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Chemotactic activity of extracellular nucleotides on human immune cells.

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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 CX_3C. Until now, C and CX_3C 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

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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 P2YR subtypes have been cloned so far ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$) [5, 9, 10]. Activation of P2YR induces phospholipase C activation, inositol triphosphate generation, Ca^{2+} release from intracellular stores, and/or stimulation/inhibition of adenylate cyclase. Extensive pharmacological studies performed in P2Y transfected cells revealed that $P2Y_1$, $P2Y_{11}$, $P2Y_{12}$ and $P2Y_{13}$ 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 $P2Y_2$, $P2Y_4$ and $P2Y_6$ 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 $P2Y_4$ and $P2Y_6$, respectively [16–18]. In addition, it has been shown that $P2Y_{14}$ 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 $P2X_1$–$P2X_7$ 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, ATPγS 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 $P2Y_2$ (formerly known as P_{2U}) receptor.

Surprisingly, the first reverse transcripaise polymerase chain reaction (RT-PCR) data revealed that human neutrophils express only $P2Y_4$ and $P2Y_6$ but not $P2Y_1$ and $P2Y_2$ receptors [38]. However, recent reports showed that human neutrophils also express messenger ribonucleic acid (mRNA) for the $P2Y_2$ and $P2Y_{11}$ receptor subtypes [39, 40]. Among the P2X receptors, so far, only the present of the $P2X_7$ 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: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{14}$, $P2X_1$, $P2X_4$ and $P2X_7$ [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 made 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 $P2X_1$ and $P2X_4$, whereas the $P2X_7$ 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 $P2Y_1$, $P2Y_2$, $P2Y_{11}$, $P2Y_{12}$ and $P2Y_{13}$ 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 $P2Y_2$ 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 chemoattractants 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 Ca^{2+} release from intracellular stores [60, 61] and the finding of $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptor-specific mRNA in lymphocytes [38]. Different studies suggest that human B cells express at least $P2X_7$ 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 $P2X_1$, $P2X_2$, $P2X_4$ and $P2X_7$ 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 $P2X_1$, $P2X_4$ and $P2X_7$, [22, 64, 65]. Functional activity of the P2X receptors on T cells has been shown by a large influx of Na^+ and Ca^{++} 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 $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$ and $P2Y_{13}$ [66]. However, a functional expression of different P2Y receptors on human T lymphocytes has not been proven.

Studies showing that activation of the $P2X_7$ 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 $P2X_7$ receptor might be involved in the transendothelial migration of lymphocytes [71, 72]. But elegant experiments from Chen and coworkers using $P2X_7$ 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: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ $P2X_1$, $P2X_4$ and $P2X_7$ [38, 66, 79]. However, other investigations found a lack of functional $P2X_7$ in these cells, whereas the receptor appears during maturation of monocytes to macrophages [22]. Besides $P2X_7$ receptors, human macrophages or macrophage cell lines have been described to express $P2Y_2$, $P2Y_4$ and $P2Y_6$ 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 naive 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 ($P2X_1$, $P2X_4$, $P2X_5$, $P2X_7$) and eight subtypes of the P2Y receptor family ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$ $P2Y_{12}$ $P2Y_{13}$, $P2Y_{14}$) [95–99].

First, studies by Liu et al. revealed that at least activation of the $P2Y_1$, $P2Y_2$ and $P2Y_4$ 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 $P2Y_2$, as well as ADP (via $P2Y_1$?) 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 Ca^{2+} mobilisation. Recently, UDP could be added to the list of chemoattractant nucleotides, as it has been shown that UDP via binding to the $P2Y_6$ 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 $G_{i/o}$ protein-coupled chemotactic P2Y receptor responsiveness during maturation. Surprisingly, immature and mature DCs expressed similar amounts of mRNA for the purinergic receptor subtypes $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2X_1$, $P2X_4$ and $P2X_7$ [100]. DC maturation encompasses a coordinated down-regulation of inflammatory chemokine receptors (CCR_1, CCR_2, CCR_5 and $CXCR_1$) and induction of CCR_7 and $CXCR_4$ [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 matured DCs stimulated with ATP gain the ability to migrate in response to CXC ligand $(L)_{12}$ and CCL_{12} [102]. However, in contrast, Schnurr et al. reported that ATP through $P2Y_{11}$ signalling could inhibit CCL_{21}, 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 CCL_{22} [macrophage-derived chemokine (MDC)] and inhibits the lipopolysaccharide (LPS)-induced secretion of $CXCL_{10}$ (IP-10) and CCL_5 (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 $P2Y_6$ 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.

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.

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