Purinergic Receptors in Thrombosis and Inflammation

Béatrice Hechler, Christian Gachet

Abstract—Under various pathological conditions, including thrombosis and inflammation, extracellular nucleotide levels may increase because of both active release and passive leakage from damaged or dying cells. Once in the extracellular compartment, nucleotides interact with plasma membrane receptors belonging to the P2 purinergic family, which are expressed by virtually all circulating blood cells and in most blood vessels. In this review, we focus on the specific role of the 3 platelet P2 receptors P2Y1, P2Y12, and P2X1, in hemostasis and arterial thrombosis. Beyond platelets, these 3 receptors, along with the P2Y1, P2Y6, and P2X7, receptors, constitute the main P2 receptors mediating the proinflammatory effects of nucleotides, which play important roles in various functions of circulating blood cells and cells of the vessel wall. Each of these P2 receptor subtypes specifically contributes to chronic or acute vascular inflammation and related diseases, such as atherosclerosis, restenosis, endotoxemia, and sepsis. The potential for therapeutic targeting of these P2 receptor subtypes is also discussed. (Arterioscler Thromb Vasc Biol. 2015;35:2307-2315. DOI: 10.1161/ATVBAHA.115.303395.)

Key Words: atherosclerosis ■ inflammation ■ nucleotides ■ platelets ■ sepsis ■ thrombosis

Extracellular nucleotides are the most ancient and widespread intercellular signaling system in living tissues and serve as intercellular or autocrine messengers regulating numerous physiological or pathological functions, including neurotransmission, muscle contraction, bone metabolism, liver glycogen metabolism, cardiac function, vascular tone, inflammation, and hemostasis and thrombosis.¹ In most cases, extracellular nucleotides act as potent costimuli, which amplify and sustain the responses to neurotransmitters, hormones, cytokines, and other agents. They also play a role as autocrine regulators of immune responses, as a find-me-signal of apoptotic cells, and as activators of the inflammasome and are involved in both acute and chronic/adaptive situations.² Extracellular nucleotides may be released through specific mechanisms involving different plasma membrane proteins, such as pannexins or connexin hemichannels or cell activation and granule secretion as in the case of blood platelets. They can also be passively released after mechanical stress or changes in the homeostatic conditions, including anoxia and ischemia-reperfusion injury. During inflammation, for example, adenosine 5′-triphosphate (ATP) released from dying cells and damaged tissues or in response to inflammatory stimuli acts as a danger signal or damage-associated molecular pattern, which displays potent immune-enhancing activity, whereas at sites of vascular injury, adenine nucleotides, massively released by activated platelets, contribute to both hemostasis and thrombosis formation by greatly amplifying most of the platelet responses. Once in the extracellular compartment, nucleotides can be metabolized by various ectonucleotidases, such as ectonucleoside triphosphate diphosphohydrolase 1 (CD39), which converts ATP and adenosine 5′-diphosphate (ADP) to adenosine monophosphate; ecto-5′-nucleotidase (CD73), which converts adenosine monophosphate to adenosine; and nucleoside diphosphate kinase, which catalyzes the exchange of phosphate groups between different nucleoside diphosphates3 (Figure 1). This complex nucleotide metabolism probably explains why it has long been difficult to assign particular cell functions to the activation of specific receptors. Furthermore, the molecular identity of the nucleotide receptors remained unknown for a long time.

The receptors for nucleotides belong to the P2 receptor family, whereas P1 receptors are activated by adenosine and comprise 4 subtypes A₁, A₂A, A₂B, and A₃. P2 receptors are subdivided into ligand-gated P2X cation channels and G protein–coupled P2Y receptors (Figure 1). The first P2 receptor subtype to be identified was cloned from a chick brain complementary DNA library in 1993. Currently, the known P2 receptor family comprises 7 subtypes of P2X receptors (P2X₁, P2X₂, P2X₃, P2X₄, P2X₅, P2X₆, and P2X₇) and 8 subtypes of P2Y receptors (P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁, P2Y₁₂, P2Y₁₃, and P2Y₁₄).⁴ These P2 receptor subtypes display some agonist selectivity. Thus, P2X receptors are activated principally by ATP, some P2Y receptors are activated by purine nucleotides alone (P2Y₁, P2Y₁₂, and P2Y₁₃), and others by both purine and pyrimidine nucleotides (P2Y₁₄), whereas P2Y₁₄ is activated by nucleotide sugars. The various cell types involved in innate immunity, inflammation, and thrombosis all express multiple P2 receptor subtypes, which may play complementary roles depending on the relative affinities for their natural ligand and the downstream signaling pathways. However, a certain extent...
of redundancy is not excluded as, for example, in the case of the many $G$-coupled P2Y receptor subtypes expressed by endothelial cells (ECs), which are involved in the regulation of vascular tone.1

In this short review, we will discuss the roles of the platelet P2 receptor subtypes in the physiological process of hemostasis and in the pathological development of arterial thrombosis. Beyond platelets, these P2 receptors, along with other P2 receptor subtypes, are present on various blood cell types and on cells of the vessel wall. We will describe in detail their known respective contributions to the pathological processes of acute and chronic inflammation involved in sepsis, atherosclerosis, restenosis, and asthma.

### Platelet P2 Receptors in Hemostasis and Thrombosis

Adenine nucleotides (both ADP and ATP) are deeply involved in the processes of hemostasis and in the development and extension of arterial thrombosis through their key roles in platelet activation.3 They act on platelets through 3 distinct P2 receptors: the P2Y$_1$ and P2Y$_{12}$ receptors are $G$ protein–coupled ADP receptor subtypes, whereas the P2X$_1$ ligand-gated cation channel is activated by ATP (Figure 2).

#### Platelet P2Y$_{12}$ Receptor

The P2Y$_{12}$ receptor, cloned in 2001, is activated by ADP, whereas ATP is an antagonist. It constitutes the canonical receptor for ADP on blood platelets and is to date the only P2 receptor subtype to be an established target for drugs in clinical use6 (see below). Long before its molecular cloning, the pharmacological importance of the P2Y$_{12}$ receptor in hemostasis and thrombosis was well recognized. This is because the thienopyridine compounds (ticlopidine and clopidogrel), of which an active liver metabolite covalently binds to the P2Y$_{12}$ receptor, were used as molecular tools to characterize the platelet responses to ADP and its role in thrombosis.7 Clopidogrel treatment was shown to inhibit platelet aggregation in response to ADP and other agents and to selectively block the ability of ADP to inhibit cAMP production while preserving the ability of ADP to trigger intracellular calcium mobilization. Furthermore, clopidogrel was effective in number of animal models of experimental thrombosis. All these findings were later confirmed in P2Y$_{12}^{-/-}$ mice.8,9

The expression of the P2Y$_{12}$ receptor was long thought to be restricted to platelets and subregions of the brain. However, it has now been shown that this receptor is also expressed on vascular smooth muscle cells (VSMCs)10,11 and dendritic cells (DCs).12 On blood platelets, the P2Y$_{12}$ receptor is entirely responsible for the role played by ADP in the amplification of aggregation, secretion, and stabilization of platelet aggregates and in enhancement of the procoagulant activity induced by agonists, such as thrombin, collagen, or thromboxane A$_2$.6 The P2Y$_{12}$ receptor is coupled to the $G_{ai2}$ subunit of the heterotrimeric G proteins, which is responsible for the activation of 2 phosphoinositide 3-kinase isoforms (PI 3-K p110$\beta$ and p110$\gamma$) and the inhibition of cAMP-dependent protein kinase and subsequent phosphorylation of various targets. Notably, one of these signaling proteins is vasodilator-stimulated phosphoprotein, an actin regulatory protein, which is used as a marker of the P2Y$_{12}$ receptor activation state, especially to monitor the effects of P2Y$_{12}$-targeting antiplatelet drugs.6

The central role of the P2Y$_{12}$ receptor in platelet activation and the growth and stabilization of a thrombus makes this receptor an attractive molecular target for antithrombotic agents.6,13 Indeed, in addition to the active metabolites of the 3 generations of thienopyridine produgs (ticlopidine, clopidogrel, and prasugrel, respectively), it is targeted by the direct-acting reversible oral P2Y$_{12}$ antagonist ticagrelor (AZD6140) and of the direct IV antagonist cangrelor.14 All these drugs are now approved for the treatment and prevention of thrombotic events in acute coronary syndromes.

One limitation of targeting the P2Y$_{12}$ receptor is related to the bleeding risk, which increases with the degree of inhibition

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**Nonstandard Abbreviations and Acronyms**

<table>
<thead>
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<th>Abbreviation</th>
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<tr>
<td>ADP</td>
<td>adenosine 5′-diphosphate</td>
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<td>ApoE</td>
<td>apolipoprotein E</td>
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<tr>
<td>ATP</td>
<td>adenosine 5′-triphosphate</td>
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<td>DCs</td>
<td>dendritic cells</td>
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<td>ECs</td>
<td>endothelial cells</td>
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<td>TF</td>
<td>tissue factor</td>
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<td>VSMCs</td>
<td>vascular smooth muscle cells</td>
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**Figure 1.** Schematic overview of the various mechanisms of nucleotide release from cells, of the ecto-enzymes (CD39 and CD73) responsible for nucleotide metabolization, and of P1R receptors for adenine (ADO) and P2 receptor subtypes (P2XR and P2YR) for nucleotides. ADP indicates adenosine 5′-diphosphate; AMP, adenosine monophosphate; and ATP, adenosine 5′-triphosphate.
of P2Y12-dependent platelet functions.15,16 Similarly, patients with severe P2Y12 deficiency can experience serious hemorrhage.13 A recent report of the crystal structure of P2Y12 has revealed how this receptor behaves when it binds agonists and antagonists and should provide valuable insights for the development of improved P2Y12 antagonists.17

Platelet P2Y1 Receptor

The P2Y1 receptor was the first P2 receptor to be cloned, as early as 1993. In contrast to P2Y12, P2Y1 is widely distributed in many tissues, including blood vessels and blood cells, smooth muscle cells, neural tissue, and the heart, testis, prostate, and ovary. Coupled to Gαq, the P2Y1 receptor triggers platelet shape change and weak, transient aggregation in response to ADP.5 Studies using P2Y1−/− mice or pharmacological inhibitors have confirmed its requirement for ADP-induced platelet shape change and aggregation.18,19 Although only responsible for weak and transient platelet responses, this receptor plays a crucial role in various models of thrombosis, including systemic thromboembolism induced by infusion of a mixture of collagen and adrenaline19 or tissue factor (TF)20 and localized thrombosis after laser- or ferric chloride–induced arterial injury.21 Because the P2Y1 receptor is also present on ECs, mice with selective invalidation of P2Y1 in platelets or ECs have been generated to elucidate the respective contributions of the receptors of these cell types to thrombosis. Thus, mice selectively deficient for the platelet P2Y1 receptor were equally well protected in a model of ferric chloride–induced thrombosis as whole body P2Y1−/− mice (unpublished data), indicating that the platelet receptor is probably entirely responsible for the contribution of P2Y1 to thrombosis. The endothelial P2Y1 receptor nevertheless plays an important role in vascular inflammation (see below), which could also be relevant to thrombosis.

Inhibition of thrombosis is observed in animals treated with the selective, high-affinity P2Y1 receptor antagonist MRS2500.22 However, because of its limited bioavailability during long-term treatment, new P2Y1 receptor antagonists with an improved pharmacokinetic profile will need to be developed. The recent report of the x-ray crystal structure of the human P2Y1 receptor, identifying 2 completely distinct ligand binding sites,23 will no doubt open up new possibilities for the development of improved P2Y1 antagonists. It is worthy of note that inhibition of the P2Y1 receptor results in only moderate prolongation of the bleeding time, which could be advantageous in terms of safety as compared with inhibition of the P2Y12 receptor.

The role of the P2Y1 receptor might also be regulated at the level of protein expression because its overexpression in megakaryocytes and platelets leads to increased ADP-induced platelet aggregation and arterial thrombosis.24 Conversely, downregulation of P2Y1 receptor expression occurs through A2B adenosine receptor–mediated increase in cAMP levels, resulting in reduced ADP-induced platelet aggregation. This may have implications in thrombosis, especially under conditions of injury or inflammation where A2B receptor is upregulated in platelets.25

Thus, consideration of the role of the P2Y1 receptor in platelet aggregation and experimental thrombosis provides the rational for suggesting that this receptor could be a relevant target for new antiplatelet compounds.

Platelet P2X1 Receptor

The third component of the panoply of platelet P2 receptors is P2X1, a ligand-gated cation channel responsible for the fast calcium entry induced by ATP.5,26 It is expressed on numerous cell types, namely neutrophils, macrophages, and VSMCs but it seems to be absent from vascular ECs.1 In platelets, the P2X1 receptor triggers transient shape change and participates in collagen- and shear-induced platelet aggregation.27–29 One characteristic of this receptor is that it desensitizes quickly, thereby hampering the in vitro study of its functions in platelet activation. This explains why most of our knowledge of the P2X1 receptor stems from its in vivo evaluation in P2X1−/− mice, which has revealed its important role in thrombosis. Thus, P2X1−/− mice display resistance to the systemic thromboembolism induced by injection of a mixture of collagen and adrenaline and to the thrombosis triggered by localized laser-induced injury of the vessel wall of mesenteric arteries.28 The latter was moreover confirmed using the P2X1 antagonist NF449.30 P2X1−/− mice are also less susceptible to the systemic thromboembolism triggered by TF, that is, in a thrombin-dependent system (unpublished data). Finally, P2X1−/− mice exhibit no prolongation of the bleeding time as compared with wild-type animals, indicating that they conserve normal hemostasis.28 Overall, the P2X1 receptor should also be considered as a potential target for safe antiplatelet drugs.

Platelet P2Y14 Receptor

Finally, platelets also express the P2Y14 receptor for uridine 5′-diphosphate-glucose,31 but this receptor has no role in their hemostatic function. P2Y14 is also present in neutrophils, DCs, B-lymphocyte, and T-lymphocyte, suggesting a physiological function in immune or inflammatory responses (see below).32
P2 Receptors in Inflammation

As mentioned in the Introduction, cells of the immune system as likewise blood and vascular cells all express various subtypes of P2 receptors, which have been shown to be involved in the modulation of inflammation and in immune responses. On the contrary, platelets themselves play an important role not only in thrombosis but also in modulating inflammatory responses through release of inflammatory mediators or compounds with trophic activity and exposure of P-selectin, CD40, and CD40 ligand. These molecules allow interaction of the platelets with immune cells and their subsequent activation with release of a range of inflammatory cytokines and exposure of TF. Therefore, in addition to acting as anti-thrombotics, antagonists and inhibitors of the platelet P2 receptors could have anti-inflammatory effects.

P2Y12 Receptor in Inflammation

The platelet P2Y12 receptor is indeed involved in modulating inflammatory responses because numerous studies have described reduced levels of circulating inflammatory mediators (tumor necrosis factor-α, C-reactive protein), decreased exposure of P-selectin and CD40 ligand, diminished formation of platelet–leukocyte aggregates, and less subsequent TF exposure in mice or patients receiving clopidogrel. In addition, the P2Y12 receptor expressed on VSMCs has been reported to promote proinflammatory and mitogenic responses through a thrombin-induced pathway in vitro. All these findings point to a potential contribution of this receptor to inflammatory diseases. Accordingly, convincing data indicate that the P2Y12 receptor plays an important role in atherosclerosis and restenosis. Specifically, studies using P2Y12−/− mice and bone marrow chimeric mice expressing either vessel wall or platelet P2Y12 indicate a contribution of the platelet P2Y12 receptor to restenosis and of the vessel wall and platelet P2Y12 receptors to the development of atherosclerosis. The platelet P2Y12 receptor has also been proposed to play an important role in allergic asthma. Platelet activation is strongly involved in this inflammatory disease because it is required for the recruitment of inflammatory cells to the lungs and remodeling of the airway wall. Paruchuri et al showed that the proasthmatic action of leukotriene E4 in mice requires the platelet P2Y12 receptor because inflammation was abrogated by platelet depletions, by treatment with clopidogrel, or by treatment in P2Y12−/− mice. The mechanism involved was the association of P2Y12 with an as yet unidentified coreceptor of the cysteinyl leukotriene family, but this nevertheless remains to be established. Additional evidence for a role of the platelet P2Y12 receptor in human asthma was provided by recent clinical observations that P2Y12 receptor variants were associated with altered lung function in a large family-based asthma cohort and that prasugrel tended to decrease the bronchial hyper-reactivity to mannitol in patients with allergic asthma in a randomized placebo-controlled trial.

In sharp contrast, a recent study reported no involvement of P2Y12 in allergic inflammation in ovalbumin-challenged mice, whereas the platelet P2Y12 receptor appeared to be pivotal (see below). Obviously, there are conflicting data concerning the part played by the P2Y12 receptor in asthma, and whether the discrepancies might be related to differences in the mouse models of allergic inflammation or to the use of P2Y12 receptor antagonists versus P2Y12−/− mice will require further investigation.

Recent findings point to a proinflammatory role of the P2Y12 receptor in acute inflammation because of sepsis or lipopolysaccharide-induced endotoxemia (Figure 5), where platelets play an important role by enhancing the pulmonary infiltration of neutrophils and exacerbating tissue damage. Specifically, the involvement of platelet P2Y12 in sepsis has been suggested on the grounds of the inhibitory effect of the P2Y12 antagonist ticagrelor on platelet–neutrophil aggregate formation, neutrophil recruitment, and lung damage in mice.

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susceptible or whether the P2Y1 receptors present on other cell types played an important part in the development of atherosclerosis in apolipoprotein E (ApoE) \(^{-/-}\) mice. Thus, studies using P2Y\(_{12}\) \(^{-/-}\) mice and bone marrow chimeric mice expressing P2Y\(_1\) on either hematopoietic- or non-hematopoietic-derived cells revealed that platelet P2Y\(_1\) was not involved in this process, suggesting a potential role of the P2Y\(_1\) receptors expressed on vessel wall cells, probably on ECs.\(^{31}\) In addition, the endothelial P2Y\(_1\) receptor has been shown to contribute to the tumor necrosis factor-\(\alpha\)-induced upregulation of adhesion molecules (P-selectin, VCAM-1 [vascular cell adhesion molecule 1], ICAM-1 [intracellular adhesion molecule 1]) in a P38 MAPK (mitogen-activated protein kinase)-dependent manner, resulting in the recruitment of monocytes in vitro and their recruitment to the vascular wall in vivo\(^{52}\) (Figure 3). This was confirmed in mice with selective invalidation of the endothelial P2Y\(_1\) receptor (unpublished data) and in mice treated with the selective P2Y\(_1\) antagonist MRS2500.\(^{52}\)

Additional evidence for a role of the endothelial P2Y\(_1\) receptor in vascular inflammation was provided recently by the finding that inorganic polyphosphate interacts with this receptor. Inorganic polyphosphate is derived from bacteria or released from activated platelets and has prothrombotic and proinflammatory effects in vivo. Its interaction with the endothelial P2Y\(_1\) receptor and with the receptor for advanced glycation end products has shown to dramatically amplify the proinflammatory responses of nuclear proteins, the high mobility group box 1, and histone H4, thereby activating NF-\(\kappa\)B, promoting the expression of cell adhesion molecules and inducing barrier-disruptive effects in vitro and in vivo.\(^{53}\)

On VSMCs, the P2Y\(_1\) receptor has been shown to contribute to their proliferation and migration in vitro, which could be relevant for the intimal hyperplasia observed in a vein graft model in mice.\(^{54}\) In this case, a contribution from the P2Y\(_1\) receptor present on circulating blood cells, not only platelets but also macrophages, cannot be excluded. Indeed macrophages seem required because their depletion abrogated hyperplasia,\(^{54}\) and recent data indicated a role of the P2Y\(_1\) receptor in macrophage phagocytic and migration activity.\(^{59}\)

As a consequence, the P2Y\(_1\) receptor could represent an attractive and original target for drugs with multiple sites of action to treat atherothrombosis and other inflammatory diseases.\(^{21}\)

**P2Y\(_1\) Receptor in Inflammation**

The platelet P2Y\(_1\) receptor contributes to leukocyte activation through its participation in platelet P-selectin exposure and in the formation of platelet–leukocyte conjugates, leading to leukocyte TF exposure.\(^{50}\) The role of the P2Y\(_1\) receptor in platelet functions along with its presence in all the blood cell types and tissues involved in inflammation raises the question of its involvement in related pathological processes. Accordingly, one recent report described an important contribution of the platelet P2Y\(_1\) receptor to inflammation of the airways in allergic mice through its role in the formation of the platelet–leukocyte complexes required for leukocyte recruitment to the lung tissue.\(^{44}\) However, it is difficult to firmly establish from these data whether the platelet receptor was entirely responsible or whether the P2Y\(_1\) receptors present on other cell types were also involved in allergic asthma.

The P2Y\(_1\) receptor is expressed on ECs, and this receptor plays an important part in the development of atherosclerosis subjected to cecal ligation and puncture.\(^{47}\) However, because ticagrelor also inhibits adenosine uptake, the possibility that its protective effect might be related at least partially to its effect on adenosine metabolism cannot be ruled out. Similarly, clopidogrel attenuated inflammation in a rat model of lipopolysaccharide-induced endotoxemia.\(^{48}\) In sharp contrast, in a slightly different endotoxemia model triggered by repeated administration of lipopolysaccharide on 4 consecutive days, P2Y\(_{12}\) \(^{−/-}\) mice displayed a higher circulating neutrophil count, an enhanced inflammatory state, and more severe lung injury as compared with wild-type mice, indicating a protective role of the P2Y\(_{12}\) receptor in this context.\(^{49}\) Because platelet consumption was, however, similar in wild-type and P2Y\(_{12}\) \(^{−/-}\) mice, the authors suggested the involvement of leukocyte P2Y\(_{12}\) rather than the platelet receptor. The reasons for the discrepant pro- or anti-inflammatory role of the P2Y\(_{12}\) receptor in various models of endotoxemia are not yet clear. The authors of the latter study proposed that they could be related to off-target effects of clopidogrel because clopidogrel treatment had a protective effect in P2Y\(_{12}\) \(^{−/-}\) mice. Alternatively, because healthy untreated P2Y\(_{12}\) \(^{−/-}\) mice displayed significantly lower numbers of neutrophils in the spleen and bone marrow, one hypothesis is that the P2Y\(_{12}\) receptor could be involved in regulation of the cellular composition of bone marrow and spleen, which might alter their responsiveness to inflammation.\(^{49}\) Further studies will be needed to resolve this question and to make all the relevant comparisons between the models, the drugs administered, and the use of receptor-deficient mice.

**P2X\(_3\) Receptor in Inflammation**

Among leukocyte subsets, the P2X\(_3\) receptor is present on neutrophils where it plays an important part in facilitating the neutrophil chemotaxis induced by various chemoattractants, possibly by favoring contraction and retraction of the trailing uropod.\(^{56}\) This is in accordance with its recently discovered role in acute inflammation, where it was found to be important for neutrophil emigration from venules during endotoxemia in mice, thereby contributing to tissue damage, systemic inflammation, and mortality\(^{57}\) (Figure 5). These findings suggest that the P2X\(_3\) receptor could represent a target for new therapeutic strategies to reduce the host tissue damage caused by neutrophils.

The neutrophil P2X\(_3\) receptor, along with the platelet P2X\(_{1}\) receptor, may contribute to thrombus formation in a context of inflammation. This has been highlighted in a specific model of neutrophil-dependent thrombosis of cremasteric arterioles triggered by laser injury, where activation of the neutrophil...
P2X receptor appeared to be dispensable for neutrophil recruitment and subsequent fibrin generation and thrombus formation at the site of vessel injury. Whether this role of the P2X receptor may be relevant under other conditions of vascular inflammation is an open question. Overall, this receptor nonetheless plays an important role in thrombosis and inflammatory processes, indicating that it could constitute an interesting target for new therapeutics in these fields.

Other P2 Receptors in Vascular Inflammation

Besides the P2Y1, P2Y12, and P2X receptors, numerous studies have highlighted the importance of other P2 receptor subtypes to inflammation. The respective roles of the P2Y2 and P2Y6 receptors in vascular inflammation have been extensively and thoroughly characterized. These 2 receptors are widely expressed on immune cells and on most blood vessel cells and display similar signaling pathways using Gq/11 to activate the PLC/IP3 (phospholipase C/inositol triphosphate) pathway and trigger the release of intracellular calcium. Their main difference lies in their native ligands, P2Y2 being activated equipotently by ATP and UTP, whereas P2Y6 is activated by uridine 5′-diphosphate. Under physiological conditions, the P2Y2 and P2Y6 receptors contribute to regulation of the vascular tone by causing vessel relaxation when expressed on the endothelium but contraction when expressed on VSMCs. Under inflammatory conditions, P2Y2 and P2Y6 play important roles in attracting inflammatory cells to the site of inflammation by inducing the release of chemotactic factors (P2Y2 and P2Y6) or driving directional cell motility (P2Y6). As a result, these receptors could be involved in various vascular inflammatory diseases. Moreover, the P2Y2, P2Y6, and P2X receptor subtypes, similar to the platelet P2Y1 and P2X1 receptors, have been shown to play a pivotal role in the pathogenesis of allergic lung inflammation in mice and humans. Finally, recent evidence of the contribution of the P2X receptor to activation of TF and thrombosis is presented.

P2Y2 Receptor

Under inflammatory conditions, the endothelial P2Y2 receptor promotes the recruitment of circulating inflammatory cells to the vessel wall through upregulation of the expression of leukocyte adhesion molecules (Figure 3). This role of P2Y2 has been shown to be important in various inflammatory processes, such as the tumor cell extravasation, leading to cancer metastasis, the intimal hyperplasia developing after placement of a collar around the carotid artery in the rabbit, and the development of atherosclerosis in mice (Figure 4). Apart from the endothelial P2Y2 receptor, a contribution from P2Y2 receptors expressed on various leukocyte subsets cannot be ruled out because P2Y2 signaling contributes to the ATP-induced release of proinflammatory chemokines and cytokines from myeloid immune cells, including monocytes, macrophages, neutrophils, and DCs.

In addition, the P2Y2 receptor displays an important specificity among P2Y receptors because it induces the chemotaxis of multiple leukocyte subsets, including eosinophils and DCs, with consequences for allergic lung inflammation (below), and also of neutrophils and macrophages. On these latter cells, the P2Y2 receptor mediates the role of ATP as a find-me-signal, which guides them to inflammatory sites and promotes the phagocytic clearance of apoptotic cells or bacteria. It thereby contributes to wound healing, but also to the deleterious accumulation of neutrophils in tissues, which causes tissue damage in sepsis (Figure 5).

P2Y6 Receptor

On inflammatory challenge, the P2Y6 receptor, like the P2Y2 receptor, contributes to the recruitment of inflammatory cells by enhancing the expression of proinflammatory cytokines and leukocyte adhesion molecules (Figure 3). In addition, P2Y6, like P2Y2, triggers the release of proinflammatory cytokines and chemokines from various leukocyte subsets. The P2Y6 receptor has been shown to be involved in the development of atherosclerosis with, however, discrepancies concerning the respective contributions of the cells expressing this receptor. On the one hand, in one study, it was reported that whole body P2Y6 deficiency failed to impact lesion formation, whereas P2Y6 deficiency on hematopoietic-derived cells. On the other hand, in another recent study, it was reported that whole body P2Y6 deficiency failed to impact lesion formation, whereas P2Y6 deficiency on hematopoietic cells reduced lesion development. The reasons for such divergent results are not yet clear. One hypothesis put forward by the authors is that the P2Y6 receptor may play differential and perhaps opposing roles in macrophages versus vascular cell types. In this line, angiotensin II–infused P2Y6−/− mice display increased aortic aneurysm formation, a process thought to primarily involve medial VSMCs. Clearly, further studies will be needed to elucidate the respective contributions of the leukocyte and vascular P2Y6 receptors to atherosclerosis.

Roles of the P2Y2, P2Y6, and P2X Receptors in Allergic Lung Inflammation

Allergic lung inflammation is mediated by DCs, eosinophils, and Th2 lymphocytes and leads to airway remodeling and declining lung function. The P2Y2 and P2Y6 receptors both contribute to asthmatic airway inflammation, as demonstrated by the reduced symptoms of experimental asthma in mice lacking either of these P2 receptor subtypes. However, the 2 receptors differ in their underlying molecular mechanisms and the respective contributions of the cell types expressing them. Specifically, as shown in experiments using P2Y6−/− and P2Y6+/− mouse chimeras, the contribution of the P2Y6 receptor to airway inflammation relies on its presence on airway epithelial cells, where it induces the production of IL-6 and IL-8, thereby favoring attraction of eosinophils and DCs (Figure 6). On these latter cells, the P2Y6 receptor plays an important role in regulating their chemotaxis to sites of allergen exposure, as likewise their production of reactive oxygen species (Figure 6).

Besides the P2Y6 receptor subtypes, the P2X receptor, an ATP-gated cation channel present on all leukocyte subsets and especially on DCs, eosinophils, and macrophages, has been identified as the only P2X receptor to make an important contribution to allergic airway inflammation (Figure 6). Among P2X receptor subtypes, the behavior of P2X, is unique in that,
on repetitive or prolonged exposure to high concentrations of ATP, it can form a large pore permeable to hydrophilic solutes of molecular mass ≤ 900 kDa. The P2X7 receptor is best known for its general proinflammatory effect, mediated by stimulating a caspase-1–activating multiprotein complex called the inflammasome, which results in an increased production of proinflammatory cytokines and their rapid release from mononuclear phagocytes. Concerning specifically allergic airway inflammation, P2X7 receptor signaling on DCs is important for the conversion of naïve T cells into Th2 effector cells through NLRP3 (nod-like receptor protein 3) inflammasome signaling complexes, which drive proteolytic maturation and the secretion of IL-1β. In addition, P2X7 receptor signaling is involved in the release of proinflammatory cytokines and reactive oxygen metabolites from eosinophils or macrophages.

Furthermore, there is convincing evidence for an important role of these P2 receptor subtypes in asthma in humans because eosinophils isolated from asthmatic individuals expressed higher levels of P2Y12, P2X7 receptors as compared with healthy control cells and were more sensitive to ATP-induced migration and production of reactive oxygen metabolites. In addition, low-functioning P2X7 receptor variants have been shown to be associated with reduced risks of childhood asthma and allergic sensitization in a birth cohort at high risk for the development of asthma.

Overall, these results suggest that the P2Y12, P2Y6, and P2X7 receptor subtypes could represent new therapeutic targets to treat allergic lung inflammation

**Role of the P2X7 Receptor in TF-Dependent Thrombosis**

A role of the P2X7 in activation of TF, the primary initiator of coagulation, has been recently highlighted. TF is typically sequestered from blood or exists in a noncoagulant encrypted form on hematopoietic cells. Stimulation of P2X7 on macrophages and VSMCs has been found to induce both the activation of TF cell surface procoagulant activity and generation of procoagulant TF-bearing microparticles. This process has been shown to involve inflammasome activation and caspase-1–dependent actin remodeling. This translates into a role of the P2X7 receptor in experimental thrombus formation after mild FeCl3 injury of the carotid artery.

**P2Y12 Receptor**

As already mentioned, this receptor is present on various leukocyte subsets, including monocytes, macrophages, DCs, and neutrophils, suggesting a role in inflammation. To date, a clear role for this receptor has only been established in human neutrophils, where it contributes to chemotaxis. Further studies will be required to evaluate its role in various inflammatory situations.

**Conclusions**

The effects of adenine nucleotides on platelets are mediated by 3 distinct receptors, P2Y12, P2Y1, and P2X7, each of which plays a specific role in platelet functions and arterial thrombosis. The platelet P2Y12 receptor is an established target for antithrombotic drugs, whereas the P2Y1 and P2X7 receptors constitute attractive targets for new antithrombotic agents. Beyond platelets, P2Y12 is present on VSMCs and may contribute, together with the platelet P2Y12 receptor, to the development of atherosclerosis, which may be relevant to the central role of P2Y12 in atherothrombosis. Conversely, the expression of P2Y12 on leukocyte subsets and its potential role in inflammatory diseases is poorly characterized and will require more detailed investigation. The other platelet P2 receptor subtypes, P2Y1 and P2X7, along with the P2Y12, P2Y6, and P2X7 receptors, constitute the main P2 receptors mediating the proinflammatory effects of nucleotides and play important roles in various functions of blood cells and cells of the vessel wall. They seem to be essential for the development of various pathological processes involving acute or chronic inflammation. However, further studies based on the generation of mice presenting tissue-specific deletion of these P2 receptor subtypes will be needed to precisely define their specific functions under physiological and pathological conditions. These P2 receptors could represent potential novel therapeutic targets for the treatment of inflammatory diseases. The development of pharmacological agonists and antagonists of P2 receptor subtypes, selective and with increased stability, will nevertheless be required for long-term treatment in vivo to allow the design of new and improved therapeutic approaches.

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**Disclosures**

None.

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