

Regulation of allergic inflammation by the ectoenzyme E-NPP3 (CD203c) on basophils and mast cells

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Abstract Adenosine 5'-triphosphate (ATP) is released from dying or damaged cells, as well as from activated cells. Once secreted, extracellular ATP induces several immune responses via P2X and P2Y receptors. Basophils and mast cells release ATP upon FcεRI-crosslinking, and ATP activates basophils and mast cells in an autocrine manner. Nucleotide-converting ectoenzymes, such as E-NTPD1, E-NTPD7, and E-NPP3, inhibit ATP-dependent immune responses by hydrolyzing ATP, thereby contributing to immune response regulation. E-NPP3 is a well-known activation marker for human basophils. E-NPP3's physiologic function has recently been disclosed in mice. E-NPP3 is rapidly induced on basophils and mast cells after FcεRI-crosslinking and hydrolyzes extracellular ATP on cell surfaces to prevent ATP-dependent excess activation of basophils and mast cells. In the absence of E-NPP3, basophils and mast cells are overactivated and mice suffer from severe chronic allergic inflammation. Thus, the ATP-hydrolyzing ectoenzymes E-NPP3 has a nonnegligible role in the regulation of basophil- and mast cell-mediated allergic responses.

Keywords Allergy · ATP · Basophil · Mast cell · E-NPP3

Introduction

Adenosine 5'-triphosphate (ATP), which is composed of adenosine and a triphosphate group, is the most common and major energy source for various cell types. ATP is produced by complex biosynthetic mechanisms to serve the demands of cellular responses. Generally, intracellular ATP concentration range is between 1 and 10 mM [1, 2], whereas extracellular compartments contain low ATP concentrations, from 1 to 10 nM under stable conditions [1]. Abundant amounts of ATP (up to hundreds of mM) are stored in secretory granules in several cell types, such as insulin-containing granules of pancreatic β cells, chromaffin granules of adrenal medulla, serotonergic granules of platelets, secretory granules of mast cells, and secretory vesicles of neurons [1, 3–5].

ATP is released in response to several kinds of stimulation. T cells release ATP via pannexin-regulated hemichannels when T cell receptors are stimulated [6]. In addition, macrophage and neutrophils release ATP in response to chemotactic mediators through pannexin-1 [7, 8]. Moreover, activated platelets and mast cells secrete ATP through exocytosis [4, 9]. Environmental changes have also been shown to induce ATP release. In conditions of low oxygen or shear stress, erythrocytes release ATP via pannexin-1 [10]. ATP release via connexin-regulated hemichannels in astrocytes is induced during swelling mediated by hypoosmotic conditions [11, 12]. In addition to active ATP release by various cell types, cell damage caused by apoptosis and necrosis has been shown to lead to passive ATP release [1]. Thus, ATP is released by various pathways in different cells in response to different stimuli or environmental conditions.

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Extracellular ATP, which is released from damaged or dying cells, has been recognized as a “danger signal” or “find me signal” [13, 14]. In addition, recent accumulating evidence has demonstrated that ATP released from physiologically stimulated cells activates immune cells and modulates immune responses in an autocrine or paracrine manner [15]. Furthermore, in accordance with elucidation of ATP’s role in immune responses, regulatory mechanisms for ATP-dependent immune responses are being elaborated. In this review, the focus is on nucleotide-converting ectoenzymes that modulate ATP-induced immune responses. In particular, the role of the ectoenzyme E-NPP3 in basophil- and mast cell-dependent allergic responses is discussed.

Purinergic receptors recognizing extracellular ATP

Extracellular nucleotides trigger several cellular responses via interactions with membrane-bound purinergic receptors. Purinergic receptors, termed P2 receptors, are main receptors that recognize extracellular nucleotides, such as ATP, UTP, and ADP [16–19]. There are two types of P2 receptors: P2X and P2Y. The former receptors are ligand-gated ion channels, consisting of seven members (P2X1–7) [16–18], whereas the latter are seven-transmembrane, G protein-coupled receptors, which are comprised of eight members (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14) [16, 17, 19]. Interactions of extracellular ATP and P2 receptors have been shown to trigger immune responses and induce tissue inflammation [20–23]. For example, neutrophils rapidly release ATP after chemotactic receptor activation, and then released ATP amplifies the chemotactic intracellular signal through activation of P2Y2 on neutrophils [7]. ATP released from damaged or dying cells has been shown to activate P2Y6, P2Y12, and P2Y13 receptors to increase microglia chemotactic and phagocytic responses [24, 25]. Low ATP concentrations (10–250 μ M) released from injured cells has been shown to activate P2Y11 that, in turn, promotes dendritic cell (DC) maturation that leads to initiation of a type 2 immune response [26, 27]. It has also been reported that high ATP concentrations (>1 mM) released from pathogenic bacteria activates P2X7 on DCs and macrophages that then induces NLRP3 inflammasome activation, leading to IL-1 β secretion in the presence of endotoxin [28–30]. In addition, ATP from intestinal commensal bacteria has been shown to promote IL-6 and TGF- β production from a unique subset of intestinal lamina propria CX3CR1⁺ CD70⁺ DCs, for induction of Th17 differentiation via P2X or P2Y receptors [31]. It has also been reported that ATP acts on T cells via P2X receptors to amplify T cell receptor signaling, thus promoting T cell activation [6, 32, 33]. Furthermore, ATP has been shown to inhibit

generation and function of regulatory T cells via P2X receptors [20]. In addition to T cells, B cells have been shown to be activated by extracellular ATP [34, 35]. Extracellular ATP has also been shown to modulate functions of other immune cell types. Indeed, ATP mediates migration of monocytes, eosinophils [15, 33], and natural killer (NK) cells [36]. Thus, extracellular ATP plays important roles in modulation of a variety of immune cells.

ATP and basophils/mast cells

Along with the fact that extracellular ATP acts on many types of immune cells, allergic responses have been shown to be modulated by ATP through activation of basophils and mast cells. Both basophils and mast cells express a high affinity IgE receptor, Fc ϵ RI. After Fc ϵ RI-crosslinking by antigen-bound IgE, basophils and mast cells are activated to release several proinflammatory cytokines and mediators, leading to induction of type 2 immune responses against allergens and pathogens. Although basophils and mast cells share some functions, such as immune responses against parasites, as well as induction in allergic diseases and autoimmune diseases [37–39], they show nonredundant and nonoverlapping functions during allergic responses. For example, basophils play a critical role in the induction of IgE-mediated chronic allergic skin inflammation [40, 41], whereas mast cells mediate intestinal allergic inflammation [22, 42, 43].

Several studies have demonstrated that mast cells secrete ATP in response to various stimuli, such as IgE-mediated Fc ϵ RI-crosslinking, mechanical stimulation, irradiation, and high temperature [4, 5, 44]. Extracellular ATP released by mast cells then activates P2 receptors to elevate cytoplasmic Ca²⁺ concentrations in mast cells by induction of Ca²⁺ influx through cellular membranes or Ca²⁺ release from intracellular storage compartments [18, 45–48]. ATP has been shown to augment degranulation activity of mast cells, which is mediated by Fc ϵ RI-crosslinking [49, 50]. It has also been shown that extracellular ATP, by itself, is capable of inducing mast cell degranulation [46, 48, 51–53]. As is the case for the role of extracellular ATP on neutrophils, eosinophils, monocytes/macrophages, NK cells, and dendritic cells, ATP is a potent chemoattractant for mast cells [54]. Moreover, ATP has been shown to enhance activation of the NLRP3 inflammasome induced by Toll-like receptors or the TNF- α receptor in mast cells [55, 56]. In accordance with immune-activating properties of extracellular ATP in mast cells, several studies have revealed that ATP mediates skin and intestinal inflammation through activation of P2 receptors on mast cells [22, 56]. Thus, ATP is critically involved in mast cell activity and, accordingly, allergic responses. Compared to ATP’s role in influencing mast cells, the effect of extracellular ATP on basophils remained relatively unresolved.

Nucleotide-converting ectoenzymes

In accordance with the fact that extracellular ATP induces or enhances various aspects of immune responses, there exists a fine system that regulates ATP-dependent immune responses. This system is mainly conducted by rapid ATP hydrolysis. Indeed, there are two families of nucleotide-converting ectoenzymes that are expressed on plasma cell membranes and hydrolyze extracellular ATP. These ectonucleotidases include the ecto-nucleoside triphosphate diphosphohydrolase (E-NTPD) and the ecto-nucleotide pyrophosphatase/phosphodiesterase (E-NPP) families [57, 58]. In addition to these families, ecto-5'-nucleotidase (known as CD73) has also been shown to modulate immune responses. E-NTPD ectoenzymes convert ATP to ADP as well as ADP to AMP, whereas E-NPP ectoenzymes hydrolyze ATP to AMP. Ecto-5'-nucleotidase generates adenosine from AMP. These nucleotide-converting ectoenzymes have been shown to modulate a variety of aspects of immune responses.

E-NTPD family

The E-NTPD family consists of eight members (E-NTPD1–8), each of which is expressed in different tissues and cells and possesses differential nucleotide substrate preferences [57–59]. Among these family members, the focus here is on E-NTPD1 and E-NTPD7, both of which have been shown to be involved in immune response modulation.

E-NTPD1

E-NTPD1, also known as CD39, is expressed in immune cells. Accordingly, E-NTPD1 has been shown to modulate functions of various immune cell types, such as regulatory T (Treg) cells, B cells, dendritic cells, neutrophils, and macrophages [60]. The ecto-5'-nucleotidase CD73 hydrolyzes AMP into adenosine, which possesses immunosuppressive activities [61]. Hence, immune responses are effectively modulated by E-NTPD1, which produces AMP from ATP, also cooperating with CD73. The coordinated function of E-NTPD1 and CD73 has been characterized in CD4⁺ Foxp3⁺ Treg cells. E-NTPD1 activity is increased by T cell receptor signaling in Treg cells [62]. Then, adenosine is generated by Treg cells through the E-NTPD1/CD73 ATP catalytic axis, which contributes to enhanced immunosuppressive capacity in Treg cells [63, 64]. Furthermore, E-NTPD1/CD73-dependent conversion of ATP to adenosine has been shown to help B cells enter into the class-switch recombination process [65]. Thus, E-NTPD1 and CD73 cooperation could be considered as an immune-converting process that changes ATP-induced inflammation to adenosine-mediated anti-inflammatory responses.

In neutrophils, E-NTPD1 has been shown to reduce ATP-mediated chemotactic effects and activation [66, 67]. In the absence of E-NTPD1, macrophages secrete large amounts of proinflammatory cytokines [68, 69]. Thus, E-NTPD1 possesses regulatory functions on lymphocytes and myeloid cells. In accordance with the fact that E-NTPD1 modulates the function of several immune cell types, several lines of evidence indicate that E-NTPD1 plays negative regulatory roles in development of autoimmune diseases, inflammatory bowel diseases, and allergic diseases [70–72].

E-NTPD7

E-NTPD7 is selectively expressed on small intestinal epithelial cells. Although it remains unclear whether E-NTPD7 is expressed on apical surfaces of the intestinal epithelial cell layer, ATP concentrations have been shown to be elevated in the small intestinal lumen of E-NTPD7 knockout mice [73]. ATP injection into the ligation loop of the small intestine of wild-type mice results in a ~10-fold increase in ATP concentration. In contrast, ATP concentrations are increased over 1000-fold after ATP injection in E-NTPD7 knockout mice, suggesting that the increased luminal ATP concentrations were caused by defective ATP clearance in the absence of E-NTPD7. Thus, E-NTPD7 is required for controlling ATP concentration in the small intestine. Intestinal lumen ATP has been shown to promote Th17 cell differentiation in small intestinal lamina propria [31]. Accordingly, E-NTPD7 knockout mice show increased number of IL-17-producing CD4⁺ Th17 cells in small intestinal lamina propria. Oral antibiotic treatment as well as intraperitoneal administration of an ATP antagonist, oxidized ATP, decreases the Th17 cell numbers in small intestinal lamina propria. Moreover, E-NTPD7 knockout mice display increased resistance to intestinal infection with *Citrobacter rodentium*. Taken together, E-NTPD7, which is selectively expressed on epithelial cells in the small intestine, has been shown to control Th17 cell responses through ATP degradation [73].

Thus far, the immunological aspects of functions in the E-NTPD family have been characterized only in E-NTPD1 and E-NTPD7. Future studies will reveal the role of other E-NTPD family members in immune responses.

E-NPP family

The E-NPP family of ectoenzymes, with seven members (E-NPP1–7), hydrolyzes ATP to AMP [57, 74–76]. E-NPP1 and E-NPP3 are type II transmembrane glycoproteins, whereas E-NPP2 is not a transmembrane protein but a secreted enzyme. E-NPP1, E-NPP2, and E-NPP3 have been shown to hydrolyze various nucleotides, including extracellular ATP. In contrast to the first three members of E-NPPs family, other E-NPP members do not harbor a domain with nuclease activity and

accordingly do not catalyze nucleotides [75]. E-NPP6 and E-NPP7 have been shown to mainly catalyze choline phosphodiester or lysophospholipids with phosphodiester bonds; however, catalytic substrates of E-NPP4 and E-NPP5 remain unknown.

E-NPP1 and E-NPP2

E-NPP1 is expressed in bone and cartilage in the high concentrations. Accordingly, E-NPP1's role in the maintenance of bone homeostasis has been characterized. E-NPP1 prevents excessive bone mineralization and controls normal soft tissue calcification via pyrophosphate generation from nucleotides [77, 78]. In contrast to E-NPP1-mediated regulatory function in bone development, E-NPP2 has been shown to play an important role in early vascular development [79]. E-NPP2 possesses catalytic activity that produces lysophosphatidic acid (LPA) from lysophosphatidylcholine as well as catalytic activity for ATP [80]. LPA activates G-protein-coupled receptors that initiate several biological responses, including cell proliferation, cell migration, and vascular development. Thus, E-NPP2 has been implicated in multiple cellular functions. In contrast to E-NPP1 and E-NPP2, the biologic function of E-NPP3 remained unclear, although a report shows that E-NPP3 is a regulator of N-acetylglucosaminyltransferase for maintaining normal concentrations of intracellular nucleotide sugars [81].

E-NPP3

E-NPP3 is well known as CD203c, particularly in the field of allergy research. CD203c is used as an activation marker of human basophils, as E-NPP3 (CD203c) expression on basophils is rapidly induced by IgE and antigen-mediated Fc ϵ RI-crosslinking [82–85]. Therefore, E-NPP3 is employed as a useful and trusty marker for clinical diagnosis of allergic diseases [84–88]. However, the role of E-NPP3 in allergic responses remained to be elucidated.

E-NPP3 in basophils and mast cells

Recently, the physiologic function of E-NPP3 was characterized in mice [89]. As in the case of human basophils, E-NPP3 expression is immediately upregulated on murine basophils upon Fc ϵ RI-crosslinking. Fc ϵ RI-crosslinking also rapidly increases E-NPP3 expression on murine mast cells, which are known to release ATP upon Fc ϵ RI-crosslinking. Similarly, basophils rapidly secrete ATP upon Fc ϵ RI-crosslinking. In culture supernatants of Fc ϵ RI-crosslinking-stimulated basophils and mast cells of E-NPP3 knockout mice, ATP concentrations are dramatically increased compared to wild-type basophils and mast cells. As E-NPP3 is a nucleotide-converting ectoenzyme, wild-type basophils, and mast cells generate pyrophosphate via ATP hydrolysis after ATP addition to a culture. The pyrophosphate concentration in culture supernatant of basophils and mast cells lacking E-NPP3 is not increased after ATP addition. Thus, E-NPP3 expression on basophils and mast cells plays a critical role in extracellular ATP hydrolysis.

Stimulation by Fc ϵ RI-crosslinking as well as ATP induces cytokine production from basophils and mast cells, and the cytokine production was increased in basophils and mast cells of E-NPP3 knockout mice. Overactivation of basophils and mast cells in E-NPP3 knockout mice in response to Fc ϵ RI-crosslinking as well as ATP is abrogated in mice lacking both E-NPP3 and P2X7. From these findings, the following model for activation of basophils and mast cells is proposed; Fc ϵ RI-crosslinking initiates activation of basophils and mast cells and production of several inflammatory and allergic mediators. ATP, included among these mediators, acts on P2X7 on basophils and mast cells in an autocrine manner and contributes to sustained activation of basophils and mast cells. Excess activation of basophils and mast cells is prevented by rapid induction of E-NPP3 on basophils and mast cells after activation, which then hydrolyzes ATP on cell surfaces to inhibit ATP-dependent responses (Fig. 1).

Fig. 1 After IgE-mediated Fc ϵ RI-crosslinking, ATP is released from basophils and mast cells. Simultaneously, E-NPP3, which converts extracellular ATP to AMP and pyrophosphate, is rapidly induced on basophils and mast cells

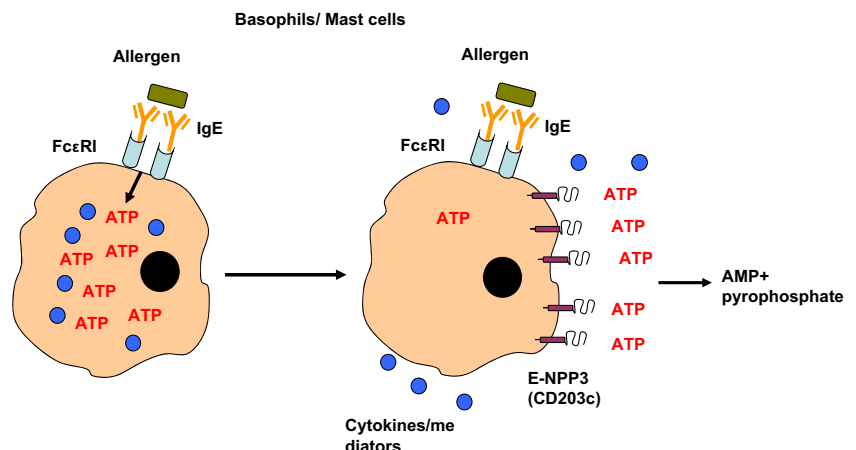
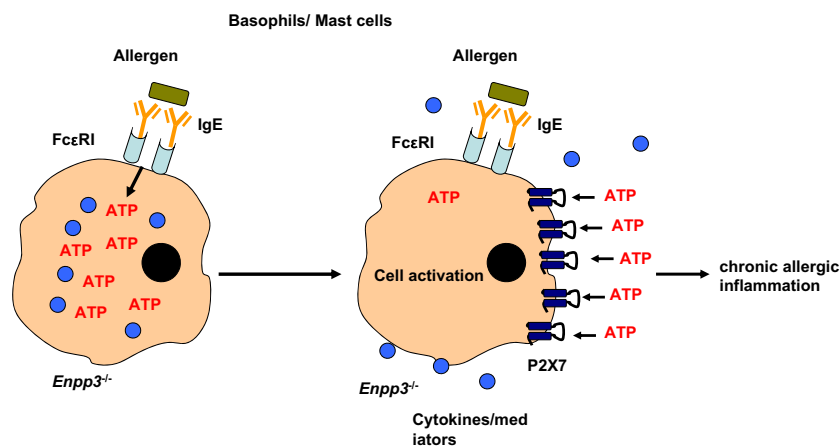


Fig. 2 In the absence of E-NPP3, ATP clearance activity is impaired in basophils and mast cells. Therefore, extracellular ATP concentration is increased and acts on P2X7, leading to basophil and mast cell overactivation. This results in enhanced chronic allergic inflammation



E-NPP3 in allergic responses

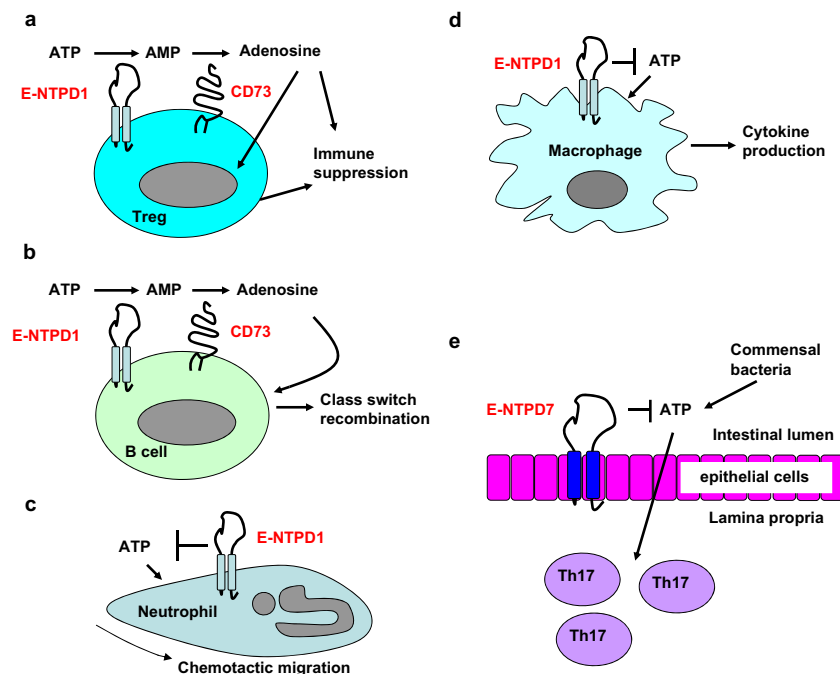
The function of E-NPP3 *in vivo* has also been analyzed. In E-NPP3 knockout mice, basophil and mast cell numbers increase and serum ATP concentration increases. E-NPP3 knockout mice do not show severe immediate allergic responses, as evidenced by normal passive cutaneous anaphylaxis. As basophils are essential for induction of chronic allergic skin inflammation [40, 41], the basophil-dependent allergic skin inflammation model has been introduced in E-NPP3 knockout mice. E-NPP3 knockout mice show severe ear swelling together with increased infiltration of basophils, eosinophils, and neutrophils.

A model for allergen-induced diarrhea has also been introduced. In this model, the number of mast cells and IL-4-producing Th2 cells is dramatically increased in small and large intestines of E-NPP3 knockout mice. Serum IgE

as well as ATP concentrations are greatly elevated in allergen-challenged E-NPP3 knockout mice.

Several studies have indicated that basophils and mast cells are involved in development of allergic asthma [39, 90–92]. Therefore, when the sensitivity to allergen-induced airway inflammation was analyzed, E-NPP3 knockout mice were found to show high sensitivity to allergen-induced airway inflammation and all E-NPP3 knockout mice died after allergen challenge. The numbers of infiltrating basophils, eosinophils, and neutrophils in lung, mucus production by airway epithelia, and serum ATP concentration are all increased in allergen-challenged E-NPP3 knockout mice. Notably, in both allergen-induced diarrhea and allergen-induced airway inflammation models, treatment with an ATP antagonist ameliorated disease severity in E-NPP3 knockout mice, indicating that

Fig. 3 **a** Cooperation of E-NTPD1 and CD73, which are highly expressed on Treg cells, leads to effective ATP hydrolysis to adenosine, which promotes immunosuppressive activity of Treg cells. **b** Adenosine generated by cooperation of E-NTPD1 and CD73 promotes class-switch recombination in B cells. **c** E-NTPD1 suppresses ATP-mediated chemotactic effect on neutrophils via ATP hydrolysis. **d** E-NTPD1 inhibits ATP-dependent cytokine production of macrophages through ATP hydrolysis. **e** E-NTPD7, which is specifically expressed on small intestinal epithelial cells, suppresses ATP-mediated Th17 responses through hydrolysis of luminal ATP



increased ATP concentration contributes to enhanced chronic allergic responses in E-NPP3 knockout mice (Fig. 2) [89]. Thus, E-NPP3 is critical to suppression of chronic allergic inflammation.

Conclusions

ATP is well known to be an essential energy source inside the cell. In addition, ATP plays various roles at the extracellular compartment. Accumulated evidence indicates that extracellular ATP is a nonnegligible mediator or regulator in the immune system. Moreover, extracellular ATP participates in development or enhancement of allergic inflammation. Basophils and mast cells, which mediate Th2 and allergic responses, release ATP during cellular activation. Thus, basophils and mast cells are supposed to be the major source of extracellular ATP during allergic responses. A study using E-NPP3 knockout mice has demonstrated that ATP augments basophil- and mast cell-mediated allergic inflammation. Therefore, reduction of extracellular ATP concentration would be useful for treatment of allergic diseases. However, this study raised an obvious question regarding whether ATP released from basophils and mast cells is beneficial for us or not. In a study by Tsai et al. [89], the focus was on chronic allergic inflammation. However, basophils and mast cells not only induce allergic inflammation but also have protective roles in host immune responses against parasite invasion. Therefore, it is supposed here that ATP is required for sustained activation of basophils and mast cells to effectively expel invading parasites. After elimination of parasites, ATP is immediately hydrolyzed by E-NPP3 to terminate the inflammation. It would be interesting to analyze the sensitivity of E-NPP3 knockout mice to parasite infection.

Nucleotide-converting ectoenzymes, which can catalyze extracellular nucleotides, have been shown to regulate ATP-mediated immune responses via several cells types (Fig. 3). E-NPP3 is essential for prevention of basophil- and mast cell-mediated chronic allergic inflammation by controlling extracellular ATP concentration. Therefore, E-NPP3 and extracellular ATP could be novel therapeutic targets for allergic disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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