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Review article

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

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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)

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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 $P2X_7$ 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 mechanisms[7-12] 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 hemopoietic 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 "P_2-purinoceptor"). P2 receptors are divided into 2 subfamilies: G protein–coupled (P2Y) and ligand-gated ion channels (P2X).[3,28-30] 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 ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$) and 7 P2X ($P2X_{1-7}$) receptors have been cloned and characterized pharmacologically[2] (Table 1). $P2Y_5$, $P2Y_7$, $P2Y_9$, and $P2Y_{10}$ have

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Table 1. P2Y and P2X receptor subtypes

P2Y	Amino acid number	P2X	Amino acid number
$P2Y_1$	362	$P2X_1$	399
$P2Y_2$	373	$P2X_2$†	472, 401
p2y3*	328	$P2X_3$	397
$P2Y_4$	352	$P2X_4$†	388, 329
$P2Y_6$	379	$P2X_5$	455
$P2Y_{11}$	371	$P2X_6$	379
		$P2X_7$	595

*p2y3 was cloned from chick brain and may be the chick homologue of the mammalian $P2Y_6$.

†$P2X_2$ and $P2X_4$ 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 $P2Y_6$.[2] $P2Y_8$ 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: $P2Y_{ADP}$.[2]

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.[2,31,32] 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, $P2Y_4$ and $P2Y_6$, UTP is more potent,[33-36] and at $P2Y_2$ ATP and UTP are equipotent.[31] At $P2Y_1$, UTP is inactive and ADP is reported to be equipotent or even more potent than ATP[37,38]; at $P2Y_{11}$ ATP is more potent than ADP and UTP is inactive.[39] With respect to the signal transduction pathway, $P2Y_1$ and $P2Y_2$ are coupled to stimulation of phospholipase C-β and inhibition of adenylate cyclase via $G_{q/11}$ and G_i proteins, respectively.[2] There are reports suggesting that $P2Y_2$ can also trigger stimulation of phospholipase D and breakdown of phosphatidylcholine, but the mechanism is unclear.[40] $P2Y_4$ and $P2Y_6$ seem to only couple to phosphoinositide breakdown, whereas $P2Y_{11}$ 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,[41] 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.[2] Reactive blue 2, trypan blue, and reactive red have also been used as P2Y antagonists, but they also block P2X-dependent responses.[2] Recently Harden and coworkers have introduced a number of nucleotide analogues as competitive $P2Y_1$ antagonists.[42,43] Pyridoxal phosphate (P5P) and pyridoxalphosphate-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[44,45] and then shown to be nearly ubiquitous[24,46,47]—that mediate fast permeability changes to monovalent and divalent cations (Na^+, K^+, and Ca^{++}). One of the members of this subfamily, $P2X_7$, 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[22,48-50] (Figures 1 and 2). All P2X receptors are likely to be multimeric structures of which 7 basic subunits have been cloned. The subunit composition of the native receptors has been resolved only in one case: rat dorsal ganglia that express a $P2X_2/P2X_3$ heteromer.[51] It is not known how P2X receptors assemble for most cell types. Subunit stoichiometry is likewise unknown but for $P2X_1$ and $P2X_3$, which have been shown to assemble as trimers or hexamers.[52] Whether other P2X receptors also assemble according to the same stoichiometry is not known. P2X receptors range from 379 to 595 amino acids and are thought to have 2 transmembrane hydrophobic domains separated by a bulky extracellular region harbouring 10 cysteines and 2 to 6 N-linked glycosylation sites.[2,30,44,45] The aminotermini and carboxytermini are both on the cytoplasmic side of the plasma membrane. This tertiary structure and membrane topology is reminiscent of that of other ion channels such as the epithelial amiloride-sensitive

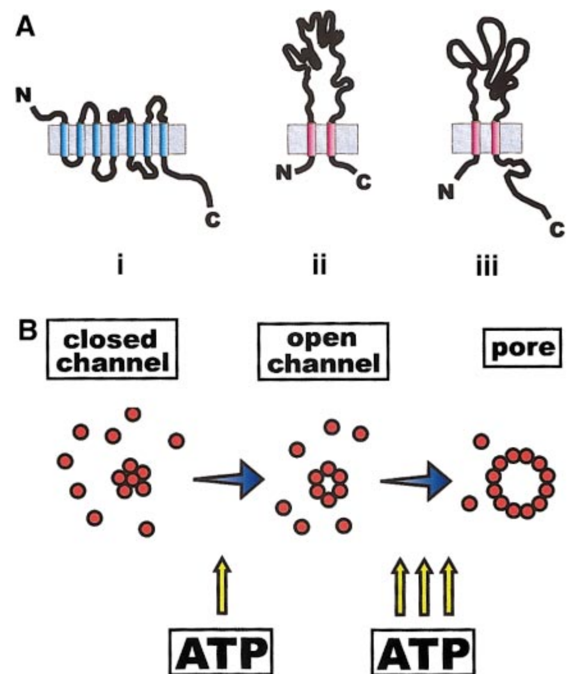

Figure 1. Membrane topology of P2Y and P2X receptor subunits. (A) P2Y receptors (i) are typical 7-membrane–spanning receptors made of a single polypeptide chain, with the N- and C-termini on the external and cytoplasmic side of the plasma membrane, respectively. P2X receptors (ii) are formed by subunits that span the plasma membrane twice and have both the N- and C-termini on the cytoplasmic side. The $P2X_7$ subunit differs from the other members of the P2X subfamily ($P2X_1$-$P2X_6$) in the extended carboxyterminal tail (iii). (B) It is hypothesized that $P2X_7$ receptor is generated by the aggregation of an unknown number of subunits (maybe 6) to form an ATP-activated channel. Recruitment of additional subunits causes formation of a nonselective pore (also see Figure 2).

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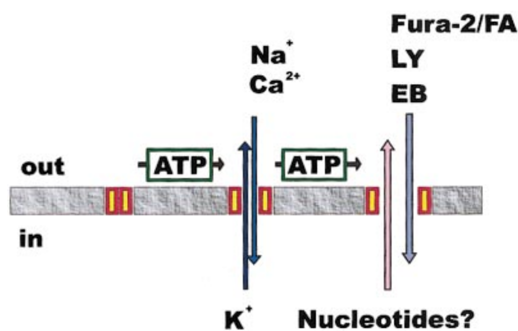

Figure 2. Permeability transition of $P2X_7$ receptor. A transient stimulation with ATP causes the opening of the $P2X_7$ channel and the concomitant Ca^{++} and Na^+ influx and K^+ efflux. However, upon sustained stimulation with ATP, the $P2X_7$ receptor undergoes a transition that generates a reversible membrane pore permeable to several low-molecular-weight hydrophylic solutes (Fura-2/FA, Fura-2 free acid; LY, lucifer yellow; EB, ethidium bromide) and to nucleotides.

Na^+ channel (ENaC), the degenerins cloned from *Caenorhabditis elegans*, and the inward rectifying K^+ channel (Kir).[30] Signal transduction occurs via fast Na^+ and Ca^{++} influx and K^+ efflux, leading to depolarization of the plasma membrane and an increase in the concentration of cytosolic Ca^{++} ($[Ca^{++}]_i$). 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.[53]

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 $P2X_1$, $P2X_4$, and $P2X_7$ can be obtained from at least 2 companies; in a few laboratories sera against all the members of the subfamily have been raised.[53,46,49,54] One monoclonal antibody selective for the human $P2X_7$ receptor has been produced and characterized by Buell and colleagues.[55] 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 $P2X_7$ agonist.[55]

The unique naturally occurring agonist of P2X receptors is ATP, albeit diadenosine polyphosphates, such as $P,^1P^4$-diadenosine tetraphosphate (Ap_4A) and $P,^1P^6$-diadenosine hexaphosphate (Ap_6A), are active at $P2X_1$ [2], and UTP has been reported to be an agonist at $P2X_3$ as well as $P2X_1$.[56,57] There is an ongoing debate, initiated by the pioneeristic experiments of Cockcroft and Gomperts in mast cells,[58,59] on whether P2X receptors recognize the bianionic (ATP^{2-}) or tetra-anionic (ATP^{4-}) form of the nucleotide. In physiologic solutions, the free acid ATP^{4-} is complexed by Mg^{++}, Ca^{++}, or H^+ to yield various mixtures of $MgATP^{2-}$, $CaATP^{2-}$, and $HATP^{3-}$, whereas a small amount (1%-10%, depending on the divalent cation concentration and the pH) is present as the fully dissociated tetra-anion. Removal of Mg^{++} and Ca^{++} and alkalinization of the medium increases the apparent affinity of ATP and BzATP for the native P2Z receptor of mast cells and other cell types[58-61] and for the recombinant $P2X_7$ receptor,[48,62] but addition of Mg^{++} quickly terminates stimulation. Interpretation of the effects of divalent cation removal is complicated by the concomitant inhibition of ecto-ATPase/ectonucleotidase activity, because these enzymes require the presence of divalent cations. Slowing of hydrolytic activity prolongs the half-life of the nucleotide, thus 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 Ca^{++} and Mg^{++} removal.[63] This ATP analogue is used to pharmacologically discriminate P2X receptor subtypes: $P2X_1$ and $P2X_3$ are sensitive to low (0.5-5 μM) concentrations, and $P2X_2$ and $P2X_{4-7}$ are activated by high (>100 μM) doses.[2] An often neglected finding is the high potency of BzATP at all P2X—not just $P2X_7$—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.[53]

Better antagonists, with better-characterized activity, are available at P2X than at P2Y receptors. PPADS is a noncompetitive inhibitor of most P2X receptors[53] and, depending on the experimental conditions, may act irreversibly. Oxidized ATP (oATP) was introduced 7 years ago as a selective P2Z ($P2X_7$) inhibitor,[64] 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.[64] 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.[65] 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 $P2X_7$ 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 $P2X_7$ receptor.[64,66-69] Refractoriness lasts several hours, until new receptors are inserted into the plasma membrane.

More recently, Wiley and colleagues have introduced another powerful blocker of $P2X_7$, compound 1-[*N,0*-bis(5-isoquinolinesulphonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62).[70] This molecule was originally used as an inhibitor of the calcium calmodulin–dependent kinase[71] and made its first appearance in the purinergic field in a study by Blanchard et al[72] aimed at investigating the role of the P2Z 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 $P2X_7$ receptor.[73] KN-62 is a very useful tool for short-term studies, but modification of long-term responses should be interpreted with caution because of concomitant inhibition of calcium calmodulin kinase. Surprenant and colleagues[74] have recently shown that Coomassie Brilliant Blue G selectively blocks rat $P2X_7$ with nanomolar affinity.

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.[60,75] Stimulation of this receptor triggered the same reversible increase in plasma membrane permeability to low-molecular-mass solutes

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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^{++} mobilization via a mechanism other than the ATP-permeabilizing receptor.[76] This was made possible by the selection by Steinberg and colleagues[75] of ATP-resistant J774 macrophage clones later shown to lack the $P2X_7$ 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 $P2X_7$ 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 $P2X_7$ is the only constitutive subunit or, rather, the native P2Z receptor is formed by the assembly of $P2X_7$ in association with other P2X subtypes. However, because $P2X_7$ reproduces all known effects of the native P2Z and cells resisting ATP-mediated permeabilization lack $P2X_7$, we will assume heretofore that the macrophage P2Z and $P2X_7$ 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 $P2X_7$.

P2Y and P2X subtype expression by macrophages

All murine macrophage lines so far investigated express P2Y receptors coupled to release of Ca^{++} from intracellular stores and IP_3 generation, but the individual subtypes have not been investigated in detail. Functional and molecular expression of $P2X_7$ 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 $P2X_7$.[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 monocytic cell line has been reported to express $P2X_7$ to a significant level.[88] However, $P2X_7$ receptor expression can differ significantly among cell batches propagated in different laboratories. Monocytes freshly isolated from peripheral blood express P2Y receptors but lack $P2X_7$, 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 $P2X_7$ expression when stimulated with ATP.[66,84] There appears to be an inverse correlation between $P2Y_2$ and $P2X_7$ expression during monocyte to macrophage maturation: $P2Y_2$ messenger RNA (mRNA) declines while $P2X_7$ mRNA increases.[89] Up-regulation of $P2X_7$ and acquisition of $P2X_7$-dependent responses are detectable within 24 hours of seeding human monocytes on plastic dishes. Up-regulation of $P2X_7$ 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 $[Ca^{++}]_i$. 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 Silverstein[60,75,76] 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^{++} from intracellular stores (P2Y) and the ATP-permeabilizing (P2Z/$P2X_7$) receptor were 2 separate entities with widely different nucleotide selectivity and affinity and likely involved in different responses.[76] In J774 macrophages, the concentration of ATP giving one half of the maximal response (EC_{50}) for Ca^{++} 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 $P2X_7$, was reported to be between 250 and 400 μM,[93] but a lower EC_{50} was reported for $P2X_7$-triggered Ca^{++} rise in thioglycollate-elicited mouse peritoneal macrophages.[94] 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 $P2X_7$ receptor.[76,95] The UTP EC_{50} for Ca^{++} release from intracellular stores is between 300 and 500 nM [76] 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 $P2X_7$.

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 chemotact 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 $[Ca^{++}]_i$ that can be closely mimicked by the addition of cell lysate or of ATP at micromolar doses.[97] 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

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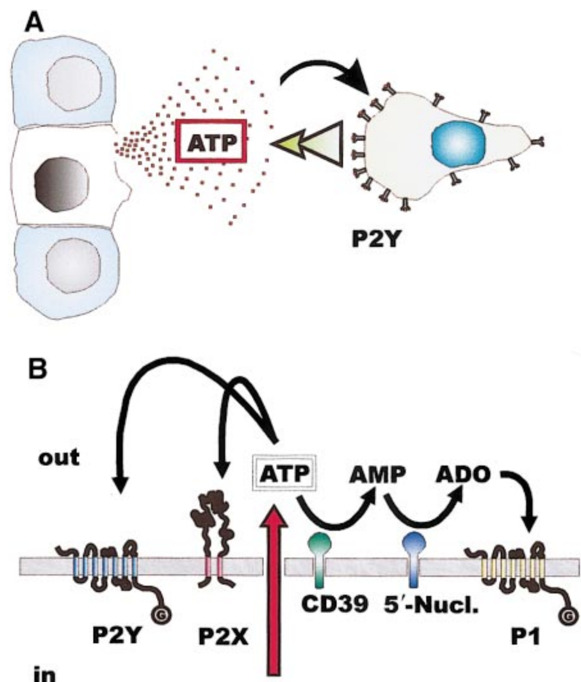

Figure 3. Fate of released ATP: possible role in leukocyte chemotaxis. (A) The intracellular ATP concentration is in the 5 to 10 mM range; thus, an ATP gradient capable of driving leukocyte chemotaxis by acting at P2Y receptors is likely to occur at sites of cell or tissue damage. (B) ATP released into the pericellular milieu can either ligate P2Y or P2X receptors or be hydrolyzed by plasma membrane ecto-ATPases or ecto-ATP diphosphohydrolase (CD39). Hydrolysis of AMP by 5′-nucleotidase generates adenosine that activates P1 purinergic receptors.

receptor. Thus it can be hypothesized that ATP and other intracellular nucleotides function as early alarm signals that alert macrophages of even minor cell and tissue damage (a response could be elicited with as little as 100 nM ATP) (Figure 3).

The $[Ca^{++}]_i$ rise could also be exploited by the macrophages for the potentiation of antimicrobial defense mechanisms. Nucleotides by themselves are unable to activate the macrophage NADPH oxidase but enhance superoxide generation stimulated by phagocytosable particles.[98] It is conceivable that P2 receptors could also be used as an amplification system to spread the alarm by generating additional inflammatory mediators. In murine and human macrophages, extracellular ATP triggers release of TNF-α and interleukin (IL)-1β. P2 receptors involved in TNF-α release have not been identified and there is accordingly little insight into the molecular mechanism involved. An early report in Raw 264.7 murine macrophages[99] suggests that ATP might act by enhancing the level of TNF-α mRNA, and this would not require priming by other proinflammatory factors, but a much more detailed investigation is clearly needed.

Participation of P2 receptors in IL-1β secretion is more firmly established and dissected mechanistically. It has been known for a while that LPS-dependent release of the proinflammatory cytokine IL-1β from macrophages and microglial cells, in contrast to peripheral blood monocytes, is a slow and inefficient process that leads to extracellular accumulation of minute amounts of this cytokine and mainly of the high-molecular-weight (31-34 kd), uncleaved, biologically inactive, procytokine form.[100,101] This finding has led many investigators to postulate that a second stimulus is needed to trigger processing and secretion of the 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,[102] and in 1992 Gabel and coworkers reported that mature IL-1β formation could be induced by the K^+ ionophore nigericin.[101] What is in common between nigericin and ATP? Perregaux and coworkers[101] reasoned that both nigericin and ATP decrease intracellular K^+ levels[60] and that perhaps this ionic perturbation was needed to activate the enzyme that cleaves pro–IL-1β into mature IL-1β, ie, IL-1β–converting enzyme (ICE), also known as caspase-1.[103] Later studies fulfilled this prediction because ATP was shown to trigger IL-1β release via a nonlytic mechanism in many different mononuclear phagocytic cells, and release was inhibited by procedures that prevented K^+ efflux [85,104-106] (Figure 4). In support of a key role for K^+ in ICE activation, Cheneval et al[107] 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.[107] 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.[106,108] Furthermore, macrophages isolated from mice deficient in ICE were unable to process pro–IL-1β upon challenge with LPS plus ATP.[109] Finally, involvement of $P2X_7$ in ATP-mediated ICE activation is supported by (*a*) agonist and antagonist profile of cytokine release,[85] (*b*) blockade by a specific anti-$P2X_7$ monoclonal antibody,[55] and (*c*) detection of ICE proteolytic fragments (p20 and p10) in ATP-stimulated microglial cells.[106,110] There are no clues as to how a decrease in K^+ concentration may activate ICE autoprocessing; nevertheless, K^+ provides a straightforward link between $P2X_7$ and ICE because opening of the $P2X_7$ channel/pore provides a very fast pathway for K^+ efflux.[60,104] 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.[111]

In human monocytes, ATP is a powerful stimulus not only for caspase-1 activation but also for the externalization of mature caspase-1 subunits.[112] 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, eg, by inducing exocytosis of IL-1β–containing specialized vesicles (late endosomes or lysosomes), as recently suggested by Rubartelli and coworkers.[113] 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,

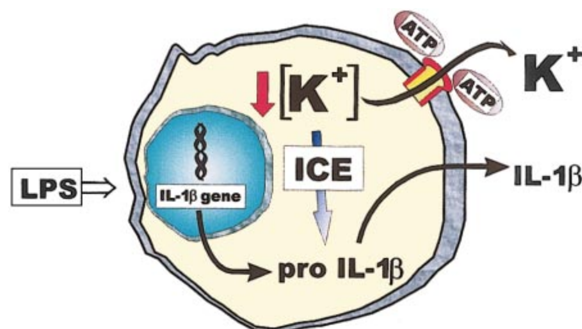

Figure 4. Model for $P2X_7$-mediated ICE/caspase-1 activation and IL-1β maturation. Stimulation with LPS triggers IL-1β gene transcription and pro–IL-1β accumulation. Opening of the $P2X_7$ pore by extracellular ATP causes a large K^+ efflux that triggers proteolytic activation of ICE and cleavage of pro–IL-1β. Mature IL-1β is then secreted through an unknown pathway.

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as suggested by studies in human and mouse macrophages and mouse microglia [9,114]

Participation of $P2X_7$ 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 colleagues[115] 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 P2Y as well as P2X purinoceptors[2,3]; however, at $P2X_7$ 2-MeS-ATP acts as a partial agonist and thus it is conceivable that it might antagonize $P2X_7$ stimulation by locally secreted ATP and reduce LPS-dependent TNF-α and IL-1β release. Altogether, these observations suggest that $P2X_7$ (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[9] or as a binding site for LPS.[115,116]

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 $P2X_7$ 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 $P2X_7$) 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 P2Y, 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.[116] In the light of the report that ATP triggers NF-κB activation via $P2X_7$ and that this activation is blocked by oATP,[110] it is likely that the P2 receptor that participates in LPS-dependent macrophage activation is $P2X_7$.

A common event observed in many reactions involving mononuclear phagocytes is multinucleation: often during chronic inflammatory reactions macrophages differentiate into epithelioid cells that eventually fuse into large polykarions 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 al[66] suggested that the $P2X_7$ 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 $[Ca^{++}]_i$ changes, chemotaxis, or expression of plasma membrane molecules thought to take part in cell fusion (eg, CD11a, CD18, and CD54) are unaffected.[66] We have extended these studies to J774 macrophages and selected several clones that either express $P2X_7$ to a very high level ($P2X_7$plus) or lack it altogether ($P2X_7$less). $P2X_7$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 $P2X_7$ 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 $P2X_7$ receptor and chronic inflammation. Experiments from Molloy et al[124] and Lammas et al[125] 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 $P2X_7$ 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 ATP[91,127,128] 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 $P2X_7$ agonists was reported as early as 1992 [83] and shown to be independent of pore formation and of the ensuing Ca^{++} 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[125]; thus it might not be a coincidence that this cytokine and other proinflammatory factors also up-regulate $P2X_7$ 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 $P2X_7$ 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 $P2X_7$ 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 Ca^{++} from intracellular stores in response to ATP, but no permeabilization of the plasma membrane, and accordingly lacked reactivity to anti-$P2X_7$ antibodies.[77] Blockade of the $P2X_7$ receptor by oATP or KN-62 abrogated ATP-dependent cytotoxicity. The role of P2 receptors in cytotoxicity is usually tested in the presence of exogenous ATP, but it cannot be excluded that ATP spontaneously released by cell monolayers may provide a chronic cytolytic stimulus by acting as an autocrine/paracrine factor. We have tested this hypothesis in $P2X_7$plus J774 macrophage cultures grown to confluence. These macrophage clones show an unusually high rate of spontaneous cell death that can be significantly reduced by

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pretreatment with oATP or coincubation with apyrase or hexokinase.[131] In contrast to the $P2X_7$plus clones, the $P2X_7$less cells have a low rate of spontaneous death that is not affected by the presence of $P2X_7$ blockers or ATP-hydrolyzing enzymes. The mechanism of ATP-dependent death can be either necrosis or apoptosis, depending 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 colloido-osmotic lysis; on the contrary, monocyte-derived human macrophages, which incidentally are more resistant to ATP-mediated cytotoxicity, are prone to die by apoptosis.[124,125] 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[132] 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 pathways 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.[106,133] In addition, the crucial transcription factors, NF-κB and NFAT (nuclear factor of activated T cells), are also stimulated.[110,134]

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,[135] but their physiologic function was never understood. In 1993 Girolomoni and coworkers[136] 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 $P2X_7$-like ATP-permeabilizing receptor,[137] but only during the last few years has a systematic study of these receptors been carried out in human and mouse dendritic cells.[138-143]

Human dendritic cells were found to express mRNA for $P2X_1$, $P2X_4$, $P2X_5$, and $P2X_7$ as well as for $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors.[140] Immunohistochemistry with an anti-$P2X_7$ monoclonal antibody performed in human tonsils shows that a cell population of the marginal zone identified as dendritic cells heavily expresses $P2X_7$.[55] Scanty pharmacologic data suggest that at least $P2Y_1$, $P2Y_2$, and $P2Y_4$ are functional and mediate a Ca^{++} signal in these cells.[139] $P2X_7$ 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 particpate in antigen presentation.[141-142] During their investigation of P2Y receptors in human dendritic cells, Liu et al[139] 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.[144] Given the high expression of $P2X_7$, it is not surprising that dendritic cells are exceedingly sensitive to the cytotoxic activity of ATP and readily die by apoptosis.[141,145] Whether this may have relevance in the overall process of modulation of the immune response is presently unknown.

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[146]; Fishman et al in 1980 observed that in human peripheral lymphocytes ATP suppressed proliferation,[147] presumably via generation of adenosine. In 1981 Ikehara et al[148] in some way reconcilied 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.[149,150] 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.[151-157]

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 Ca^{++} release from intracellular stores.[158] 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 $P2X_7$ 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).[154,159-161] 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.[159-162] 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.[163] Unpublished data from our laboratory show that these cells express at the mRNA level $P2X_1$, $P2X_4$, and $P2X_7$, although a significant variability is observed among samples from different subjects. ATP and BzATP cause in T lymphocytes a large influx of Na^+ and Ca^{++} 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 $P2X_7$.[48,62] This might be due to the assembly of $P2X_1$ or $P2X_4$ subunits together with $P2X_7$ 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 $P2X_1$, $P2Y_1$, and $P2Y_2$ receptors and accordingly undergo Ca^{++} release from intracellular stores and an increase in plasma membrane permeability to external cations when challenged with ATP.[164-166] Steroid hormones or cross-linking of T-cell receptor (TCR) by anti-TCR monoclonal antibody causes a transient enhancement of $P2Y_2$ mRNA, suggesting that this could be an early event in response to a variety of activatory stimuli.[167] Sensitivity to ATP in thymocytes changes

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depending on the stage of maturation: $CD4^+CD8^-TCR^{high}$ thymocytes were found to be very sensitive to ATP-mediated lysis[166]; large double-positive proliferating thymocytes were much less responsive compared with those terminally differentiated.[168] As in the case of human lymphocytes, the cut-off of the ATP-gated plasma membrane channel for mouse lymphocytes appears to be slightly over 300 d (ethidium is admitted, propidium is excluded).[166] Functional and pharmacologic data support expression of $P2X_7$ by mouse lymphocytes,[155,156,166] but there is little molecular evidence to corroborate this claim. At variance with human lymphocytes, mouse thymocytes are readily killed by ATP.[155,156] In double-positive ($CD4^+CD8^+$) immature thymocyte populations, expression of $P2X_1$ correlated with susceptibility to undergo apoptosis upon dexamethasone treatment,[164] and incubation in the presence of apyrase blocked the process, as if dexamethasone induced $P2X_1$ activation via autocrine/paracrine release of ATP. Ability of extracellular ATP to cause apoptosis of mouse cell lines was initially documented in P-815 mastocytoma and YAC lymphoid cells and subsequentely extended to thymocytes.[132,169] Among mouse lymphocytes, cytotoxic T-lymphocyte clones, cytotoxic T lymphocytes from peritoneal exudates, and lymphokine-activated killer cells turned out to be fully insensitive to ATP.[132,155] Whether this is due to lack of P2 purinergic receptors or to high expression of ecto-ATPase/ectonucleotidases it is not known. The potent cytotoxic activity of extracellular ATP and the remarkable resistance of cytotoxic T lymphocytes and lymphokine-activated killer cells instigated speculations on the possible participation of ATP receptors in target cell killing, as a pathway alternative or parallel to perforin or lymphotoxin.[156,157,170] However, it has been very difficult to provide sound experimental support to this hypothesis.[171]

The role of P2X receptors in the control of lymphocyte proliferation could be more complex than just being effectors of cell death. We have recently examined the effect of transduction of $P2X_7$less human B-lymphoid cells with the $P2X_7$ receptor complementary DNA and have surprisingly found that its expression confers a proliferation advantage in the absence of serum.[172] We have not yet dissected the biochemical mechanism underlying the enhanced growth rate of the $P2X_7$ transfectants, but we believe that it involves autocrine stimulation by ATP, because incubation with apyrase or hexokinase or pretreatment with oATP abrogated proliferation of $P2X_7$ transfectants in the absence of serum.[172] Whether these observations are relevant for tumors arising from hemopoietic cells is under investigation.

Stimulation with extracellular ATP causes shedding of the cell adhesion molecule L-selectin (CD62L) and the low-affinity receptor for IgE (CD23) from B chronic lymphocytic leukemic cells.[173,174] These cells express $P2X_7$, and agonist/antagonist studies suggest that the receptor involved is $P2X_7$.[162] CD23 and L-selectin are normally found in high amounts in sera from patients with B chronic lymphocytic leukemia, and this could be due to ATP-dependent shedding via $P2X_7$ stimulation.

P2 receptors in polymorphonuclear granulocytes

Scattered evidence for a role of extracellular nucleotides in granulocyte responses has been present for a while,[175,176] but a systematic investigation was only started at the end of the 1980s.[177-181] Most studies concentrated on neutrophils, showing that ATP was able to trigger an increase in $[Ca^{++}]_i$, stimulation of phosphoinositide breakdown, superoxide anion generation, and granule exocytosis (both specific and azurophilic).[182,183] In human neutrophils, ATP and UTP were reported to be equipotent for both the $[Ca^{++}]_i$ increase and superoxide anion formation,[178,179] and ATP was also shown to stimulate phospholipase C and diacylglycerol generation as well as protein kinase C activity.[183,184] 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.[185] 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,[186] 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.[187,188] Of course, it cannot be excluded that the ATP effect could be at least in part mediated by its hydrolysis to adenosine,[189] but recent data suggest that ecto-ATPase activity has an inhibitory rather than stimulatory effect on granulocyte-endothelium interaction.[190] Dubyak and coworkers reported that chronic stimulation with ATPγS or UTP could drive differentiation of myelomonocytic progenitor cells (HL-60 and U937).[191] Later studies showed that myeloid progenitors express $P2Y_1$ at earlier and $P2Y_2$ at later stages of differentiation.[192] The myeloid progenitors HL-60 cells also express P2 Y_{11}.[39]

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 $P2Y_4$ and $P2Y_6$ but not $P2Y_1$ or $P2Y_2$ receptors.[5] Among P2X receptors, the presence of $P2X_7$ was shown by Northern blotting and immunocytochemistry.[49] It has been suggested that human neutrophils might express receptors for diadenosine polyphosphates,[193] but evidence for this is preliminary. Besides neutrophils, eosinophils also express P2 receptors coupled to $[Ca^{++}]_i$ increases, actin reorganization, and stimulation of the NADPH oxidase.[194,195] Interestingly, eosinophils show locomotive activity in response to ATP, ADP, and GTP.[194] 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,[25,196-198] but the receptors involved have been, at least partially, identified only during the last 5 years. Stimulation with ADP causes release of Ca^{++} from intracellular stores, Ca^{++} influx, phospholipase C activation, inhibition of stimulated adenylate cyclase, shape change, activation of fibrinogen receptors, and aggregation.[199-201] ATP and ATP analogues are potent inhibitors of these responses. It has also been shown that ADP causes granule release and thromboxane A_2 production.[202,203] 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: $P2X_1$ [204-207] and $P2Y_1$.[208,209] With the availability of more selective platelet $P2Y_1$ and $P2X_1$ 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

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possible to block ADP-mediated inhibition of stimulated adenylate cyclase activity without decreasing the ADP-dependent $[Ca^{++}]_i$ rise. Thus it is postulated that ADP-triggered platelet activation is mediated by 3 receptors: One not yet cloned receptor ($P2T_{AC}$) is coupled to inhibition of stimulated adenylate cyclase activity; a second ($P2Y_1$) to phospholipase C activation, $InsP_3$ formation, and Ca^{++} release from intracellular stores; and a third one ($P2X_1$) to fast Ca^{++} influx across the plasma membrane.[210,211] According to this proposal, the $P2T_{AC}$ receptor would coincide with the platelet $P2Y_{ADP}$ (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 $P2Y_{ADP}$. 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′-(β,γ-methylene)-diphosphonate [2-MeS-β,γ-Me-ATP]; 2-propylthioadenylyl 5′-(β,γ-difluoromethylene)-diphosphonate [ARL 66096]; and 2-propylthioadenylyl 5′-(β,γ-dichloromethylene)-diphosphonate [ARL 67085]) are selective competitive antagonists at $P2Y_{ADP}$, and 3′-substituted AMP analogues (eg, adenosine 3′-phosphate 5′-phosphosulphate [A3P5PS]) are selective $P2Y_1$ antagonists.[212] The pharmacology of the platelet $P2Y_1$ 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 $P2Y_1$, because $P2Y_1$-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 $p2y1^{-/-}$ mice. A $P2X_1$-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 $P2Y_{ADP}$ receptor expression[215] and, on the other hand, expression of $P2Y_1$ (and $P2X_1$) 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 $P2X_1$ 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 $P2X_1$ 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*/ATP diphosphohydrolase knockout ($cd39^{-/-}$) 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 $P2Y_1$ 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 Na^+ influx and K^+ efflux paralleled by an increase in water content. As later demonstrated in other cell types, ion fluxes were prevented by 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 $P2X_7$-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 P2X, erythrocyte P2Y receptors are more thoroughly characterized. Avian red blood cells express a typical $P2Y_1$ receptor coupled to phospholipase β 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

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Table 2. P2 receptors expressed by blood cells

Cell type	P2Y	P2X	Reference no.
Rat/mouse peritonel macrophages	P2Y	$P2X_7$	49, 77, 79-82, 94
BAC1.2F5 macrophages	P2Y	$P2X_7$-like	83
RAW 264.7 macrophages	P2Y	$P2X_7$-like	81, 116
J774 macrophages	P2Y	$P2X_7$	60, 75-77
THP-1 macrophages	$P2Y_2$	$P2X_7$	5, 88
KG-1 myeloblastic cells	$P2Y_1$		192
HL-60 myeloid cells	$P2Y_2$, $P2Y_{11}$	$P2X_1$	39, 192, 232
U937 monocytes	$P2Y_2$, $P2Y_6$		5
Human monocytes	$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$		5, 89
Human macrophages	P2Y	$P2X_7$	77, 79, 89
FSDC	P2Y	$P2X_7$	141
Mouse dendritic cells*		$P2X_7$	141
Human dendritic cells†	$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$	$P2X_1$, $P2X_4$, $P2X_5$, $P2X_7$	139, 140, 142
Human Langerhans' cells‡		$P2X_7$-like	136
Human dendritic cells§		$P2X_7$	55, 137
P-815 mastocytoma		$P2X_7$-like	132
YAC lymphoma cells		$P2X_7$-like	132
Mouse lymphocytes		$P2X_7$	49, 155, 166
Murine thymocytes	$P2Y_1$, $P2Y_2$	$P2X_1$	164, 165, 167, 168
Human B lymphocytes	P2Y	$P2X_7$	154, 158, 160, 161, 162
Human T lymphocytes		$P2X_1$, $P2X_4$, $P2X_7$	163, O.R.B. et al, unpublished data, 1999
Human PMN	$P2Y_4$, $P2Y_6$	$P2X_7$	5, 49
Human platelets	$P2Y_1$	$P2X_1$	204-209
Erythrocytes	$P2Y_1$	$P2X_7$-like	224, 225
RBL and rat mast cells	P2Y	$P2X_7$-like	58, 59, 230, 231
Mouse hemopoietic precursors		$P2X_7$-like	228, 229

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, "$P2X_7$-like" means that functional and pharmacologic evidence strongly suggest expression of $P2X_7$, 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, polimorphonuclear; RBL, rat basophilic leukemia.

*Derived from bone marrow.

†Derived from blood precursors.

‡Derived from epidermis.

§Derived from tonsils and lymph nodes.

by $P2X_7$ activation and can be significantly enhanced by including in the reaction medium a low-molecular-weight nonpermeant poisonous agent such as potassium thiocyanate.[228,229] 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 $P2Y_{ADP}$ antagonism are already in use as antithrombotic agents, and $P2Y_1$ 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), or cytotoxic responses and cytokine secretion (via $P2X_7$), 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 $P2X_7$ antagonists. These compounds might prove beneficial to reduce IL-1β release and granuloma formation. Finally, high expression of $P2X_7$ 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 $P2X_7$ receptor coupled to IL-1β and IL-18 release.[233,234] In addition, two papers show that exogeneous ATP can be a differentiation factor for human dendritic cells.[235,236]

References

1. Burnstock G. A basis for distinguishing two types of purinergic receptors. In: Straub RW, Bolis L, eds. Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach. New York, NY: Raven Press; 1978:107-119.
2. Ralevic V, Burnstock G. Receptors for purines and pyrimidines. Pharmacol Rev. 1998;50:413-492.
3. Abbracchio MP, Burnstock G. Purinoceptors: are there families of P2X and P2Y purinoceptors? Pharmacol Ther. 1994;64:445-475.
4. Gordon JL. Extracellular ATP: effects, sources and fate. Biochem J. 1986;233:309-319.
5. Jin J, Dasari VR, Sistare FD, Kunapuli SP. Distribution of P2Y receptor subtypes on hematopoietic cells. Br J Pharmacol. 1998;123:789-794.
6. Burnstock G. The past, present and future of purine nucleotides as signalling molecules. Neuropharmacology. 1997;36:1127-1139.
7. Filippini A, Taffs RE, Sitkovsky MV. Extracellular ATP in T-lymphocyte activation: possible role in effector functions. Proc Natl Acad Sci U S A. 1990;87:8267-8271.
8. Jorgensen NR, Geist ST, Civitelli R, Steinberg TH. ATP and gap junction-dependent intercellular calcium signalling in osteoblastic cells. J Cell Biol.1997;139:497-506.
9. Ferrari D, Chiozzi P, Falzoni S, Hanau S, Di Virgilio F. Purinergic modulation of interleukin-1β release from microglial cells stimulated with bacterial endotoxin. J Exp Med. 1997;185:579-582.

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10. Mitchell CH, Carrè DA, McGlinn AM, Stone RA, Civan MM. A release mechanism for stored ATP in ocular ciliary epithelial cells. Proc Natl Acad Sci U S A. 1998;95:7174-7178.
11. Cotrina ML, Lin JH, Alves-Rodriguez A, et al. Connexins regulate calcium signalling by controlling ATP release. Proc Natl Acad Sci U S A. 1998; 95:15735-15740.
12. Jiang Q, Mak D, Devidas S, et al. Cystic fibrosis transmembrane conductance regulator-associated ATP release is controlled by a chloride sensor. J Cell Biol. 1998;143:645-657.
13. Holmsen H, Storm E, Day HJ. Determination of ATP and ADP in blood platelets. Anal Biochem. 1972;46:489-501.
14. Meyers KM, Holmsen H, Seachord CL. Comparative study of platelet dense granule constituents. Am J Physiol. 1982;243:R454–R461.
15. Kaczmarek E, Koziak K, Sevigny J, et al. Identification and characterization of CD39 vascular ATP diphosphohydrolase. J Biol Chem. 1996;271: 33116-33122.
16. Wang TF, Guidotti G. CD39 is an ecto-(Ca^{2+}, Mg^{2+})-apyrase. J Biol Chem. 1996;271:9898-9901.
17. Zimmermann H, Braun N. Ecto-nucleotidases: molecular structures, catalytic properties and functional roles in the nervous system. Prog Brain Res. 1999;120:371-385.
18. Beaudoin AR, Grondin G, Gendron FP. Immunolocalization of ATP diphosphohydrolase in pig and mouse brains and sensory organs of the mouse. Prog Brain Res. 1999;120:387-395.
19. Redegeld FA, Caldwell CC, Sitkovsky MV. Ecto-protein kinases: ecto-domain phosphorylation as a novel target for pharmacological manipulation? Trends Pharmacol Sci. 1999;20:453-459.
20. Dubyak GR. Signal transduction by P2-purinergic receptors for extracellular ATP. Am J Respir Cell Mol Biol. 1991;4:295-300.
21. Dubyak GR, el-Moatassim C. Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. Am J Physiol. 1993;265: C577–C606.
22. Di Virgilio F. The P2Z purinoceptor: an intriguing role in immunity, inflammation and cell death. Immunol Today. 1995;16:524-528.
23. Fredholm BB. Purines and neutrophil leukocytes. Gen Pharmacol. 1997;28:345-350.
24. Di Virgilio F, Chiozzi P, Falzoni S, et al. Cytolytic P2X purinoceptors. Cell Death Differ. 1998;5:191-199.
25. Kunapuli SP, Daniel JL. P2 receptor subtypes in the cardiovascular system. Biochem J. 1998;336: 513-523.
26. Hourani S, Hall DA. P2T purinoceptors: ADP receptors on platelets. Ciba Found Symp. 1996; 198:52-69.
27. IUPHAR Compendium on Receptor Characterization and Classification. International Union of Pharmacology. London: IUPHAR Media; 1998.
28. Burnstock G, Kennedy C. Is there a basis for distinguishing two types of P2-purinoceptor? Gen Pharmacol.1985;16:433-440.
29. Barnard EA, Burnstock G, Webb TE. G protein-coupled receptors for ATP and other nucleotides: a new receptor family. Trends Pharmacol Sci. 1994;15:67-70.
30. North RA. Families of ion channels with two hydrophobic segments. Curr Opin Cell Biol. 1996;8: 474-483.
31. Lustig KD, Shiau AK, Brake AJ, Julius D. Expression cloning of an ATP receptor from mouse neuroblastoma cells. Proc Natl Acad Sci U S A. 1993; 90:5113-5117.
32. Webb TE, Simon J, Krishek BJ, et al. Cloning and functional expression of a brain G-protein-coupled ATP receptor. FEBS Lett. 1993;324:219-225.
33. Communi D, Motte S, Boeynaems J-M, Pirotton S. Pharmacological characterization of the human $P2Y_4$ receptor. Eur J Pharmacol. 1996;317: 383-389.
34. Charlton SJ, Brown CA, Weisman GA, Turner JT, Erb L, Boarder ML. Cloned and transfected P2Y4 receptors: characterization of suramin and PPADS-insensitive response to UTP. Br J Pharmacol. 1996;119:1301-1303.
35. Chang K, Hanaoka K, Kumada M, Takuwa Y. Molecular cloning and functional analysis of a novel P2 nucleotide receptor. J Biol Chem. 1995;270: 26152-26158.
36. Communi D, Parmentier M, Boeynaems JM. Cloning, functional expression and tissue distribution of the human $P2Y_6$ receptor. Biochem Biophys Res Commun. 1996;222:303-308.
37. Henderson DJ, Elliot DG, Smith GM, Webb TE, Dainty IA. Cloning and characterization of a bovine P2Y receptor. Biochem Biophys Res Commun.1995;212:648-656.
38. Ayyanathan K, Webb TE, Sandhu AK, Athwal RS, Barnard EA, Kunapuli SP. Cloning and chromosomal localization of the human $P2Y_1$ purinoceptor. Biochem Biophys Res Commun. 1996;218: 783-788.
39. Communi D, Govaerts C, Parmentier M, Boeynaems JM. Cloning of a human purinergic P2Y receptor coupled to phospholipase and adenylyl cyclase. J Biol Chem. 1997;272:31969-31973.
40. Purkiss JR, Boarder MR. Stimulation of phosphatidate synthesis in endothelial cells in response to P2-receptor activation. Evidence for phospholipase C and phospholipase D involvement, phosphatidate and diacylglycerol interconversion and the role of protein kinase C. Biochem J. 1992;287:31-36.
41. Dunn PM, Blakeley AGH. Suramin: a reversible P2-purinoceptor antagonist in the mouse vas deferens. Br J Pharmacol. 1988;93:243-245.
42. Boyer JL, Romero-Avila T, Schachter JR, Harden TK. Identification of competitive antagonists of the P2Y1 receptor. Mol Pharmacol. 1996;50: 1323-1329.
43. Kim YC, Gallo-Rodriguez C, Jang SY, et al. Acyclic analogues of deoxyadenosine 3′,5′-bisphosphates as $P2Y_1$ receptor antagonists. J Med Chem. 2000;43:746-755.
44. Brake AJ, Wagenbach MJ, Julius D. New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. Nature. 1994;371: 519-523.
45. Valera S, Hussy N, Evans RJ, et al. A new class of ligand-gated ion channels defined by P2X receptor for extracellular ATP. Nature. 1994;371: 516-519.
46. Buell G, Collo G, Rassendren F. P2X receptors: an emerging channel family. Eur J Neurosci. 1996;8:2221-2228.
47. Soto F, Garcia-Guzman M, Stuhmer W. Cloned ligand-gated channels activated by extracellular ATP (P2X receptors). J Membr Biol. 1997;160:91-100.
48. Surprenant A, Rassendren F, Kawashima E, North RA, Buell G. The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor ($P2X_7$). Science. 1996;272:735-738.
49. Collo G, Neidhart S, Kawashima E, Kosco-Vilbois M, North RA, Buell G. Tissue distribution of the $P2X_7$ receptor. Neuropharmacology. 1997;36: 1277-1283.
50. Di Virgilio F, Falzoni S, Mutini C, Sanz JM, Chiozzi P. Purinergic $P2X_7$ receptor: a pivotal role in inflammation and immunomodulation. Drug Dev Res. 1998;45:207-213.
51. Lewis C, Neidhart S, Holy C, North RA, Buell G, Surprenant A. Co-expression of P2X2 and P2X3 receptor subunits can account for ATP-gated currents in sensory neurons. Nature. 1995;377:432-435.
52. Nicke A, Baumert HG, Rettinger J, et al. $P2X_1$ and $P2X_3$ form stable trimers: a novel structural motif of ligand-gated ion channels. EMBO J. 1998;17: 3016-3028.
53. Evans RJ, Surprenant A, North RA. P2X receptors. In: Turner JT, Weisman GA, Fedan. JS, eds. The P2 Nucleotide Receptors. Totowa, NJ: Humana Press; 1998:43-61.
54. Afework M, Burnstock G. Distribution of P2X receptors in the rat adrenal gland. Cell Tissue Res. 1999;298:449-456.
55. Buell G, Chessell IP, Michel D, et al. Blockade of human $P2X_7$ receptor function with a monoclonal antibody. Blood. 1998;92:3521-3528.
56. Chen C-C, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G, Wood JN. A P2X purinoceptor expressed by a subset of sensory neurons. Nature. 1995;377:428-431.
57. Hashimoto M, Kokubun S. Contribution of P2-purinoceptors to neurogenic contraction of rat urinary bladder smooth muscle. Br J Pharmacol. 1995;115:636-640.
58. Cockcroft S, Gomperts BD. ATP induces nucleotide permeability in rat mast cells. Nature. 1979; 279:541-542.
59. Cockcroft S, Gomperts BD. The ATP^{4-} receptor of rat mast cells. Biochem J. 1980;188:789-798.
60. Steinberg TH, Silverstein SC. Extracellular ATP^{4-} promotes cation fluxes in the J774 mouse macrophage cell line. J Biol Chem. 1987;262:3118-3122.
61. Pizzo P, Zanovello P, Bronte V, Di Virgilio F. Extracellular ATP causes lysis of mouse thymocytes and activates a plasma membrane ion channel. Biochem J. 1991;274:139-144.
62. Rassendren F, Buell GN, Virginio C, Collo G, North RA, Surprenant A. The permeabilizing ATP receptor, $P2X_7$. Cloning and expression of a human cDNA. J Biol Chem.1997;272:5482-5486.
63. Trezise DJ, Bell NJ, Kennedy I, Humphrey PPA. Effects of divalent cations on the potency of ATP and related agonists in the rat isolated vagus nerve: implications for P_2 purinoceptor classification. Br J Pharmacol.1994;113:463-470.
64. Murgia M, Hanau S, Pizzo P, Rippa M, Di Virgilio F. Oxidized ATP: an irreversible inhibitor of the macrophage purinergic P_{2Z} receptor. J Biol Chem. 1993;268:8199-8203.
65. Redegeld FA, Smith P, Apasov S, Sitkovsky MV. Phosphorylation of T-lymphocyte plasma membrane-associated proteins by ectoprotein kinases: implications for a possible role for ecto-phosphorylation in T-cell effector functions. Biochim Biophys Acta. 1997;1328:151-165.
66. Falzoni S, Munerati M, Ferrari D, Spisani S, Moretti S, Di Virgilio F. The purinergic P2Z receptor of human macrophage cells: characterization and possible physiological role. J Clin Invest. 1995; 95:1207-1216.
67. Wiley JS, Chen JR, Snook MB, Jamieson GP. The P2Z- purinoceptor of human lymphocytes: actions of nucleotide agonists and irreversible inhibition by oxidized ATP. Br J Pharmacol. 1994; 112:946-950.
68. Schulze-Lohoff E, Hugo C, Rost S, et al. Extracellular ATP causes apoptosis and necrosis of cultured mesangial cells via P2Z/$P2X_7$ receptors. Am J Physiol. 1998;275:F962–F971.
69. Solini A, Chiozzi P, Morelli A, Fellin R, Di Virgilio F. Human primary fibroblasts in vitro express a purinergic $P2X_7$ receptor coupled to ion fluxes, microvesicle formation and IL-6 release. J Cell Sci. 1999;112:297-305.
70. Gargett CE, Wiley JS. The isoquinoline derivative KN-62: a potent antagonist of the P2Z-receptor of human lymphocytes. Br J Pharmacol. 1997;120: 1483-1490.
71. Kato M, Hagiwara M, Nimura Y, Shionoya S, Hidaka H. Purification and characterization of calcium-calmodulin kinase II from human parathyroid glands. J Endocrinol. 1991;131:155-162.

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72. Blanchard DK, Wei S, Duan C, Pericle F, Diaz JI, Djeu JY. Role of extracellular adenosine triphosphate in the cytotoxic T lymphocyte-mediated lysis of antigen presenting cells. Blood. 1995;85: 3173-3178.

73. Humphreys BD, Virginio C, Surprenant A, Rice J, Dubyak GR. Isoquinolines as antagonists of the $P2X_7$ nucleotide receptor: high selectivity for the human versus rat receptor homologues. Mol Pharmacol. 1998;54:22-32.

74. Jiang LH, Mackenzie AB, North RA, Surprenant A. Brilliant blue G selecticely blocks ATP-gated rat $P2X_7$ receptors. Mol Pharmacol. 2000;58:82-88.

75. Steinberg TH, Newman AS, Swanson JA, Silverstein SC. ATP^{4-} permeabilizes the plasma membrane of mouse macrophages to fluorescent dyes. J Biol Chem.1987;262:8884-8888.

76. Greenberg S, Di Virgilio F, Steinberg TH, et al. Extracellular nucleotides mediate Ca^{2+} fluxes in J774 macrophages by two distinct mechanisms. J Biol Chem. 1988;263:10337-10343.

77. Chiozzi P, Sanz JM, Ferrari D, et al. Spontaneous cell fusion in macrophage cultures expressing high levels of the $P2Z/P2X_7$ receptor. J Cell Biol. 1997;138:697-706.

78. Ferrari D, Chiozzi P, Falzoni S, et al. ATP-mediated cytotoxicity in microglial cells. Neuropharmacology. 1997;36:1295-1301.

79. Di Virgilio F, Meyer BC, Greenberg S, Silverstein SC. Fc receptor-mediated phagocytosis occurs in macrophages at exceedingly low cytosolic Ca^{2+} levels. J Cell Biol. 1988;106:657-666.

80. Naumov AP, Kaznacheyeva EV, Kiselyov KI, Kuryshev YA, Mamin AG, Mozhayeva GN. ATP-activated inward currents and calcium-permeable channels in rat macrophage plasma membranes. J Physiol. 1995;486:323-337.

81. Lin WW, Lee YT. Pyrimidinoceptor-mediated activation of phospholipase C and phospholipase A2 in RAW 264.7 macrophages. Br J Pharmacol. 1996;119:261-268.

82. Ichinose M. Modulation of phagocytosis by P2-purinergic receptors in mouse peritoneal macrophages. Jpn J Physiol. 1995;45:707-721.

83. el-Moatassim C, Dubyak GR. A novel pathway for the activation of phospholipase D by P2Z purinergic receptors in BAC1.2F5 macrophages. J Biol Chem. 1992;267:23664-23673.

84. Hickman SE, El Khoury J, Greenberg S, Schieren I, Silverstein SC. P2Z adenosine triphosphate receptor activity in cultured human monocyte-derived macrophages. Blood. 1994;84:2452-2456.

85. Ferrari D, Chiozzi P, Falzoni S, et al. Extracellular ATP triggers IL-1β release by activating the purinergic P2Z receptor of human macrophages. J Immunol. 1997;159:1451-1458.

86. Cowen DS, Lazarus HM, Shurin SB, Stoll SE, Dubyak GR. Extracellular adenosine triphosphate activates calcium mobilization in human phagocytic leukocytes and neutrophil/monocyte progenitor cells. J Clin Invest. 1989;83:1651-1660.

87. Fredholm BB, Abbracchio MP, Burnstock G, et al. Towards a revised nomenclature for P1 and P2 receptors. Trends Pharmacol Sci. 1997;18:79-82.

88. Humphreys BD, Dubyak GR. Induction of the $P2z/P2X_7$ nucleotide receptor and associated phospholipase D activity by lipopolysaccharide and IFNγ in the human THP-1 monocytic cell line. J Immunol. 1996;157:5627-5637.

89. Dubyak GR, Clifford EE, Humphreys BD, Kertesy SB, Martin KA. Expression of multiple ATP receptor subtypes during the differentiation and inflammatory activation of myeloid leukocytes. Drug Dev Res. 1996;39:269-278.

90. Martin KA, Kertesy SB, Dubyak GR. Down-regulation of P2U-purinergic nucleotide receptor messenger RNA expression during in vitro differentiation of human myeloid leukocytes by phorbol esters or inflammatory activators. Mol Pharmacol. 1997;51:97-108.

91. Cohn ZA, Parks E. The regulation of pinocytosis in mouse macrophages, III: the induction of vesicle formation by nucleosides and nucleotides. J Exp Med. 1967;125:457-466.

92. Sung SS, Young JD, Origlio AM, Heiple JM, Kaback HR, Silverstein SC. Extracellular ATP perturbs transmembrane ion fluxes, elevates cytosolic $[Ca^{2+}]_i$, and inhibits phagocytosis in mouse macrophages. J Biol Chem. 1985;260: 13442-13449.

93. Buisman HP, Steinberg TH, Fischbarg J, et al. Extracellular ATP induces a large nonselective conductance in macrophage plasma membranes. Proc Natl Acad Sci U S A. 1988;85:7988-7992.

94. Alonso-Torre SR, Trautmann A. Calcium responses elicited by nucleotides in macrophages. Interaction between two receptor subtypes. J Biol Chem. 1993;268:18640-18647.

95. Murgia M, Pizzo P, Steinberg TH, Di Virgilio F. Characterization of the cytotoxic effect of extracellular ATP in J774 mouse macrophages. Biochem J. 1992;288:897-901.

96. McCloskey MA, Fan Y, Luther S. Chemotaxis of rat mast cells toward adenine nucleotides. J Immunol. 1999;163:970-977.

97. Oshimi Y, Miyazaki S, Oda S. ATP-induced Ca^{2+} response mediated by P2U and P2Y purinoceptors in human macrophages: signalling from dying cells to macrophages. Immunology. 1999;98: 220-227.

98. Schmid-Antomarchi H, Schmid-Alliana A, Romey G, et al. Extracellular ATP and UTP control the generation of reactive oxygen intermediates in human macrophages through the opening of a charybdotoxin-sensitive Ca^{2+}-dependent K^+ channel. J Immunol. 1997;159:6209-6215.

99. Tonetti M, Sturla L, Giovine M, Benatti U, De Flora A. Extracellular ATP enhances mRNA levels of nitric oxice synthase and TNFα in lipopolysaccharide-treated RAW 264.7 murine macrophages. Biochem Biophys Res Commun. 1995; 214:125-130.

100. Hogquist KA, Unanue ER, Chaplin DD. Release of IL-1 from mononuclear phagocytes. J Immunol. 1991;147:2181-2186.

101. Perregaux D, Barberia J, Lanzetti AJ, Geoghegan KF, Carty TJ, Gabel CA. IL-1β maturation: evidence that mature cytokine formation can be induced specifically by nigericin. J Immunol. 1992; 149:1294-1303.

102. Hogquist KA, Nett MA, Unanue ER, Chaplin DD. Interleukin 1 is processed and released during apoptosis. Proc Natl Acad Sci U S A. 1991;88: 8485-8489.

103. Budihardo I, Oliver H, Lutter M, Luo X, Wang X. Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol. 1999;15: 269-290.

104. Perregaux D, Gabel CA. Interleukin-1β maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. J Biol Chem.1994;269:15195-15203.

105. Ferrari D, Villalba M, Chiozzi P, Falzoni S, Ricciardi-Castagnoli P, Di Virgilio F. Mouse microglial cells express a plasma membrane pore gated by extracellular ATP. J Immunol. 1996;156:1531-1539.

106. Sanz JM, Di Virgilio F. Kinetics and mechanism of ATP-dependent IL-1β release from microglial cells. J Immunol. 2000;164:4893-4898.

107. Cheneval D, Ramage P, Kastelic T, et al. Increased mature interleukin-1β secretion from THP-1 cells induced by nigericin is a result of activation of p45 IL-1β-converting enzyme processing. J Biol Chem. 1998;273:17846-17851.

108. Perregaux DG, Gabel CA. Post-translational processing of murine IL-1: evidence that ATP-induced release of IL-1α and IL-1β occurs via a similar mechanism. J Immunol. 1998;160:2469-2477.

109. Li P, Allen H, Banerjee S, et al. Mice deficient in IL-1β-convering enzyme are defective in production of mature IL-1β and resistant to endotoxic shock. Cell. 1995;80:401-411.

110. Ferrari D, Wesselborg S, Bauer MKA, Schulze-Osthoff K. Extracellular ATP activates transcription factor NF-κB through the P2Z purinoceptor by selectively targeting NF-κB p65. J Cell Biol. 1997;139:1635-1643.

111. Dallaporta B, Marchetti P, de Pablo MA, et al. Plasma membrane potential in thymocyte apoptosis. J Immunol. 1999;162:6534-6542.

112. Laliberte RE, Eggler J, Gabel CA. ATP treatment of human monocytes promotes caspase-1 maturation and externalization. J Biol Chem. 1999; 274:36944-36951.

113. Andrei C, Dazzi C, Lotti L, Torrisi MR, Chimini G, Rubartelli A. The secretory route of the leaderless protein interleukin 1β involved exocytosis of the endolysosome-related vesicles. Mol Biol Cell. 1999;10:1463-1475.

114. Sperlagh B, Hasko G, Nemeth Z, Vizi ES. ATP released by LPS increases nitric oxide production in Raw 264.7 macrophage cell line via $P2Z/P2X_7$ receptors. Neurochem Int. 1998;33:209-215.

115. Proctor RA, Denlinger LC, Leventhal PS, et al. Protection of mice from endotoxic death by 2-methylthio-ATP. Proc Natl Acad Sci U S A.1994; 91:6017-6020.

116. Hu Y, Fisette PL, Denlinger LC, et al. Purinergic receptor modulation of lipopolysaccharide signalling and inducible nitric-oxide synthase expression in Raw 264.7 macrophages. J Biol Chem. 1998;273:27170-27175.

117. Denlinger LC, Fisette PL, Garis KA, et al. Regulation of inducible nitric oxide synthase expression by macrophage purinoreceptors and calcium. J Biol Chem. 1996;271:337-342.

118. Tonetti M, Sturla L, Bistolfi T, Benatti U, De Flora A. Extracellular ATP potentiates nitric oxide synthase expression induced by lipopolysaccharide in RAW 264.7 murine macrophages. Biochem Biophys Res Commun. 1994;203:430-435.

119. Sikora A, Liu J, Brosnan C, Buell G, Chessel I, Bloom BR. Cutting edge: purinergic signaling regulates radical-mediated bacterial killing mechanisms in macrophages through a $P2X_7$-independent mechanism. J Immunol. 1999;163: 558-561.

120. Fais S, Burgio VL, Capobianchi MR, Gessani S, Pallone F, Belardelli F. The biological relevance of polykarions in the immune response. Immunol Today. 1997;18:522-527.

121. Takashima T, Ohnishi K, Tsuyuguchi I, Kishimoto S. Differential regulation of formation of multinucleated giant cells from concanavalin A–stimulated human blood monocytes by IFN-γ and IL-4. J Immunol. 1993;150:3002-3010.

122. Di Virgilio F, Falzoni S, Chiozzi P, Sanz JM, Ferrari D, Buell GN. ATP receptors and giant cell formation. J Leukoc Biol. 1999;66:723-726.

123. Falzoni S, Chiozzi P, Ferrari D, Buell G, Di Virgilio F. $P2X_7$ receptor and polykarion formation. Mol Biol Cell. 2000;11:3169-3176.

124. Molloy A, Laochumroonvorapong P, Kaplan G. Apoptosis, but not necrosis, of infected monocytes is coupled with killing of intracellular bacillus Calmette-Guerin. J Exp Med. 1994;180:1499-1509.

125. Lammas DA, Stober C, Harvey CJ, Kendrick N, Panchalingam S, Kumararatne DS. ATP-induced killing of mycobacteria by human macrophages is mediated by purinergic $P2Z/P2X_7$ receptors. Immunity. 1997;7:433-444.

126. Kusner DJ, Adams J. ATP-induced killing of virulent *Mycobacterium tuberculosis* within human macrophages requires phospholipase D. J Immunol. 2000;164:379-388.

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127. Steinberg TH, Buisman HP, Greenberg S, Di Virgilio F, Silverstein SC. Effects of extracellular ATP on mononuclear phagocytes. Ann N Y Acad Sci. 1990;603:120-129.

128. Coutinho-Silva R, Alves LA, Savino W, Persechini PM. A cation non-selective channel induced by extracellular ATP in macrophages and phagocytic cells of the thymic reticulum. Biochim Biophys Acta. 1996;1278:125-130.

129. el-Moatassim C, Dubyak GR. Dissociation of the pore-forming and phospholipase D activities stimulated via P2Z purinergic receptors in BAC1.2F5 macrophages. Product inhibition of phospholipase D enzyme activity. J Biol Chem. 1993;268:15571-15581.

130. Humphreys BD, Dubyak GR. Modulation of $P2X_7$ nucleotide receptor expression by pro- and anti-inflammatory stimuli in THP-1 monocytes. J Leukoc Biol.1998;64:265-273.

131. Chiozzi P, Murgia M, Falzoni S, Ferrari D, Di Virgilio F. Role of the purinergic P2Z receptor in spontaneous cell death in J774 macrophage cultures. Biochem Biophys Res Commun. 1996;218:176-181.

132. Zanovello P, Bronte V, Rosato A, Pizzo P, Di Virgilio F. Responses of mouse lymphocytes to extracellular ATP. II. Extracellular ATP causes cell type-dependent lysis and DNA fragmentation. J Immunol. 1990;145:1545-1550.

133. Ferrari D, Los M, Bauer MK, Vandenabeele P, Wesselborg S, Schulze-Osthoff K. P2Z purinoceptor ligation induces activation of caspases with distinct roles in apoptotic and necrotic alterations of cell death. FEBS Lett. 1999;447:71-75.

134. Ferrari D, Stroh C, Schulze-Osthoff K. $P2X_7$/P2Z purinoceptor-mediated activation of transcription factor NFAT in microglial cells. J Biol Chem.1999;274:13205-13210.

135. Chaker MB, Tharp MD, Bergstresser PR. Rodent epidermal Langerhans cells demonstrate greater histochemical specificity for ADP than for ATP and AMP. J Invest Dermatol. 1984;82:496-500.

136. Girolomoni G, Santantonio ML, Pastore S, Bergstresser PR, Giannetti A, Cruz PD Jr. Epidermal Langerhans cells are resistant to the permeabilizing effects of extracellular ATP: *in vitro* evidence supporting a protective role of membrane ATPase. J Invest Dermatol. 1993;100:282-287.

137. Coutinho-Silva R, Alves LA, Campos-de-Carvalho AC, Savino W, Persechini PM. Characterization of P2Z purinergic receptors on phagocytic cells of the thymic reticulum in culture. Biochim Biophys Acta. 1996;1280:217-222.

138. Alves LA, Coutinho-Silva R, Savino W. Extracellular ATP: a further modulator in neuroendocrine control of the thymus. Neuroimmunomodulation. 1999;6:81-89.

139. Liu Q, Bohlen H, Titzer S, et al. Expression and a role of functionally coupled P2Y receptors in human dendritic cells. FEBS Lett. 1999;445:402-408.

140. Berchtold S, Ogilvie AL, Bogdan C, et al. Human monocyte derived dendritic cells express functional P2X and P2Y receptors as well as ecto-nucleotidases. FEBS Lett. 1999;458:424-428.

141. Mutini C, Falzoni S, Ferrari D, et al. Mouse dendritic cells express the $P2X_7$ purinergic receptor: characterization and possible participation in antigen presentation. J Immunol. 1999;163:1958-1965.

142. Ferrari D, La Sala A, Chiozzi P, et al. The P2 purinergic receptors of human dendritic cells: identification and coupling to cytokine release. FASEB J. In press.

143. Nihei OK, de Carvalho AC, Savino W, Alves LA. Pharmacological properties of P2Z/$P2X_7$ receptor characterized in murine dendritic cells: role on the induction of apoptosis. Blood. 2000;96:996-1005.

144. Marriott I, Inscho EW, Bost KL. Extracellular uridine nucleotides initiate cytokine production by murine dendritic cells. Cell Immunol. 1999;195:147-156.

145. Coutinho-Silva R, Persechini PM, Bisaggio RD, et al. P2Z/$P2X_7$ receptor-dependent apoptosis of dendritic cells. Am J Physiol. 1999;276:C1139–C1147.

146. Gregory SH, Kern M. Adenosine and adenine nucleotides are mitogenic for mouse thymocytes. Biochem Biophys Res Commun.1978;83:1111-1116.

147. Fishman RF, Rubin AL, Novogrodsky A, Stenzel KH. Selective suppression of blastogenesis induced by different mitogens: effect of noncyclic adenosine-containing compounds. Cell Immunol. 1980;54:129-139.

148. Ikehara S, Pahwa RN, Lunzer DG, Good RA, Modak MJ. Adenosine 5′-triphosphate (ATP)-mediated stimulation and suppression of DNA synthesis in lymphoid cells, I: characterization of ATP responsive cells in mouse lymphoid organs. J Immunol. 1981;127:1834-1838.

149. Schmidt A, Ortaldo JR, Herberman RB. Inhibition of human natural killer cell reactivity by exogenous adenosine 5′-triphosphate. J Immunol. 1984;132:146-150.

150. Lin J, Krishnaraj R, Kemp RG. Exogenous ATP enhances calcium influx in intact thymocytes. J Immunol. 1985;135:3403-3410.

151. el-Moatassim C, Dornand J, Mani JC. Extracellular ATP increases cytosolic free calcium in thymocytes and initiates the blastogenesis of the phorbol 12-myristate 13-acetate-treated medullary population. Biochim Biophys Acta. 1987;927:437-444.

152. el-Moatassim C, Maurice T, Mani JC, Dornand J. The $[Ca^{2+}]_i$ increase induced in murine thymocytes by extracellular ATP does not involve ATP hydrolysis and is not related to phosphoinositide metabolism. FEBS Lett. 1989;242:391-396.

153. el-Moatassim C, Bernad N, Mani JC, Dornand J. Extracellular ATP induces a non-specific permeability of thymocyte plasma membranes. Biochem Cell Biol. 1989;67:495-502.

154. Wiley JS, Dubyak GR. Extracellular adenosine triphosphate increases cation permeability of chronic lymphocytic leukemic lymphocytes. Blood. 1989;73:1316-1323.

155. Di Virgilio F, Bronte V, Collavo D, Zanovello P. Responses of mouse lymphocytes to extracellular adenosine 5′-triphosphate (ATP). Lymphocytes with cytotoxic activity are resistant to the permeabilizing effect of ATP. J Immunol. 1989;143:1955-1960.

156. Filippini A, Taffs RE, Agui T, Sitkovsky MV. Ecto-ATPase activity in cytolytic T lymphocytes. Protection from the cytolytic effects of extracellular ATP. J Biol Chem. 1990;265:334-340.

157. Di Virgilio F, Pizzo P, Zanovello P, Bronte V, Collavo D. Extracellular ATP as a possible mediator of cell-mediated cytotoxicity. Immunol Today. 1990;11:274-277.

158. Padeh S, Cohen A, Roifman CM. ATP-induced activation of human B lymphocytes via P_2-purinoceptors. J Immunol. 1991;146:1626-1632.

159. Wiley JS, Chen R, Jamieson GP. The ATP^{4-} receptor-operated channel (P2Z class) of human lymphocytes allows Ba^{2+} and $ethidium^{+}$ uptake: inhibition of fluxes by suramin. Arch Biochem Biophys. 1993;305:54-60.

160. Ferrari D, Munerati M, Melchiorri L, Hanau S, Di Virgilio F, Baricordi OR. Responses to extracellular ATP of lymphoblastoid cell lines from Duchenne muscular dystrophy patients. Am J Physiol. 1994;267:C886–C892.

161. Markwardt F, Lohn M, Bohm T, Klapperstuck M. Purinoceptor-operated cationic channels in human B-lymphocytes. J Physiol. 1997;498:143-151.

162. Wiley JS, Gargett CE, Zhang W, Snook MB, Jamieson GP. Partial agonists and antagonists reveal a second permeability state of human lymphocyte P2Z/$P2X_7$ channel. Am J Physiol. 1998;275:C1224–C1231.

163. Baricordi OR, Ferrari D, Melchiorri L, et al. An ATP-activated channel is involved in mitogenic stimulation of human T lymphocytes. Blood. 1996;87:682-690.

164. Chvatchko Y, Valera S, Aubry JP, Renno T, Buell G, Bonnefoy JY. The involvement of an ATP-gated ion channel, $P2X_1$, in thymocyte apoptosis. Immunity. 1996;5:275-283.

165. Apasov SG, Koshiba M, Chused TM, Sitkovsky MV. Effects of extracellular ATP and adenosine on different thymocyte subsets. Possible role of ATP-gated channels and G protein-coupled purinergic receptor. J Immunol. 1997;158:5095-5105.

166. Chused TM, Apasov S, Sitkovsky M. Murine T lymphocytes modulate activity of an ATP-activated P2Z-type purinoceptor during differentiation. J Immunol. 1996;157:1371-1380.

167. Koshiba M, Apasov S, Sverdlov V, et al. Transient up-regulation of P2Y2 nucleotide receptor mRNA expression is an immediate early gene response in activated thymocytes. Proc Natl Acad Sci U S A. 1997;94:831-836.

168. Ross PE, Ehring GR, Cahalan MD. Dynamics of ATP-induced calcium signalling in single mouse thymocytes. J Cell Biol. 1997;138:987-998.

169. Zheng LM, Zychlinsky A, Liu CC, Ojcius DM, Young JD. Extracellular ATP as a trigger for apoptosis or programmed cell death. J Cell Biol. 1991;112:279-288.

170. Steinberg TH, Di Virgilio F. Cell-mediated cytotoxicity: ATP as an effector and the role of target cells. Curr Opin Immunol. 1991;3:71-75.

171. Zambon A, Bronte V, Di Virgilio F, et al. Role of extracellular ATP in cell-mediated cytotoxicity: a study with ATP-sensitive and ATP-resistant macrophages. Cell Immunol. 1994;156:458-467.

172. Baricordi OR, Melchiorri L, Adinolfi E, et al. Increased proliferation rate of lymphoid cells transfected with the $P2X_7$ ATP receptor. J Biol Chem. 1999;274:33206-33208.

173. Jamieson GP, Snook MB, Thurlow PJ, Wiley JS. Extracellular ATP causes loss of L-selectin from human lymphocytes via occupancy of P2Z purinoceptors. J Cell Physiol. 1996;166:637-642.

174. Gu B, Bendall LG, Wiley JS. Adenosine triphosphate-induced shedding of CD23 and L-selectin (CD62L) from lymphocytes is mediated by the same receptor but different metalloproteases. Blood. 1998;92:946-951.

175. Tou JS, Maier C. Phospholipid metabolism and lysosomal enzyme secretion by leukocytes. Effects of dibutyryl cyclic adenosine 3′:5′-monophosphate and ATP. Biochim Biophys Acta. 1976;451:353-362.

176. LeRoy EC, Ager A, Gordon JL. Effects of neutrophil elastase and other proteases on porcine aortic endothelial prostaglandin I2 production, adenine nucleotide release, and responses to vasoactive agents. J Clin Invest. 1984;74:1003-1010.

177. Ward PA, Cunningham TW, McCulloch KK, Phan SH, Powell J, Johnson KJ. Platelet enhancement of O^{2-} responses in stimulated human neutrophils. Identification of platelet factor as adenine nucleotides. Lab Invest. 1988;58:37-47.

178. Ward PA, Cunningham TW, McCulloch KK, Johnson KJ. Regulatory effects of adenosine and adenine nucleotides on oxygen radical responses of neutrophils. Lab Invest. 1988;58:438-447.

179. Kuroki M, Minakami S. Extracellular ATP triggers superoxide production in human neutrophils. Biochem Biophys Res Commun. 1989;162:377-380.

180. Kuroki M, Takeshige K, Minakami S. ATP-induced calcium mobilization in human neutrophils. Biochim Biophys Acta. 1989;1012:103-106.

181. Dubyak GR, Cowen DS. Activation of inositol phospholipid-specific phospholipase C by P2-purinergic receptors in human phagocytic leukocytes: role of pertussis toxin-sensitive G proteins. Ann N Y Acad Sci. 1990;603:227-245.

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182. Cockcroft S, Stutchfield J. ATP stimulates secretion in human neutrophils and HL60 cells via a pertussis toxin-sensitive guanine nucleotide-binding protein coupled to phospholipase C. FEBS Lett. 1989;245:25-29.

183. Balazovich KJ, Boxer LA. Extracellular adenosine nucleotides stimulate protein kinase C activity and human neutrophil activation. J Immunol. 1990;144:631-637.

184. Cowen DS, Sanders M, Dubyak G. P2-purinergic receptors activate a guanine nucleotide-dependent phospholipase C in membranes from HL-60 cells. Biochim Biophys Acta. 1990;1053:195-203.

185. Freyer DR, Boxer LA, Axtell RA, Todd RF. Stimulation of human neutrophil adhesive properties by adenine nucleotides. J Immunol. 1988;141:580-586.

186. Robson SC, Kaczmarek E, Siegel JB, et al. Loss of ATP diphosphohydrolase activity with endothelial cell activation. J Exp Med. 1997;185:153-163.

187. Dawicki DD, McGowan-Jordan J, Bullard S, Pond S, Round S. Extracellular nucleotides stimulate leukocyte adherence to cultured pulmonary artery endothelial cells. Am J Physiol. 1995;268:L666–L673.

188. Rounds S, Likar LL, Harrington EO, et al. Nucleotide-induced PMN adhesion to cultured epithelial cells: possible role of MUC1 mucin. Am J Physiol. 1999;277:L874–L880.

189. Cronstein BN, Van de Stouwe M, Druska L, Levin RI, Weismann G. Nonsteroidal antiinflammatory agents inhibit stimulated neutrophil adhesion to endothelium: adenosine dependent and independent mechanisms. Inflammation. 1994;18:323-325.

190. Sud'ina GF, Mirzoeva OK, Galkina SI, Pushkareva MA, Ullrich V. Involvement of ecto-ATPase and extracellular ATP in polymorphonuclear granulocyte-endithelial interactions. FEBS Lett. 1998;423:243-248.

191. Cowen DS, Berger M, Nuttle L, Dubyak GR. Chronic treatment with P2-purinergic receptor agonists induces phenotypic modulation of the HL-60 and U937 human myelogenous leukemia cell lines. J Leukoc Biol. 1991;50:109-122.

192. Clifford EE, Martin KA, Dalal P, Thomas R, Dubyak GR. Stage-specific expression of P2Y receptors, ecto-apyrase and ecto-5′-nucleotidase in myeloid leukocytes. Am J Physiol. 1997;273: C973–C987.

193. Gasmi L, McLennan AG, Edwards SW. Diadenosine polyphosphates induce intracellular Ca^{2+} mobilization in human neutrophils via a pertussis toxin sensitive G-protein. Immunology. 1997;90: 154-159.

194. Saito H, Ebisawa M, Reason DC, et al. Extracellular ATP stimulates interleukin-dependent cultured mast cell and eosinophils through calcium mobilization. Int Arch Allergy Appl Immunol. 1991; 94:68-70.

195. Dichmann S, Idzko M, Zimpfer U, et al. Adenosine triphosphate-induced oxygen radical production and CD11b up-regulation: Ca^{2+} mobilization and actin reorganization in human neutrophils. Blood. 2000;95:973-978.

196. Gaarder A, Jonsen A, Laland S, Hellem AJ, Owren P. Adenosine diphosphate in red cells as a factor in the adhesiveness of human blood platelets. Nature. 1961;192:531-532.

197. Born GV. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature. 1962; 194:927-928.

198. Born GV, Kratzer MAA. Source and concentration of extracellular adenosine triphosphate during haemostasis in rats, rabbits and man. J Physiol. 1984;354:419-429.

199. Gachet C, Hechler B, Leon C, et al. Activation of ADP receptors and platelet function. Thromb Haemost. 1997;78:271-275.

200. Hourani S, Hall DA. Receptors for ADP of human blood platelets. Trends Pharmacol Sci. 1994;15: 103-108.

201. Mills DC. ADP receptors on platelets. Thromb Haemost. 1996;76:835-856.

202. Packham MA, Bryant NL, Guccione MA, Kinlough-Rathbone RL, Mustard JF. Effect of the concentration of Ca^{2+} in the suspending medium on the responses of human and rabbit platelets to aggregating agents. Thromb Haemost. 1989;62: 968-976.

203. Pengo V, Boschello M, Marzari A, Baca M, Schivazappa L, Dalla Volta S. Adenosine diphosphate (ADP)-induced alpha granules release from platelets of native whole blood is reduced by ticlopidine but not by aspirin or dipyridamole. Thromb Haemost. 1986;56:147-150.

204. MacKenzie AB, Mahaut-Smith MP, Sage SO. Activation of receptor-operated cation channels via $P2X_1$ not P_{2T} purinoceptors in human platelets. J Biol Chem. 1996;271:2879-2881.

205. Vial C, Hechler B, Leon C, Cazenave JP, Gachet C. Presence of $P2X_1$ purinoceptors in human platelets and megakarioblastic cell lines. Thromb Haemost. 1997;78:1500-1504.

206. Sun B, Li J, Okahara K, Kambayashi J. $P2X_1$ purinoceptor in human platelets. J Biol Chem. 1998; 273:11544-11547.

207. Clifford EE, Parker K, Humphreys BD, Kertesy SB, Dubyak GR. The $P2X_1$ receptor, an adenosine triphosphate-gated cation channel, is expressed in human platelets but not in human blood leukocytes. Blood. 1998;91:3172-3181.

208. Leon C, Hechler B, Vial C, Leray C, Cazenave JP, Gachet C. The $P2Y_1$ receptor is an ADP receptor antagonized by ATP and expressed in platelets amd megakarioblastic cells. FEBS Lett.1997;403: 26-30.

209. Savi P, Beauverger P, Labouret C, et al. Role of $P2Y_1$ purinoceptor in ADP-induced platelet activation. FEBS Lett. 1998;422:291-295.

210. Daniel JL, Dangelmaier C, Jin J, Ashby B, Smith JB, Kunapuli SP. Molecular basis for ADP-induced platelet activation, I: evidence for three distinct ADP receptors on human platelets. J Biol Chem. 1998;273:2024-2029.

211. Park HS, Hourani SM. Differential effects of adenine nucleotide analogues on shape change and aggregation induced by adenosine 5-diphosphate (ADP) in human platelets. Br J Pharmacol. 1999;127:1359-1366.

212. Hourani SMO. Pharmacology of the platelet ADP receptors: agonist and antagonist. Haematologica. 2000;85:58-65.

213. Fabre JE, Nguyen M, Latour A, et al. Decreased platelet aggregation, increased bleeding time and resistance to thromboembolism in $P2Y_1$-deficient mice. Nat Med. 1999;5:1199-1202.

214. Mulryan K, Gitterman DP, Lewis CJ, et al. Reduced vas deferens contraction and male infertility in mice lacking $P2X_1$ receptors. Nature. 2000; 403:86-89.

215. Cattaneo M, Gachet C. ADP receptors and clinical bleeding disorders. Arterioscler Thromb Vasc Biol. 1999;19:2281-2285.

216. Leon C, Vial C, Gachet C, et al. The P2Y1 receptor is normal in a patient presenting a severe deficiency of ADP-induced platelet aggregation. Thromb Haemost.1999;81:775-781.

217. Cusack NJ, Hourani SMO. Platelet P2 receptors: from curiosity to clinical targets. J Auton Nerv Syst. 2000;81:37-43.

218. Lages B, Weiss HJ. Secreted dense granule adenine nucleotides promote calcium influx and the maintenance of elevated cytosolic calcium levels in stimulated human platelets. Thromb Haemost. 1999;81:286-292.

219. Vischer UM, Wollheim CB. Purine nucleotides induce regulated secretion of von Willebrand factor: involvement of cytosolic Ca^{2+} and cyclic adenosine monophosphate-dependent signaling in endothelial exocytosis. Blood. 1998;91:118-127.

220. Enjyoji K, Sevigny J, Lin Y, et al. Targeted disruption of *cd39*/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation. Nat Med. 1999;5:1010-1017.

221. Parker JC, Snow RL. Influence of external ATP on permeability and metabolism of dog red blood cells. Am J Physiol.1972;223:888-893.

222. Trams EG, Kaufman H, Burnstock G. A proposal for the role of ecto-enzymes and adenylates in traumatic shock. J Theor Biol. 1980;87:609-621.

223. Bergfeld GR, Forrester T. Release of ATP from human erythrocytes in response to a brief period of hypoxia and hypercapnia. Cardiovasc Res. 1992;26:40-47.

224. Berrie CO, Hawkins PT, Stephens LR, Harden TK, Downes CP. Phosphatidylinositol 4,5-bisphosphate hydrolysis in turkey erythrocytes is regulated by P2Y purinoceptors. Mol Pharmacol. 1989;35:526-532.

225. Boyer JL, Downes CP, Harden TK. Kinetics of activation of phospholipase C by P2 purinergic agonists and guanine nucleotides. J Biol Chem. 1989;264:884-890.

226. Boeynaems J-M, Pearson JD. P2 purinoceptors on vascular endothelial cells: physiological significance and transduction mechanisms. Trends Pharmacol Sci. 1990;11:34-37.

227. Boarder MR, Hourani SM. The regulation of vascular function by P2 receptors: multiple sites and multiple receptors. Trends Pharmacol Sci. 1998; 19:99-107.

228. Modderman WE, Vrijheid-Lammers T, Lowik CW, Nijweide PJ. Removal of hematopoietic cells and macrophages from mouse bone marrow cultures: isolation of fibroblast-like stromal cells. Exp Hematol. 1994;22:194-201.

229. Nijweide PJ, Modderman WE, Hagenaars CE. Extracellular adenosine triphosphate: a shock to hemopoietic cells. Clin Orthop. 1995;313:92-102.

230. Osipchuk Y, Cahalan M. Cell-to-cell calcium signals mediated by ATP receptors in mast cells. Nature. 1992;359:241-244.

231. Qian YX, McCloskey MA. Activation of mast cell K^{+} channels through multiple G protein-linked receptors. Proc Natl Acad Sci U S A. 1993;90: 7844-7848.

232. Buell G, Michel AD, Lewis C, Collo G, Humphrey PP, Surprenant A. $P2X_1$ receptor activation in HL-60 cells. Blood. 1996;87:2659-2664.

233. Perregaux DG, McNiff P, Laliberte R, Conklyn M, Gabel G. ATP acts as an agonist to promote stimulus-induced secretion of IL-1β and IL-18 in human blood. J Immunol. 2000;165:4615-4623.

234. Mehta VB, Hart J, Wewers D. ATP stimulated release of IL-1β and IL-18 requires priming by LPS and is independent of caspase-1 cleavage. J Biol Chem. In press.

235. Schnurr M, Then F, Galambos P, Scholz C, Siegmund B, Endres S, Eigler A. Extracellular ATP and TNF-α synergize in the activation of human dendritic cells. J Immunol. 2000;165:4704-4709.

236. La Sala A, Ferrari D, Corinti S, Cavani A, Di Virgilio F, Girolomoni G. Extracellular ATP induces a distorted maturation of dendritic cells and inhibits their capacity to initiate T-helper 1 responses. J Immunol. In press.

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