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Shaping immune responses through the activation of dendritic cells' P2 receptors

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Abstract Dendritic cells (DCs) activate and shape the adaptive immune response by capturing antigens, migrating to peripheral lymphoid organs where naïve T cells reside, expressing high levels of MHC and costimulatory molecules and secreting cytokines and chemokines. DCs are endowed with a high degree of functional plasticity and their functions are tightly regulated. Besides initiating adaptive immune responses, DCs play a key role in maintaining peripheral tolerance toward self-antigens. On the basis of the information gathered from the tissue where they reside, DCs adjust their functional activity to ensure that protective immunity is favoured while unwanted or exaggerated immune responses are prevented. A wide variety of signals from neighbouring cells affecting DC functional activity have been described. Here we will discuss the complex role of extracellular nucleotides in the regulation of DC function and the role of P2 receptors as possible tools to manipulate immune responses.

Key words antigen presentation · ATP · autoimmunity · chemokine · dendritic cells · immune deviation · immunological tolerance · inflammation · interleukin-12

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Abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
CCR	Chemokine CC motif receptor
CCL	Chemokine CC motif ligand
CD	Cluster of differentiation
CXCL	Chemokine CXC motif ligand
CXCR	Chemokine CXC motif receptor
DCs	Dendritic cells
IDO	Indoleamine 2,3-dioxygenase
IFN	Interferon
IL	Interleukin
LN	Lymph node
NK	Natural killer
pDC	Plasmacytoid dendritic cell
STAT	Signal transducer and activator of transcription
TGF	Transforming growth factor
Th	T helper
TSP	Thrombospondin

Introduction

Dendritic cells (DCs) are a heterogeneous bone marrow-derived leukocyte population of specialised antigen-presenting cells functioning as initiators and regulators of T cell responses and influencing the activity of B lymphocytes and natural killer cells [1]. In humans, DC subsets include interstitial DCs found in peripheral tissues, Langerhans cells of the skin and plasmacytoid dendritic cells (pDCs) mostly present in the blood and in lymphoid organs. While interstitial DCs and Langerhans cells derive from a myeloid precursor, pDCs were initially considered of

lymphoid origin. Later studies have shown that pDCs can be differentiated from either common lymphoid or common myeloid precursors in both humans and mice. For simplicity we will hereafter refer to interstitial DCs and Langerhans cells as myeloid DCs. In the mouse, at least six different DC subsets have been identified. The existence of diverse DC populations specialised in particular tasks ensures efficient induction of host defense to multiple pathogens and tumor cells as well as the preservation of self-tolerance. In addition, each DC subtype is endowed with a certain degree of functional plasticity making the same cell able to work as a promoter of inflammation or a regulator of immune responses, depending on the nature of the stimulus and on the environmental conditions in which the cell has been activated [2]. Recent studies on the function of P2 receptors on DCs have revealed that the presence of nucleotides (in particular ATP) in the extracellular milieu surrounding DCs can significantly modify DC functions.

P2 receptors

P2 receptors are a class of plasma membrane receptors expressed by virtually all cell types. Their activation elicits diverse responses depending on cell type, receptors expressed and nucleotide concentration. Two P2 receptor subfamilies have been described so far and are named P2Y and P2X. Members of the two groups differ in protein structure, pharmacology and function [3–5].

P2Y receptors are seven membrane-spanning, G-protein-coupled receptors whose activation triggers generation of inositol 1,4,5-trisphosphate and release of Ca^{2+} from the intracellular stores [6]. Eight P2Y subtypes have been cloned so far and are named $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ [6, 7].

Neurons, heart, skeletal muscle, platelets, liver and digestive tract express $P2Y_1$ mRNA [6]. Stimulation of this subtype has been linked to platelet aggregation and nitric oxide (NO) release [8]. $P2Y_1$ is potently activated by ADP. The $P2Y_2$ receptor is expressed in skeletal muscle, heart, lung, spleen, placenta and kidney [6, 9]. Its function has been linked to ion transport in epithelia [10]. $P2Y_2$ is activated with similar efficiency by ATP and UTP. $P2Y_4$ is present in the intestine, lung and placenta [6]. UTP is a potent agonist at $P2Y_4$. Expression of $P2Y_6$ has been found in many human tissues, including spleen, thymus, placenta, intestine, lung and brain [6, 11, 12]. UDP is very active at $P2Y_6$. The $P2Y_{11}$ subtype has been found in corneal epithelia, endothelial and pancreatic duct cells, promyelocytic HL-60 cells, dendritic cells and lymphocytes; its activation is associated with increased intracellular concentration of cyclic AMP [13–16]. ATP is the preferred ligand at $P2Y_{11}$ [17].

$CD34^+$ stem cells, mast cells, vascular smooth muscle cells and platelets express the $P2Y_{12}$ subtype [18–20]. It is potently activated by ADP and linked to ADP-induced shape changes in platelets [21].

$P2Y_{13}$ is expressed in bone marrow, spleen, liver, brain, airway epithelial cells, red blood cells, monocytes, dendritic and T cells [18–20, 22, 23]. $P2Y_{13}$ has recently been linked to the regulation of hepatic high-density lipoprotein (HDL) endocytosis [24]. Its preferred agonist is ADP [25]. The recently identified $P2Y_{14}$ subtype has been found in hematopoietic cells, monocyte-derived dendritic cells and human airway epithelial cells. The $P2Y_{14}$ subtype responds to UDP-glucose and related sugar nucleotides [23, 26, 27].

P2X proteins are membrane receptors that form ion channels upon activation by extracellular ATP. Ligation of the agonist induces oligomerization and formation of homo—or in some cases hetero—multimer ion selective channels, being permeable to monovalent and divalent cations [28–30]. The P2X subunit is formed by an extracellular loop, two transmembrane domains and two (amino- and carboxyl-terminal) cytoplasmic domains. Seven P2X subtypes have been cloned so far ($P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_6$, and $P2X_7$). They were originally identified in mammalian neurons and smooth muscle cells, and subsequently also found in fibroblasts, lymphocytes, macrophages, and dendritic cells [5]. All P2X subtypes are activated by ATP.

The $P2X_1$ subtype is expressed by smooth muscle cells, megakariocytes, platelets, lymphocytes, dendritic cells, epithelial cells, ventricular myocardium, and neurons [31–34]. 2′,3′-(4-benzoyl)benzoyl-ATP (BzATP) and α,β-methylene ATP are good agonists at this subtype.

$P2X_2$ has different functional splice variants. 2meSATP is a better agonist than ATP for this subtype. It is expressed in pancreatic cells and neurons, where along with $P2X_3$, it is involved in nociceptive responses after nerve injury [35]. $P2X_3$ receptor is expressed by neurons and its activation has been linked to nociceptive signaling [35, 36]; mRNA expression of this subtype has also been found in keratinocytes, and $CD43^+$ hematopoietic cell precursors [32]. $P2X_4$ has been found in neurons, hematopoietic cell precursors, macrophages, monocyte-derived dendritic cells and fibroblasts, keratinocytes, and placenta [9, 23, 37]. $P2X_5$ and $P2X_6$ mRNAs have been detected in neurons, keratinocytes and thyrocytes [38–40]. The $P2X_7$ receptor is expressed in macrophages, microglia, dendritic cells and placenta [7, 9]. It is a non-desensitising receptor with the peculiar capacity to undergo a permeability transition from a cationic selective channel to a plasma membrane pore upon stimulation with high or pulsed ATP doses. BzATP is more potent than ATP at this subtype.

What extracellular nucleotides signal to DCs?

Nucleotides are present at relatively high amounts in the cytoplasm of cells where their concentration ranges from 1–10 mM. In the extracellular space their concentration is considerably lower ranging between 1 and 10 nM. Due to the steep concentration gradient, their small size and their high mobility in the extracellular compartment, nucleotides can be rapidly released along with other cellular components following mechanical stress, cell damage or death. Increased nucleotide concentration in the extracellular space is therefore closely associated with tissue stress or damage [41–43]. However non-lytic nucleotide release may occur in many cell types under a variety of conditions. Activated platelets represent a relevant source of ATP released concomitantly with several inflammatory mediators during clot formation [43]. ATP is released from exercising skeletal muscle as well as from vascular endothelial cells and smooth muscle cells in conditions of increased blood flow or upon mechanical stimulus [44–47]. Moreover, ATP secretion from endothelial cells and leukocytes may be induced by pathogen-associated molecules such as LPS [48, 49].

The ability of DCs to sense tissue stress is a cardinal point of the danger theory [50]. In this model, rather than be activated solely by the recognition of foreign pathogens, DCs react to the presence of environmental molecules associated with tissue stress, the so-called danger signals. Danger signals can be classified into endogenous and exogenous. Endogenous danger signals can be further subdivided into constitutive (ATP, adenosine, some heat shock proteins) and inducible (type I interferons, some other heat shock proteins). Exogenous danger signals include microbial-associated molecules recognised by Toll-like receptors expressed on a variety of cells including DCs. The ability to recognise endogenous danger signals allows the immune system to discriminate between harmless (e.g., commensal flora at mucosal surfaces) and pathogenic organisms by assessing their effect (damage) on the host. However, tissue damage might also be secondary to the intrinsic toxicity of sustained inflammation, which can ultimately be as harmful as the infection itself. In order to restore homeostasis, a timely termination of inflammatory processes is required. Extracellular ATP, in fact, has been suggested to be a signal of danger whose function is to activate DCs as well as to limit excessive inflammatory responses and promote tolerance.

Dendritic cells circulate in the bloodstream or reside in peripheral tissues where they are specialised in the uptake of potential antigens. In this life-cycle stage, DCs are considered “immature” and express chemokine receptors for inflammatory chemokines such as CXCR1, CCR1, CCR2 and CCR5 and for other inflammatory factors enabling them to migrate from the blood to inflamed tissues [51–53]. Recent work by Idzko and colleagues shows that ATP released from dying cells can be included in the list of mediators able to recruit dendritic cells. *In vitro*, exposure to low concentrations (100 nM) of extracellular ATP, through the activation of P2Y receptors, induces intracellular calcium mobilisation, actin polymerisation and chemotaxis of immature, but not mature, monocyte-derived DCs [54].

Additional evidence suggesting a role for P2Y receptors in cell trafficking comes from the observation of monocytes/macrophages from CD39-deficient mice that have impaired ability to metabolise extracellular ATP and display P2Y signaling pathway desensitisation associated with reduced chemotactic responses [55]. In addition, exposure to ATP gradients has been reported to inhibit chemokine-elicited migration of monocyte-derived DCs and freshly isolated $CD1a^+$ dermal dendritic cells but not circulating peripheral blood $CD1c^+$ DCs or plasmacytoid DCs through a $P2Y_{11}$-dependent mechanism [16]. While all four DC populations express discrete amounts of mRNA encoding $P2Y_{11}$, only monocyte-derived DCs and dermal $CD1a^+$ DCs display responsiveness to $P2Y_{11}$ agonists, consistent with the susceptibility to ATP gradients.

Whether circulating DCs need some unidentified signal present in peripheral tissues (such as the skin) or in the *in vitro* cultures to express functional $P2Y_{11}$ protein on cell membrane is unknown. These findings suggest a complex role for ATP in the regulation of DC trafficking: ATP might work as a chemoattractant for DCs toward the site of tissue damage; on the other hand ATP gradients might prolong the permanence of immature DCs at the site of the antigen encounter (Figure 1).

Dendritic cells exposed to pathogen-associated molecules engaging Toll-like receptors undergo maturation, a complex process turning immature DCs into efficient antigen-presenting cells. Additional stimuli driving DC maturation include inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) or the engagement of CD40 by CD40 ligand expressed on activated T lymphocytes.

Early after activation, DCs produce inflammatory cytokines including TNF-α, IL-1 and IL-6 and downregulate the expression of inflammatory chemokine receptors and of $P2Y_{11}$ [16], while upregulating lymphoid chemokine receptors CCR7 and CXCR4 [53, 56]. This sets DCs to leave the site where activation occurred, enter into lymphatic circulation and migrate to draining lymph nodes.

Maturing DCs progressively reduce antigen uptake activity, while upregulating the expression of molecules involved in antigen presentation such as major histocompatibility complexes (MHC) I and II, the co-stimulatory molecules CD80 and CD86 providing signals 1 and 2 for T cell activation. Other surface molecules involved in the

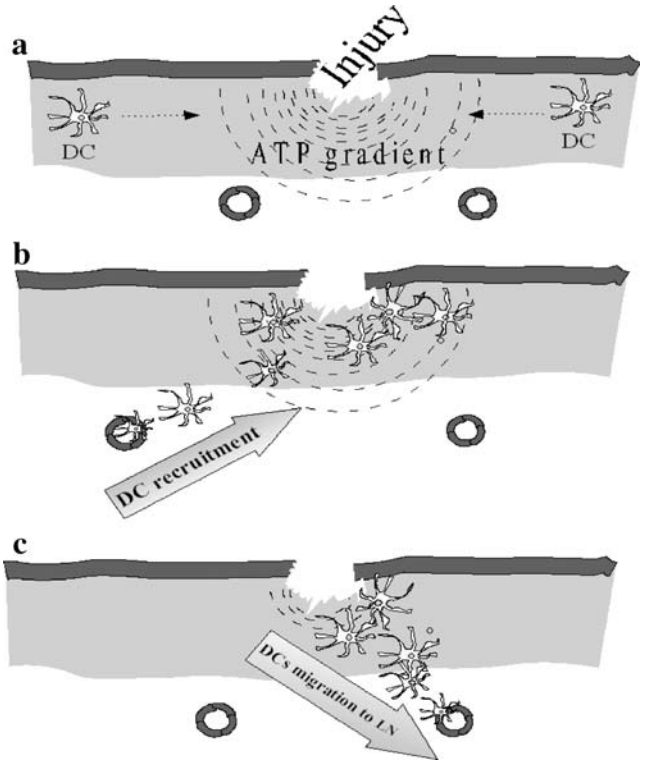

Figure 1 (a–c) Proposed role of extracellular ATP in the regulation of DC trafficking. Due to cell death, the extracellular space surrounding sites of tissue injury is characterised by increased ATP concentration. Circulating DCs might follow the ATP gradient to traffic to perilesional area where an antigen encounter is more likely to occur (a) and where ATP might reach concentrations in the micromolar range. $P2Y_{11}$ activation transiently inhibits DCs migration, prolonging their persistence at the site of antigen encounter (b). At later stages, due to the action of ecto-nucleotidases, extracellular ATP levels drop down, and the chemokines CCR7 and CXCR4 are upregulated by $P2Y_{11}$ signaling. This sets DCs for efficient migration from peripheral tissues toward regional lymph nodes (c).

interaction with T cells, such as CD54 and CD40, and OX40 ligand are also upregulated. In addition chemokines released by DCs at early stages of maturation, such as CCL2, CCL3, CCL4, CCL5, CXCL8 and CXCL10 recruit circulating monocytes, immature DCs, T cells and neutrophils at the site of the antigen encounter [52, 53]. Migrating DCs upregulate the production of lymphoid chemokines including CCL17, CCL19 and CCL22, providing chemotactic signals for naïve T cells and mature DCs in the lymph nodes.

Besides presenting antigens and necessary co-stimulatory signals for the activation of T lymphocytes, DCs instruct T helper cells to differentiate into IFN-γ−(Th 1 phenotype) or IL-4-producing cells (Th 2). Th 1 differentiation is driven by the presence of IL-12 in the microenvironment where antigen presentation occurs [57, 58]. DCs are a major source of IL-12, which is also an important stimulatory factor for NK cells. Activated DCs migrating to the lymph node induce NK recruitment through a CXCR3-dependent mechanism [59]. DCs are a relevant source of the CXCR3 ligands CXCL9, CXCL10 and CXCL11, and both myeloid DC and pDC supernatants have been shown to induce NK cell migration *in vitro* [60]. NK trafficking into inflamed lymph node has been proven necessary for efficient priming of Th 1 lymphocytes *in vivo* [59]. In fact, IFN-γ released by NK cells at the site of antigen presentation induces STAT-1 activation, which in turn elicits the expression of the transcription factor t-bet in naïve T lymphocytes. Subsequently t-bet promotes Th 1 differentiation by driving the expression of IL-12 receptor [61]. In this context, IL-12 produced by DCs by triggering its cognate receptor on naïve T cells, induces STAT-4 activation, a key event for Th 1 differentiation. Conversely, the absence of IL-12 and signals leading to t-bet expression favours Th 2 development (Figure 2).

Immature monocyte-derived DCs exposed to chronic stimulation with low micromolar concentrations of ATP but not UTP undergo partial membrane maturation and upregulate CD83, CD54, CD86 as well as the lymphoid chemokine receptor CCR7 conferring functional responsiveness to lymphoid chemokines such as CCL19 and CXCL12 [56, 62]. Dendritic cells stimulated with ATP alone fail to produce detectable cytokines [62]. However, when DCs are stimulated with the prototypic stimulus bacterial endotoxin (LPS) in the presence of ATP, profound changes in the maturation program are induced. While LPS-treated DCs produce high amounts of proinflammatory (IL-12, IL-23, TNF-α, IL-1, IL-6) and regulatory (IL-10 and IL-1 receptor antagonist) cytokines, when stimulated with LPS in the presence of low concentrations of ATP, DC production of IL-12, TNF-α, and IL-1 is completely abrogated. Conversely, production of the regulatory cytokine IL-10 is unaffected and IL-1 receptor antagonist is increased. As a result of the blocked IL-12 production, DCs exposed to extracellular ATP have reduced ability to induce Th1 differentiation *in vitro* [62, 63]. In addition, the production of chemokines preferentially attracting Th 1 polarised lymphocytes such as CXCL10 and CCL5 is also blocked, yet DCs still produce high amounts of the Th 2-attracting chemokines CCL-22 and CCL-17 [56]. The lack of CXCL10 (a ligand for CXCR3) production might also impair DC contribution to the recruitment of NK cells into the lymph node thus further favouring Th 2 development.

Altered DC maturation induced by the presence of extracellular ATP is reminiscent of the effects of treatment with cyclic adenosine monophosphate (cAMP)-inducing agents. Dendritic cells in which intracellular concentration of cAMP is increased following ligand-activation of Gs protein-coupled receptors, such as EP2 prostaglandin receptor [64], H_2 histamine receptor [65, 66], β-adrenergic receptors [67], or A2 adenosine receptor [68], display blocked TNF-α and IL-12p70 production but unaffected or even increased IL-10 together with augmented expression

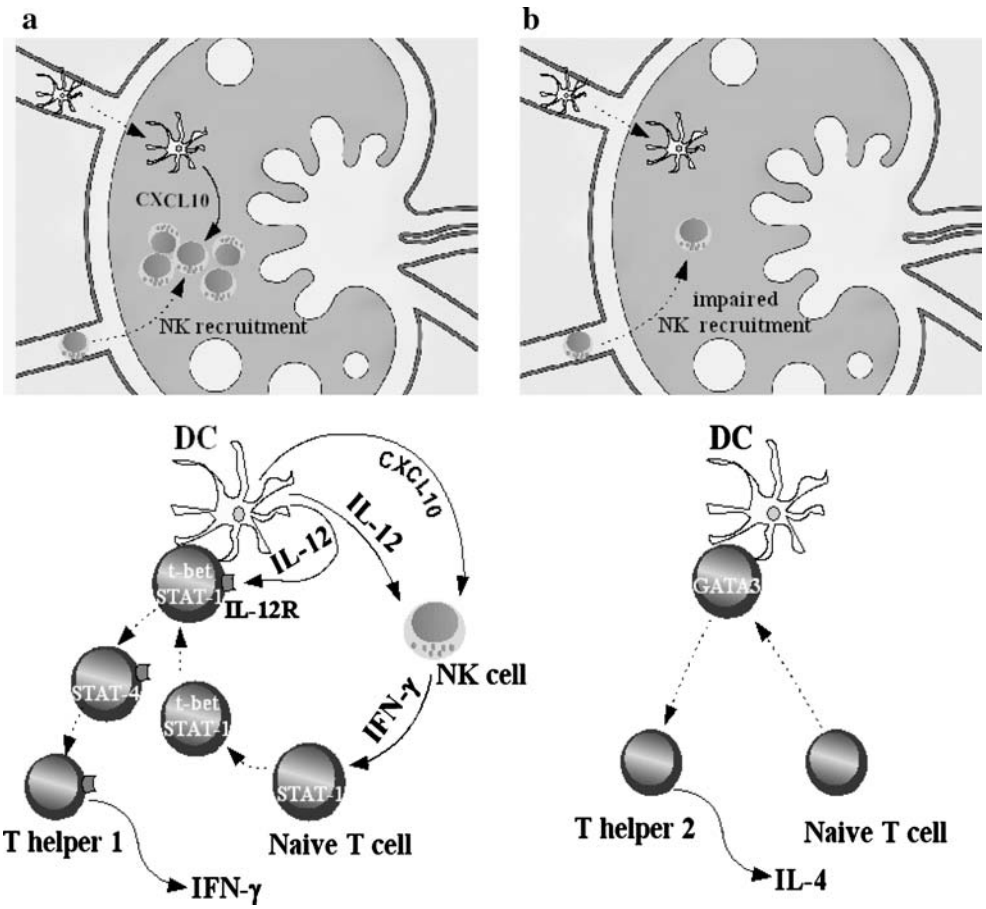

Figure 2 (a, b) Dendritic cells shape T lymphocyte responses. (a) DCs migrating to the lymph node participate in NK recruitment by producing the CXCR3 ligand CXCL10. NK trafficking into inflamed lymph nodes is necessary for efficient priming of type 1 T lymphocytes. During antigen presentation, IFN-γ released by NK allows naïve T lymphocytes to be activated by IL-12 through the induction of IL-12 receptor. Under these conditions, DCs deliver signal 1 (MHC-peptide complex engaging TCR), signal 2 (DC costimulatory molecules CD80 or CD86 engaging CD28 on T lymphocyte surface) and signal 3 (STAT-4 activation by IL-12). (b) In ATP-conditioned DCs, simultaneous inhibition of IL-12 and CXCL10, but not IL-10 production, impairs the development of type 1 responses: reduced NK recruitment and antigen presentation in the absence of IL-12, but in the presence of IL-10, favours T helper 2 and/or T regulatory 1 differentiation.

of CD83 and CD86. Similar effects were observed by increasing intracellular cAMP by ADP ribosylation of the Gαs subunit of G proteins by cholera toxin [69, 70], by stimulation of adenylyl cyclases through forskolin administration [67] and by treatment with membrane-permeable cAMP analogues such as dibutyryl cAMP [67] or 8-bromocyclic AMP [71]. Extracellular ATP can increase the intracellular level of cAMP through the activation of the $P2Y_{11}$ receptor that is coupled to the adenylyl cyclase and the phosphoinositide pathways [72]. Moreover $P2Y_{11}$ has been suggested to be the P2 receptor mediating the ATP-induced DC maturation [13]. The stimulation of the cAMP pathway is also involved in the reported synergy between extracellular ATP and TNF-α or suboptimal doses of LPS in the induction of IL-12p40 by DCs. Either TNF-α or suboptimal doses of LPS are unable to elicit IL-12 production unless this stimulation takes place in the presence of extracellular ATP [13, 73]. However the synergistic effect is limited to the IL-12 p40 chain; no IL-12p70 heterodimer is produced by DCs exposed to extracellular ATP irrespective of the stimulus used [63].

Interestingly, under optimal stimulation conditions, the concomitant activation of the cAMP pathway by either extracellular ATP or prostaglandin E_2, besides inhibiting IL-12p70, results in enhanced production of IL-23, a heterodimeric cytokine, a member of the small IL-12 family, composed of the p19 and IL-12p40 proteins. While IL-12 p70 is essential for the polarisation of naïve T cells toward Th 1 phenotype, IL-23 is thought to act preferentially on memory T lymphocytes stimulating IFN-γ production [74]. Consistently, DCs stimulated with *E. coli* in the presence of ATP display decreased ability to prime Th 1 responses but are still efficient in promoting IFN-γ production by memory T lymphocytes [75]. This might have important consequences for the regulation of immune responses as follows: in the presence of excessive tissue damage, large amounts of self peptides usually confined in the intracellular compartment become available to surrounding DCs. These DCs are limited in their ability to prime Th 1 responses by the concomitant presence of extracellular ATP, thus reducing the risk of activating potentially self-reactive IFN-γ producing cells. Concurrently, DCs are still able to support IFN-γ production by memory T cells, whose priming likely does not occur in the presence of overwhelming amounts of self-antigens and that therefore might be instrumental for pathogen eradication.

Triggering the $P2Y_{11}$ receptor, besides inhibiting Th 1 priming, can also confer immunosuppressive activity to

DCs. Marteau and colleagues, in a very recent report, showed that extracellular ATP induces DCs to produce large amounts of thrombospondin-1 (TSP-1) and to express indoleamine 2,3 dioxygenase [76]. Thrombospondin-1 exerts immunoregulatory activity by different mechanisms: (1) inhibition of T cell proliferation by binding to its receptor (CD47) on the cell membrane; (2) autocrine inhibition of IL-12 production by activating the phosphoinositol 3 kinase pathway in DCs [77]; (3) autocrine inhibition of DC activation [78]; (4) activation of the potent immunosuppressive cytokine TGF-β1 [79]. In addition Marteau and colleagues showed that ATP synergizes with IFN-γ to induce the expression of indoleamine 2,3 dioxygenase (IDO) [76]. Dendritic cells expressing IDO, an intracellular enzyme involved in the catabolism of the essential amino acid tryptophan, suppress T cell proliferation and promote tolerance [80–83]. In summary, while chronic exposure to very low (nanomolar) concentrations of extracellular ATP induces chemotactic activity of DCs, micromolar concentrations modify DC function to promote less self-harmful type 2 responses and/or tolerance by a variety of mechanisms due to adenylyl cyclase activation.

Another set of studies addressed the effects on DC physiology of high extracellular ATP doses showing that in the millimolar range, ATP causes the opening of $P2X_7$ ion channel across the DC membrane and consequently increases permeability to low molecular weight solutes. Due to the availability of specific antibodies and inhibitors, $P2X_7$ is the best characterised P2 receptor. It is expressed at very high levels on both murine and human DCs [31, 84]. Enhanced DC membrane permeability following treatment with ATP doses suggestive of activation of the $P2X_7$ pore triggers rapid secretion of IL-1β and TNF-α by mature dendritic cells [31]. It has been shown that in mononuclear phagocytes, $P2X_7$-mediated IL-1β release is due to the activation of interleukin-1-converting enzyme/caspase-1, which cleaves pre-stored IL-1 precursor to produce the mature form of IL-1β [85]. High membrane expression of $P2X_7$ by DCs correlates with sensitivity to cytotoxic effects of extracellular ATP inducing apoptosis or necrosis depending on the dose and length of exposure [84, 86, 87]. Moreover it has been suggested that during antigen presentation, macrophages that upon activation have upregulated $P2X_7$ expression might be lysed by ATP released from cytotoxic T lymphocytes [88]. Inhibition of $P2X_7$ activation by oxidised ATP results in the prevention of $P2X_7$-induced cell death as well as down-modulation of LPS-induced signaling. In particular, decreased activation of nuclear factor-κB, and of extracellular signal-regulated kinases 1 and 2 are observed in macrophages stimulated with LPS in the presence of $P2X_7$ antagonist, pointing to a role of $P2X_7$ signaling in cell activation induced by Toll-like receptor engagement [89].

Although there is a substantial discrepancy between physiological ATP levels detected in the extracellular space and the concentration needed to trigger $P2X_7$ *in vitro*, it is important to consider that the average extracellular nucleotide concentrations might represent significantly different local distributions. In close proximity to leaking plasma membranes of damaged cells, as well as of healthy actively secreting cells, nucleotides might locally reach molar concentration. Moreover, upon activation of pore-forming $P2X_7$, the egress of intracellular nucleotides through the pore can trigger $P2X_7$ on the membrane of adjacent cells, resulting in the amplification of local release of intracellular nucleotides in the extracellular milieu. Furthermore, the microbicidal peptide LL37, representing the C terminus of the cathelicidin family member cationic peptide 18 produced by neutrophils and epithelial cells, has been reported to be an endogenous $P2X_7$ activator that triggers maturation and release of IL-1β from LPS-primed monocytes [90].

The concentration of ATP needed for P2X7 activation *in vitro* might not reflect the physiological situation. For example, recent evidence provided by Seman and coworkers showed that $P2X_7$ activation can be triggered by nicotinamide-adenine dinucleotide (NAD)-dependent purinoceptor ADP-ribosylation [91]. Although NAD itself is not a ligand for $P2X_7$, NAD released upon tissue injury and inflammation represents the substrate for ecto-ADP ribosyltransferase-2 (ART-2) catalyzing ADP-ribosylation of $P2X_7$. Interestingly, NAD derived from cell lysates is sufficient to activate $P2X_7$ and in the presence of NAD, $P2X_7$ activation can be triggered by low ATP concentrations. Similarly, we showed that otherwise ineffective concentrations of ATP induce $P2X_7$-mediated cytotoxicity in the presence of the antibiotic polymyxin B [92]. These studies demonstrate that (1) $P2X_7$ and possibly other purinergic receptors might be activated by non-nucleotide agonists, and (2) *in vivo*, different agonists can cooperate and activate purinoceptors at significantly lower concentrations than those needed *in vitro*.

To date no study has specifically addressed the modulation of dendritic cell activity by extracellular nucleotides *in vivo*. However the *in vivo* administration of the ATP analogue 2-methylthio-ATP inhibits the release of TNF-α and IL-1 and protects mice from endotoxin shock [93, 94]. Although these studies did not specifically address the contribution of DCs to the inflammatory response to LPS, their results are in keeping with what was observed in human monocyte-derived DCs stimulated *in vitro* with LPS in the presence of extracellular ATP [62, 63].

Mizumoto and colleagues studying contact hypersensitivity to haptens in CD39-deficient mice showed that the impairment of DC ability to metabolise extracellular nucleotides significantly influences their function *in vitro* and *in vivo*. $CD39^{-/-}$ Langerhans cells and bone marrow-

derived DCs lack ecto-diphosphohydrolase activity with consequent accumulation of extracellular nucleotides in the pericellular space. Under these conditions, DCs and Langerhans cells are unresponsive to ATP due to P2 receptor desensitisation and display impaired antigen-presenting capacity. Moreover T lymphocytes increase pericellular ATP concentration upon activation, suggesting that nucleotides have an important role in DC–T cell communication during antigen presentation [55].

Conclusions

Nucleotides, in particular ATP, have been proposed as endogenous signals of tissue stress. In addition, ATP actively secreted by T lymphocytes might work as an important mediator for cell–cell communication during antigen presentation. In fact extracellular nucleotides, by stimulating P2 receptors, can profoundly influence DC functions and have a great impact on the outcome of immune response. Chronic exposure to low (micromolar) concentrations of extracellular ATP might work as negative feedback to limit DC contribution to exacerbated inflammation, mainly through the activation of $P2Y_{11}$ and the following rise in the intracellular cAMP concentration. Concentration gradients of extracellular ATP attract immature DCs into injured tissues and prolong their permanence at the site of antigen encounter. In the proximity of damaged cells, where the concentration may be in the micromolar range, ATP blocks the synthesis by DCs of proinflammatory cytokines and chemokines for the recruitment of NK cells and type 1 polarised T lymphocytes and limits DCs' capacity to promote type 1 responses. As a result, the development of less self-harmful type 2 responses is favoured. In addition, expression of regulatory molecules such as IDO and thrombospondin-1 might turn DCs into active regulatory cells promoting tolerance rather than immunity.

On the other hand, high concentrations of extracellular ATP causes DC death through $P2X_7$ activation and might represent a mechanism for the elimination of antigen-presenting cells into the lymph node by activated T lymphocytes.

Although further *in vivo* studies are required to confirm the observations made on monocyte DCs *in vitro*, targeting P2 receptor function might represent a promising approach to enhance immune responses for increased vaccination efficacy or conversely, to promote the tolerogenic functions of DCs for the treatment of inflammatory and autoimmune diseases.

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