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Editorial overview: “Purinergic immune cell regulation reveals novel pharmacological targets.”

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Short title: Purinergic immune cell regulation

Introduction

Purinergic signaling networks have emerged as central control mechanisms that regulate a wide spectrum of physiological processes. They involve autocrine and paracrine systems that transmit signals that control individual cell functions and facilitate cell-to-cell communications. Purinergic signaling mechanisms consist of several critical components that are triggered by various cues such as mechanical stress, hormones, cytokines, or bacterial stimuli. The receptors that sense such cues often trigger mitochondrial production of adenosine triphosphate (ATP) that not only fuels intracellular signaling mechanisms but that can also act as a second messenger, namely when ATP is released into the extracellular space. Cells can release ATP via vesicular transport, pannexin channels, or connexin hemichannels in a fashion that targets cell surface regions where specific purinergic receptors are located. These can either amplify or downmodulate functional cell responses, depending on which purinergic receptor subsets are present at those subcellular regions. The purinergic receptors include three families, namely the P2X, P2Y, and P1 receptors. The seven P2X receptor subtypes function as ATP-gated Ca2+ channels, while the eight P2Y receptor subtypes belong to the G protein–coupled receptor (GPCR) superfamily [1,2]. Most P2Y receptors respond to ATP or its breakdown product adenosine diphosphate (ADP), but others recognize different nucleotides. Finally, the four P1 receptor subtypes that are also GPCR superfamily members recognize the ATP breakdown product adenosine and can either inhibit or stimulate cell responses [3]. Thus, the distribution of the different purinergic receptor subtypes across cell surfaces and the concentrations of their respective ligands are equally important in the functional responses of cells to purinergic stimuli.

The concentrations of ATP, ADP, adenosine monophosphate and adenosine are defined by several classes of ectonucleotidases that are capable of hydrolyzing ATP and its breakdown products [4]. These ectonucleotidase families include eight different ecto-nucleoside triphosphate diphosphohydrolase members (e.g., ENTPD1, also known as CD39), seven ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP) members (e.g., ENPP1 and ENPP3) [5], four alkaline phosphatase subtypes [6], as well as ecto-5-nucleotidase (NT5E, also known as CD73) [7]. Cells can redistribute their mitochondria, ATP release sites, purinergic receptors, and ectonucleotidases in a

spatiotemporal manner that allows localized finetuning of functional responses by rearranging the different components of these purinergic signaling networks. Purinergic signaling events come to an end when the ATP breakdown product adenosine is removed. Adenosine levels can be diminished by two adenosine deaminase isoforms that deaminate adenosine to inosine, which is incapable of participating in purinergic signaling [8]. Adenosine and its breakdown products can, however, also be taken up by cells to be recycled to ATP. This uptake occurs through equilibrative nucleoside transporter or concentrative nucleoside transporter family members [9]. The (re)distribution, colocalization, translocation, and activity levels of all these different purinergic- signaling components provide the necessary flexibility to regulate the complex physiological processes that are essential for multicellular life.

The immune system comprises different cell populations that eliminate infectious invaders as well as damaged and malignant host cells without causing harm to healthy host tissues. Recent work has shown that immune cell subsets heavily depend on purinergic signaling networks to control their varied roles in host defense. Neutrophil granulocytes, for example, need the mammalian target of rapamycin signaling networks, mitochondrial translocation, pannexin-mediated ATP release, as well as autocrine P2Y2 and A2a receptor stimulation to detect danger signals that guide them to sources of infection [10–13]. Similar to neutrophils, T cells also depend on mitochondrial trafficking, mitochondrial ATP production, pannexin-mediated ATP release, and autocrine feedback via purinergic receptors to screen antigen-presenting cells and to form immune synapses that T cells use to communicate with those antigen-presenting cells [14–16].

Much effort has been focused on defining the purinergic signaling networks that control the multitude of functional roles of immune cells because a better understanding of these networks holds great promise for the development of novel therapeutic interventions to treat a wide range of diseases including microbial and viral infections, acute and chronic inflammation, autoimmune diseases, transplant rejection, and cancer. In this collection of review articles, current knowledge of several of these topics has been summarized.

The contribution of Masayuki Miyasaka et al. focuses on the purinergic signaling networks that regulate the complex processes of T- cell migration in lymphoid organs [17]. This article provides an excellent summary of current knowledge about how autocrine purinergic signaling regulates the movements of lymphocytes and other immune cells into, within, and out of lymph nodes to induce adaptive immune responses. The group has developed highly sophisticated matrix-assisted laser desorption/ionization time-of-flight mass spectrometry imaging methods that provide unprecedented insights into the spatiotemporal changes in ATP signaling and the metabolic processes that control immune cell migration. The authors highlight the complexity and the overlapping functions of the purinergic signaling networks that control immune cells in lymphoid tissues. In addition, they describe the need for further in-depth studies to gain a more complete understanding of how these networks control normal immune responses and how their disruption contributes to immune dysfunction.

The contributions by Talia Swartz, Eliseo Eugenin, and Lourdes Arruvito focus on the timely concepts of how purinergic signaling contributes to viral infections. This is an aspect of purinergic immunomodulation that has been largely neglected but that has attracted increasing attention in the wake of the recent COVID-19 pandemic.

Talia Swartz et al. begin by discussing the mechanisms whereby extracellular ATP signaling through P2X receptors contributes to the pathogenesis of HIV-1 [18]. HIV-1-infected cells release ATP into the extracellular space, where it induces the production and release of proinflammatory cytokines and the activation of apoptotic pathways, particularly through stimulation of P2X7 receptors in surrounding immune cells. It is thought that these purinergic signaling–driven events contribute to chronic inflammation in HIV-1. Furthermore, recent studies suggest that HIV-1 utilizes P2X receptors for the infection of cells and that P2X receptor inhibitors can prevent viral entry and replication. Targeting P2X receptors might therefore be a promising new therapeutic approach in two respects: first, to block the viral life cycle, and second, to reduce HIV-associated chronic inflammation and comorbidities such as neurocognitive impairment or cardiovascular disease.

The review by Christian Hernandez and Eliseo Eugenin focuses on pannexin-1 (Panx-1) channels as another interesting component of purinergic signaling in the pathogenesis of HIV infections [19]. They describe the recent findings that the binding of HIV envelope protein gp120 to CD4 and CCR5-CXCR4 receptors on T cells or monocytes/macrophages leads to opening of Panx-1 and the release of ATP. Importantly, aberrant Panx-1 channel activity in HIV patients under antiretroviral therapy is associated with increased circulating ATP levels and correlates with cognitive impairments in NeuroHIV, suggesting that ATP and Panx-1 could serve as potential therapeutic targets in HIV/NeuroHIV patients. Eugenin et al. recently found that SARS-CoV-2 S protein induces prolonged opening of Panx-1 and release of ATP in a fashion akin to that associated with HIV infections. These authors argue that inhibition of Panx-1 channels could be a potential treatment for COVID-19 by preventing viral entry as well as the excessive proinflammatory response that is caused by elevated extracellular ATP concentrations. These conclusions may also hold true for other pulmonary infections such as influenza [20].

Indeed, several recent studies have led to the conclusion that purinergic- signaling pathways are activated in severe COVID-19 [21,22]. This evidence suggests that purinergic- signaling mechanisms are potential targets to prevent the devastating inflammatory response in COVID-19 patients. The review by Lourdes Arruvito et al. represents a concise and comprehensive summary of our current understanding of this rapidly evolving field [23]. High levels of ATP and ADP combined with reduced levels of adenosine have been found in the plasma of patients with severe COVID-19 and are thought to favor systemic inflammation and thrombotic events. Furthermore, recent data indicate that alterations in the CD39/CD73 axis, which is a major regulator of extracellular ATP and adenosine levels, contribute to the pathogenesis of COVID-19. While exacerbated inflammatory and thrombotic processes are well established characteristics of severe COVID-19, more work is necessary to clearly define the roles

of purinergic signaling mechanisms and their potential as therapeutic targets in COVID-19.

The contribution of Ronald Sluyter et al. summarizes current knowledge of purinergic signaling and its role in graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation, another example of a disease complex with a strong inflammatory component that is influenced by purinergic signaling in multiple ways [24]. Their own studies with a humanized mouse model provide important evidence for both GVHD-enhancing as well as GVHD-suppressing effects. P2X7 receptors are thought to promote GVHD progression through activation of host antigen–presenting cells and a P2X7 receptor–dependent loss of suppressive regulatory T cells. On the other hand, the ectoenzymes CD39 and CD73, which reduce extracellular ATP levels and induce the formation of adenosine, may reduce GVHD development by promoting adenosine A2a receptor–mediated anti-inflammatory responses. The authors propose that purinergic- signaling molecules could therefore be attractive pharmaceutic targets to prevent or reduce the life-threatening clinical impact of GVHD.

Finally, Shyni Varghese and Hunter Newman highlight in their contribution the role of extracellular adenosine and adenosine-mediated immune modulation in bone homeostasis and bone tissue repair [25]. Osteoblasts and osteoclasts express all four adenosine receptors. While A1 receptors promote osteoclast differentiation and bone resorption, A2a and A2b receptor activation stimulates osteoblastogenesis and angiogenesis and inhibits osteoclast function. Moreover, adenosine plays an important role in inflammation-induced bone remodeling and bone healing through the anti-inflammatory effects it elicits in immune cells through A2a and A2b receptors. Therefore, adenosine receptors are promising therapeutic targets in the treatment of bone injuries and osteoporosis. Varghese et al. summarize encouraging results from first experimental studies that have tested various local and systemic drug- delivery strategies aimed at reducing the side- effects of systemic adenosine application while locally prolonging the half-life of adenosine molecules.

In summary, these review articles show that purinergic signaling networks have major impacts on immune cell regulation and inflammation with wide-ranging clinical implications. The topics discussed in this special issue suggest that drugs targeting purinergic receptors, ATP release mechanisms, or ectoenzymes that define the extracellular ATP/adenosine homeostasis are promising candidates for treating inflammation and other immune-related disorders.

However, the review articles presented here can cover only part of the important work that has been done in this field, and we would like to highlight some of the many other excellent articles that have been published on those interesting research topics. These include the complex roles of the purinergic- signaling mechanisms that regulate immune cell responses in inflammation and cancer [26–28], kidney and liver diseases [29,30], and wound healing [31] to name just a few. Progress in our understanding of how these signaling networks regulate immune cell functions will surely reveal exciting new avenues to target these and many other immune-related diseases [32].

Conflict of interest statement

Nothing to declare.

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References

1. von Kügelgen I: **Pharmacological characterization of P2Y receptor subtypes - an update**. *Purinergic Signal* 2023; Sep 12. doi: 10.1007/s11302-023-09963-w. Epub ahead of print. PMID: 37697211..
2. Illes P, Müller CE, Jacobson KA, Grutter T, Nicke A, Fountain SJ, Kennedy C, Schmalzing G, Jarvis MF, Stojilkovic SS, et al.: **Update of P2X receptor properties and their pharmacology: IUPHAR Review 30**. *Br J Pharmacol* 2021, **178**:489-514.
3. Vincenzi F, Pasquini S, Contri C, Cappello M, Nigro M, Travagli A, Merighi S, Gessi S, Borea PA, Varani K: **Pharmacology of Adenosine Receptors: Recent Advancements**. *Biomolecules* 2023, **13**.
4. Zimmermann H, Zebisch M, Sträter N: **Cellular function and molecular structure of ecto-nucleotidases**. *Purinergic Signal* 2012, **8**:437-502.
5. Zimmermann H: **Ectonucleoside triphosphate diphosphohydrolases and ecto-5'-nucleotidase in purinergic signaling: how the field developed and where we are now**. *Purinergic Signal* 2021, **17**:117-125.
6. Millán JL: **Alkaline Phosphatases : Structure, substrate specificity and functional relatedness to other members of a large superfamily of enzymes**. *Purinergic Signal* 2006, **2**:335-341.
7. Yegutkin GG: **Enzymes involved in metabolism of extracellular nucleotides and nucleosides: functional implications and measurement of activities**. *Crit Rev Biochem Mol Biol* 2014, **49**:473-497.
8. Zavialov AV, Engström A: **Human ADA2 belongs to a new family of growth factors with adenosine deaminase activity**. *Biochem J* 2005, **391**:51-57.
9. Boswell-Casteel RC, Hays FA: **Equilibrative nucleoside transporters-A review**. *Nucleosides Nucleotides Nucleic Acids* 2017, **36**:7-30.
10. Chen Y, Corriden R, Inoue Y, Yip L, Hashiguchi N, Zinkernagel A, Nizet V, Insel PA, Junger WG: **ATP release guides neutrophil chemotaxis via P2Y2 and A3 receptors**. *Science* 2006, **314**:1792-1795.
11. Bao Y, Ledderose C, Graf AF, Brix B, Birsak T, Lee A, Zhang J, Junger WG: **mTOR and differential activation of mitochondria orchestrate neutrophil chemotaxis**. *J Cell Biol* 2015, **210**:1153-1164.
12. Junger WG: **Purinergic regulation of neutrophil chemotaxis**. *Cell Mol Life Sci* 2008, **65**:2528-2540.

13. Wang X, Chen D: **Purinergic Regulation of Neutrophil Function**. *Front Immunol* 2018, **9**:399.
14. Ledderose C, Junger WG: **Mitochondria Synergize With P2 Receptors to Regulate Human T Cell Function**. *Front Immunol* 2020, **11**:549889.
15. Ledderose C, Bromberger S, Slubowski CJ, Sueyoshi K, Junger WG: **Frontline Science: P2Y11 receptors support T cell activation by directing mitochondrial trafficking to the immune synapse**. *J Leukoc Biol* 2021, **109**:497-508.
16. Woehrle T, Ledderose C, Rink J, Slubowski C, Junger WG: **Autocrine stimulation of P2Y1 receptors is part of the purinergic signaling mechanism that regulates T cell activation**. *Purinergic Signal* 2019, **15**:127-137.
17. Kobayashi D, Umemoto E, Miyasaka M: **The role of extracellular ATP in homeostatic immune cell migration**. *Curr Opin Pharmacol* 2023, **68**:102331.
18. Rodriguez NR, Fortune T, Vuong T, Swartz TH: **The role of extracellular ATP and P2X receptors in the pathogenesis of HIV-1**. *Curr Opin Pharmacol* 2023, **69**:102358.
19. Hernandez CA, Eugenin EA: **The role of Pannexin-1 channels, ATP, and purinergic receptors in the pathogenesis of HIV and SARS-CoV-2**. *Curr Opin Pharmacol* 2023, **73**:102404.
20. Ledderose C, Valsami EA, Elevado M, Junger WG: **ATP release from influenza-infected lungs enhances neutrophil activation and promotes disease progression**. *J Infect Dis* 2023. Oct 6:jiad442. doi: 10.1093/infdis/jiad442. Epub ahead of print. PMID: 37804120..
21. Korb VG, Schultz IC, Beckenkamp LR, Wink MR: **A Systematic Review of the Role of Purinergic Signalling Pathway in the Treatment of COVID-19**. *Int J Mol Sci* 2023, **24**.
22. Hasan D, Shono A, van Kalken CK, van der Spek PJ, Krenning EP, Kotani T: **A novel definition and treatment of hyperinflammation in COVID-19 based on purinergic signalling**. *Purinergic Signal* 2022, **18**:13-59.
23. Arruvito L, Sananez I, Seery V, Russo C, Geffner J: **Purinergic signaling pathway in severe COVID-19**. *Curr Opin Pharmacol* 2023, **70**:102379.
24. Sluyter R, Cuthbertson P, Elhage A, Sligar C, Watson D: **Purinergic signalling in graft-versus-host disease**. *Curr Opin Pharmacol* 2023, **68**:102346.
25. Newman H, Varghese S: **Extracellular adenosine signaling in bone health and disease**. *Curr Opin Pharmacol* 2023, **70**:102378.
26. Di Virgilio F, Sarti AC, Coutinho-Silva R: **Purinergic signaling, DAMPs, and inflammation**. *Am J Physiol Cell Physiol* 2020, **318**:C832-c835.
27. Kepp O, Bezu L, Yamazaki T, Di Virgilio F, Smyth MJ, Kroemer G, Galluzzi L: **ATP and cancer immunosurveillance**. *Embo j* 2021, **40**:e108130.
28. Idzko M, Ferrari D, Eltzschig HK: **Nucleotide signalling during inflammation**. *Nature* 2014, **509**:310-317.
29. Dwyer KM, Kishore BK, Robson SC: **Conversion of extracellular ATP into adenosine: a master switch in renal health and disease**. *Nat Rev Nephrol* 2020, **16**:509-524.
30. Vuerich M, Robson SC, Longhi MS: **Ectonucleotidases in Intestinal and Hepatic Inflammation**. *Front Immunol* 2019, **10**:507.

31. Ferrari D, Gambari R, Idzko M, Müller T, Albanesi C, Pastore S, La Manna G, Robson SC, Cronstein B: **Purinergic signaling in scarring**. *Faseb j* 2016, **30**:3-12.
32. Antonioli L, Blandizzi C, Pacher P, Haskó G: **The Purinergic System as a Pharmacological Target for the Treatment of Immune-Mediated Inflammatory Diseases**. *Pharmacol Rev* 2019, **71**:345-382.