Regulation of Neutrophil Function by Adenosine

Kathryn E. Barletta, Klaus Ley, Borna Mehrad

Abstract—Adenosine is an endogenously released purine nucleoside that signals via 4 widely expressed G protein-coupled receptors: A1, A2A, A2B, and A3. In the setting of inflammation, the generation and release of adenosine is greatly enhanced. Neutrophils play an important role in host defense against invading pathogens and are the cellular hallmark of acute inflammation. Neutrophils both release adenosine and can respond to it via expression of all 4 adenosine receptor subtypes. At low concentrations, adenosine can act via the A1 and A3 adenosine receptor subtypes to promote neutrophil chemotaxis and phagocytosis. At higher concentrations, adenosine acts at the lower-affinity A2A and A2B receptors to inhibit neutrophil trafficking and effector functions such as oxidative burst, inflammatory mediator production, and granule release. Modulation of neutrophil function by adenosine is relevant in a broad array of disease models, including ischemia reperfusion injury, sepsis, and noninfectious acute lung injury. This review will summarize relevant research in order to provide a framework for understanding how adenosine directly regulates various elements of neutrophil function. (Arterioscler Thromb Vasc Biol. 2012;32:856-864.)

Key Words: blood cells ■ cytokines ■ chemotaxis ■ adhesion ■ host defense

Neutrophils are professional phagocytes that play a critical role in host defense against infection and are important to the pathogenesis of many inflammatory diseases. Neutrophils are the most abundant leukocyte in human blood and are among the first cells recruited in response to invading pathogens. They are short-lived cells that do not replicate once differentiated in the bone marrow, and ultimately undergo one of several cell death programs, which can contribute to their antimicrobial functionality in tissue targets. Neutrophil antimicrobial effector mechanisms include phagocytosis and intracellular killing, release of extracellular enzymes and antimicrobial granule contents, and neutrophil extracellular trap (NET) formation. Two critical mechanisms to neutrophil effector functions are generation of oxidative burst and resultant elaboration of reactive oxygen species, and the generation of an extracellular chromatin fibrillar matrix, known as NETs, which serve to colocalize microbes and antimicrobial molecules. In addition to their well-recognized role as effector cells in antimicrobial immunity and inflammatory tissue damage, neutrophils are increasingly recognized as a multi-faceted array of immunoregulatory functions, including release of soluble mediators and cross-talk with other leukocytes.

Adenosine is an endogenous purine nucleoside with a very short tissue half-life and potent signaling functions. Under baseline conditions, adenosine is released constitutively from multiple cell types, with extracellular concentrations in the nanomolar range.1–4 In settings of inflammation and tissue injury, adenosine release and generation is greatly augmented and tissue levels can rise 100-fold.5 Adenosine is a breakdown product of ATP, a process mediated intracellularly by the soluble 5′-nucleotidase CD73. CD73 also exists as an ectoenzyme and functions with the apyrase CD39 to produce adenosine from ATP in the extracellular space. Once generated, adenosine is rapidly degraded by adenosine deaminase into inosine, or phosphorylated by adenosine kinase into AMP, resulting in a biological half-life of under 10 seconds.6 Adenosine signals via 4 G protein-coupled receptors: A1, A2A, A2B, and A3, encoded by Adora1, Adora2a, Adora2b, and Adora3, respectively. The A1 receptor is G\textsubscript{i/o}-coupled and inhibits formation of cAMP, whereas the A2A and A2B receptors are G\textsubscript{s}-coupled and promote formation of cAMP. The A1 receptor is both G\textsubscript{i/o} and G\textsubscript{o11}-coupled, inhibiting cAMP production and enhancing inositol P3 production. The A2A and A3 receptors have high affinity for adenosine, with EC\textsubscript{50} values between 0.2 to 0.5 μmol/L, whereas the A2A receptor has a somewhat lower affinity (EC\textsubscript{50} 0.6–0.9 μmol/L).7 The A2B receptor exhibits much lower affinity for adenosine than all other receptor subtypes, with an EC\textsubscript{50} between 16 and 64 μmol/L.7 The effects of adenosine in a given tissue are therefore complex, being determined by the interplay of the dynamics of production and clearance of adenosine and the receptor repertoire of the cells present.

Neutrophils both produce adenosine and can respond to it via all 4 adenosine receptors.7–12 Neutrophils can be a major source of tissue adenosine, and adenosine can stimulate or inhibit...
neutrophil function depending on its concentration in the microenvironment and the receptor profile of the neutrophil.² ⁵ ⁷

Neutrophils as a Source of Adenosine
Activated neutrophils secrete adenosine and its precursors, which are converted to adenosine and can subsequently act in an autocrine fashion to regulate neutrophil function (Figure). Early work demonstrated that adenosine is continuously generated in unstimulated neutrophils independent of ectoenzymes and then secreted,¹³ and that this intracellular production is mediated by a cytosolic ⁵’ nucleotidase.¹⁴ Endogenous adenosine production by neutrophils is also inducible after stimulation by a variety of stimulants, including phorbol 12-myristate 13-acetate, N-formyl-methionine-leucine-phenylalanine (fMLP), calcium ionophore, and complement C5a.¹⁵–²⁰

In addition to adenosine, neutrophils release ATP after stimulation, thereby providing a substrate for extracellular generation of adenosine. ATP is released from activated neutrophils via connixin 43 hemichannels and is rapidly converted to ⁵’ AMP and adenosine by the ectoenzymes CD39 and CD73, respectively, expressed on the neutrophil surface.²¹,²² ATP can act independently of adenosine to regulate neutrophil function; recent reviews have characterized these effects in detail.²³–²⁵ Neutrophil-derived ⁵’ AMP promotes chloride secretion from epithelial cells and enhances endothelial barrier function; however, both functions are mediated after extracellular conversion to adenosine by CD73.²⁴–²⁶

Several studies have provided insight into how adenosine levels are maintained in the extracellular space during inflammation. Stimulation of neutrophils enhances extracellular adenosine accumulation in part by inactivation of extracellular adenosine deaminase, an enzyme that converts adenosine into inosine.²⁰ Adenosine is cleared from the extracellular space in part by passive uptake by equilibrative nucleoside transporters. Their expression is inhibited during hypoxic conditions, thus promoting extracellular adenosine accumulation.²⁷,²⁸

Modulation of Neutrophil Movement by Adenosine
Recruitment
Adenosine produced by neutrophils can act in an autocrine and paracrine fashion to promote or inhibit neutrophil chemotaxis. The early literature was dominated by inhibitory effects of adenosine on neutrophils.¹⁷,²⁰–³¹ One of the first reports of adenosine promoting neutrophil function attributed enhanced neutrophil chemotaxis toward complement C5 fragments and fMLP to the A₂ (later A₂A) receptor³²; but a follow-up study determined that adenosine promotes chemotaxis by acting at the A₁ receptor by using N⁶-cyclopentyladenosine (Table 1), a selective A₁ receptor agonist.³³ The A₁ receptor was also noted to have a higher affinity for adenosine than the A₂ receptor, and therefore, at early stages of inflammation, lower local concentrations of adenosine promoted neutrophil recruitment, whereas later high concentrations of adenosine limit neutrophil recruitment by action at A₂ receptors.⁹

The role of adenosine A₃ receptors in neutrophil chemotaxis was addressed more recently.³⁴ Human neutrophils subjected to a chemotactic gradient of fMLP were shown to release ATP from their leading edge. The extracellular ATP was then converted to adenosine by CD73 on the neutrophil cell surface, generating an adenosine gradient that subsequently acts on neutrophil A₃ receptors, also concentrated at the leading edge, to augment chemotaxis.³⁵ The relevance of A₃-directed neutrophil chemotaxis was demonstrated in a mouse model of intra-abdominal infection in A₃ receptor
knockout mice (Adora3−/−). Using the cecal ligation and puncture model of sepsis, Adora3−/− mice exhibited decreased recruitment of neutrophils to the lung and peritoneum and increased numbers of circulating neutrophils compared to wild-type treated mice.34

In addition to CD73, the ecto-nucleoside triphosphate diphosphohydrolase I (E-NTPDase 1, CD39) is capable of converting extracellular ATP to adenosine and has also been found to associate with the leading edge of neutrophils migrating along a chemotactic gradient. Neutrophils from CD39-deficient mice or neutrophils in which CD39 was inhibited pharmacologically exhibited impaired chemotaxis but not polarization.35 It has therefore been postulated that release of ATP and conversion to adenosine by migrating neutrophils, along with polarized expression of A2A receptors, contribute to neutrophil chemotaxis.

Global 2-chloroadenosine Agonist
Global NECA Agonist
Global 8-(p-sulfophenyl)theophylline Antagonist
Global Theophylline Antagonist
A1 CPA Agonist
A1 COPA Agonist
A2A CGS-21680* Agonist
A2A WRC-0470* Agonist
A2A ATL146e Agonist
A2A ATL193 Agonist
A2A ATL202 Agonist
A2A ZM-241385* Antagonist
A2A/A2B CPCA Agonist
A2B BAY 60–6583* Agonist
A2B ZM-241385* Antagonist
A2A/A2B CGS-21680* Agonist
A2B COPA Agonist
A3 Cl-IB-MECA Agonist
A3 MRS-1220* Antagonist
A3 ATL146e Agonist
A3 CGS-21680* Agonist
A3 WRC-0470* Agonist
A3 CPX-997 Agonist
A3 PCX-997 Antagonist

Table 1. Pharmacological Ligands of Adenosine Receptors

<table>
<thead>
<tr>
<th>Receptor Target</th>
<th>Ligand</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td>2-chloroadenosine</td>
<td>Agonist</td>
</tr>
<tr>
<td>Global</td>
<td>NECA</td>
<td>Agonist</td>
</tr>
<tr>
<td>Global</td>
<td>8-(p-sulfophenyl)theophylline</td>
<td>Antagonist</td>
</tr>
<tr>
<td>Global</td>
<td>Theophylline</td>
<td>Antagonist</td>
</tr>
<tr>
<td>A1</td>
<td>CPA</td>
<td>Agonist</td>
</tr>
<tr>
<td>A1</td>
<td>COPA</td>
<td>Agonist</td>
</tr>
<tr>
<td>A2A</td>
<td>CGS-21680*</td>
<td>Agonist</td>
</tr>
<tr>
<td>A2A</td>
<td>WRC-0470*</td>
<td>Agonist</td>
</tr>
<tr>
<td>A2A</td>
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<td>A2A</td>
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<td>A2A</td>
<td>ATL202</td>
<td>Agonist</td>
</tr>
<tr>
<td>A2A</td>
<td>ZM-241385*</td>
<td>Antagonist</td>
</tr>
<tr>
<td>A2A/A2B</td>
<td>CPCA</td>
<td>Agonist</td>
</tr>
<tr>
<td>A2B</td>
<td>BAY 60–6583*</td>
<td>Agonist</td>
</tr>
<tr>
<td>A2B</td>
<td>CI-IB-MECA</td>
<td>Agonist</td>
</tr>
<tr>
<td>A3</td>
<td>MRS-1220*</td>
<td>Antagonist</td>
</tr>
</tbody>
</table>

Taken together, these data suggest that submicromolar concentrations of adenosine can augment neutrophil chemotaxis toward inflammatory stimuli by autocrine action at the high-affinity A1 and A3 adenosine receptors. In addition, neutrophil chemotaxis is inhibited by the interaction of netrin-1 and the A2B receptor.

Adhesion and Transmigration

Neutrophil adhesion and transmigration across the endothelial cell barrier is a multistep process. Neutrophils are captured from the circulation and loosely tethered to the vascular endothelium near sites of injury by selectins, which facilitate “rolling” of neutrophils along the endothelial surface, allowing local inflammatory stimuli to interact with and further activate the neutrophils. These stimuli, including ELR-containing CXC chemokine ligands such as CXCL8, activate integrins on the surface of neutrophils. Neutrophil integrins, such as β2 (CD11a/CD18 and CD11b/CD18) and very late antigen-4 (VLA-4) tightly bind cell adhesion molecules on the vascular endothelium (ICAMs and VCAMs). This is followed by diapedesis into the interstitium and further migration toward the site of injury. Adenosine acts on both neutrophils and endothelial cells to inhibit neutrophil adhesion and transmigration.43

Several studies have demonstrated that adenosine attenuates adhesion and neutrophil-induced damage to the endothelium. The adenosine analog 2-chloroadenosine inhibits adherence of fMLP-stimulated neutrophils to endothelial monolayers and inhibits neutrophil-mediated endothelial cell damage.29 A similar effect was shown with the pan-adenosine receptor agonist N-ethylcarboxamidoadenosine (NECA).44 NECA and the A2A receptor agonist CGS-21680 decreased adherence of phorbol 12-myristate 13-acetate-stimulated neutrophils to porcine aortic endothelium and adenosine and CGS-21680 inhibited neutrophil adhesion and damage to endothelial cells in isolated canine coronary arteries.46 These effects were attributed to inhibition of selectin-based adhesion.47

Additional studies revealed that adenosine inhibits both the shedding of L-selectin and expression of β2 integrins (mainly CD11b/CD18) on the surface of neutrophils, thus limiting adhesion.12 These effects are potentiated by the addition of dipryridamole, a nucleoside uptake inhibitor that enhances extracellular concentrations of adenosine, and attenuated by addition of adenosine deaminase.12 Neutrophil adhesion to fibrinogen, a ligand for CD11b/CD18, can also be inhibited by NECA.48 NECA was also shown to attenuate CD11b/CD18 expression on fMLP-stimulated neutrophils in a dose-dependent manner.12,49 The inhibitory effects of adenosine on
neutrophil adhesion appear to be at least partially mediated via the A2A receptor, since ATL146e, a selective A2A receptor agonist, inhibits human neutrophil CD49d expression, a component of the VLA-4 integrin complex, and adhesion to a VCAM-1 coated surface, whereas the A2A receptor antagonist, ZM-241385, blocked this inhibition.50 Similarly, preincubation of endothelial cells with the anti-inflammatory drug sulfasalazine resulted in a dose-dependent inhibition of fMLP-stimulated neutrophil adhesion that was lost with the addition of adenosine deaminase or an A1 receptor antagonist.51

Several in vivo models demonstrate that A2A-mediated inhibition of neutrophil adhesion can have anti-inflammatory effects. Infusion of ATL146e in a model of canine myocardial infarction reduced P-selectin expression and neutrophil infiltration and was correlated with reduction in infarct size after reperfusion.52 Similarly, ATL146e infusion in a murine model of carotid ligation and repair resulted in reduced neutrophil recruitment, VCAM-1, ICAM-1, and P-selectin expression, an effect that correlated with sustained reduction in neointimal tissue formation and subsequent vessel constriction.53

The adenosine A1 receptor has also been implicated in the regulation of neutrophil adhesion. Activation of the A1 receptor with the selective agonist 2-chloro-N6-(3-iodobenzyl)adenosine-5’-N-methyluronamide (CI-IB-MECA) reduced platelet activating factor (PAF)-stimulated neutrophil adherence to coronary endothelium. This effect was reversed by MRS-1220, an A3-selective antagonist.54 Furthermore, in a model of reperfusion injury in isolated rabbit hearts CI-IB-MECA attenuated the neutrophil-mediated reduction in cardiac contractile recovery. Thus agonism of A1 promoted recovery after reperfusion in part by inhibiting neutrophil adhesion.54

Adenosine also attenuates neutrophil accumulation though actions on the endothelium: Adenosine inhibits release of the neutrophil chemoattractant CXCL8 from endothelial cells and reduces expression of adhesion molecules endothelial-selectin (E-selectin) and VCAM-1 on the endothelial cell surface.38 Endogenous adenosine and 5’ AMP induce surface expression of CD73 on endothelial cells, generating additional adenosine in a positive feedback loop while promoting endothelial barrier function.55 This enhanced barrier function is due in part to adenosine A2B receptor activation on endothelial cells,56 which is upregulated on posthypoxic endothelial cells along with CD39 and CD73. Furthermore, activated neutrophils can promote barrier function in these posthypoxic cells, an effect inhibitable by A2B antagonism.56 Taken together, these studies suggest paracrine regulation of neutrophil accumulation by adenosine at the endothelial cell barrier; production of adenosine by activated neutrophils enhances adenosine production by endothelial cells, and acts on endothelial A2B receptors to enhance barrier function and limit neutrophil transmigration.

In contrast, the A1 receptor has been shown to enhance neutrophil adhesion to the endothelium. The A1 receptor-specific agonist NECA enhances neutrophil adhesion to gelatin-coated plates, via a CD11b/CD18-independent mechanism.48 A different A1 receptor agonist, 2-chloro-N6-cyclopentyladenosine (COPA), promoted phorbol 12-myristate 13-acetate-stimulated adhesion of neutrophils to cultured porcine aortic endothelial cells.49

Thus, A1 receptors may promote adhesion via an integrin-independent mechanism, while A2A receptors attenuate adhesion via inhibition of integrins. Alternatively, it is possible that A1-mediated adhesion functions similarly to A2A and A2B-mediated chemotaxis; submicromolar concentrations of adenosine promote neutrophil recruitment during early stages of inflammation. Once the inflammatory reaction is under way, elevated concentrations of adenosine can activate the lower-affinity A2A and A2B receptors, inhibiting neutrophil adhesion and transmigration, respectively.

### Modulation of Neutrophil Effector Mechanisms by Adenosine

#### Inflammatory Mediators

Activated neutrophils release numerous cytokines, chemokines, and arachidonic acid-derived lipid mediators, with diverse effects on the ongoing inflammatory reaction. Adenosine receptor activation inhibits proinflammatory mediator release from activated neutrophils, while promoting release of anti-inflammatory mediators. The adenosine A1 agonist CPA, and the combined A2A/A2B agonist 5’-(N-cyclopropyl)-carboxamido-adenosine (CPA), both inhibit the release of TNF from LPS-stimulated human neutrophils, with A1 agonism 1000× more effective than A2A agonism.57 Similarly, activation of the A2A receptor with CGS-21680 inhibited release of TNF and inflammatory chemokines from LPS-stimulated neutrophils.58

Induction of cyclooxygenase-2 activity converts arachidonic acid into prostaglandin E2 and thromboxane A2. Prostaglandin E2 is a potent anti-inflammatory agent capable of inhibiting neutrophil chemotaxis, aggregation, and superoxide production, while thromboxane A2 is a proinflammatory mediator that activates platelet aggregation and clotting. A series of studies reported that leukocyte cyclooxygenase-2 induction is attenuated in A2A receptor-deficient mice (Adora2a(−/−)). Furthermore, CGS-21680 activation of A2A receptors on fMLP-stimulated human neutrophils potentiates the induction of cyclooxygenase-2 and enhances prostaglandin E2 generation without affecting production of thromboxane A2.59,60

Arachidonic acid can also be converted into leukotrienes via the 5-lipoxygenase pathway. Leukotriene B4 (LTB4) is a potent neutrophil chemoattractant and can also stimulate oxidative burst and degranulation. Early studies found that adenosine analogs inhibit fMLP-induced synthesis of LTB4 in whole blood.61 A subsequent study examined ligand-stimulated neutrophils in isolation and found that removal of endogenous adenosine or blockade of A2A receptors enhanced LTB4 synthesis.62 Similarly, activated neutrophils are unable to transform arachidonic acid into 5-lipoxygenase products without removal of adenosine or addition of an A2A antagonist.53 Finally, endogenous adenosine inhibits the ability of neutrophils to produce LTA4, a precursor of LTB4, LTC4, and lipoxins, which modulate functional responses of phagocytes.64,65

Taken together, these studies provide evidence that activation of A2A receptors on neutrophils can influence the broader...
inflammatory response by modulating production and release of pro- and anti-inflammatory mediators, such as chemokines, leukotrienes, and prostaglandins.

**Phagocytosis**

Neutrophil phagocytosis is facilitated by opsonization of microbes by complement, antibodies, and other opsonins. As such, the neutrophil Fc receptors (FcR) and complement receptors are critical to their phagocytic activity. After fusion of the phagosome with lysosomes, pathogens in the resulting phagolysosome are killed by superoxide radical production as well as nonoxidative microbial granule components.

The regulation of neutrophil phagocytosis by adenosine is concentration- and receptor-dependent. A1 receptor agonism enhances FcRγ-mediated phagocytosis in human neutrophils at picomedicmicromolar concentrations of adenosine.66,67 In contrast, micromolar concentrations of adenosine or NECA inhibit FcR-mediated phagocytosis.67 Few subsequent studies have specifically examined effects of adenosine receptors on neutrophil phagocytosis, but it can be postulated that, similar to regulation of neutrophil chemotaxis, low concentrations of adenosine promote phagocytosis via A1 receptor binding, while elevated concentrations of adenosine inhibit phagocytosis via activation of A2A receptors.

**Degranulation**

Neutrophils contain primary, secondary, and tertiary granules that contain antimicrobial molecules and enzymes that degrade extracellular matrix components. Primary or azurophilic granules are defined by containing myeloperoxidase and CD63; they also contain a number of antimicrobial molecules including neutrophil elastase, acid hydrolase, defensins and bacterial permeability increasing protein. Secondary (or specific) granules contain lactoferrin whereas tertiary (or gelatinase) granules no not; secondary and tertiary granules otherwise contain similar compounds including gelatinase, lysozyme, lipocalin, collagenase, as well as components of the NADPH oxidase complex. In general, engagement of adenosine receptors on neutrophil inhibits granule release, limiting neutrophil-mediated injury. Early work demonstrated that micromolar concentrations of adenosine inhibit degranulation of human neutrophils, as measured by lactoferrin secretion in response to fMLP68 and release of bacterial permeability increasing protein, neutrophil elastase, and defensins in response to LPS and TNFα.11 Subsequent studies showed that agonism of the A2A and A3 receptor, but not the A1 receptor, inhibit elastase release from neutrophils in response to fMLP,69 which was associated with cAMP-dependent sequestration of cytosolic calcium.69,70 Consistent with this, in vivo administration of the A2A agonist WRC-0470 inhibited degranulation of secondary neutrophil granules in a rat model of meningitis, as measured by extracellular lysozyme concentration.71 These data suggest that adenosine appears to inhibit neutrophil granule release, which is at least in part mediated via binding to the A2A receptor.

**Oxidative Burst**

Neutrophil oxidative burst requires assembly of the NADPH oxidase subunits and culminates in transfer of electrons to molecular oxygen to produce superoxide, O2•−, a reactive free radical that both spontaneously and enzymatically dismutates to generate hydrogen peroxide, the substrate for the generation of additional reactive oxygen intermediaries including hypochlorous acid, singlet oxygen, ozone, hypohalous acids, chloramine, and hydroxyl radical.72 These reactive oxygen species mediate oxidative damage against invading pathogens as well as host tissues and also have a subtler immunomodulatory role.

Regulation of oxidative burst is among the earliest and best characterized effects of adenosine on neutrophils, first described by Cronstein and colleagues who found that oxidative burst activity in fMLP-stimulated neutrophils is inhibited by micromolar concentrations of adenosine by nearly 50%,18 an effect that could be replicated by the adenosine analog NECA and antagonized by theophylline.73 A similar effect was seen when adenosine was administered in vivo in a porcine model of endotoxemia.74 Subsequent studies have linked inhibition of oxidative burst to adenosine action at the neutrophil A2A receptor: the A2A agonists WRC-0470, CGS-21680, ATL193, and ATL1466 inhibit neutrophil oxidative burst in response to a variety of neutrophil stimuli, including fMLP, TNFα, PAF, and IgG70,75-78, after exposure to complement C3b-coated zymosan particles79; and during rat bacterial meningitis,71 The mechanism of adenosine-mediated inhibition of neutrophil oxidative burst was examined in fMLP-stimulated human neutrophils and was found to correlate with a reduction of flavocytochrome b (the heterodimer of gp91phox and p22phox) content in neutrophil plasma membranes and primary granules.80 Activation of A2A receptors increases cAMP and intracellular calcium, but inhibition of protein kinase A does not restore superoxide anion synthesis,81 suggesting additional pathways, such as EPAC signaling, may also be involved in these effects. In this context, phospholipase D may be an important mediator of adenosine regulation of neutrophil function, because adenosine signaling via A2A inhibits phospholipase D activation by blocking membrane recruitment of small GTPases.82

Regulation of neutrophil oxidative burst by other adenosine receptors has been less thoroughly characterized. The A2B receptor agonist BAY 60-6583 inhibited superoxide production in fMLP-stimulated murine neutrophils with a peak effect of approximately 50%, an effect that was absent in neutrophils from A2B-deficient mice (Adora2b−/−).83 Interestingly, the inhibitory effect of this agonist on neutrophils that were first primed with TNF before stimulation with fMLP or neutrophils harvested from LPS-treated mice was much more modest.83 In contrast, the A1 receptor agonist CPA enhanced superoxide generation during FcRγ-mediated stimulation of human neutrophils, an effect that could be blocked by the adenosine antagonist 8-(p-Sulfophenyl)theophylline and by pertussis toxin.66 The activation of neutrophil A1 receptors with Cl-IIB-MECA had no effect on superoxide production by canine neutrophils stimulated with PAF.54 A careful study of A3 agonists and antagonists could not rule out the contribution of A3 receptors to inhibition of oxidative burst but concluded inhibition is predominantly mediated through A2A activation.10
### Table 2. Brief Summary of Neutrophil-Adenosine Receptor Literature

<table>
<thead>
<tr>
<th>Receptor</th>
<th>System</th>
<th>Stimulus</th>
<th>Manipulation</th>
<th>Effect on Neutrophil</th>
<th>Ref.</th>
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<tr>
<td>Unspecified</td>
<td>Human neutrophil</td>
<td>fMLP</td>
<td>2-chloroadenosine</td>
<td>Inhibits adherence to endothelium</td>
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<td>fMLP</td>
<td>NECA</td>
<td>Inhibits neutrophil-mediated endothelial damage</td>
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<td>Human neutrophil</td>
<td>fMLP</td>
<td>Adenosine kinase inhibition</td>
<td>Inhibits selectin-based adhesion</td>
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<td>A1</td>
<td>Human neutrophil</td>
<td>fMLP</td>
<td>NECA</td>
<td>Promotes chemotaxis</td>
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<td>Human neutrophil</td>
<td>fMLP</td>
<td>CPA</td>
<td>Promotes adhesion to gelatin-coated plates</td>
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<tr>
<td>A1</td>
<td>Porcine neutrophil</td>
<td>PMA</td>
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<td>A1</td>
<td>Human neutrophil</td>
<td>Antibody-coated erythrocytes</td>
<td>CPA</td>
<td>Promotes FC&lt;sub&gt;γ&lt;/sub&gt;-mediated phagocytosis</td>
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<td>A1</td>
<td>Human neutrophil</td>
<td>FcR&lt;sub&gt;γ&lt;/sub&gt; stimulation</td>
<td>CPA</td>
<td>Enhances superoxide production</td>
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<td>COPA, CPCA</td>
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<td>Complement C5, fMLP</td>
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<td>Dipyrimidole, NECA</td>
<td>Inhibits selectin shedding, expression of β&lt;sub&gt;2&lt;/sub&gt; integrins</td>
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<tr>
<td>A2</td>
<td>Human neutrophil</td>
<td>fMLP</td>
<td>NECA</td>
<td>Inhibits adhesion to fibrinogen-coated plates</td>
<td>48</td>
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<tr>
<td>A2</td>
<td>Human neutrophil</td>
<td>Antibody-coated yeast</td>
<td>NECA</td>
<td>Inhibits FcγR&lt;sub&gt;γ&lt;/sub&gt;-mediated phagocytosis</td>
<td>66, 67</td>
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<tr>
<td>A2A</td>
<td>Porcine neutrophil</td>
<td>PMA</td>
<td>NECA, CGS-21680</td>
<td>Inhibits adhesion</td>
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<td>A2A</td>
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<td>A2A</td>
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<td>fMLP, TNF</td>
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<td>Myocardial infarction</td>
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<td>Inhibits p-selectin expression, infiltration</td>
<td>52</td>
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<td>A2A</td>
<td>Murine neutrophil</td>
<td>Carotid ligation</td>
<td>ATL146e</td>
<td>Inhibits recruitment, integrin and selectin expression</td>
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<tr>
<td>A2A</td>
<td>Human neutrophil</td>
<td>LPS</td>
<td>CGS-21680</td>
<td>Inhibits inflammatory chemokine release</td>
<td>58</td>
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<tr>
<td>A2A</td>
<td>Adora&lt;sub&gt;2A&lt;/sub&gt;&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>Air pouch inflammation</td>
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<td>Inhibits COX-2 induction</td>
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<tr>
<td>A2A</td>
<td>Human neutrophil</td>
<td>fMLP</td>
<td>CGS-21680</td>
<td>Promotes COX-2, PGE&lt;sub&gt;2&lt;/sub&gt; generation</td>
<td>59, 60</td>
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<tr>
<td>A2A</td>
<td>Whole blood, human neutrophil</td>
<td>fMLP</td>
<td>NECA, CGS-21680</td>
<td>Inhibits LTB&lt;sub&gt;4&lt;/sub&gt; synthesis</td>
<td>61, 62</td>
</tr>
<tr>
<td>A2A</td>
<td>Human neutrophil</td>
<td>fMLP</td>
<td>CGS-21680</td>
<td>Inhibits leukotriene synthesis</td>
<td>63</td>
</tr>
<tr>
<td>A2A</td>
<td>Human neutrophil</td>
<td>Various</td>
<td>CGS-21680</td>
<td>Inhibits LTA&lt;sub&gt;4&lt;/sub&gt; production</td>
<td>64, 65</td>
</tr>
<tr>
<td>A2A</td>
<td>Rat neutrophil</td>
<td>Meningitis</td>
<td>WRC-0470</td>
<td>Inhibits degranulation</td>
<td>71</td>
</tr>
<tr>
<td>A2A</td>
<td>Human, murine, rat neutrophil</td>
<td>fMLP, TNF, PAF, IgG, C3b-Zymosan, bacterial meningitis</td>
<td>WRC-0470, CGS-21680, ATL193, ATL146e</td>
<td>Inhibits oxidative burst</td>
<td>70, 75–79</td>
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<tr>
<td>A2A</td>
<td>Human neutrophil</td>
<td></td>
<td>CGS-21680</td>
<td>Inhibits neutrophil apoptosis</td>
<td>84, 85</td>
</tr>
<tr>
<td>A2A, A3</td>
<td>Human neutrophil</td>
<td>fMLP, TNF, LPS</td>
<td></td>
<td>Inhibits degranulation</td>
<td>68–70</td>
</tr>
<tr>
<td>A2B</td>
<td>Murine, human neutrophil</td>
<td>LPS, hypoxia, colitis</td>
<td>Netrin-1</td>
<td>Inhibits recruitment, transepithelial migration</td>
<td>39–41</td>
</tr>
<tr>
<td>A2B</td>
<td>Murine neutrophil</td>
<td>fMLP</td>
<td>BAY 60–6583</td>
<td>Inhibits superoxide production</td>
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<tr>
<td>A2</td>
<td>Human neutrophil</td>
<td>fMLP</td>
<td></td>
<td>Promotes chemotaxis</td>
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<tr>
<td>A2</td>
<td>Adora&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
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<td></td>
<td>Inhibits recruitment</td>
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<tr>
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<td>Canine, rabbit neutrophil</td>
<td>PAF</td>
<td>Cl-IB-MECA</td>
<td>Inhibits adhesion to endothelium</td>
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N-formyl-methionine-leucine-phenylalanine (fMLP); NECA, N-ethylcarboxamidoadenosine; CPA, N<sup>-</sup>-cyclopentyladenosine; CPCA, 2-chloro-N<sup>-</sup>-cyclopentyladenosine; CPCA, 5<sup>-</sup>-(N-cyclopopyr)-carboxamido-adenosine; COX, cyclooxygenase; PMA, phorbol myristate acetate; LPS, lipopolysaccharide; PAF, platelet activating factor; PG, prostaglandin; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; AA, arachidonic acid; LT, leukotriene; LO, lipoxygenase; Cl-IB-MECA, 2-chloro-N<sup>-</sup>-(3-iodobenzyl)adenosine-5<sup>-</sup>-N-methyluronamidine.

See Table 1 for ligand information.
Neutrophil Death

Cell death is essential to both homeostatic turnover of neutrophils in the resting state and during tissue inflammation. Neutrophil death occurs via several distinct apoptosis subroutines, by necrosis, and as the end-result of NETosis. Several studies have demonstrated that adenosine analogs delay apoptosis of resting human neutrophils in culture. Adenosine agonists with higher affinity for the A<sub>2A</sub> receptor had more potency in inhibiting neutrophil apoptosis. Similarly, theophylline and its analogs promote apoptosis of resting neutrophils in culture. The role of adenosine signaling in neutrophil death in the context of inflamed tissues has not been investigated to our knowledge.

Applications in Preclinical Models of Human Disease

Ischemia-reperfusion injury (IRI) is an important pathogenic mechanism relevant to many diseases, including myocardial infarction, sickle cell crises and early graft dysfunction in solid organ transplantation. Neutrophil infiltration is a prominent feature of IRI and mediates tissue injury in part due to oxidative damage to the endothelium. Inhibition of neutrophil adherence and superoxide production by adenosine has been shown to attenuate IRI. In this context, administration of the A<sub>2A</sub> agonists CGS-21680 or ATL146e reduce infarct size in a canine model of myocardial IRI using coronary ligation. Adenosine A<sub>2A</sub> receptor agonists have also been shown to improve hepatic IRI and sickle cell disease-induced IRI by inhibiting iNKT cell activation, thought to be upstream of neutrophil-mediated tissue damage.

Adenosine receptor modulation has also been investigated in the context of several other inflammatory diseases. In a model of LPS-induced acute lung injury, chimeric mice lacking the A<sub>2A</sub> receptor on bone marrow-derived cells exhibited more neutrophil recruitment into the alveolar space. In addition, pretreatment of wild-type mice with the A<sub>2A</sub> agonist ATL202 reduced neutrophil recruitment and cytokine release, an effect that required the expression of A<sub>2A</sub> receptors on myeloid cells. In the context of antimicrobial host defense, agonism of the A<sub>2A</sub> receptor with ATL146e resulted in improved survival after intraperitoneal administration of LPS; conversely, genetic deletion of the A<sub>2A</sub> receptor or its pharmacological antagonism enhanced bacterial clearance and survival in a models of intraabdominal infection. Thus the modulation of A<sub>2A</sub> receptor activity to inhibit neutrophil function may be beneficial in noninfectious inflammatory diseases but may impair defense against infections.

Conclusions

Neutrophils are involved in generation of tissue adenosine, and adenosine can activate or inhibit various neutrophil functions. Adenosine regulation of neutrophils is highly dependent on the inflammatory microenvironment and, in part, regulated by expression of adenosine receptors on neutrophils and the affinity of these receptors for adenosine. Nanomolar concentrations of adenosine act via A<sub>1</sub> and A<sub>2</sub> receptors to promote neutrophil chemotaxis toward inflammatory stimuli and phagocytosis, whereas micromolar concentrations result in activation of the low-affinity A<sub>2A</sub> and A<sub>2B</sub> receptors, which inhibits neutrophil phagocytosis, granule release, and oxidative burst; limits excessive tissue damage; and promotes endothelial barrier function and repair (Table 2).

There are numerous avenues for additional research in the field of neutrophil-adenosine biology. Recent pharmacological developments have resulted in potent, selective A<sub>2B</sub> and A<sub>3</sub> receptor agonists and antagonists, which should facilitate study of these receptors. The impact of A<sub>2B</sub> and A<sub>3</sub> receptor signaling on neutrophil effector functions, including phagocytosis and oxidative burst, granule release, and inflammatory mediator production, requires further characterization. The downstream signaling pathways that couple adenosine receptor activation with inhibition or activation of neutrophil function should be further elucidated. Adenosine receptor modulation of neutrophil cell death pathways, including the recently characterized process of NETosis, would also benefit from additional study. Insight gained into the basic biology of adenosine–neutrophil interactions should be applied to the study of relevant disease models; adenosine receptor biology has numerous therapeutic applications that can be advanced through translational research.

Finally, there are numerous factors involved in the regulation of neutrophil activation and function, eg, ATP and the neuronal guidance molecule netrin-1. Study of the interplay between adenosine and these molecules is important to achieve a more complete understanding of neutrophil biology.

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Disclosures

None.

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Regulation of Neutrophil Function by Adenosine
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