutamated. The polyglutamated derivatives accumulate in cells and tissues where they may still actively inhibit folate-dependent enzymes (1, 7, 17). Among other effects, methotrexate promotes the accumulation of dihydrofolate polyglutamates (1, 2). Both dihydrofolate polyglutamates and methotrexate polyglutamate inhibit the enzyme 5-aminopterin and 4-carboxamide ribonucleotide (AICAR) transformylase, leading, potentially, to intracellular accumulation of AICAR (1, 2). The intracellular accumulation of AICAR enhances adenosine release from stressed cells (8, 9), which leads to the interesting hypothesis that adenosine may actually be the mediator of the anti-inflammatory effects of methotrexate. Cronstein et al. (24) have tested this hypothesis in an in vitro system and observed that treatment of endothelial cells or fibroblasts with methotrexate led to a modest increase in adenosine release. However, a much greater increase in adenosine release was observed when the methotrexate-treated cells were exposed to a stress (in this case activated neutrophils). Fewer stimulated neutrophils adhered to the methotrexate-treated endothelial cells and fibroblasts in this in vitro model of inflammation, and the diminished neutrophil adhesion was due to the increase in adenosine release from the methotrexate-treated cells. A similar increase in adenosine release and an adenosine-mediated decrease in inflammatory cell-to-cell interactions were observed when fibroblasts and endothelial cells were treated with AICARiboside, a cell-soluble precursor of AICAR. Recent studies by Asako et al. (4) confirmed this hypothesis in vivo; methotrexate applied topically (and in relatively high concentration) diminished leukocyte extravasation from the vasculature via a mechanism that is dependent on the presence of adenosine; adenosine deaminase reversed the anti-inflammatory effect of methotrexate. Moreover, using specific antagonists, these investigators demonstrated that the anti-inflammatory effects of methotrexate, acting via adenosine, were due to interaction with adenosine A$\_2$ receptors (presumably on the leukocytes). More recently it was demonstrated that weekly treatment of mice with pharmacologically relevant doses of methotrexate increased splenocyte AICAR content, increased adenosine concentration in inflammatory exudates, and inhibited leukocyte accumulation in inflammatory exudates (murine air pouch model of inflammation) by an adenosine A$\_2$-receptor-mediated effect (32). Similarly, Gruber et al. (46) have shown that intravenous infusions of AICARiboside also diminished leukocyte accumulation and cardiac injury in a model of ischemic injury (reperfusion injury), although the role of adenosine in the inhibition of cardiac injury is less clear in this model. Because sulfasalazine, an anti-inflammatory agent developed to treat rheumatoid arthritis and more commonly used to treat inflammatory bowel disease, may also interfere with AICAR metabolism (44), Baggott et al. (6) have suggested that sulfasalazine may diminish inflammation via adenosine release in a manner similar to methotrexate.

ADENOSINE RECEPTORS AND INFLAMMATION, A LOOK INTO THE FUTURE

Strong evidence from in vitro and in vivo studies indicates that adenosine can act as a potent inhibitor of inflammation. The medicinal use of adenosine is limited by its rapid metabolism and by its potent cardiovascular effects; however, it is possible that new, more highly specific agonists may be developed that can diminish inflammation without the unwanted side effects (hypotension, bradycardia) experienced by individuals treated with adenosine. More recently attention has been directed at developing agents that promote the release of adenosine at inflamed sites for use in the treatment of inflammatory diseases. Even now the therapeutic potential of enhanced release of adenosine is being tested in the clinic; trials are currently underway on the use of AICAR in the prevention of cardiac injury during coronary artery bypass grafting. Recent preliminary studies in animals indicate that adenosine kinase inhibitors, presumably by enhancing adenosine release at inflamed sites, may also be potent anti-inflammatory and antirheumatic agents (39). If clinical trials with AICAR prove the efficacy of this agent, then adenosine, adenosine receptor agonists, and agents that promote adenosine release at sites of inflammation will provide fertile ground for development of new, safer, and more effective anti-inflammatory agents.

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BRIEF REVIEW


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48. Hirschhorn, R., V. Rogneger-Maniscalco, L. Kuritsky, and P. S. Rosen. Bone marrow transplantation only partially re-

49. Hughes, P. J., and M. K. Church. Separate purinoceptors medi-

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53. Jesaitis, A. J., G. M. Bokoch, J. O. Tolley, and R. A. Allen. Lateral segregation of neutrophil chemotactic receptors into ac-


skeleton interactions and membrane traffic may regulate che-
moattractant-induced superoxide production in human granulo-

56. Jesaitis, A. J., J. O. Tolley, G. M. Bokoch, and R. A. Allen. Regulation of chemotactic receptor interaction with trans-

57. Kaminski, P. M., and K. G. Proctor. Attenuation of no-flow phenomenon, neutrophil activation, and reperfusion injury in in-


tion of human neutrophils and HL-60 cells. Pertussis toxin re-

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64. Leonard, E. J., A. Shenai, and A. Skeel. Dynamics of chem-
tactic peptide-induced superoxide generation by human mono-

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67. Londos, C., D. M. F. Cooper, and J. Wolff. Subclasses of ex-

68. Maenhaut, C., J. Van Sande, F. Libert, M. Abramowitz, M. Parmentier, J.-J. Vanderhaegen, J. E. Dumont, G. Vars-

69. Martini, C., S. Di Sacco, P. Tacchi, L. Cecca-

70. Marquardt, D. L., C. W. Parker, and T. J. Sullivan. Poten-

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74. McGarrity, S. T., A. H. Stephenson, and R. O. Webster. Regulation of human neutrophil functions by adenine nucleo-


77. Newby, A. C., A. C. Holmquist, J. Illingworth, and J. D. Pearson. The control of adenosine concentration in polymor-

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