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## Purinergic Signaling during Inflammation

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Purines are heterocyclic aromatic molecules that are among the oldest and most influential biochemical compounds in evolutionary history.<sup>1</sup> The purine nucleotide adenosine triphosphate (ATP) is the universal energy currency of intracellular biologic reactions on which mammalian life is based. Here we examine the roles that purines play as extracellular signaling molecules, with a particular focus on ATP, adenosine diphosphate (ADP), and adenosine.

The first reports of purinergic signaling date back to 1929, when scientists intravenously injected extracts from cardiac tissues into intact animals and observed transient slowing of the heart rate; they subsequently identified the biologic agent as an “adenine compound.”<sup>2</sup> Transient heart block induced by intravenous injection of adenosine remains an important clinical application of purinergic signaling (Table 1).

Additional research in recent decades has led to the discovery of many biologic effects of ATP, ADP, and adenosine signaling. These mediators function through the activation of G-protein-coupled or ligand-gated ion-channel receptors. Adenosine is derived from ATP and ADP through the actions of enzymes on cellular surfaces, which also express the receptors for these mediators. Since the receptors of ATP and ADP and the receptors of adenosine often transduce opposite effects, the resulting cellular response is attributable to both the ratio of ADP and ATP to adenosine concentrations and the relative levels of expression and signaling intensity of their receptors.<sup>3</sup> Here we discuss the functional role of purinergic signaling in inflammatory diseases and the contribution of disordered purinergic signaling to the mechanisms of acute and chronic disease.

### EXTRACELLULAR NUCLEOTIDE RELEASE AND SIGNALING

In their physiologic state, mammalian cells contain high concentrations of ATP (5 to 8 mM). Pathologic conditions such as inflammation or ischemia cause the release of ATP. Cellular necrosis is associated with ATP egress from intracellular stores.<sup>4</sup> During apoptosis, controlled ATP release can occur through pannexin hemichannels, where ATP functions as a chemotactic signal for phagocytes.<sup>5</sup> Endothelial cells or activated inflammatory cells such as polymorphonuclear neutrophils release ATP through connexin hemichannels.<sup>6–8</sup> Platelets release purines in the form of ADP from intracellular granules (Fig. 1).<sup>4</sup>

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Two decades ago, Burnstock described the existence of ATP receptors that he referred to as “P2” receptors.<sup>3</sup> Subsequently, they were divided on the basis of their chemical properties into P2X receptors (ligand-gated ion channels) and P2Y receptors (G-protein-coupled receptors). Gene-targeted mice with deleted P2 receptors are typically viable, indicating redundancy of the signaling system in physiologic conditions. However, in pathologic conditions such as asthma, vascular inflammation, or graft-versus-host disease, P2-receptor knockout mice are protected from inflammatory diseases.<sup>9,11,12</sup> Genetic studies directly implicate ATP signaling in human inflammatory or neoplastic diseases. For example, in genetic studies involving humans, mutations in the P2X7-receptor gene have been linked to susceptibility to tuberculosis<sup>13</sup> and the clinical outcomes of chronic lymphocytic leukemia,<sup>14</sup> and genetic abnormalities in T-cell-dependent P2Y11 signaling have been implicated in narcolepsy.<sup>15</sup> Pharmacologic P2-receptor antagonists inhibit inflammation such as that which occurs in inflammatory bowel disease (IBD), lung inflammation, and ischemia and reperfusion.<sup>4,11,16</sup>

## EXTRACELLULAR CONVERSION OF ATP AND ADP TO ADENOSINE

In the extracellular space, ATP and ADP are rapidly metabolized to adenosine monophosphate (AMP), which in turn is metabolized to adenosine. This nucleotide phosphohydrolysis involves a two-step enzymatic process regulated by ectoenzymes. In the first step, ATP and ADP are both converted to AMP through the ectonucleoside triphosphate diphosphohydrolase 1 (CD39). Mice with genetic deletion of *Cd39* are viable, indicating that CD39-dependent phosphohydrolysis is not vital under physiologic conditions. Elevations in extracellular ATP and ADP levels in conjunction with attenuated adenosine levels account for increased susceptibility to the development of pathologic inflammation during disease states in *Cd39*<sup>-/-</sup> mice.<sup>17-20</sup> In humans, polymorphisms of noncoding regions of *CD39* decrease ectonucleotidase expression, leading to increased susceptibility to IBD and multiple sclerosis.<sup>21,22</sup>

In the second step of the extracellular generation of adenosine, ecto-5'-nucleotidase (CD73) converts extracellular AMP to adenosine. Mice with a genetic deletion of *Cd73* are viable, indicating that CD73-dependent phosphohydrolysis of AMP is not vital under physiologic conditions. However, a recent study involving humans identified loss-of-function mutations of the gene encoding CD73 as the genetic basis of familial peripheral-artery calcifications.<sup>23</sup> During pathophysiological conditions, *Cd73*<sup>-/-</sup> mice have attenuated adenosine signaling, whereas extracellular ATP and ADP levels remain almost unchanged. The lack of extracellular adenosine signaling in *Cd73*<sup>-/-</sup> mice causes susceptibility to hypoxia-induced inflammation<sup>24</sup> and barrier dysfunction of the vasculature and the intestine.<sup>25,26</sup> The antiinflammatory actions of methotrexate, as well as sulfasalazine, are mediated at least in part through CD73-dependent production of adenosine.<sup>27,28</sup> Pharmacologic studies in a laboratory or a clinical setting using pharmacologic compounds that increase extracellular ATP and ADP conversion to adenosine show therapeutic effects (Table 1) in patients with inflammatory disease or ischemia.<sup>17,29-32</sup>

## EXTRACELLULAR ADENOSINE SIGNALING

Adenosine can signal through four distinct G-protein-coupled receptors: the adenosine A<sub>1</sub> receptor (ADORA1), the adenosine A<sub>2A</sub> receptor (ADORA2A), the adenosine A<sub>2B</sub> receptor (ADORA2B), and the adenosine A<sub>3</sub> receptor (ADORA3) (Fig. 2).<sup>40-42</sup> Adenosine-receptor subtypes are distributed differently on each adenosine target cell. For example, ADORA2B is highly expressed on vascular endothelial cells,<sup>36,43</sup> whereas ADORA2A is highly expressed on immune cells such as neutrophils<sup>33</sup> and lymphocytes.<sup>34</sup> Specific human disease states relating to defects or mutations of any of the four defined adenosine receptors

are currently unknown. Adenosine-receptor–knockout mice are viable, indicating redundancy of the system under physiologic conditions.

In contrast, many specific biologic and cell-specific functions have been identified for each receptor under pathologic conditions, and a few select examples are discussed below. The chronotropic effects of adenosine that are critical in the treatment of supraventricular tachycardia are dependent on ADORA1.<sup>44</sup> Pharmacologic studies show antiinflammatory functions of ADORA2A signaling in human neutrophils<sup>33</sup>; ADORA2A plays a critical role in attenuating inflammatory-cell activation at multiple tissue sites.<sup>33,45</sup> Pharmacologic studies show therapeutic benefits of ADORA2A antagonists in Parkinson's disease.<sup>46</sup> ADORA2B plays a role in tissue adaptation to conditions of hypoxia, inflammation, or ischemia,<sup>32,47–49</sup> whereas ADORA3A has been linked to chloride transport in the production of aqueous humor by the nonpigmented epithelium of the eye.<sup>50</sup> A recent randomized clinical trial showed salutary effects of an ADORA3 agonist in the treatment of the dry-eye syndrome.<sup>39</sup>

## TERMINATION OF ADENOSINE SIGNALING

During the termination of signaling, adenosine is transported from the extracellular space to the intracellular compartment (Fig. 2). This transport involves equilibrative nucleoside transporters — diffusion-limited channels that allow adenosine to freely cross the cellular membrane according to its concentration gradient.<sup>43,51</sup> Mice with genetic deletion of equilibrative nucleoside transporters are viable, but they have elevated adenosine levels during disease states which help to protect them during organ ischemia.<sup>43</sup> Pharmacologic inhibition of equilibrative nucleoside transporters with dipyridamole and concomitant elevations of extracellular adenosine levels can be used to induce coronary-artery vasodilatation during stress echocardiography (thereby revealing coronary lesions),<sup>52</sup> to prevent recurrent stroke by inhibiting platelet aggregation,<sup>53</sup> and to conserve the patency of hemodialysis grafts.<sup>54</sup>

Within the intracellular compartment, adenosine is rapidly metabolized to inosine through adenosine deaminase (ADA)<sup>55</sup> or to AMP through adenosine kinase.<sup>56</sup> Inhibition of adenosine kinase by cyclosporine — leading to enhanced extracellular adenosine concentrations — may contribute, at least in part, to the antiinflammatory effects of cyclosporine.<sup>57</sup> Studies in mice with genetic deletion of *Ada* show elevated extracellular adenosine levels, leading to severe lung inflammation.<sup>58</sup> Cytotoxic effects of adenosine metabolites on lymphocytes in patients with a defect in the gene encoding ADA cause severe combined immunodeficiency (SCID). Gene therapy with autologous CD34+ bone marrow cells transduced with a retroviral vector containing the ADA gene has been used for the treatment of patients with ADA-associated SCID.<sup>59</sup>

## PURINERGIC SIGNALING AS A REGULATOR OF PLATELET FUNCTION

Inflammation is closely linked to thrombosis, and platelets are critical mediators in this process. ATP and ADP signaling plays an essential role during platelet activation (Fig. 3). ADP initiates platelet aggregation by simultaneous activation of P2Y1 and P2Y12. Activation of P2Y1, in turn, activates phospholipase C and triggers shape changes, whereas P2Y12 couples to the G protein G<sub>i</sub> to reduce adenylyl cyclase activity and inactivate the fibrinogen-receptor glycoprotein IIb/IIIa (GPIIb/IIIa) receptor, which is critical for platelet aggregation.<sup>62</sup> Thienopyridine drugs such as ticlopidine and clopidogrel exert their antithrombotic effect through inhibition of the platelet P2Y12 receptor.<sup>63,64</sup> Patients with a defect in the P2Y12-receptor gene have a congenital bleeding disorder.<sup>65</sup> In contrast, gain-of-function mutations of the P2Y12 gene are associated with increased ADP-induced platelet aggregation, and a case–control study suggested that persons with such mutations

have an increased risk of arteriosclerosis.<sup>66,67</sup> However, a large clinical trial that examined genetic determinants of the response to clopidogrel and cardiovascular events showed that gene mutations influencing drug absorption and metabolic activation, rather than *P2Y12* mutations, are more clinically relevant for pharmacologic effects.<sup>68</sup> Platelets also express P2X1 receptors, which alter platelet aggregation and shape change with ADP and ATP activation in vivo.<sup>61</sup>

Experimental studies involving mutant mice with induced defects in the extracellular conversion of ATP and ADP to adenosine have shown that termination of nucleotide signaling and concomitant generation of adenosine inhibit platelet activation. Thus, *Cd39*<sup>-/-</sup> mice had increased cardiac ischemic injury,<sup>20</sup> increased cerebral infarct volumes, and reduced postischemic perfusion after experimental stroke induction, whereas treatment with soluble ectonucleotidases corrected the phenotype and was therapeutic in wild-type mice.<sup>69</sup>

Ex vivo treatment of human blood with soluble nucleotidase — which enhances the conversion of AMP to adenosine — is associated with elevated adenosine levels and inhibition of platelet aggregation.<sup>70</sup> Signaling events through ADORA2A<sup>71</sup> and ADORA2B<sup>72</sup> inhibit platelet aggregation. Similarly, the adenosine uptake inhibitor dipyridamole is used as a platelet inhibitor for the prevention of recurrent stroke<sup>53</sup> or to improve the patency of hemodialysis grafts in patients.<sup>54</sup> Moreover, the P2Y12-receptor antagonist ticagrelor inhibits platelet function — at least in part — by inhibiting adenosine uptake, thereby elevating extracellular adenosine levels.<sup>73</sup>

## EXTRACELLULAR ATP AND ADENOSINE DURING ISCHEMIA AND REPERFUSION

Ischemia–reperfusion injury is linked to important clinical conditions such as organ transplantation and cardiovascular disease characterized by the activation of inflammatory pathways.<sup>4,74</sup> Extracellular ATP release can elicit an immune response during ischemia and reperfusion, acting as a chemotactic signal for phagocytes,<sup>5</sup> activating the NLRP3 inflammasome,<sup>75</sup> and have chemotactic effects on inflammatory cells.<sup>76</sup> Pharmacologic strategies to block ATP release or P2-receptor signaling are thought to be useful in attenuating sterile inflammation during ischemia and reperfusion.<sup>4</sup>

In contrast, extracellular adenosine has been referred to as a “safety signal” that dampens hypoxia-induced inflammation during ischemia and reperfusion.<sup>77</sup> Extracellular conversion of ATP to adenosine has a central role in attenuating sterile inflammation during ischemia–reperfusion injury. Experimental studies have shown that pharmacologic strategies to increase the breakdown of ATP to adenosine are effective in attenuating tissue injury and sterile inflammation during ischemia and reperfusion.<sup>17,19,20,31,32,78,79</sup> In addition, several experimental studies provide evidence of a protective role of adenosine signaling in models of ischemia and reperfusion (e.g., through activation of ADORA2A on inflammatory cells<sup>33,34,45</sup> or activation of ADORA2B on vascular endothelium, epithelium, or cardiac myocytes<sup>32,36,37,80</sup>). For example, a recent study has identified a regulatory circuit in the heart through which ADORA2B signaling controls expression of the circadian protein PER2, which stabilizes the transcription factor hypoxia-inducible factor (HIF) 1 (Hif-1), promotes glycolytic metabolism, and has cardioprotective effects.<sup>38</sup> Exposure of mice to intense light stabilized Per2 in the heart and reduced cardiac injury after myocardial ischemia.<sup>38</sup> Activation of Adora2a on T cells attenuates ischemia and reperfusion in experimental models of sickle cell disease.<sup>34</sup>

## P1- AND P2-RECEPTOR SIGNALING DURING ACUTE LUNG INJURY

Acute lung injury is among the leading causes of morbidity and mortality associated with critical illness. Experimental studies show elevated levels of pulmonary ATP after exposure to lung injury<sup>81</sup> and indicate that activation of P2 receptors such as P2Y6 or P2X7 result in enhanced inflammation and vascular leakage.<sup>9,10</sup> Studies of lung injury in mice with genetic defects in *Cd39* or *Cd73* have shown increased levels of pulmonary edema, lung inflammation, and attenuated gas exchange.<sup>29,30</sup> Genetic and pharmacologic studies in mice point to the potential role of adenosine-receptor signaling in the treatment of acute lung injury.<sup>45</sup> For example, the Adora2b agonist BAY 60-6583 increases clearance of alveolar fluid and attenuates capillary–alveolar leakage during lung injury induced by mechanical ventilation in mice.<sup>47</sup>

Other experimental studies suggest that iatrogenic hyperoxia — used for the treatment of patients with acute lung injury and shock like states — may suppress protective adenosine-signaling effects. One of us has noted that mice exposed to acute lung injury while breathing air with an oxygen concentration of 60 to 100% — thereby mimicking therapeutic oxygenation — have higher mortality than mice kept at sea level while breathing ambient air with an oxygen concentration of 21%. Inhalation of ADORA2A agonists compensates for the oxygenation-associated loss of generation of endogenous adenosine and preserves ADORA2A-mediated tissue protection while allowing the oxygenation of hypoxic tissues.<sup>82</sup>

## PURINERGIC SIGNALING IN IBD

IBD is associated with excessive inflammation of the bowel, and purinergic signaling has been implicated in IBD. Intestinal inflammation is associated with a severe shift in metabolic supply and demand for oxygen, resulting in profound hypoxia of the inflamed mucosa.<sup>83</sup> This metabolic alteration is associated with the post-translational stabilization of hypoxia-dependent transcription factors such as HIF — the key transcription factor for adaptation to hypoxia.<sup>84</sup> Studies have shown that hypoxia signaling transcriptionally induces *CD39* and *CD73* during intestinal inflammation, thereby shifting the balance from ATP to adenosine signaling.<sup>16–18</sup> Moreover, pharmacologic studies in mice suggest that HIF activators can attenuate intestinal inflammation<sup>85,86</sup> and that this protection involves enhancement of extracellular adenosine production and signaling (Fig. 4).<sup>91</sup>

ATP signaling has been implicated in long-term gut dysmotility and enteric-nerve injury in IBD, findings that are consistent with a proinflammatory role of P2-receptor signaling in this disorder. P2X7, pannexin-1 channels, the Asc adapter protein, and caspases are all involved in the ATP-induced signaling pathways that drive enteric-nerve death during intestinal inflammation.<sup>87</sup> In addition, regulatory T cells — a subset of CD4+ T lymphocytes that are critical in suppressing experimentally induced intestinal inflammation<sup>92</sup> — require both CD39- and CD73-dependent production of extracellular adenosine for their suppressor functions in mouse models.<sup>93</sup> In general, mice in which *Cd39*<sup>92</sup> or *Cd73*<sup>94</sup> has been knocked out have a more severe course of experimentally induced intestinal inflammation than do normal mice, a finding that is consistent with this notion. Adenosine-receptor signaling appears to have antiinflammatory and barrier-protective effects during experimentally induced intestinal inflammation, through adenosine signaling events involving Adora2a or Adora2b receptors.<sup>89,90,95</sup> Moreover, the antiinflammatory effects of sulfasalazine and methotrexate — both commonly used to treat IBD — involve the release of extracellular adenosine.<sup>27,28</sup> Together, these findings highlight the therapeutic potential of pharmacologic strategies that shift the balance from proinflammatory activation of P2 receptors to antiinflammatory activation of adenosine receptors (particularly Adora2a and Adora2b)<sup>89,90</sup> for the treatment of IBD. For example, such a shift can be achieved with the use of HIF

activators, soluble forms of apyrase (which converts ATP and ADP to AMP) or ectonucleotidases (which convert AMP to adenosine), or adenosine-receptor agonists.

## IMMUNOTHERAPY OF CANCER

Although studies have implicated purinergic signaling in the rate of cancer-cell growth,<sup>96</sup> purinergic signaling has immunologic consequences in patients with neoplastic disease. Recent advances in cancer treatment have included adoptive T-cell therapy, pharmacologic use of antibodies, and boosting of innate immune responses, with encouraging results in lymphoproliferative disease and “immune-responsive tumors” such as renal-cell cancer and melanoma. However, effective immunotherapeutic targeting of other common solid cancers remains elusive.

Damage-associated molecular pattern molecules are released by injured tissue and by cancer cells to initiate adaptive and innate immune responses. These molecules include purines such as ATP, which mediates inflammasome activation<sup>75</sup> and activation of dendritic cells, thereby potentiating tumor antigen presentation and tumor clearance. Enhancement of ATP-mediated effects on the immune system may constitute a new and effective means of inducing anticancer activity. Indeed, ATP conversion to adenosine is immunosuppressive. For example, a subset of type 17 helper T cells express CD39 and CD73, thereby leading to adenosine release, regulatory functions, and the subsequent suppression of CD4+ and CD8+ T-cell effector functions.<sup>97</sup> T-cell signaling through ADORA2A generates immunosuppressive mechanisms, limiting tissue injury.<sup>45,98</sup> In contrast, these processes preclude autoimmunity and provide protection for malignant tumors in hypoxic environments characterized by high levels of adenosine.<sup>99</sup> On the basis of these observations, induction of ectonucleotidases and consequent enhancement of adenosine-dependent Adora2a signaling has been thought to play a role in the pathophysiological inhibition of T lymphocytes. For example, genetic deletion or pharmacologic inhibition of ADORA2A or CD39 strongly augments tumor immune injury and rejection by T cells.<sup>100,101</sup> Strategies for manipulating the tumor microenvironment with adenosine-receptor antagonists or inhibition of ectonucleotidases warrant investigation as potential cancer treatment.

## CONCLUSIONS

Purinergic signaling is an important regulatory mechanism in a wide range of inflammatory diseases. There are many instances in which signaling events initiated by adenosine P1 receptors and those initiated by nucleotide P2 receptors have opposing effects in biologic systems, and shifting the balance between purinergic P1 and P2 signaling is an emerging therapeutic concept in efforts to dampen pathologic inflammation and promote healing. Several drugs that affect purinergic signaling — such as adenosine, caffeine, clopidogrel, and dipyridamole — are already used in patients. Increasing developments in this arena will open up several new avenues for the treatment of patients with inflammatory diseases.

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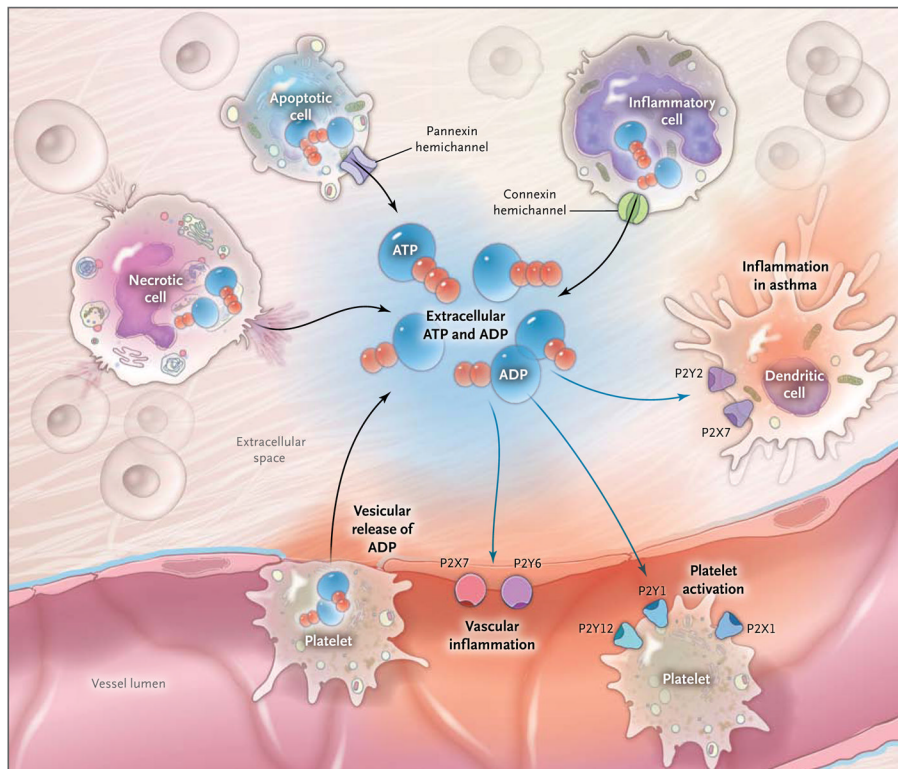
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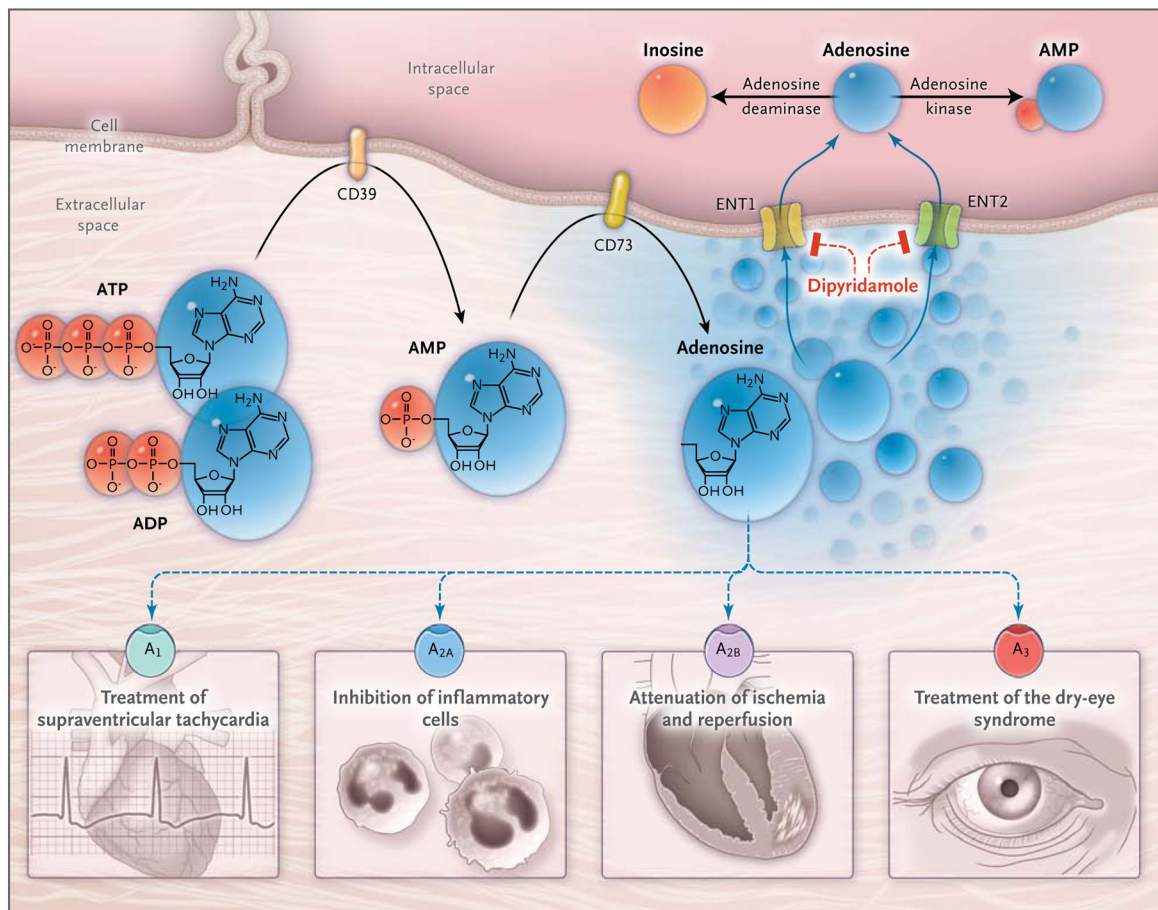
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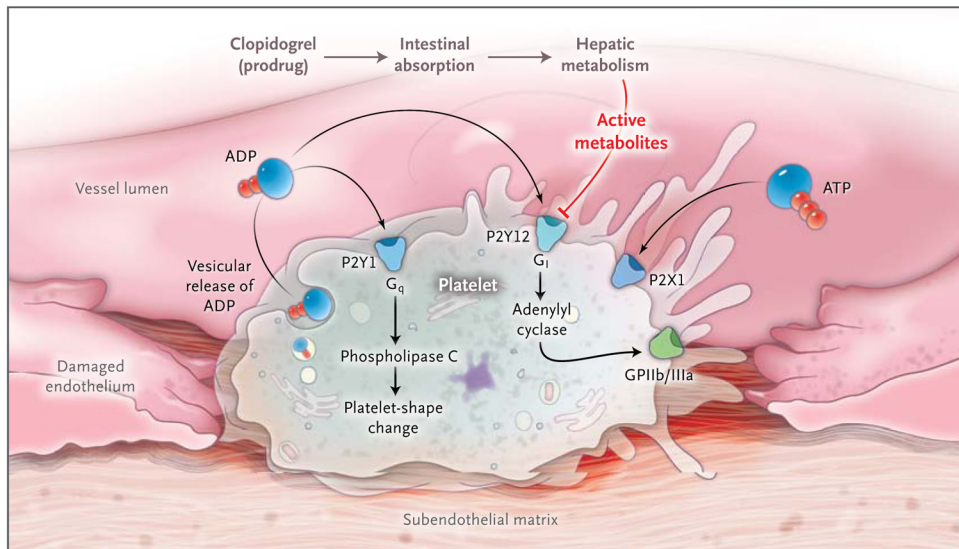
**Figure 1. Release of Extracellular Adenosine Triphosphate (ATP) and Adenosine Diphosphate (ADP) and Activation of ATP (P2) Receptors during Inflammation**

During inflammatory conditions that occur in vascular thrombosis, hypoxia, ischemia, inflammatory bowel disease, and acute lung injury, multiple cell types release nucleotides, typically in the form of ATP or ADP, from the intracellular compartment into the extracellular space. The release of nucleotides includes release of ATP from necrotic cells, pannexin-hemichannel– dependent release of ATP during apoptosis, and release of ATP through connexin hemichannels from activated inflammatory cells such as polymorphonuclear granulocytes (neutrophils).<sup>4,5,8</sup> In addition, release of extracellular ATP has been shown to occur through vesicular exocytosis or connexin hemichannels from endothelial<sup>7</sup> and urothelial cells, osteoblasts, and astrocytes, as well as nerves (not shown).<sup>3</sup> An additional source of extracellular nucleotides in inflammatory conditions is provided by activated platelets, which release ATP and ADP through the release of granules and exocytosis. In the extracellular space, these nucleotides function as signaling molecules that can activate P2Y receptors (G-protein– coupled receptors) or P2X receptors (ligand-gated ion channels). Examples of nucleotide-receptor signaling in inflammatory conditions include P2Y6- or P2X7-receptor signaling, which mediates vascular inflammation,<sup>9,10</sup> and P2Y1-, P2X1-, and P2Y12-receptor signaling, which mediates platelet activation. Activation of P2 receptors of the P2Y2 and P2X7 family that are expressed on dendritic cells is thought to play a role in promoting lung inflammation in chronic lung diseases such as asthma.<sup>11</sup>



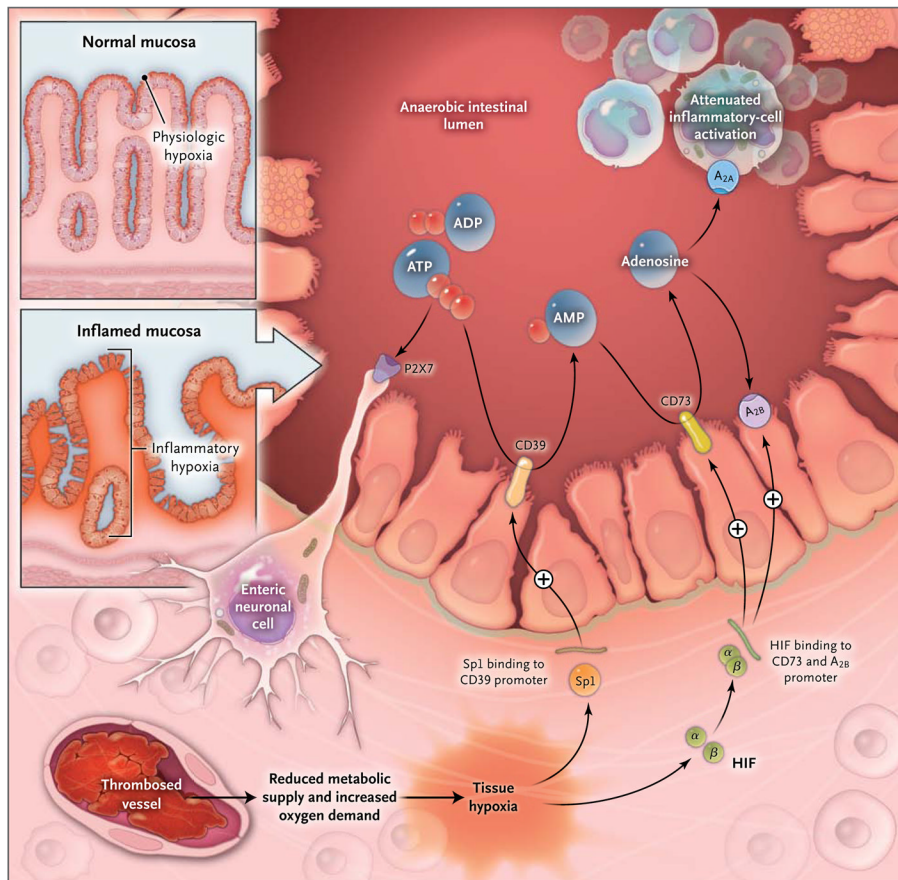
**Figure 2. Extracellular Adenosine Signaling and Its Termination**

In inflammatory conditions, extracellular adenosine is derived predominantly from the enzymatic conversion of the precursor nucleotides ATP and ADP to AMP through the enzymatic activity of the ectonucleoside triphosphate diphosphohydrolase 1 (CD39) and the subsequent conversion of AMP to adenosine through ecto-5'-nucleotidase (CD73). Extracellular adenosine can signal through four distinct adenosine receptors: ADORA1 ( $A_1$ ), ADORA2A ( $A_{2A}$ ), ADORA2B ( $A_{2B}$ ), and ADORA3 ( $A_3$ ). An example of the functional role of extracellular adenosine signaling is  $A_1$ -receptor activation during intravenous administration of adenosine for the treatment of supraventricular tachycardia. In addition, experimental studies implicate activation of  $A_{2A}$  that is expressed on inflammatory cells such as neutrophils<sup>33</sup> or lymphocytes in the attenuation of inflammation.<sup>34,35</sup> Other experimental studies provide evidence of signaling events through  $A_{2B}$  in tissue adaptation to hypoxia and attenuation of ischemia and reperfusion.<sup>36–38</sup> A clinical trial has shown that an oral agonist of the  $A_3$  adenosine receptor may be useful in the treatment of the dry-eye syndrome.<sup>39</sup> Adenosine signaling is terminated by adenosine uptake from the extracellular space toward the intracellular space, predominantly through equilibrative nucleoside transporter 1 (ENT1) and equilibrative nucleoside transporter 2 (ENT2), followed by metabolism of adenosine to AMP through the adenosine kinase or to inosine through the adenosine deaminase. Blockade of equilibrative nucleoside transporters by dipyridamole is associated with increased extracellular adenosine concentrations and signaling (e.g., during pharmacologic stress echocardiography or in protection of tissue from ischemia).



### Figure 3. Purinergic Signaling in Platelet Function and Thrombosis

Purinergic signaling is an important link among platelet activation, vascular thrombosis, and inflammation. Platelets mediate primary hemostasis through adhesion, aggregation, and subsequent thrombus formation. Extracellular nucleotides are continuously released from cells associated with the blood. When the vessel wall is injured, platelets roll and become tethered to the subendothelial matrix. These interactions cause changes in the shape of platelets and vesicular release of ADP. Platelet responses to ADP require the coordinated activation of P2Y1 and P2Y12, which function as guanosine triphosphate-binding protein (G protein)-coupled receptors.<sup>60</sup> ADP-dependent activation of the P2Y1 receptor causes activation of phospholipase C mediated through the G protein  $G_q$ , leading to subsequent changes in the shape of platelets. ADP also activates P2Y12 receptors, with subsequent activation of the G protein  $G_i$ , thereby contributing to fibrinogen-receptor activation (the glycoprotein IIb/IIIa [GPIIb/IIIa] receptor) and platelet aggregation. The platelet inhibitor clopidogrel has pharmacologic effects on the P2Y12 receptor once it has been absorbed and converted from a prodrug to active metabolites. Both intestinal absorption of the prodrug and hepatic metabolism are controlled by specific gene products. Active clopidogrel metabolites irreversibly antagonize the P2Y12 receptor, which in turn inactivates the GPIIb/IIIa-fibrinogen receptor. Clinical trials that examined genetic determinants of the response to clopidogrel and cardiovascular events indicate that gene mutations affecting drug absorption and metabolic activation, rather than *P2Y12* mutations, account for the pharmacologic effects of clopidogrel. Platelets also express P2X1 receptors, which mediate modulation of platelet aggregation and shape changes after activation by ADP or ATP *in vivo*.<sup>61</sup>



**Figure 4. Hypoxia Control of Extracellular Adenosine Generation and Signaling in Intestinal Inflammation**

Histologic staining of intestinal sections for hypoxia shows that hypoxia is present within the apical surface of the intestinal mucosa (orange area in upper insert). This presence is most likely due to the fact that the intestinal lumen is anaerobic, which results in a steep oxygen gradient across the epithelial monolayer. In patients with intestinal inflammation such as that which occurs in the course of inflammatory bowel disease, a decrease in metabolic supply (e.g., due to thrombosed vessels) and profound increases in oxygen demand result in an imbalance in oxygen availability. This imbalance causes severe hypoxia of the inflamed mucosa, as indicated by histologic staining for tissue hypoxia (as shown in the lower insert, the orange staining that extends from the apical aspects of the mucosa into the crypts and submucosal tissues indicates severe tissue hypoxia).<sup>83</sup> Release of ATP or ADP from inflammatory cells, platelets, or epithelial cells results in the activation of P2 receptors such as the P2X7 receptor, expressed on enteric neurons, thereby promoting tissue inflammation and injury.<sup>87</sup> Hypoxia causes the activation of transcriptional programs that result in an Sp1-dependent induction of CD39<sup>17</sup> and a hypoxia-inducible factor (HIF)-dependent induction of CD73<sup>25</sup> and the ADORA2B (A<sub>2B</sub>) adenosine receptor.<sup>88</sup> These transcriptional changes lead to an increased rate of turnover of the extracellular nucleotides ATP and ADP to AMP (through CD39) and subsequently to adenosine (through CD73). Experimental studies indicate that adenosine-receptor activation — particularly through Adora2a (A<sub>2A</sub>)<sup>89</sup> and A<sub>2B</sub><sup>90</sup> — dampens intestinal inflammation and promotes epithelial integrity during intestinal inflammation.

Table 1

Clinical Therapeutic Agents Targeting Purinergic Signaling.

Drug	Target	Indication	Mechanism of Action	Potential Side Effects
Clopidogrel	P2Y <sub>12</sub> nucleotide receptors	Acute coronary syndrome	Inhibition of P2Y <sub>12</sub> ; direct and irreversible inhibition of platelet function	Hemorrhage, neutropenia, thrombotic thrombocytopenic purpura (rare)
Adenosine	Cardiac A <sub>1</sub> adenosine receptors	Supraventricular tachycardia, pharmacologic stress echocardiography	Adenosine receptor agonist; induction of transient complete heart block (agonist on A <sub>1</sub> adenosine receptors), coronary vasodilatation (agonist on A <sub>2A</sub> adenosine receptors)	Prolonged heart block, metallic taste
Caffeine	Adenosine receptors	Headache, fatigue	Inhibition of cerebral adenosine A <sub>2A</sub> receptors	CNS excitation, caffeine intoxication
Theophylline	Adenosine receptors	Bronchodilatation	Unclear (phosphodiesterase inhibition or antagonism of adenosine receptors)	Narrow therapeutic index, CNS excitation, seizures
Methotrexate	Enhanced CD73-dependent adenosine production	Rheumatoid arthritis, IBD, autoimmune diseases	Enhancement of adenosine-mediated inhibition of inflammation	Ulcerative stomatitis, low white-cell count, high teratogenicity
Sulfasalazine	Enhanced CD73-dependent adenosine production	Rheumatoid arthritis, IBD, autoimmune diseases	Inhibition of 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside transferase, enhancement of adenosine release at an inflamed site, and diminished inflammation through adenosine occupancy of A <sub>2</sub> receptors on inflammatory cells	Gastrointestinal distress, headache, dizziness
Dipyridamole	Equilibrative nucleoside transporters ENT1 and ENT2	Platelet inhibition, coronary vasodilatation during stress echocardiography	Inhibition of ENT1 and ENT2; increased extracellular adenosine levels due to inhibition of adenosine uptake	Oral drug: bleeding tendency; intravenous drug: chest pain, angina in patients with coronary artery disease
Regadenoson	Adenosine A <sub>2A</sub> receptors	Stress echocardiography	Agonist on A <sub>2A</sub> adenosine receptors; adenosine A <sub>2A</sub> -receptor-mediated coronary vasodilatation	Dyspnea, headache

\* CNS denotes central nervous system, and IBD inflammatory bowel disease.