

## Purinergic receptors in gastrointestinal inflammation

Vasantha L. Kolachala, Rahul Bajaj, Meghana Chalasani, and Shanthi V. Sitaraman

Division of Digestive Diseases, Emory University School of Medicine, Atlanta, Georgia

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**Kolachala VL, Bajaj R, Chalasani M, Sitaraman SV.** Purinergic receptors in gastrointestinal inflammation. *Am J Physiol Gastrointest Liver Physiol* 294: G401–G410, 2008. First published December 6, 2007; doi:10.1152/ajpgi.00454.2007.—Purinergic receptors comprise a family of transmembrane receptors that are activated by extracellular nucleosides and nucleotides. The two major classes of purinergic receptors, P1 and P2, are expressed widely in the gastrointestinal tract as well as immune cells. The purinergic receptors serve a variety of functions from acting as neurotransmitters, to autocrine and paracrine signaling, to cell activation and immune response. Nucleosides and nucleotide agonist of purinergic receptors are released by many cell types in response to specific physiological signals, and their levels are increased during inflammation. In the past decade, the advent of genetic knockout mice and the development of highly potent and selective agonists and antagonists for the purinergic receptors have significantly advanced the understanding of purinergic receptor signaling in health and inflammation. In fact, agonist/antagonists of purinergic receptors are emerging as therapeutic modalities to treat intestinal inflammation. In this article, the distribution of the purinergic receptors in the gastrointestinal tract and their physiological and pathophysiological role in intestinal inflammation will be reviewed.

adenosine receptor; P2 receptor; inflammation

PURINERGIC RECEPTORS COMPRISE a family of transmembrane receptors, which are activated by extracellular nucleosides and nucleotides. This large family of receptors has been subdivided into two major classes, P1 and P2, that have preferential affinity for adenosine and ATP, respectively. Since the original purinergic receptor hypothesis and classification by Burnstock (16, 17), extensive investigation has established their indispensable role in cellular homeostasis and raised excitement over the potential of developing therapeutic targets in many pathological conditions.

Purinergic receptors are expressed widely in the gastrointestinal (GI) tract and liver and serve a variety of functions from acting as neurotransmitters to autocrine and paracrine signaling to cell activation and immune response. These receptors are also expressed widely on immune cells. Indeed, the original impetus for targeting purine analogs as therapeutic agents was their noted anti-inflammatory effects on neutrophils (28). Recent data suggest that anti-inflammatory effects of drugs such as methotrexate (30) and sulfasalazine (49) are, in part, mediated through their ability to release adenosine. ATP (143) and UTP (75) are released by many cell types in response to specific physiological signals. Consequently, low concentrations of nucleotides and nucleosides are present in blood, bile, and interstitial fluids under normal conditions. However, during inflammation ATP levels in the vicinity of the cell rises rapidly. It is estimated that up to 1% of intracellular ATP can be released in response to stimuli. Given that intracellular ATP is around 3–6 mM, such an increase is highly consequential in the kinetics of the P2 receptor (46, 113). ATP is rapidly

metabolized into adenosine by ectonucleotidases, and adenosine in turn acts on the P1 receptor to elicit additional cellular responses. In this article, the distribution of purinergic receptors P1 and P2 in the GI tract and their physiological and pathophysiological role will be reviewed.

### P1 Receptors

P1 or adenosine receptors represent a family of G protein-coupled receptors that are expressed in a wide variety of tissues. This family contains four receptor subtypes: A1 and A3, which mediate inhibition of adenylate cyclase, and A2a and A2b, which mediate stimulation of adenylyl cyclase. A growing body of literature for each specific receptor has elucidated important functions in intestinal and liver pathophysiology.

### A1 Receptor

A1 receptor is ubiquitous in the central and peripheral nervous system and is abundantly expressed in enteric neurons. Its mRNA is also present in peripheral tissues including the stomach, spleen, lung, kidney, small intestine, and liver at varying levels (35, 110). A1 receptor mRNA has been detected at low levels in the large intestine and mediates secretory reflexes (24, 27, 67, 69). In addition, A1 receptors are expressed on a variety of immune cells, including neutrophils and monocytes (116). A1 receptor responses are mediated through its coupling to the Gi and Go family of G proteins (48, 90). The activation of A1 receptor accordingly inhibits adenylate cyclase activity, causing a decrease in second messenger cAMP (80, 134). Stimulation of A1 receptor activates phospholipase C, leading to phosphoinositide metabolism and an increase in inositol 1,4,5-trisphosphate and diacylglycerol and Ca<sup>2+</sup> mobilization (64, 87).

Address for reprint requests and other correspondence: S. V. Sitaraman, Division of Digestive Diseases, Rm. 201-F, 615, Michael St., Whitehead Research Bldg., Emory Univ., Atlanta, GA 30322 (e-mail: ssitar2@emory.edu).

A1 receptors promote G protein-dependent neutrophil migration, activation, and vascular attachment (117) and monocyte phagocytosis (116). Adenosine acts on neutrophil A1 receptor to generate superoxide anions essential in defense against microorganisms or removal of cellular debris. An upregulation of A1 receptor activation on neutrophils and monocytes is thought to underlie the proinflammatory effects of A1 receptor in some tissues. This initial cascade appears essential as a cellular defense mechanism. Studies using A1 receptor gene knockout mice and antagonists have shown that A1 receptor activation mediates protective anti-inflammatory effects in the liver. In a model of murine sepsis using cecal ligation and puncture, A1 receptor knockout mice were assessed for degree of hepatic injury and cytokine secretion (51). The A1 receptor knockout mice were highly susceptible to increased hepatic injury and showed elevated levels of TNF- $\alpha$  and mortality, providing evidence that A1 receptor activity plays a protective role in this model of sepsis. These effects of A1 receptor on sepsis were reproduced with pharmacological blockade of the receptor in mice, confirming its protective effects on inflammation.

The mechanism underlying the anti-inflammatory effect of A1 receptor is unknown but it may modulate the inflammatory response through its effect on immune cell function. For example, dendritic cells are important in the initiation and regulation of immune responses (119, 133). A1 receptors are highly expressed on immature dendritic cells and may account for initial dendritic cell recruitment to inflamed sites. Activated mature dendritic cells, on the other hand, also highly express A2a receptors (119), which stimulate several anti-inflammatory pathways. The A1 receptor may thus serve as the initial gateway to the inflammatory cascade promoting immune cell migration and activation required for primary host defense.

In addition to its effect on immune cell migration and function, A1 receptor has been shown to play a significant role in luminal adenosine-induced chloride secretion in human jejunum (54). In a study using rigorous electrophysiological techniques, the investigators demonstrated that luminal adenosine-induced chloride secretion was mediated by A1 receptor even though A2b receptor was the most abundant adenosine receptor expressed in jejunal mucosa. A1 receptor is abundantly expressed in the myenteric plexus in the GI tract, wherein it is thought to play an important role in intestinal motility (24). Some studies have shown that A1 receptor mediates cholinergic nerve activity in ileum and the impaired cholinergic neurotransmission may mediate altered motility during inflammation (33, 67, 130). One study has demonstrated a beneficial effect of A1 receptor agonist in ischemic intestinal injury (103). As more information regarding the underlying physiology of the A1 receptors is gathered, further studies are required to define its role in intestinal inflammation if therapeutic targets are to be developed.

### A2a Receptor

A2a receptor is widely expressed in intestinal mucosa, enteric neurons (24), and hepatocytes (22) as well as in a variety of immune cells, including neutrophils (29), monocyte/macrophages (61), dendritic cells, lymphocytes (53), eosinophils (144), and mast cells (131). In the intestine, it is expressed in the jejunum, ileum, and cecum (24). It is positively

coupled to G $\alpha$ s and activates adenylate cyclase. Activation of A2a receptors produces a constellation of effects on a variety of inflammatory cell types that can attenuate injury as a result of mucosal inflammation, ischemia, or sepsis and thus A2a receptor is viewed as anti-inflammatory. Indeed, A2a receptor may be the dominant receptor in mediating adenosine's function as a retaliatory metabolite (93, 94) and anti-inflammatory agent (121).

A2a receptor and its agonists modify the activity, cytokine, and chemokine responses of neutrophils and other immune cells. Such modulation of cytokine secretion, in part, underlies its anti-inflammatory response (28, 53, 128, 129). The activation of the A2a receptor inhibits the neutrophil oxidative burst and acts principally through cAMP-dependent protein kinase A (29, 129). Lipopolysaccharide (LPS)-activated neutrophils from A2a<sup>-/-</sup> mice had higher levels of TNF- $\alpha$ , MIP-1a, and MIP-1b compared with neutrophils from wild-type mice expressing A2a receptor. Engagement of the adenosine A2a receptor selectively prevents the expression and release of TNF- $\alpha$ , MIP-1a, Cel3, MIP-1b/ccl4, MIP-2a/cxcl2, and MIP-3a/cel20 (86).

A2a receptor on human monocytes is cAMP dependent (68) and inhibits the secretion of IL-12, a proinflammatory cytokine and a major inducer of Th1 responses. A2a receptor activation also enhances IL-10 production, an anti-inflammatory cytokine, by monocytes, and thus adenosine binding at A2a receptors may also contribute to the resolution of an inflammatory response. Finally, a recent study has suggested that heterologous desensitization of A2a receptors attenuated chemokine receptor signaling and consequent neutrophil chemotaxis induced by IL-8 and MCP-1 (145). Together, these studies suggest that adenosine, when released during inflammation or tissue injury, may contribute to the selective suppression of Th1 responses and the cellular immune response demonstrating the potent anti-inflammatory role of the A2a receptor.

The beneficial effects of A2a agonists have been shown in a number of inflammatory diseases such as gastritis, colitis, and ischemic-reperfusion injury in the liver. Stress-induced gastritis correlates with gastric neutrophil accumulation, inflammatory cytokine production, free radical production, mucosal blood flow, and increased acid secretion (60, 66, 102). ATL-146e, a specific A2a agonist, inhibits both stress-induced and aspirin-induced gastric inflammation and damage (96, 99). Odashima et al. have demonstrated that an injection of ATL-146e given prior to water-immersion stress and aspirin-induced injury reduces the extent of gastric mucosal lesions in mice (96, 145). This may occur, in part, by inhibition of neutrophil infiltration into the gastric mucosal tissue and inhibiting the production of proinflammatory cytokines in gastric mucosal tissue (96, 99).

The role of A2a receptor in infectious colitis has been studied with both specific receptor agonists and A2a gene knockout mice. In a model of colitis mediated by *Clostridium difficile* toxin A, ATL-313, another A2a receptor-specific agonist, reduced tissue injury and inflammation in mice. In these studies, A2a stimulation decreased ileal MPO activity, edema, hemorrhage, inflammatory cell infiltration, TNF- $\alpha$  production, and mucosal disruption, and increased adenosine deaminase activity (21).

Inflammatory bowel disease (IBD) is an important example of dysregulated immune responses from Th1/2 cells (43). A2a

receptor has clearly shown beneficial effects on the development of intestinal inflammation in a variety of animal models of IBD. A2a<sup>-/-</sup> mice are highly susceptible to severe colitis in these animal models. Formalin immune complex-induced colitis in rabbits resembles human ulcerative colitis with a similar cytokine and Th2 profile (25, 26, 98). Odashima et al. (97) demonstrated that ATL-146e ameliorates acute and chronic colitis with improvement in organ inflammation, edema, and necrosis and reduction in cellular infiltration. SamP1/YitFC mice develop spontaneous ileitis that resembles Crohn's disease with a similar clinical, histological, and immunological profile to human disease (72, 111). A similar striking result comparable to steroid therapy was seen with reduction in active inflammation and villous injury in SamP1/YitFC mice treated with ATL-146e. These effects may be in part due to effect of A2a-mediated suppression of cytokine production, namely decreasing TNF- $\alpha$  and IFN- $\gamma$ . The effect of A2a stimulation on Th profile was supported further in another model of colitis induced by the transfer of CD45RB high donor cells into mice with severe combined immunodeficiency. In this model, transfer of regulatory wild type T cells blocks colitis induced from CD45RB high cells, and these effects were lost when regulatory T cells were obtained from A2a knockout mice. TNF- $\alpha$ , IFN- $\gamma$ , and IL-2 mRNA levels in T cells from control mice were significantly decreased by A2a agonists whereas no effect on cytokine mRNA levels were seen when CD4<sup>+</sup> T cells were derived from A2a<sup>-/-</sup> mice (91). These data support the modulation of immune cell cytokine expression as a mechanism by which A2a receptors exert their anti-inflammatory effect.

A brief period of ischemia followed by reperfusion has been shown to be protective against reperfusion injury in the heart (88), central nervous system (15), and liver (105). This ischemic preconditioning has been shown to improve graft survival during liver transplantation, and early damage is thought to be due to loss of viability of sinusoidal endothelial cells or nonparenchymal cell injury. Adenosine has been shown to mimic this preconditioning, with levels increasing to three times the control level in the reperfusion phase (92). Pharmacological preconditioning with A2a receptor agonist, CGS 21680, attenuates postischemic apoptotic cell death in rat liver transplant models (9) and preserves hepatic tissue blood flow during ischemia (3, 60, 105). This effect was nearly abolished by selective receptor blockade with adenosine A<sub>2</sub> receptor antagonist 3,7-dimethyl-1-propargylxanthine, whereas no change was seen with A1 receptor blockade. These effects correlated with an increase in cAMP levels in sinusoidal endothelial cells, and exogenous dibutyryl cAMP simulated the protective effects on nonparenchymal cell killing (3).

A2a receptor also mediates protective effects in other models of hepatic injury. Genetic knockout of A2a receptor increases the severity of hepatic injury from toxins (concanavalin A, LPS) and duration of cell-mediated proinflammatory cytokine production. Liver injury by concanavalin A is dependent on CD4 Th cells and macrophages and proinflammatory cytokine production such as TNF- $\alpha$  (96). ATL-146e limits LPS-induced liver injury with suppression of TNF- $\alpha$ , IL-6, and IFN- $\gamma$  (32, 98). The striking phenotype of markedly enhanced liver damage and sepsis in A2a-deficient mice suggests that no other mechanism of downregulation of inflammation *in vivo* is able to fully compensate for the lack of A2a receptors in T cells and cells of the innate immune system (101).

Mechanisms of beneficial effects of drugs are being linked to adenosine receptors as well. The protective effects of antioxidants, such as S-adenosylmethionine (SAME), in the liver are thought to be mediated by the A2a receptor (122). SAME is the first product in methionine metabolism, a precursor for GSH as well as a methyl donor in most transmethylation reactions. In a rat liver ischemia model, SAME flush protected parenchymal injury from cold and rewarming ischemia and improved hemodynamic and functional abnormalities as well as bile flow. This effect was blocked with 8-PT, an A2a receptor antagonist, by blocking vasodilation effects (39).

Together, these data suggest that targeting A2a receptors have many clinical applications to arrest inappropriate or excessive inflammatory responses in various portions of the GI tract.

### A2b Receptor

A2b receptor mRNA, highly expressed in the cecum and colon, is also found in the esophagus, stomach, and jejunum but appears to be absent in the ileum (124). A2b receptor is also expressed in the glial cells and myenteric plexus of the jejunum where it plays a role in the motility of the GI tract (24). Northern blot analysis of mRNA from human colon has shown that A2b receptor is the only adenosine receptor present in the human colonic epithelia (125, 126) and is the predominant adenosine receptor in the jejunum (24). A2b receptor is the only adenosine receptor in colonic epithelial-derived cell lines such as T84. In intestinal epithelial cells, A2b receptor positively couples to G $\alpha$ s and activates adenylate cyclase in contrast to coupling with Gq in other tissues (6, 41). The predominant signaling pathway of A2b receptor in the intestine is through increased cAMP, phosphorylation of cAMP response element-binding protein (CREB; 56) as well as protein kinase A (PKA) activation (40, 129).

Adenosine regulates ion transport in a variety of epithelia (36, 57, 107). In colonic epithelium, A2b receptor plays a prominent role in regulating chloride secretion, through the activation of apical cystic fibrosis conductance regulator (CFTR; 7, 8, 13, 126, 127). The chloride secretory pathway results in movement of isotonic fluid into the lumen, a process that naturally serves to hydrate the mucosal surface, thereby protecting the intestine by preventing the translocation of bacteria, bacterial products, and antigens to lamina propria (5, 47). This secretory mucosal flush often parallels active inflammatory responses elicited by luminal pathogens and serves as a crude form of mucosal defense (11, 74). However, an upregulation of ion secretion during inflammation is considered to be an important component of inflammation-associated diarrhea (12, 59). Recent studies in our laboratory demonstrated that A2b receptor expression is upregulated during human IBD as well as in murine models of colitis (70) and potentially plays a role in inflammation-associated diarrhea.

A2b receptor mediates a variety of cellular signals and protein synthesis potentially involved in inflammation. IL-6 is an important proinflammatory cytokine that is consistently seen in high levels in the serum and tissue of patients with active IBD (63, 81, 109). Stimulation of A2b receptor causes an increase in IL-6 secretion, which is polarized to the apical (luminal) compartment (120). This appears to be transcriptionally mediated by activating transcription factor and CREB



elements (120). The apical secretion of IL-6 may also contribute to immune regulation at the mucosal surface through neutrophil activation. Crypt abscesses are central to the pathogenesis of intestinal inflammation characterized by a collection of neutrophils intimately located with the apical membrane of intestinal epithelial cells. A2b receptor-mediated IL-6 secretion has been shown to increase intracellular calcium in neutrophils, which is correlated with neutrophil degranulation (120). A2b receptors may therefore provide epithelial-derived paracrine signals to neutrophils as an additional means of regulation of inflammatory responses by intestinal epithelia.

Adenosine also mediates polarized fibronectin synthesis and secretion in colonic epithelial cells (137). Fibronectin, an extracellular matrix protein, is upregulated and secreted into the apical compartment when T84 cells are stimulated with adenosine. The apical secretion of fibronectin is rather surprising given that fibronectin is present in the lamina propria. Interestingly, cAMP has been shown to traffic proteins to the apical surface, and the locus of cAMP action on the secretory pathway is at least in part at the level of the trans-Golgi network (89). Functionally, adenosine-induced fibronectin significantly enhances the adherence and invasion of *Salmonella typhimurium* to cultured epithelial cells as well as consequent IL-8 secretion (137). It is possible that A2b receptor-mediated cAMP increase may play a role in trafficking of IL-6 and fibronectin from their classical localization on the basolateral side to the apical surface. In combination with apically driven chloride secretion, adenosine-induced fibronectin and IL-6 secretion may serve as critical host factors that modulate adherence and invasion of bacteria, thus playing a key role in mucosal immune responses during inflammation.

Yet the overall outcome of A2b receptor function during inflammation seems to depend on the cytokine profile in the intestinal milieu. We have demonstrated that TNF- $\alpha$ , an important cytokine whose levels are increased in the intestinal mucosa, serum, and stools of patients with IBD, is an important regulator of A2b receptor and upregulates A2b receptor mRNA and protein expression (70). IFN- $\gamma$ , another cytokine that plays an important role in IBD, downregulates adenosine-mediated signaling by directly inhibiting adenylate cyclase expression and affecting global cAMP-mediated responses in the intestinal epithelia (71). TNF- $\alpha$  and IFN- $\gamma$  are increased at various stages of inflammation, and it is possible that a premature inhibitory effect of IFN- $\gamma$  on adenylate cyclase may further aggravate the proinflammatory process in acute or chronic colitis whereas a late and sustained increase in TNF- $\alpha$  may contribute to inflammation through chloride, IL-6, and fibronectin secretion.

The upregulation of A2b receptor during colitis and its functional effects discussed above suggest that A2b receptor may play a largely proinflammatory role in the development of colonic inflammation. A2b receptor antagonists may therefore serve as potential therapeutic targets for treating intestinal inflammation. Indeed, ongoing studies in our laboratory indicate that orally administered A2b receptor antagonists ameliorate clinical symptoms and histological signs of colitis in two animal models of IBD. Our data demonstrated that A2b receptor specific antagonist ATL 801 given in the diet showed improved histological and clinical score in dextran sodium sulfate (DSS)-induced colitis in mice. It also inhibited the proinflammatory cytokine IL-6 and keratinocyte-derived che-

mokine in colonic cultures in mice fed with A2b receptor antagonist (unpublished data). Recent studies of Ryzhov et al. (115) demonstrated the proinflammatory effects of A2b receptor and showed that stimulation of A2b receptors upregulates the proinflammatory cytokine IL-6.

The physiological role of A2b receptor in other portions of the GI tract is still being determined. In the liver, there is renewed interest in its possible role in tumor progression. A2b receptor is highly expressed in human hepatocarcinoma cells, implicating a role in proliferation, invasion, and malignant progression (123, 139). The study of the intestinal A2b receptor, both structural and biochemical aspects as well as biological contributions to colitis, has an exciting future with the development of highly specific antagonists and possibly agonists as therapeutic targets.

### A<sub>3</sub> Receptor

A<sub>3</sub>, the fourth adenosine receptor, couples to G<sub>i</sub> and inhibits adenylate cyclase activity similar to A<sub>1</sub> receptor (1, 104, 135, 146). Several studies suggest that it couples to G<sub>q/11</sub> proteins (58, 104), although intracellular signaling mechanisms are not known in the intestine. A<sub>3</sub> receptor stimulates phospholipase C and intracellular Ca<sup>2+</sup> and has been shown to activate phosphoinositide-3-kinase in various tissues (4). A<sub>3</sub> mRNA is expressed in jejunum and proximal colon and most abundantly in the liver (35, 79, 118, 146). A<sub>3</sub> receptor has been described as mediating anti-inflammatory effects in the intestine. Mice stimulated with the A<sub>3</sub> receptor agonist, N<sup>6</sup>-(3-iodobenzyl)-adenosine-5'-N-methyluronamide (IB-MECA), in DSS and IL-10<sup>-/-</sup> models of colitis show reduced infiltration of immune cells, reduced oxidative damage, and attenuated cytokine and chemokine production (82). Lee et al. (76) demonstrated the protective effect of the A<sub>3</sub> receptor in sepsis models as well. Activation of A<sub>3</sub> receptor in a murine model of septic peritonitis using cecal ligation reduced mortality and renal and hepatic injury. Antagonism of the receptor, and genetic A<sub>3</sub><sup>-/-</sup> mice demonstrated increased acute renal and hepatic injury. In addition, studies have demonstrated that activation of A<sub>3</sub> receptor by IB-MECA inhibits LPS-induced TNF- $\alpha$  and nitric oxide levels in murine macrophage (RAW 264.7) cells (83). These effects are mediated through inhibition of intracellular Ca<sup>2+</sup>, which further suppresses NF- $\kappa$ B and ERK1/2 pathways. Continued investigation is needed to delineate the mechanism underlying anti-inflammatory effects of A<sub>3</sub> receptor in these models of sepsis.

A novel role for A<sub>3</sub> receptor in neutrophil chemotaxis has been recently described (23). One of the secretory products released by activated neutrophils is ATP. These investigators have elegantly demonstrated that ATP is crucial for neutrophil chemotaxis. Importantly, extracellular adenosine that accumulates as a result of enzymatic conversion from ATP causes the polarized localization and activation of A<sub>3</sub> receptors at the leading edge of migrating neutrophils. The effect of the adenosine gradient is to increase the speed of chemotaxis. Consistent with this observation, the authors report a reduction in chemotaxis speed by pharmacological blockade of the A<sub>3</sub> receptor. The possibility that A<sub>3</sub> agonist could inhibit neutrophil chemotaxis and thus prevent unwanted inflammation is exciting in terms of A<sub>3</sub> receptor as a therapeutic consideration for a wide range of diseases wherein neutrophil accumulation underlies their pathogenesis. This novel role for A<sub>3</sub> receptor

Table 1. *Distribution and function of the purinergic receptors in the gastrointestinal tract*

Purinergic Receptor	Distribution: Tissue and Cellular Source	Signaling	Expression Levels in Inflammation	Function	
				Normal	Inflammation
<b>P1</b>					
A1	Liver, stomach, small intestine, ileum (35), monocytes, mononuclear phagocytes, neutrophils (87, 116), enteric neurons	Gi/o (48, 90), ↓ cAMP PLC (87)	Upregulated (130) in colitis	Involved in ion secretion in jejunum (54), intestinal motility (24) cholinergic nerve function in the ileum (33, 130)	Proinflammatory in the intestine, promotes migration and activation of neutrophils (117), monocyte (116); anti-inflammatory in liver (51)
A2a	Jejunum, ileum, cecum, liver, (24), neutrophils (29), monocyte/macrophages (61), dendritic cells and lymphocytes (53), eosinophils (144), and mast cells (131)	Gsα ↑ cAMP (108)	Upregulated (44)	Inhibits overactive immune cells and protects normal tissue from excessive damage (100)	Anti-inflammatory (121); inhibits the secretion of TNF-α, FN-γ, IL-12 (91) and enhances IL-10 production by monocytes; inhibits neutrophil infiltration, secretion of proinflammatory cytokines in gastric mucosal tissue (96, 97)
A2b	Esophagus, stomach, duodenum, jejunum, colon, cecum (124), mast cells, glial cells, myenteric plexus neutrophils (44), lymphocytes (88a), macrophages (138a)	Gsα ↑ cAMP (126)	Upregulated during colitis (70)	Chloride secretion in intestine (126); participates in glycogenolysis in the liver (19)	Proinflammatory in intestine, secretes IL-6, fibronectin (115, 120, 137)
A3	Stomach, jejunum, colon ileum, cecum, liver (118), monocytes, macrophages, mast cells	Gi/o ↓ cAMP, Gq/11, PLC	Upregulated (130) during colitis	?	Anti-inflammatory in intestine and liver (76, 82); inhibits neutrophil chemotaxis
<b>P2</b>					
P2X 1-7	Hepatocytes, cholangiocytes (42), immune cells, macrophages, lymphocytes, mast cells (34), P2X2, P2X3, P2X5 expressed in enteric nervous system (20, 106, 114, 136) P2X2, P2X3, P2X4 in circular myocytes, P2X3, P2X5 are expressed in longitudinal myocytes (51)	None	P2X3 upregulated in colitis (142)	Smooth muscle relaxation, gastrointestinal motor reflexes (50)	Conveys nociceptive information from gastrointestinal to central nervous system (62)
P2Y 1-12	Intestine, liver, hepatocytes, bile duct epithelium, P2Y1 (52), P2Y2 (140), P2Y6 (141), P2Y12 (141) are expressed in enteric neurons	Gq/11, Gi, PLC (108)	?	Chloride and potassium secretion (54, 84), involved in enteric neuronal function (10)	?

open avenues for further research on the mechanism by which it inhibits neutrophil movement as well as further dissection of interplay between adenosine receptors in neutrophils that determine the final outcome of their function.

### *P2 Receptor*

The P2 class of purinergic receptors are molecularly distinct from P1 and further subdivided into P2X, ligand-gated ion channel receptors, and P2Y, seven transmembrane G protein-coupled receptors (113). These receptors preferentially bind ATP and metabolites such as UTP, ADP, and UDP (18) with varying agonist profiles. Both P2X and P2Y are expressed widely throughout the intestinal tract (77, 113, 132) and in the liver on hepatocytes and cholangiocytes (42) and immune cells (34), where they mediate physiological functions.

### *P2X Receptors*

P2X receptors are widely distributed in the enteric nervous system wherein ATP is an important neurotransmitter, but their

role in intestinal function and inflammation is largely unknown. P2X receptors are present on dorsal root ganglia and sensory neurons in the intestine. P2X2 (20), P2X3 (106, 136), and P2X5 (114) have been shown to be expressed in the enteric nervous system. A common denominator to all P2X subtypes is a Ca<sup>2+</sup> influx across nonselective cation channels (also permeable for Na<sup>+</sup> and/or K<sup>+</sup>) promoted by purines acting through the receptor channel itself. This then causes membrane depolarization leading to a secondary opening of voltage-gated Ca<sup>2+</sup> channels. The effect of P2X receptor on ion channel opening does not depend on activation of second messengers and, therefore, the cellular response time is generally very rapid (within 10 ms). Some P2X receptors also induce cytokine secretion in immune cells in addition to their effect on ion channels, through second messenger activation. For example, P2X7 activation induces IL-1β secretion by macrophages, and P2X7 gene knockout mice develop less severe arthritis (14). Recently, P2X4 receptors were shown to be expressed on biliary epithelium wherein they mediate biliary secretion (37).

The role of purinergic receptors in the enteric nervous system is an intense area of research. Sensory neurons appear to play a role in the inflammatory processes in the gut (85). Signal transduction appears to be enhanced in the presence of ATP during inflammation, and several receptors are upregulated in chronic inflammatory conditions (95, 142). In a rat model of colitis, Wynn et al. (138) demonstrated that P2X3 receptors are upregulated in dorsal root ganglia and that ATP released from distension is increased, highlighting the purinergic receptor contribution to mechanosensory transduction. Purinergic agonists also increased neuronal activity, whereas P2 antagonists reduced it. Sensitivity to afferent excitation in response to ATP is greater in colitic conditions and with P2 agonists, whereas antagonists reduced this effect. Interestingly, the upregulation was also in adjacent dorsal root ganglia, and the authors suggest that inflammation in one area may affect sensory pathways in others. Indeed, interactions of the immune system and neurohormonal control of the GI motility are being investigated as part of the pathophysiology of irritable bowel syndrome (IBS). Modifying the transmission of nociceptive stimuli through these receptors has been postulated as a therapy for IBS (50, 65), which is plagued by visceral hypersensitivity.

#### *P2Y Receptors*

P2Y4 has the highest expression in the intestinal epithelial cells. P2Y1 receptors have also been identified in intrinsic enteric neurons in both myenteric and submucosal plexuses. Like P2X receptors, P2Y receptors are present on dorsal root ganglia and sensory neurons in the intestine. P2Y1 (52), P2Y2 (140), P2Y6, and P2Y12 (141) have been demonstrated to be expressed in the enteric nervous system. P2Y 1, 2, 4, 6, 13 receptors have been described in hepatocytes and may serve to regulate gluconeogenesis and glycolysis. P2Y 2, 4, 6 receptors are present on bile duct epithelium; in addition, P2Y1 receptor mRNA is present in gallbladder epithelium. The epithelial secretion of chloride in response to UTP is mediated exclusively by the P2Y4 subtype (55) whereas both P2Y2 and P2Y4 are involved in the UTP-induced secretion of potassium (84). P2Y6 receptors are expressed on the basolateral surface of intestinal epithelial cells, and UDP acts through P2Y6 to elicit chloride secretion (73). P2Y1 and P2Y11 receptors mediate the purinergic component of nonadrenergic, noncholinergic relaxation of smooth muscle in the GI tract, whereas P2Y4 plays a major role in chloride secretion in the intestinal epithelial cells. UTP has been shown to stimulate chloride secretion and modulate bile secretion by acting through P2Y1 receptors (112).

#### *P2 Receptor and Intestinal Inflammation*

Despite a well-known role in a variety of homeostatic functions in the GI tract, little is known about the role of P2 receptor in intestinal inflammation. In situ hybridization showed the presence of P2Y6 mRNA in T cells infiltrating the lesions of patients with IBD. ATP levels are present in low concentrations in blood, bile, and interstitial fluids (46). Levels can increase dramatically in a variety of settings including cell injury, metabolic stress, cell volume, and hypoxia (113). The downstream effects of this local extracellular ATP are still

being investigated and have been shown to modify inflammation, healing (2), and enteric nervous system signal transduction. In a model of hepatic injury, ATP exacerbates chemically induced liver damage, and this is blocked with the P2 receptor antagonist suramin (31). ATP itself can induce cell death in hepatocytes (147), which is mediated by P2X7 receptors. ATP may mediate other responses to injury such as scar formation. Activated hepatic stellate cells demonstrate increased P2Y signaling and regulate procollagen-1 transcription and may serve as a target for treatment of liver fibrosis (38).

#### *Summary and Perspectives*

The role of purinergic receptor signaling during the course of inflammatory and immune responses in the intestine appears to be extremely complex since tissues and immune cells often express multiple purinergic receptors and receptors on the same cell may have opposing effects on the inflammatory response (Table 1). Several tiers of regulation exist that possibly modulate cellular function and determine the final inflammatory response from the differing receptor levels at baseline and during inflammation, varying potencies of agonists and secondary messenger systems, and the distribution of ectoenzymes that process nucleotides. With the advent of genetic knockout mice and the development of highly potent and selective agonists and antagonists for the purinergic receptors, our understanding of the concerted action of purinergic receptors on cells and tissue is being further refined. An example of such interdependent and multifaceted action of multiple purinergic receptors has been described recently (23). The purinergic signaling circuitry, summarized by Linden (78), suggests that the inflammatory response to infection or tissue damage depends on the coordination of ATP metabolism and recognition by purinergic receptors expressed on neutrophils. A neutrophil migrating toward a chemotactic stimulus (fMLP) releases ATP from its leading edge. Such ATP levels are 1,000-fold higher during inflammation. ATP is dephosphorylated by ectoenzymes (CD39 and CD73) to ADP and adenosine. Gradients of ATP and adenosine initiate and accelerate directional chemotaxis via P2Y2 and A<sub>3</sub> adenosine receptors, respectively, on neutrophils. Other adenosine receptors (A<sub>2a</sub> and A<sub>2b</sub>) inhibit neutrophil chemotaxis and adhesion to endothelial cells, as well as platelet aggregation.

Based on the role of purinergic receptor signaling, therapeutic strategies have been implemented for some central nervous system disorders and asthma (45). Important advances have been made on the role of P1 (especially A<sub>2a</sub> and A<sub>2b</sub>) and some P2Y receptors in intestinal inflammation. As our understanding of the underlying pathophysiology of these receptors evolves, the potential for therapies will expand. Thus the future for P1/P2 receptor-based therapy is highly promising with treatment of inflammatory conditions of the gut and cholestatic and fibrotic liver diseases in the distant horizon.

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