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REVIEW

# Physiologic roles of P2 receptors in leukocytes

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## Abstract

Since their discovery in the 1970s, purinergic receptors have been shown to play key roles in a wide variety of biologic systems and cell types. In the immune system, purinergic receptors participate in innate immunity and in the modulation of the adaptive immune response. In particular, P2 receptors, which respond to extracellular nucleotides, are widely expressed on leukocytes, causing the release of cytokines and chemokines and the formation of inflammatory mediators, and inducing phagocytosis, degranulation, and cell death. The activity of these receptors is regulated by ectonucleotidases—expressed in these same cell types—which regulate the

**Abbreviations:** 2-AG, 2-arachidonoylglycerol; 2meSATP, 2-methylthio-adenosine triphosphate; AA, arachidonic acid; AC, adenylate cyclase; AD, Alzheimer disease; ADA, adenosine deaminase; ADO, adenosine; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; ATPγS, adenosine-5′-o-(3-thio-triphosphate); Aβ, amyloid β; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; BCG, bacilli Calmette-Guerin; BD, Behçet's disease; BMDMs, bone marrow derived-macrophages; BzATP, 2′(3′)-o-(4-benzoylbenzoyl) adenosine triphosphate; CALHM1, calcium homeostasis modulating channel 1; CFA, complete Freund's adjuvant; CFTR, cystic fibrosis transmembrane conductance regulator; CLL, chronic lymphocytic leukemia; COX-2, cyclooxygenase-2; cP2X7, chicken P2X7; Cx43, connexin 43; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; ECP, eosinophil cationic protein; E-NPP, ectonucleotide pyrophosphate/phosphodiesterase; E-NTPDase, ectonucleoside triphosphate diphosphohydrolase; GALT, gut-associated lymphoid tissue; GM-CSF, granulocyte-macrophage colony stimulating factor; GPCR, G protein-coupled receptor; gpP2X7, giant panda P2X7; GTP, guanosine triphosphate; HDM, house dust mite; HIV, human immunodeficiency virus; HLA-G, human leukocyte antigen G; HMGB1, high mobility group box 1; hP2X3, human P2X3; IBD, inflammatory bowel disease; $IP_3$, inositol triphosphate; KC, keratinocyte chemoattractant; KO, knockout; LPS, lipopolysaccharide; $LTB_4$, leukotriene $B_4$; $LTC_4$, leukotriene $C_4$; MACs, maxi-anion channels; MCP-1, monocyte chemoattractant protein-1; MGCs, multinucleated giant cells; MIP-1α, macrophage inflammatory protein-1α; MIP-1MIP-2, macrophage inflammatory protein 2, ted giant cells; MIP-3α, macrophage inflammatory protein-3α; MPS, mononuclear phagocytic system; NAADP, nicotinic acid adenine dinucleotide phosphate; NAD, nicotinamide adenine dinucleotide; NETs, neutrophil extracellular traps; N; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; OA, oleic acid; oATP, oxidized ATP; OGD, oxygen-glucose deprivation; OVA, ovalbumin; P1R, P1 receptor; P2R, P2 receptor; PAMPs, pathogen-associated molecular patterns; $PGE_2$, prostaglandin $E_2$; $PLA_2$, phospholipase $A_2$; PLC, phospholipase C; PLD, phospholipase D; PMN, polymorphonuclear neutrophil; PNP, purine nucleotide phosphorylase; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; RB-2, Reactive Blue-2; ROS, reactive oxygen species; rP2X7, rat P2X7; TACE, TNF-α converting enzyme; TM, transmembrane; $TXB_2$, thromboxane $B_2$; UDP, uridine diphosphate; UTP, uridine triphosphate; VASP, vasodilator-stimulated phosphoprotein; VRACs, volume-regulated anionic channels; WT, wildtype; zfP2X4, zebrafish P2X4; α,β-meATP, α,β-methylene adenosine triphosphate.

availability of nucleotides in the extracellular environment. In this article, we review the characteristics of the main purinergic receptor subtypes present in the immune system, focusing on the P2 family. In addition, we describe the physiologic roles of the P2 receptors already identified in leukocytes and how they can positively or negatively modulate the development of infectious diseases, inflammation, and pain.

**KEYWORDS**
ATP, glia, immune cells, P2X, P2Y

## 1 | INTRODUCTION

Leukocytes have a critical role in immune surveillance and protection against viruses, bacteria, fungi, and others. These cells are mainly produced in the bone marrow and can be subdivided into several subtypes according to the proteins expressed on their cell membranes, including receptors that help them sense the environment.[1]

Purinergic receptors are transmembrane proteins that respond to extracellular purines and pyrimidines, and are divided into 2 families: P1 (activated by adenosine [ADO]) and P2 (activated by nucleotides).[2] The physiologic agonists of P2 receptors include adenosine triphosphate (ATP), adenosine diphosphate (ADP), uridine triphosphate (UTP), uridine diphosphate (UDP), UDP-glucose, nicotinamide adenine dinucleotide (NAD), and nicotinic acid adenine dinucleotide phosphate (NAADP), among others.[3–6] P2 receptors are further subdivided into 2 classes: P2X (ligand-gated ion channels) and P2Y (G-protein coupled receptors (GPCRs)).[2]

P2 receptors display a widespread distribution in mammalian tissues. Cells of the immune system express several P2 subtypes, which play important roles on chemotaxis, cytokine release and formation of proinflammatory mediators, among others.[7,8] In this article, we review the main characteristics of the purinergic receptors in the immune system, focusing on the P2 family. In addition, we describe important discoveries on the physiologic roles of P2 receptors in leukocytes and how they can positively or negatively modulate the development of infectious diseases, inflammation, and pain.

### 1.1 | Historical background

1979, Cockroft and Gomperts reported that $ATP^{4-}$, that is, ATP uncoupled to $Mg^{2+}$, induced histamine release from mast cells, and also turned their plasma membrane permeable to large solutes.[9,10] This observation followed the description, by Cohn and Parks, of the role of ATP in the formation of pinocytic vesicles in cultured macrophages[11] and the descriptions by Burnstock of purinergic receptors in a variety of tissues,[10] led Cockcroft and Gomperts to propose that the effects of extracellular ATP on mast cells were mediated by purinergic receptors.[12] These observations were followed by the description of several physiological responses to ATP in a variety of tissues, including smooth muscle contraction and relaxation, neuron polarization, and membrane permeabilization.[13–15]

The current classification of P2 receptors was conceived in the 1990s, with the advancement of molecular cloning studies of these proteins and their pharmacologica characterization with the patch-clamp electrophysiologic technique.[16–18] In the current classification, the P2X class comprises the ionotropic receptors, whereas the P2Y class are GPCRs.

### 1.2 | P2 receptors and the current understanding of their general role in the immune system

The idea that extracellular nucleotides can play an important modulatory role in the immune system has long been proposed,[19–21] and P1 and P2 receptors, as well as ectoenzymes that interact with their natural ligands, have been identified in many immune cell types, pointing to complex immune-neuro-endocrine interactions.[22–27] Tissue injury situations, in particular, result in massive release of ATP and other nucleotides, leading to the activation of P2 receptors. The activation of these receptors induces proinflammatory responses, contributing to the development of acute inflammation. In turn, ectonucleotidases, by converting ATP to ADO, counteract the maintenance of acute inflammatory responses, as ADO activates P1 receptors, which have anti-inflammatory effects and are involved in the resolution of inflammation.[28] In this review, we will focus on the effects mediated by P2 receptors on leukocytes.

## 2 | P2 RECEPTOR

### 2.1 | P2X receptors

The sequencing of P2X receptors revealed a new class of receptors, with no significant homology to other purinergic receptors based on sequence and mutagenesis analysis.[29,30] The P2X receptor class comprises 7 members, named P2X1–7. The sequence homologies between them ranges from 35 to 48%.[31]

Each P2X receptor subunit displays 2 hydrophobic transmembrane segments, with 1 extracellular loop and intracellular N- and C-terminals.[30,32–34] The C-terminal domain has binding sites to interact with protein kinases. The two transmembrane (TM) domains, TM1 and TM2, are involved in the ion channel and pore formation, respectively. The extracellular loop has cysteine residues that form disulfide bridges.[35]

**TABLE 1** Homomeric and heteromeric P2X receptors

| P2X receptor | Homomultimer | Heteromultimer(s) |
|---|---|---|
| **P2X1** | Yes | P2X1/2 |
| | | P2X1/4 |
| | | P2X1/5 |
| **P2X2** | Yes | P2X1/2 |
| | | P2X2/3 |
| | | P2X2/6 |
| **P2X3** | Yes | P2X2/3 |
| **P2X4** | Yes | P2X1/4 |
| | | P2X4/6 |
| **P2X5** | Yes | P2X1/5 |
| **P2X6** | No | P2X2/6 |
| | | P2X4/6 |
| **P2X7** | Yes | No |

References [40–43].

All P2X receptors described so far, except for P2X6, can form homomultimer channels in vitro, since purinergic analogs activate currents when each subtype is expressed in oocytes or cell lines (P2X6, in contrast, only works as a heteromultimer).[32,33] However, in vivo more than one P2X subtype may be found in the same cell type, including neurons and muscle cells, and these receptors also appear to form heteromultimers.[36–38] Indeed, the coexpression of P2X2 and P2X3 results in a channel with characteristics that differ from an exogenous expression of P2X2 or P2X3 alone; and the same can be observed for P2X1 and P2X5.[39] Other heteromeric receptors described so far were P2X4/6[40] and P2X2/6[41] in the CNS. Table 1 summarizes the homomultimers and heteromultimers formed by P2X.

Regarding the exact number of subunits that form the cation channel, Nicke and colleagues[42] showed that P2X receptors present a trimeric arrangement, based on chemical cross-linking and blue native PAGE experiments. The trimeric assembly of these receptors was confirmed in 2009 by Kawate and colleagues,[44] who resolved the crystal structure of the zebrafish P2X4 receptor in its closed state.

Several studies have focused on clarifying the structural motifs responsible for P2X channel formation. These studies revealed that a 25 amino acid sequence, including the second transmembrane domain, is determinant for subunit co-assembly, whereas the extracellular region by itself is not sufficient for the assembly of the full-sized subunits.[41] Additionally, mutations in the transmembrane domains of P2X receptors can alter pore dilation.[45,46]

The P2X4 subunits resemble the shape of a dolphin: in this analogy, the extracellular domain represents the upper body, and the transmembrane domains represent the fluke (for a detailed explanation of the dolphin's shape, see Ref.[44]). Additionally, there are some lateral fenestrations. The N- and C-terminals were not present in the crystalized receptor, preventing a more detailed study of the pore structure. Interestingly, the subunits are connected only by the upper portion of the extracellular domain, and the region immediately above the membrane is not connected. This conformation allows the transmembrane domains, which are in contact, to move in the gating (opening and closing) of the channel. Moreover, the residues that form the body domain are conserved, which suggests that body-to-body interactions are a common feature among all P2X receptors.[44]

The crystallographic data of the zebrafish P2X4 (zfP2X4) receptor complexed with ATP (in the open state) demonstrated that the three ATP-binding pockets are located in conserved residues distant 40 Å above the membrane border. These binding pockets are formed by the intersection of the three subunits and have several positively charged residues.[47] The transition from a closed state to an open state occurs when ATP binds the agonist site. In this situation, a small fold forms in the lower part of the extracellular loop and the transmembrane domains enlarge, causing pore dilation. The hydrated ions flow into the pore through the lateral fenestrations.[47]

The crystal structure of human P2X3 (hP2X3) revealed that this receptor also presents a dolphin-like architecture. The transmembrane domains in hP2X3 are longer than those in zfP2X4, and the antagonists of the P2X3 receptor seem to fasten to the orthosteric binding pocket. hP2X3 has an intracellular motif, named "cytoplasmic cap," which is formed by a network of three $\beta$-sheets that cap the cytoplasmic surface of the pore. This structure is only observed in the ATP-bound (open state) and generates lateral fenestrations for water and ion flux. So far, this "cytoplasmic cap" was only observed in P2X3 receptors.[48]

More recently, the P2X7 structure was elucidated by X-ray crystallography. The crystal structure of chicken P2X7 (cP2X7) in complex with the antagonist TNP-ATP again revealed a dolphin-like architecture, with the Asp319 to Leu344 residues in the TM2 helices lining the pore. The binding site for TNP-ATP was the same as for the ATP molecule, allowing interaction with some parts of the structure of adjacent subunits.[49,50] In contrast, the crystal structure of the P2X7 receptor from giant panda (gpP2X7) exposed the allosteric site, distinct from the ATP-binding site. This allosteric site was formed between two adjacent subunits and was able to accommodate different small molecules through hydrophobic interactions.[51] Finally, the elucidation of the rat P2X7 (rP2X7) structure by cryo-electron microscopy, uncovered characteristics that distinguishes this receptor from other P2X receptors. rP2X7 contains a cytoplasmic segment with a distinctive folding with guanosine and zinc-binding sites as well as a C-cys linked to the membrane with some palmitoyl groups, which explains the reason why this receptor does not desensitize.[52]

In terms of pharmacology, P2X receptors are physiologically activated by ATP with an $EC_{50}$ between 0.5 and 12 $\mu$M, with the exception of P2X7, which has an $EC_{50}$ value greater than 100 $\mu$M. Other general agonists for P2X receptors include 2-methylthio-adenosine triphosphate (2-MeSATP), adenosine-5′-o-(3-thio-triphosphate) (ATP$\gamma$S), $\alpha,\beta$-methylene adenosine triphosphate ($\alpha,\beta$-MeATP), and 2′(3′)-o-(4-benzoylbenzoyl) adenosine triphosphate (BzATP). Regarding antagonists, Suramin, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS), Reactive Blue-2 (RB-2), and TNP-ATP are considered nonselective antagonists. Selective antagonists have been identified for all P2X subtypes except P2X5 and P2X6, but those molecules may act on other subtypes at higher concentrations. Gefapixant, a P2X3 receptor antagonist, has recently completed phase

**TABLE 2** P2Y-associated main effector systems

| P2Y receptor | G-protein coupled | Main effector systems |
|---|---|---|
| $P2Y_1$ | $G_q$ | PLC, Rac, and Rho activation |
| $P2Y_2$ | $G_q$; Gi; $G_{12/13}$ | PLC, Rac, and Rho activation |
| $P2Y_4$ | $G_q$; $G_i$ | PLC activation |
| $P2Y_6$ | $G_q$; $G_{12/13}$ | PLC and Rho activation |
| $P2Y_{11}$ | $G_q$; $G_s$ | PLC and AC activation |
| $P2Y_{12}$ | $G_i$ | PLC and Rho activation; AC inhibition |
| $P2Y_{13}$ | $G_i$ | PLC and Rho activation; AC inhibition |
| $P2Y_{14}$ | $G_i$ | PLC activation; AC inhibition |

References [66] and [67].

3 in clinical trials, and the P2X7 receptor antagonists AZD9056 and CE-224,535, have also advanced to the stage of clinical trials, aiming at the treatment of rheumatoid arthritis; however, they did not show superior efficacy to the conventional treatment.[53,54] Meanwhile, the P2X7 antagonist JNJ-54175446 is currently being tested for depression treatment.

In addition, knockout (KO) models have already been developed for in vivo studies with P2X receptors (for more updates on the pharmacology of these receptors, see Ref. [55]).

## 2.2 | P2Y receptors

P2Y receptors belong to the GPCR superfamily. Since the initial cloning in the 1990s, eight subtypes of P2Y have been characterized, named $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11-14}$. Missing numbers consist of subtypes incorrectly classified as P2Y receptors or nonmammalian orthologues.[17,56,57] As other GPCRs, these receptors present the following putative structure: seven transmembrane domains known as TM1–7, three intracellular loops, three extracellular loops, an intracellular C-terminal, and an extracellular N-terminal.[35] The agonist binding site is located between TM6-7 domains near the extracellular face of the plasma membrane.[58–61]

Until now, only two P2Y receptors have had their structures elucidated by X-ray crystallography. The first was the $P2Y_{12}$ receptor, whose structure revealed the existence of two possible sub-pockets for ligands, and that the agonist binding promotes conformational changes in helices 6 and 7.[62,63] Similar to $P2Y_{12}$, the crystal structure of $P2Y_1$ receptor also has two distinct ligand binding sites that differ in terms of location and shape, and the nucleotide-binding site is situated in the extracellular loop region.[64,65]

Regarding the associated G protein, P2Y receptors may be coupled to $G_q$ protein ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$), whose activation results in the mobilization of intracellular calcium via phospholipase C (PLC)/inositol triphosphate ($IP_3$), or $G_i$ protein ($P2Y_2$, $P2Y_4$, and $P2Y_{12-14}$), inhibiting the adenylate cyclase (AC) enzyme. The $P2Y_{11}$ receptor can couple to both the $G_q$ protein as well as the $G_s$ protein, which activates AC.[66] Table 2 summarizes the major G proteins associated with P2Y receptors and their main effector systems.

Regarding their pharmacology, human P2Y receptors are activated by different nucleotides, including ADP ($P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$), ATP ($P2Y_2$ and $P2Y_{11}$), UDP ($P2Y_6$ and $P2Y_{14}$), UTP ($P2Y_2$ and $P2Y_4$), UDP-glucose ($P2Y_{14}$), diadenosine tetraphosphate ($P2Y_2$, $P2Y_4$, $P2Y_{12}$ and $P2Y_{13}$), and diuridine tetraphosphate ($P2Y_2$, $P2Y_4$, and $P2Y_6$). P2Y receptors have a number of selective antagonists for each of their subtypes (for more information, see Ref. [66]). The development of selective antagonists allowed the replacement of nonselective antagonists such as Suramin, PPADS, and RB-2 in experimental tests that seek to understand the physiologic roles of these receptors.[68] The discovery of new antagonists has been boosted with the advancement of elucidation of the crystallographic structure of P2Y receptors and the use of chem-informatics techniques by research groups that study these receptors. Finally, it is important to emphasize that $P2Y_{12}$ receptor antagonists have been in the pharmaceutic market for over 20 years, being used to prevent platelet aggregation and acting as antithrombotic agents. These drugs include clopidogrel, prasugrel, ticagrelor, and cangrelor.[66]

# 3 | PHYSIOLOGIC ACTIVATION OF P2 RECEPTORS IN IMMUNE CELLS

## 3.1 | Sources of extracellular nucleotides and nucleosides

A central question in the study of purinergic receptors in the immune system is how endogenously released nucleosides and nucleotides might reach concentrations sufficient to activate these receptors. Indeed, 20 years ago there was some skepticism that ATP and ADO could play a decisive role in immune-cell-specific phenomena, due to the lack of quantitative data about nucleotide and nucleoside species, sources, and concentrations in situations that might be relevant for the development of immune response. However, over the years, evidence has accumulated that these molecules can be selectively released from almost all cells studied and can indeed reach sufficient concentrations in many immunologically relevant circumstances. Another source of skepticism regarding the role of purinergic receptors in the immune system may be attributed to the formerly widespread idea

that the immune system is self-contained and exploits only "specific" molecules generated within itself, such as cytokines and chemokines, as its main soluble intercellular mediators. This scenario has changed as multiple and complex immuno-neuro-endocrine interactions have been the object of intense research over the last 20 years.

Currently, the main sources of extracellular nucleotides and nucleosides are believed to be: (a) corelease of nucleotides with other neurotransmitters by sympathetic and parasympathetic nerves; (b) exocytotic release from intracellular vesicles in non-neural cells such as platelets, astrocytes, and suprarenal cells; (c) release mediated by membrane transporters, such as the ATP-binding cassette protein superfamily; (d) electrochemically driven efflux through anion channels and transmembrane pores, such as connexin hemichannels and pannexin channels; (e) lytic release from injured/damaged cells, as might be the case in trauma, in the site of acute infection, and due to lysis by effector cells. In each of these cases, much attention has been given to the release of ATP. However, UTP, ADP, UDP, ADO, and other nucleotides and nucleosides are also released in many situations. The immune system might be potentially affected by all of these sources, as described below.[69–73]

### 3.1.1 | Sympathetic and parasympathetic nerve release

Several research groups have demonstrated innervation by sympathetic and parasympathetic nerve fibers of primary and secondary lymphoid organs, including the thymus, bone marrow, spleen, lymph nodes, and gut-associated lymphoid tissue (GALT).[74,75] In these organs, the innervation predominantly follows the vasculature, but intense nerve fiber ramification is also seen in lymphoid parenchyma—including the thymic cortex, splenic white pulp, the periarterial lymphatic sheath, lymph node paracortical regions, and T-dependent regions of GALT. In some cases, direct contact between the nerve terminal and immune cells such as lymphocytes and macrophages was evident.[76] In all these sites, a possible release of ATP conjointly with classical neurotransmitters could be expected as demonstrated in other targets of sympathetic innervation.[77,78] Similar to what occurs in other organs, the release of vesicular contents from sympathetic nerve terminals in the thymus is regulated at the presynaptic terminal by P1 receptors, as well as by other mediators.[79]

### 3.1.2 | Exocytotic release

The release of nucleotides stored within intracellular vesicles by exocytosis also occurs in cells of non-neural origin. Those include platelets, mast cells, and suprarenal cells. After an appropriate stimulus, these cells release their vesicular nucleotide contents, modulating neighboring cells in a paracrine fashion.[8] Evidence of nonlytic release of ATP was also reported for lymphocytes, macrophages, and microglial cells.[36,80–82] Some data suggest that ATP can be released by both cytotoxic lymphocytes and target cells during the cytolytic effector function. This phenomenon was first reported in 1973 by Heney, who recognized that the total amount of ATP released during a cytolytic reaction could be larger than what could be contained in the target cell pool, suggesting the release of ATP from both the effector and target cells.[83] Whereas target cells probably release ATP as a consequence of pores formed by perforin, the effector cells are engaged in an active process possibly involving the release of lytic granules.[80,84]

### 3.1.3 | Release mediated by membrane transporters

Nonvesicular release of cytosolic nucleotides has also been proposed, as the concentration of cytoplasmic ATP in healthy eukaryotic cells ranges from 3 to 5 mM. As a strong anion, ATP cannot diffuse through the plasma membrane lipid bilayer; accordingly, membrane transporters have been proposed as mediators of nonvesicular release in healthy cells. Two possible candidates have been described, the P-glycoprotein and the cystic fibrosis transmembrane conductance regulator (CFTR), both belonging to the ATP-binding cassette protein superfamily.[85,86] Although the ATP-transporter nature of CFTR is highly controversial, its possible indirect importance in ATP release could not be excluded.[85,87–92]

### 3.1.4 | Release mediated by anion channels and transmembrane pores

Several pore-forming proteins have a pore diameter larger than the ATP molecule in cross-section (1.14–1.22 nm). These include connexins, pannexins, calcium homeostasis modulating channel 1 (CALHM1), volume-regulated anionic channels (VRACs), and maxi-anion channels (MACs).[93,94]

Connexins are a family of hemichannels that form gap junctions, whose diameter of the narrowest pore region is 1.4 nm. Among all connexin family members, connexin 43 (Cx43) was observed to play essential role in ATP release in leukocytes.[95,96] For example, Cx43 mediates ATP release from macrophages during sepsis.[96]

Another family of hemichannels, pannexins, does not form gap junctions but can allow molecules up to 1 kDa to permeate through them. Pannexins are a pathway of ATP release in various conditions, such as shear stress in leukocytes, immunogenic cell death with chemotherapeutics, and others.[97–100]

CALHM1, a protein belonging to the CALHM family, is the only family member that forms a functional ion channel. This voltage- and extracellular calcium-sensitive and calcium-permeable ion channel regulates intracellular calcium homeostasis. It was also shown to allow the flow of ATP to the extracellular medium and to have a diameter at the narrowest region of its pore of 1.42 nm. Although its role in ATP release in airway epithelial cells, taste bud cells, and bladder has been observed, no role for CALHM1 in ATP release from immune cells has been described yet.[93,94,101–104]

VRACs carry negatively charged organic and inorganic osmolytes to the extracellular medium. ATP, besides flowing through these channels, binds to them directly to trigger their activities. VRACs have a pore size

of 1.2–1.4 nm and are expressed in RAW 264.7 murine macrophages, which were observed to release ATP.[93,94,105]

MACs are high-conductance, ATP-permeable, voltage-dependent channels that are expressed in all cells investigated so far, including T and B cells. These channels regulate cell volume and fluid secretion through the transport of chloride, anions, and ATP through their pores, whose diameter is estimated to range from 1.1 to 1.5 nm. These channels are expressed in cardiomyocytes, for example, where they mediate the release of ATP to the extracellular medium in a physiologically significant manner (at micromolar level). On the other hand, millimolar concentrations of extracellular ATP block these channels, in a kind of negative feedback.[93,94,104,106–108]

Finally, as ATP can induce the opening of large P2X7-associated pores, it is also conceivable that ATP, as well as other nucleotides can leak out of the cells through these channels. Such a mechanism could create a positive-feedback mechanism in which the release of intracellular ATP could trigger the release of even more ATP from the cytosol of the same cell and activate a similar process in neighboring cells. This process may be involved in the propagation of intracellular calcium waves observed in microglia and mast cells.[109] However, this positive feedback mechanism could also lead to the sustained opening of the P2X7 pore and cell death.

#### 3.1.5 | Lytic release

Another important source of extracellular nucleotides comes from injured cells that undergo irreversible cell membrane damage. This may occur in different types of tissue trauma and may represent an important stimulus triggering the activation of P2, and P1, receptors in endothelial cells, platelets, polymorphonuclear cells, and other leukocytes. Extracellular nucleotides constitute a "find me" signal, via activation of purinergic receptors (e.g., $P2Y_2$), to attract phagocytes (monocytes, macrophages, dendritic cells [DCs], and neutrophils) and promote the clearance of necrotic or apoptotic cell bodies.[72,110]

#### 3.1.6 | Other sources

Other nonlytic ATP release mechanisms, independent of P-glycoprotein and CFTR, have been described,[111] including some triggered by cell volume alterations and mechanical stimuli.[112,113] An example is the release of ATP from blood vessel endothelial cells by shear stress. The autocrine action of ATP leads to NO release and consequent smooth muscle relaxation. Another example, described by Lazarowski et al.,[114] is a CFTR-independent, mechanically induced release of cytoplasmic UTP in the human 1321N1 astrocytoma. In both cases, the mechanism of ATP and UTP release is still unknown.

### 3.2 | Physiologic removal of purinergic agonists

Regardless of the primary source of nucleotides and nucleosides, ectoenzymes quickly alter the final composition of the extracellular milieu. Therefore, the final physiologic effect of nucleotide release depends on the interplay between the agonists, these enzymes, and the subtypes of P2 receptors expressed on the surface of the cells. Figure 1 summarizes the main nucleotide release pathways and the action of these molecules on purinergic receptors.

There are at least four families of ectonucleotidases: (a) ectonucleoside triphosphate phosphohydrolase (E-NTPDase), which hydrolyze tri and diphosphate nucleotides such as ATP and ADP into adenosine monophosphate (AMP); (b) ectonucleotide pyrophosphate/phosphodiesterase (E-NPP), which perform hydrolysis of pyrophosphate and phosphodiester bonds, thereby converting ATP or ADP to AMP; (c) alkaline phosphatases, which cleave tri, di, or monophosphate nucleotides into nucleoside and phosphate molecule; and (d) ecto-5′-nucleotidases, which convert monophosphate nucleotides (e.g., AMP) to ADO.[115,116]

In addition, the resulting ADO molecules can be further deaminated into inosine and, subsequently, hypoxanthine, by reactions mediated by adenosine deaminase (ADA) and purine nucleotide phosphorylase (PNP), respectively.[115,116] Table 3 summarizes the main enzymes responsible for the cleavage of extracellular nucleotides and their degradation products.

## 4 | P2 RECEPTORS IN DIFFERENT COMPONENTS OF THE IMMUNE SYSTEM

A specific set of P2 subtypes are expressed in leukocytes, which sometimes act synergistically, inducing the secretion of inflammatory mediators, stimulating chemotaxis, and activating signaling pathways such as the MAPK. In the following sections, we will describe the main roles of P2 receptors in these cells.

### 4.1 | Mononuclear phagocyte system (MPS) cells

#### 4.1.1 | Monocytes

Monocytes are derived from the bone marrow and are present in blood. These cells recognize damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), triggering a series of responses that include phagocytosis, antigen presentation, chemokine secretion, and migration to tissues, where they differentiate into macrophages and DCs.[117]

ATP acts as an immunomodulating agent in monocytes. ATP activation inhibits human leukocyte antigen G (HLA-G) and IL-10 secretion in lipopolysaccharide (LPS)-activated human monocytes in vitro.[118] On the other hand, in cells stimulATP stimulation after LPS treatment induces IL-1$\beta$ release from monocytes and potentiates cytokine release from cells stimulated with different PAMPs and DAMPs. Additionally, several PAMPs and DAMPs stimulate ATP release with autocrine effects.[119] UDP, another nucleotide, stimulates macrophage inflammatory protein-3$\alpha$ (MIP-3$\alpha$) release from human monocytes from peripheral blood.[120]

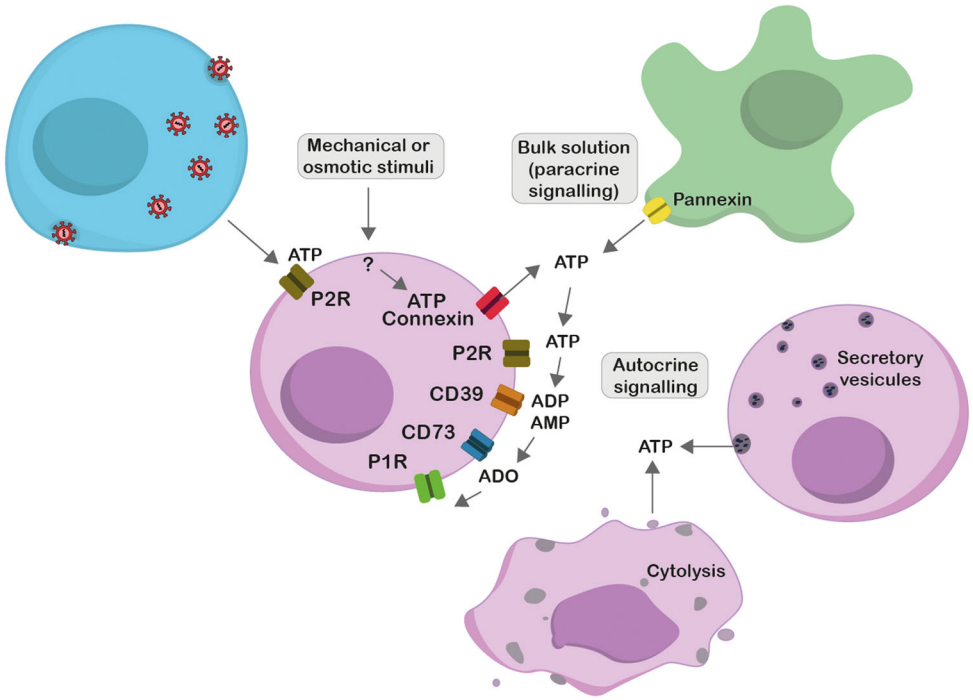


**FIGURE 1** ATP signaling in the immune system cells. ATP is stored in cells within intracellular vesicles, and thus may be released via exocytosis, through membrane pores such as connexin and pannexin-1, and by dying cells. The endogenously released ATP provides autocrine and paracrine signaling, acting upon nearby P2 receptors (P2R). ATP is also degraded by soluble and membrane-associated ectonucleotidases such as ecto-apyrases (CD39) and ecto-5′-nucleotidases (CD73), originating ADP/AMP and ADO, respectively. ADO, in turn, acts upon P1 receptors (P1R)

**TABLE 3** Main ectonucleotidases and their products

| Enzyme | The final product(s) |
|---|---|
| E-NTPDase | ATP → ADP + Pi |
| | ADP → AMP + Pi |
| E-NPP | ATP → AMP + PPi |
| Alkaline phosphatase | TP → ADP + Pi |
| | ADP → AMP + Pi |
| | AMP → ADO + Pi |
| Ecto-5′-nucleotidase | AMP → ADO + Pi |

References [115] and [116].

P2 receptors expressed in human monocytes are involved in intracellular calcium mobilization,[121,122] and P2X7 activation induces the formation of a membrane pore permeable to large solutes. However, in contrast to what is observed in macrophages, this pore only allows dye uptake when the extracellular medium contains low sodium and chloride levels.[123]

P2X7 involvement has been demonstrated in several immunologic responses mediated by monocytes. P2X7 activation induces surface CD86 expression in human monocytes, whereas P2X7 blockade diminishes its expression.[124] In patients with sepsis, an increase in P2X7 expression in monocytes and its activation could be implicated in mitochondrial depolarization.[125] Patients with Behçet's disease (BD), an immune-inflammatory syndrome, present higher levels of P2X7 on the surface of monocytes, despite no difference in P2X7 mRNA expression, compared with controls. Moreover, in monocytes from BD patients, ATP triggers augmented calcium influx, pore formation, and IL-1$\beta$ release.[126] In contrast, monocytes in primary Sjögren's syndrome patients, also express significantly higher levels of P2X7 than those from control individuals, but, in this case, higher levels of P2X7 mRNA are also observed.[127]

A summary of the P2 receptors expressed in monocytes and their functions is shown in Table 4.

### 4.1.2 | Macrophages

Macrophages are phagocytic cells that are present in tissues and body cavities even in the absence of inflammation, where they contribute to surveillance and protection against infection. Their functions include phagocytosis of pathogens, toxins, and dead cells; cytokine and chemokine secretion; activation and recruitment of leukocytes to injured tissue; antigen presentation to T cells; and oxygen and nitrogen reactive species formation.[117]

Most of the information about the effects of ATP in immune system cells was obtained in macrophages. In 1985, Sung et al.[138] demonstrated that exogenous ATP interferes with transmembrane ion flux and inhibits phagocytosis in mouse macrophages. Afterward,

**TABLE 4** P2 receptors in monocytes

| Cell source | P2 receptor | Effects | References |
|---|---|---|---|
| **Human monocytes** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, P2X1, P2X4, and P2X7 | Calcium mobilization, ↑CD86, and cyclooxygenase-2 (COX-2) expression, CCL20, HLA-G, IL-1$\beta$, and IL-10 release, MAPK and NF-$\kappa$B activation, and pore formation | 118,120–122,124,128–131 |
| **THP-1 cell line*** | P2X1, P2X2, P2X4, P2X5, P2X6, and P2X7a | Calcium mobilization, chemotaxis, dye uptake, IL-1$\beta$, and IL-8 release, and ROS formation | 119,132–135 |
| **U937 cell line** | Some P2X and P2Y receptors | Calcium mobilization, ERK1/2 phosphorylation, and IL-8 release | 136,137 |

[a]Only the expression of P2X receptors was investigated.

several research groups showed that ATP induces plasma membrane permeabilization to fluorescent dyes up to 900 Da as well as a non-selective plasma membrane conductance in macrophages, which can be observed through the patch-clamp technique in whole-cell configuration.[139–142] Both these effects were concentration and temperature dependent and were modulated by $Mg^{2+}$ and blocked by P2X7 antagonists.[143–145] Due to its role in ATP-triggered permeabilization, P2X7 is the most studied purinergic receptor in macrophages. This receptor was first named P2Z, but in the 1990s, it was named P2X7 according to cloning and homology data.[29] A number of effects of ATP on macrophages have been attributed to P2X7 activation, either in studies using specific antagonists or KO mice. Despite the importance of P2X7 KO mice in discovering new functions associated with this receptor, some of these models display incomplete inactivation of the P2X7 gene. As a result, the Glaxo $P2X7^{-/-}$ mouse lines display a functional P2X7 receptor in T cells; and the Pfizer $P2X7^{-/-}$ mice express a P2X7-like protein in the brain (reviewed in[146]).

ATP and BzATP activate caspase-1 and MEK1/2, and promote IFN-$\beta$ expression in macrophages. ATP-activated macrophages secrete several cytokines, including TNF-$\alpha$, keratinocyte chemoattractant (KC), IL-1$\beta$, and macrophage inflammatory protein 2 (MIP-2), as well as eicosanoids, CD14, and cathepsin.[147–154] ATP also promotes reactive oxygen species (ROS) production, cell migration, and the shedding of microvesicles containing IL-18.[150,152,155] All of those effects are significantly inhibited by P2X7 blockage or KO.[147–150,152,156,157]

ATP exposure can induce lytic cell death in human macrophages, and this effect is enhanced by IFN-$\gamma$ treatment. On the other hand, ATP-mediated cytotoxicity is reverted by granulocyte-macrophage colony-stimulating factor (GM-CSF) and P2X7 antagonists.[80,158,159]

Murine peritoneal macrophages also form multinucleated cells upon GM-CSF cytokine stimulation. This effect can be inhibited by the P2X7 antagonists such as oxidized ATP (oATP) and A740003, by P2X7 KO, or by pannexin-1 blockage, suggesting the participation of P2X7 and pannexin-1 in cell fusion.[160]

P2X4 is another receptor that plays important role in macrophages. P2X4 activation was connected to macrophage recruitment to the peritoneal cavity and inflammatory response, since it induces cytosolic phospholipase $A_2$ ($PLA_2$) activation and prostaglandin $E_2$ ($PGE_2$) release.[161,162] P2X4 seems to act together with P2X7 in regulating macrophages, through facilitating cytokine release and suppressing autophagy during inflammation.[163,164] Recently, it was demonstrated that both apyrase and P2X4/P2X7 antagonists significantly diminish macrophage phagocytic activity, in an extracellular calcium-dependent manner, suggesting their role in paracrine and autocrine stimulation of macrophage phagocytosis.[165]

Macrophages also express P2Y receptors, which mediate LPS-induced expression of iNOS and cytokine release such as IL-6, MIP-2, and TNF-$\alpha$ in murine macrophages.[166–169] P2Y receptors also augment phagocytosis and endocytosis, chemotaxis, and induce $PGE_2$ and leukotriene $C_4$ ($LTC_4$) synthesis.[170–173] In a peritonitis murine model evoked by *Escherichia coli*, $P2Y_6$ activation resulted in monocyte chemoattractant protein-1 (MCP-1)-induced chemotaxis of both monocytes and macrophages, and in clearance of bacteria.[173]

A summary of the P2 receptors expressed in various types of macrophages and their functions is shown in Table 5. Osteoclasts, a resident bone cell involved in bone resorption, are also from myeloid origin and can be derived from macrophages; therefore, they are also included in this summary.[174]

### 4.1.3 | Microglia

Microglia represent the main immune effector cell population of the CNS.[201] Microglia are known to release various cytokines, NO, and ROS.[202]

ATP induces different effects on microglial cells, including ion channel opening, intracellular calcium mobilization, apoptosis, phagocytosis, NF-$\kappa$B activation, ATP release, cytokine release, and chemokine secretion (e.g., CXCL2).[203–212] Many of these effects have been attributed to P2X7 activation.[213]

P2X7 activation triggers CXCL2 production, NFAT and MAPK pathway activation, cytokine release, and ROS production.[214–216] LPS augments P2X7 expression in microglia and contributes to its activation in rats.[217,218]

P2X7 was overexpressed in microglia of Alzheimer's disease (AD) patients. Amyloid $\beta$ $(A\beta)_{1-42}$ peptide stimulates P2X7 expression in fetal human microglia, suggesting an important role for this receptor in the development of AD.[219] P2X7 silencing enhanced $A\beta_{1-42}$ accumulation at extracellular medium, by diminishing microglia phagocytosis in vitro, suggesting a protective role of P2X7 in AD. P2X7 blockage

**TABLE 5** P2 receptors in macrophages

| Cell source | P2 receptor | Effects | References |
|---|---|---|---|
| **Human macrophages** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, P2X1, P2X4, P2X5, and P2X7 | Calcium mobilization, cell death, cathepsin release, chemokine and cytokine (CCL2, CCL4, CXCL5, IFN-$\gamma$, IL-1$\beta$, IL-6, IL-23, TNF-$\alpha$) release, microvesicle shedding, mycobacterium killing, and ↓ leishmanial infection | 151,153–155,158,175–179 |
| **Human alveolar macrophages** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{14}$, P2X1, P2X4, P2X5, and P2X7 | Calcium mobilization, induction of membrane currents and cytokine (IL-1$\beta$, IL-6, and TNF-$\alpha$) secretion | 170,180 |
| **Human monocyte-derived macrophages** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{13}$, P2X1, P2X4, P2X5, and P2X7 | Calcium mobilization and CXCL5 release | 154 |
| **Human monocyte-derived osteoclasts** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, P2X1, P2X4, P2X5, P2X6, and P2X7 | Multinucleated cells formation and ↑osteoclast resorption | 181,182 |
| **Murine peritoneal macrophages** | $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{12}$, $P2Y_{14}$, P2X1, P2X4, and P2X7 | Calcium mobilization, cell death, chemotaxis, cytokine (IL-1$\alpha$, IL-1$\beta$, IL-6, KC, TNF-$\alpha$, MIP-2) release, induction of membrane currents, membrane permeabilization, microbicide actions, multinucleated cells formation, oleic acid (OA) and arachidonic acid (AA) release, phagocytosis and endocytosis, $PGE_2$ and $LTC_4$ synthesis, ROS production, and ↓ leishmanial infection | 150,152,156,157,160, 161,172,173,180,183–186 |
| **Murine bone marrow derived-macrophages (BMDMs)** | $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{12}$, $P2Y_{14}$, P2X1, P2X2, P2X4, and P2X7 | Caspase-1 and MEK1/2 activation, cathepsin release, IFN-$\beta$ expression, inflammatory mediators (IL-1$\beta$, leukotriene $B_4$ ($LTB_4$), $PGE_2$, and thromboxane $B_2$ ($TXB_2$)) release, phagocytosis, and ↑ TNF-$\alpha$ converting enzyme (TACE) activity | 147–151,165,187 |
| **Mouse alveolar macrophages*** | P2X1, P2X3, P2X4, P2X5, and P2X7[a] | Induction of membrane currents | 188 |
| **Murine osteoclasts** | $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, P2X1, P2X2, P2X3, P2X4, P2X5, and P2X7 | ↑ area resorbed per osteoclast and ↑ osteoclast number | 189 |
| **THP-1 derived macrophages cell line** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, P2X1, P2X2, P2X4, P2X5, P2X6, and P2X7 | IL-1$\beta$ release | 190,191 |
| **RAW 264.7 cell line** | $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{12}$, $P2Y_{14}$, P2X2, P2X4, and P2X7 | Actin reorganization, cell death, chemotaxis, expression of proinflammatory mediators (COX-2, GM-CSF, high mobility group box 1 (HMGB1), IFN-$\beta$, IL-1$\alpha$, IL-1$\beta$, iNOS, MCP-1, NO, $PGE_2$, and TNF-$\alpha$), NF-$\kappa$B, MAPK, caspase-3, and Ras activation, NO and ROS production, membrane blebbing, and membrane permeabilization | 149,165,173,192–196 |
| **J774 cell line** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, P2X1, P2X2, and P2X7 | Calcium mobilization, induction of membrane currents, IL-1$\beta$ release, membrane permeabilization, and ↓ bacilli Calmette-Guerin (BCG) viability | 139–142,195,197,198 |
| **KUP5 cell line** | $P2Y_2$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, P2X4, and P2X7 | IL-1$\beta$ and IL-6 release | 199,200 |

[a]Only the expression of P2X receptors was investigated.

or KO also prevents IL-1$\beta$ and TNF-$\alpha$ released by microglia.[220,221] A$\beta_{1-42}$ aggregates stimulate murine primary microglia to release ATP and augment $P2Y_2$ expression in these cells. Both ATP and UTP induce cell migration and A$\beta_{1-42}$ uptake and degradation, which were not observed in $P2Y_2^{-/-}$ cells, suggesting an important role for this receptor in the modulation of AD.[222]

Uridine nucleotides induce important physiologic effects in microglia. UDP stimulation promotes actin polymerization in primary rat microglia and vasodilator-stimulated phosphoprotein (VASP) phosphorylation, suggesting a possible role of VASP in UDP-induced actin aggregation.[223] $P2Y_6$ activation induces MCP-1 and macrophage inflammatory protein-1$\alpha$ (MIP-1$\alpha$) secretion by rat primary microglia in a calcium-dependent manner.[224] UTP is also involved in microglia phagocytosis, CCL2 expression and ERK1/2 phosphorylation in rat spinal microglia.[207,225] $P2Y_2$ and $P2Y_4$ receptors also mediate particle pinocytosis in the presence of ATP and UTP.[226]

Other P2Y receptors exert important functions in microglia. $P2Y_{13}$ activation causes intracellular calcium mobilization in dorsal spinal cord microglia in a concentration-dependent manner.[227] $P2Y_{12}$ and $P2Y_{13}$ augment IL-1$\beta$, IL-6, and TNF-$\alpha$ release in microglia from the rat dorsal spinal cord.[228] ADP and ATP stimulate process extension and adhesion in microglial cells from rats.[229,230] Loss of $P2Y_{12}$ receptor diminishes the number of microglial projections and could be responsible for the conversion from ramified to amoeboid cell state. ADP also stimulates membrane ruffling and chemotaxis of rat and murine microglia. However, $P2Y_{12}$ expression in LPS-activated microglia in vivo was not observed.[231–234] Microglial $P2Y_{12}$ KO prevented neuronal cell death and reduced cell migration and NF-$\kappa$B expression in oxygen-glucose deprivation (OGD), an in vitro model of ischemia.[235]

A summary of the P2 receptors expressed in microglia and their functions are shown in Table 6.

In summary, the activation of P2 receptors in MPS cells results in proinflammatory responses. Both P2X and P2Y receptors induce the release of cytokines, chemokines, and the formation of inflammatory mediators, but many of these effects are attributed to P2X7 activation. Its activation further results in the formation of multinucleated giant cells (MGCs) by macrophages. On the other hand, migration and chemotaxis activities, as well as phagocytosis, endocytosis, and pinocytosis, seem to be more related to the activation of P2Y receptors, although P2X receptors can also mediate these effects.

## 4.2 | Dendritic cells

DCs act as sentinels of the immune system, and they are the most important APCs of the body, being responsible for the initiation of adaptive immune responses, inducing the activation of Th1, Th2, or Th17 profiles.[251]

P2 receptors are implicated in the maturation of DCs through ATP stimulation.[252,253] Human immature and mature DCs respond to ATP, UTP, and ADP through calcium mobilization. However, mature DCs seem to be less sensitive to UTP and ADP. All of these nucleotides induce actin polymerization and chemotaxis in immature DCs, but fail to do so in mature cells, suggesting that these nucleotides have chemotactic activity on immature cells, attracting them to inflammatory sites.[254,255]

Nucleotides increase the production of cytokines such as IL-6, IL-10, and IL-12.[256,257] Moreover, P2 activation increases antigen presentation by DCs.[258] Taken together, these data suggest that ATP analogs might be useful in the treatment of some infectious diseases. Supporting this idea, it was demonstrated that an ATP analog protects mice infected with gram-negative bacteria.[259]

The P2X7 receptor plays several roles in DCs, including intracellular calcium mobilization, endocytosis and apoptosis.[260] This receptor is also involved in antigen presentation and IL-1$\beta$ release.[258,261] In addition, P2X7 displays a crucial role in DC-mediated cancer control. Dying tumor cells release ATP, which acts on the P2X7 receptor in DCs and triggers the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome, allowing the secretion of IL-1$\beta$ cytokine, which is important to control cancer growth and spread.[262]

Other P2 receptors are expressed in DCs and may be associated with different functions. P2Y receptors induce intracellular calcium mobilization and modulate cytokine expression and secretion.[256,263,264] P2Y receptors could be implicated in human DC migration in peripheral blood, as well as in pinocytosis.[265–267] P2Y activation also favors Th2 and Th17 responses.[253,268,269]

A summary of the P2 receptors expressed in DCs and their functions are shown in Table 7.

In summary, the role of P2 receptors on DCs is associated with chemotaxis to inflamed tissues for the capture of antigens and their presentation to lymphocytes, initiating adaptive immune responses. Furthermore, high concentrations of ATP seem to decrease the release of proinflammatory cytokines and activation of P2Y receptors seems to modulate the Th2 or Th17 response profile.

## 4.3 | Lymphocytes

### 4.3.1 | T cells

T cells are a subpopulation of lymphocytes whose precursors originate in the bone marrow and differentiate in the thymus. These cells are responsible for antigen recognition after its presentation through sentinel cells via the HLA. Once activated (with the help of costimulatory signals), T cells stimulate IL-2 secretion, clonal expansion, and can differentiate into effector and memory cells. According to their subset, they have different roles in immunity, such as cytokine production, aiding B cells in antibody production ($CD4^+$ cells), and destroying malignant or virus-infected cells ($CD8^+$ cells).[276]

Nucleotides have important effects on T cells. It has long been known that ATP may induce the blastogenesis of murine medullar thymocytes.[277] ATP also induces intracellular calcium mobilization, sustained depolarization of the plasma membrane, and membrane permeabilization to low molecular weight molecules (~300 Da). In contrast, cytotoxic T cells are resistant to the permeabilizing effects of ATP.[278–280] These findings opened the possibility that ATP could be an

**TABLE 6** P2 receptors in microglia

| Cell source | P2 receptor | Effects | References |
|---|---|---|---|
| **Human** | $P2Y_1$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, P2X4, and P2X7 | Calcium mobilization and COX-2, IL-6, IL-1$\beta$, IL-12, MCP-1, and TNF-$\alpha$ expression | 236 |
| ***Rhesus* macaque** | $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, P2X1, P2X4, P2X5, and P2X7 | Not determined | 237 |
| **Rat** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$[a] | Actin polymerization, adhesion, CCL2, CCL3, IL-1$\beta$, IL-6 and TNF-$\alpha$ expression and release, chemotaxis, ERK1/2 and VASP phosphorylation, $H_2O_2$ and nitrite production, membrane ruffling, ↑ or ↓phagocytosis, and process extension | 207,215,216,223,225,228,230–234,238–240 |
| **Mouse** | $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, P2X1, P2X4, and P2X7 | 2-Arachidonoylglycerol (2-AG) production, ATP, IL-1$\beta$ and TNF-$\alpha$ release, chemotaxis, membrane ruffling, and phagocytosis | 222,231–234,236,241–243 |
| **BV-2 cell line** | $P2Y_2$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14a}$ | Chemotaxis and CXCL2 production | 214,234,244 |
| **C8B4 cell line** | $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$[a] | Calcium mobilization | 245 |
| **N9 cell line** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, P2X1, P2X2, P2X3, P2X4, P2X6 and P2X7 | Not determined | 246–248 |
| **GL261 glioma-derived CD11b$^+$ cells** | $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, P2X1, P2X3, P2X4, P2X5, P2X6, and P2X7 | ↓ cell proliferation and ↓ IL-6 release | 246 |
| **N13 cell line** | P2X7[a] | ATP and IL-1$\beta$ release, cell death, cell swelling, and dye uptake | 249 |
| **MG-5 cell line** | P2X7[b] | CCL3 release | 238 |
| **EOC13 cell line** | P2X7[c] | Cell death, pore formation, and ROS and NO production | 250 |

[a]Only the expression of P2Y receptors was investigated. [b]Receptor expression was evaluated by functional assays. [c]Only the expression of P2X7 receptor was evaluated.

additional cytotoxic molecule used by T cells in lytic events,[82,84,281] but this was refuted by others.[282]

ATP may induce differentiation, growth, or death of CD4$^+$ T cells. It has been shown that whereas ATP concentrations below 50 nM in the extracellular milieu does not alter CD4$^+$ T cells activity, 250 nM can trigger proliferation, and 1 mM can induce cell death (via P2X4 and P2X7 receptors). However, exposure to 1 mM of ATP can also increase proliferation and immunoregulatory effects of T regulatory cells.[283] ATP may also control the migration of T cells within lymph nodes in homeostatic situations.[284]

Some ATP-mediated effects on T cells have been attributed to the expression of P2X7 receptor.[285] P2X7 receptor was initially characterized in T cells by pharmacologic and physiologic means, as a P2X-activated channel was implicated in the mitogenic stimulation and cell death of human T cells.[286–290] Later, it was demonstrated that CD4/CD8-defined single positive thymocytes express more P2X7 receptor than double negative or double-positive cells.[290,291] FasL, an apoptosis inductor, stimulates ATP release via pannexin-1 hemichannels, and can induce P2X7-mediated apoptosis.[292] Pannexin hemichannels also contribute to the activation of P2 receptors in T cells. Both murine CD4$^+$ and CD8$^+$ T cells express pannexins 1 and 2 in the plasma membrane, and after TCR activation, ATP is released from T cell via pannexin channels and may activate this cell autocrinelly.[293,294]

Besides cell death, P2X7 has also been implicated in CD62L and CD27 shedding, lymphocyte proliferation, caspase-1 activation, and IL-1$\beta$ release.[295–297] P2X7 stimulation regulates T cell activation, initiating effector immunity.[296] Also, the participation of P2X7 and $P2Y_6$ receptors in T cell activation via TCR has been observed.[298] Finally, studies with P2X7 KO mice have revealed that P2X7 activation contributes to $\gamma\delta$ T cell lineage commitment and the development of peripheral $\gamma\delta$ T cells.[299]

P2X7 activation also seems to inhibit Treg function. P2X7$^{-/-}$ Tregs display lower levels of released ATP and phosphorylated ERK than

**TABLE 7** P2 receptors in dendritic cells

| Cell source | P2 receptor | Effects | References |
|---|---|---|---|
| **Human from peripheral blood** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, P2X1, P2X4, and P2X7 | Actin polymerization, chemotaxis, CCL20 release, ↓IL-12, ↓ IL-23, ↑IL-1$\beta$, ↑ IL-27, intracellular calcium mobilization, and NLRP3 inflammasome activation | 120,254,260–262,265,270,271 |
| **Human monocyte-derived dendritic cells** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{14}$, P2X1, P2X4, P2X5, and P2X7 | Intracellular calcium mobilization, dye uptake and CD23 shedding | 264,272 |
| **Human plasmacytoid dendritic cells*** | $P2Y_4$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$[a] | Intracellular calcium mobilization and IFN-$\alpha$ release | 263 |
| **Human Langerhans cells*** | P2X7[b] | Resistance to lytic effects of ATP and dye uptake | 273 |
| **Murine phagocytic cells of the thymic reticulum*** | P2X7[b] | Intracellular calcium mobilization | 141,274 |
| **Murine bone marrow dendritic cells*** | P2X7[b] | ↑IL-1$\beta$, ↑IL-23, NLRP3 inflammasome activation, silica nanoparticles uptake, and ROS production | 269,275 |
| **Murine D2SC/1 cell line*** | P2X7[c] | Cell permeabilization and cell death | 258,260 |

[a]Only the expression of P2Y receptors was evaluated. [b]Receptor expression was evaluated by functional assays. [c]Only the expression of P2X7 receptor was evaluated.

controls.[300] P2X7 activation in murine macrophages diminishes the expression of MHC I, and ATP treatment diminishes antigen presentation, reducing $CD8^+$ T cell activation.[301]

P2X4 is involved in Th17 activation and its inhibition impacts IL-17 release. Since Th17 is involved in the development of autoimmune arthritis, P2X4 blockade might alleviate the severity of this disease.[302]

$P2Y_6$ expression was described in T cells in inflammatory bowel disease (IBD).[303] In an experimental model of colitis, $P2Y_6^{-/-}$ animals showed an increase of cytokines involved in Th17 differentiation, such as IL-1$\beta$, IL-6, TGF-$\beta$1, IL-17, and IL-23. An increase of Th17 cells in the gut was also observed, suggesting that $P2Y_6$ has a protective role in intestinal inflammation.[304]

Besides P2X7 and $P2Y_6$, the role of other P2 receptors on T cells has also been demonstrated. ADP induces intracellular calcium mobilization in T cells, while UDP-glucose slightly inhibits T cell proliferation.[305]

A summary of the P2 receptors expressed in T cells and their functions is shown in Table 8.

### 4.3.2 | B cells

B cells are a subgroup of lymphocytes developed in bone marrow, which are responsible for antibody production. These cells can recognize different antigens through the B cell receptor. Once maturated, these cells differentiate into several subsets, including follicular, marginal zone, and B-1 B cells.[276,313]

Extracellular ATP exerts many effects on B cells. In human B cells isolated from patients with chronic lymphocytic leukemia (CLL), ATP induces cation permeability, intracellular calcium mobilization, and membrane pore opening.[314–317] Human B cells express functional P2X7 receptors, although, in B cells, this receptor promotes less dye uptake than in monocytes and macrophages, and the membrane pore present a lower molecular weight cut-off (~300 Da).[280] This permeability response is further reduced in some B-CLL patients, suggesting that this receptor could be nonfunctional because of mutations.[318,319]

B cells may modulate the expression of P2 receptors depending on the site or the situation (naive or activated). P2X7 activation in human leukemic lymphocytes has been associated with stimulation of phospholipase D (PLD) activity and modulation of L-selectin and the low-affinity IgE receptor (named CD23) by interfering with different proteases, suggesting some importance of this receptor in cellular adhesive processes.[320–322] P2X7 receptor is also important to IgM release, lymphocyte proliferation, cell migration and shedding of CD21, CD23, and CD62L.[323–326]

A summary of the P2 receptors expressed in B cells and their functions is shown in Table 9.

In summary, ATP activation of T cells promotes activation, proliferation, and differentiation of $CD4^+$ T cells with the participation of the P2X7 receptor. The activation of P2Y receptors can inhibit the proliferation of these cells and induce differentiation to the Th17 subtype. Meanwhile, in B cells, activation of P2 receptors is associated with adhesion, migration and IgM secretion.

## 4.4 | NK cells

NK cells are cytotoxic lymphocytes that do not carry classical markers for B or T lymphocytes. They are pivotal cells of the innate immunity, acting in the initial immune response, especially in virus infections, exerting direct cytotoxicity against infected cells, and producing cytokines to activate other cells. NK cells also appear to be involved in tumor surveillance, since NK deficiency can lead to increased

**TABLE 8** P2 receptors in T cells

| Cell source | P2 receptor | Effects | References |
|---|---|---|---|
| **Human from peripheral blood** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, P2X1, P2X4, and P2X7 | Caspase-1 activation, cell motility reduction, differentiation, IL-1$\beta$ release, and proliferation | 297,303,306–308 |
| **Human $CD4^+$ T cells** | $P2Y_2$, P2X1, P2X4, P2X5, P2X6, and P2X7 | Cell death, differentiation, and growth | 283,309 |
| **Jurkat cell line*** | P2X1, P2X4, and P2X7 | Cell death | 292,309,310 |
| **Murine T cells** | $P2Y_6$, $P2Y_{14}$, and P2X7 | CD27 and CD62L shedding, CD25 expression, cell death, IL-2 production, and inhibition of cell proliferation | 287,295,305 |
| **Murine thymocytes** | $P2Y_1$, $P2Y_2$, P2X1, P2X2, P2X5, P2X6, and P2X7 | Apoptosis, CD27 shedding, differentiation and T cell activation | 287,288,290,296,298,308,311 |
| **Murine splenic $CD4^+$ and $CD8^+$ cells** | P2X7[b] | Cell death and differentiation | 291 |
| **Rat thymocytes** | $P2Y_2$, P2X1, and P2X4 | Not determined | 312 |

[a]Only the expression of P2X receptors was evaluated. [b]Only the expression of P2X7 receptor was evaluated.

**TABLE 9** P2 receptors in B cells

| Cell source | P2 receptor | Effects | References |
|---|---|---|---|
| **Human B cell** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, P2X1, P2X2, P2X3, P2X4, P2X5, P2X6, and P2X7 | CD21, CD23, and CD62L shedding, and IgM release | 324–328 |
| **Epstein-Barr immortalized B cells** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, P2X1, P2X2, P2X4, P2X5, P2X6, and P2X7 | Not determined | 328 |
| **Human leukemic B lymphocytes*** | P2X7[a] | Bromide ethidium uptake, phospholipase D activation and modulation of L-selectin and CD23 | 314–316,318,320–322,329 |
| **Mouse and rat bone marrow B cells*** | P2X7[a] | Not determined | 37 |
| **Murine splenic B cells*** | P2X7[a] | CD23 shedding | 325 |

[a] Only the expression of P2X7 receptor was evaluated.

susceptibility to neoplasia. They are also able to kill different tumor cells in vitro.[330]

Besides a possible role as a cytotoxin secreted by NK cells, ATP seems to be involved in the modulation of NK cell responses. The first evidence of the presence of purinergic receptors in NK cells was reported in 1983 by Henriksson, who showed the inhibition of natural killing activity by ADO ribonucleotides; these findings were later reproduced and confirmed.[331–333] It was postulated that this ATP-related activity was due to post recognition signaling events mediated by P2 receptors.[332,334]

ATP also inhibits the proliferation of NK cells, probably acting through a lineage-specific receptor.[333] The fact that UTP failed to affect NK cells, associated with a lack of inhibition of the effects of adenine nucleotides by pertussis toxin, may indicate the involvement of a P2X receptor, but this hypothesis lacks experimental confirmation.[333] The possible influence of phosphorylation reactions taking place on NK cell surfaces in these phenomena should not be discarded.[335]

Surprisingly, ATP inhibited NK cell migration in response to CX3CL1 and abolished the CX3CL1-dependent NK killing of endothelial cells. These effects were attributed to $P2Y_{11}$ according to pharmacologic blockers used to elucidate the receptor activation.[336] Recently, it was demonstrated that $P2Y_6$ receptor expression diminishes NK cell maturation and activation. On the other hand, $P2Y_6$ deficiency increases the NK cytotoxicity and antimetastatic activities, suggesting a new niche for cancer therapy.[337]

Taken together, the findings suggest that, in NK cells, the activation of P2 receptors generates inhibitory responses on the proliferation and chemotaxis of these cells, in contrast to what was observed in other cells of the immune system.

A summary of the P2 receptors expressed in NK cells and their functions is shown in Table 10.

**TABLE 10** P2 receptors in NK cells

| Cell source | P2 receptors | Effects | References |
|---|---|---|---|
| **Human blood** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, P2X1, P2X4, P2X5, P2X6, and P2X7 | ↓ cell proliferation, ↓ chemotaxis, and ↓ killing activity | 332–334,336 |

## 4.5 | Granulocytes

### 4.5.1 | Neutrophils

Neutrophils, also called polymorphonuclear neutrophils (PMN), are myeloid cells with multilobulated nuclei. They normally represent 70% of the leukocyte population, being the most abundant granulocytes. Neutrophils are the first cells to arrive at an inflammatory site to deal with tissue damage and microorganisms. Marked neutropenia can lead to overwhelming infection.[338]

Important neutrophil functions are migration from the blood to the injured tissue, the release of granule contents, and oxidative burst, which results in the production of free radicals.[339] Over the last decades, a growing body of evidence indicates the action of purine nucleotides on these functions and in neutrophils' intracellular signaling pathways.

Several studies were published in the 1980s demonstrated the effect of extracellular ATP on neutrophils. In these works, the authors showed that extracellular ATP and its analogs were able to increase both intracellular calcium and the respiratory burst after stimulation with formylated chemotactic peptides.[21,340–343]

$P2Y_2$ is predominantly expressed in neutrophils. The activation of this receptor is involved in elastase release, cell degranulation, ROS production, MAPK phosphorylation, and intracellular calcium mobilization.[344,345] $P2Y_2$ is also associated with neutrophil migration toward the IL-8 gradient.[346–348] Epithelial cells incubated with peptides from human neutrophils or with nucleotides (ATP, ADP, UTP, and UDP) secreted the chemokine IL-8 in a concentration-dependent manner, suggesting the participation of P2Y receptors in this process.[349] $P2Y_4$ and $P2Y_6$ could also contribute to UTP and UDP-induced neutrophil migration in vivo.[350,351] In an experimental model of colitis in mice, intrathecal administration of UDP recruited neutrophils to the bowel, aggravating the symptoms of inflammation such as stool consistency, blood in stool, and rectal bleeding.[351]

Furthermore, $P2Y_6$ activation is associated with neutrophil extracellular traps (NETs) formation, IL-8 release, migration, phagocytosis, superoxide production, and calcium mobilization.[352]

$P2Y_1$ and $P2Y_{14}$ receptors are involved to chemotaxis in vitro and neutrophil migration in vivo.[353–356] This latter receptor also mediates elastase release.[357] Some recent evidence suggests that $P2Y_{12}$ antagonists used in thrombosis prevention, such as clopidogrel, prasugrel, and ticagrelor, could modulate neutrophil functions in vitro and in vivo. However, this role remains uncharacterized.[358–360]

P2X1 is another receptor involved in the chemotaxis of neutrophils. Genetic deletion of P2X1 slightly reduced neutrophil migration in vitro, but significantly reduced the migration of these cells to the peritoneum in a peritonitis experimental model.[361] In addition, P2X1 plays a protective role in endotoxemia, as $P2X1^{-/-}$ animals survived less than wildtype (WT) animals, and produced higher levels of ROS and neutrophil infiltration.[362] $P2X1^{-/-}$ animals also present lower neutrophil and fibrin accumulation at the injury site.[363]

P2X7 activation in neutrophils causes cell depolarization, membrane pore opening, intracellular calcium mobilization, and secretion of IL-1$\beta$ in an NLRP3 inflammasome-dependent manner.[364,365] The P2X7 receptor is up-regulated in both blood and bronchoalveolar lavage fluid (BALF) neutrophils after exposure to cigarette smoke. Mice exposed to cigarette smoke have increased neutrophil counts and higher cytokine levels on BALF neutrophils compared with control animals, which may indicate a role of purinergic signaling in neutrophils during lung inflammation.[366] In contrast, Martel-Gallegos et al.[367] demonstrated through different techniques (patch-clamp, dye uptake, ROS production, RT-PCR, Western blotting, and immunofluorescence) that human neutrophils do not express P2X7 receptor. This fact may suggest that neutrophils respond to ATP via another P2 receptor.[367] In this sense, ATP could activate purinergic P2 receptors in other cell types, such as macrophages or fibroblasts, which could release chemokines to recruit neutrophils to the injury site.[152,368]

A summary of the P2 receptors expressed in neutrophils and their functions is shown in Table 11.

### 4.5.2 | Mast cells

Mast cells can be found in almost all vascularized tissues of the body, especially in mucosa and epithelia. They are characterized by the presence, in their cytoplasm, of many metachromatic granules rich in chemical mediators, including histamine and heparin. Upon activation, mast cells not only release granule contents, but also begin the synthesis of other chemical mediators, such as arachidonic acid (AA) metabolites and cytokines.[371] Another characteristic of mast cells is the presence of a high-affinity receptor for IgE on their surface. Mast cells are involved in inflammation, anaphylactic reactions, and in some parasite infections.[372]

Mast cells were one of the first cells of the immune system to have the presence of purinergic receptors characterized, with the description of the effect of ATP causing permeabilization of the plasma membrane to large solutes, through the activation of the P2X7 receptor.[373,374] Since then, extracellular nucleotides were found to have a large number of biologic effects on mast cells. These include the release of histamine by mast cells upon stimulation with ATP, as well as ADP and guanosine triphosphate (GTP). However, this effect was observed only on rat and mouse mast cells, and not in human or

**TABLE 11** P2 receptors in neutrophils

| Cell source | P2 receptor | Effects | References |
|---|---|---|---|
| **Human** | $P2Y_1$, $P2Y_2$, $P2Y_{14}$, P2X1, P2X4, P2X5, and P2X7? | Elastase release, intracellular calcium mobilization, MAPK phosphorylation, migration, NET formation, phagocytosis, and ROS production | 344,348,352,357,365,369,370 |
| **Mouse** | $P2Y_1$, $P2Y_4$, $P2Y_6$, $P2Y_{14}$, and P2X1 | Migration | 350,351,353,361 |
| **Rat** | $P2Y_2$, $P2Y_{11}$, P2X1, P2X4, P2X5, and P2X7 | Migration | 356 |
| **HL-60 cell line** | $P2Y_2$, P2Y14, and P2X7 | Chemotaxis and dye uptake | 354,364 |

guinea pig cells.[375,376] On the other hand, ATP, 2MeSATP, and UTP enhanced the anti-IgE-induced histamine release by human lung mast cells.[385]

P2X and some P2Y receptors are associated with an increase of intracellular calcium levels, suggesting a role for these receptors in calcium mobilization and mast cell degranulation.[109,377–379] Conversely, histamine induces ATP release to the extracellular medium via pannexin-1, which could represent a positive feedback response.[380]

P2 receptors seem also to be involved in triggering other mast cell responses, such as differentiation to mucosal and serosal phenotypes and chemoattraction.[375,381] Indeed, ADP, ATP, and UTP are effective chemoattractants, apparently through the activation of P2Y receptors.[382] These nucleotides were also reported to inhibit TNF-$\alpha$ generation dependent of TLR2 ligand, and abolish TNF-$\alpha$, IL-8, and MIP-1 assembly in response to $LTB_4$.[383] Finally, ATP induces mast cell apoptosis, cytokine production (IL-4, IL-6, IL-13, and TNF-$\alpha$), and is involved in mast cell-induced inflammation.[384,385]

A summary of the P2 receptors expressed in mast cells and their functions is shown in Table 12.

### 4.5.3 | Eosinophils

Eosinophils are granulocytes involved mainly in the inflammatory response to allergens and infection with helminthic parasites. They are characterized by cytoplasmic granules containing lysosomal hydrolases, as well as cationic proteins.[390]

The first work that suggested the expression of P2 receptors in eosinophils was published by Dichmann et al. in 2000, describing ATP-induced ROS generation, CD11b up-regulation, calcium mobilization, and actin polymerization.[391] Since then, extracellular nucleotides, such as ATP, UTP, and UDP, were found to induce IL-8 and eosinophil cationic protein (ECP) release by eosinophils. The involvement of P2Y receptors in granule release is suggested by the fact that this effect was blocked by pertussis toxin. On the other hand, IL-8 release was blocked by KN-62, indicating P2X involvement.[392] Among P2 receptors, $P2Y_2$ was the main receptor linked to chemotaxis in vitro and migration of eosinophils in vivo.[393]

In an asthma animal model, treatment with KN-62, a P2X7 receptor antagonist, significantly inhibited eosinophilia as well as lymphocytosis in bronchoalveolar lavage (BAL). On the other hand, the same response was observed using $P2X7^{-/-}$ animals. Considering the incomplete inactivation of the P2X7 gene observed in some P2X7 KO models (reviewed in[146]), these apparently contradictory results do not allow a clear definition of the role, or lack of it, for P2X7 in eosinophilia in asthma models. Indeed, an up-regulation of P2X7 expression in asthmatic patients compared to healthy individuals has been reported, as well as a slight increase in oxygen radical production by these cells, although the number of individuals evaluated was small ($n = 8$).[394] In contrast, some groups did not observe the expression of P2X7 in asthmatic patients.[369,395]

$P2Y_{14}$ is another P2 receptor overexpressed in eosinophils, especially in the asthma context, and is also involved in chemotaxis of eosinophils to airway.[396]

A summary of the P2 receptors expressed in eosinophils and their functions is shown in Table 13.

Despite their important effects on allergic diseases, there is still much to be studied regarding purinergic signaling in this cell type. In addition, there are no studies on the possible role of purinergic signaling in helminthic diseases, which may represent a new direction for research.

### 4.5.4 | Basophils

Basophils are the least abundant granulocyte found in peripheral blood. These cells contain histamine-rich cytoplasmic granules and are the circulating counterparts of mast cells, apparently having similar properties and functions.[399]

Despite their similarity to mast cells, research on basophils is still scarce, including in the field of purinergic receptors. Nevertheless, UDP stimulation has been reported to augment intracellular calcium mobilization and IgE-dependent degranulation in basophils.[400,401]

A summary of the P2 receptors expressed in basophils and their functions is shown in Table 14.

**TABLE 12** P2 receptors in mast cells

| Cell source | P2 receptors | Effects | References |
|---|---|---|---|
| **Human lung** | $P2Y_1$, $P2Y_2$, P2X1, P2X2, and P2X7 | Enhance the anti-IgE-induced histamine | 386,387 |
| **Cord blood derived-human mast cell** | $P2Y_1$, $P2Y_2$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, P2X1, and P2X4 | Reduce cytokine production (TNF-$\alpha$, IL-8, and macrophage inflammatory protein-1$\beta$ (MIP-1$\beta$)) | 383 |
| **Murine peritoneal mast cells** | P2X1, P2X3, P2X4, and P2X7[a] | Intracellular calcium mobilization, induction of membrane currents, histamine, and chemokine release | 388 |
| **Bone marrow-derived mast cells (BMMC)** | $P2Y_1$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, P2X1, P2X2, P2X3, P2X4, P2X6, and P2X7 | Apoptosis, calcium influx, cell permeabilization, and cytokine production (IL-4, IL-6, IL-13, and TNF-$\alpha$) | 384,389 |
| **LAD2** | $P2Y_1$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, and P2X7 | Cell degranulation | 109,379 |
| **MC/9** | $P2Y_1$, $P2Y_6$, $P2Y_{13}$, $P2Y_{14}$, P2X1, P2X2, P2X4, P2X6, and P2X7 | Apoptosis, calcium influx, cell permeabilization, and cytokine production (IL-4, IL-6, IL-13, and TNF-$\alpha$) | 384 |
| **P815** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, P2X1, P2X2, P2X3, P2X4, P2X5, P2X6 and P2X7 | Apoptosis, calcium influx, and cell permeabilization | 384 |
| **RBL-2H3** | $P2Y_1$, $P2Y_2$, $P2Y_{13}$, and $P2Y_{14}$[b] | Cell degranulation | 377,378 |

[a]Only the expression of P2X receptors was investigated.
[b]Only the expression of P2Y receptors was investigated.

**TABLE 13** P2 receptors in eosinophils

| Cell Source | P2 receptors | Effects | References |
|---|---|---|---|
| **Human** | $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, P2X1, P2X4, and P2X7 | Actin polymerization, CD11b up-regulation, chemotaxis, IL-8 and ECP release, and ROS generation | 369,391,392,394,395,397,398 |
| **Rat** | $P2Y_1$, $P2Y_2$, $P2Y_4$, P2X1, P2X2, P2X4, and P2X7 | Chemotaxis and cell migration | 393 |

**TABLE 14** P2 receptors in basophils

| Cell Source | P2 receptors | Effects | References |
|---|---|---|---|
| **Human** | $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$[a] | Intracellular calcium mobilization and IgE-dependent degranulation | 401 |

[a]Only the expression of P2Y receptors was investigated.

## 5 | ROLE OF P2 RECEPTORS IN DISEASES AND THERAPEUTIC PERSPECTIVES

The data discussed here indicates that P2 receptors are widely expressed in the cells of the immune system, where they play important roles in innate immune response, as well as in the initiation of adaptive immune response. However, the exacerbation of proinflammatory responses promoted by P2 receptors also seems to contribute to the development of inflammatory diseases and pain. In this scenario, the use of selective agonists and antagonists of P2 receptors can significantly contribute to the management of these conditions.

### 5.1 | Infectious diseases

A potential therapeutic use of P2 agonists in the treatment of some infectious diseases has emerged from the studies of their effects on the immune system. Macrophages are capable of killing intracellular *Mycobacterium tuberculosis* or BCG through a mechanism dependent on nitrogen and oxygen free radicals activated by P2 receptors.[81,402,403] ATP induces apoptosis and autophagy of mycobacterium-infected macrophages, reducing BCG viability.[176,177,404] Another ATP-activated pathway that reduces BCG viability is associated with PLD activity and phagosome–lysosome fusion.[197]

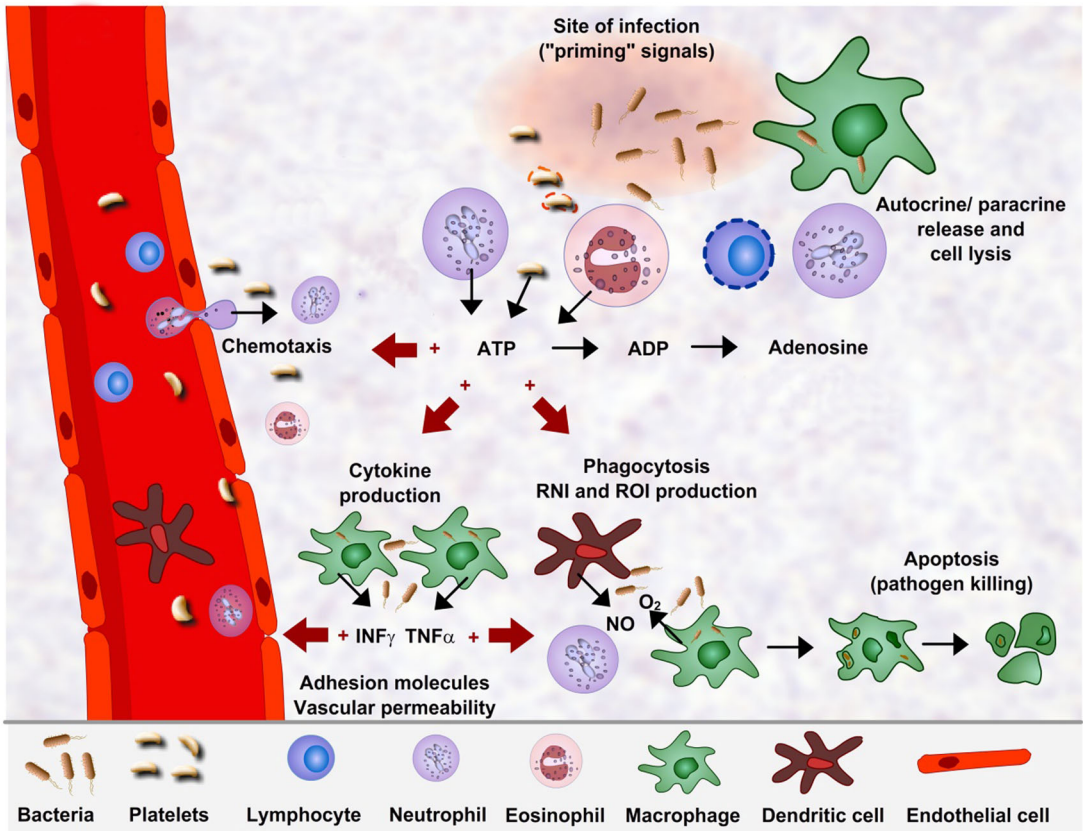


**FIGURE 2** Main roles of P2 receptors expressed on leukocytes in infection. At the site of infection, cells release higher ATP concentrations, which functions as a "find me" signal to activate leukocytes. Extracellular ATP activates P2 receptors expressed on leukocytes, promoting cytokines production such as IFN-$\gamma$ and TNF-$\alpha$, phagocytosis, and the production of NO and ROS. Those, together with cell apoptosis caused by P2X7 activation, mediate both extracellular and intracellular pathogen killing. The activation of P2 receptors, especially P2Y, stimulates chemokine release, production of adhesion molecules and augments vascular permeability, favoring chemotaxis of leukocytes to the site of infection.

P2X7 KO increases mycobacterial viability, suggesting that P2X7 is important to protect cells from mycobacterial infection.[197] P2X7 receptor could also play a dual role in tuberculosis depending on the mycobacterium strain. P2X7 activation contributes to the resolution of tuberculosis in mice infected with laboratory strain, but it could aggravate the disease in mice infected with a hypervirulent strain.[405,406]

Polymorphisms in the P2X7 genes have been associated with loss of macrophage activity and the development of tuberculosis.[467-469] Individuals presenting the rs3751143 polymorphism, in which Glu496 is exchanged to Ala, have reduced P2X7 expression and decreased apoptosis when infected with BCG, favoring mycobacteria survival.[407] The same effect is observed in individuals presenting heterozygosis in polymorphism Thr357 to Ser.[408]

Inflammasome activation is another strategy to fight pathogens. The inflammasome is a multiprotein complex associated with inflammation and cell death. It is composed of three proteins: a molecular pattern sensing protein, (generally NLRP3), the ASC adapter protein, and the pro-caspase-1 enzyme, which is cleaved and activated upon integration into the inflammasome complex. Active caspase-1 promotes the activation of proinflammatory cytokines IL-1$\beta$ and IL-18, and may also mediate pyroptosis cell death, contributing to pathogen killing.[409–411] Some studies have demonstrated that infected macrophages and DCs use this strategy in infection control.[412–414]

In chlamydia infection, P2X7-induced apoptosis, as well as membrane permeabilization and calcium mobilization, is inhibited in macrophages. In turn, the treatment of macrophages with ATP reduces infection levels, via PLD activation and vesicle fusion.[415]

Similarly to the P2X7 receptor, $P2Y_2$ activation plays a role in microbial elimination. $P2Y_2$ activation augments the acidification of mycobacterial phagosomes in a calcium-dependent manner, killing the mycobacteria without causing macrophage death. This effect was mediated by the $P2Y_2$ receptor even in the absence of P2X7.[416]

ATP treatment significantly reduces infection with *Toxoplasma gondii* in macrophages from swiss, C57Bl/6, and Balb/c mice strains. Brilliant blue G treatment or P2X7 KO abolished this protection, suggesting a role for P2X7 in *T. gondii* elimination.[186] UTP and UDP treatment, in addition to ATP treatment, in peritoneal macrophages infected with *T. gondii* also reduced the percentage of infected cells and the number of parasites per cell in a concentration-dependent manner, suggesting the participation of P2Y receptors in this effect.[417]

Several P2 receptors are involved in the reduction of *Leishmania amazonensis* infection of macrophages by nucleotide treatment. The microbicidal actions of P2X7 on macrophages against *L. amazonensis*

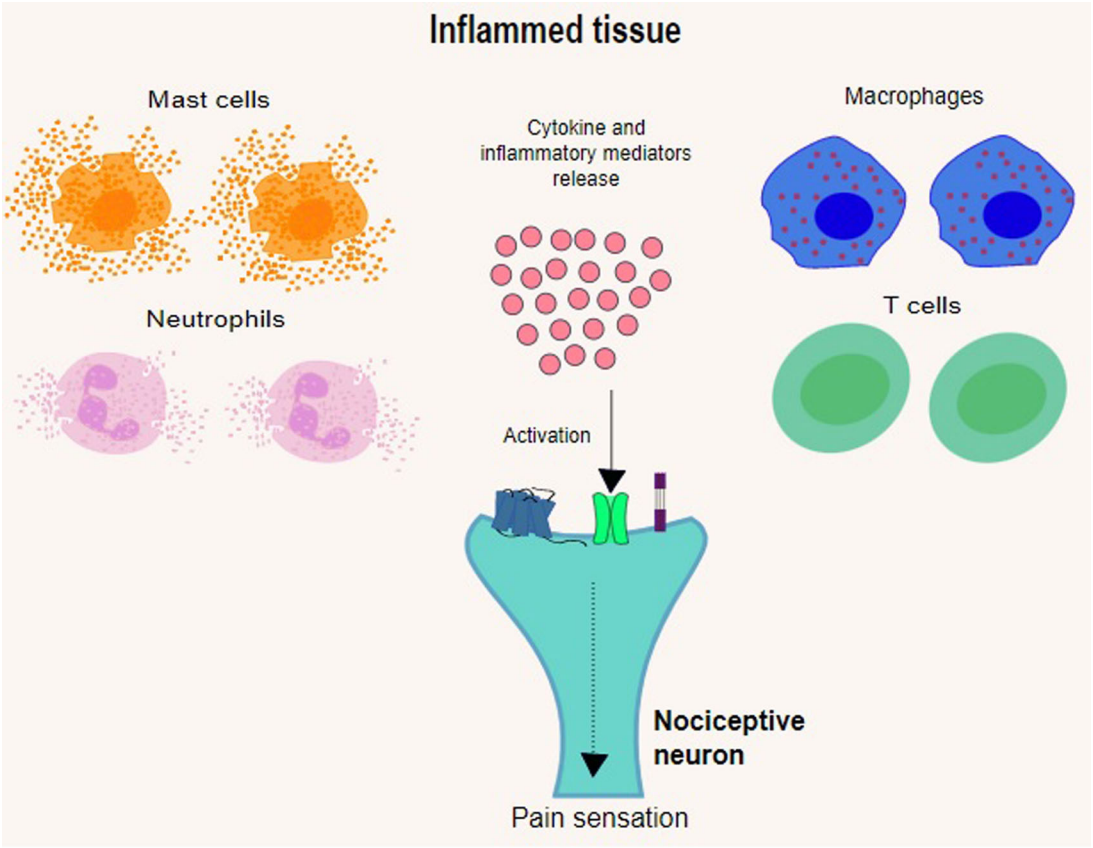


**FIGURE 3** Schematic illustration of the P2 receptors in pain. At inflamed tissue, immune cells, including macrophages, neutrophils, mast cells, and T cells, are activated by extracellular nucleotides. Once activated, these cells release several cytokines, including IL-1$\beta$, IL-6, and TNF-$\alpha$, as well as prostaglandins, histamine, and other inflammatory mediators, contributing to sensitization of nociceptor sensory neurons via ion channels, cytokine receptors and GPCRs, and promoting pain sensation

involve the production of lipid mediators and $LTB_4$ secretion.[418–420] Among P2Y receptors, $P2Y_2$ may be one of the most important to control infection, since inhibition of this receptor augments it. UTP stimulation induces ATP and $LTB_4$ release, contributing to autocrine signaling and parasite death, respectively.[184,185,421] Figure 2 illustrates the role of P2 receptors expressed on leukocytes in infectious diseases.

Recently, several studies have demonstrated the benefits of blocking P2 receptors in infectious diseases such as acquired immunodeficiency syndrome. Hazleton et al.[422] demonstrated that oATP and Suramin inhibited human immunodeficiency virus (HIV) p24 production and release as well as the percentage of infected cells. These authors suggested the participation of at least three receptors in these processes: $P2Y_1$, $P2X_1$, and $P2X_7$.[422] Giroud et al.[423] also observed that P2X1 antagonists blocked virus fusion.

## 5.2 | Inflammatory diseases

Nucleotides act as a "find me" signal to leukocytes, stimulating their migration to inflammatory sites. P2 receptors are activated as a result of the accumulation of extracellular nucleotides at these sites and promote the formation and release of inflammatory mediators, such as cytokines, chemokines, ROS, among others, contributing to local inflammation, tissue damage, and pain sensation. In this scenario, P2 receptors emerged as a promising target for therapeutics in inflammatory diseases.[110,424–426]

The participation of the P2X7 in inflammatory diseases is related to its ability to activate the inflammasome, a target in the treatment of inflammatory disorders.[426] Despite many studies, the exact mechanism by which P2X7 promotes inflammasome activation is still unknown. Hypotheses proposed include a decrease in intracellular $K^+$ concentration, ROS production, and the destabilization of lysosomes. This will likely be an important field of study in the coming years, as a strategy to identify new anti-inflammatory agents.[409–411]

In animal models of arthritis, P2X7 antagonists alleviate joint inflammation, including type II collagen-induced joint damage.[306,427,428] P2X7 KO mice developed fewer joint cartilage lesions than controls, and lower proteoglycan content and collagen degradation, reducing the severity of the disease.[429] P2X7 antagonists also inhibit the release of cathepsins (lysosomal proteases), suggesting a possible role in chronic inflammation.[151]

The $P2Y_2$ receptor role in cell recruitment to the inflammation site is well recognized.[430] This receptor senses the nucleotide gradient, promoting neutrophil migration to the site of inflammation.[431,432] Impairment of $P2Y_2$ activity could be beneficial in certain models of lung inflammatory disease. However, one exception is infection with the pneumonia virus of mice, as $P2Y_2^{-/-}$ mice had lower counts of leukocytes in BALF, and had higher mortality than WT animals.[433]

$P2Y_1$ and $P2Y_6$ play important roles in vascular inflammation. $P2Y_1$ participates in vascular inflammation by recruiting leukocytes. $P2Y_1^{-/-}$ mice present lower levels of adhesion molecules and of leukocytes. $P2Y_1$ blockage also reduced the number of rolling leukocytes in vivo.[434] $P2Y_6$ overexpression was accompanied by the increased expression of VCAM-1 in LPS-injected animals.[435] In ovalbum (OVA) and house dust mite (HDM) models of allergic inflammation, $P2Y_6$ is up-regulated and modulates the classical allergic features such as eosinophilia, airway remodeling, Th2 cytokine secretion, and bronchial hyperesponsiveness, which was alleviated by the use of antagonists or in $P2Y_6$ receptor KO animals.[436,437]

Regarding pain, P2X receptors seem to play a more important role (or their participation in pain processes is more studied) than P2Y receptors. P2X receptors promote the release of several inflammatory mediators, such as IL-1$\beta$ and prostaglandins, which are involved in inflammatory hyperalgesia.[438]

P2X2/3 activation is associated with inflammatory hyperalgesia induced by carrageenan, bradykinin, prostaglandins, and sympathomimetic amines, which could be reverted with the use of their specific antagonists.[439–442] Moreover, P2X3 and the heteromeric P2X2/3 were shown, by functional assays, to be key receptors involved in pain in primary sensory neurons, via $PLA_2$ activation.[443–445] Recently, it was demonstrated that TNF-$\alpha$ released from macrophages induce P2X3 expression in neurons, modulating allodynia.[446] In turn, P2X4 is related to mechanical allodynia after nerve injury.[447–449] Additionally, P2X4 is overexpressed in microglia after nerve injury and $P2X4^{-/-}$ mice have abolished tactile allodynia.[447,448]

Selective blockers of the P2X7 receptor diminished allodynia in different models of rat neuropathic pain.[450] P2X7 blockage relieved inflammatory pain and thermal hyperalgesia.[451,452] KO of IL-1$\alpha\beta$ in mice abolished the hyperalgesic effects of the complete Freund's adjuvant (CFA) model, indicating that hyperalgesia relief by the blockage of the P2X7 is due to the blockage of IL-1$\beta$.[453]

P2Y receptors also participate in neuropathic pain. $P2Y_{12}$ receptor inactivation, both pharmacologically and through receptor KO, abolished the pain after nerve injury.[454,455] In contrast, other pharmacologic $P2Y_{12}$ inhibitors failed to block pain, indicating that the role of $P2Y_{12}$ in pain must be better explored. Figure 3 illustrates the role of P2 receptors activation in pain.

## 6 | CONCLUDING REMARKS

This review compiles broad evidence of the wide distribution of P2 receptors in cells of the immune system. In leukocytes, these receptors play important roles in the innate immune response and in the initiation of the adaptive immune response, contributing significantly to pathogen death and infection control. However, the activation of these receptors is also related to the development of inflammatory diseases and pain, making them a promising target for the development of anti-inflammatory drugs and analgesics.

Purinergic therapy is still in its infancy, but it has several fields of application in which it can mature significantly. In this review we show that, over the approximately 50 years of the purinergic field, much has been discovered regarding the presence of P2 receptors and their roles in the organism; still, much more remains to be explored, especially concerning the immune system and CNS. Yet, the work on P2 receptor role in the immune system strongly suggests that they are a promising target for drug development over the next decades.

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## AUTHORSHIP

A. V. P. A., N. C. S. F., C. A., and L. A. A. conceived the idea. A. V. P. A., N. C. S. F., A. G. C. B., O. K. N., F. P. F., R. C. B., C. A., W. S., R. C. S., P. M. P., and L. A. A. wrote or contributed to the writing of the manuscript. A. V. P. A. and N. C. S. F. contributed equally to this work.

## DISCLOSURE

The authors declare no conflict of interest.

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