

## Regulation of Neutrophil Function by Adenosine

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**Abstract**—Adenosine is an endogenously released purine nucleoside that signals via 4 widely expressed G protein-coupled receptors: A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub>. 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 A<sub>1</sub> and A<sub>3</sub> adenosine receptor subtypes to promote neutrophil chemotaxis and phagocytosis. At higher concentrations, adenosine acts at the lower-affinity A<sub>2A</sub> and A<sub>2B</sub> 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 fibrillary 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 for 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.<sup>1-4</sup> In settings of inflammation and tissue

injury, adenosine release and generation is greatly augmented and tissue levels can rise 100-fold.<sup>5</sup> 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.<sup>6</sup> Adenosine signals via 4 G protein-coupled receptors: A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub>, encoded by *Adora1*, *Adora2a*, *Adora2b*, and *Adora3*, respectively. The A<sub>1</sub> receptor is G<sub>i/o</sub>-coupled and inhibits formation of cAMP, whereas the A<sub>2A</sub> and A<sub>2B</sub> receptors are G<sub>s</sub>-coupled and promote formation of cAMP. The A<sub>3</sub> receptor is both G<sub>i/o</sub> and G<sub>q/11</sub>-coupled, inhibiting cAMP production and enhancing inositol P<sub>3</sub> production. The A<sub>1</sub> and A<sub>3</sub> receptors have high affinity for adenosine, with EC<sub>50</sub> values between 0.2 to 0.5 μmol/L, whereas the A<sub>2A</sub> receptor has a somewhat lower affinity (EC<sub>50</sub> 0.6–0.9 μmol/L). The A<sub>2B</sub> receptor exhibits much lower affinity for adenosine than all other receptor subtypes, with an EC<sub>50</sub> between 16 and 64 μmol/L.<sup>7</sup> 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.<sup>7-12</sup> Neutrophils can be a major source of tissue adenosine, and adenosine can stimulate or inhibit

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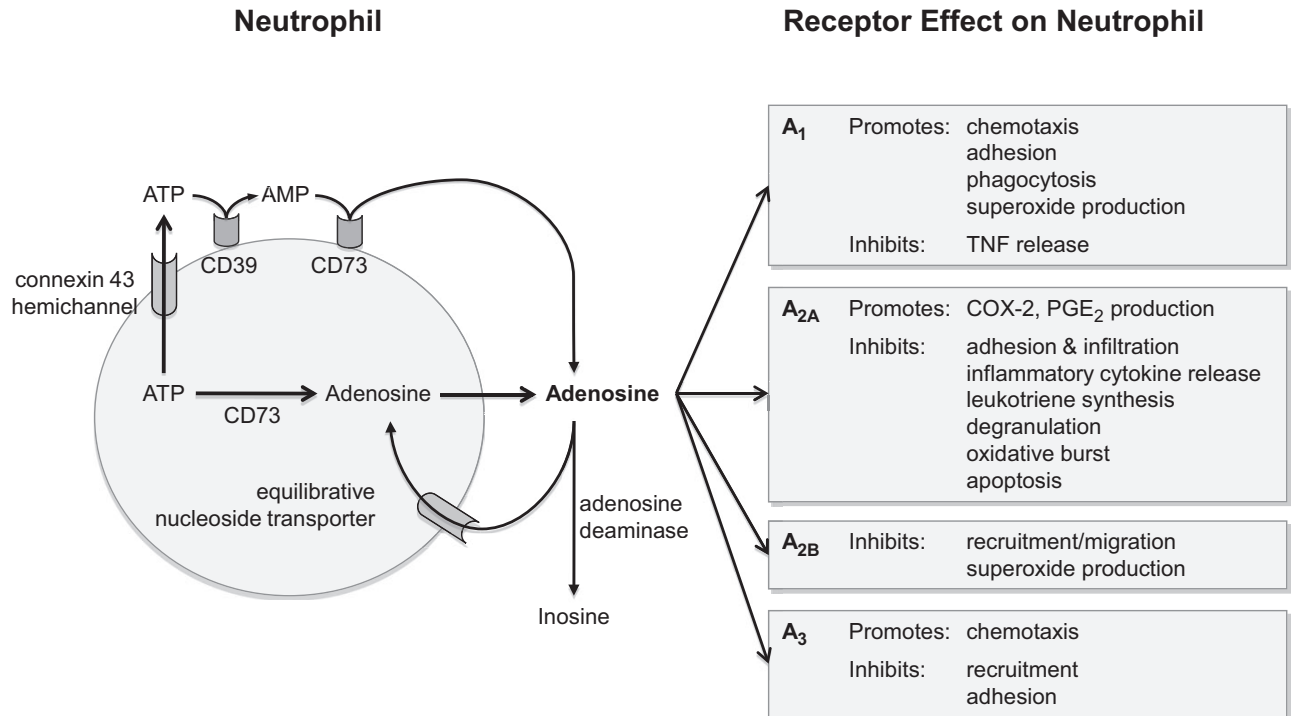
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**Figure.** Diagrammatic overview of adenosine regulation of neutrophils. PGE<sub>2</sub> indicates prostaglandin E<sub>2</sub>.

neutrophil function depending on its concentration in the microenvironment and the receptor profile of the neutrophil.<sup>2,5,7</sup>

### 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,<sup>13</sup> and that this intracellular production is mediated by a cytosolic 5' nucleotidase.<sup>14</sup> 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.<sup>15–20</sup>

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 connexin 43 hemichannels and is rapidly converted to 5' AMP and adenosine by the ectoenzymes CD39 and CD73, respectively, expressed on the neutrophil surface.<sup>21,22</sup> ATP can act independently of adenosine to regulate neutrophil function; recent reviews have characterized these effects in detail.<sup>2,7,20,23</sup> 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.<sup>24–26</sup>

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.<sup>20</sup> 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.<sup>27,28</sup>

### 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.<sup>17,29–31</sup> One of the first reports of adenosine promoting neutrophil function attributed enhanced neutrophil chemotaxis toward complement C5 fragments and fMLP to the A<sub>2</sub> (later A<sub>2A</sub>) receptor<sup>32</sup>; but a follow-up study determined that adenosine promotes chemotaxis by acting at the A<sub>1</sub> receptor by using N<sup>6</sup>-cyclopentyladenosine (Table 1), a selective A<sub>1</sub> receptor agonist.<sup>9</sup> The A<sub>1</sub> receptor was also noted to have a higher affinity for adenosine than the A<sub>2</sub> 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 A<sub>2</sub> receptors.<sup>9</sup>

The role of adenosine A<sub>3</sub> receptors in neutrophil chemotaxis was addressed more recently.<sup>33</sup> 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 A<sub>3</sub> receptors, also concentrated at the leading edge, to augment chemotaxis.<sup>33</sup> The relevance of A<sub>3</sub>-directed neutrophil chemotaxis was demonstrated in a mouse model of intra-abdominal infection in A<sub>3</sub> receptor

**Table 1. Pharmacological Ligands of Adenosine Receptors**

Receptor Target	Ligand	Effect
Global	2-choloradenosine	Agonist
Global	NECA	Agonist
Global	8-(p-sulfophenyl)theophylline	Antagonist
Global	Theophylline	Antagonist
A <sub>1</sub>	CPA	Agonist
A <sub>1</sub>	COPA	Agonist
A <sub>2A</sub>	CGS-21680*	Agonist
A <sub>2A</sub>	WRC-0470*	Agonist
A <sub>2A</sub>	ATL146e	Agonist
A <sub>2A</sub>	ATL193	Agonist
A <sub>2A</sub>	ATL202	Agonist
A <sub>2A</sub>	ZM-241385*	Antagonist
A <sub>2A</sub> /A <sub>2B</sub>	CPCA	Agonist
A <sub>2B</sub>	BAY 60-6583*	Agonist
A <sub>3</sub>	CI-IB-MECA	Agonist
A <sub>3</sub>	MRS-1220*	Antagonist

CGS-21680, 3-[4-[2-[[6-amino-9-[(2R,3R,4S,5S)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]purin-2-yl]amino]ethyl]phenyl]propanoic acid; WRC-0470, 2-cyclohexylmethylidenehydrazinoadenosine; ZM-241385, 4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol; BAY 60-6583, 2-[6-amino-3,5-dicyano-4-[4-(cyclopropylmethoxy)phenyl]pyridin-2-ylsulfanyl]acetamide; MRS-1220, N-[9-Chloro-2-(2-furanyl)[1,2,4]-triazolo[1,5-c]quinazolin-5-yl]benzene acetamide; NECA, N-ethylcarboxamidoadenosine; CPA, N<sup>6</sup>-cyclopentyladenosine; COPA, 2-chloro-N<sup>6</sup>-cyclopentyladenosine; CPCA, 5'-(N-cyclopropyl)-carboxamido/adenosine; CI-IB-MECA, 2-chloro-N<sup>6</sup>-(3-iodobenzyl)adenosine-5'-N-methyluronamide.

knockout mice (*Adora3*<sup>-/-</sup>). Using the cecal ligation and puncture model of sepsis, *Adora3*<sup>-/-</sup> mice exhibited decreased recruitment of neutrophils to the lung and peritoneum and increased numbers of circulating neutrophils compared to wild-type treated mice.<sup>34</sup>

In addition to CD73, the ecto-nucleoside triphosphate diphosphohydrolase 1 (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.<sup>35</sup> It has therefore been postulated that release of ATP and conversion to adenosine by migrating neutrophils, along with polarized expression of A<sub>3</sub> receptors, CD73 and CD39 at the leading edge, represents an autocrine positive feedback mechanism that amplifies chemotactic signals and promotes forward movement of neutrophils.<sup>7,36</sup> In contrast, due to its uniform distribution across the surface of neutrophils, the A<sub>2A</sub> receptor may act as a “global inhibition” signal that promotes chemotaxis by inhibiting backward movement.<sup>7,37</sup> Neutrophil-derived adenosine can also inhibit release of the potent neutrophil chemoattractant, CXCL8, from endothelial cells, potentially allowing extravasated neutrophils to migrate away from endothelial cells, further into the damaged tissue region.<sup>38</sup>

Recently, the neuronal guidance molecule netrin-1 has been implicated in the regulation of neutrophil chemotaxis in

an adenosine A<sub>2B</sub> receptor-dependent fashion. Exogenous netrin-1 dampens infiltration of neutrophils in models of lipopolysaccharide (LPS)-induced acute lung injury, hypoxia-induced lung inflammation, and acute experimental colitis, effects dependent on the expression of the A<sub>2B</sub> receptor.<sup>39–41</sup> In addition, netrin-1 engagement of the A<sub>2B</sub> receptor in vitro limits neutrophil transepithelial migration.<sup>41</sup> However, it has been proposed that netrin-1 does not bind A<sub>2B</sub> directly, but instead activation of the A<sub>2B</sub> receptor closely regulates expression of the netrin-1 receptor UNC5B, producing the observed anti-inflammatory effects.<sup>42</sup>

Taken together, these data suggest that submicromolar concentrations of adenosine can augment neutrophil chemotaxis toward inflammatory stimuli by autocrine action at the high-affinity A<sub>1</sub> and A<sub>3</sub> adenosine receptors. In addition, neutrophil chemotaxis is inhibited by the interaction of netrin-1 and the A<sub>2B</sub> 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  $\beta_2$  (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.<sup>43</sup>

Several studies have demonstrated that adenosine attenuates adhesion and neutrophil-induced damage to the endothelium. The adenosine analog 2-choloradenosine inhibits adherence of fMLP-stimulated neutrophils to endothelial monolayers and inhibits neutrophil-mediated endothelial cell damage.<sup>29</sup> A similar effect was shown with the pan-adenosine receptor agonist N-ethylcarboxamidoadenosine (NECA).<sup>44</sup> NECA and the A<sub>2A</sub>-receptor agonist CGS-21680 decreased adherence of phorbol 12-myristate 13-acetate-stimulated neutrophils to porcine aortic endothelium<sup>45</sup> and adenosine and CGS-21680 inhibited neutrophil adhesion and damage to endothelial cells in isolated canine coronary arteries.<sup>46</sup> These effects were attributed to inhibition of selectin-based adhesion.<sup>47</sup>

Additional studies revealed that adenosine inhibits both the shedding of L-selectin and expression of  $\beta_2$  integrins (mainly CD11b/CD18) on the surface of neutrophils, thus limiting adhesion.<sup>12</sup> These effects are potentiated by the addition of dipyridamole, a nucleoside uptake inhibitor that enhances extracellular concentrations of adenosine, and attenuated by addition of adenosine deaminase.<sup>12</sup> Neutrophil adhesion to fibrinogen, a ligand for CD11b/CD18, can also be inhibited by NECA.<sup>48</sup> NECA was also shown to attenuate CD11b/CD18 expression on fMLP-stimulated neutrophils in a dose-dependent manner.<sup>12,49</sup> The inhibitory effects of adenosine on

neutrophil adhesion appear to be at least partially mediated via the  $A_{2A}$  receptor, since ATL146e, a selective  $A_{2A}$  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  $A_{2A}$  receptor antagonist, ZM-241385, blocked this inhibition.<sup>50</sup> 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  $A_{2A}$  receptor antagonist.<sup>51</sup>

Several *in vivo* models demonstrate that  $A_{2A}$ -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.<sup>52</sup> 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.<sup>53</sup>

The adenosine  $A_3$  receptor has also been implicated in the regulation of neutrophil adhesion. Activation of the  $A_3$  receptor with the selective agonist 2-chloro- $N^6$ -(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  $A_3$ -selective antagonist.<sup>54</sup> 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  $A_3$  promoted recovery after reperfusion in part by inhibiting neutrophil adhesion.<sup>54</sup>

Adenosine also attenuates neutrophil accumulation through 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.<sup>38</sup> 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.<sup>55</sup> This enhanced barrier function is due in part to adenosine  $A_{2B}$  receptor activation on endothelial cells,<sup>26</sup> 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  $A_{2B}$  antagonism.<sup>56</sup> 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  $A_{2B}$  receptors to enhance barrier function and limit neutrophil transmigration.

In contrast, the  $A_1$  receptor has been shown to enhance neutrophil adhesion to the endothelium. The  $A_1$  receptor-specific agonist NECA enhances neutrophil adhesion to gelatin-coated plates, via a CD11b/CD18-independent mechanism.<sup>48</sup> A different  $A_1$  receptor agonist, 2-chloro- $N^6$ -cyclopentyladenosine (COPA), promoted phorbol 12-

myristate 13-acetate-stimulated adhesion of neutrophils to cultured porcine aortic endothelial cells.<sup>45</sup>

Thus,  $A_1$  receptors may promote adhesion via an integrin-independent mechanism, while  $A_{2A}$  receptors attenuate adhesion via inhibition of integrins. Alternatively, it is possible that  $A_1$ -mediated adhesion functions similarly to  $A_1$  and  $A_3$ -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  $A_{2A}$  and  $A_{2B}$  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  $A_1$  agonist COPA, and the combined  $A_{2A}/A_{2B}$  agonist 5'-(N-cyclopropyl)-carboxamido-adenosine (CPCA), both inhibit the release of TNF from LPS-stimulated human neutrophils, with  $A_2$  agonism 1000 $\times$  more effective than  $A_1$  agonism.<sup>57</sup> Similarly, activation of the  $A_{2A}$  receptor with CGS-21680 inhibited release of TNF and inflammatory chemokines from LPS-stimulated neutrophils.<sup>58</sup>

Induction of cyclooxygenase-2 activity converts arachidonic acid into prostaglandin  $E_2$  and thromboxane  $A_2$ . Prostaglandin  $E_2$  is a potent anti-inflammatory agent capable of inhibiting neutrophil chemotaxis, aggregation, and superoxide production, while thromboxane  $A_2$  is a proinflammatory mediator that activates platelet aggregation and clotting. A series of studies reported that leukocyte cyclooxygenase-2 induction is attenuated in  $A_{2A}$  receptor-deficient mice (*Adora2a*<sup>-/-</sup>). Furthermore, CGS-21680 activation of  $A_{2A}$  receptors on fMLP-stimulated human neutrophils potentiates the induction of cyclooxygenase-2 and enhances prostaglandin  $E_2$  generation without affecting production of thromboxane  $A_2$ .<sup>59,60</sup>

Arachidonic acid can also be converted into leukotrienes via the 5-lipoxygenase pathway. Leukotriene  $B_4$  (LT) $B_4$  is a potent neutrophil chemoattractant and can also stimulate oxidative burst and degranulation. Early studies found that adenosine analogs inhibit fMLP-induced synthesis of LTB $_4$  in whole blood.<sup>61</sup> A subsequent study examined ligand-stimulated neutrophils in isolation and found that removal of endogenous adenosine or blockade of  $A_{2A}$  receptors enhanced LTB $_4$  synthesis.<sup>62</sup> Similarly, activated neutrophils are unable to transform arachidonic acid into 5-lipoxygenase products without removal of adenosine or addition of an  $A_{2A}$  antagonist.<sup>63</sup> Finally, endogenous adenosine inhibits the ability of neutrophils to produce LTA $_4$ , a precursor of LTB $_4$ , LTC $_4$ , and lipoxins, which modulate functional responses of phagocytes.<sup>64,65</sup>

Taken together, these studies provide evidence that activation of  $A_{2A}$  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 microbicidal granule components.

The regulation of neutrophil phagocytosis by adenosine is concentration- and receptor-dependent.  $A_1$  receptor agonism enhances FcR $\gamma$ -mediated phagocytosis in human neutrophils at pico- to nanomolar concentrations of adenosine.<sup>66,67</sup> In contrast, micromolar concentrations of adenosine or NECA inhibit FcR-mediated phagocytosis.<sup>67</sup> 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  $A_1$  receptor binding, while elevated concentrations of adenosine inhibit phagocytosis via activation of  $A_{2A}$  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 do 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 fMLP<sup>68</sup> and release of bacterial permeability increasing protein, neutrophil elastase, and defensins in response to LPS and TNF $\alpha$ .<sup>11</sup> Subsequent studies showed that agonism of the  $A_{2A}$  and  $A_3$  receptor, but not the  $A_1$  receptor, inhibit elastase release from neutrophils in response to fMLP,<sup>69</sup> which was associated with cAMP-dependent sequestration of cytosolic calcium.<sup>69,70</sup> Consistent with this, in vivo administration of the  $A_{2A}$  agonist WRC-0470 inhibited degranulation of secondary neutrophil granules in a rat model of meningitis, as measured by extracellular lysozyme concentration.<sup>71</sup> These data suggest that adenosine appears to inhibit neutrophil granule release, which is at least in part mediated via binding to the  $A_{2A}$  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,  $O_2^-$ , 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.<sup>72</sup> 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%,<sup>18</sup> an effect that could be replicated by the adenosine analog NECA and antagonized by theophylline.<sup>73</sup> A similar effect was seen when adenosine was administered in vivo in a porcine model of endotoxemia.<sup>74</sup> Subsequent studies have linked inhibition of oxidative burst to adenosine action at the neutrophil  $A_{2A}$  receptor: the  $A_{2A}$  agonists WRC-0470, CGS-21680, ATL193, and ATL146e inhibit neutrophil oxidative burst in response to a variety of neutrophil stimuli, including fMLP, TNF $\alpha$ , PAF, and IgG<sup>70,75–78</sup>; after exposure to complement C3b-coated zymosan particles<sup>79</sup>; and during rat bacterial meningitis.<sup>71</sup> 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 gp91<sup>phox</sup> and p22<sup>phox</sup>) content in neutrophil plasma membranes and primary granules.<sup>80</sup> Activation of  $A_{2A}$  receptors increases cAMP and intracellular calcium, but inhibition of protein kinase A does not restore superoxide anion synthesis,<sup>81</sup> 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  $A_{2A}$  inhibits phospholipase D activation by blocking membrane recruitment of small GTPases.<sup>82</sup>

Regulation of neutrophil oxidative burst by other adenosine receptors has been less thoroughly characterized. The  $A_{2B}$  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  $A_{2B}$ -deficient mice (*Adora2b*<sup>-/-</sup>).<sup>83</sup> 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.<sup>83</sup> In contrast, the  $A_1$  receptor agonist CPA enhanced superoxide generation during FcR $\gamma$ -mediated stimulation of human neutrophils, an effect that could be blocked by the adenosine antagonist 8-(p-Sulfophenyl)theophylline and by pertussis toxin.<sup>66</sup> The activation of neutrophil  $A_3$  receptors with Cl-IB-MECA had no effect on superoxide production by canine neutrophils stimulated with PAF.<sup>54</sup> A careful study of  $A_3$  agonists and antagonists could not rule out the contribution of  $A_3$  receptors to inhibition of oxidative burst but concluded inhibition is predominantly mediated through  $A_{2A}$  activation.<sup>10</sup>

**Table 2.    Brief Summary of Neutrophil-Adenosine Receptor Literature**

Receptor	System	Stimulus	Manipulation	Effect on Neutrophil	Ref.
Unspecified	Human neutrophil	fMLP	2-chloroadenosine	Inhibits adherence to endothelium	29
Unspecified	Human neutrophil	fMLP	NECA	Inhibits neutrophil-mediated endothelial damage	29, 44
Unspecified	Human neutrophil	fMLP	Adenosine kinase inhibition	Inhibits selectin-based adhesion	47
Unspecified	Human neutrophil	fMLP	NECA	Inhibits oxidative burst	18, 73
A <sub>1</sub>	Human neutrophil	fMLP	CPA	Promotes chemotaxis	9
A <sub>1</sub>	Human neutrophil	fMLP	CPA	Promotes adhesion to gelatin-coated plates	48
A <sub>1</sub>	Porcine neutrophil	PMA	COPA	Promotes adhesion	45
A <sub>1</sub>	Human neutrophil	Antibody-coated erythrocytes	CPA	Promotes FcR $\gamma$ -mediated phagocytosis	66, 67
A <sub>1</sub>	Human neutrophil	FcR $\gamma$ stimulation	CPA	Enhances superoxide production	66
A <sub>1</sub> , A <sub>2A</sub> /A <sub>2B</sub>	Human neutrophil	LPS	COPA, CPCA	Inhibits TNF release	57
A <sub>2</sub>	Human neutrophil	Complement C5, fMLP	NECA	Promotes chemotaxis	32
A <sub>2</sub>	Human neutrophil	fMLP	Dipyrimidole, NECA	Inhibits selectin shedding, expression of $\beta_2$ integrins	12, 49
A <sub>2</sub>	Human neutrophil	fMLP	NECA	Inhibits adhesion to fibrinogen-coated plates	48
A <sub>2</sub>	Human neutrophil	Antibody-coated yeast	NECA	Inhibits FcR $\gamma$ -mediated phagocytosis	66, 67
A <sub>2A</sub>	Porcine neutrophil	PMA	NECA, CGS-21680	Inhibits adhesion	45
A <sub>2A</sub>	Canine neutrophil	PAF	CGS-21680	Inhibits adhesion	46
A <sub>2A</sub>	Human neutrophil	fMLP, TNF	ATL146e	Inhibits adhesion to VCAM-1 coated surface	50
A <sub>2A</sub>	Canine neutrophil	Myocardial infarction	ATL146e	Inhibits p-selectin expression, infiltration	52
A <sub>2A</sub>	Murine neutrophil	Carotid ligation	ATL146e	Inhibits recruitment, integrin and selectin expression	53
A <sub>2A</sub>	Human neutrophil	LPS	CGS-21680	Inhibits inflammatory chemokine release	58
A <sub>2A</sub>	<i>Adora2a</i> <sup>-/-</sup> mice	Air pouch inflammation		Inhibits COX-2 induction	59
A <sub>2A</sub>	Human neutrophil	fMLP	CGS-21680	Promotes COX-2, PGE <sub>2</sub> generation	59, 60
A <sub>2A</sub>	Whole blood, human neutrophil	fMLP	NECA, CGS-21680	Inhibits LTB <sub>4</sub> synthesis	61, 62
A <sub>2A</sub>	Human neutrophil	fMLP	CGS-21680	Inhibits leukotriene synthesis	63
A <sub>2A</sub>	Human neutrophil	Various	CGS-21680	Inhibits LTA <sub>4</sub> production	64, 65
A <sub>2A</sub>	Rat neutrophil	Meningitis	WRC-0470	Inhibits degranulation	71
A <sub>2A</sub>	Human, murine, rat neutrophil	fMLP, TNF, PAF, IgG, C3b-Zymosan, bacterial meningitis	WRC-0470, CGS-21680, ATL193, ATL146e	Inhibits oxidative burst	70, 75–79
A <sub>2A</sub>	Human neutrophil		CGS-21680	Inhibits neutrophil apoptosis	84, 85
A <sub>2A</sub> , A <sub>3</sub>	Human neutrophil	fMLP, TNF, LPS		Inhibits degranulation	68–70
A <sub>2B</sub>	Murine, human neutrophil	LPS, hypoxia, colitis	Netrin-1	Inhibits recruitment, transepithelial migration	39–41
A <sub>2B</sub>	Murine neutrophil	fMLP	BAY 60–6583	Inhibits superoxide production	83
A <sub>3</sub>	Human neutrophil	fMLP		Promotes chemotaxis	33
A <sub>3</sub>	<i>Adora3</i> <sup>-/-</sup> mice	Sepsis		Inhibits recruitment	34
A <sub>3</sub>	Canine, rabbit neutrophil	PAF	CI-IB-MECA	Inhibits adhesion to endothelium	54

N-formyl-methionine-leucine-phenylalanine (fMLP); NECA, N-ethylcarboxamidoadenosine; CPA, N<sup>6</sup>-cyclopentyladenosine; COPA, 2-chloro-N<sup>6</sup>-cyclopentyladenosine; CPCA, 5'-(N-cyclopropyl)-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; CI-IB-MECA, 2-chloro-N<sup>6</sup>-(3-iodobenzyl)adenosine-5'-N-methyluronamide.

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 NET production (NETosis). Several studies have demonstrated that adenosine analogs delay apoptosis of resting human neutrophils in culture.<sup>84,85</sup> Adenosine agonists with higher affinity for the A<sub>2A</sub> receptor had more potency in inhibiting neutrophil apoptosis.<sup>84</sup> Similarly, theophylline and its analogs promote apoptosis of resting neutrophils in culture.<sup>85</sup> 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.<sup>86,87</sup> 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.<sup>88–91</sup> 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,<sup>89,92</sup> 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.<sup>90</sup> 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<sup>91</sup>; 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.<sup>91,93</sup> 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>3</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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## References

1. Harkness RA, Simmonds RJ, Coade SB. Purine transport and metabolism in man: the effect of exercise on concentrations of purine bases, nucleosides and nucleotides in plasma, urine, leucocytes and erythrocytes. *Clin Sci*. 1983;64:333–340.
2. Bours MJL, Swennen ELR, Di Virgilio F, Cronstein BN, Dagnelie PC. Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. *Pharmacol Ther*. 2006;112:358–404.
3. Ontyd J, Schrader J. Measurement of adenosine, inosine, and hypoxanthine in human plasma. *J Chromatogr*. 1984;307:404–409.
4. Yoneyama Y, Suzuki S, Sawa R, Araki T. Plasma adenosine concentrations increase in women with hyperemesis gravidarum. *Clin Chim Acta*. 2004;342:99–103.
5. Fredholm BB. Purines and neutrophil leukocytes. *Gen Pharmacol*. 1997;28:345–350.
6. Möser GH, Schrader J, Deussen A. Turnover of adenosine in plasma of human and dog blood. *Am J Physiol*. 1989;256:C799–C806.
7. Junger WG. Immune cell regulation by autocrine purinergic signalling. *Nat Rev Immunol*. 2011;11:201–212.
8. Fredholm BB, Zhang Y, van der Ploeg I. Adenosine A<sub>2A</sub> receptors mediate the inhibitory effect of adenosine on formyl-Met-Leu-Phe-stimulated respiratory burst in neutrophil leukocytes. *Naunyn-Schmiedeberg Arch Pharmacol*. 1996;354:262–267.
9. Cronstein BN, Daguma L, Nichols D, Hutchison AJ, Williams M. The adenosine/neutrophil paradox resolved: human neutrophils possess both A<sub>1</sub> and A<sub>2</sub> receptors that promote chemotaxis and inhibit O<sub>2</sub> generation, respectively. *J Clin Invest*. 1990;85:1150–1157.
10. Gessi S, Varani K, Merighi S, Cattabriga E, Iannotta V, Leung E, Baraldi PG, Borea PA. A(3) adenosine receptors in human neutrophils and promyelocytic HL60 cells: a pharmacological and biochemical study. *Mol Pharmacol*. 2002;61:415–424.

11. Bouma MG, Jeunhomme TM, Boyle DL, Dentener MA, Voitenok NN, van den Wildenberg FA, Buurman WA. Adenosine inhibits neutrophil degranulation in activated human whole blood: involvement of adenosine A2 and A3 receptors. *J Immunol*. 1997;158:5400–5408.
12. Thiel M, Chambers JD, Chouker A, Fischer S, Zourelidis C, Bardenheuer HJ, Arfors KE, Peter K. Effect of adenosine on the expression of beta(2) integrins and L-selectin of human polymorphonuclear leukocytes in vitro. *J Leukoc Biol*. 1996;59:671–682.
13. Newby AC, Holmquist CA. Adenosine production inside rat polymorphonuclear leukocytes. *Biochem J*. 1981;200:399–403.
14. Worku Y, Newby AC. The mechanism of adenosine production in rat polymorphonuclear leukocytes. *Biochem J*. 1983;214:325–330.
15. Bengtsson T, Zalavary S, Stendahl O, Grenegård M. Release of oxygen metabolites from chemoattractant-stimulated neutrophils is inhibited by resting platelets: role of extracellular adenosine and actin polymerization. *Blood*. 1996;87:4411–4423.
16. Mann JS, Renwick AG, Holgate ST. Release of adenosine and its metabolites from activated human leukocytes. *Clin Sci*. 1986;70:461–468.
17. Cronstein BN, Kramer SB, Weissmann G, Hirschhorn R. A new physiological function for adenosine: regulation of superoxide anion production. *Trans Assoc Am Physicians*. 1983;96:384–391.
18. Cronstein BN, Kramer SB, Weissmann G, Hirschhorn R. Adenosine: a physiological modulator of superoxide anion generation by human neutrophils. *J Exp Med*. 1983;158:1160–1177.
19. Kitakaze M, Hori M, Morioka T, Takashima S, Minamino T, Sato H, Inoue M, Kamada T. Attenuation of ecto-5'-nucleotidase activity and adenosine release in activated human polymorphonuclear leukocytes. *Circ Res*. 1993;73:524–532.
20. van Waeg G, Van den Berghe G. Purine catabolism in polymorphonuclear neutrophils. Phorbol myristate acetate-induced accumulation of adenosine owing to inactivation of extracellularly released adenosine deaminase. *J Clin Invest*. 1991;87:305–312.
21. Eltzschig HK, Eckle T, Mager A, Küper N, Karcher C, Weissmüller T, Boengler K, Schulz R, Robson SC, Colgan SP. ATP release from activated neutrophils occurs via connexin 43 and modulates adenosine-dependent endothelial cell function. *Circ Res*. 2006;99:1100–1108.
22. Eltzschig HK, Macmanus CF, Colgan SP. Neutrophils as sources of extracellular nucleotides: functional consequences at the vascular interface. *Trends Cardiovasc Med*. 2008;18:103–107.
23. Grassi F. Purinergic control of neutrophil activation. *J Mol Cell Biol*. 2010;2:176–177.
24. Madara JL, Parkos C, Colgan S, MacLeod RJ, Nash S, Matthews J, Delp C, Lencer W. Cl<sup>-</sup> secretion in a model intestinal epithelium induced by a neutrophil-derived secretagogue. *J Clin Invest*. 1992;89:1938–1944.
25. Madara JL, Patapoff TW, Gillece-Castro B, Colgan SP, Parkos CA, Delp C, Mrsny RJ. 5'-adenosine monophosphate is the neutrophil-derived paracrine factor that elicits chloride secretion from T84 intestinal epithelial cell monolayers. *J Clin Invest*. 1993;91:2320–2325.
26. Lennon PF, Taylor CT, Stahl GL, Colgan SP. Neutrophil-derived 5'-adenosine monophosphate promotes endothelial barrier function via CD73-mediated conversion to adenosine and endothelial A2B receptor activation. *J Exp Med*. 1998;188:1433–1443.
27. Baldwin SA, Beal PR, Yao SYM, King AE, Cass CE, Young JD. The equilibrative nucleoside transporter family, SLC29. *Pflugers Arch*. 2004;447:735–743.
28. Eltzschig HK, Abdulla P, Hoffman E, Hamilton KE, Daniels D, Schönfeld C, Löffler M, Reyes G, Duzenko M, Karhausen J, Robinson A, Westerman KA, Coe IR, Colgan SP. HIF-1-dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia. *J Exp Med*. 2005;202:1493–1505.
29. Cronstein BN, Levin RI, Belanoff J, Weissmann G, Hirschhorn R. Adenosine: an endogenous inhibitor of neutrophil-mediated injury to endothelial cells. *J Clin Invest*. 1986;78:760–770.
30. Cronstein BN, Kubersky SM, Weissmann G, Hirschhorn R. Engagement of adenosine receptors inhibits hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) release by activated human neutrophils. *Clin Immunol Immunopathol*. 1987;42:76–85.
31. Schrier DJ, Imre KM. The effects of adenosine agonists on human neutrophil function. *J Immunol*. 1986;137:3284–3289.
32. Rose FR, Hirschhorn R, Weissmann G, Cronstein BN. Adenosine promotes neutrophil chemotaxis. *J Exp Med*. 1988;167:1186–1194.
33. Chen Y, Corriden R, Inoue Y, Yip L, Hashiguchi N, Zinkernagel A, Nizet V, Insel PA, Junger WG. ATP release guides neutrophil chemotaxis via P2Y<sub>2</sub> and A<sub>3</sub> receptors. *Science*. 2006;314:1792–1795.
34. Inoue Y, Chen Y, Hirsh MI, Yip L, Junger WG. A<sub>3</sub> and P2Y<sub>2</sub> receptors control the recruitment of neutrophils to the lungs in a mouse model of sepsis. *Shock*. 2008;30:173–177.
35. Corriden R, Chen Y, Inoue Y, Beldi G, Robson SC, Insel PA, Junger WG. Ecto-nucleoside triphosphate diphosphohydrolase 1 (E-NTP-Dase1/CD39) regulates neutrophil chemotaxis by hydrolyzing released ATP to adenosine. *J Biol Chem*. 2008;283:28480–28486.
36. Junger WG. Purinergic regulation of neutrophil chemotaxis. *Cell Mol Life Sci*. 2008;65:2528–2540.
37. Linden J. Cell biology. Purinergic chemotaxis. *Science*. 2006;314:1689–1690.
38. Bouma MG, van den Wildenberg FA, Buurman WA. Adenosine inhibits cytokine release and expression of adhesion molecules by activated human endothelial cells. *Am J Physiol*. 1996;270:C522–C529.
39. Mirakaj V, Thix CA, Laucher S, Mielke C, Morote-Garcia JC, Schmit MA, Henes J, Unertl KE, Köhler D, Rosenberger P. Netrin-1 dampens pulmonary inflammation during acute lung injury. *Am J Respir Crit Care Med*. 2010;181:815–824.
40. Aherne CM, Collins CB, Masterson JC, Tizzano M, Boyle TA, Westrich JA, Parnes JA, Furuta GT, Rivera-Nieves J, Eltzschig HK. Neuronal guidance molecule netrin-1 attenuates inflammatory cell trafficking during acute experimental colitis [epub ahead of print August 3]. *Gut*. 2011.
41. Rosenberger P, Schwab JM, Mirakaj V, Masekowsky E, Mager A, Morote-Garcia JC, Unertl K, Eltzschig HK. Hypoxia-inducible factor-dependent induction of netrin-1 dampens inflammation caused by hypoxia. *Nat Immunol*. 2009;10:195–202.
42. Linden J. Regulation of leukocyte function by adenosine receptors. *Adv Pharmacol*. 2011;61:95–114.
43. Sæve S, Mohlin C, Vumma R, Persson K. Activation of adenosine A<sub>2</sub>A receptors inhibits neutrophil transendothelial migration. *Infect Immun*. 2011;79:3431–3437.
44. Gunther GR, Herring MB. Inhibition of neutrophil superoxide production by adenosine released from vascular endothelial cells. *Ann Vasc Surg*. 1991;5:325–330.
45. Felsch A, Stöcker K, Borchard U. Phorbol ester-stimulated adherence of neutrophils to endothelial cells is reduced by adenosine A<sub>2</sub> receptor agonists. *J Immunol*. 1995;155:333–338.
46. Zhao ZQ, Sato H, Williams MW, Fernandez AZ, Vinten-Johansen J. Adenosine A<sub>2</sub>-receptor activation inhibits neutrophil-mediated injury to coronary endothelium. *Am J Physiol*. 1996;271:H1456–H1464.
47. Firestein GS, Bullough DA, Erion MD, Jimenez R, Ramirez-Weinhouse M, Barankiewicz J, Smith CW, Gruber HE, Mullane KM. Inhibition of neutrophil adhesion by adenosine and an adenosine kinase inhibitor. The role of selectins. *J Immunol*. 1995;154:326–334.
48. Cronstein BN, Levin RI, Phillips M, Hirschhorn R, Abramson SB, Weissmann G. Neutrophil adherence to endothelium is enhanced via adenosine A<sub>1</sub> receptors and inhibited via adenosine A<sub>2</sub> receptors. *J Immunol*. 1992;148:2201–2206.
49. Wollner A, Wollner S, Smith JB. Acting via A<sub>2</sub> receptors, adenosine inhibits the upregulation of Mac-1 (CD11b/CD18) expression on FMLP-stimulated neutrophils. *Am J Respir Cell Mol Biol*. 1993;9:179–185.
50. Sullivan GW, Lee DD, Ross WG, DiVietro JA, Lappas CM, Lawrence MB, Linden J. Activation of A<sub>2</sub>A adenosine receptors inhibits expression of alpha 4/beta 1 integrin (very late antigen-4) on stimulated human neutrophils. *J Leukoc Biol*. 2004;75:127–134.
51. Gadangi P, Longaker M, Naime D, Levin RI, Recht PA, Montesinos MC, Buckley MT, Carlin G, Cronstein BN. The anti-inflammatory mechanism of sulfasalazine is related to adenosine release at inflamed sites. *J Immunol*. 1996;156:1937–1941.
52. Glover DK, Riou LM, Ruiz M, Sullivan GW, Linden J, Rieger JM, Macdonald TL, Watson DD, Beller GA. Reduction of infarct size and postischemic inflammation from ATL-1466, a highly selective adenosine A<sub>2</sub>A receptor agonist, in reperfused canine myocardium. *Am J Physiol Heart Circ Physiol*. 2005;288:H1851–H1858.
53. McPherson JA, Barringhaus KG, Bishop GG, Sanders JM, Rieger JM, Hesselbacher SE, Gimple LW, Powers ER, Macdonald T, Sullivan G, Linden J, Sarembock IJ. Adenosine A<sub>2</sub>(A) receptor stimulation reduces inflammation and neointimal growth in a murine carotid ligation model. *Arterioscler Thromb Vasc Biol*. 2001;21:791–796.
54. Jordan JE, Thourani VH, Auchampach JA, Robinson JA, Wang NP, Vinten-Johansen J. A(3) adenosine receptor activation attenuates neutrophil function and neutrophil-mediated reperfusion injury. *Am J Physiol*. 1999;277:H1895–H1905.



55. Narravula S, Lennon PF, Mueller BU, Colgan SP. Regulation of endothelial CD73 by adenosine: paracrine pathway for enhanced endothelial barrier function. *J Immunol.* 2000;165:5262–5268.
56. Eltzschig HK, Ibla JC, Furuta GT, Leonard MO, Jacobson KA, Enjyoji K, Robson SC, Colgan SP. Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidases and adenosine A2B receptors. *J Exp Med.* 2003;198:783–796.
57. Thiel M, Chouker A. Acting via A2 receptors, adenosine inhibits the production of tumor necrosis factor- $\alpha$  of endotoxin-stimulated human polymorphonuclear leukocytes. *J Lab Clin Med.* 1995;126:275–282.
58. McColl SR, St-Onge M, Dussault A-A, Laflamme C, Bouchard L, Boulanger J, Pouliot M. Immunomodulatory impact of the A2A adenosine receptor on the profile of chemokines produced by neutrophils. *FASEB J.* 2006;20:187–189.
59. Cadieux J-S, Leclerc P, St-Onge M, Dussault A-A, Laflamme C, Picard S, Ledent C, Borgeat P, Pouliot M. Potentiation of neutrophil cyclooxygenase-2 by adenosine: an early anti-inflammatory signal. *J Cell Sci.* 2005;118:1437–1447.
60. Pouliot M, Fiset M-E, Massé M, Naccache PH, Borgeat P. Adenosine up-regulates cyclooxygenase-2 in human granulocytes: impact on the balance of eicosanoid generation. *J Immunol.* 2002;169:5279–5286.
61. Krump E, Lemay G, Borgeat P. Adenosine A2 receptor-induced inhibition of leukotriene B4 synthesis in whole blood ex vivo. *Br J Pharmacol.* 1996;117:1639–1644.
62. Krump E, Picard S, Mancini J, Borgeat P. Suppression of leukotriene B4 biosynthesis by endogenous adenosine in ligand-activated human neutrophils. *J Exp Med.* 1997;186:1401–1406.
63. Surette ME, Krump E, Picard S, Borgeat P. Activation of leukotriene synthesis in human neutrophils by exogenous arachidonic acid: inhibition by adenosine A(2a) receptor agonists and crucial role of autocrine activation by leukotriene B(4). *Mol Pharmacol.* 1999;56:1055–1062.
64. Flamand N, Surette ME, Picard S, Bourgoin S, Borgeat P. Cyclic AMP-mediated inhibition of 5-lipoxygenase translocation and leukotriene biosynthesis in human neutrophils. *Mol Pharmacol.* 2002;62:250–256.
65. Flamand N, Boudreault S, Picard S, Austin M, Surette ME, Plante H, Krump E, Vallée MJ, Gilbert C, Naccache P, Laviolette M, Borgeat P. Adenosine, a potent natural suppressor of arachidonic acid release and leukotriene biosynthesis in human neutrophils. *Am J Respir Crit Care Med.* 2000;161:S88–S94.
66. Salmon JE, Cronstein BN. Fc gamma receptor-mediated functions in neutrophils are modulated by adenosine receptor occupancy. A1 receptors are stimulatory and A2 receptors are inhibitory. *J Immunol.* 1990;145:2235–2240.
67. Zalavary S, Stendahl O, Bengtsson T. The role of cyclic AMP, calcium and filamentous actin in adenosine modulation of Fc receptor-mediated phagocytosis in human neutrophils. *Biochim Biophys Acta.* 1994;1222:249–256.
68. Richter J. Effect of adenosine analogues and cAMP-raising agents on TNF-, GM-CSF-, and chemotactic peptide-induced degranulation in single adherent neutrophils. *J Leukoc Biol.* 1992;51:270–275.
69. Visser SS, Theron AJ, Ramafi G, Ker JA, Anderson R. Apparent involvement of the A(2A) subtype adenosine receptor in the anti-inflammatory interactions of CGS 21680, cyclopentyladenosine, and IB-MECA with human neutrophils. *Biochem Pharmacol.* 2000;60:993–999.
70. Anderson R, Visser SS, Ramafi G, Theron AJ. Accelerated resequestration of cytosolic calcium and suppression of the pro-inflammatory activities of human neutrophils by CGS 21680 in vitro. *Br J Pharmacol.* 2000;130:717–724.
71. Sullivan GW, Linden J, Buster BL, Scheld WM. Neutrophil A2A adenosine receptor inhibits inflammation in a rat model of meningitis: synergy with the type IV phosphodiesterase inhibitor, rolipram. *J Infect Dis.* 1999;180:1550–1560.
72. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol.* 2006;6:173–182.
73. Cronstein BN, Rosenstein ED, Kramer SB, Weissmann G, Hirschhorn R. Adenosine: a physiologic modulator of superoxide anion generation by human neutrophils. Adenosine acts via an A2 receptor on human neutrophils. *J Immunol.* 1985;135:1366–1371.
74. Thiel M, Holzer K, Kreimeier U, Moritz S, Peter K, Messmer K. Effects of adenosine on the functions of circulating polymorphonuclear leukocytes during hyperdynamic endotoxemia. *Infect Immun.* 1997;65:2136–2144.
75. Sullivan GW, Rieger JM, Scheld WM, Macdonald TL, Linden J. Cyclic AMP-dependent inhibition of human neutrophil oxidative activity by substituted 2-propynylcyclohexyl adenosine A(2A) receptor agonists. *Br J Pharmacol.* 2001;132:1017–1026.
76. Stewart AG, Harris T. Adenosine inhibits platelet-activating factor, but not tumour necrosis factor- $\alpha$ -induced priming of human neutrophils. *Immunology.* 1993;78:152–158.
77. Zalavary S, Bengtsson T. Adenosine inhibits actin dynamics in human neutrophils: evidence for the involvement of cAMP. *Eur J Cell Biol.* 1998;75:128–139.
78. Sullivan GW, Carper HT, Mandell GL. The specific type IV phosphodiesterase inhibitor rolipram combined with adenosine reduces tumor necrosis factor- $\alpha$ -primed neutrophil oxidative activity. *Int J Immunopharmacol.* 1995;17:793–803.
79. Kubersky SM, Hirschhorn R, Broekman MJ, Cronstein BN. Occupancy of adenosine receptors on human neutrophils inhibits respiratory burst stimulated by ingestion of complement-coated particles and occupancy of chemoattractant but not Fc receptors. *Inflammation.* 1989;13:591–599.
80. Swain SD, Siemsen DW, Nelson LK, Sipes KM, Hanson AJ, Quinn MT. Inhibition of the neutrophil NADPH oxidase by adenosine is associated with increased movement of flavocytochrome b between subcellular fractions. *Inflammation.* 2003;27:45–58.
81. Cronstein BN, Haines KA, Kolasinski S, Reibman J. Occupancy of G alpha s-linked receptors uncouples chemoattractant receptors from their stimulus-transduction mechanisms in the neutrophil. *Blood.* 1992;80:1052–1057.
82. Thibault N, Harbour D, Borgeat P, Naccache PH, Bourgoin SG. Adenosine receptor occupancy suppresses chemoattractant-induced phospholipase D activity by diminishing membrane recruitment of small GTPases. *Blood.* 2000;95:519–527.
83. van der Hoeven D, Wan TC, Gizewski ET, Kreckler LM, Maas JE, Van Orman J, Ravid K, Auchampach JA. A role for the low-affinity A2B adenosine receptor in regulating superoxide generation by murine neutrophils. *J Pharmacol Exp Ther.* 2011;338:1004–1012.
84. Walker BA, Rocchini C, Boone RH, Ip S, Jacobson MA. Adenosine A2a receptor activation delays apoptosis in human neutrophils. *J Immunol.* 1997;158:2926–2931.
85. Yasui K, Agematsu K, Shinozaki K, Hokibara S, Nagumo H, Nakazawa T, Komiyama A. Theophylline induces neutrophil apoptosis through adenosine A2A receptor antagonism. *J Leukoc Biol.* 2000;67:529–535.
86. Thiagarajan RR, Winn RK, Harlan JM. The role of leukocyte and endothelial adhesion molecules in ischemia-reperfusion injury. *Thromb Haemost.* 1997;78:310–314.
87. Polanowska-Grabowska R, Wallace K, Field JJ, Chen L, Marshall MA, Figler R, Gear ARL, Linden J. P-selectin-mediated platelet-neutrophil aggregate formation activates neutrophils in mouse and human sickle cell disease. *Arterioscler Thromb Vasc Biol.* 2010;30:2392–2399.
88. Jordan JE, Zhao ZQ, Sato H, Taft S, Vinten-Johansen J. Adenosine A2 receptor activation attenuates reperfusion injury by inhibiting neutrophil accumulation, superoxide generation and coronary endothelial adherence. *J Pharmacol Exp Ther.* 1997;280:301–309.
89. Wallace KL, Linden J. Adenosine A2A receptors induced on iNKT and NK cells reduce pulmonary inflammation and injury in mice with sickle cell disease. *Blood.* 2010;116:5010–5020.
90. Reutershan J, Cagnina RE, Chang D, Linden J, Ley K. Therapeutic anti-inflammatory effects of myeloid cell adenosine receptor A2a stimulation in lipopolysaccharide-induced lung injury. *J Immunol.* 2007;179:1254–1263.
91. Sullivan GW, Fang G, Linden J, Scheld WM. A2A adenosine receptor activation improves survival in mouse models of endotoxemia and sepsis. *J Infect Dis.* 2004;189:1897–1904.
92. Lappas CM, Day Y-J, Marshall MA, Engelhard VH, Linden J. Adenosine A2A receptor activation reduces hepatic ischemia reperfusion injury by inhibiting CD1d-dependent NKT cell activation. *J Exp Med.* 2006;203:2639–2648.
93. Németh ZH, Csóka B, Wilmanski J, Xu D, Lu Q, Ledent C, Deitch EA, Pacher P, Spolarics Z, Haskó G. Adenosine A2A receptor inactivation increases survival in polymicrobial sepsis. *J Immunol.* 2006;176:5616–5626.