The platelet P2 receptors in inflammation

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Keywords
P2Y12, asthma, inflammation, platelets, cysteinyl leukotriens

Summary
In addition to their well characterized and established role in haemostasis and thrombosis, platelets contribute to the pathogenesis of inflammation. Adenine nucleotides are signalling molecules that regulate the function of virtually every cell in the body, by interacting with P2 receptors. Their important role in inflammation is well established. In the last few years, the pro-inflammatory roles of adenine nucleotides interacting with their platelet P2 receptors has emerged. In particular, it was shown that the platelet P2Y12 receptor for ADP significantly contributed to the pro-inflammatory effects of cysteinyl leukotrienes (CysLT) in experimental models of asthma in mice. More importantly, it was recently shown that P2Y12 variants were associated with lung function in a large family-based asthma cohort and that the P2Y12 antagonist prasugrel tended to decrease bronchial hyper-reactivity to mannitol in patients with allergic bronchial asthma in a randomized, placebo controlled trial. Conclusion: These data strongly suggest that P2Y12 may represent an important pharmacological target for the treatment of patients with allergic bronchial asthma.

Platelets play a well established role in haemostasis and thrombosis, but also have inflammatory functions, influence innate and adaptive immune responses, play a role in tumorigenesis, cancer growth and dissemination (1–5).

The role of platelets in inflammation has been documented in many inflammatory conditions in several studies. Platelets express toll-like receptors, which initiate the innate immune response. Moreover, they interact with activated endothelium, undergo chemotaxis, “prime” leukocytes for efficient tissue recruitment, activate other inflammatory cells, exert phagocytosis, release biologically active substances, including adhesive proteins, vasoactive and pro-inflammatory mediators (1–5). Major platelet-derived chemokines and cytokines include PF4/CXCL4, pro-platelet basic protein (ppbp), RANTES, inorganic polyphosphate and IL-1β, among a large number of inflammatory molecules (6–8). Platelet-derived microparticles also contribute to the inflammatory process (9, 10).

Platelets either initiate or accelerate the immune response in diverse inflammatory diseases, including neurodegenerative diseases (11), atherosclerosis (12–14), trans-fusion-related lung injury (15), rheumatoid arthritis (16), SLE (16), transplant rejection (7) and allergic bronchial asthma (17–20).

Many molecules expressed on the platelet membrane, or contained in platelet granules and secreted upon platelet activation play a role in the inflammatory process. Among them, adenine nucleotides, which are secreted by platelet dense granules or by other cells, contribute to the inflammatory process by interacting with their receptors, which are expressed on several cell lines, including platelets.

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Der Plättchen-P2-Rezeptor bei Entzündungen
Hämostaseologie 2015;35: 262–266
http://dx.doi.org/10.5482/HAMO-14-09-0044
received: September 15, 2014
accepted in revised form: December 18, 2014
epub ahead of print: January 12, 2015
Nucleotides and their receptors in inflammation

Purine and pyrimidine nucleotides are extracellular signalling molecules that regulate the function of virtually every cell in the body. During inflammatory, ischaemic and hypoxic conditions, they are released from damaged cells or secreted via non-lytic mechanisms and interact with specific receptors on the cell plasma membranes, which are named P2 receptors (21).

P2Y receptors are 7-membrane-spanning, whose carboxyl terminal domain is on the cytoplasmic side, while the amino terminal domain is exposed to the extracellular environment. Common mechanisms of signal transduction include activation of phospholipase C and/or regulation of adenyl cyclase activity. Eight P2Y receptors have been identified so far: P2Y1, P2Y2, P2Y4, P2Y11, P2Y12, P2Y13, and P2Y14. From a phylogenetic and structural point of view, two distinct P2Y receptor subgroups with a relatively high level of structural divergence have been identified:

- the first subgroup includes the G<sub>i</sub>-coupled subtypes (P2Y1, P2Y2, P2Y4, P2Y11, P2Y12, P2Y13, and P2Y14).
- the second subgroup includes the G<sub>v</sub>-coupled subtypes (P2Y12, P2Y13, and P2Y14) (21).

P2X receptors are ligand-gated ion channels that mediate rapid changes in the membrane permeability of monovalent and divalent cations. P2X receptors have 2 transmembrane domains separated by a large extracellular region; both the amino terminal and the carboxyl terminal domains are on the cytoplasmic side of the plasma membrane (21). Seven P2X receptors have been identified so far (P2X<sub>1</sub> – P2X<sub>7</sub>). They exist as homo- or hetero-oligomers (trimers or hexamers) and are primarily ATP receptors.

Several studies indicated that inflammatory disease conditions are associated with the extracellular release of nucleotides and highlighted fundamental roles for P2Y receptors during inflammatory and infectious diseases (22).

Experimental studies in mice demonstrated that the endothelial P2Y<sub>1</sub> receptor plays a major pathogenic role in acute arterial inflammation, which is considered to be the link between risk factors for atherosclerosis and the biology underlying its complications, among which the life threatening condition of arterial thrombosis, which causes tissue ischaemia and infarction (23).

The activation of P2Y<sub>2</sub> by ATP contributes to the defence against bacterial infections, the promotion of wound healing or the enhancement of mucociliary clearance mechanisms (22). In contrast, it can also lead to uncontrolled inflammation, promotion of chronic inflammatory disease states and fibrotic remodelling (24). P2Y<sub>1</sub> antagonists could evolve as useful drugs for the treatment for chronic inflammatory diseases (25,26).

Inappropriate P2Y<sub>2</sub> signalling, predominantly on stromal cells, can drive detrimental immune responses in chronic inflammatory disorders such as atherosclerosis, chronic lung disease and inflammatory bowel disease (22).

Dendritic cells (DC) express the P2Y<sub>12</sub> ADP receptor and DC P2Y<sub>12</sub> activation increases antigen endocytosis and processing (27). P2Y<sub>12</sub> receptors expressed on platelets also play an important role in inflammation.

ATP signalling through T-cell P2X<sub>7</sub> increases differentiation of CD4<sup>+</sup> T-helper cells towards a pro-inflammatory Th17 cell type (28). P2X<sub>7</sub> receptors contribute to chronic inflammatory disease states, such as chronic lung disease (25,29), asthma (30, 31) or inflammatory bowel diseases (32, 33), when activated inappropriately.

Termination of signalling through P2 receptors is closely linked to the conversion of adenosine nucleotides (ADP and ATP) to adenosine by ectonucleotidases. ATP and ADP are converted to AMP by CD39 (34, 35), and AMP is converted to adenosine by CD73 (36). CD39 on endothelial cells and leukocytes (37) reduces inflammatory cell trafficking and platelet reactivity, with a consequent reduction in tissue injury following cerebral ischaemic challenge (38). Adenosine modulates the inflammatory responses to a variety of stressful conditions (39). High concentrations of adenosine, activating A<sub>2A</sub>R, inhibit neutrophil trafficking, granule release, and the production of reactive oxygen species and inflammatory mediators (40). Genetic deficiency of A<sub>2A</sub>R increases mortality in mice with sepsis and reduced levels of inflammatory markers (41). Thus, elevation of endogenous adenosine concentrations may reduce the inflammatory responses (22, 39, 42). Disruption of CD39 or CD73 in mice rendered them more susceptible to tissue injury during inflammatory conditions such as acute lung injury or intestinal inflammation (43,44).

P2 receptors on platelets

Human platelets express at least three distinct receptors that interact with ADP or ATP: P2Y<sub>1</sub>, P2Y<sub>11</sub> and P2X<sub>7</sub>. They have the following order of expression: P2Y<sub>12</sub> > P2X<sub>7</sub> > P2Y<sub>1</sub> (21). They also express small amounts of P2Y<sub>14</sub> mRNA and protein but no contribution of this receptor to several measures of platelet function has yet been demonstrated. Human platelets also express two subtypes of adenosine receptors: A<sub>2A</sub> and A<sub>2B</sub> (21).

Both P2Y receptors expressed on platelets have been shown to potentially play a role in inflammation. The platelet P2Y<sub>1</sub> receptor contributes to P-selectin exposure, the formation of platelet-leukocyte aggregates and tissue factor exposure when platelets are stimulated with ADP, collagen or low concentrations of thrombin receptor agonist peptides (29, 31). The potential role of the platelet P2Y<sub>12</sub> receptor in inflammation has been shown in studies of the effects of drugs inhibiting the receptor, such as the thienopyridines ticlopidine and clopidogrel. Decreased exposure of P-selectin, diminished formation of platelet-leukocyte aggregates and less subsequent tissue factor exposure have been documented after treatment with thienopyridines (45, 46). The inhibition of CD40L exposure and release (47) and the reduction of circulating levels of C-reactive protein (48) in response to P2Y<sub>12</sub> antagonists further support the role of P2Y<sub>12</sub> in the inflammatory process. However, the most abundant and convincing data on the pro-inflammatory role of the platelet P2Y<sub>12</sub> receptor focused on the important pathogenic role of platelet P2Y<sub>12</sub> in allergic bronchial asthma.
The platelet P2Y\textsubscript{12} receptor in inflammation

Vascular inflammation

A critical role for platelets in vascular inflammation and its inhibition by P2Y\textsubscript{12} antagonists has been demonstrated both in humans and experimental animals. In a murine model of abdominal aortic aneurism, treatment with clopidogrel, an inhibitor of P2Y\textsubscript{12}, significantly suppressed aneurysm formation, inflammatory cytokine expression, infiltration of macrophages and production of matrix metalloproteinases (49).

Clopidogrel has also been shown to exhibit pleiotropic anti-inflammatory properties in humans (50–52). However, the inflammation-reducing effects of clopidogrel have not been a persistent finding in all trials and the results of different studies in this field are sometimes contradictory (53–59). Clopidogrel withdrawal was associated with an increase in platelet and inflammatory biomarkers in diabetic patients (60).

P2Y\textsubscript{12} is also expressed on smooth muscle cells (SMC) (61). Thrombin increases expression of P2Y\textsubscript{12} in human vascular SMC, which results in an enhanced proinflammatory and mitogenic response to ADP (62).

Allergic bronchial asthma

Allergic bronchial asthma is a chronic inflammatory disease that impairs the quality of life and is associated with significant mortality rate. In 1981, it was shown that platelets are activated during antigen-induced bronchoconstriction (17). More recently, it was shown that platelets accumulate in lungs of asthmatic patients, are required for airway wall remodelling and recruitment of inflammatory cells in murine allergic lung inflammation (18, 19) and migrate into the lungs of ovalbumin-sensitized and -challenged mice by an IgE-dependent mechanism (20).

Cysteinyl-leukotrienes (LTs) LTC\textsubscript{4}, LTD\textsubscript{4} and LTE\textsubscript{4} are biologically active lipids that play a role in allergic asthma (63). They are formed in eosinophils, basophils and mast cells by 5-lipoxygenase, which catalyses the conversion of arachidonic acid into LTA\textsubscript{4}, an unstable intermediate that is enzymatically converted into either LTB\textsubscript{4} or LTC\textsubscript{4}, which is transported into the extracellular space where a gamma-glutamyl-transpeptidase forms LTD\textsubscript{4}, which is finally converted into the stable metabolite LTE\textsubscript{4} (64). Cysteinyl-LTs interact with G protein-coupled receptors, CysLT\textsubscript{1}R, CysLT\textsubscript{2}R and GPR99 (65–67). Platelets adhere to leukocytes and amplify the production of cysteinyl-LTs (63), express CysLT\textsubscript{1}R and CysLT\textsubscript{2}R and, when exposed to LTD\textsubscript{4} or LTE\textsubscript{4}, release RANTES, a powerful eosinophil chemoattractant (68).

Recently, it was shown that LTE\textsubscript{4} enhances inflammatory cell recruitment in lungs of sensitized mice, which is abrogated by platelet depletion, by treatment with the anti-P2Y\textsubscript{12} thienopyridine drug clopidogrel (69), and in mice lacking P2Y\textsubscript{12}, but not in mice lacking CysLT\textsubscript{1}R and CysLT\textsubscript{2}R (70). Moreover, intranasal administration of LTC\textsubscript{4} in sensitized mice before ovalbumin challenges potentiated the recruitment of eosinophils in the bronchoalveolar lavage, which was dependent on CysLT\textsubscript{1}R, but also on P2Y\textsubscript{12} and platelets (71).

The mechanism by which the platelet P2Y\textsubscript{12} contributes to the effects of cysteinyl-LT is uncertain. Because LTE\textsubscript{4} shows negligible activity at CysLT\textsubscript{1}R and CysLT\textsubscript{2}R, its biological effects are likely mediated by a third, elusive receptor, which was tentatively identified with P2Y\textsubscript{12}, based on computer modelling and the demonstration that LTE\textsubscript{4} signals through P2Y\textsubscript{12} in transfected cells (72). However, more recently, GPR99 was identified as the elusive receptor for LTE\textsubscript{4} (67). Moreover, studies that demonstrated the important role played by platelet P2Y\textsubscript{12} in LTE\textsubscript{4}- or LTC\textsubscript{4}-induced enhanced recruitment of inflammatory cells in the lungs of sensitized mice failed to show that the CysLT interact directly with the platelet P2Y\textsubscript{12}, which might therefore play an indirect, albeit important role in the process (63, 70, 71).

Independently of whether it plays a direct or an indirect role in the inflammatory process, these data clearly suggest that the platelet P2Y\textsubscript{12} represents an ideal pharmacological target for the treatment of allergic asthma.

The demonstration that P2Y\textsubscript{12} variants are associated with lung function in a large family-based asthma cohort and that house dust mite modulates these associations through gene-by-environment effects provided the first human evidence supporting a role for P2Y\textsubscript{12} in this disorder (73).

In addition, in a randomized, placebo-controlled, cross-over study it was recently shown that treatment with the thienopyridine P2Y\textsubscript{12} antagonist prasugrel of patients with allergic asthma for 15 days tended to reduce bronchial hyper-reactivity to mannitol (74). This effect of prasugrel likely reflects a reduction in airway inflammation, because the mannitol test, like other forms of indirect airway challenge, more closely reflects active airway inflammation than the direct challenges, such as the metacholine test (75). The greater specificity of the mannitol test for detecting changes in airway hyper-responsiveness in asthma patients is likely explained by the fact that it mimics the normal pathophysiology of bronchial asthma, causing the release of various mediators of bronchoconstriction (76). Although the results of this study cannot clarify whether the effect of prasugrel was mediated by its interaction with P2Y\textsubscript{12} on platelets or other cells, the former hypothesis is supported by the results of experimental studies that demonstrated the important role of platelet P2Y\textsubscript{12} in the recruitment of inflammatory cells in lungs of sensitized mice challenged with cysteinyl-LT (70, 71). Moreover, due to the very short half-life of the active metabolite of prasugrel, inhibition of P2Y\textsubscript{12} on nucleated cells, such as leukocytes, would likely be very short lived and probably insufficient to exert any clinically relevant effect (77). In contrast, the effect of the active metabolite of prasugrel on the anucleated platelet lasts for the entire life of the cell, because the active metabolite irreversibly inhibits the receptor and platelets are unable of the novo synthesis of non-inhibited receptors (77).

Conclusion

Several lines of evidence indicate that platelets play an important pathogenic role not only in haemostasis and thrombosis, but also in...
The role of platelets in inflammatory processes, in particular, has gained particular attention in the last three decades. Among the many platelet receptors and molecules that are involved in inflammation, the platelet P2Y12 receptor for ADP has recently been implicated in the pathogenesis of allergic asthma, through its direct or indirect interactions with cysteinyL LT. Both an observational, epidemiologic study and, more recently, a small, proof-of-concept randomized clinical trial supported the hypothesis that P2Y12 may represent an important pharmacological target for the treatment of patients with allergic bronchial asthma.

Conflicts of interest
MC received lecture honoraria, advisory board honoraria & research grants from Eli Lilly, Daiichi Sankyo and AstraZeneca.

References
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