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Extracellular ATP in the Immune System: More Than Just a “Danger Signal”

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Extracellular adenosine 5'-triphosphate (eATP) is ubiquitously used for cell-to-cell communication. The low concentration of eATP ([eATP]) that exists in a “halo” surrounding resting cells signals the presence of neighboring living cells. Transient increases in [eATP] are used for basic physiological signaling, namely, in the nervous and vascular systems. Larger increases in [eATP] that are associated with cell death serve as a key “danger” signal in inflammatory processes. Two studies now point to roles for ATP in the immune system: providing a costimulatory signal to T cells and driving the differentiation of intestinal T helper 17 (T_H17) cells.

The importance of extracellular adenosine 5'-triphosphate (eATP) for cell-to-cell communication in the nervous and vascular systems has been thoroughly studied for years, but its role in the immune system is less well known. Two studies now demonstrate that eATP is also important for cell-to-cell communication in the immune system. In the first study, Grassi and collaborators propose that ATP released by T cells is a key autocrine player in T cell receptor (TCR) signaling (1). In the second study, the team of Takeda and colleagues proposes that ATP released by commensal bacteria drives the differentiation of intestinal T helper 17 (T_H17) cells (2).

Various aspects of purinergic signaling have been reviewed previously (3–6). The steady-state cytosolic concentration of ATP is 3 to 10 mM, whereas [eATP] is ~10 nM, which is maintained as a result of the activities of extracellular ecto-ATPases and ecto-adenosine triphosphatases (ecto-ATPases), which metabolize ATP into adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), and adenosine. Because of these enzymes, there is a 10⁶-fold gradient for ATP efflux, and the release of a very small fraction of cellular ATP is sufficient to activate some purinoreceptors (P2Rs). The cells' ability to detect eATP has been maintained throughout evolution in animals and plants. Among the purinoreceptors, P1Rs (now known as A1, A2, and A3 receptors) respond to adenosine but not to ATP, whereas all P2Rs (P2XR or P2YR) respond to ATP, with some also responding to ADP, uridine 5'-triphosphate, or uridine 5'-diphosphate. P2XRs are

Ca²⁺-permeable, nonselective cation channels. On activation, most P2YRs couple to heterotrimeric guanine nucleotide-binding proteins (G proteins) and phospholipase C-β, isoforms, which leads to a rise in intracellular [Ca²⁺] on activation; P2Y₁₁ leads to a rise in the intracellular concentration of cyclic AMP. Most P2Rs are sensitive to micromolar concentrations of eATP (Table 1). However, P2X₇ requires a very high concentration (>100 μM) of ATP for its activation and is thus a specific detector of large increases in [eATP], such as those that occur on cell death. P2X₁, P2X₄, and P2X₇ are found on T cells (7), as are several functional P2YRs (1). Dendritic cells (DCs) express mRNA for at least four P2XRs (including P2X₇) and eight P2YRs.

Resting cells release ATP at basal rates (8). Despite the presence of ecto-ATPases, this leak creates an ATP “halo” (an area of low [ATP]) around the cell, which may be in the micromolar range. Such a halo has been reported at the surface of platelets (9) and Jurkat T cells (a human CD4⁺ T cell line) (10). One may consider this halo as a low-intensity signal to immediate neighboring cells informing them of the presence of other living cells and constituting a first type of proprioceptive, ATP-mediated intercellular communication. A second type of communication is triggered by modest increases in [eATP] triggered by various mechanical (e.g., shear stress or stretching) and chemical (e.g., Ca²⁺-mobilizing agonists) stimuli.

The increase in [eATP] may be due to the release of vesicles in which ATP is concentrated, as, for example, in some nerve terminals, mast cells, platelets, and Jurkat T cells. However, other cells, such as primary T cells (1) or red blood cells (RBCs), do not have such ATP-rich granules. In these cells, ATP is released through pannexin-1 (panx1) channels

(1, 11, 12). Panx1 channels are nonselective and permeable to molecules up to 900 daltons. They may be activated by mechanical stimulation, depolarization, and changes in intracellular [Ca²⁺] [including that triggered by P2YR (13)], but for the most part, their opening is controlled by P2X₇ (12). In a few tens of seconds following P2X₇ activation by large [eATP], cells are effectively permeabilized because of the P2X₇-dependent opening of panx1 channels. This is rapidly followed by the formation of membrane blebs and the appearance of phosphatidylserine at the cell surface. These phenomena are reversible (termed pseudoapoptosis) if P2X₇ activation only lasts for a few minutes (14, 15). However, a longer activation leads to cytochrome c release by mitochondria and apoptosis.

Fully controlled, transient increases in [eATP] occur at purinergic synapses, for example. In the nervous system, eATP may be used in neuron-neuron, neuron-glia, and gli-glia communication (16). This ATP-dependent exchange of information often depends on two other molecules: Ca²⁺ and nitric oxide (NO) (17). Limited production of NO through the action of neuronal NO synthase (nNOS) and endothelial NOS (eNOS) in a Ca²⁺- and calmodulin-dependent manner is used for physiological responses, whereas large concentrations of NO produced by inducible NOS (iNOS) in a Ca²⁺-independent manner are mainly toxic. An example of how this trio of molecules (ATP, Ca²⁺, and NO) functions can be found in the vascular system. When RBCs are subjected to hypoxia or to a mechanical or shear stress, they release ATP (18). This eATP is detected by P2Rs on endothelial cells, which leads to an increase in intracellular [Ca²⁺] in these cells that stimulates eNOS to produce NO. The guanosine monophosphate (GMP) cyclase of neighboring smooth muscle cells is activated by NO, and the cyclic GMP thus produced leads to relaxation of smooth muscle cell and vasodilatation. A positive feedback is inserted in this communication system, as the increase in intracellular [Ca²⁺] leads to an increased production of ATP by mitochondria. This in turn may lead to the release of ATP and sustained Ca²⁺ signaling. The ATP released by deformed RBCs may help these cells to communicate not only with endothelial cells but also with other neighbors, such as platelets. Platelets that sense a local increase in [eATP] (due to RBCs or other platelets) also produce NO (19), and this is key to preventing the further recruitment of other platelets to the activated platelets and thus averting obstruction of narrow vessels.

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Cell type	Response to low [eATP]	Response to high [eATP]
Eosinophils	Chemotaxis; induction of IL-8 secretion	Induction of IL-8 secretion
Neutrophils	Chemotaxis; increased degranulation and ROS production	Increased adhesion to endothelium
Monocytes or macrophages	Chemotaxis; reduced secretion of inflammatory cytokines	Increased secretion of inflammatory cytokines
Immature DCs	Chemotaxis; maturation	Maturation
Mature DCs	Reduced secretion of inflammatory cytokines, including IL-1 β , IL-6, IL-12, and TNF- α	Increased secretion of inflammatory cytokines, including IL-1 β , TNF- α , and IL-23; induction of T _H 17 cells
T and B cells	Costimulation for antigenic stimulation	Costimulation for antigenic stimulation; shedding of L-selectin

Table 1. How eATP modulates immune responses in various cell types. Responses to low [eATP] are mediated by P2R with high or intermediate affinity for ATP. High affinity [median effective concentration (EC₅₀) < 1 μ M]: P2X₁, P2X₃, P2Y₂, and P2Y₁₃; intermediate affinity (EC₅₀ = 1 to 20 μ M): P2X₂, P2X₄, P2X₅,

P2X₆, P2Y₁, P2Y₄, and P2Y₁₁. Responses to high [eATP] are mediated by P2X₇ (EC₅₀ > 100 μ M). ROS, reactive oxygen species; TNF- α , tumor necrosis factor- α . [All the articles used to build this table are quoted in the review of Bours *et al.* (6), except the induction of T_H17 cells (2).]

A third type of cell-to-cell communication is that which occurs when [eATP] is strongly increased, for example, as happens after cell damage. This may lead both to activation of an inflammatory response and to the death of P2X₇-expressing cells. After tissue invasion by pathogenic bacteria, the bacterial product lipopolysaccharide triggers the cytoplasmic accumulation of pro-interleukin-1 β in mononuclear phagocytes. In the context of cellular damage, ATP-induced activation of P2X₇ receptors and panx1 triggers the assembly of the NALP3 inflammasome and the activation of caspase-1, which leads to the processing and release of interleukin 1 β (IL-1 β) (20). If the stimulation of P2X₇ is protracted, the phagocyte then dies. This third type of communication thus detects the process of cell death and leads to an inflammatory response and, potentially, to further cell death. Another aspect of the ATP-dependent inflammatory response is chemotaxis. ATP exerts a direct chemotactic effect on eosinophils, neutrophils, and immature (but not mature) DCs (21). In addition, ATP stimulates eosinophils to secrete IL-8, which is itself a potent chemoattractant of eosinophils and neutrophils (7).

In most cases, eATP is used for communication between different cells (paracrine signaling); however, ATP may also be used in an autocrine manner. For instance, presynaptic

P2XRs that sense ATP that was just released as a cotransmitter trigger a presynaptic increase in [Ca²⁺] that facilitates the release of neurotransmitter in response to the action potential that follows (22).

Grassi and colleagues propose that such an autocrine loop also functions in T cells to facilitate antigen recognition (1). In their model, the initial Ca²⁺ response triggered by the recognition by the TCR of a cognate peptide-loaded major histocompatibility complex molecule leads to Ca²⁺ uptake by mitochondria. This stimulates the synthesis of ATP, the release of ATP through panx1 channels (as this release was inhibited by carbenoxolone, a nonspecific inhibitor of panx1), and the activation of various P2XRs. Thus, ATP released by the T cell would constitute a costimulatory signal. Indeed, accumulation of micromolar concentrations of ATP was detected in the pericellular space of T cells treated with antibody against CD3 (to activate the T cells). The release of ATP was inhibited by oxidized ATP (oATP, an irreversible inhibitor of P2XRs); this inhibition demonstrated the involvement of P2XRs in this phenomenon. In addition, the sustained activation of extracellular signal-regulated kinase, observed 16 hours after stimulation of T cells with antibody against CD3, was not observed in the presence of inhibitors

of P2XRs. The authors suggested that T cell activation in the absence of purinergic costimulation induced T cell anergy, more precisely the “clonal anergy” that may be obtained in vitro and that should be distinguished from the more physiological “T cell tolerance” observed in vivo following chronic stimulation of the TCR (23). T cells that were stimulated for 16 hours with antibodies against CD3 and CD28 (to provide costimulation) expressed genes characteristic of clonal anergy only when stimulation was performed in the presence of oATP. In two mouse models of experimental autoimmune diseases, Grassi and colleagues proposed that eATP contributed to exacerbation of disease, because inflammation was milder in mice treated with oATP than it was in untreated mice.

The influence of bacterially released ATP on the differentiation of T_H17 cells in the lamina propria was examined by Takeda and colleagues (2). The intestinal mucosa is a very important interface between the body and the external world. Not only must the intestinal immune system be ready to rapidly fight against infectious agents, it must also be prevented from mounting an inappropriate inflammatory response against commensal bacteria in the gut. This delicate balance requires a number of cellular players, in particular T_H17 cells, which mediate an inflammatory

response (24) and are normally controlled by the simultaneous presence of regulatory T cells (Tregs) (25). Takeda and colleagues showed that commensal bacteria released large amounts of ATP (although not enough to deliver a “danger” inflammatory signal to the intestinal mucosa). The number of T_H17 cells in bacteria-free mice was increased by treating them with adenosine 5'-O-(3-thiotriphosphate), a nonhydrolyzable form of ATP, or decreased by treatment with apyrase, which degrades ATP. The percentage of T_H17 cells measured in a coculture of T cells and DCs was strongly increased by the addition of bacterial supernatant, and this effect was apyrase-sensitive. Colitis (an inflammatory bowel disorder) triggered by the transfer of naïve CD4⁺ T cells was worse in mice treated with αβ-ATP than in untreated mice.

The observations of Atarashi *et al.* (2) are quite consistent with those of Schenk *et al.* (1). With the same experimental model, they observed milder symptoms in mice treated with inhibitors of purinergic transmission than in untreated mice. However, the interpretations of the two groups are different. Schenk *et al.* suggested that most of their observations were due to an autocrine effect of ATP on T cells, without considering potential effects of ATP on DCs or other cell types. However, Atarashi *et al.* suggested that the effects of bacterially derived ATP were mediated by a subpopulation of DCs that are responsible for the differentiation of T_H17 cells, without taking other cell types into account.

As various purinergic receptors are ubiquitously expressed and may serve multiple modes of cell-to-cell communication—depending on the context, the amplitude, and the duration of the rise in [eATP]—such reductionist interpretations (attributing the effects of ATP to one type of purinergic receptor expressed by one type of cell) are unlikely to be fully correct. The situation is even more complex than presented thus far, as pathological situations that lead to large increases in [eATP] (for example, in tumors) (26) also lead, because of ATP degradation, to the formation of adenosine, which is a potent immunosuppressor of cells that express A2 and A3 receptors, such as lymphocytes (27). Thus, one has to keep in mind the complexity of the cellular and molecular environment in which ATP-dependent phenomena are observed. Schenk *et al.* have shown convincingly that ATP released by T cells (and presumably other cell types) may deliver a costimulatory signal to T cells, which confirmed previous observations (28–31). Atarashi *et al.* have clearly demonstrated that

T_H17 cell differentiation is strongly influenced by bacterially dependent ATP in the lamina propria. However, the cellular networks responsible for these phenomena are still to be discovered. We are far from having a comprehensive view of how physiological and pathological local variations in [eATP] modulate the global functioning of the immune system, and it is important to consider that eATP may play a role not only as a danger signal (when released by damaged cells), but in a larger range of situations.

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