Chemotactic activity of extracellular nucleotides on human immune cells.

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Received: 19 September 2006 / Accepted: 10 October 2006 / Published online: 8 March 2007
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Abstract Purinergic P2 receptors are a class of plasma membrane receptors that are express in many tissues and are ligated by extracellular nucleotides [such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), uridine 5′-triphosphate (UTP) and uridine 5′-diphosphate (UDP)], which are released as a consequence of cell damage, cell stress, bacterial infection or other noxious stimuli. According to the molecular structure, P2 receptors are divided into two subfamilies: P2X and P2Y receptors. The P2X receptors are ligand-gated channels, whereas P2Y receptors are G-protein-coupled seven-membrane-spanning receptors. Several studies indicate that nucleotides play an important role in immune response modulation through their action on multiple cell types, including monocytes, mast cells, dendritic cells, neutrophils, and eosinophils. Recent work by our group and others identified extracellular nucleotides as chemotaxins for various human immune cells, including eosinophils, neutrophils and dendritic cells. In this review, we summarise recent findings in this field and put forward a hypothesis on the role of P2 receptors in the early recruitment of human immune cells to the site of inflammation.

Keywords Chemotaxins · Immune cells · Nucleotides · Purinergic receptors

Introduction

Cell migration plays a key role in a wide variety of biological processes, such as embryogenesis, development, angiogenesis, haematopoiesis, immune response and inflammation. In inflammation and host defence, the targeted trafficking of immune cells to tissues and/or lymphoid organs is one of the essential steps. Migration of the different leukocytes is tightly controlled by chemokines. These chemotactic cytokines are secreted proteins with a molecular weight of 8–10 kDa, which direct cellular traffic along ingeniously regulated concentration gradient in the extracellular space. Based on amino acid alignments, chemokines are divided into four families. According to the position and the spacing of the first two conserved cysteines or the lack of them, these families are distinguished as either C, CC, CXC or CX3C. Until now, C and CX3C are composed of only one member each, lymphotactin and fractalkine, respectively, whereas CC and CXC each consist of numerous, well-characterised members [1, 2]. The chemotactic effects of these molecules are mediated due to their interactions with different specific serpentine receptors that span the plasma membrane seven times and belong to the G-protein-coupled receptor family.

Besides chemokines, many constitutive molecules can regulate the function of leukocytes and thereby modulate immune responses, e.g. following tissue damage, intracellular localised substances can be released into the extracellular space. For this reason, an increase of the extracellular concentration of certain molecules can be a very simple sign of cell damage. However, for constitutive molecules to function as chemotaxins, it is essential that they are recognised by immune system migration cells. In the past few years, evidence has accumulated strongly suggesting that nucleotides fulfil these requirements. They are present at high concentrations (5–10 mM) in the cytoplasm of all cells, whereas in the extracellular compartment, their concentration is in the nanomolar range. Nucleotides can be released into the extracellular space via nonlytic
mechanisms through regulated transport; e.g. adenosine triphosphate (ATP) has been reported to be secreted by different cell types in a broad variety of conditions, such as shear stress, endotoxin stimulation, or at sites of platelet aggregation [3, 4]. Hence, in tissues, nucleotides are able to generate concentration-dependent gradients, which can serve as chemotactic signals for different immune cells, causing migration.

P2 receptors

P2 receptors are subdivided on the basis of pharmacological, functional and cloning data into two families: the P2YR and P2XR [5–8]. P2YR are seven-membrane-spanning, G-protein-coupled receptors, and eight different P2Y subtypes have been cloned so far (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13 and P2Y14) [5, 9, 10]. Activation of P2YR induces phospholipase C activation, inositol triphosphate generation, Ca\(^{2+}\) release from intracellular stores, and/or stimulation/inhibition of adenylyl cyclase. Extensive pharmacological studies performed in P2Y transfected cells revealed that P2Y1, P2Y11, P2Y12 and P2Y13 selectively interact with ATP and/or adenosine diphosphate (ADP), whereas uridine 5′-triphosphate (UTP) and uridine 5′-diphosphate (UDP) are inactive [11–15]. In contrast the P2Y2, P2Y4 and P2Y6 subtypes are responsive to uridine nucleotides [16–18]. Whereas ATP and UTP activate P2Y2 with similar efficiency, UTP and UDP are most the potent agonists at P2Y4 and P2Y6, respectively [16–18]. In addition, it has been shown that P2Y14 specifically responds to UDP glucose and related sugar nucleotides but not to ATP, ADP, UTP or UDP [5, 9, 19, 20]. P2YR have been shown to modulate multiple cell function of various human immune cells, including cytokine release from dendritic cells, reactive oxygen metabolite production from neutrophils and eosinophils or chemokine release from airway epithelial cells [20–23].

P2XR are multimeric ligand-gated plasma-membrane ion channels activated by extracellular ATP and selective for monovalent and divalent cations [8, 24, 25]. At this time, seven different monomers have been cloned: the P2X1–P2X7 subtypes. Activation of P2XR leads to increased plasma membrane permeability to ions (Na\(^{+}\), K\(^{+}\), and Ca\(^{2+}\)) and induction of apoptosis in human immune cells [22, 26]. In contrast to P2YR, the only currently known physiological ligand for all P2XR subtypes is ATP.

P2 receptors and migration of neutrophils

The first report that extracellular nucleotides can modulate human neutrophil function was a paper by Ward et al. showing that ATP and ADP can induce superoxide anion formation [27, 28]. Furthermore, it has been shown that human neutrophils or human promyelocytic HL60 cells respond to ATP, ATP\(\gamma\)S and UTP with an increase in intracellular Ca\(^{2+}\) concentration via a pertussis toxin-sensitive G-protein receptor that coupled to the inositol phospholipid signaling system, suggesting involvement of P2Y subtypes [27, 29–33]. Activation of these intracellular signal-transduction systems is of great interest in the light of neutrophil recruitment to the site of inflammation, as increase in Ca\(^{2+}\) concentration is an important step in human neutrophil migration [34–36]. Furthermore, it has been shown that ATP also increases membrane expression of CD11b/CD18 and adhesion to albumin-coated polystyrene latex beads [37]. Up-regulation of these molecules enhances the adhesion of neutrophils to other cells, e.g. between neutrophils and pulmonary endothelial cells, and could be of relevance for neutrophil migration across the vessel wall. However, after earlier observations suggesting that nucleotides might be chemoattractant for human neutrophils, it was not until the end of the 1990s that ATP and UTP were shown, by Verghese et al. [103] to induce actin polymerisation and chemotaxis in human neutrophils via the activation of P2Y2 (formerly known as P2U) receptor.

Surprisingly, the first reverse transcription polymerase chain reaction (RT-PCR) data revealed that human neutrophils express only P2Y4 and P2Y6 but not P2Y1 and P2Y2 receptors [38]. However, recent reports showed that human neutrophils also express messenger ribonucleic acid (mRNA) for the P2Y2 and P2Y1 receptor subtypes [39, 40]. Among the P2X receptors, so far, only the present of the P2X7 receptor has been shown by Northern blotting and immunocytochemistry [41]. Because increased extracellular nucleotide concentrations are associated with cell damage/injury and human neutrophils are the initial cell type found at tissue injury sites, the conclusion could be drawn that extracellular nucleotides among other mediators are involved in the recruitment of neutrophils to the site of inflammation.

P2 receptors and recruitment of human eosinophils

Besides neutrophils, eosinophils also express P2 receptors. Several studies showed that human eosinophils express mRNA for the following P2Y and P2X subtypes: P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y14, P2X1, P2X4 and P2X7 [23, 42–44]. Stimulation of P2 receptors expressed by eosinophils induces multiple cell responses, including production of reactive oxygen metabolites and secretion of eosinophil cationic protein (ECP) [23, 26].

Burgers and colleagues [45] showed that ATP, secreted by thrombin-activated platelets, was able to raise eosinophil intracellular Ca\(^{2+}\) concentration and make the cells chemo-
tact towards platelets. These seminal studies were later confirmed by the identification of eosinophil P2Y and P2X receptors [42, 44] and the observations that nucleotides also induce up-regulation of adhesion molecule CD11b and actin polymerisation to important features involved in blood eosinophil recruitment to tissue [23, 42, 44].

In addition to direct chemotactic influences, ATP might also have indirect effects on eosinophil recruitment. We recently showed that ATP and UDP induce secretion of interleukin (IL)-8 by eosinophils [26]. This chemokine is a potent attractor for eosinophils themselves (and neutrophils), i.e. they are able to recruit more cells to inflammation sties. Increased secretion of IL-8 has been described in eosinophils from patients with bronchial asthma or atopic dermatitis [46]. Moreover, IL-8 concentration in bronchoalveolar fluids from asthmatic patients is increased significantly in comparison with that of healthy subjects [46]; therefore, one can suggest the involvement of different nucleotides in the direct or IL-8-mediated recruitment of eosinophils and thus in the development and maintenance of allergic diseases.

**P2 receptors and mast cells**

Mast cells are situated around blood vessels and nerves, especially at interfaces with the external environment, emphasising their role in immunity. They express several mRNAs that encode P2XR and P2YR subtypes [47, 48]. Human mast cells express P2X1 and P2X4, whereas the P2X7 receptor subtype is only expressed by human-cord-blood-derived mast cells when activated with anti-immunoglobulin (Ig)E [47]. Among the P2Y receptors, the presence of P2Y1, P2Y2, P2Y11, P2Y12 and P2Y13 subtypes were shown by RT-PCR [48]. Moreover, ATP and UTP enhance histamine release by human lung mast cells stimulated by cross-linkage of the fragment crystallisable (Fc)εRI [49]. This effect is attributed to the P2Y2 receptor. Data on chemotactic effects of nucleotides on mast cells are rare, but there is evidence that they might effect the migration of these cells. For example, McCloskey and colleagues showed that the nucleotides ADP, ATP and UTP are effective chemotactants for rat-bone-marrow-cultured mast cells [50]. However, whether nucleotides can also induce migration of human mast cells remains to be elucidated.

**P2 receptors and lymphocytes**

The first evidence for a role of extracellular nucleotides in human lymphocyte responses has been present for some time [51–53], but a systemic analysis of the expression and function of P2 receptors in human lymphocytes was only started at the end of the 1980s [30, 54–57]. Human B lymphocytes express both P2X and P2Y receptors [58, 59]. The presence of different P2Y receptors is indicated by the ability of ATP and many other nucleotides to trigger Ca2+ release from intracellular stores [60, 61] and the finding of P2Y1, P2Y2, P2Y4 and P2Y6 receptor-specific mRNA in lymphocytes [38]. Different studies suggest that human B cells express at least P2X7 receptor [22], but the identification of other P2X receptor subtypes is limited by the absence of specific antibodies [62, 63]. Nevertheless, confocal microscopy studies using anti-P2X polyclonal antibodies suggest the presence of P2X1, P2X2, P2X3 and P2X7 subtypes on human B lymphocytes [59]. However, despite the presence of functional P2 receptors, data about chemotactic effects on B lymphocytes induced by nucleotides are still missing.

Functional and pharmacologic studies revealed that human peripheral T lymphocytes express P2X-like ATP-activated channels, most likely P2X1, P2X4 and P2X7, [22, 64, 65]. Functional activity of the P2X receptors on T cells has been shown by a large influx of Na+ and Ca2+ from the extracellular medium caused by ATP and 3′-O-(4-benzoyl)benzoyl-ATP (Bz-ATP) [65]. The expression of P2Y receptors subtypes is still unclear, and whereas Baricordi and coworkers described a lack of functional P2Y receptors expression [65], recent studies using complete lymphocyte populations (T and B cells) could detected all the target genes for P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12 and P2Y13 [66]. However, a functional expression of different P2Y receptors on human T lymphocytes has not been proven.

Studies showing that activation of the P2X7 receptor by ATP and Bz-ATP induced shedding of CD23 and L-selectin from B and T lymphocytes of a B-chronic lymphocytic leukaemia (B-CLL) patient and from normal subjects, a classic effect of chemoattractants [67–70], leads to the assumption that the P2X7 receptor might be involved in the transendothelial migration of lymphocytes [71, 72]. But elegant experiments from Chen and coworkers using P2X7 antagonist in in vitro migration assays indicated that ATP is neither a chemoattractant that stimulates transmigration of lymphocytes nor an agonist that mediates the global L-selectin loss during transendothelial migration [73]. In any case a direct effect of extracellular nucleotides on T and B lymphocyte migration has not yet been investigated and, therefore, the role of nucleotides in the recruitment of lymphocytes remains unclear.

**P2 receptor in monocyte/macrophages**

Although the first report on a potential role of exogenous nucleotides on mouse macrophage function was a paper by Cohn and Parks from 1967 [74], a systemic investigation
on human monocytes/macrophages was only started in the 1990s [30, 38, 75–78]. Freshly isolated blood monocytes have been shown to express mRNA for the following P2Y and P2X subtypes: P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, P2X1, P2X4 and P2X7 [38, 66, 79]. However, other investigations found a lack of functional P2X7 in these cells, whereas the receptor appears during maturation of monocytes to macrophages [22]. Besides P2X7 receptors, human macrophages or macrophage cell lines have been described to express P2Y2, P2Y4 and P2Y6 receptors [30, 80, 81].

Activation of P2 receptors expressed by human monocytes/macrophages induces multiple cell responses, including increase of intracellular calcium concentration, induction of apoptosis, generation of reactive oxygen intermediates, NO generation and secretion of IL-1β, tumour necrosis factor (TNF)-α or IL-18 [22, 30, 77, 79, 82, 83]. In the light of migration, it is of great interest that extracellular ADP causes increased surface expression of MAC-1 (alpha M beta 2 integrin, CD11b/CD18) on monocytes [84] and that nucleotides can induce the adherence of monocytes to surfaces [38], which could be of relevance for monocyte migration across the vessel wall. Accordingly, migration of the mouse macrophage line J774 towards ADP has been demonstrated [50]. Interestingly, Warny et al. demonstrated that UDP activates IL-8 gene expression and IL-8 release in human monocytic cells [85]. Because IL-8 is a central mediator in inflammation and an important chemotactic factor for various cells, including neutrophils, eosinophils and CD16+ natural killer (NK) cells [46, 86–89], UDP [through its action on dendritic cells (DC)] might be indirectly involved in the recruitment of these cells to the side of inflammation. However, at this time, no information on the chemotactic activity of nucleotides on human monocytes/macrophages is available.

**P2 receptors in dendritic cells**

DCs are powerful antigen-presenting cells that circulate in the bloodstream or reside in peripheral tissues. They are characterised by a high antigen uptake capacity, recognition of constitutive or inducible endogenous “danger signals” provided by surrounding cells and a high responsiveness to chemotactic signals. The migratory ability of DCs is one of the main features in the initiation of immune responses. After acquiring antigens in the peripheral tissue, DCs migrate to the draining mediastinal lymph nodes to activate naïve T cells [90–92]. Besides this “classic” action, some evidence also suggests that tissue-resident DCs are able to uptake tissue antigens and to migrate to the afferent lymph nodes, even in the absence of inflammatory conditions, thus contributing to tolerance maintenance [93, 94].

In the last few years, DCs came into the focus of researchers of the purinergic field, and the role of P2 receptors in DC migration came to the fore. RT-PCR analysis revealed that human DCs express a broad variety mRNAs for at least four subtypes of the P2X receptor family (P2X1, P2X4, P2X4, P2X7) and eight subtypes of the P2Y receptor family (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, P2Y14) [95–99].

First, studies by Liu et al. revealed that at least activation of the P2Y1, P2Y2 and P2Y4 mediates calcium release from intracellular storage. Furthermore, the observation that DCs redirect their dendrites towards a nearby patch pipette leaking ATP suggested that P2YR might mediate DC chemotactic response [96]. Indeed, ATP and UTP, probably via activation of the P2Y2, as well as ADP (via P2Y1?) turned out to be potent chemotactic stimuli for immature but not for mature DCs [100]. In contrast, P2X receptor activation had only marginal chemotactic activity in both immature and mature DCs. Chemotaxis was paralleled by other intracellular signalling events, such as actin polymerisation and intracellular Ca2+ mobilisation. Recently, UDP could be added to the list of chemoattractant nucleotides, as it has been shown that UDP via binding to the P2Y6 receptor increased intracellular calcium, induced actin polymerisation and migration of immature, but again not mature, DCs [101]. The discrepancy between the responsiveness to extracellular nucleotides of immature and mature DCs could be explained by functional studies. They revealed a selective down-regulation of the Gί/o protein-coupled chemotactic P2Y receptor responsiveness during maturation. Surprisingly, immature and mature DCs expressed similar amounts of mRNA for the purinergic receptor subtypes P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2X4, P2X7 and P2X7 [100]. DC maturation encompasses a coordinated down-regulation of inflammatory chemokine receptors (CCR1, CCR2, CCR5 and CXCR4) and induction of CCR7 and CXCR4 [2]. In addition, during maturation, functional down-regulation of chemotaxis-regulating P2Y receptors are uncoupled to chemotaxis-associated signal transduction pathways. As a result, DCs lose sensitivity to inflammatory chemokines as well as to the nucleotides ATP, ADP, UTP and UDP.

In addition, nucleotides can modulate the migration of DCs to chemokines, as immature and mature DCs stimulated with ATP gain the ability to migrate in response to CXCL ligand (L)12 and CCL12 [102]. However, in contrast, Schnurr et al. reported that ATP through P2Y13 signalling could inhibit CCL21, induce migration of immature and mature monocyte-derived DCs and CD1a+ dermal DCs but not of CD1c+ peripheral blood DCs or IL-3R+ plasmacytoid DCs [98]. This controversy could be due to differences in the blood donors or preparation of monocyte-derived DCs in the different laboratories.
Besides direct chemotactic effects on DCs, extracellular nucleotides are also involved indirectly via their action on DCs in the trafficking of other leukocytes through the release of chemokines. For example, ATP up-regulates the constitutive production of CCL22 [macrophage-derived chemokine (MDC)] and inhibits the lipopolysaccharide (LPS)-induced secretion of CXCL10 (IP-10) and CCL5 (RANTES), resulting in selectively impaired recruitment of type 1 but not type 2 T cells, suggesting a nucleotide-mediated communication between DCs and T cells—an important event during antigen presentation in vivo [102]. In accordance, UDP can enhance the LPS-mediated release of chemotactic factor IL-8 via P2Y6 from mature DCs [101], so again, UDP might be indirectly involved in the recruitment of neutrophils, eosinophils and CD16+ NK cells to the side of inflammation.

In sum, activation of DCs by extracellular nucleotides leads to multiple cell responses, which results in a direct migration of DCs and also maybe indirect (DC-mediated) recruitment of other immune cells to the site of inflammation. Over the last few years, several studies have implied that extracellular nucleotides—which are actively released or diffuse out of mechanically stressed, infected or injured cells—might be involved in the early recruitment of immune cells to the site of inflammation/cell damage. In these in vitro experiments, it has been shown that extracellular nucleotides (mainly by activating P2YR subtypes) are direct chemoattractants for human neutrophils, eosinophils and DCs and/or they can modulate the chemokine production of eosinophils, monocytes and DCs, which might then influence the migration capacity of other immune cells. However, whether nucleotides play a role in the migration of immune cells in vivo still remains to be elucidated.

Conclusion

Over the last few years, several studies have implied that extracellular nucleotides—which are actively released or diffuse out of mechanically stressed, infected or injured cells—might be involved in the early recruitment of immune cells to the site of inflammation/cell damage. In these in vitro experiments, it has been shown that extracellular nucleotides (mainly by activating P2YR subtypes) are direct chemoattractants for human neutrophils, eosinophils and DCs and/or they can modulate the chemokine production of eosinophils, monocytes and DCs, which might then influence the migration capacity of other immune cells. However, whether nucleotides play a role in the migration of immune cells in vivo still remains to be elucidated.

References

1. Sallusto F, Mackay CR (2004) Chemoattractants and their receptors in homeostasis and inflammation. Curr Opin Immunol 16(6):724–731
2. Sallusto F, Mackay CR, Lanzavecchia A (2000) The role of chemokine receptors in primary, effector, and memory immune responses. Annu Rev Immunol 18:593–620
3. Cotrina ML et al (1998) Connexins regulate calcium signaling by controlling ATP release. Proc Natl Acad Sci USA 95(26):15735–15740
4. Mitchell CH et al (1998) A release mechanism for stored ATP in ocular ciliary epithelial cells. Proc Natl Acad Sci USA 95(12):7174–7178
5. Abbracchio MP et al (2003) Characterization of the UDP-glucose receptor (re-named here the P2Y14 receptor) adds diversity to the P2Y receptor family. Trends Pharmacol Sci 24(2):52–55
6. Dubyak GR, el-Moatassim C (1993) Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. Am J Physiol 265(3 Pt 1):C577–C606
7. Fredholm BB et al (1994) Nomenclature and classification of purinoceptors. Pharmacol Rev 46(2):143–156
8. North RA, Surprenant A (2000) Pharmacology of cloned P2X receptors. Annu Rev Pharmacol Toxicol 40:563–580
9. Lee BC et al (2003) P2Y-like receptor, GPR105 (P2Y14), identifies and mediates chemotaxis of bone-marrow hematopoietic stem cells. Genes Dev 17(13):1592–1604
10. von Kugelgen I, Wetter A (2000) Molecular pharmacology of P2Y-receptors. Naunyn Schmiedebergs Arch Pharmacol 362(4–5):310–323
11. Communi D et al (1997) Cloning of a human purinergic P2Y receptor coupled to phospholipase C and adenylyl cyclase. J Biol Chem 272(51):31969–31973
12. Foster CJ et al (2001) Molecular identification and characterization of the platelet ADP receptor targeted by thienopyridine antithrombotic drugs. J Clin Invest 107(12):1591–1598
13. Marteau F et al (2003) Pharmacological characterization of the human P2Y13 receptor. Mol Pharmacol 64(1):104–112
14. Schachter JB et al (1996) Second messenger cascade specificity and pharmacological selectivity of the human P2Y1-purinoceptor. Br J Pharmacol 118(1):167–173
15. van der Weyden L et al (2000) Pharmacological characterization of the P2Y11 receptor in stably transfected hematological cell lines. Mol Cell Biochem 213(1–2):75–81
16. Communi D et al (1996) Pharmacological characterization of the human P2Y4 receptor. Eur J Pharmacol 317(2–3):383–389
17. Nicholas RA et al (1996) Uridine nucleotide selectivity of three phospholipase C-activating P2 receptors: identification of a UDP-selective, a UTP-selective, and an ATP- and UTP-specific receptor. Mol Pharmacol 50(2):224–229
18. Ralevic V et al (1997) Characterization of P2 receptors for purine and pyrimidine nucleotides in human placental cytendylos. Br J Pharmacol 121(6):1121–1126
19. Chambers JK et al (2000) A G protein-coupled receptor for UDP-glucose. J Biol Chem 275(15):10767–107671
20. Muller T et al (2005) The P2Y14 receptor of airway epithelial cells: coupling to intracellular Ca2+ and IL-8 secretion. Am J Respir Cell Mol Biol 33(6):601–609
21. la Sala A et al (2003) Alerting and tuning the immune response by extracellular nucleotides. J Leukoc Biol 73(3):339–343
22. Di Virgilio F et al (2001) Nucleotide receptors: an emerging family of G-protein-coupled receptors: novel findings and future perspectives. J Leukoc Biol 70(6):447
23. Ferrari D et al (2006) Activation of human eosinophils via P2Y receptors: functional implications. Pharmacol Rev 46(2):143–156
29. Dubyak GR, Cowen DS, Meuller LM (1988) Activation of inositol phospholipid breakdown in HL60 cells by P2-purinergic receptors for extracellular ATP. Evidence for mediation by both pertussis toxin-sensitive and pertussis toxin-insensitive mechanisms. J Biol Chem 263(34):18108–18117
30. Cowen DS et al (1989) Extracellular adenosine triphosphate activates calcium mobilization in human phagocytic leukocytes and neutrophil/modecy progenitor cells. J Clin Invest 83(5):1651–1660
31. Cowen DS, Lazarus HM, Dubyak GR (1988) Flow cytometric measurements of cytosolic [Ca2+] in normal and leukemic progenitor cells. Ann N Y Acad Sci 551:273–276
32. Cockcroft S, Stutchfield J (1989) ATP stimulates secretion in human neutrophils and HL60 cells via a pertussis toxin-sensitive guanine nucleotide-binding protein coupled to phospholipase C. FEBS Lett 245(1–2):25–29
33. Cowen DS, Sanders M, Dubyak G (1990) P2-purinergic receptors activate a guanine nucleotide-dependent phospholipase C in membranes from HL-60 cells. Biochim Biophys Acta 1052(2–3):195–203
34. Lynn WS, Mukherjee C (1978) Motility of human polymorphonuclear leukocytes. Roles of hydroxy fatty acids, other lipids, and cations. Am J Pathol 91(3):581–594
35. Cramer EB, Gallin JI (1979) Localization of submembranous cations to the leading end of human neutrophils during chemotaxis. J Cell Biol 82(2):369–379
36. Metzner B et al (1994) [Ca2+]i-transients and actin polymerization in human neutrophils under stimulation with GRO alpha and complement fragment C5a. Agents Actions 42(3–4):101–106
37. Freyer DR et al (1988) Stimulation of human neutrophil adhesive properties by adenosine nucleotides. J Immunol 141(2):580–586
38. Jin J et al (1998) Distribution of P2Y receptor subtypes on haematopoietic cells. Br J Pharmacol 123(5):789–794
39. Chen Y et al (2004) A putative osmoreceptor system that inhibits cytokine secretion by human peripheral blood monocytes and neutrophils. J Leukoc Biol 74(6):950–954
40. Sak K, Boeynaems JM, Everaerts H (2003) Involvement of P2 receptors in the differentiation of haematopoietic cells. J Leukoc Biol 73(4):442–447
41. Collo G et al (1997) Tissue distribution of the P2X7 receptor. Neuropharmacology 36(9):1277–1283
42. Dichtmann S et al (2000) Adenosine triphosphate-induced oxygen radical production and CD11b up-regulation: Ca(2+)-mobilization and actin reorganization in human eosinophils. Blood 95(3):973–978
43. Ferrari D et al (2000) P2 purinergic receptors of human eosinophils: characterization and coupling to oxygen radical production. FEBS Lett 486(3):217–224
44. Idzko M et al (2001) Functional characterization of P2Y and P2X receptors in human eosinophils. J Cell Physiol 188(3):329–336
45. Burgers JA et al (1993) Human platelets secrete chemotactic activity for eosinophils. Blood 81(1):49–55
46. Yousefi S et al (1995) IL-8 is expressed by human peripheral blood eosinophils. Evidence for increased secretion in asthma. J Immunol 154(4):5481–5490
47. Bradding P et al (2003) Ion channel gene expression in human lung, skin, and cord blood-derived mast cells. J Leukoc Biol 73(5):614–620
48. Feng C et al (2004) Adenine nucleotides inhibit cytokine generation by human mast cells through a Gs-coupled receptor. J Immunol 173(2):7539–7547
49. Schulman ES et al (1999) ATP modulates anti-IgE-induced release of histamine from human lung mast cells. Am J Respir Cell Mol Biol 20(3):530–537
50. McCloskey MA, Fan Y, Luther S (1999) Chemotaxis of rat mast cells toward adenosine nucleotides. J Immunol 163(2):970–977
51. Hallak GJ, Wilkinson JH (1977) Action of adenosine phosphates on the release of intracellular lactate dehydrogenase from human and rat lymphocytes. Enzyme 22(6):361–369
52. Estes G, Solomon SS, Norton WL (1971) Inhibition of lymphocyte activation by cyclic and non-cyclic nucleotides. J Immunol 107(5):1485–1492
53. Fishman RF et al (1980) Selective suppression of blastogenesis induced by different mitogens: effect of noncyclic adenosine-containing compounds. Cell Immunol 54(1):129–139
54. Filippini A et al (1990) Ecto-ATPase activity in cytolytic T-lymphocytes. Protection from the cytolytic effects of extracellular ATP. J Biol Chem 265(1):334–340
55. Filippini A, Tafel RE, Sitkovsky MV (1990) Extracellular ATP in T-lymphocyte activation: possible role in effector functions. Proc Natl Acad Sci U S A 87(21):8267–8271
56. Wiley JS, Dubyak GR (1989) Extracellular adenosine triphosphate increases cation permeability of chronic lymphocytic leukemic lymphocytes. Blood 73(5):1316–1323
57. Wiley JS et al (1990) Extracellular ATP stimulates an amiloride-sensitive sodium influx in human lymphocytes. Arch Biochem Biophys 280(2):263–268
58. Markwardt F et al (1999) Purinoreceptors in human B-lymphocytes. Prog Brain Res 123(29):345–353
59. Sluyter R, Barden JA, Wiley JS (2001) Detection of P2X purinergic receptors on human B lymphocytes. Cell Tissue Res 304(2):231–236
60. Padhe S, Cohen A, Roisman CM (1991) ATP-induced activation of human B lymphocytes via P2-purinoceptors. J Immunol 146(5):1626–1632
61. Biffen M, Alexander DR (1994) Mobilization of intracellular Ca2+ by adenosine nucleotides in human T-leukaemia cells: evidence for ADP-specific and P2y-purinergic receptors. Biochem J 304(3):769–774
62. Markwardt F et al (1997) Purinoreceptor-operated cationic channels in human B lymphocytes. J Physiol 498(5):143–151
63. Lohn M et al (2001) Sodium block and depolarization diminish P2Z-dependent Ca2+ entry in human B lymphocytes. Cell Calcium 29(6):395–408
64. Wiley JS et al (1994) The P2Z-purinoceptor of human lymphocytes: actions of nucleotide agonists and irreversible inhibition by oxidized ATP. Br J Pharmacol 112(3):946–950
65. Baricordi OR et al (1996) An ATP-activated channel is involved in mitogenic stimulation of human T lymphocytes. Blood 87(2):682–690
66. Wang L et al (2004) P2 receptor mRNA expression profiles in human lymphocytes, monocytes and CD34+ stem and progenitor cells. BMC Immunol 5:16
67. Ellerbroek PM et al (2004) Effects of the capsular polysaccharides of Cryptococcus neoformans on phagocyte migration and inflammatory mediators. Curr Med Chem 11(2):253–266
68. Griffin JD et al (1990) Granulocyte-macrophage colony-stimulating factor and other cytokines regulate surface expression of the leukocyte adhesion molecule-1 on human neutrophils, monocytes, and their precursors. J Immunol 145(2):576–584
69. Khan AI, Kubes P (2003) L-selectin: an emerging player in chemokine function. Microcirculation 10(3–4):351–358
70. Sperti O et al (1991) Leukocyte adhesion molecule-1 (LAM-1, L-selectin) interacts with an inducible endothelial cell ligand to support leukocyte adhesion. J Immunol 147(8):2565–2573
71. Jamieson GP et al (1996) Extracellular ATP causes loss of L-selectin from human lymphocytes via occupancy of P2Z purinoceptors. J Cell Physiol 166(3):637–642
72. Gu B, Bendall LJ, Wiley JS (1998) Adenosine triphosphate-induced shedding of CD23 and L-selectin (CD62L) from
lymphocytes is mediated by the same receptor but different metalloproteases. Blood 92(3):946–951
73. Chen JR et al (1999) Transendothelial migration of lymphocytes in chronic lymphocytic leukaemia is impaired and involved down-regulation of both L-selectin and CD23. Br J Haematol 105(1):181–189
74. Cohn ZA, Parks E (1967) The regulation of pinocytosis in mouse macrophages. 3. The induction of vesicle formation by nucleosides and nucleotides. J Exp Med 125(3):457–466
75. Falzoni S et al (1995) The purinergic P2Z receptor of human macrophage cells. Characterization and possible physiological role. J Clin Invest 95(3):1207–1216
76. Hickman SE et al (1994) P2Z adenosine triphosphate receptor activity in cultured human monocyte-derived macrophages. Blood 84(8):2452–2456
77. Ferrari D et al (1997) Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages. J Immunol 159(3):1451–1458
78. Sakamoto H, Firkin F (1984) Characterization of leucocyte phagocytic stimulatory material released by activated human platelets. Br J Haematol 57(1):49–60
79. Perregaux DG et al (2000) ATP acts as an agonist to promote stimulus-induced secretion of IL-1 beta and IL-18 in human blood. J Immunol 165(8):4615–4623
80. Fredholm BB et al (1997) Towards a revised nomenclature for P1 and P2 receptors. Trends Pharmacol Sci 18(3):79–82
81. Siober CB et al (2001) ATP-mediated killing of Mycobacterium bovis bacille Calmette-Guerin within human macrophages is calcium dependent and associated with the acidification of mycobacteria-containing phagosomes. J Immunol 166(10):6276–6286
82. Schmid-Antomarchi H et al (1997) Extracellular ATP and UTP control the generation of reactive oxygen intermediates in human macrophages through the opening of a charybdotoxin-sensitive Ca2+–dependent K+ channel. J Immunol 159(12):6209–6215
83. Mehta VB, Hart J, Wewers MD (2001) ATP-stimulated release of interleukin (IL)-1beta and IL-18 requires priming by lipopolysaccharide and is independent of caspase-1 cleavage. J Biol Chem 276(6):3820–3826
84. Altieri DC, Edgington TS (1988) The saturable high affinity association of factor X to ADP-stimulated monocytes defines a novel function of the Mac-1 receptor. J Biol Chem, 1988. 263 (15):7007–7015
85. Wamby M et al (2001) P2Y(6) nucleotide receptor mediates interleukin-8 production in response to UDP or lipopolysaccharide. J Biol Chem 276(28):26051–26056
86. Teran LM et al (1996) Leukocyte recruitment after local endobronchial allergen challenge in asthma. Relationship to procedure and to airway interleukin-8 release. Am J Respir Crit Care Med 154(2 Pt 1):469–476
87. Campbell JJ et al (2001) Unique subpopulations of CD56+ NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. J Immunol 166 (11):6477–6482
88. Mukaida N (2000) Interleukin-8: an expanding universe beyond neutrophil chemotaxis and activation. Int J Hematol 72(4):391–398
89. Baggioni M (2001) Chemokines in pathology and medicine. J Intern Med 250(2):91–104
90. Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392(6673):245–252
91. Steinman RM (1991) The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 9:271–296
92. Hart DN (1997) Dendritic cells: unique leukocyte populations which control the primary immune response. Blood 90(9):3245–3287
93. Heath WR, Carbone FR (2001) Cross-presentation, dendritic cells, tolerance and immunity. Annu Rev Immunol 19:47–64
94. Steinman RM et al (2000) The induction of tolerance by dendritic cells that have captured apoptotic cells. J Exp Med 191(3):411–416
95. Berchtold S et al (1999) Human monocyte derived dendritic cells express functional P2X and P2Y receptors as well as ecto-nucleotidases. FEBS Lett 458(3):424–428
96. Liu QH et al (1999) Expression and a role of functionally coupled P2Y receptors in human dendritic cells. FEBS Lett 445 (2–3):402–408
97. Ferrari D et al (2000) The P2 purinergic receptors of human dendritic cells: identification and coupling to cytokine release. J Immunol 164(1):121–126
98. Schnurr M et al (2003) ATP gradients inhibit the migratory capacity of specific human dendritic cell types: implications for P2Y11 receptor signaling. Blood 102(2):613–620
99. Skelton L et al (2003) Human immature monocyte-derived dendritic cells express the G protein-coupled receptor GPR105 (KIAA0001, P2Y14) and increase intracellular calcium in response to its agonist, uridine diphosphoglucone. J Immunol 171(4):1941–1949
100. Idzko M et al (2002) Nucleotides induce chemotaxis and actin polymerization in immature but not mature human dendritic cells via activation of pertussis toxin-sensitive P2y receptors. Blood 100(3):925–932
101. Idzko M et al (2004) Characterization of the biological activities of uridine diphosphate in human dendritic cells: Influence on chemotaxis and CXCL5 release. J Cell Physiol 201(2):286–293
102. la Sala A et al (2002) Dendritic cells exposed to extracellular adenosine triphosphate acquire the migratory properties of mature cells and show a reduced capacity to attract type 1 T lymphocytes. Blood 99(5):1715–1722
103. Verghese MW et al (1996) P2U agonists induce chemotaxis and actin polymerization in human neutrophils and differentiated HL60 cells. J Biol Chem, 1996. 271(26):15597–15601