

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of May 27, 2015):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/345/6202/1254009.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2014/08/20/science.1254009.DC1.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/345/6202/1254009.full.html#related>

This article **cites 56 articles**, 27 of which can be accessed free:

<http://www.sciencemag.org/content/345/6202/1254009.full.html#ref-list-1>

This article has been **cited by** 3 articles hosted by HighWire Press; see:

<http://www.sciencemag.org/content/345/6202/1254009.full.html#related-urls>

This article appears in the following **subject collections**:

Immunology

<http://www.sciencemag.org/cgi/collection/immunology>

Review Article

Pathophysiological Role of Extracellular Purinergic Mediators in the Control of Intestinal Inflammation

Yosuke Kurashima,^{1,2,3} Hiroshi Kiyono,^{2,3,4} and Jun Kunisawa^{1,2,4,5,6}

¹ Laboratory of Vaccine Materials, National Institute of Biomedical Innovation, Osaka 567-0085, Japan

² Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan

³ Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Tokyo 102-0075, Japan

⁴ International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan

⁵ Department of Microbiology and Immunology, Kobe University School of Medicine, Kobe 650-0017, Japan

⁶ Graduate School of Pharmaceutical Sciences and Graduate School of Dentistry, Osaka University, Osaka 565-0871, Japan

Correspondence should be addressed to Jun Kunisawa; kunisawa@nibio.go.jp

Received 5 August 2014; Accepted 30 September 2014

Academic Editor: Ishak O. Tekin

Copyright © Yosuke Kurashima et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Purinergic mediators such as adenosine 5'-triphosphate (ATP) are released into the extracellular compartment from damaged tissues and activated immune cells. They are then recognized by multiple purinergic P2X and P2Y receptors. Release and recognition of extracellular ATP are associated with both the development and the resolution of inflammation and infection. Accumulating evidence has recently suggested the potential of purinergic receptors as novel targets for drugs for treating intestinal disorders, including intestinal inflammation and irritable bowel syndrome. In this review, we highlight recent findings regarding the pathophysiological role of purinergic mediators in the development of intestinal inflammation.

1. General Features and Metabolism of ATP in the Intestinal Compartment

Damage, trauma, and pathogenic infection cause inflammatory responses in tissues. Clinical pathologic responses involve the release of a series of inflammatory mediators, including cytokines (e.g., IL-1 β , IL-6, and TNF α), lipid mediators (e.g., leukotrienes, platelet activating factor, and prostaglandins), and chemical mediators (e.g., histamine).

Accumulating evidence clearly demonstrates the importance of purinergic mediators, especially adenosine 5'-triphosphate (ATP), in the development of various inflammatory disorders [1]. In general, ATP is generated during glycolysis and the tricarboxylic acid cycle in the intracellular compartment and acts as an energy source. However, ATP is occasionally released into the extracellular compartment as so-called extracellular ATP (eATP). Biological roles of eATP were first reported in synaptic neurotransmission and

neuromodulation [2]. eATP is released from nerves as a transmitter or cotransmitter and causes pain [2]. In the intestine, purinergic signaling is important for synaptic transmission in the enteric nervous system [2]. The excitatory postsynaptic potential of myenteric neurons is mediated by eATP together with nicotinic acetylcholine [3, 4]. Thus, stimulation by eATP is important for maintaining physiological intestinal motility.

In addition to nerve cells, dead, activated, or infected cells release eATP, recruiting and activating both innate and acquired immunity [5]. For instance, bacterial stimulation leads to eATP release from monocytes and enhances the production of cytokines in an autocrine manner [6]. Some gap junction hemichannels, such as pannexin and connexin hemichannels, are important for ATP release during cell activation [7]. In the steady state intestine some commensal bacteria also have the potential to release eATP [8]; thus, germ-free mice have lower luminal ATP levels than do specific pathogen-free mice. This commensal-derived eATP

stimulates CD70⁺ CD11c^{low} cells in the intestinal compartment and recruits Th17 cells into the colon [9].

Hydrolysis of the released eATP is catalyzed by cell surface-located enzymes, such as ectonucleoside triphosphate diphosphohydrolase family enzymes (e.g., e-NTPDase I (CD39), ectonucleotidase, and NT5E (CD73)). Consistent with the activity of eATP in the induction of intestinal Th17 cells, a deficiency of eATP-degrading enzymes elevates the concentration of luminal eATP and subsequently enhances the generation of Th17 cells in the gut [10]. By the sequential enzymatic activity of CD39 and CD73, eATP is hydrolyzed to adenosine in the extracellular compartment [11] (Figure 1). Finally, adenosine is metabolized by two pathways: one is intracellular uptake by equilibrative nucleoside transporters and the other is metabolism to AMP or inosine by adenosine kinase and adenosine deaminase, respectively [11].

Recognition of eATP is mediated by purinergic receptors, which comprise P2X (P2X₁₋₇) and P2Y receptors (P2Y_{1,2,4,6,11-14}). P2X₁₋₇ receptors are ATP-gated ion channels and are specific for ATP, whereas P2Y receptors are G protein-coupled receptors that are specific for ADP, UTP, and ATP [5]. Each eATP-specific purinergic receptor requires a different concentration of eATP for activation. For instance, activation of P2X₇ receptors requires a high concentration (mM level) of eATP, whereas other P2X receptors require lower concentrations (nM to μ M) [5]. In addition, heterooligomeric assembly occurs within P2X receptor subunits (e.g., P2X₁₋₃, P2X₁₋₄, and P2X₂₋₄₋₅) and alters their functional properties, providing versatile signaling pathways mediated by eATP [12, 13].

Among several P2X and P2Y receptors, P2X₇ is involved mainly in the induction of inflammatory responses. P2X₇ uniquely has 200 amino acid residues in its C-terminus, which is longer than that of other P2X receptors [14]. C-terminal residues are important for receptor localization at the cell surface [14]. Stimulation of P2X₇ by prolonged high concentrations of eATP induces pore formation in the cell membrane and increases membrane permeability [14, 15]. These pores allow influx and efflux of particles with molecular masses of up to 800 Da [11]. These changes also mediate the production of reactive oxygen species and activate inflammasome, a key molecule in the production of inflammatory cytokines such as IL-1 β and IL-18 [5] that is responsible for inducing inflammatory responses. In addition, eATP-P2X₇ pathways are involved in molecular shedding. Molecules responsible for adhesion (e.g., CD44 and CD62L) are shed from the cell surface by P2X₇ activation; stimulation by eATP is thus involved in cell migration [16, 17].

2. Role of eATP in Prevention and Development of Infectious Diseases

Some kinds of pathogens use intestinal tissues as invasion sites. Upon infection, pathogenic components from the microorganisms stimulate innate immune cells such as macrophages and neutrophils via innate receptors such as toll-like receptors (TLRs). This stimulation induces the

release of eATP through pannexin-1 hemichannels and subsequently activates P2Y₂ and P2X₇ receptors in an autocrine or paracrine manner and enhances cytokine production [6, 18]. In microglial cells and macrophages, initial stimulation of lipopolysaccharide- (LPS-) TLR4 pathways with subsequent signaling by the P2X₇ pathway induces Ca²⁺ influx and IL-1 β secretion [19]. In fact, eATP-P2X₇ pathways play important roles in eliminating intracellular pathogens. Activation of P2X₇ by selective agonists induces effective clearance of *Toxoplasma gondii* from infected macrophages and of chlamydia from epithelial cells [20, 21]. These signals are required for protective immunity against pathogens. In addition, a recent study found that eATP production was induced by administration of vaccine adjuvant, which is required for an effective response in vaccination against infectious agents and cancer [22].

Reciprocally, the pathogenicity of some pathogens is determined by their ability to induce eATP release. For instance, enteropathogenic *Escherichia coli* induces eATP release from host cells by killing them via type III secretion systems as well as cell-permeable cystic fibrosis transmembrane conductance regulator-mediated pathways [23]. Similarly, cholera toxin from *Vibrio cholerae* is capable of inducing eATP production [24]. Another study in colon epithelial cell lines found that adenosine, a metabolite of eATP, bound to A_{2B} receptors, resulting in short-circuit current responses causing diarrhea [23, 24].

Some kinds of pathogens have unique systems that inhibit eATP release from host cells and thus prevent the spread of infection to the host's immune system. For instance, infection of epithelial cells with *Shigella flexneri* induces eATP release via connexin hemichannels in the early phase of infection, and this release alerts the host to the pathogenic infection. However, prolonged infection with *Shigella* is accompanied by the production of PtdIns5P, a lipid mediator, to close the connexin hemichannels [25]. Another example is that of *Streptococcus agalactiae*, a commensal bacterium that resides in the intestine or vaginal mucosa but occasionally shows pathogenicity, causing neonatal pneumonia. *Streptococcus agalactiae* releases ecto-5'-nucleoside diphosphate phosphohydrolase and degrades extracellular nucleotides, including eATP; it thus turns off the eATP-mediated alerting of the host defenses to danger [26, 27].

3. Pathological Aspects of eATP in the Mucosal Compartment

eATP-purinergic receptor-mediated pathways are now considered to be targets for the treatment of inflammatory disorders in the systemic compartment, including inflammatory pain and rheumatoid arthritis [28]. Accumulating evidence suggests that eATP-purinergic receptor-mediated pathways are also potential targets for the treatment of inflammatory diseases of mucosal tissues in, for example, the respiratory and gastrointestinal tracts [4, 5, 29]. In the asthma model, migration of eosinophils, dendritic cells, and Th2 cells into the inflamed lung is mediated by the P2Y₂ receptor; therefore, P2Y₂-deficient mice show reduced inflammatory

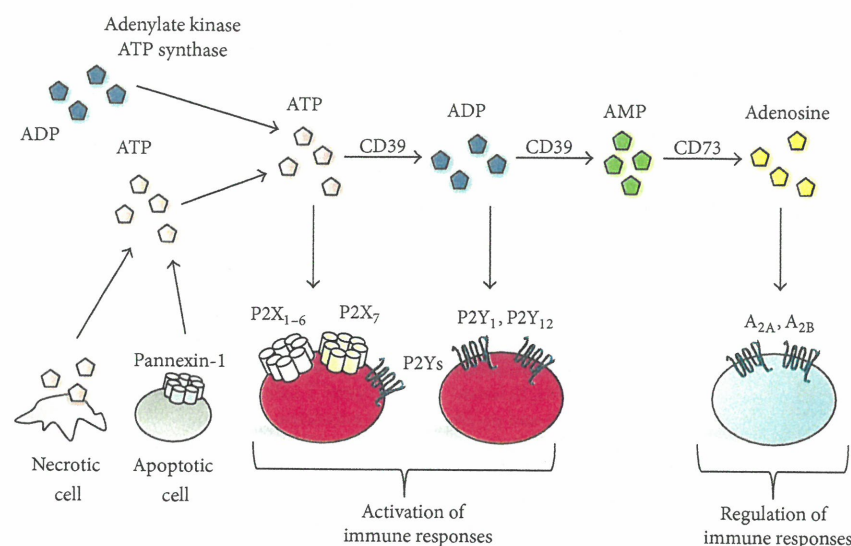


FIGURE 1: ATP is released from necrotic and apoptotic cells as extracellular ATP (eATP). Also, adenylate kinase and synthase mediate the generation of ATP in the extracellular compartment. Extracellular purines (e.g., ATP and ADP) stimulate their receptors and modulate various biological processes. Once eATP is released, the ATP is soon hydrolyzed to AMP and adenosine by the ectonucleotidases CD39 and CD73. Adenosine binds to adenosine receptors (e.g., A₁, A_{2A}, A_{2B}, and A₃).

responses [30]. Th2-type immune responses are also induced by dendritic cells expressing P2X₇. Indeed, depletion of eATP by apyrase treatment or P2X₇ deficiency reduces signs of inflammation in the upper respiratory tract [31]. It was recently found that the functional capacity of P2X₇ (i.e., its ability to promote pore formation) is associated with asthma risk or disease severity in humans [32]. Moreover, *in vivo* imaging analysis has revealed eATP release in the intestinal compartment and peritoneal cavity of mice with acute graft-versus-host disease (GVHD) [33]. Treatment with apyrase or with inhibitors of various purinergic receptors inhibits GVHD-associated intestinal inflammation. In this case, the eATP-P2X₇ pathway activates dendritic cells and consequently induces Th1 immune responses (e.g., IFN γ production) and expansion of donor T cells, thus contributing to the onset of inflammation.

Several studies have revealed the pathologic roles of eATP and purinergic receptors (especially P2X₇) in the development of intestinal disorders, including irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) [1, 34] (Table 1). IBS is a common gastrointestinal disorder characterized by discomfort, chronic abdominal pain, and altered bowel habit. Sometimes it occurs after intestinal infection. One meta-analysis has demonstrated that the risk of IBS increases 600% after gastrointestinal infection [35]. Consistently, it has been reported that transient intestinal infection with *Trichinella spiralis* in mice causes postinflammatory visceral hypersensitivity, which is associated with IL-1 β production mediated by eATP-P2X₇ pathways [34] (Table 1). Because mast cells are considered to play a critical role in the development of IBS and express high levels of P2X₇, it is possible that the eATP-P2X₇ pathway in mast cells is involved in the development of IBS [36].

The eATP-purinergic receptor pathway, especially the eATP-P2X₇ pathway, is also involved in the development of IBD. Overexpression of P2X₇ receptors has been observed in the intestinal mucosa of patients with IBD—especially Crohn's disease [44]. Experimentally, P2X₇-deficient mice do not develop experimental colitis, and inhibition of P2X₇ by A-740003, Brilliant Blue G, or KN-62 ameliorates experimental colitis by reducing the recruitment of neutrophils, T cells, and macrophages, as well as collagen deposition [44] (Table 1). The eATP-P2X₇ pathway is therefore now considered to be a novel therapeutic target in the treatment of IBD [43, 44] (Table 1).

Several mechanisms of eATP-mediated inflammation in the development of IBD have been proposed. First, eATP from damaged intestinal epithelial cells, which are frequently observed in IBD patients, and inflammatory cells (e.g., neutrophils and macrophages) stimulates dendritic cells to produce IL-6, IL-12, and IL-23 and TGF β , thus inducing the production of inflammatory Th1 and Th17 cells [42, 43] (Figure 2) (Table 1). Enteric neuronal cell death is frequently observed in intestinal inflammation and causes colonic motor dysfunction. The eATP-P2X₇ pathway is involved in enteric neuronal cell death through the pannexin-inflammasome cascade, and thus colonic motor dysfunction during colitis is prevented by targeting these pathways [41] (Table 1). We previously established mast cell-specific antibody libraries and showed that P2X₇ is expressed at high levels in mast cells in the colonic tissues [40]. eATP stimulates mast cells to induce the production of inflammatory chemokines (e.g., CCL2, CCL4, CCL7, CXCL1, and CXCL2), cytokines (IL-1 β , IL-6, and TNF α), and mediators (histamines and leukotriene). Thus, blockade of P2X₇ by a specific antibody (IF11 monoclonal antibody) inhibits mast cell activation in the colonic

TABLE 1: Recent reports indicating the critical roles of eATP in the adverse conditions of intestines (inflammatory bowel diseases and irritable bowel syndrome).

Enteric diseases	Receptors	Functions	Reference
Inflammatory bowel disease	P2R/A2BR	Enhance co-transmigration of neutrophils and platelets across intestinal epithelial cells in IBD patients. Platelets release large amount of ATP in the lumen metabolite to adenosine via CD73 and ecto-NTPDases expressed in epithelial cells. Adenosine-A2BR pathway induces electrogenic Cl ⁻ secretion with water movement to lumen.	[37]
	P2XR	T cell receptor stimulation induces ATP synthesis and release from activated T cells through pannexin-1 hemichannels. Released ATP activates T cells and produce IL-2 and proliferation in autocrine manner. Blockage of P2X receptors (oxidative ATP) impairs the development of colitis in mice.	[38]
	P2R	ATP released from commensal bacteria acts on CD70+ CD11c+ cells reside in the intestinal lamina propria and induces Th17 cells in mice; degradation of ATP (by apyrase treatments) ameliorates colitis in mice.	[9]
	P2Y2	Increase of P2Y2 expression in epithelial cells is observed during colitis. P2Y2 stimulation induces release of prostaglandin E2 release from the cells and promotion of intestinal microtubule stabilization and mucosal reepithelization. Those pathways take part in the wound healing during colonic inflammation. Treatment with P2Y2 agonist improves recovery from colitis in mice.	[39]
	P2X7	ATP induces activation of mast cells and enhances inflammatory responses, upregulation of P2X7 in mast cells of Crohn's disease patients, anti-P2X7 antibody treatment inhibits colitis in mice.	[40]
	P2X7	Induction of enteric neuronal cell death and alteration of intestinal motility.	[41]
	P2R	ATP induces IL-6 and CXCL1 productions from epithelial cells; ATP influences the response of epithelial cells to various TLR ligands and induces inflammatory T cells by affecting DC maturations.	[42]
	P2X7	Prophylactic systemic P2X7 blockade (A740003 and brilliant blue G) reduces inflammatory cytokines in rats.	[43]
	P2X7	Upregulation of P2X7 in epithelium, macrophage, and dendritic cells of Crohn's disease patients, P2X7-deficient mice did not develop colitis.	[44]
Irritable bowel syndrome	P2X7	Induction of IL-1 β and the development of postinflammatory visceral hypersensitivity in the <i>Trichinella spiralis</i> -infected mouse	[34]

tissues and consequently prevents the development of intestinal inflammation [40] (Table 1). In this pathway, P2X₇ expression on mast cells is important for the development of colitis, because mast cell-deficient mice reconstituted with P2X₇-deficient mast cells show amelioration of inflammatory signs. Of clinical relevance, we have found that the number of P2X₇⁺ mast cells is increased at sites of inflammation in Crohn's disease patients [40]. eATP is produced by injured epithelial cells and inflammatory cells, including neutrophils, via gap junction molecules such as connexin 43 [45]. It was reported that P2Y2 and P2X7 receptors are important for the migration of neutrophils and macrophages. In the

inflammatory condition, neutrophils transmigrated between epithelial cells to the luminal part of the intestine. In this condition, platelets translocate along with neutrophils and released eATP at the mucosal surface (Figure 2) (Table 1). Additionally, mast cells express ectoadenylate kinase and ATP synthase to mediate the extracellular conversion of ADP to ATP, which in turn promotes mast cell activation in an autocrine and paracrine manner (Figure 2). We have recently found that, in contrast to the abundance of P2X₇ expression on mast cells in the colon, there are limited levels of P2X₇ expression on skin mast cells, which is regulated by skin fibroblasts [46]. Skin fibroblasts uniquely express Cyp26b1 to

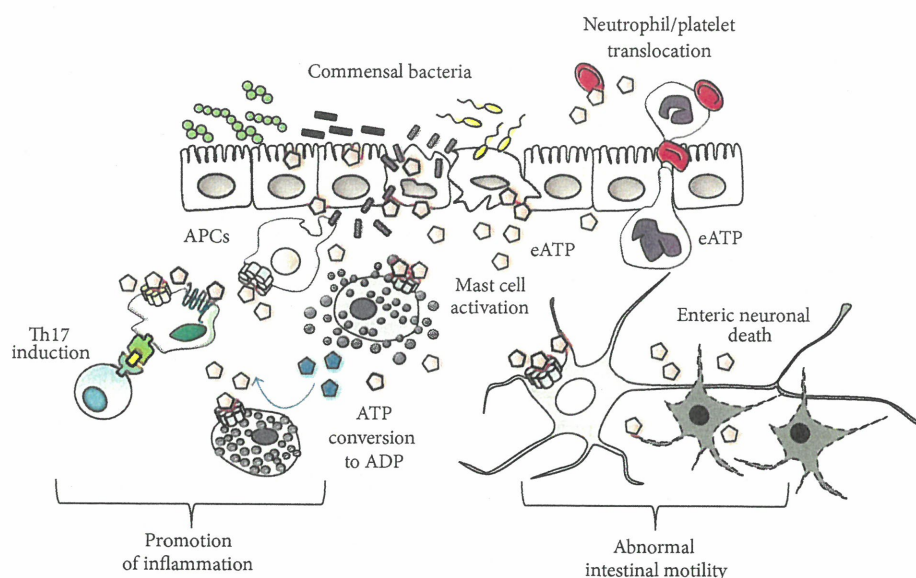


FIGURE 2: In the intestinal compartment, extracellular ATP (eATP) is released from damaged epithelial cells and commensal bacteria. Macrophages, platelets, mast cells, and neutrophils are potential source of eATP upon their activation. Neutrophils facilitate translocation of platelets across intestinal epithelium. eATP also induces Th17 cell generation, activation of mast cells, and neuronal cell death, promoting intestinal inflammation. APCs: antigen-presenting cells. eATP stimulates mast cells to induce the production of inflammatory chemokines (e.g., CCL2, CCL4, CCL7, CXCL1, and CXCL2), cytokines (IL-1 β , IL-6, and TNF α), and mediators (histamines and leukotrienes).

degrade retinoic acid within tissues or microenvironments; Cyp26b1 is responsible for inhibiting P2X₇ expression [46]. Thus, unique tissue environments determine P2X₇ expression on mast cells, which is a critical factor in the development of local inflammation.

4. Resolution of eATP-Mediated Inflammation for Maintenance of Mucosal Homeostasis

Once eATP is released, it is soon hydrolyzed to ADP, AMP, and adenosine by the ectonucleotidases CD39 and CD73; this is essential for resolving inflammatory responses (Figure 1). Indeed, CD39-deficient mice, as well as humans who have CD39 polymorphism and thus low levels of CD39 expression, have increased susceptibility to IBD [47]. Similarly, CD73 deficiency or administration of CD73 inhibitor (e.g., α , β -methylene ADP) enhances susceptibility to intestinal inflammation in mice [48–50].

Adenosine, which is derived from the dephosphorylation of eATP via CD39 and CD73 or diffuses directly from the intracellular compartment via equilibrative nucleoside transporters, binds to adenosine receptors such as A_{2A} and A₃ receptors, which are involved in both the promotion and the resolution of inflammatory responses [51–53]. A_{2A} and A₃ receptor expression on T cells and myeloid cells is a prerequisite for the inhibition of intestinal inflammation [54]. In fact, A_{2A} and A₃ adenosine receptor-selective agonists (e.g., ATP-146e and IB-MECA, resp.) ameliorate intestinal inflammation by impairing the recruitment of inflammatory cells and the production of inflammatory cytokines [55, 56].

In addition, cyclosporine, salicylates, methotrexate, and sulfasalazine, which are used to treat IBD in humans, all decrease eATP levels and increase adenosine production, partly via the stimulation of CD73-dependent adenosine production [57]. Similarly, upregulation of CD39 expression induced on dendritic cells by IL-27 hampers Th1 and Th17 cell production and consequently prevents eATP-mediated inflammation [58]. All of this evidence indicates that inhibition of eATP signaling, together with the promotion of adenosine-mediated regulatory pathways by targeting receptors or ectoenzymes, would be a beneficial strategy for the treatment of intestinal inflammation.

5. Closing Remarks

The importance of purinergic signaling was recognized almost 70 years ago. Accumulating evidence has since revealed the underlying molecular and cellular mechanisms of purinergic signal-mediated maintenance and disruption of mucosal homeostasis. Currently, the clinical relevance of some of the drugs used to treat intestinal inflammation is explained by their regulation of eATP-adenosine balance. Additionally, drugs that target purinergic receptors have now undergone clinical trials [11]. Notably, ATP-adenosine balance, as well as receptor expression levels and the cells expressing these receptors, differs among tissues and environmental conditions. Further investigations using new technologies such as *in vivo* monitoring of eATP release [59, 60] will clarify the complex mechanisms of purinergic signal-mediated immune regulation. This in turn will provide further advances in the design of drugs for preventing and

treating inflammatory diseases and maintaining immunologic health.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

Some of the work featured in this review article was supported by grants from the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries, and the Food Industry; the Program for Promotion of Basic and Applied Research for Innovations in Bio-Oriented Industry; Japan Science and Technology Agency's Core Research for Evolutional Science and Technology (CREST); the Ministry of Education, Culture, Sports, Science, and Technology of Japan; the Ministry of Health and Welfare of Japan; the Yakult Bio-Science Foundation; Kowa Life Science Foundation; a Kishimoto Foundation Research Grant; and The Naito Foundation.

References

- [1] M. Idzko, D. Ferrari, and H. K. Eltzschig, "Nucleotide signalling during inflammation," *Nature*, vol. 509, no. 7500, pp. 310–317, 2014.
- [2] G. Burnstock, "Purinergic signalling and disorders of the central nervous system," *Nature Reviews Drug Discovery*, vol. 7, no. 7, pp. 575–590, 2008.
- [3] G. Burnstock, "The journey to establish purinergic signalling in the gut," *Neurogastroenterology and Motility*, vol. 20, no. 1, pp. 8–19, 2008.
- [4] J. A. Roberts, M. K. Lukewich, K. A. Sharkey, J. B. Furness, G. M. Mawe, and A. E. Lomax, "The roles of purinergic signaling during gastrointestinal inflammation," *Current Opinion in Pharmacology*, vol. 12, no. 6, pp. 659–666, 2012.
- [5] W. G. Junger, "Immune cell regulation by autocrine purinergic signalling," *Nature Reviews Immunology*, vol. 11, no. 3, pp. 201–212, 2011.
- [6] A. Piccini, S. Carta, S. Tassi, D. Lasiglié, G. Fossati, and A. Rubartelli, "ATP is released by monocytes stimulated with pathogen-sensing receptor ligands and induces IL-1 β and IL-18 secretion in an autocrine way," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 23, pp. 8067–8072, 2008.
- [7] S. E. Adamson and N. Leitinger, "The role of pannexin1 in the induction and resolution of inflammation," *FEBS Letters*, vol. 588, no. 8, pp. 1416–1422, 2014.
- [8] T. Iwase, H. Shinji, A. Tajima et al., "Isolation and identification of ATP-secreting bacteria from mice and humans," *Journal of Clinical Microbiology*, vol. 48, no. 5, pp. 1949–1951, 2010.
- [9] K. Atarashi, J. Nishimura, T. Shima et al., "ATP drives lamina propria T_H17 cell differentiation," *Nature*, vol. 455, no. 7214, pp. 808–812, 2008.
- [10] T. Kusu, H. Kayama, M. Kinoshita et al., "Ecto-nucleoside triphosphate diphosphohydrolase 7 controls Th17 cell responses through regulation of luminal ATP in the small intestine," *Journal of Immunology*, vol. 190, no. 2, pp. 774–783, 2013.
- [11] H. K. Eltzschig, M. V. Sitkovsky, and S. C. Robson, "Purinergic signaling during inflammation," *The New England Journal of Medicine*, vol. 367, no. 24, pp. 2322–2333, 2012.
- [12] L.-H. Jiang, M. Kim, V. Spelta, X. Bo, A. Surprenant, and R. A. North, "Subunit arrangement in P2X receptors," *The Journal of Neuroscience*, vol. 23, no. 26, pp. 8903–8910, 2003.
- [13] G. E. Torres, T. M. Egan, and M. M. Voigt, "Hetero-oligomeric assembly of P2X receptor subunits. Specificities exist with regard to possible partners," *The Journal of Biological Chemistry*, vol. 274, no. 10, pp. 6653–6659, 1999.
- [14] H. M. Costa-Junior, F. S. Vieira, and R. Coutinho-Silva, "C terminus of the P2X7 receptor: treasure hunting," *Purinergic Signalling*, vol. 7, no. 1, pp. 7–19, 2011.
- [15] H. L. Wilson, S. A. Wilson, A. Surprenant, and R. North, "Epithelial membrane proteins induce membrane blebbing and interact with the P2X7 receptor C terminus," *The Journal of Biological Chemistry*, vol. 277, no. 37, pp. 34017–34023, 2002.
- [16] F. Scheuplein, N. Schwarz, S. Adriouch et al., "NAD⁺ and ATP released from injured cells induce P2X7-dependent shedding of CD62L and externalization of phosphatidylserine by Murine T cells," *Journal of Immunology*, vol. 182, no. 5, pp. 2898–2908, 2009.
- [17] C. Lin, S. Ren, L. Zhang, H. Jin, J. Sun, and Y. Zuo, "Extracellular ATP induces CD44 shedding from macrophage-like P388D1 cells via the P2X7 receptor," *Hematological Oncology*, vol. 30, no. 2, pp. 70–75, 2012.
- [18] Y. Chen, Y. Yao, Y. Sumi et al., "Purinergic signaling: a fundamental mechanism in neutrophil activation," *Science Signaling*, vol. 3, no. 125, p. ra45, 2010.
- [19] D. Ferrari, P. Chiozzi, S. Falzoni, S. Hanau, and F. Di Virgilio, "Purinergic modulation of interleukin-1 β release from microglial cells stimulated with bacterial endotoxin," *Journal of Experimental Medicine*, vol. 185, no. 3, pp. 579–582, 1997.
- [20] G. Corrêa, C. Marques da Silva, A. C. de Abreu Moreira-Souza, R. C. Vommaro, and R. Coutinho-Silva, "Activation of the P2X₇ receptor triggers the elimination of *Toxoplasma gondii* tachyzoites from infected macrophages," *Microbes and Infection*, vol. 12, no. 6, pp. 497–504, 2010.
- [21] T. Darville, L. Welter-Stahl, C. Cruz, A. A. Sater, C. W. Andrews Jr., and D. M. Ojcius, "Effect of the purinergic receptor P2X₇ on *Chlamydia* infection in cervical epithelial cells and vaginally infected mice," *The Journal of Immunology*, vol. 179, no. 6, pp. 3707–3714, 2007.
- [22] M. Vono, M. Taccone, P. Caccin et al., "The adjuvant MF59 induces ATP release from muscle that potentiates response to vaccination," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 52, pp. 21095–21100, 2013.
- [23] J. K. Crane, R. A. Olson, H. M. Jones, and M. E. Duffey, "Release of ATP during host cell killing by enteropathogenic *E. coli* and its role as a secretory mediator," *American Journal of Physiology: Gastrointestinal and Liver Physiology*, vol. 283, no. 1, pp. G74–G86, 2002.
- [24] J. K. Crane, T. M. Naeher, S. S. Choudhari, and E. M. Giroux, "Two pathways for ATP release from host cells in enteropathogenic *Escherichia coli* infection," *American Journal of Physiology: Gastrointestinal and Liver Physiology*, vol. 289, no. 3, pp. G407–G417, 2005.
- [25] A. Puhar, H. Tronchère, B. Payrastre, G. Tran van Nhieu, and P. J. Sansonetti, "A *Shigella* effector dampens inflammation by regulating epithelial release of danger signal ATP through

- production of the lipid mediator PtdIns5P," *Immunity*, vol. 39, no. 6, pp. 1121–1131, 2013.
- [26] A. Firon, M. Dinis, B. Raynal, C. Poyart, P. Trieu-Cuot, and P. A. Kaminski, "Extracellular nucleotide catabolism by the Group B *Streptococcus* ectonucleotidase NudP increases bacterial survival in blood," *The Journal of Biological Chemistry*, vol. 289, no. 9, pp. 5479–5489, 2014.
- [27] R. Coutinho-Silva and D. M. Ojcius, "Role of extracellular nucleotides in the immune response against intracellular bacteria and protozoan parasites," *Microbes and Infection*, vol. 14, no. 14, pp. 1271–1277, 2012.
- [28] S. Brumfield, J. J. Matasi, D. Tulshian et al., "Synthesis and SAR development of novel P2X7 receptor antagonists for the treatment of pain: part 2," *Bioorganic and Medicinal Chemistry Letters*, vol. 21, no. 24, pp. 7287–7290, 2011.
- [29] L. Antonioli, R. Colucci, C. Pellegrini et al., "The role of purinergic pathways in the pathophysiology of gut diseases: pharmacological modulation and potential therapeutic applications," *Pharmacology & Therapeutics*, vol. 139, no. 2, pp. 157–188, 2013.
- [30] T. Müller, B. Robaye, R. P. Vieira et al., "The purinergic receptor P2Y2 receptor mediates chemotaxis of dendritic cells and eosinophils in allergic lung inflammation," *Allergy*, vol. 65, no. 12, pp. 1545–1553, 2010.
- [31] T. Müller, R. P. Vieira, M. Grimm et al., "A potential role for P2X₇R in allergic airway inflammation in mice and humans," *American Journal of Respiratory Cell and Molecular Biology*, vol. 44, no. 4, pp. 456–464, 2011.
- [32] D. M. Manthei, D. J. Jackson, M. D. Evans et al., "Protection from asthma in a high-risk birth cohort by attenuated P2X₇ function," *Journal of Allergy and Clinical Immunology*, vol. 130, no. 2, pp. 496–502, 2012.
- [33] K. Wilhelm, J. Ganesan, T. Müller et al., "Graft-versus-host disease is enhanced by extracellular ATP activating P2X₇R," *Nature Medicine*, vol. 16, no. 12, pp. 1434–1439, 2010.
- [34] C. Keating, P. Pelegrin, C. M. Martínez, and D. Grundy, "P2X₇ receptor-dependent intestinal afferent hypersensitivity in a mouse model of postinfectious irritable bowel syndrome," *The Journal of Immunology*, vol. 187, no. 3, pp. 1467–1474, 2011.
- [35] M. Thabane, D. T. Kottachchi, and J. K. Marshall, "Systematic review and meta-analysis: the incidence and prognosis of post-infectious irritable bowel syndrome," *Alimentary Pharmacology & Therapeutics*, vol. 26, no. 4, pp. 535–544, 2007.
- [36] L. Ohman and M. Simren, "Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions," *Nature Reviews Gastroenterology & Hepatology*, vol. 7, no. 3, pp. 163–173, 2010.
- [37] T. Weissmüller, E. L. Campbell, P. Rosenberger et al., "PMNs facilitate translocation of platelets across human and mouse epithelium and together alter fluid homeostasis via epithelial cell-expressed ecto-NTPDases," *Journal of Clinical Investigation*, vol. 118, no. 11, pp. 3682–3692, 2008.
- [38] U. Schenk, A. M. Westendorf, E. Radaelli et al., "Purinergic control of T cell activation by ATP released through pannexin-1 hemichannels," *Science Signaling*, vol. 1, no. 39, article ra6, 2008.
- [39] E. Degagné, J. Degrandmaison, D. M. Grbic, V. Vienne, G. Arguin, and F.-P. Gendron, "P2Y2 receptor promotes intestinal microtubule stabilization and mucosal re-epithelization in experimental colitis," *Journal of Cellular Physiology*, vol. 228, no. 1, pp. 99–109, 2013.
- [40] Y. Kurashima, T. Amiya, T. Nochi et al., "Extracellular ATP mediates mast cell-dependent intestinal inflammation through P2X7 purinoceptors," *Nature Communications*, vol. 3, article 1034, 2012.
- [41] B. D. Gulbransen, M. Bashashati, S. A. Hirota et al., "Activation of neuronal P2X7 receptor-pannexin-1 mediates death of enteric neurons during colitis," *Nature Medicine*, vol. 18, no. 4, pp. 600–604, 2012.
- [42] Y. Yao, M. K. Levings, and T. S. Steiner, "ATP conditions intestinal epithelial cells to an inflammatory state that promotes components of DC maturation," *European Journal of Immunology*, vol. 42, no. 12, pp. 3310–3321, 2012.
- [43] C. C. Marques, M. T. Castelo-Branco, R. G. Pacheco et al., "Prophylactic systemic P2X7 receptor blockade prevents experimental colitis," *Biochimica et Biophysica Acta: Molecular Basis of Disease*, vol. 1842, no. 1, pp. 65–78, 2014.
- [44] A. R. Neves, M. T. L. Castelo-Branco, V. R. Figliuolo et al., "Overexpression of ATP-activated P2X7 receptors in the intestinal mucosa is implicated in the pathogenesis of Crohn's disease," *Inflammatory Bowel Diseases*, vol. 20, no. 3, pp. 444–457, 2014.
- [45] H. K. Eltzschig, T. Eckle, A. Mager et al., "ATP release from activated neutrophils occurs via connexin 43 and modulates adenosine-dependent endothelial cell function," *Circulation Research*, vol. 99, no. 10, pp. 1100–1108, 2006.
- [46] Y. Kurashima, T. Amiya, K. Fujisawa et al., "The Enzyme Cyp26b1 mediates inhibition of mast cell activation by fibroblasts to maintain skin-barrier homeostasis," *Immunity*, vol. 40, no. 4, pp. 530–541, 2014.
- [47] D. J. Friedman, B. M. Künzli, Y. I. A-Rahim et al., "CD39 deletion exacerbates experimental murine colitis and human polymorphisms increase susceptibility to inflammatory bowel disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 39, pp. 16788–16793, 2009.
- [48] M. S. Bynoe, A. T. Waickman, D. A. Mahamed, C. Mueller, J. H. Mills, and A. Czopik, "CD73 is critical for the resolution of murine colonic inflammation," *Journal of Biomedicine and Biotechnology*, vol. 2012, Article ID 260983, 13 pages, 2012.
- [49] N. A. Louis, A. M. Robinson, C. F. MacManus, J. Karhausen, M. Scully, and S. P. Colgan, "Control of IFN- α A by CD73: implications for mucosal inflammation," *The Journal of Immunology*, vol. 180, no. 6, pp. 4246–4255, 2008.
- [50] K. Synnestvedt, G. T. Furuta, K. M. Comerford et al., "Ecto-5'-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia," *Journal of Clinical Investigation*, vol. 110, no. 7, pp. 993–1002, 2002.
- [51] S. P. Colgan and H. K. Eltzschig, "Adenosine and hypoxia-inducible factor signaling in intestinal injury and recovery," *Annual Review of Physiology*, vol. 74, pp. 153–175, 2012.
- [52] L. Antonioli, P. Pacher, E. S. Vizi, and G. Haskó, "CD39 and CD73 in immunity and inflammation," *Trends in Molecular Medicine*, vol. 19, no. 6, pp. 355–367, 2013.
- [53] L. Antonioli, R. Colucci, C. la Motta et al., "Adenosine deaminase in the modulation of immune system and its potential as a novel target for treatment of inflammatory disorders," *Current Drug Targets*, vol. 13, no. 6, pp. 842–862, 2012.
- [54] J. H. Ye and V. M. Rajendran, "Adenosine: an immune modulator of inflammatory bowel diseases," *World Journal of Gastroenterology*, vol. 15, no. 36, pp. 4491–4498, 2009.
- [55] M. Odashima, G. Bamias, J. Rivera-Nieves et al., "Activation of A2A adenosine receptor attenuates intestinal inflammation in animal models of inflammatory bowel disease," *Gastroenterology*, vol. 129, no. 1, pp. 26–33, 2005.

- [56] C. C. Kurtz, "Extracellular adenosine regulates colitis through effects on lymphoid and nonlymphoid cells," *The American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 307, no. 3, pp. G338–G346, 2014.
- [57] F. Ochoa-Cortes, A. Liñán-Rico, K. A. Jacobson, and F. L. Christofi, "Potential for developing purinergic drugs for gastrointestinal diseases," *Inflammatory Bowel Disease*, vol. 20, no. 7, pp. 1259–1287, 2014.
- [58] I. D. Mascanfroni, A. Yeste, S. M. Vieira et al., "IL-27 acts on DCs to suppress the T cell response and autoimmunity by inducing expression of the immunoregulatory molecule CD39," *Nature Immunology*, vol. 14, no. 10, pp. 1054–1063, 2013.
- [59] T. Takahashi, Y. Kimura, K. Niwa et al., "In vivo imaging demonstrates ATP release from murine keratinocytes and its involvement in cutaneous inflammation after tape stripping," *Journal of Investigative Dermatology*, vol. 133, no. 10, pp. 2407–2415, 2013.
- [60] P. Pellegatti, L. Raffaghello, G. Bianchi, F. Piccardi, V. Pistoia, and F. Di Virgilio, "Increased level of extracellular ATP at tumor sites: in vivo imaging with plasma membrane luciferase," *PLoS ONE*, vol. 3, no. 7, Article ID e2599, 2008.

Regulation of Intestinal IgA Responses by Dietary Palmitic Acid and Its Metabolism

Jun Kunisawa,^{*,†,‡,§,||} Eri Hashimoto,^{*,†} Asuka Inoue,^{‡,||} Risa Nagasawa,^{*,†}
Yuji Suzuki,[†] Izumi Ishikawa,[†] Shiori Shikata,^{*,†} Makoto Arita,^{‡,*,*,1} Junken Aoki,^{††,||} and
Hiroshi Kiyono^{†,‡,††,‡‡,§§}

Enhancement of intestinal IgA responses is a primary strategy in the development of oral vaccine. Dietary fatty acids are known to regulate host immune responses. In this study, we show that dietary palmitic acid (PA) and its metabolites enhance intestinal IgA responses. Intestinal IgA production was increased in mice maintained on a PA-enriched diet. These mice also showed increased intestinal IgA responses against orally immunized Ag, without any effect on serum Ab responses. We found that PA directly stimulates plasma cells to produce Ab. In addition, mice receiving a PA-enriched diet had increased numbers of IgA-producing plasma cells in the large intestine; this effect was abolished when serine palmitoyltransferase was inhibited. These findings suggest that dietary PA regulates intestinal IgA responses and has the potential to be a diet-derived mucosal adjuvant. *The Journal of Immunology*, 2014, 193: 000–000.

High levels of IgA are present in the intestine, where they protect the host against pathogenic microorganisms by preventing their attachment to and entrance into epithelial cells, as well as by neutralizing their toxins (1). Some patients with IgA deficiency show increased susceptibility to infectious pathogens, including *Giardia lamblia*, *Campylobacter*, *Clostridium*, *Salmonella*, and rotavirus (2). Given the immunologic importance of IgA in

immunosurveillance in the intestine, the primary goal of effective oral vaccines is the efficient induction of Ag-specific IgA responses (3).

An efficient intestinal IgA response requires host-derived factors, including cytokines (e.g., IL-5, IL-6, IL-10, IL-15, APRIL, BAFF) and chemokines (e.g., CCL25/CCR9) (4, 5), as well as immunologic cross-talk with environmental factors (e.g., commensal bacteria and dietary materials) (6). Indeed, germ-free mice have decreased intestinal IgA responses because of the immature structure of Peyer's patches (PPs) and isolated lymphoid follicles (7, 8). We recently identified a unique subset of intestinal IgA-producing plasma cells (PCs) in the murine intestine; these cells expressed CD11b, required microbial stimulation and mature PP structure, proliferated vigorously, and produced high amounts of IgA (9).

In addition to commensal bacteria, nutritional molecules, such as vitamins, are essential for the development, maintenance, and regulation of intestinal immune responses (6, 10, 11). Therefore, nutritional deficiencies and inappropriate dietary intake increase the risk for infectious, allergic, and inflammatory diseases (12, 13). Among various dietary factors, oils are known to influence host immune function and inflammatory responses (14–16). Overnutrition due to a high-fat diet leads to the development of inflammation in adipose tissue, which is frequently associated with obesity and atherosclerosis (17). Recent findings suggest that, in addition to the quantity of oil ingested, the fatty acid (FA) composition of the oil is an important factor in various immunologic and inflammatory conditions (14–16). Dietary oil is generally composed of long-chain saturated FAs (e.g., C16:0 palmitic acid [PA] and C18:0 stearic acid) and mono- or polyunsaturated FAs (PUFAs; e.g., C18:1 oleic acid, C18:2 linoleic acid, and C18:3 α -linolenic acid). α -Linolenic acid and linoleic acid are precursors of ω 3 and ω 6 PUFAs, respectively; ω 3 FAs are metabolized into anti-inflammatory molecules, whereas ω 6 FAs are converted into proinflammatory lipid mediators (16, 18). Therefore, the ratio of α -linolenic acid/linoleic acid in dietary oils is thought to determine the onset of various immunologic conditions. In addition to modulating the ω 3– ω 6 PUFA balance, saturated FAs, such as PA, stimulate host immune responses by promoting the production of proinflammatory cytokines, including IL-6 and TNF- α (19, 20). Although these proinflammatory cytokines are prerequisite factors for the efficient induction of IgA

^{*}Laboratory of Vaccine Materials, National Institute of Biomedical Innovation, Osaka 567-0085, Japan; [†]Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan; [‡]International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan; [§]Department of Microbiology and Immunology, Kobe University School of Medicine, Kobe 650-0017, Japan; ^{||}Graduate School of Pharmaceutical Sciences and Graduate School of Dentistry, Osaka University, Osaka 565-0871, Japan; ^{||}Laboratory of Molecular and Cellular Biochemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan; ^{††}Department of Health Chemistry, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo 113-0033, Japan; ^{‡‡}Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Saitama 332-0012, Japan; ^{§§}Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Saitama 332-0012, Japan; ¹Department of Medical Genome Science, Graduate School of Frontier Science, University of Tokyo, Chiba 277-8561, Japan; and ^{§§}Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan

¹Current address: Laboratory for Metabolomics, RIKEN Center for Integrative Medical Sciences, Yokohama, Kanagawa, Japan.

Received for publication November 1, 2013. Accepted for publication June 11, 2014.

This work was supported by grants from the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries, and Food Industry (to J.K.); the Program for Promotion of Basic and Applied Research for Innovations in Bio-oriented Industry (to J.K.); the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to J.K., M.A., J.A., and H.K.); the Ministry of Health and Welfare of Japan (to J.K. and H.K.); the Yakult Bio-Science Foundation (to J.K.); the Ono Medical Research Foundation (to J.K.); the Japan Science and Technology Agency, Core Research for Evolutional Science and Technology (to J.A. and H.K.); and Precursory Research for Embryonic Science and Technology (to M.A.).

Address correspondence and reprint requests to Dr. Jun Kunisawa, Laboratory of Vaccine Materials, National Institute of Biomedical Innovation, 7-9-8 Asagi Saito, Osaka 567-0085, Japan. E-mail address: kunisawa@nibio.go.jp

The online version of this article contains supplemental material.

Abbreviations used in this article: CT, cholera toxin; FA, fatty acid; iLP, intestinal lamina propria; PA, palmitic acid; PC, plasma cell; PP, Peyer's patch; PUFA, polyunsaturated fatty acid; S1P, sphingosine 1 phosphate; SPT, serine palmitoyltransferase.

Copyright © 2014 by The American Association of Immunologists, Inc. 0022-1767/14/\$16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1302944