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Mini Review

Regulation of immune responses by ATP-hydrolyzing ecto-enzymes

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Extracellular adenosine 5'-triphophate (ATP) mediates the immune response. Several ecto-enzymes hydrolyze ATP, including the ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) and ecto-nucleotide pyrophosphate/phosphodiesterase (E-NPP) protein families. Among these, E-NTPD1, E-NTPD7, and E-NPP3 have been shown to regulate the immune response. E-NTPD1 is expressed in lymphocytes and myeloid cells and modulates their function. E-NTPD7, which is selectively expressed in the epithelial cells of the small intestine, regulates Th17 responses in the small intestine by controlling ATP levels. E-NPP3 is rapidly induced on activated basophils and mast cells, and regulates ATP-dependent activation in basophils and mast cells to prevent chronic allergic inflammation. Thus, ATP-hydrolyzing ecto-enzymes modulate the immune response through ATP hydrolysis.

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Introduction

Adenosine 5'-triphosphate (ATP) plays a role in many cellular functions by providing energy through its hydrolysis. Extracellular ATP was first shown to mediate cell-to-cell communication in the nervous system, where ATP is released at the neuronal synapse[1]. Several purinergic receptors, such as P2X and P2Y, which sense extracellular ATP, have been characterized[2].

Extracellular ATP-dependent cell-to-cell communication was then demonstrated in the immune system[3]. ATP,

which is released from damaged cells, is recognized as a "danger-associated molecular pattern" by P2X7 to activate the inflammasome, leading to secretion of mature IL-1β[4]. ATP is also secreted by activated immune cells such as T lymphocytes and neutrophils[5, 6]. In the intestine, symbiotic/pathogenic bacteria, such as *Enterococcus mundtii*, *Enterococcus gallinarum*, *Escherichia coli*, and *Salmonella*, secrete ATP[7-9]. Furthermore, purinergic P2X and P2Y receptors are expressed in a variety of immune cells and regulate their immune functions[10-12]. Thus, extracellular ATP mediates several biological functions, including the immune responses.

Accordingly, extracellular ATP concentration is finely regulated by ATP-hydrolyzing ecto-enzymes expressed on the plasma membrane of immune cells, including a family of ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases), which convert ATP to ADP as well as ADP to AMP, and a family of ecto-nucleotide pyrophosphatase/phosphodiestrases (E-NPPs), which hydrolyze ATP to AMP[6, 13-42]. In this review, we describe the function of these ecto-enzymes, which modulate various aspects of the immune response.

E-NTPDases

There are eight E-NTPDases, which catalyze ATP to ADP or ADP to AMP, and that show distinct tissue distribution (Table 1). Of these, the immunological role of E-NTPD1 and E-NTPD7 has been characterized.

1)E-NTPD1

E-NTPD1, also called CD39, is expressed on several immune cells, such as T cells, neutrophils, macrophages, and dendritic cells[13]. Among T cells, E-NTPD1 is highly expressed on regulatory T (Treg) cells. The activity of E-NTPD1 is enhanced by TCR stimulation of Treg cells[14], which correlates with the immunosuppressive function of Treg cells[15].

Neutrophil chemotactic activity is enhanced by ATP[6], but negatively regulated by E-NTPD1, which is expressed on neutrophils[16, 17]. Neutrophils and macrophages from mice lacking E-NTPD1 (*Entpd1*$^{-/-}$ mice) produce elevated amounts of cytokines, indicating that E-NTPD1 modulates cytokine production through ATP hydrolysis[18, 19]. In accordance with the immunomodulatory function of E-NTPD1, *Entpd1*$^{-/-}$ mice are highly susceptible to intestinal inflammation[20].

2)E-NTPD7

E-NTPD7 is not expressed on immune cells, but mediates the immune responses through ATP hydrolysis in the small intestinal lumen. It has been shown previously that extracellular ATP in the intestinal lumen mediates Th17 responses through activation of a unique subset of intestinal dendritic cells[10]. Based on this finding, we searched for ATP-hydrolyzing ecto-enzymes expressed in intestinal epithelial cells, and found that E-NTPD7 is highly expressed in the small intestine.

ATP concentration in the luminal contents of the small intestinal is markedly increased in *Entpd7*$^{-/-}$ mice, owing to defective ATP clearance activity. Because ATP mediates T-cell responses, the number of CD4^{+} T cells producing IL-17, IFN-γ, and IL-10 in the lamina propria of the small and large intestines was then analyzed. The number of IL-17-producing Th17 cells in the small intestinal lamina propria was found to be markedly increased in *Entpd7*$^{-/-}$ mice. Notably, the increased number of Th17 cells in

Table 1 Overview of E-NTPDases and E-NPPs

E-NTPDases and E-NPPs are expressed in several cell types and responsible for maintenance of tissue homeostasis.

E-NTPD family	Expression indifferent tissue/cell types	Physiological functions and Diseases	ref.
ENTPD1	T cell,neutrophil, macophage, dendritic cell	Intestinal inflammation, multiple sclerosis	6, 13-20
ENTPD2	Astrocyte, taste buds	Taste responses	23, 42
ENTPD3	Tendon cell, pancreatic b- cell		31, 32
ENTPD5	Osteoblast, tumor cell	Bone mineralization, cancer	24, 29, 30
ENTPD6	Vestibular hair cell		28
ENTPD7	Epithelial cell	Intestinal inflammation	43
ENTPD8	Renal epithelial cell	Diabetic nephropathy	25
E-NPP family	Expression in tissue/cell types	Physiological function and Disease	
ENPP1	Osteoblast, chondrocyte	Bone mineralization, type2 diabetes, obesity	26, 27, 33
ENPP2	Synovial fibroblast, white addipose tissue, high endothelial venule	Rheumatoid arthritis, obesity	34, 37, 38
ENPP3	Mast cell, basophil	Chronic allergic inflammation	35
ENPP4	Vascular endothelium		36
ENPP6	Kidney, Brain		41
ENPP7	Intestine	Colonic cancer	39, 40

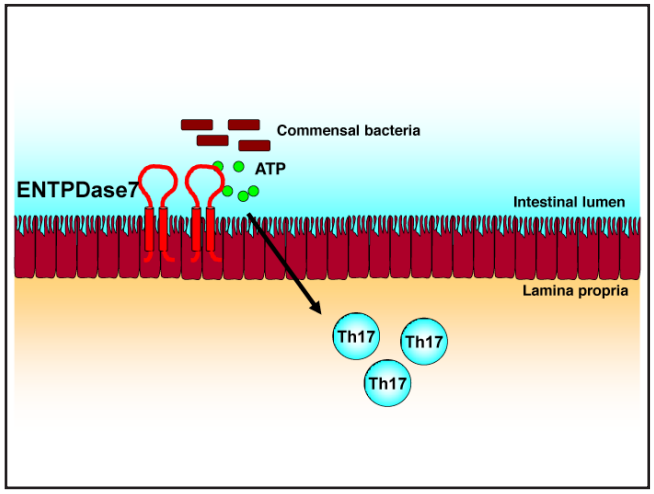

Fig. 1 E-NTPD7 regulates the Th17 responses in the small intestine

Luminal ATP is increased in a commensal bacteria-dependent manner. E-NTPD7, which is highly expressed in small intestine epithelial cells, controls ATP levels, and thereby regulates ATP-dependent Th17 responses.

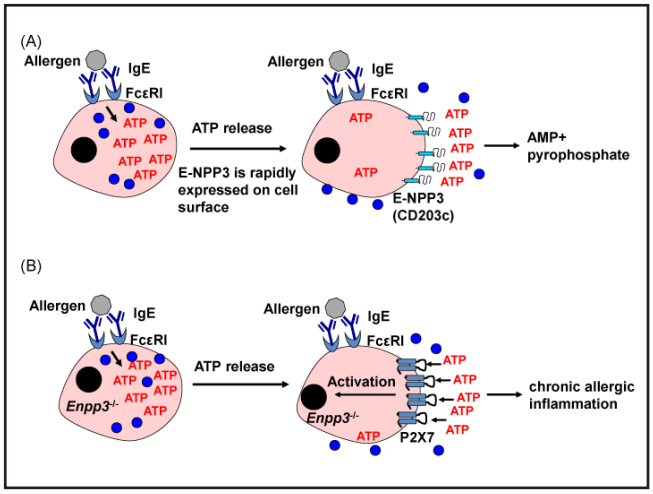

Fig. 2 E-NPP3 regulates chronic allergic responses by basophils and mast cells

(A)Basophils and mast cells release ATP upon FcεRI crosslinking. Simultaneously, E-NPP3 is induced on the surface of basophils and mast cells and hydrolyzes extracellular ATP.

(B)In the absence of E-NPP3, ATP clearance is impaired in basophils and mast cells. Surplus extracellular ATP activates P2X7 in an autocrine manner to induce basophils and mast cells, leading to chronic allergic inflammation.

Entpd7$^{-/-}$ mice depends on commensal bacteria and ATP, because treatment of *Entpd7*$^{-/-}$ mice with antibiotics and an ATP antagonist reduced the number of Th17 cells (Fig. 1). In accordance with the increased number of Th17 cells in the intestine, *Entpd7*$^{-/-}$ mice were resistant to intestinal infection with *Citrobacter rodentium*. Thus, *Entpd7*$^{-/-}$ mice revealed that E-NTPD7 is expressed in the epithelial cells of the small intestine, and regulates the intestinal Th17 responses by controlling luminal ATP levels in the small intestine[43].

E-NPPs

There are seven E-NPPs[44, 45] (Table 1). Among these members, E-NPP1, 2, and 3 have been shown to hydrolyze ATP. E-NPP2 is a secreted enzyme, whereas E-NPP1 and 3 are transmembrane proteins. In contrast to E-NPP1, which inhibits bone mineralization, the function of E-NPP3 remains unclear[44].

1)E-NPP3

E-NPP3, which is also called CD203c, is well known as an activation marker of human basophils, and thus used as a diagnostic marker for allergic diseases[46]. Murine basophils and mast cells stimulated with IgE and antigen (FcεRI crosslinking) showed enhanced expression of E-NPP3. In mice lacking E-NPP3 (*Enpp3*$^{-/-}$ mice), the number of peripheral blood and spleen basophils, as well as masts cells in the small and large intestines, increased. Although mast cells regulate immediate allergic responses, the sensitivity to passive cutaneous anaphylaxis was not altered in *Enpp3*$^{-/-}$ mice. Interestingly, a previous study indicated that basophils mediate chronic allergic skin inflammation[47], consistent with our observation that the sensitivity to basophil-dependent allergic skin inflammation was enhanced in *Enpp3*$^{-/-}$ mice. The sensitivity to allergen-induced airway inflammation as well as allergen-induced diarrhea was also increased in *Enpp3*$^{-/-}$ mice. In a model of allergen-induced diarrhea, the number of mast cells as well as IL-4-producing Th2 cells in the small and large intestines increased in *Enpp3*$^{-/-}$ mice. In addition, serum ATP levels were greatly elevated in allergen-challenged *Enpp3*$^{-/-}$ mice. Treatment with an ATP antagonist during allergen challenge reduced the number of mast cells and Th2 cells. Thus, *Enpp3*$^{-/-}$ mice showed enhanced chronic allergic inflammation with increased ATP levels.

Enpp3$^{-/-}$ basophils and mast cells produced elevated amounts of IL-4 and IL-6 after FcεRI crosslinking, whereas wild-type basophils and mast cells secreted small amounts

of ATP upon FcεRI crosslinking. In *Enpp3*$^{-/-}$ cell culture, ATP levels were enhanced. E-NPP3 is an ecto-enzyme that hydrolyzes ATP to produce pyrophosphate. Indeed, in the culture of wild-type basophils and mast cells, pyrophosphate levels increased after ATP addition, but not in *Enpp3*$^{-/-}$ cells. Thus, ATP clearance was impaired in *Enpp3*$^{-/-}$ mice, leading to increased ATP levels. Wild-type basophils and mast cells, when stimulated with ATP, produced IL-4 and IL-6, respectively. In addition, ATP-dependent cytokine production increased in *Enpp3*$^{-/-}$ cells. In mice lacking both E-NPP3 and P2X7, which senses extracellular ATP, ATP-induced and FcεRI crosslinking-induced cytokine production decreased. The number of peripheral basophils and mast cells also decreased in mice lacking both E-NPP3 and P2X7.

Taken together, *Enpp3*$^{-/-}$ cells reveal a novel mechanism for regulation of allergic responses. Basophils and mast cells secrete several mediators that induce immediate allergic responses upon FcεRI crosslinking. ATP is also secreted after FcεRI crosslinking. Simultaneously, E-NPP3 is rapidly induced and hydrolyzes ATP to suppress ATP-dependent activation of basophils and mast cells (Fig. 2A). In the absence of E-NPP3, ATP, which is increased in basophils and mast cells, acts via P2X7 in an autocrine manner, leading to enhanced chronic allergic inflammation (Fig. 2B).

Conclusion

A series of studies on ATP-hydrolyzing ecto-enzymes have revealed that E-NTPD1, E-NTPD7, and E-NPP3 play key roles in regulating the immune response. In addition to E-NTPDases and E-NPPs, ecto-5'-nucleotidases such as CD73, which produce adenosine from AMP, have also been shown to regulate immune responses[13]. Although much progress has been made, the role of other E-NTPDase and E-NPP family members in regulating the immune response remains to be elucidated.

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Conflict of interests

The authors declare they have no conflicts of interest.

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