REVIEW

Adenosine: An immune modulator of inflammatory bowel diseases

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Abstract

Inflammatory bowel disease (IBD) is a common and lifelong disabling gastrointestinal disease. Emerging treatments are being developed to target inflammatory cytokines which initiate and perpetuate the immune response. Adenosine is an important modulator of inflammation and its anti-inflammatory effects have been well established in humans as well as in animal models. High extracellular adenosine suppresses and resolves chronic inflammation in IBD models. High extracellular adenosine levels could be achieved by enhanced adenosine absorption and increased *de novo* synthesis. Increased adenosine concentration leads to activation of the A2a receptor on the cell surface of immune and epithelial cells that would be a potential therapeutic target for chronic intestinal inflammation. Adenosine is transported *via* concentrative nucleoside transporter and equilibrative nucleoside transporter transporters that are localized in apical and basolateral membranes of intestinal epithelial cells, respectively. Increased extracellular adenosine levels activate the A2a receptor, which would reduce cytokines responsible for chronic inflammation.

Key words: Crohn's disease; Ulcerative colitis; Inflammatory bowel diseases; Epithelial cells; Membrane transporters; Immuno-modulator

INTRODUCTION

Adenosine is a purine molecule necessary for normal cell metabolism and growth. Recently, adenosine has been recognized as a potential anti-inflammatory molecule. In general, cellular adenosine is produced by both *de novo* synthesis and by absorption from the diet into the body through transporters in the gastrointestinal tract. It is thought that activation of adenosine receptors deactivates the synthesis of critical components necessary for activation of chronic inflammatory diseases, including inflammatory bowel disease (IBD). Many reviews have focused on the general aspects of adenosine activation of its receptors in inflamed tissues. This review focuses on the identification of the role of intestinal epithelial cell adenosine transporters during IBD.

CURRENT UNDERSTANDING OF INFLAMMATORY BOWEL DISEASES

IBD, including Crohn's disease (CD) and ulcerative colitis (UC), is a common and lifelong disabling gastrointestinal disease[1,2]. It has highest incidence and prevalence in the developed countries. The worldwide incidence varies greatly with that of UC ranging from 0.5-24.5/100 000 and that of CD ranging from 0.1-16/100 000 in different populations. There are more than 2 million IBD patients in the United States[3]. The precise mechanism of IBD is still unknown. CD and UC differ in their histological presentation and cytokine profile. The accumulated data indicate that IBD results from a complex interplay of genetic, environmental, and immunologic factors. The presence of one or more genetically determined defects leads to an over-reaction of the host mucosal

immune system to normal constituents of the mucosal microflora. The genetically determined alterations of gut epithelial barrier function enhance exposure of the mucosal immune system to microflora components. The over-reaction causes either a Th1-type T cell-mediated inflammation (Crohn's disease) or a Th2-type T cell-mediated inflammation (ulcerative colitis). Multiple cytokines are released in the inflammatory process. The most important factors are tumor necrosis factor (TNF)-α, interleukin (IL)-1, interferon (INF)-γ, IL-6, 12, 13 and 17, monocyte chemotactic protein (MCP)-1 and IL-8[4]. These cytokines attract and activate neutrophils, eosinophils, mast/plasma cells and macrophages. These inflammatory cells produce large amounts of unstable chemical species such as reactive oxygen species (ROS) or oxyradicals (i.e. superoxide anions, hydrogen peroxide, hydroxyl radicals, peroxynitrite), resulting in tissue injury[5,6].

Since the cause of IBD is still unknown, currently available treatments for the disease are non-specific and may cause side effects such as osteoporosis and suppression of the immune system. Many patients respond and maintain remission with existing therapy. Thus, at present, there is no cure for IBD. But for some patients, the available therapeutic options for IBD are still inadequate. The conventional treatments use corticosteroids, mesalamine, and immunosuppressants. These either nonspecifically block downstream inflammatory events, such as the secretion of cytokines and activation of immunocytes and neutrophils, or increase tissue adenosine levels, regardless of the nature of the underlying T cell response that generated these events. These agents have been used for treatment of mild and moderate IBD with some success for many years despite shortcomings and toxicities. The newer therapies using biologics, such as antibodies against TNF-α and α-integrin molecules, eliminate a specific major inflammatory cytokine or act by disrupting accumulation of cells at areas of inflammation. Both strategies have been successful in subsets of IBD patients but have also been associated with significant complications including fatal infections[7-12].

Emerging treatments are being developed to target the hierarchy of the inflammatory cytokine effect including IL-12/IL-23[13], IFN-γ[14], IL-6[15], and IL-10 levels[16]. Several antibodies currently on clinic trial include: anti-IL-12p40, an antibody against IL-12 and IL-23, the master cytokines underlying the Th1 response, for Crohn's disease[13]; anti-IL-23p19, a potentially useful treatment for patients with resistance to anti-TNF therapy which acts by targeting IL-23 and IL-17 rather than IL-12 and IFN-γ in experimental colitis. Other approaches to the treatment of IBD currently under investigation are leukocytapheresis to eliminate effector cells[17,18], administration of probiotics, use of GM-CSF to enhance innate immune function[19], administration of microbe-derived agents or intestinal parasites to activate the innate immune system by inducing counter-regulatory immune responses to quell established inflammation[20], administration of anti-CD3 antibodies[21], autologous hematopoietic stem cell transplant[22], extracorporeal photophoresis to restore immunoregulation[23], and adipose stem cell infusion[24].

ADENOSINE MODULATES CHRONIC INFLAMMATION IN IBD

Adenosine exerts broad biologic effects, including smooth muscle contraction, neurotransmission in the peripheral and central nervous systems, platelet aggregation, pain, exocrine and endocrine secretion, lipolysis, glycogenesis, immune system development and response (e.g. severe combined immunodeficiency is due to lack of adenosine-deaminase), cardiac conduction and contractility, and anti-inflammation[25]. It has long been reported that adenosine, a purine nucleoside that is released at injured and inflamed sites, plays a central role in the regulation of inflammatory responses and in limiting inflammatory tissue destruction[26]. Early after the injurious or infectious signal, high concentrations of extracellular adenosine favor a transition from neutrophil infiltration to macrophage recruitment, to facilitate a highly efficient specific immune response carried out by macrophages. At later stages of immune or inflammatory processes, adenosine contributes to the resolution of inflammation, both by down-regulating macrophage activation and by advancing Th2 *vs* Th1-cell response[26]. Some anti-inflammatory and immunomodulating drugs, such as salicylates, methotrexate and purine analogs like 6-MP and cyclosporine, exert their therapeutic actions in inflammatory diseases, at least in part, by decreasing intracellular adenosine 5'-triphosphate (ATP) concentrations and increasing extracellular adenosine levels[27].

There are numerous reports that have demonstrated the ability of adenosine to exert anti-inflammatory actions in a variety of animal models. The anti-inflammatory effects can be achieved by increasing intracellular or extracellular adenosine levels through the mechanism of either enhanced production or inhibition of adenosine catabolism. The majority of work has been focusing on inhibition of adenosine catabolism or direct activation of adenosine receptors. A recent article by Antonioli et al[28] reported that inhibition of adenosine deaminase can attenuate mucosal inflammation in experimental colitis through the mechanism of reducing mucosal myeloperoxidase activity, production of malondialdehyde and TNF-α levels as well as plasma TNF-α and interleukin-6 levels. Other studies have demonstrated that adenosine acting on the A2a receptor of T-lymphocytes can selectively suppress the expression of pro-inflammatory cytokines while sparing anti-inflammatory activity mediated by IL-10 and TGF-β[29]. The tissue injury and inflammation in mice with enteritis induced by *Clostridium difficile* toxin A can be alleviated by a new A2a receptor agonist, ATL 313, through the mechanism of inhibiting neutrophil infiltration, TNF-α production and adenosine deaminase activity[30]. Adenosine can down-regulate neutrophil functions by

decreasing their adhesion, degranulation, and oxidant activities[25]. An increase in endogenous adenosine levels by inhibition of adenosine kinase ameliorates colitis by suppression of IFN-γ in colonic tissue and CD69 expression in splenocytes, as well as maintaining tissue integrity by reducing energy demand, increasing nutrient availability, and modulating the immune system[31]. At a molecular level, adenosine has been demonstrated to be a negative regulator of NF-κB and MAPK signaling in human intestinal epithelial cells[32]. Based on these findings, the adenosine system can represent a very promising target for therapies of inflammatory bowel diseases.

PHYSIOLOGY OF ADENOSINE METABOLISM BY INTESTINAL EPITHELIUM CELLS

Intracellular adenosine level is maintained by constant synthesis and degradation, as well as by trans-membrane transport through nucleoside transporters. The intracellular adenosine is produced by *de novo* synthesis from amino acids, CO_2, carbon-1-tetrahydrofolate and ribose-5-phosphate, salvage of endogenous adenine, dephosphorylation of ATP, ADP and AMP, as well as by transportation of exogenous nucleobases and nucleosides through concentrative nucleoside transporters (CNTs) or equilibrative nucleoside transporters (ENTs). Adenosine is metabolized to inosine by adenosine-deaminase, to either an end product uric acid or phosphorylated to ATP by adenosine-kinase or diffused into the extracellular space *via* ENTs[33,34].

Most tissues and cells have *de novo* synthesis capacity to produce adenine endogenously from amino acids, CO_2 and carbon-1-tetrahydrofolate for their own use. However, some tissues and cells either lack or have very limited *de novo* synthesis capacity. These tissues and cells rely largely on exogenous nucleoside supply and salvage of endogenous nucleobases and nucleosides. Bone marrow, lymphocytes, leukocytes and intestinal epithelial cells are among them[35-39]. The liver is the major organ supplying the nucleobases to these tissue and cells. The dietary nucleobases and nucleosides are absorbed by intestinal villous epithelial cells and degraded to end products such as uric acid. The uric acid is brought to the liver by blood and is taken up by hepatocytes and transformed into nucleobases. The synthesized nucleobases are released into the blood stream and carried to the tissues.

The intestinal epithelial cells have a very limited capacity for *de novo* synthesis; the villous cells can directly use the absorbed dietary nucleosides but the cryptal cells depend on blood supply. The impact of lack or limitation of nucleoside supply to cryptal cells on epithelial repair and barrier function during IBD has not been fully investigated[40,41]. It is reasonable to speculate that poor absorption in the intestine or suppression of synthesis in the liver will ultimately result in disruption of epithelial barrier function in chronic bowel inflammatory diseases

like IBD, which in turn will lead to over-exposure of the innate immune system to intraluminal bacterial antigens and cause persistent inflammation or exacerbation of the diseases.

In general, the extracellular adenosine is produced by dephosphorylation of ATP by an enzymatic cascade consisting of Ntpases and ecto-5'-nucleotidase (Ecto 5'NTase), and direct diffusion of intracellular adenosine through ENTs. It is removed by enzymatic degradation by adenosine deaminase to inosine or by adenosine kinase to AMP. It can also be transported back into cells by membranous transporters like CNTs/ENTs. The extracellular adenosine level is believed to be lower than 1 μmol/L in normal tissue but can be as high as 100 μmol/L in inflamed or ischemic tissues[25]. Only a high adenosine level can exert immunomodulatory and immunosuppressive effects. Luminal adenosine level is estimated to be 5 mmol/L in normal intestine, while it is 6 mmol/L in inflamed intestine due to ATP and adenosine secretion in inflammatory and other cell types[42].

It is not entirely clear which cell types are the most important producer of extracellular adenosine, but endothelial cells, neutrophils, nerve terminal and epithelial cells have been identified in the literature[43,44]. Extracellular adenosine binds to adenosine receptors (AR) 1, 2a, 2b and 3, all of which are expressed on the surface of immune cells. Low level expression of A1R is demonstrated in small intestine. A2bR is the only receptor expressed in epithelial cells of cecum and colon. A3R can be detected in jejunum and proximal colon[45,46]. Adenosine receptors are members of the G protein-coupled family of receptors[47]. A1 and A3 receptors are usually coupled with Gi proteins that inhibit adenylate cyclase, whereas the A2aR and A2bR receptors are coupled with Gs proteins that activate adenylate cyclase. Several studies have demonstrated that adenosine attenuates intestinal inflammation predominantly through the effects of the A2aR receptor of neutrophils and T-lymphocytes[29,30,48]. However, Yang *et al*[49] found that activation of the A2bR can also have anti-inflammatory effects, using a gene knock-out method to delete this gene in order to show a pro-inflammatory phenotype.

MOLECULAR MECHANISM OF ADENOSINE TRANSPORT

The significance of exogenous adenosine transport by intestinal epithelial cells in the treatment of IBD and its impact on epithelial cell barrier function has not been explored. Concentrative nucleoside transporters (CNTs) have been identified as the major transporters for absorption of exogenous nucleosides from the diet. Three distinct CNTs (CNT1, CNT2 and CNT3) that exhibit different substrate specificity have been cloned and characterized from humans, rats and mice[34]. CNT1 predominantly transports pyrimidines. CNT2 transports purine and uridine, while CNT3 transports purines and

pyrimidines. The expression of CNTs and their substrate specificity vary among species. CNT3 was not found in intestinal epithelial cells of human and rat[34,50,51]. CNTs belong to the solute carrier family 28 (SLC-28). CNTs are expressed in the apical membranes of intestinal epithelial cells, as well as in other cell types including hepatocytes, endothelial cells, neutrophils, lymphocytes and macrophages.

CNT2 has been cloned and characterized in humans, mice, rats and rabbits. The rat CNT2 cDNA is 2.9 Kb and encodes a 659 amino acid protein with molecular weight of 72 kDa[52]. The apparent molecular weight on western blot is usually around 60 kDa due to high hydrophobicity of membrane protein. Fourteen putative transmembrane domains were identified by hydropathy analysis. The presence of several consensus sequences for protein kinase-C (PKC) and protein kinase-A (PKA) phosphorylation sites on both N- and C-termini, and an ATP/GTP binding motif in N-terminus, suggest that CNT2 may be regulated by protein kinases and intracellular ATP and GTP. CNT2 may be a glycoprotein as there are five possible N-linked glycosylation sites. Na^+-adenosine cotransport in brush-border membranes from rabbit ileum was identified and partially characterized in one previous study[53]. *In vitro* expressed CNT2 in *Xenopus laevis* oocytes exhibited Na^+-dependent adenosine uptake with an apparent Km for adenosine of 6 μmol/L, with substrate selectivity to purine and uridine[54].

REGULATION OF ADENOSINE TRANSPORT AND CNT2 EXPRESSION IN GENERAL

The CNT2 expression and adenosine uptake are highly regulated processes and are species and tissue specific[55]. Sub-cellular trafficking (i.e. internalization of membrane transporters to sub-cellular storage vesicles) has been shown to be a regulatory mechanism for CNT2 in several cell lines including adrenal chromaffin cells, reticulocytes and cholangiocytes[56-58]. Although CNT1 has been shown to be up-regulated in intestine and down-regulated in hepatocytes of pyrimidine-free diet fed animals, the dietary effects of adenosine on CNT2 activity and molecular expressions are not known[59,60]. Several studies have shown that nucleoside transport functions and expressions are regulated by hormones[61-66]. Tyrosine and glucagon have been shown to stimulate adenosine transport and CNT2 expression in both *in vitro* and *in vivo* models[61-63]. Studies with insulin and glucose have yielded different results regarding adenosine transport and molecular expression. Insulin regulates adenosine transport through different signaling pathways that involve PI3K, MAPK, NO synthase, PKC and MAP kinase[64-66]. One study showed that adenosine transport is up-regulated by activation of the A1 adenosine receptor through ATP-sensitive K-channels in hepatocytes, suggesting some positive feedback regulation among adenosine receptors and the adenosine transporter[67].

The effects of proliferative (EGF and TGF-α) and differentiating (glucocorticoid) hormones were demonstrated in IEC-6 cells (a rat intestinal cryptal cell line). A four-fold increase of adenosine transport activity and CNT2 molecular expression after treatment with dexamethasone was observed while no significant impact was noted from treatment with proliferative hormones[68]. However, a more recent paper by the same research group found that TGF-β can transcriptionally up-regulate *CNT2* gene expression in rat hepatocytes[69]. Adenosine transport and CNT2 expression are altered during cell growth cycle and differentiation. Hepatocarcinogenesis is accompanied by loss of CNT2 expression and increased expression of ENTs. CNT2 mRNA and protein levels were increased right before the peak of incorporation of thymidine into DNA and during liver regeneration after partial hepatectomy[70,71].

CNT2 expression was up-regulated by lipopo-lysaccharide (LPS), NO, INF-α and TNF-α in macrophages and B-lymphocytes in several studies[72-74]. The LPS-induced increase of adenosine transport and molecular expression is TNF-α-dependent but not iNOs-dependent. cNOs is required for maintaining the basal transport activities of adenosine in activated B-cells. The CNT2 is recognized as an important regulator of extracellular adenosine concentrations. CNT2 expression is suppressed in inflamed tissue as a mechanism to maintain high extracellular adenosine concentration. The CNT2 expression is suppressed in neutrophils and macrophages during inflammation[75]. There is controversy between the *in vivo* and *in vitro* studies about the functional status of adenosine transport and CNT2 expression during inflammation and the effects of inflammatory mediators.

MECHANISM OF REGULATION OF ADENOSINE TRANSPORT AND CNT2 EXPRESSION IN INTESTINAL EPITHELIAL CELLS

A systematic study for the mechanism of regulation of adenosine transport and CNT2 expression in intestinal epithelial cells is not available. Adenosine functions as a nutrient for nucleic acid metabolism, an energy carrier molecule for cell energy metabolism, and a second messenger in autocrine and paracrine hormone regulation. Adenosine transport is regulated differently from other nutrient transporters such as glucose and amino acid transporters. Luminal adenosine and ATP have been reported to regulate glucose and bile acid transport in intestine, proximal renal tubule cells and cholangiocytes[42,76,77]. The role of the activation of purinergic receptors on adenosine uptake was also studied in vascular endothelial cells and chromaffin cells. ATP has been shown to up-regulate adenosine transport and protein expression in vascular endothelial cells and chromaffin cells[78-80]. However, the effect of ATP on

adenosine transport in normal and inflamed intestine remains to be elucidated.

MECHANISM OF REGULATION OF NA⁺-CO-TRANSPORT IN INTESTINAL EPITHELIAL CELLS DURING CHRONIC INTESTINAL INFLAMMATION

The regulation of intestinal epithelial transporters during chronic inflammation is a very complex process, involving many cell types and immune-inflammatory mediators. The cells involved in chronic enteritis include all inflammatory cells (neutrophils, basophils, eosinophils, macrophages, lymphocytes), fibroblasts, vascular endothelial cells, nerve cells and epithelial cells. These cells produce numerous cytokines and immune-inflammatory mediators such as prostaglandins, leukotrienes, reactive oxidative metabolites (ROMs) and nitric oxide[1-6]. So far, there are no perfect animal models for IBD though several different models exist with chemically-induced and genetically-induced enteritis. It is very difficult to fully understand the regulation of nutrient and electrolyte transport in the chronically inflamed intestine.

The mast cell has been implicated as an important player in chronic intestinal inflammation. It releases multiple inflammatory mediators including histamine, serotonin, cytokines, prostaglandins, leukotrienes and ROMs. The released mediators are very important regulators of transporters in epithelial cells. It was consistently demonstrated that blocking mast cell degranulation prevented down-regulation of a number of transporters including Na⁺-glucose and Na⁺-amino acid cotransport as well as electrolyte transporters such as Cl^-/HCO_3^- exchange[81].

Nitric oxide has been identified as an important regulating factor in the intestinal tract. Constitutive nitric oxide synthase (cNOs) plays an important role in the regulation of transporters in normal intestine. It is essential for some transporters to function properly under physiologic conditions. Inducible nitric oxide synthase (iNOS) is activated during chronic inflammation, ischemia and tissue injury. iNOS produces large amounts of nitric oxide (NO) during chronic inflammation, which is generally considered as detrimental to tissue and cells because of formation of peroxynitrous acid, though controversy continues about its role in inflammation. The mechanism of cNOS and iNOS effects on nutrient co-transporters in epithelial cells is possibly through direct and indirect effects, by stimulating the production and/or release of other inflammatory mediators such as arachidonic acid metabolites (e.g. prostaglandins and leukotrienes)[82-84].

Prostaglandins have been shown to be very important inflammatory mediators in chronic intestinal inflammation. A large amount of prostaglandins are produced in the intestinal tissue during IBD. Prostaglandins inhibit electrolyte absorption and Na⁻
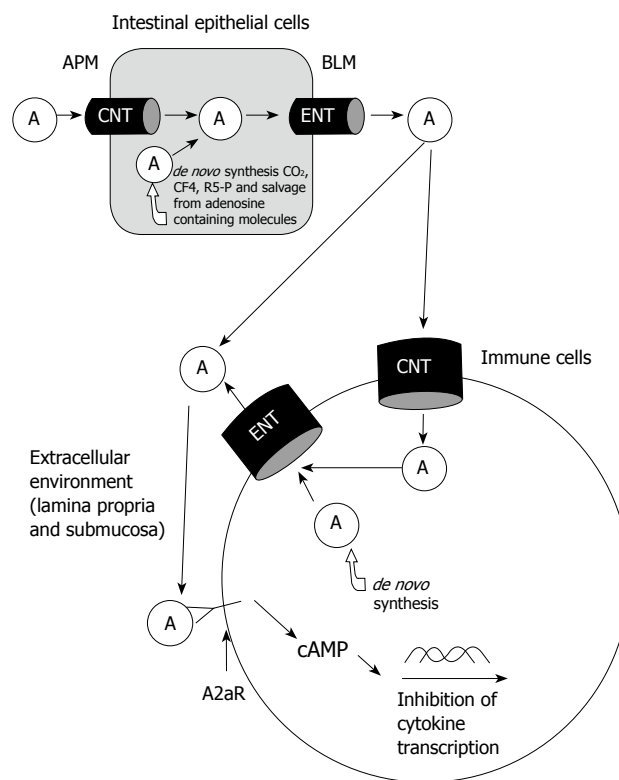

Figure 1 Pathways and roles of adenosine. Absorbed and *de novo* synthesized adenosine delivered to extracellular space. Adenosine binds to A2aR of immune cells and activates signaling pathways to inhibit the production of inflammatory mediators. A: Adenosine; APM: Apical membrane; BLM: Basolateral membrane; CNT: Concentrative nucleoside transporter; ENT: Equilibrative nucleoside transporter.

nutrient co-transporter functions[85-89]. In addition, they also promote mucous secretion and cytoprotection in the gastrointestinal tract during IBD[85,88]. Prostaglandin E2 (PGE2) was also reported to suppress glucose transport in the ovine intestine[90]. Leukotrienes have been demonstrated to inhibit electrolyte transport in a similar pattern to prostaglandins[91-98].

Corticosteroids are the most frequently used broad spectrum immunomodulators in IBD for blocking the production of most major inflammatory mediators. Corticosteroids prevent mast cell degranulation and block the phospholipase A2 (PLA2) enzyme pathway. They also down-regulate arachidonic acid release and production of prostaglandins and leukotrienes. In addition, corticosteroids suppress the production of iNOS and inducible cyclooxygenase (COX-2) during chronic inflammation[99].

It is possible that mast cells, nitric oxide, arachidonic acid metabolites and steroids are all involved in the regulation of adenosine transport and CNT2 expression during chronic enteritis. It should be noted that adenosine itself is an inflammatory modulator. Extracellular adenosine can suppress all these above-mentioned inflammatory cells and mediators. The interplay among adenosine, inflammatory cells and mediators can be very complex and is also very interesting for further study.

CONCLUSION

The pathways of adenosine metabolism and transport are fully illustrated in Figure 1. Luminal adenosine is absorbed by intestinal epithelial cells through CNT and ENT transporters and can also be synthesized *de novo*. The increase in intracellular levels of adenosine leads to extracellular transport along with the release of adenosine from damaged cells during inflammation. Using various therapies during IBD, it may be possible to eventually increase the extracellular levels of adenosine so that the appropriate receptors can be activated to inhibit and reduce the effects of chronic inflammation on the gut. IBD is a common and lifelong disabling gastrointestinal disease. At present, there is no cure for IBD. Emerging treatments are being developed to target cytokines that perpetuate the chronic inflammatory response. Adenosine is an important modulator of inflammation and its anti-inflammatory effects have been well established in humans as well as in animal models. Therapeutic targeting of receptors such as the A2a receptor could reduce cytokine levels and thus reduce the effects of chronic inflammation during IBD.

REFERENCES

1   **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429
2   **Lichtenstein GR**, Abreu MT, Cohen R, Tremaine W. American Gastroenterological Association Institute technical review on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. *Gastroenterology* 2006; **130**: 940-987
3   **Lakatos PL**. Recent trends in the epidemiology of inflammatory bowel diseases: up or down? *World J Gastroenterol* 2006; **12**: 6102-6108
4   **Strober W**, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest* 2007; **117**: 514-521
5   **Oz HS**, Chen TS, McClain CJ, de Villiers WJ. Antioxidants as novel therapy in a murine model of colitis. *J Nutr Biochem* 2005; **16**: 297-304
6   **Martin GR**, Wallace JL. Gastrointestinal inflammation: a central component of mucosal defense and repair. *Exp Biol Med* (Maywood) 2006; **231**: 130-137
7   **Adelman B**, Sandrock A, Panzara MA. Natalizumab and progressive multifocal leukoencephalopathy. *N Engl J Med* 2005; **353**: 432-433
8   **Blonski W**, Lichtenstein GR. Complications of biological therapy for inflammatory bowel diseases. *Curr Opin Gastroenterol* 2006; **22**: 30-43
9   **Feagan BG**, Greenberg GR, Wild G, Fedorak RN, Paré P, McDonald JW, Dubé R, Cohen A, Steinhart AH, Landau S, Aguzzi RA, Fox IH, Vandervoort MK. Treatment of ulcerative colitis with a humanized antibody to the alpha4beta7 integrin. *N Engl J Med* 2005; **352**: 2499-507
10   **Hanauer SB**, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Rutgeerts P. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002; **359**: 1541-1549
11   **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476
12   **Sandborn WJ**, Colombel JF, Enns R, Feagan BG, Hanauer SB, Lawrance IC, Panaccione R, Sanders M, Schreiber S, Targan S, van Deventer S, Goldblum R, Despain D, Hogge GS, Rutgeerts P. Natalizumab induction and maintenance therapy for Crohn's disease. *N Engl J Med* 2005; **353**: 1912-1925
13   **Mannon PJ**, Fuss IJ, Mayer L, Elson CO, Sandborn WJ, Present D, Dolin B, Goodman N, Groden C, Hornung RL, Quezado M, Yang Z, Neurath MF, Salfeld J, Veldman GM, Schwertschlag U, Strober W. Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med* 2004; **351**: 2069-2079
14   **Hommes DW**, Mikhajlova TL, Stoinov S, Stimac D, Vucelic B, Lonovics J, Zákuciová M, D'Haens G, Van Assche G, Ba S, Lee S, Pearce T. Fontolizumab, a humanised anti-interferon gamma antibody, demonstrates safety and clinical activity in patients with moderate to severe Crohn's disease. *Gut* 2006; **55**: 1131-1137
15   **Ito H**, Takazoe M, Fukuda Y, Hibi T, Kusugami K, Andoh A, Matsumoto T, Yamamura T, Azuma J, Nishimoto N, Yoshizaki K, Shimoyama T, Kishimoto T. A pilot randomized trial of a human anti-interleukin-6 receptor monoclonal antibody in active Crohn's disease. *Gastroenterology* 2004; **126**: 989-996; discussion 947
16   **Braat H**, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JP, van Deventer SJ, Neirynck S, Peppelenbosch MP, Steidler L. A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. *Clin Gastroenterol Hepatol* 2006; **4**: 754-759
17   **Nishioka C**, Aoyama N, Maekawa S, Shirasaka D, Nakahara T, Tamura T, Fukagawa M, Umezu M, Abe T, Kasuga M. Leukocytapheresis therapy for steroid-naïve patients with active ulcerative colitis: its clinical efficacy and adverse effects compared with those of conventional steroid therapy. *J Gastroenterol Hepatol* 2005; **20**: 1567-1571
18   **Sands BE**, Sandborn WJ, Wolf DC, Katz S, Safdi M, Schwartz DA, Hanauer SB. Pilot feasibility studies of leukocytapheresis with the Adacolumn Apheresis System in patients with active ulcerative colitis or Crohn disease. *J Clin Gastroenterol* 2006; **40**: 482-489
19   **Korzenik JR**, Dieckgraefe BK, Valentine JF, Hausman DF, Gilbert MJ. Sargramostim for active Crohn's disease. *N Engl J Med* 2005; **352**: 2193-2201
20   **Weinstock JV**. Helminths and mucosal immune modulation. *Ann N Y Acad Sci* 2006; **1072**: 356-364
21   **Plevy S**, Salzberg B, Van Assche G, Regueiro M, Hommes D, Sandborn W, Hanauer S, Targan S, Mayer L, Mahadevan U, Frankel M, Lowder J. A phase I study of visilizumab, a humanized anti-CD3 monoclonal antibody, in severe steroid-refractory ulcerative colitis. *Gastroenterology* 2007; **133**: 1414-1422
22   **Oyama Y**, Craig RM, Traynor AE, Quigley K, Statkute L, Halverson A, Brush M, Verda L, Kowalska B, Krosnjar N, Kletzel M, Whitington PF, Burt RK. Autologous hematopoietic stem cell transplantation in patients with refractory Crohn's disease. *Gastroenterology* 2005; **128**: 552-563
23   **Reinisch W**, Nahavandi H, Santella R, Zhang Y, Gasché C, Moser G, Waldhör T, Gangl A, Vogelsang H, Knobler R. Extracorporeal photochemotherapy in patients with steroid-dependent Crohn's disease: a prospective pilot study. *Aliment Pharmacol Ther* 2001; **15**: 1313-1322
24   **García-Olmo D**, García-Arranz M, Herreros D, Pascual I, Peiro C, Rodríguez-Montes JA. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum* 2005; **48**: 1416-1423
25   **Haskó G**, Cronstein BN. Adenosine: an endogenous regulator of innate immunity. *Trends Immunol* 2004; **25**: 33-39
26   **Lawrence T**, Willoughby DA, Gilroy DW. Anti-inflammatory lipid mediators and insights into the resolution of inflammation. *Nat Rev Immunol* 2002; **2**: 787-795
27   **Cronstein BN**, Montesinos MC, Weissmann G. Sites of action for future therapy: an adenosine-dependent

mechanism by which aspirin retains its antiinflammatory activity in cyclooxygenase-2 and NFkappaB knockout mice. *Osteoarthritis Cartilage* 1999; **7**: 361-363

28   **Antonioli L**, Fornai M, Colucci R, Ghisu N, Da Settimo F, Natale G, Kastsiuchenka O, Duranti E, Virdis A, Vassalle C, La Motta C, Mugnaini L, Breschi MC, Blandizzi C, Del Taca M. Inhibition of adenosine deaminase attenuates inflammation in experimental colitis. *J Pharmacol Exp Ther* 2007; **322**: 435-442

29   **Naganuma M**, Wiznerowicz EB, Lappas CM, Linden J, Worthington MT, Ernst PB. Cutting edge: Critical role for A2A adenosine receptors in the T cell-mediated regulation of colitis. *J Immunol* 2006; **177**: 2765-2769

30   **Cavalcante IC**, Castro MV, Barreto AR, Sullivan GW, Vale M, Almeida PR, Linden J, Rieger JM, Cunha FQ, Guerrant RL, Ribeiro RA, Brito GA. Effect of novel A2A adenosine receptor agonist ATL 313 on Clostridium difficile toxin A-induced murine ileal enteritis. *Infect Immun* 2006; **74**: 2606-2612

31   **Siegmund B**, Rieder F, Albrich S, Wolf K, Bidlingmaier C, Firestein GS, Boyle D, Lehr HA, Loher F, Hartmann G, Endres S, Eigler A. Adenosine kinase inhibitor GP515 improves experimental colitis in mice. *J Pharmacol Exp Ther* 2001; **296**: 99-105

32   **Jijon HB**, Walker J, Hoentjen F, Diaz H, Ewaschuk J, Jobin C, Madsen KL. Adenosine is a negative regulator of NF-kappaB and MAPK signaling in human intestinal epithelial cells. *Cell Immunol* 2005; **237**: 86-95

33   **Baldwin SA**, Beal PR, Yao SY, King AE, Cass CE, Young JD. The equilibrative nucleoside transporter family, SLC29. *Pflugers Arch* 2004; **447**: 735-743

34   **Gray JH**, Owen RP, Giacomini KM. The concentrative nucleoside transporter family, SLC28. *Pflugers Arch* 2004; **447**: 728-734

35   **Savaiano DA**, Clifford AJ. Adenine, the precursor of nucleic acids in intestinal cells unable to synthesize purines de novo. *J Nutr* 1981; **111**: 1816-1822

36   **Savaiano DA**, Ho CY, Chu V, Clifford AJ. Metabolism of orally and intravenously administered purines in rats. *J Nutr* 1980; **110**: 1793-1804

37   **Ho CY**, Miller KV, Savaiano DA, Crane RT, Ericson KA, Clifford AJ. Absorption and metabolism of orally administered purines in fed and fasted rats. *J Nutr* 1979; **109**: 1377-1382

38   **Sonoda T**, Tatibana M. Metabolic fate of pyrimidines and purines in dietary nucleic acids ingested by mice. *Biochim Biophys Acta* 1978; **521**: 55-66

39   **LeLeiko NS**, Bronstein AD, Baliga BS, Munro HN. De novo purine nucleotide synthesis in the rat small and large intestine: effect of dietary protein and purines. *J Pediatr Gastroenterol Nutr* 1983; **2**: 313-319

40   **Nuñez MC**, Ayudarte MV, Morales D, Suarez MD, Gil A. Effect of dietary nucleotides on intestinal repair in rats with experimental chronic diarrhea. *JPEN J Parenter Enteral Nutr* 1990; **14**: 598-604

41   **Uauy R**, Stringel G, Thomas R, Quan R. Effect of dietary nucleosides on growth and maturation of the developing gut in the rat. *J Pediatr Gastroenterol Nutr* 1990; **10**: 497-503

42   **Kimura Y**, Turner JR, Braasch DA, Buddington RK. Lumenal adenosine and AMP rapidly increase glucose transport by intact small intestine. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G1007-G1014

43   **Sperlágh B**, Dóda M, Baranyi M, Haskó G. Ischemic-like condition releases norepinephrine and purines from different sources in superfused rat spleen strips. *J Neuroimmunol* 2000; **111**: 45-54

44   **Martin C**, Leone M, Viviand X, Ayem ML, Guieu R. High adenosine plasma concentration as a prognostic index for outcome in patients with septic shock. *Crit Care Med* 2000; **28**: 3198-3202

45   **Yaar R**, Jones MR, Chen JF, Ravid K. Animal models for the study of adenosine receptor function. *J Cell Physiol* 2005; **202**:

9-20

46   **Kolachala VL**, Bajaj R, Chalasani M, Sitaraman SV. Purinergic receptors in gastrointestinal inflammation. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G401-G410

47   **Fredholm BB**, IJzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* 2001; **53**: 527-552

48   **Odashima M**, Bamias G, Rivera-Nieves J, Linden J, Nast CC, Moskaluk CA, Marini M, Sugawara K, Kozaiwa K, Otaka M, Watanabe S, Cominelli F. Activation of A2A adenosine receptor attenuates intestinal inflammation in animal models of inflammatory bowel disease. *Gastroenterology* 2005; **129**: 26-33

49   **Yang D**, Zhang Y, Nguyen HG, Koupenova M, Chauhan AK, Makitalo M, Jones MR, St Hilaire C, Seldin DC, Toselli P, Lamperti E, Schreiber BM, Gavras H, Wagner DD, Ravid K. The A2B adenosine receptor protects against inflammation and excessive vascular adhesion. *J Clin Invest* 2006; **116**: 1913-1923

50   **Meier Y**, Eloranta JJ, Darimont J, Ismair MG, Hiller C, Fried M, Kullak-Ublick GA, Vavricka SR. Regional distribution of solute carrier mRNA expression along the human intestinal tract. *Drug Metab Dispos* 2007; **35**: 590-594

51   **Lu H**, Chen C, Klaassen C. Tissue distribution of concentrative and equilibrative nucleoside transporters in male and female rats and mice. *Drug Metab Dispos* 2004; **32**: 1455-1461

52   **Che M**, Ortiz DF, Arias IM. Primary structure and functional expression of a cDNA encoding the bile canalicular, purine-specific Na(+)-nucleoside cotransporter. *J Biol Chem* 1995; **270**: 13596-13599

53   **Betcher SL**, Forrest JN Jr, Knickelbein RG, Dobbins JW. Sodium-adenosine cotransport in brush-border membranes from rabbit ileum. *Am J Physiol* 1990; **259**: G504-G510

54   **Gerstin KM**, Dresser MJ, Wang J, Giacomini KM. Molecular cloning of a Na+-dependent nucleoside transporter from rabbit intestine. *Pharm Res* 2000; **17**: 906-910

55   **Podgorska M**, Kocbuch K, Pawelczyk T. Recent advances in studies on biochemical and structural properties of equilibrative and concentrative nucleoside transporters. *Acta Biochim Pol* 2005; **52**: 749-758

56   **Torres M**, Delicado EG, Fideu MD, Miras-Portugal MT. Down-regulation and recycling of the nitrobenzylthioinosine-sensitive nucleoside transporter in cultured chromaffin cells. *Biochim Biophys Acta* 1992; **1105**: 291-299

57   **Liang L**, Johnstone RM. Evidence for an internal pool of nucleoside transporters in mammalian reticulocytes. *Biochim Biophys Acta* 1992; **1106**: 189-196

58   **Fernández-Veledo S**, Huber-Ruano I, Aymerich I, Duflot S, Casado FJ, Pastor-Anglada M. Bile acids alter the subcellular localization of CNT2 (concentrative nucleoside cotransporter) and increase CNT2-related transport activity in liver parenchymal cells. *Biochem J* 2006; **395**: 337-344

59   **López-Navarro AT**, Ortega MA, Peragón J, Bueno JD, Gil A, Sánchez-Pozo A. Deprivation of dietary nucleotides decreases protein synthesis in the liver and small intestine in rats. *Gastroenterology* 1996; **110**: 1760-1769

60   **Valdés R**, Ortega MA, Casado FJ, Felipe A, Gil A, Sánchez-Pozo A, Pastor-Anglada M. Nutritional regulation of nucleoside transporter expression in rat small intestine. *Gastroenterology* 2000; **119**: 1623-1630

61   **Fideu MD**, Miras-Portugal MT. Long term regulation of nucleoside transport by thyroid hormone (T3) in cultured chromaffin cells. *Neurochem Res* 1992; **17**: 1099-1104

62   **Fideu MD**, Arce A, Esquifino AI, Miras-Portugal MT. Thyroid hormones modulate both adenosine transport and adenosine A1 receptors in rat brain. *Am J Physiol* 1994; **267**: C1651-C1656

63   **Gomez-Angelats M**, del Santo B, Mercader J, Ferrer-Martinez A, Felipe A, Casado J, Pastor-Anglada M. Hormonal regulation of concentrative nucleoside transport in liver parenchymal cells. *Biochem J* 1996; **313** (Pt 3): 915-920

64 **Sakowicz M**, Szutowicz A, Pawelczyk T. Differential effect of insulin and elevated glucose level on adenosine transport in rat B lymphocytes. *Int Immunol* 2005; **17**: 145-154

65 **Montecinos VP**, Aguayo C, Flores C, Wyatt AW, Pearson JD, Mann GE, Sobrevia L. Regulation of adenosine transport by D-glucose in human fetal endothelial cells: involvement of nitric oxide, protein kinase C and mitogen-activated protein kinase. *J Physiol* 2000; **529** Pt 3: 777-790

66 **Aguayo C**, Casado J, González M, Pearson JD, Martín RS, Casanello P, Pastor-Anglada M, Sobrevia L. Equilibrative nucleoside transporter 2 is expressed in human umbilical vein endothelium, but is not involved in the inhibition of adenosine transport induced by hyperglycaemia. *Placenta* 2005; **26**: 641-653

67 **Duflot S**, Riera B, Fernández-Veledo S, Casadó V, Norman RI, Casado FJ, Lluís C, Franco R, Pastor-Anglada M. ATP-sensitive K(+) channels regulate the concentrative adenosine transporter CNT2 following activation by A(1) adenosine receptors. *Mol Cell Biol* 2004; **24**: 2710-2719

68 **Aymerich I**, Pastor-Anglada M, Casado FJ. Long term endocrine regulation of nucleoside transporters in rat intestinal epithelial cells. *J Gen Physiol* 2004; **124**: 505-512

69 **Valdés R**, Fernández-Veledo S, Aymerich I, Casado FJ, Pastor-Anglada M. TGF-beta transcriptionally activates the gene encoding the high-affinity adenosine transporter CNT2 in rat liver parenchymal cells. *Cell Mol Life Sci* 2006; **63**: 2527-2537

70 **Dragan Y**, Valdés R, Gomez-Angelats M, Felipe A, Javier Casado F, Pitot H, Pastor-Anglada M. Selective loss of nucleoside carrier expression in rat hepatocarcinomas. *Hepatology* 2000; **32**: 239-246

71 **del Santo B**, Tarafa G, Felipe A, Casado FJ, Pastor-Anglada M. Developmental regulation of the concentrative nucleoside transporters CNT1 and CNT2 in rat liver. *J Hepatol* 2001; **34**: 873-880

72 **Soler C**, Felipe A, Casado FJ, Celada A, Pastor-Anglada M. Nitric oxide regulates nucleoside transport in activated B lymphocytes. *J Leukoc Biol* 2000; **67**: 345-349

73 **Soler C**, Valdés R, Garcia-Manteiga J, Xaus J, Comalada M, Casado FJ, Modolell M, Nicholson B, MacLeod C, Felipe A, Celada A, Pastor-Anglada M. Lipopolysaccharide-induced apoptosis of macrophages determines the up-regulation of concentrative nucleoside transporters Cnt1 and Cnt2 through tumor necrosis factor-alpha-dependent and -independent mechanisms. *J Biol Chem* 2001; **276**: 30043-30049

74 **Soler C**, Felipe A, Mata JF, Casado FJ, Celada A, Pastor-Anglada M. Regulation of nucleoside transport by lipopolysaccharide, phorbol esters, and tumor necrosis factor-alpha in human B-lymphocytes. *J Biol Chem* 1998; **273**: 26939-26945

75 **Pastor-Anglada M**, Casado FJ, Valdés R, Mata J, García-Manteiga J, Molina M. Complex regulation of nucleoside transporter expression in epithelial and immune system cells. *Mol Membr Biol* 2001; **18**: 81-85

76 **Lee YJ**, Park SH, Han HJ. ATP stimulates Na+-glucose cotransporter activity via cAMP and p38 MAPK in renal proximal tubule cells. *Am J Physiol Cell Physiol* 2005; **289**: C1268-C1276

77 **Minagawa N**, Nagata J, Shibao K, Masyuk AI, Gomes DA, Rodrigues MA, Lesage G, Akiba Y, Kaunitz JD, Ehrlich BE, Larusso NF, Nathanson MH. Cyclic AMP regulates bicarbonate secretion in cholangiocytes through release of ATP into bile. *Gastroenterology* 2007; **133**: 1592-1602

78 **Casillas T**, Delicado EG, Miras-Portugal MT. Adenosine 5'-triphosphate modulation of nitrobenzylthioinosine binding sites in plasma membranes of bovine chromaffin cells. *Neurosci Lett* 1993; **164**: 51-54

79 **Parodi J**, Flores C, Aguayo C, Rudolph MI, Casanello P, Sobrevia L. Inhibition of nitrobenzylthioinosine-sensitive adenosine transport by elevated D-glucose involves activation of P2Y2 purinoceptors in human umbilical vein endothelial cells. *Circ Res* 2002; **90**: 570-577

80 **Delicado EG**, Casillas T, Sen RP, Miras-Portugal MT. Evidence that adenine nucleotides modulate nucleoside-transporter function. Characterization of uridine transport in chromaffin cells and plasma membrane vesicles. *Eur J Biochem* 1994; **225**: 355-362

81 **Schreiber S**, Raedler A, Stenson WF, MacDermott RP. The role of the mucosal immune system in inflammatory bowel disease. *Gastroenterol Clin North Am* 1992; **21**: 451-502

82 **Sharma JN**, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology* 2007; **15**: 252-259

83 **Persichini T**, Cantoni O, Suzuki H, Colasanti M. Cross-talk between constitutive and inducible NO synthase: an update. *Antioxid Redox Signal* 2006; **8**: 949-954

84 **Kubes P**, McCafferty DM. Nitric oxide and intestinal inflammation. *Am J Med* 2000; **109**: 150-158

85 **Racusen LC**, Binder HJ. Effect of prostaglandin on ion transport across isolated colonic mucosa. *Dig Dis Sci* 1980; **25**: 900-904

86 **Stenson WF**. Role of eicosanoids as mediators of inflammation in inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1990; **172**: 13-18

87 **Thierauch KH**, Dinter H, Stock G. Prostaglandins and their receptors: II. Receptor structure and signal transduction. *J Hypertens* 1994; **12**: 1-5

88 **Phillips TE**, Stenson WF, Neutra MR. Lipoxygenase metabolites of arachidonic acid do not induce mucus secretion from rabbit intestinal goblet cells in vitro. *Prostaglandins Leukot Essent Fatty Acids* 1989; **37**: 51-55

89 **Al-Awqati Q**, Greenough WB 3rd. Prostaglandins inhibit intestinal sodium transport. *Nat New Biol* 1972; **238**: 26-27

90 **Wilson DE**. Role of prostaglandins in gastroduodenal mucosal protection. *J Clin Gastroenterol* 1991; **13** Suppl 1: S65-S71

91 **Grönroos E**, Thodeti CK, Sjölander A. Leukotriene D4 induces a rapid increase in cAMP in the human epithelial cell line, Int 407: a potential role for this signal in the regulation of calcium influx through the plasma membrane. *Cell Calcium* 1998; **24**: 9-16

92 **Feuerstein G**, Hallenbeck JM. Leukotrienes in health and disease. *FASEB J* 1987; **1**: 186-192

93 **Henderson WR Jr**. The role of leukotrienes in inflammation. *Ann Intern Med* 1994; **121**: 684-697

94 **Samuelsson B**, Dahlén SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 1987; **237**: 1171-1176

95 **Smith PL**, Montzka DP, McCafferty GP, Wasserman MA, Fondacaro JD. Effect of sulfidopeptide leukotrienes D4 and E4 on ileal ion transport in vitro in the rat and rabbit. *Am J Physiol* 1988; **255**: G175-G183

96 **Elton E**, Chiossone DC, McCafferty GP, Ryan FM, Smith PL. SK&F 104353: selective antagonism of peptidoleukotrine-induced changes in electrolyte transport by rat ileal mucosa in vitro. *J Pharmacol Exp Ther* 1989; **251**: 484-489

97 **Smith PL**, Chiossone DC, McCafferty GP. Characterization of LTC4 effects on rabbit ileal mucosa in vitro. *Naunyn Schmiedebergs Arch Pharmacol* 1990; **341**: 94-100

98 **Wallace JL**, Keenan CM. An orally active inhibitor of leukotriene synthesis accelerates healing in a rat model of colitis. *Am J Physiol* 1990; **258**: G527-G534

99 **Barnes PJ**. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. *Br J Pharmacol* 2006; **148**: 245-254