Nucleotide receptors: an emerging family of regulatory molecules in blood cells

Francesco Di Virgilio, Paola Chiozzi, Davide Ferrari, Simonetta Falzoni, Juana M. Sanz, Anna Morelli, Maurizia Torboli, Giorgio Bolognesi, and O. Roberto Baricordi

Nucleotides are emerging as an ubiquitous family of extracellular signaling molecules. It has been known for many years that adenosine diphosphate is a potent platelet aggregating factor, but it is now clear that virtually every circulating cell is responsive to nucleotides. Effects as different as proliferation or differentiation, chemotaxis, release of cytokines or lysosomal constituents, and generation of reactive oxygen or nitrogen species are elicited upon stimulation of blood cells with extracellular adenosine triphosphate (ATP). These effects are mediated through a specific class of plasma membrane receptors called purinergic P2 receptors that, according to the molecular structure, are further subdivided into 2 subfamilies: P2Y and P2X. ATP and possibly other nucleotides are released from damaged cells or secreted via nonlytic mechanisms. Thus, during inflammation or vascular damage, nucleotides may provide an important mechanism involved in the activation of leukocytes and platelets. However, the cell physiology of these receptors is still at its dawn, and the precise function of the multiple P2X and P2Y receptor subtypes remains to be understood. (Blood. 2001;97:587-600)

Introduction

In 1978 the existence of plasma membrane receptors for extracellular nucleotides, the P2 purinergic receptors, was formally recognized.1 At that time, this identification was only based on pharmacologic and functional evidence and on the prophetic intuition of Geoff Burnstock. To date, 12 mammalian P2 receptors have been cloned, characterized, and recognized as responsible for the diverse cellular responses to stimulation with extracellular nucleotides.2,3 The P2 receptor family also includes receptors for extracellular pyrimidines. The new classification based on the molecular structure is rapidly replacing the previous one (P2Y, P2X, P2U, P2T, and P2Z) based on the pharmacologic profile,4 although doubts remain on whether functional responses of the native P2Z receptor of immune cells can be entirely explained by the cloned P2X7 subunit. A similar uncertainty also concerns the platelet P2T receptor, which is likely to arise from the combination of P2Y and P2X-dependent responses.2,5 Extracellular effects of nucleotides were initially recognized in smooth muscle contraction, neurotransmission, regulation of cardiac function, and platelet aggregation.6 However, over the last 10 years it has become clear that the intercellular mediator role of these molecules is widespread, and blood cells have emerged as one of the most interesting targets.

Contrary to a widely held opinion, adenosine triphosphate (ATP) and possibly also uridine triphosphate (UTP) are often released into the extracellular environment via nonlytic mechanisms7-12 and also more frequently as a consequence of cell damage or acute cell death. Furthermore, platelet-dense granules are a relevant source of secreted ATP.13,14 Once in the pericellular environment, ATP can serve as a ligand for P2 receptors or be quickly hydrolyzed by powerful ubiquitous ecto-ATPases and ectonucleotidases.15-18 ATP can also be used as a phosphate donor by poorly characterized ectokinases.19 Thus, ATP possesses all the properties of a bona fide fast-acting intercellular messenger: (a) it is released in a controlled fashion, (b) ligates specific plasma membrane receptors coupled to intracellular signal transduction, and (c) is quickly degraded to terminate its action.

Outside excitable tissues, P2 receptors have an obvious relevance in platelet aggregation, but immunity and inflammation are providing some of the most exciting developments in this evolving field. A few reviews covering different aspects of P2 receptor distribution and function in hematopoietic cells have appeared and have been an invaluable source of information for the present work.20-26

P2 receptors: what are they?

According to the International Union of Pharmacology (IUPHAR) Committee on Receptor Nomenclature and Drug Classification,27 receptors for extracellular nucleotides are termed P2 receptors (this nomenclature replaces the older “P2-purinoceptor”). P2 receptors are divided into 2 subfamilies: G protein–coupled (P2Y) and ligand-gated ion channels (P2X).28-30 Current P2Y/P2X nomenclature is based on the molecular structure and has replaced the previous one based on pharmacologic and functional criteria. In mammalian cells, 5 P2Y (P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11) and 7 P2X (P2X1-7) receptors have been cloned and characterized pharmacologically7 (Table 1). P2Y5, P2Y7, P2Y8, and P2Y10 have
Table 1. P2Y and P2X receptor subtypes

<table>
<thead>
<tr>
<th>P2Y</th>
<th>P2X</th>
<th>Amino acid number</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2Y1</td>
<td></td>
<td>362</td>
</tr>
<tr>
<td>P2Y2</td>
<td></td>
<td>373</td>
</tr>
<tr>
<td>p2y3</td>
<td></td>
<td>328</td>
</tr>
<tr>
<td>P2Y4</td>
<td></td>
<td>352</td>
</tr>
<tr>
<td>P2Y6</td>
<td></td>
<td>379</td>
</tr>
<tr>
<td>P2Y11</td>
<td></td>
<td>371</td>
</tr>
</tbody>
</table>

*P2Y3 was cloned from chick brain and may be the chick homologue of the mammalian P2Y6.†P2X7 and P2X8 are present in two splice variants.

been purged from this sequence because they are primarily non-nucleotide receptors (although they may also bind extracellular nucleotides). A p2y3 (lower case to indicate that it has not been cloned from mammals) receptor has been cloned from chick brain and suggested to be a homologue of the mammalian P2Y6. P2Y8 has so far only been cloned from Xenopus neural plate; thus it is not included in the list of mammalian receptors. The adenosine diphosphate (ADP)-activated, G protein–coupled receptor of platelets that triggers inhibition of stimulated adenylate cyclase has not yet been cloned; thus it is recommended that this receptor should be given in italics: P2Y_{ADP}.

P2Y receptors

P2Y receptors are 7-membrane–spanning proteins, numbering from 328 to 379 amino acids, for a molecular mass of 41 to 53 kd after glycosylation. The aminoterminal domain faces the extracellular environment, and the carboxyterminal is on the cytoplasmic side of the plasma membrane (Figure 1). Signal transduction occurs via the classical pathways triggered by most 7-membrane–spanning receptors: activation of phospholipase C and/or stimulation/inhibition of adenylate cyclase. All of the P2Y receptors are activated by ATP, but at 2 of them, P2Y4 and P2Y6, UTP is more potent, and at P2Y2, ATP and UTP are equipotent. At P2Y1, UTP is inactive and ADP is reported to be equipotent or even more potent than ATP; at P2Y11, ATP is more potent than ADP and UTP is inactive. With respect to the signal transduction pathway, P2Y1 and P2Y2 are coupled to stimulation of phospholipase C-β and inhibition of adenylate cyclase via G_{q/11} and G proteins, respectively. There are reports suggesting that P2Y2 can also trigger stimulation of phospholipase D and breakdown of phosphatidylcholine, but the mechanism is unclear. P2Y4 and P2Y11 seem to only couple to phosphoinositide breakdown, whereas P2Y12 rather surprisingly stimulates activation of both the phosphoinositide and the adenylate cyclase pathways.

Investigation of P2Y receptors has been severely hindered by the lack of specific antibodies, whether polyclonal or monoclonal. Likewise, few selective agonists, besides naturally occurring nucleotides, or antagonists are available. A widely used P2Y antagonist is suramin, a drug originally developed for the treatment of tripanosomiasis. However, suramin does not discriminate between P2Y and P2X and has been reported to inhibit other receptors such as the nicotinic, glutamate, GABA, and 5-hydroxytryptamine receptors as well as the activity of diverse growth factors. Reactive blue 2, trypan blue, and reactive red have also been used as P2Y antagonists, but they also block P2X-dependent responses. Recently Harden and coworkers have introduced a number of nucleotide analogues as competitive P2Y_{1} antagonists. Pyridoxal phosphate (P5P) and pyridoxal phosphate-6-azophenyl 2',4'-disulfonic acid (PPADS) are also sometimes used to inhibit P2Y-dependent responses, but they are more widely employed to block P2X receptors.

P2X receptors

P2X receptors are ATP-gated ion channels—originally cloned and characterized in excitable cells—and then shown to be nearly ubiquitous—that mediate fast permeability changes to monovalent and divalent cations (Na^{+}, K^{+}, and Ca^{2+}). One of the members of this subfamily, P2X7, has sparked vivid interest for its peculiar ability to undergo a progressive increase in size that leads to the generation of a nonselective membrane pore to inhibit P2Y-dependent responses, but they are more widely employed to block P2X receptors.

![Figure 1. Membrane topology of P2Y and P2X receptor subunits.](image)
Figure 2. Permeability transition of P2X receptor. A transient stimulation with ATP causes the opening of the P2X channel and the concomitant Ca٢⁺ influx and K⁺ efflux. However, upon sustained stimulation with ATP, the P2X7 receptor undergoes a transition that generates a reversible membrane pore permeable to Na⁺ channel (ENaC), the degenerins cloned from Caenorhabditis elegans, and the inward rectifying K⁺ channel (Kir). Signal transduction occurs via fast Na⁺ and Ca٢⁺ influx and K⁺ efflux, leading to depolarization of the plasma membrane and an increase in the concentration of cytosolic Ca٢⁺ ([Ca٢⁺]i). It is likely that the drastic upset in intracellular ion homeostasis caused by P2X receptor opening activates several additional intracellular messengers and enzyme pathways, but few studies are available on this novel and exciting field of P2X receptor biochemistry. Electrophysiologic investigation of recombinant P2X receptor subunits transfected into mammalian recipient cells has allowed identification of fast desensitizing and slowly desensitizing (or nondesensitizing) P2X receptors.

Although still on a limited basis, a few anti-P2X antibodies were made available over the last 2 years by single laboratories or commercial sources. Polyclonal antibodies against P2X1, P2X3, and P2X7 can be obtained from at least 2 companies; in a few laboratories sera against all the members of the subfamily have been raised. One monoclonal antibody selective for the human P2X7 receptor has been produced and characterized by Buell and colleagues. Interestingly, this monoclonal antibody, which recognizes an as yet to be identified epitope on the extracellular domain, inhibits activation of human macrophages by 3'-O-(4-benzoyl)benzoyl-ATP (BzATP), a P2X7 agonist.


P2 receptors in monocyte/macrophages

Early studies by Steinberg and Silverstein showed that the J774 mouse macrophage cell line expressed a plasma membrane receptor selectively activated by ATP and a few analogues. Stimulation of this receptor triggered the same reversible increase in plasma membrane permeability to low-molecular-mass solutes increasing its apparent potency. This may explain the finding that the potency of stable ATP analogues, such as α, β-methylene ATP (α, β MeATP), is unaffected by Ca٢⁺ and Mg٢⁺ removal. This ATP analogue is used to pharmacologically discriminate P2X receptor subtypes: P2X1 and P2X4 are sensitive to low (0.5-5 μM) concentrations, and P2X3 and P2X4,7 are activated by high (>100 μM) doses. An often neglected finding is the high potency of BzATP at all P2X—not just P2X7—receptors and its agonist activity at P2Y receptors, a feature that makes this ATP analogue one of the most useful tools for the study of native and recombinant P2X receptors.

Better antagonists, with better-characterized activity, are available at P2X than at P2Y receptors. PPADS is a noncompetitive inhibitor of most P2X receptors and, depending on the experimental conditions, may act irreversibly. Oxidized ATP (oATP) was introduced 7 years ago as a selective P2Z (P2X7) inhibitor, but it is likely to show the same P2X antagonist selectivity of PPADS, although no detailed investigation has been carried out. At the effective concentrations (100-300 μM), oATP shows little or no inhibitory activity at P2Y receptors and at ectonucleotidases.

Action of oATP on ectokinases has not been tested in depth; thus it cannot be excluded that some effects of this compound may be due to inhibition of ectophosphorylation. PPADS and oATP likely share the same mechanism of action. Both compounds have aldehyde groups (1 PPADS, 2 oATPs) that can react with unprotonated lysines to form Schiff’s bases. It is assumed that they preferentially modify lysine residues in the vicinity of the ATP binding site, but this assumption is yet to be proved. Although PPADS has been used as a P2 blocker for some time, it was only after the introduction of oATP that attention has been paid to the time-dependent and irreversible inhibitory effect of this P5P derivative. Time-dependent and irreversible block is extensively documented for oATP at the P2X7 receptor: A 1- to 2-hour preincubation with this inhibitor, even if followed by extensive rinsing, makes all cells so far investigated fully refractory to ATP stimulation via the P2X7 receptor. Refractoriness lasts several hours, until new receptors are inserted into the plasma membrane.

More recently, Wiley and colleagues have introduced another powerful blocker of P2X7, compound 1-[N,0-bis(5-isoquinolinesulphonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62). This molecule was originally used as an inhibitor of the calcium calmodulin–dependent kinase and made its first appearance in the purinergic field in a study by Blanchard et al aimed at investigating the role of the P2Z7 receptor in cell-mediated cytotoxicity. KN-62 acts as a competitive inhibitor at nanomolar concentrations and shows a striking species specificity: It is active only at the human and not at the rat or mouse P2X7 receptor.

Refs: 60-66-69 Refractoriness lasts several hours, until new receptors are inserted into the plasma membrane.
originally described by Cockcroft and Gomperts in rat mast cells.58,59 An intriguing finding of these studies was that stimulation of the ATP-permeabilizing receptor eventually led to cell death.75 This incidental observation stirred interest in the possible physiologic meaning of ATP-dependent cytotoxicity and fostered subsequent studies on the role of P2 receptors in the immune system. At about the same time, Greenberg et al demonstrated that J774 macrophages also expressed P2Y-like receptors coupled to Ca$^{2+}$ mobilization via a mechanism other than the ATP-permeabilizing receptor.76 This was made possible by the selection by Steinberg and colleagues75 of ATP-resistant J774 macrophage clones later shown to lack the P2X$\gamma$ receptor.77,78

According to the nomenclature proposed by Gordon,4 the macrophage-permeabilizing receptor was named P2Z, analogously to the mast cell and lymphocyte ATP receptor. The receptor responsible for ATP-dependent permeabilization has been referred to as P2Z until very recently and, even after the cloning of P2X$\gamma$, and the demonstration that its transfection confers susceptibility to ATP-dependent permeabilization, some investigators prefer the P2Z nomenclature to indicate the native ATP-permeabilizing receptor, because it is not clear whether P2X$\gamma$ is the only constitutive subunit or, rather, the native P2Z receptor is formed by the assembly of P2X$\gamma$ in association with other P2X subtypes. However, because P2X$\gamma$ reproduces all known effects of the native P2Z and cells resisting ATP-mediated permeabilization lack P2X$\gamma$, we will assume hereafter that the macrophage P2Z and P2X$\gamma$ receptors are the same molecule. As seen below, the picture is more complex in lymphocytes and other cells that do not undergo the typical ATP-dependent permeabilization, although they may express P2X$\gamma$.

P2Y and P2X subtype expression by macrophages

All murine macrophage lines so far investigated express P2Y receptors coupled to release of Ca$^{2+}$ from intracellular stores and IP$_3$ generation, but the individual subtypes have not been investigated in detail. Functional and molecular expression of P2X$\gamma$ has been shown in some murine cell lines and in mouse and rat peritoneal macrophages.60,75,79-83 Monocyte-derived human macrophages are susceptible to ATP-mediated permeabilization and express P2X$_1$,66,84,85 Among human macrophage lines, THP-1 and U937 cells express P2Y receptors (P2Y$_2$, P2Y$_4$, and P2Y$_6$),5,86-88 but only the THP-1 monocyte cell line has been reported to express P2X$_\gamma$ to a significant level.86 However, P2X$_\gamma$ receptor expression can differ significantly among cell batches propagated in different laboratories. Monocytes freshly isolated from peripheral blood express P2Y receptors but lack P2X$_\gamma$, whether investigated at the molecular or at the functional level. Although a few studies are available, it is generally agreed that, at the most, 15% to 17% of human monocytes undergo the plasma membrane permeability transitions diagnostic of P2X$_\gamma$ expression when stimulated with ATP.66,84 There appears to be an inverse correlation between P2Y$\gamma$ and P2X$_\gamma$ expression during monocyte to macrophage maturation: P2Y$_2$ messenger RNA (mRNA) declines while P2X$_\gamma$ mRNA increases.89 Up-regulation of P2X$_\gamma$ and acquisition of P2X$_\gamma$-dependent responses are detectable within 24 hours of seeding human monocytes on plastic dishes. Up-regulation of P2X$_\gamma$ and down-regulation of P2Y$_2$ by the inflammatory mediators interferon-γ and tumor necrosis factor (TNF)-α and by lipopolysaccharide (LPS) have been reported.66,89 In addition, phorbol myristate acetate causes a decrease in P2Y$_2$ mRNA in THP-1 cells.90

Role of P2 receptors in monocyte/macrophage physiology

The first report on the effect of exogenous nucleotides on macrophage function was a paper by Cohn and Parks.91 In this study the authors showed that addition of adenine nucleotides to a mouse macrophage culture resulted in a dramatic increase in pinocytic vesicle formation. After this early study, exogenous nucleotides as a stimulant for macrophages were basically neglected for several years and resurrected only in 1985 by Silverstein and coworkers,92 who reported that extracellular ATP inhibited Fc receptor–mediated phagocytosis and at the same time caused influx of Na$^+$, efflux of K$^+$, and an increase in [Ca$^{2+}$]. In this study it was also for the first time suggested that macrophages expressed receptors specific for ATP. The possibility that these ATP effects could be due to ATP hydrolysis by plasma membrane ecto-ATPase was ruled out by subsequent papers by Steinberg and Silverstein60,75,76 that reported an in-depth characterization of the macrophage-permeabilizing ATP receptor. It was also soon clear that the ATP receptor coupled to release of Ca$^{2+}$ from intracellular stores (P2Y) and the ATP-permeabilizing (P2Z/P2X$_\gamma$) receptor were 2 separate entities with widely different nucleotide selectivity and affinity and likely involved in different responses.93 In J774 macrophages, the concentration of ATP giving one half of the maximal response (EC$_{50}$) for Ca$^{2+}$ release from intracellular stores (and which therefore reflects activation of P2Y) is in the range of 50 to 70 μM. In microelectrode impalement experiments, the ATP EC$_{50}$ for depolarization, presumably reflecting opening of P2X$_\gamma$, was reported to be between 250 and 400 μM,94 but a lower EC$_{50}$ was reported for P2X$_\gamma$-triggered Ca$^{2+}$ rise in thioglycollate-elicited mouse peritoneal macrophages.95 However, determinations based on the measurement of uptake of fluorescent markers give higher EC$_{50}$ (1.0-1.5 mM ATP) for the activation of the native mouse P2X$_\gamma$ receptor.76,95 The UTP EC$_{50}$ for Ca$^{2+}$ release from intracellular stores is between 300 and 500 nM76 and thus much lower than the ATP EC$_{50}$.76,94 This suggests that macrophages express P2Y$_4$ or P2Y$_6$ or an endogenous yet to be identified uridine nucleotide–specific receptor. Therefore, it is clear that should ATP release occur in a tissue, macrophage P2Y receptors are likely to be activated more easily and more frequently than P2X$_\gamma$.

An early and, with hindsight, obvious proposal was that macrophages and, in general, inflammatory cells, could use P2Y receptors as very sensitive sensors of cell and tissue damage.76 After all, mammalian cells contain huge amounts (5-10 mM) of ATP in their cytosol; thus, any event that causes even a transient break in the plasma membrane will cause release of ATP into the pericellular environment. Furthermore, it is becoming apparent that frank cell injury or death might not even be necessary for ATP release because shear stress forces and stretching are also powerful stimuli for ATP leakage.8-12 J774 macrophages chemoattract in response to micromolar concentrations of ADP but, rather intriguingly, not of UTP.96 Human macrophages in the vicinity of dying K562 cells have been shown in vitro to undergo an increase in [Ca$^{2+}$], that can be closely mimicked by the addition of cell lysate or of ATP at micromolar doses.57 Precedent treatment with the cell lysate made the macrophages refractory to the subsequent application of ATP, suggesting, although not proving, that a substance contained in the lysate and ATP might converge on the same
extracellular accumulation of minute amounts of this cytokine in its low-molecular-weight (17 kd) biologically active form, but the identity of this second stimulus has remained elusive. In 1991 Hogquist and colleagues observed that extracellular ATP triggered IL-1β processing and release, and in 1992 Gabel and coworkers reported that mature IL-1β formation could be induced by the K⁺ ionophore nigericin. What is in common between nigericin and ATP? Perregaux and coworkers reasoned that both nigericin and ATP decrease intracellular K⁺ levels and that perhaps this ionic perturbation was needed to activate the enzyme that cleaves pro–IL-1β into mature IL-1β, i.e., IL-1β-converting enzyme (ICE), also known as caspase-1. Later studies fulfilled this prediction because ATP was shown to trigger IL-1β release via a nontoxic mechanism in many different mononuclear phagocytic cells, and release was inhibited by procedures that prevented K⁺ efflux. In support of a key role for K⁺ in ICE activation, Cheneval et al. have shown that a reduction in the K⁺ concentration leads to proteolytic cleavage of isolated recombinant ICE. Interestingly, although proteolytic activation of the isolated enzyme could be induced by a reduction in the concentration of other cations besides K⁺, autoprocessing of cytoplasmic ICE showed an absolute requirement for K⁺ depletion. That ATP acts via ICE is also demonstrated by the ability of a specific ICE inhibitor, the tetrapeptide YVAD (Tyr-Val-Ala-Asp), to block ATP-dependent IL-1β maturation. Furthermore, macrophages isolated from mice deficient in ICE were unable to process pro–IL-1β upon challenge with LPS plus ATP. Finally, involvement of the P2X₇ receptor in ATP-mediated ICE activation is supported by (a) agonist and antagonist profile of cytokine release, (b) blockade by a specific anti-P2X₇ monoclonal antibody, and (c) detection of ICE proteolytic fragments (p20 and p10) in ATP-stimulated microglial cells. There are no clues as to how a decrease in K⁺ concentration may activate ICE autoprocessing; nevertheless, K⁺ provides a straightforward link between P2X₇ and ICE because opening of the P2X₇ channel/pore provides a very fast pathway for K⁺ efflux. It would be interesting to test whether the same K⁺-based mechanism of activation also applies to other caspases and how this may be involved in apoptosis.

In human monocytes, ATP is a powerful stimulus not only for caspase-1 activation but also for the externalization of mature caspase-1 subunits. The meaning of this novel observation is elusive, but it may point to a possible function of activated caspase-1 either in the extracellular space or on the outer leaflet of the plasma membrane. In addition, ATP might trigger IL-1β release by alternative mechanisms, e.g., by inducing exocytosis of IL-1β–containing specialized vesicles (late endosomes or lysosomes), as recently suggested by Rubartelli and coworkers. It is possible that the LPS signal for IL-1β release consists, at least in part, in an autocrine/paracrine stimulation mediated by ATP secretion.

![Figure 3. Fate of released ATP: possible role in leukocyte chemotaxis.](image)

![Figure 4. Model for P2X₇-mediated ICE/caspase-1 activation and IL-1β maturation.](image)
as suggested by studies in human and mouse macrophages and mouse microglia.9,114 Participation of P2X7 in LPS-dependent activation of immune cells might have very interesting and far-reaching practical applications in the treatment of sepsis caused by gram-negative bacteria. In 1994 Proctor and colleagues115 showed that the ATP analogue, 2-methylthio-ATP (2-MeS-ATP), inhibited endotoxin-stimulated release of toxic mediators such as TNF-α and IL-1β and protected mice from endotoxin-induced death. Interpretation of this early experiment is not straightforward, because 2-MeS-ATP is an agonist at P2Y2 as well as P2X purinoreceptors2,23; however, at P2X7, 2-MeS-ATP acts as a partial agonist and thus it is conceivable that it might antagonize P2X7 stimulation by locally secreted ATP and reduce LPS-dependent TNF-α and IL-1β release. Altogether, these observations suggest that P2X7 (and maybe other P2 receptors) take part in some crucial but as yet unknown steps in the complex chain of events leading to septic shock, either as a component of a paracrine/autocrine loop or as a binding site for LPS.116,117

Stimulation with extracellular nucleotides also switches on the inducible nitric oxide synthase (iNOS),116-118 a key enzyme for the bactericidal activity of macrophages. Nucleotides per se are ineffective, but coexposure to low doses of ATP (or UTP) and LPS produces a much higher stimulation of iNOS activity compared with LPS alone. In murine Raw 264.7 macrophages a prolonged (18 hours) incubation was needed to elicit nitrite release, suggesting that P2 stimulation acted by increasing iNOS gene expression rather than by increasing enzyme activity. Other data suggest that P2 receptors are involved in NO generation in a rather more complex fashion. Denlinger and coworkers showed that pretreatment with 2-MeS-ATP prevented iNOS expression and NO generation due to the subsequent addition of LPS,117 raising the issue of the possible participation of P2 receptors in LPS-dependent signaling.116,117 In addition, it has been recently shown that NO production due to Mycobacterium tuberculosis infection also occurs in P2X7 knockout mice and it is inhibited by P2 blockers,119 thus pointing to the participation of other P2X and P2Y receptors. There are an increasing number of papers suggesting that P2 receptors (namely P2X7) might have a role in endotoxin- or parasite-mediated macrophage stimulation. Besides the studies carried out in our laboratory showing that incubation of macrophages or microglia with oATP or apyrase inhibited LPS-dependent IL-1β release,9 other groups have reported that LPS-dependent NO release and nuclear factor (NF)-κB and mitogen-associated protein kinase (MAPK) activation are profoundly inhibited by oATP or by PPADS.116 MAPK in Raw 264.7 macrophages can also be stimulated via P2Y2, but the putative purinergic receptor involved in LPS-dependent activation does not seem to be a member of the P2Y family because oATP or PPADS, which block LPS-dependent stimulation, do not affect MAPK stimulation by UTP.118 In the light of the report that ATP triggers NF-κB activation via P2X7 and that this activation is blocked by oATP,119 it is likely that the P2 receptor that participates in LPS-dependent macrophage activation is P2X7.

A common event observed in many reactions involving mononuclear phagocytes is multinucleation: often during chronic inflammatory reactions macrophages differentiate into epitheloid cells that eventually fuse into large polykaryons named multinucleated giant cells (MGCs).120 Furthermore, in the bone, osteoclast precursors normally fuse to generate large elements with increasing bone resorption activity. MGCs are a common finding of widespread infectious diseases such as tuberculosis, but little is known about the molecular mechanism underlying fusion. In 1995, Falzoni et al120 suggested that the P2X7 receptor could be involved in MGC formation. Monocyte-derived human macrophages can be induced to fuse in vitro by incubation with concanavalin A or phytohemagglutinin, provided that contaminating lymphocytes are also present.121 Pretreatment with oATP fully inhibits this process, although other responses such as concanavalin A–dependent [Ca2+]i changes, chemotaxis, or expression of plasma membrane molecules thought to take part in cell fusion (eg, CD11a, CD18, and CD54) are unaffected.56 We have extended these studies to J774 macrophages and selected several clones that either express P2X7 to a very high level (P2X7-plus) or lack it altogether (P2X7-less). P2X7-plus cells spontaneously fuse in culture to form MGCs of different size and shape, containing from a few to 20 or more nuclei.77 A monoclonal antibody raised against the P2X7 outer domain prevents fusion of human macrophages in culture.122,123

The participation in ICE activation and IL-1β release, and in MGC formation establishes an intriguing link between the P2X7 receptor and chronic inflammation. Experiments from Molloy et al124 and Lammas et al125 further strengthen this link. Both groups investigated the effect of extracellular ATP on macrophage cultures infected with Mycobacterium bovis (bacille Calmette-Guérin) and reported that P2X7 activation caused killing of the phagocyte as well as of the intracellular pathogen. The mechanism involved is unknown, but a recent paper suggests that it might require activation of phospholipase D.126 Another possibility is that the known vesiculating activity of ATP91,127,128 affects viability of the intracellular pathogen by increasing phagosome-lysosome fusion, as suggested by some of the electron microscopy images reported by Molloy et al.124 In macrophages, stimulation of a phosphatidylcholine-selective phospholipase D by P2X7 agonists was reported as early as 1992 83 and shown to be independent of pore formation and of the ensuing Ca2+ influx.129 The mechanism by which an activated phospholipase D might partake in parasite killing is unknown but might be related to the enhanced rate of vesicle fusion observed in ATP-stimulated phagocytes. Ability of macrophages to eliminate intracellular parasites is enhanced upon activation with interferon-γ122; thus it might not be a coincidence that this cytokine and other proinflammatory factors also up-regulate P2X7 expression.66,130 It is also intriguing that to kill the intraphagosomal parasite, ATP concentrations cytotoxic for the macrophage need to be used or, in other words, parasite killing appears to be a consequence of macrophage death, as if intracellular parasite elimination via P2X7 was not a primary function of the receptor but rather an accessory consequence of its primary cytotoxic activity.

That extracellular ATP is a potent cytotoxic factor for macrophages was immediately apparent as soon as a thorough investigation of ATP receptors was started in these cells, and P2X7 was quickly identified as the culprit. Initially in Silverstein’s and later in our laboratory, murine macrophage clones were selected that showed an almost absolute refractoriness to ATP-mediated cytotoxicity.60,75,76,95 These cells showed a normal mobilization of Ca2+ influx.129 The mechanism by which an activated phospholipase D might partake in parasite killing is unknown but might be related to the enhanced rate of vesicle fusion observed in ATP-stimulated phagocytes. Ability of macrophages to eliminate intracellular parasites is enhanced upon activation with interferon-γ; thus it might not be a coincidence that this cytokine and other proinflammatory factors also up-regulate P2X7 expression.66,130
pretreatment with oATP or coincubation with apyrase or hexoki-
nase. In contrast to the P2X-plus clones, the P2X-less cells have a low rate of spontaneous death that is not affected by the presence of P2X7 blockers or ATP-hydrolyzing enzymes. The mechanism of ATP-dependent death can be either necrosis or apoptosis, depend-
ing on the length of incubation in the presence of the nucleotide and the dose. In our hands, ATP-pulsed J774 macrophages appear to die mostly by colloid-osmotic lysis; on the contrary, monocyte-
derived human macrophages, which incidentally are more resistant to ATP-mediated cytotoxicity, are prone to die by apoptosis. It is possible that the propensity of these cells to die by apoptosis is related to their lower susceptibility to ATP because we have previously observed that, to set in motion the complex machinery involved in apoptosis, a certain amount of time is needed that is clearly unavailable in those cells that are so sensitive to ATP as to decease quickly. An in-depth investigation of the apoptotic path-
ways triggered by ATP in macrophages has not been yet carried out, but we know from work in microglial cells that caspase-1, -3, and -8 are activated with the subsequent cleavage of the caspase substrates PARP (poly[ADP-ribose] polymerase) and lamin B. In addition, the crucial transcription factors, NF-κB and NFAT (nuclear factor of activated T cells), are also stimulated.

P2 receptors in dendritic cells

Dendritic cells are a newcomer in the purinergic field. It has been known for a while that epidermal Langerhans’ cells posses a powerful plasma membrane formalin-resistant ecto-ATPase that has been used as a histochemical marker, but their physiologic function was never understood. In 1993 Girolomoni and cowork-
ers demonstrated that human epidermal Langerhans’ cells can be permeabilized by ATP, albeit to a lesser degree than human keratinocytes or J774 macrophages. However, inhibition of ecto-
ATPase greatly enhanced sensitivity to ATP, and this led these authors to suggest that one of the possible physiologic functions of this ectoenzyme was protection of Langerhans’ cells against the noxious effects of extracellular ATP. Scattered reports have then followed suggesting that phagocytic cells of the thymus reticulum express a P2X7-like ATP-permeabilizing receptor, but only during the last few years has a systematic study of these receptors been carried out in human and mouse dendritic cells. Human dendritic cells were found to express mRNA for P2X1, P2X4, P2X5, and P2X7 as well as for P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11 receptors. Immunohistochemistry with an anti-P2X monoclonal antibody performed in human tonsils shows that a cell population of the marginal zone identified as dendritic cells heavily expresses P2X7, Scanty pharmacologic data suggest that at least P2Y1, P2Y2, and P2Y4 are functional and mediate a Ca2+ signal in these cells. P2X7 functions have been investigated in detail in human and mouse dendritic cells, and available evidence suggests that this receptor mediates cytokine release and might also participate in antigen presentation. During their investigation of P2Y receptors in human dendritic cells, Liu et al. observed that dendritic cells redirect their dendrites toward a nearby patch pipette leaking ATP, an incidental finding that might suggest that dendritic cells, like other mononuclear phagocytes, exhibit a P2Y-mediated chemotactic response to ATP. In addition, it has been shown that stimulation with UTP or uridine diphosphate (but surprisingly not with ATP) provided a potent stimulus for the cytokine gene transcription and secretion. Given the high expression of P2X7, it is not surprising that dendritic cells are exceedingly sensitive to the cytotoxic activity of ATP and readily die by apoptosis.

P2 receptors in lymphocytes

Lymphocyte responsivity to nucleotides has been known for many years: In 1978 Gregory and Kern reported that extracellular ATP stimulated proliferation of mouse thymocytes; Fishman et al in 1980 observed that in human peripheral lymphocytes ATP suppressed proliferation; presumably via generation of adenosine. In 1981 Ikehara et al. in some way reconciled these contrasting observations by showing that ATP stimulation of DNA synthesis was observed in lymphoid cells from the thymus and inhibition in cells from spleen, lymph nodes, and peripheral blood. These early observations were followed by a few other studies that overall were of little help in building a coherent picture of the responses of lymphoid cells to extracellular nucleotides, and they remained basically anecdotal. It was not until the end of the 1980s that a systematic approach to the study of purinergic receptor expression and function in lymphocytes was started.

Human B lymphocytes express P2Y receptors, as indicated by the ability of ATP and many other nucleotides (UTP, GTP, CTP, ITP, ADP, adenosine 5′-O-(3′-thiotriphosphate), ATPγS) to trigger Ca2+ release from intracellular stores. Human B lymphocytes also express P2X receptors, although of which particular subtype is still uncertain. Pharmacologic, electrophysiologic, and reverse transcriptase–polymerase chain reaction (RT-PCR) data suggest that the P2X7 receptor is present in these cells and might be up-regulated in chronic leukocytic leukemia cells (quite intriguingly it appears to be also up-regulated in lymphoblastoid cells from patients with Duchenne dystrophy). Rather surprisingly, however, B lymphocytes do not undergo the typical increase in permeability to aqueous solutes up to 900 d, suggesting that a pore of a smaller size, permeable to molecules up to 320 d, is generated by ATP: although ethidium bromide (314 d) is admitted, propidium bromide (414 d) is excluded. Furthermore, B lymphocytes are also poorly susceptible to ATP-mediated cytotoxicity. Human peripheral T lymphocytes lack P2Y receptors according to functional and pharmacologic studies but express a P2X-like ATP-activated channel. Unpublished data from our laboratory show that these cells express at the mRNA level P2X1, P2X4, and P2X7, although a significant variability is observed among samples from different subjects. ATP and BzATP cause in T lymphocytes a large influx of Na+ and Ca2+ from the extracellular medium that is fully prevented by pretreatment with oATP. Like in B lymphocytes, ATP treatment of T lymphocytes generates a pore smaller than that seen in macrophages or in HEK293 cells transfected with the recombinant P2X7. This might be due to the assembly of P2X1 or P2X4 subunits together with P2X7 into the receptor complex, but this is purely speculative.

Expression of P2 receptors in mouse lymphocytes has been more thoroughly investigated. RT-PCR data show that murine thymocytes express the message for P2X1, P2Y1, and P2Y2 receptors and accordingly undergo Ca2+ release from intracellular stores and an increase in plasma membrane permeability to external cations when challenged with ATP. Steroid hormones or cross-linking of T-cell receptor (TCR) by anti-TCR monoclonal antibody causes a transient enhancement of P2Y2 mRNA, suggesting that this could be an early event in response to a variety of activatory stimuli. Sensitivity to ATP in thymocytes changes
P2 receptors in polymorphonuclear granulocytes

Scattered evidence for a role of extracellular nucleotides in granulocyte responses has been present for a while, but a systematic investigation was only started at the end of the 1980s. Most studies concentrated on neutrophils, showing that ATP was able to trigger an increase in [Ca\textsuperscript{2+}], stimulation of phosphoinositide breakdown, superoxide anion generation, and granule exocytosis (both specific and azurophilic). In human neutrophils, ATP and UTP were reported to be equipotent for both the [Ca\textsuperscript{2+}], increase and superoxide anion formation, and ATP was also shown to stimulate phospholipase C and diacylglycerol generation as well as protein kinase C activity. It is of great interest in the light of the proposed proinflammatory role of extracellular ATP that this nucleotide also increases membrane expression of CD11b/CD18 and adhesion to albumin-coated latex beads. Because ATP is released by the endothelium and its local concentration is likely to increase during inflammation as a consequence of inactivation of ecto-ATPases by oxygen radicals, up-regulation of adhesion molecules by this nucleotide could be of relevance for leukocyte migration across the vessel wall. ATP also enhances the adhesion between neutrophils and pulmonary endothelial cells, a mechanism that might be relevant in syndromes such as adult respiratory distress syndrome and septic shock.

P2 subtype expression has not been thoroughly investigated in neutrophils, mainly because of the lack of selective antibodies. RT-PCR data show that human polymorphonuclear granulocytes express P2Y\textsubscript{4} and P2Y\textsubscript{6} but not P2Y\textsubscript{1} or P2Y\textsubscript{2} receptors. Among P2X receptors, the presence of P2X\textsubscript{7} was shown by Northern blotting and immunocytochemistry. It has been suggested that human neutrophils might express receptors for diadenosine polyphosphates, but evidence for this is preliminary. Besides neutrophils, eosinophils also express P2X receptors coupled to [Ca\textsuperscript{2+}], increases, actin reorganization, and stimulation of the NADPH oxidase. Interestingly, eosinophils show locomotive activity in response to ATP, ADP, and GTP. No data are available as to the P2 subtypes expressed.

P2 receptors in platelets

ADP is one of the best-known activators of platelet aggregation, but the receptors involved have been, at least partially, identified only during the last 5 years. Stimulation with ADP causes release of Ca\textsuperscript{2+} from intracellular stores, Ca\textsuperscript{2+} influx, phospholipase C activation, inhibition of stimulated adenylate cyclase, shape change, activation of fibrinogen receptors, and aggregation. ATP and ATP analogues are potent inhibitors of these responses. It has also been shown that ADP causes granule release and thromboxane A\textsubscript{2} production. It was initially thought that these effects were mediated by only one receptor named P2T; however, later studies led to the molecular and pharmacologic characterization in platelets of at least 2 of the known members of the P2 family: P2X\textsubscript{1} and P2Y\textsubscript{1}. With the availability of more selective platelet P2Y\textsubscript{1} and P2X\textsubscript{1} agonists and antagonist, it is becoming evident that the view that these are the P2 receptors solely responsible of ADP-mediated platelet activation is an oversimplification. It is clear that it is
possible to block ADP-mediated inhibition of stimulated adenylate cyclase activity without decreasing the ADP-dependent \([\text{Ca}^{2+}]\) rise. Thus it is postulated that ADP-triggered platelet activation is mediated by 3 receptors: One not yet cloned receptor (P2Y\text{ADP}) is coupled to inhibition of stimulated adenylate cyclase activity; a second (P2Y1) to phospholipase C activation, \(\text{InsP}_3\) formation, and \(\text{Ca}^{2+}\) release from intracellular stores; and a third one (P2X1) to fast \(\text{Ca}^{2+}\) influx across the plasma membrane.\[^{210,211}\] According to this proposal, the P2Y\text{ADP} receptor would coincide with the platelet P2Y\text{ADP} receptor (written in italics to signify that it is not yet cloned) receptor of the nomenclature established by IUPHAR.\[^{2,27}\] Development of selective platelet P2 receptor antagonists has progressed further than in other cell types, and some have already reached clinical applications. Two thienopyridine compounds, ticlopidine and clopidogrel, inhibit ADP-triggered platelet aggregation presumably by selectively blocking P2Y\text{ADP}. The limited structure-relationship analysis so far carried out suggests that 2-alkylthio-substituted analogues of ATP and AMP (eg, 2-MeS-ATP; 2-methylthioadenylyl 5\'-\text{phosphate} \(5'\)-\text{phosphosulphate} \[A3P5PS\]) are selective P2Y1 antagonists.\[^{21,22}\] The pharmacology of the platelet P2Y1 receptor was clarified when only high-performance liquid chromatography–purified nucleotides were used and care was taken to avoid degradation of triphosphate analogues to the corresponding diphosphates. These precautions are seldom taken in the analysis of nucleotide effects in other cells, and this may lead to a re-evaluation of the agonist activity of ATP in other cell models. It is likely that full platelet activation requires stimulation and cooperative signaling of all 3 receptors, but the initial data from knockout mice suggest a central role for P2Y1, because P2Y1-deficient animals showed increased bleeding time and reduced collagen- and ADP-induced thromboembolism.\[^{213}\] Interestingly, ADP-mediated adenylate cyclase inhibition was not reduced in platelets from the \(p2y1^{-/-}\) mouse. A P2X1-deficient \((p2x1^{-/-})\) mouse is also available,\[^{214}\] but no data on platelet function in this animal have been published. A patient was described who was affected by what appears to be a selective deficit in P2Y\text{ADP} receptor expression\[^{215}\] and, on the other hand, expression of P2Y1 (and P2X1) was found to be normal in a patient affected by a severe deficiency of ADP-triggered platelet activation.\[^{216}\] Presence of a functional ATP-activated P2X1 receptor raises intriguing questions on the interplay among different P2 receptors in platelet physiology, because ATP or ATP analogues were never shown to cause platelet activation. It might be that the P2X1 receptor is chronically desensitized in vivo due to continuous leakage of ATP/ADP from blood or endothelial cells, but this issue clearly needs further scrutiny.\[^{217}\] Injury to blood cells or to the vessel wall releases ATP that is quickly dephosphorylated to ADP by ecto-ATPases expressed on the endothelium. Furthermore, platelets themselves are a major source of ATP and ADP that are stored within dense granules to a concentration of about 1 M. Thus, ADP-triggered secretion activates an autocatalytic cycle of autocrine/paracrine stimulation by released nucleotides.\[^{218}\] Release of ATP from platelets can also feed back on the endothelial cells, inducing secretion of other factors involved in hemostasis and inflammation, such as von Willebrand factor.\[^{219}\] The key role of extracellular ATP and P2 receptors in hemostasis has been underscored by the surprising phenotype of \(cd39^{-/-}\) mice.\[^{220}\] It was expected that these mice showed a thrombotic diathesis due to enhanced platelet aggregation, because CD39 has been considered an inhibitor of platelet activation. On the contrary, \(cd39^{-/-}\) mice displayed prolonged bleeding times and failure to aggregate. These deficits were shown to be due to P2Y1 receptor desensitization dependent on an increased accumulation of extracellular ATP and were largely corrected by apyrase.

### P2 receptors in erythrocytes

Effects of extracellular ATP on erythrocytes were initially reported in 1972 by Parker and Snow,\[^{221}\] who showed that this nucleotide caused \(\text{Na}^+\) influx and \(\text{K}^+\) efflux paralleled by an increase in water content. As later demonstrated in other cell types, ion fluxes were prevented by \(\text{Mg}^{2+}\) or hexokinase plus glucose and potentiated by ethylenediaminetetraacetic acid. All other nucleotides tested were ineffective. An increase in plasma membrane permeability of erythrocytes was also reported by Trams,\[^{222}\] who showed a dramatic accumulation of extracellular adenylates in the presence of extracellular ATP. These authors concluded that ATP caused a permeability change in erythrocyte plasma membrane that allowed for leakage of cytoplasmic ATP (‘ATP-induced ATP release’). These data would suggest the expression by erythrocytes of a P2X-like receptor, but no further characterization of this phenomenon was carried out. Release of ATP under hypoxic conditions has also been reported,\[^{223}\] but the pathway involved was not elucidated. At variance with P2X2, erythrocyte P2Y receptors are more thoroughly characterized. Avian red blood cells express a typical P2Y1 receptor coupled to phospholipase \(\beta\) activation via a \(G\) protein of the \(G_q\) family.\[^{224,225}\] Erythrocytes are an ideal “integrator unit” in the blood because they express P2 receptors and at the same time readily release ATP. These properties, on the one hand, make these cells sensitive to ATP released by other blood elements (eg, platelets) and, on the other hand, endow them with the ability to modulate the function of circulating or endothelial cells by secreting large amounts of this nucleotide. It has been proposed that ATP release from erythrocytes could contribute to regulation of local blood flow by acting at P2Y receptors on vascular endothelium.\[^{226,227}\] ATP has a well-known NO releasing activity; thus, under ischemic conditions, when release from erythrocytes is maximal, ATP could be one of the local factors that counteract the decreased blood flow by inducing vasodilatation.

### P2 receptors on bone marrow hemopoietic precursors

According to the few available studies, all hemopoietic precursors isolated from mouse bone marrow, as opposed to stromal cells, are highly sensitive to the cytotoxic effect of ATP.\[^{228,229}\] This phenotypic property has made available a very efficient procedure for the isolation of highly purified marrow stromal cells or the deletion of hemopoietic cell precursors. The cytotoxic mechanisms appear to be dependent on the known pore-forming ability of ATP mediated
by P2X<sub>7</sub> activation and can be significantly enhanced by including in the reaction medium a low-molecular-weight nonpermeant poisonous agent such as potassium thiocyanate. This procedure might turn out helpful for the local treatment of tumors of hemopoietic origin.

Conclusions

For many years it was thought that receptors for extracellular nucleotides had a physiologic role only in excitable tissues; however, it is now increasingly clear that they are widespread and involved in signal transduction in several other tissues, including blood cells (Table 2). Drugs based on P2Y<sub>ADP</sub> antagonism are already in use as antithrombotic agents, and P2Y<sub>1</sub> blockers are being developed for this same purpose. Besides thrombosis, another promising field of application of P2 agonist/antagonist is inflammation. Ability of P2 receptors to mediate chemotaxis (via P2Y<sub>7</sub>), or cytotoxic responses and cytokine secretion (via P2X<sub>7</sub>), opens an entirely new perspective for the development of anti-inflammatory drugs. Chronic inflammatory diseases might be one of the first targets for the clinical application of selective P2X<sub>7</sub>-antagonists. These compounds might prove beneficial to reduce IL-1β release and granuloma formation. Finally, high expression of P2X<sub>7</sub> by lymphocytic leukemia cells, and its participation in the control of cell death and proliferation, suggests a novel and as yet fully unexplored approach to the treatment of lymphoproliferative disorders.

Note added in proof. Two recent papers suggest that circulating human monocytes express a functional P2X<sub>7</sub>-receptor coupled to IL-1β and IL-18 release. In addition, two papers show that exogenous ATP can be a differentiation factor for human dendritic cells.

References


Table 2. P2 receptors expressed by blood cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>P2Y</th>
<th>P2X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat/mouse peritoneal macrophages</td>
<td>P2Y</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
</tr>
<tr>
<td>BAC1.2F5 macrophages</td>
<td>P2Y</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;-like</td>
</tr>
<tr>
<td>RAW 264.7 macrophages</td>
<td>P2Y</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;-like</td>
</tr>
<tr>
<td>J774 macrophages</td>
<td>P2Y</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
</tr>
<tr>
<td>THP-1 macrophages</td>
<td>P2Y&lt;sub&gt;2&lt;/sub&gt;</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
</tr>
<tr>
<td>KG-1 myeloblastic cells</td>
<td>P2Y&lt;sub&gt;1&lt;/sub&gt;</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
</tr>
<tr>
<td>HL-60 myeloid cells</td>
<td>P2X&lt;sub&gt;5&lt;/sub&gt;, P2Y&lt;sub&gt;2&lt;/sub&gt;</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
</tr>
<tr>
<td>U937 monocytes</td>
<td>P2Y&lt;sub&gt;1&lt;/sub&gt;, P2Y&lt;sub&gt;2&lt;/sub&gt;, P2Y&lt;sub&gt;6&lt;/sub&gt;, P2Y&lt;sub&gt;4&lt;/sub&gt;</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
</tr>
<tr>
<td>Human macrophages</td>
<td>P2Y</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;-like</td>
</tr>
<tr>
<td>Mouse dendritic cells*</td>
<td>P2Y&lt;sub&gt;1&lt;/sub&gt;, P2Y&lt;sub&gt;2&lt;/sub&gt;</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;-like</td>
</tr>
<tr>
<td>Human dendritic cells†</td>
<td>P2Y&lt;sub&gt;1&lt;/sub&gt;, P2Y&lt;sub&gt;2&lt;/sub&gt;, P2Y&lt;sub&gt;6&lt;/sub&gt;, P2Y&lt;sub&gt;4&lt;/sub&gt;, P2Y&lt;sub&gt;11&lt;/sub&gt;</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;-like</td>
</tr>
<tr>
<td>Human Langerhans’ cells‡</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;-like</td>
<td></td>
</tr>
<tr>
<td>Human dendritic cells§</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>P-815 mastocytoma</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;-like</td>
<td></td>
</tr>
<tr>
<td>YAC lymphoma cells</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;-like</td>
<td></td>
</tr>
<tr>
<td>Mouse lymphocytes</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Murine thymocytes</td>
<td>P2Y&lt;sub&gt;1&lt;/sub&gt;, P2Y&lt;sub&gt;2&lt;/sub&gt;</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
</tr>
<tr>
<td>Human B lymphocytes</td>
<td>P2Y</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
</tr>
<tr>
<td>Human T lymphocytes</td>
<td>P2X&lt;sub&gt;6&lt;/sub&gt;, P2X&lt;sub&gt;1&lt;/sub&gt;, P2X&lt;sub&gt;7&lt;/sub&gt;</td>
<td>O.R.B. et al, unpublished data, 1999</td>
</tr>
<tr>
<td>Human PMN</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>P2Y&lt;sub&gt;1&lt;/sub&gt;</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;-like</td>
</tr>
<tr>
<td>RBL and rat mast cells</td>
<td>P2Y</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;-like</td>
</tr>
<tr>
<td>Mouse hemopoietic precursors</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;-like</td>
<td></td>
</tr>
</tbody>
</table>

P2 receptor expression is based on functional and pharmacologic evidence, mRNA detection by reverse transcriptase–polymerase chain reaction, or reactivity with specific antibodies. For P2Y receptors, lack of a subscript indicates that, although functional and pharmacologic data show expression of P2Y receptors, the individual P2Y subtypes have not been yet identified. For P2X receptors, “P2X<sub>7</sub>-like” means that functional and pharmacologic evidence strongly suggest expression of P2X<sub>7</sub>, but molecular data are missing. Failure to list a P2Y or P2X receptor for a given cell type means that there is lack of evidence for its expression, whether at the functional, pharmacologic, or molecular level.

FSCD indicates fetal skin–derived dendritic cell; PMN, polymorphonuclear; RBL, rat basophilic leukemia.

*Derived from bone marrow.
†Derived from blood precursors.
‡Derived from epidermis.
§Derived from tonsils and lymph nodes.


Nucleotide receptors: an emerging family of regulatory molecules in blood cells

Francesco Di Virgilio, Paola Chiozzi, Davide Ferrari, Simonetta Falzoni, Juana M. Sanz, Anna Morelli, Maurizia Torboli, Giorgio Bolognesi and O. Roberto Baricordi

Updated information and services can be found at:
http://www.bloodjournal.org/content/97/3/587.full.html

Articles on similar topics can be found in the following Blood collections
- Hemostasis, Thrombosis, and Vascular Biology (2485 articles)
- Review Articles (712 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml