

# *The Function of eATP/Adenosine Metabolism in Immune Modulation*

Siyi Wan, Gaiping Wu<sup>a,\*</sup>

*Department of Emergency Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, 430022, China*

*<sup>a</sup>wgp66829@163.com*

*\*Corresponding author*

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**Abstract:** The adenosine metabolic pathway is a key player in molecular interactions that control physiological processes, complexly regulating immune response, inflammation, and vital functions. Enzymes like CD39 and CD73 regulate the balance between ATP, ADP, and adenosine, affecting a variety of biological processes. Purinergic signaling is mediated by P1/ adenosine and P2 receptors and influences immune cells such as neutrophils, mononuclear macrophages, dendritic cells, and lymphocytes to form their bidirectional function. The complex dance of purinergic signaling extends its influence on thrombosis, graft rejection, and biological barrier function. In diseases such as thrombosis and graft rejection, dysregulation of CD39 activity may lead to vascular damage and inflammation. In addition, ATP and adenosine have been found to play a role in tumor progression, affecting immune cell subsets and showing potential for therapeutic intervention. By understanding the adenosine metabolic pathway, we can gain insight into its multiple effects on health and disease, paving the way for future biomedical research.

## **1. Introduction**

In the intricate symphony of molecular interactions that govern physiological processes, the adenosine metabolic pathway emerges as a central player, orchestrating a cascade of events that regulate immune responses, inflammation, and various vital functions in the body. This complex network involves the release of purine and pyrimidine compounds, the activation of purinergic receptors, enzymatic hydrolysis of nucleotides, and the production of adenosine. Key enzymes such as CD39 and CD73 act as maestros, finely tuning the balance between ATP, ADP, and adenosine, thereby influencing a myriad of biological processes. This journey through the adenosine metabolic pathway unfolds as a captivating exploration into the molecular dance that shapes our physiological responses.

## **2. Adenosine metabolic pathway**

Purinergic signaling mediates different effects in almost all organs and tissues, including regulation of immune responses and leukocyte trafficking, activation and migration, vascular tone

and angiogenesis, developmental responses, degenerative neurological diseases, neurotransmission in the central nervous system, exocrine and endocrine secretion, regeneration, wound healing, epithelial cell turnover, cancer, ageing as well as smooth muscle contractility<sup>[1-15]</sup>. The realization of these complex functions is mediated by a series of reactions<sup>[16]</sup>, including (a) the release of purine and pyrimidine compounds, including ATP, ADP, UTP, UDP, UDP-glucose, and some additional nucleotide sugars, some dinucleoside polyphosphates, and the nucleoside adenosine<sup>[12]</sup> (b) purine and pyrimidine compounds act on the corresponding receptors to trigger biological responses ; (c) the metabolic enzymatic hydrolysis of purine and pyrimidine compounds and production of adenosine<sup>[17,18]</sup>; (d) adenosine binds to the corresponding receptor to stimulate biological function and the metabolic hydrolysis of adenosine . Additionally, this cascade of reactions is regulated by numerous metabolic enzymes<sup>[16-20]</sup>. Among them, ENTPDases and eN/CD73 were considered to be the key nucleotidases, playing a vital role in purinergic signaling mediation. The ENTPDase family consists of eight members, each with a different cellular distribution and function 16, 17. Among them, the ENTPDase 1/CD39 encoded by ENTPD1, is the most critical and the most widely studied, and was first identified as a nucleotide triphosphatase as the expression of CD39 was consistent with the activity of extracellular-apyrase on immune cells. Now, CD39 has been considered to be a plasma membrane rate-limiting enzyme in the cascade that degrade ATP into ADP and AMP. CD73/eN' main function is to dephosphorylate extracellular AMP to generate adenosine. Collectively, CD39/CD73 regulates purinergic signaling by controlling ATP, ADP metabolism and adenosine production, thus affecting a range of biological processes.

### 3. The signal transduction mechanism of P1/adenosine receptors

According to their ligands, the purinergic receptors are mainly divided into type 1 purinergic (P1) receptors and type 2 purinergic (P2) receptors with different functions. P1 receptors, also known as adenosine binding receptors, including A1, A2A, A2B, and A3, all of which belong to G protein-coupled receptors with seven putative transmembrane domains of hydrophobic amino acids. Adenosine regulates multiple biological functions by binding to different adenosine receptors and activating corresponding signaling pathways. The primary function of A1 receptor is inhibition of adenylate cyclase resulting a decrease of the second messenger cAMP. Second, stimulation of the A1 receptor activates phospholipase C, leading to membrane phospholipid metabolism and increased production of diacylglycerol and 1,4,5-trisphosphatidylinositol, which in turn stimulates the release of Ca<sup>2+</sup> from intracellular stores and stimulates a variety of signalling pathways, including protein kinase C, phospholipase A2, Ca<sup>2+</sup>-dependent K<sup>+</sup> channels and nitric oxide synthase. Adenosine activated adenylate cyclase is the most common signalling mechanism for A2A receptors. However, cAMP-independent signaling pathway has also been reported for A2A receptors in quite a few studies 2. For instance, the adenosine receptor-mediated inhibition of neutrophils superoxide anion generation via a cAMP-independent activation of a serine/threonine protein phosphatase in the plasma membrane 31. According to current reports, A2B receptors are involved in multiple signaling pathways, including activation of adenylate cyclase, phospholipase C activation and the subsequent phosphoinositide hydrolysis, increase of DAG and IP3, calcium mobilization and protein kinase C activation. In earlier studies, A3 receptors have been reported to inhibit adenylate cyclase activity, activate PLC, and increase IP3 levels and intracellular Ca<sup>2+</sup>. However, subsequent studies have shown that activation of the A3 receptor led to an increase in cytoplasmic calcium, but this effect was not inhibited by inhibitors of PLC and antagonists of IP3.

### 4. The signal transduction mechanism of P2 receptor

P2 receptors are divided into two classes according to their receptor type, including ligand-gated ion channel receptors (P2XRs) and G protein-coupled receptors (P2YRs). To date, there are seven subunits of P2X receptors (P2X1–7) and eight subtypes of P2Y receptors have been identified. In

addition to the P2X and P2Y receptors, P2 receptors also include the P2U, P2T, and P2Z purinergic receptor subtypes according to earlier, now rarely used nomenclature. According to the available literature, P2XRs are activated by ATP and its synthetic derivatives, while P2YRs are activated by ATP, ADP, uridine triphosphate, uridine diphosphate, and its sugar derivatives. The stoichiometry of P2X receptors involves three subunits, which mediate rapid and selective permeability to cations. P2XRs activation results influx of extracellular Na<sup>+</sup> and Ca<sup>2+</sup> into the cell and efflux of intracellular K<sup>+</sup>, leading increased intracellular Na<sup>+</sup> and Ca<sup>2+</sup> levels, decreased K<sup>+</sup>, and depolarization of the cell membrane. Most importantly, the P2X receptor activation mediates NLRP3 inflammasome activation, which in turn leads to caspase-1 activation and ultimately to enhanced IL-1 $\beta$  and IL-18, two important pro-inflammatory cytokines. In addition, the transmembrane flux of Ca<sup>2+</sup> ions can trigger a variety of intracellular signaling events, including mitogen activated kinases, protein kinase C and calmodulin. In contrast, P2YRs mediate the hydrolysis of inositol phospholipids, mobilization of intracellular Ca<sup>2+</sup> stores, inhibition of adenylate cyclase, and also the regulation of other second-messenger signaling cascades.

## 5. Effects of ATP and adenosine on immune cell function

### 5.1 Neutrophils

Neutrophils are derived from bone marrow and have chemotaxis, phagocytosis and sterilization effects. Their main function is to resist and prevent infection. Neutrophils have been shown to express all four P1 receptor subtypes and most P2 receptor subtypes (P2Y<sub>1,2,4,6,11,14</sub> and P2X<sub>1,4,5,7</sub> receptors) 6. Activation of the P2 receptor has been shown to stimulate neutrophil migrate to sites of inflammation or tissue injury, adhesion to endothelial cells 46, and to delay neutrophil apoptosis, thus enhance its bactericidal, anti-infection effect. In contrast, extracellular adenosine at higher concentrations (micromolar levels) has been shown to stimulate neutrophil migrate away from the endothelium, inhibit adhesion to vascular endothelial cells, inhibit the production of pro-inflammatory mediators, granule release, and oxidative burst via A<sub>2A</sub> and A<sub>2B</sub> receptors, thereby inhibiting the activation of neutrophils. However, lower concentrations (nanomolar) of adenosine has been reported to promote directed neutrophil migration and phagocytosis via A<sub>1</sub> and A<sub>3</sub> receptors. Taken together, we know that during tissue damage and subsequent early inflammatory processes, ATP is released extracellular, the neutrophils produce inflammatory response under the action of high extracellular ATP and low adenosine concentration. At a late stage of inflammation, extracellular ATP is hydrolyzed and subsequently produces large amounts of adenosine. Then adenosine inhibits the pro-inflammatory action of neutrophils through A<sub>2A</sub> and A<sub>2B</sub> receptors, thereby preventing superabundant accumulation of inflammatory neutrophils within tissues.

### 5.2 Monocytes-macrophages

Monocytes-macrophages include pre-monocytes in bone marrow, monocytes in peripheral blood, and macrophages in tissues. Macrophages are derived from monocytes in the blood, which in turn are derived from precursor cells in bone marrow. They play an important role in anti-infection, anti-tumor and immune regulation. Both monocytes and macrophages have been shown to express all four P1 receptors and most P2 receptor subtypes (P2Y<sub>1,2,4,6,11,12,13</sub> and P2X<sub>1,4,5,7</sub> receptors in monocytes, the same receptor subtypes except for P2Y<sub>13</sub> in macrophages) 6. Previous studies have shown that extracellular ATP and ADP could increase adhesiveness of human promonocytic U-937 cells. Notably, the extracellular ATP could trigger endothelial cell apoptosis or shrinkage through P2X<sub>7</sub> receptors and P2Y receptors, respectively, which may disrupt the integrity of the endothelial barrier and increase the permeability of endothelial macromolecules. Thus, under the action of extracellular ATP, mononuclear cells can adhere to the vascular endothelial with increased permeability, and then migrate to the inflammatory site outside the blood vessel, and then release a

variety of inflammatory factors, to activate immune cells and enhance immune function. For example, activation of P2X7 by extracellular ATP triggers maturation and release of the pro-inflammatory cytokine interleukin-1 (IL-1) in monocytes and macrophages, and the overexpression of CD39 might lead to the hydrolysis of extracellular ATP and then inhibit the secretion of IL-1. The activation of P2Y receptor increases the transcription of IL-6. In human THP-1 mononuclear cell line, lipopolysaccharide (LPS)/IFN-gamma and TNF-alpha/IFN-gamma up-regulated P2X7R function, while cAMP increased negatively regulated the P2X7R expression. Additionally, the extracellular ATP may determine the quality and the extent of the activation of the innate immune system, including the inflammatory activation of macrophages. The main functions of macrophages are phagocytosis and digestion of cell debris and pathogens, and activation of lymphocytes or other immune cells to make them respond to pathogens. Earlier reviews have shown that ATP regulates the function of macrophages bidirectionally. At high concentration, ATP inhibits phagocytosis and promotes the secretion of lysosomal enzyme through P2X7 receptor; at low concentration, ATP stimulates phagocytosis and inhibits the secretion of proteolytic enzyme through P2Y2 receptor. However, long-term stimulation of P2X7 by high concentrations of ATP can also lead to macrophage death.

In contrast, adenosine has the opposite effect on macrophages as ATP. Extracellular adenosine inhibits the expression of adhesion molecules by activating A1, A2A and A2B receptors, and restricts the adhesion of monocytes to the vascular endothelium, thus inhibiting the migration and recruitment of macrophages, and CD73 knockout can significantly reverse this phenomenon. Activation of A2a, A2b, and A3 in monocytes-macrophages lead to inhibition of the NFκB pathway, which in turn might inhibit the secretion of pro-inflammatory cytokines IL-12, IFNγ, IL-1β, and TNFα. However, adenosine has been shown to promote IL-6, IL-10 production by activating A2A and A2B receptors. Since IL-6 and IL-10 are known to have anti-inflammatory effects, and IL-6 could directly increase IL-10 production in CD4+ T cells, B cells, and macrophages, we can conclude that extracellular adenosine suppresses monocytes-macrophages mediated inflammation and immune response. In addition, adenosine regulates macrophage phagocytosis in a bidirectional way, enhancing phagocytosis by activation of A1 receptors and inhibiting phagocytosis by activation of A2A and A2B receptors, suggesting that adenosine also regulates the immune response in a bidirectional way.

### 5.3 Dendritic cells

Dendritic cells (DCs) are a class of bone-marrow-derived cells arising from lympho-myeloid haematopoiesis, and could be found in blood, tissues and lymphoid organs. They get their name from the many nerve cell-like dendrites that protrude from their cell membranes. They are the most powerful full-time antigen-presenting cells in the body, capable of efficiently ingesting, processing and presenting antigens complexed to either major histocompatibility complex (MHC) class I or MHC class II and thereby can activate naive T cells. They play a central role in innate sensing of pathogens, initiating, regulating and maintaining the immune response in the body. Dendritic cells are divided into three subsets: plasmacytoid DC (pDC), myeloid/conventional DC1 (cDC1) and myeloid/conventional DC2 (cDC2), and each DC subset develops under the control of a specific repertoire of transcription factors involving differential levels of IRF8 and IRF4 in collaboration with PU.1, ID2, E2-2, ZEB2, KLF4, IKZF1 and BATF3. In earlier studies, DCs have been shown to express all four P1 receptors in varying degrees and are correlated with their own maturation. DCs were found to express mRNA for several P2X (P2X1, P2X4, P2X5, P2X7) and P2Y (P2Y1, P2Y2, P2Y4, P2Y5, P2Y6, P2Y10, P2Y11) receptors, triggering of P2 receptors resulted in an increase in free intracellular Ca<sup>2+</sup>. In combination with TNF-α, ATP increased the expression of the DC surface markers CD80, CD83 and CD86 indicating a maturation promoting effect. DCs expressed the ecto-ATPase CD39 and the ecto-5'-nucleotidase CD73 as demonstrated by RT-PCR. Extracellular ATP was rapidly hydrolyzed by these ecto-enzymes as shown by separation of 3H-labeled ATP metabolites using a thin layer technique. These data suggest that ATP acts as a costimulatory factor on DC maturation.

## 5.4 Lymphocyte

Earlier reports indicated that CD8+CD39+ homogen-sensitized cells predominantly mediate specific killer activity, whereas CD8+CD39- cells mainly exhibit NK-like reactivity. Fractionation of the large B lymphocytes into CD39+, surface IgD+, and CD39-, surface IgD- cells showed that the latter, but not the former, cell type produced granulocyte colony-stimulating factor (G-CSF) spontaneously in culture, indicating that CD39- cells, but not CD39+ B cells, could promote the proliferation and differentiation of granulocyte progenitor cells and enhance the functions of mature neutrophils (including chemotaxis, phagocytosis and killing, etc.). Anti-CD39 mAb could induced homotypic adhesion in a CD11/CD18-EBV (Epstein-Barr Virus)-transformed B cell line derived from a patient with severe leukocyte adhesion deficiency.

## 6. The role of ATP and adenosine in different diseases

### 6.1 Thrombosis

It is reported that CD39 hydrolyses extracellular ATP and ADP to AMP, also prevents ADP-induced platelet activation associated with vascular inflammation, and follow-up studies have shown that recombinant soluble CD39 could blocked ADP-induced platelet aggregation in vitro, and inhibited collagen-induced platelet reactivity. Previous studies have shown that CD39 on the surface of human umbilical vein endothelial cells was significant changed under the stimulation of cytokines TNF-alpha and IFN-gamma, as well as the proteolytic enzyme thrombin. Suppression of the expression of CD39 may lead to elevated concentrations of ATP and ADP at the vascular interface that could predispose to thrombosis and inflammation.

### 6.2 Graft rejection

And the disordered thromboregulation may be associated with the progression of inflammatory events in delayed xenograft rejection (DXR) and the disseminated intravascular coagulation (DIC) seen in primate recipients of porcine renal xenografts. The loss of vascular CD39 activity following endothelial cell (EC) activation responses would potentiate any procoagulant changes within the xenograft and eventually exacerbated vascular damage from whatever cause and enhance the activation of platelets and coagulation pathways within xenografts resulting in graft infarction and loss. Another study showed that CD39 influences pathways of vascular injury in cardiac xenografts, and the augmentation of CD39 activity may be an important adaptive response for graft survival. However, CD39-deficient mice had prolonged bleeding times with minimally perturbed coagulation parameters.

## 7. Enhance biological barrier function

P2Y2 receptors are activated by equivalent concentrations of ATP and UTP 15. As reported in earlier studies, P2Y2 receptor expression in smooth muscle cells is upregulated by the stimulation of interleukin-1 $\beta$ , interferon- $\gamma$  and tumor necrosis factor- $\alpha$ , while the activation of P2Y2 receptors can inhibit Na<sup>+</sup> absorption, restore chloride conductance, rehydrate the airway surface, enhance ciliary beat frequency and increase mucociliary clearance in patients with Cystic Fibrosis lung disease.

## 8. The role of ATP and adenosine in tumors

Previous studies have shown that IL-2 promotes the proliferation and enhances the secretory capacity of T cells, B cells, and NK cells. A clinical study involving 103 cancer patients (77 renal cell carcinomas, 13 melanomas, 8 colorectal carcinomas, 2 mesotheliomas, 1 B-cell lymphoma, 1 malignant fibrous histiocytoma, and 1 malignant schwannoma) showed that recombinant IL-2 (rIL-



2) administered subcutaneously significantly increased the count of circulating CD39+ T cells and NK cells. Similarly, numbers of peripheral blood mononuclear cell (PBMC) subsets from renal cell carcinoma (RCC) patients analyzed by fluorescence activating cell sorter (FACS) revealed significant expression of CD39+ ( $p < 0.03$ ) cell numbers by vaccine therapy (Irradiated autologous or allogeneic tumor cells). Our study identified the potential relevance of CD39 for monitoring chronic immune disease, infection, and immune activation in cancer patients CD39 and CD73 showed a marked upregulation during myeloid maturation of HL60 cells.

## 9. Conclusion

The adenosine metabolic pathway stands as a linchpin in the intricate tapestry of purinergic signaling, exerting profound effects on diverse physiological functions. The orchestration of immune responses, inflammation, and cellular activities by ATP, ADP, and adenosine reveals the remarkable versatility of this molecular symphony. From the bidirectional regulation of immune cell functions to the modulation of thrombosis, graft rejection, and even the enhancement of biological barrier functions, the impact of purinergic signaling extends across a spectrum of health and disease. As we delve deeper into the nuances of these molecular interactions, we uncover potential avenues for therapeutic interventions in various conditions, marking the adenosine metabolic pathway as a frontier of exploration in the ever-evolving landscape of biomedical research.

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