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Platelets in patients with aspirin-exacerbated respiratory disease

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Activity Objectives

1. To review the basic pathophysiology of aspirin-exacerbated respiratory disease (AERD).
2. To understand the role of platelets and the mechanism of their contribution in patients with AERD.
3. To understand how medication altering platelet function might relate to the pathology of AERD.

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Aspirin-exacerbated respiratory disease (AERD) is a chronic inflammatory disease characterized clinically by the triad of asthma, nasal polyposis, and pathognomonic respiratory reactions after ingestion of aspirin. It is a distinct syndrome associated with eosinophilic infiltration of respiratory tissues and excessive production of cysteinyl leukotrienes. Despite the consistent clinical phenotype of the respiratory disease, the underlying pathogenesis of the disease remains unclear. In addition to their role in hemostasis, platelets have the capacity to influence the activation state and function of other immune cells during inflammation and to facilitate granulocyte recruitment into the tissues. Platelets also possess a repertoire of potent preformed mediators of inflammation that are released on activation and are a rich source of newly synthesized lipid mediators that alter vascular permeability and smooth muscle tone. Accordingly, platelet activity has been linked to diverse inflammatory diseases, including asthma. Both human and animal studies strongly suggest that platelet activity is uniquely associated with the pathophysiology of AERD. This article summarizes the evidence supporting an effector role for platelets in asthmatic patients in general and in patients with AERD in particular and considers the potential therapeutic implications. (J Allergy Clin Immunol 2015;135:1407-14.)

Key words: *Platelet, samter triad, aspirin-exacerbated respiratory disease, asthma, nasal polyp, leukotriene, thromboxane, prostaglandin, eosinophil*

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Aspirin-exacerbated respiratory disease (AERD) is an acquired syndrome that is irreversible, frequently debilitating, and usually presents with an onset in young adulthood. It is present in approximately 7% of all adults with asthma, overrepresented in studies of severe asthma and refractory nasal polyposis, and it is estimated that there are approximately 1.2 million adults in the United States living with AERD.[1,2] The pathognomonic feature of the disease is a respiratory reaction that occurs on ingestion of aspirin or any other drug that inhibits COX-1. These reactions classically involve bronchoconstriction, acute nasal congestion

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Abbreviations used
AERD: Aspirin-exacerbated respiratory disease
BAL: Bronchoalveolar lavage
cysLT: Cysteinyl leukotriene
$CysLT_1R$: Type 1 cysteinyl leukotriene receptor
$CysLT_2R$: Type 2 cysteinyl leukotriene receptor
5-LO: 5-Lipoxygenase
LT: Leukotriene
LTC_4S: Leukotriene C_4 synthase
mPGES-1: Microsomal PGE_2 synthase-1
PAF: Platelet-activating factor
PF4: Platelet factor 4
PG: Prostaglandin
TP: T prostanoid
TXA_2: Thromboxane A_2

and rhinorrhea, and ocular vasodilation. Histologically, AERD is characterized by eosinophilic inflammation in the sinonasal and bronchial mucosa, along with the presence of degranulated mast cells. The biochemical hallmark of AERD is profound overproduction of cysteinyl leukotrienes (cysLTs), a potent class of lipid inflammatory mediators generated by the 5-lipoxygenase (5-LO)/leukotriene C_4 synthase (LTC_4S) pathway. Urinary levels of leukotriene (LT) E_4, the stable metabolite of the cysLTs, are typically 3- to 4-fold higher in patients with AERD than levels found in urine from aspirin-tolerant asthmatic control subjects.[3] Clinical reactions to COX-1 inhibitors are characterized by dramatic further increases in urinary LTE_4 levels,[3] as well as marked increases in the numbers of eosinophils and basophils recruited to the sinonasal mucosa.[4] The 5-LO inhibitor zileuton and the type 1 cysteinyl leukotriene receptor ($CysLT_1R$) inhibitor montelukast both attenuate the signs and symptoms of clinical reactions, indicating the pathobiologic relevance of the cysLTs in AERD.

Although no unifying thesis explains all features of AERD, it seems very likely that its pathogenesis involves disturbances in the mechanisms that regulate tissue recruitment of immune effector cells and activity of the 5-LO/LTC_4S pathway. There is a substantial body of evidence that platelets play a central role in these mechanisms.

PLATELET CIRCULATION AND ACTIVATION

Platelets, which lack a nucleus and have no DNA, are derived from cytoplasmic fragments of megakaryocytes and have a lifespan in the circulation of approximately 8 to 10 days before being removed from the circulation by the spleen.[5] Platelets play a critical role in repair of damaged vessels. The main function of platelets is interaction with the vessel wall and subsequent platelet activation and thrombus formation in response to vessel wall injury. However, despite their lack of a nucleus, they are functional cells, and platelet activation might also sometimes be critical to the development of inflammation. The outermost glycoprotein layer of the platelet surface has a variety of activating receptors. Platelet activation responses are mediated by the binding of diverse stimuli (eg, collagen, integrins, thrombin, and other soluble mediators) to specific platelet receptors and can result in shape change, aggregation, expression of adhesion receptors, release of cytoplasmic granule contents, and generation of lipid mediators.[6] For example, on platelet activation and degranulation, the cell adhesion molecule P-selectin redistributes from cytoplasmic granules to the extracellular surface of the outer glycoprotein layer.[7] Therefore, increased membrane expression of P-selectin is indicative of platelet activation.[8] P-selectin then mediates platelet adhesion to leukocytes, which constitutively express the P-selectin counterligand P-selectin glycoprotein ligand 1. P-selectin–dependent adhesion to leukocytes facilitates the recruitment of both platelets and leukocytes to sites of tissue injury and inflammation.[9]

PLATELET MEDIATORS AND RELEVANCE TO ASTHMA

Chemokines and eosinophil-active mediators

Platelets secrete a long list of inflammatory mediators (Table I),[10-23] many of which are likely to be relevant in patients with asthma and allergic inflammation. Several powerful bronchoconstricting agents, including histamine, serotonin, thromboxane A_2 (TXA_2), and platelet-activating factor (PAF), are all released by activated platelets.[10-12,24] Platelets might also contribute to the selective accumulation of eosinophils at sites of allergic inflammation through their release of eosinophil chemotactic factors, such as thymus and activation-regulated chemokine (CCL17),[25,26] RANTES (CCL5),[27] PAF,[13] and platelet factor 4 (PF4/CXCL4).[14,15]

In addition to the production of eosinophil chemotactic factors, platelets also release GM-CSF, which delays eosinophil apoptosis and enhances eosinophil survival. The cytoplasm of resting platelets contains GM-CSF, which is then released on activation at concentrations high enough to prevent eosinophil apoptosis. An *in vitro* study demonstrated that platelet-derived GM-CSF was present in sufficient quantities to suppress the induction of eosinophil apoptosis by dexamethasone. Thus platelets could support a mechanism for relative steroid resistance in patients with eosinophilic inflammation.[16]

Nucleotides

Platelets store high concentrations of nucleotides (ATP and ADP) in their dense granules, which are released in response to activation.[17] ADP plays a crucial autocrine/paracrine role in amplifying aggregation responses by acting at platelet-associated P2Y1 and P2Y12 receptors,[28] the latter being the target of thienopyridine drugs. ATP can also serve as an endogenous danger signal to prime dendritic cells for T_H2 responses and to induce the release of IL-33, an alarmin-like cytokine that is a component of the type 2 innate immune response linked to the pathophysiology of asthma.[29] Given the abundance of nucleotides in platelet granules, it is tempting to speculate that platelets could play a role in initiating these functions as well.

Lipid mediators and transcellular arachidonic acid metabolism

Activated platelets rapidly liberate arachidonic acid from membrane lipids for conversion to eicosanoids in response to activation.[18] The major platelet-derived eicosanoids are 12-lipoxygenase–derived hydroperoxyeicosatetraenoic acid and the COX-derived prostanoids TXA_2 and prostaglandin (PG) D_2.[19] TXA_2 is a powerful short-lived mediator that signals

TABLE I. Platelet mediators with actions relevant to asthma and AERD

Mediator	Localization/formation within platelets	Presumed role of mediator
Histamine[10,21]	Dense granules	Bronchoconstriction, vasodilation, eosinophil chemotaxis, upregulation of eosinophil adhesion molecules
Serotonin[11,22]	Dense granules	Bronchoconstriction, eosinophil chemotaxis
Nucleotides (ATP/ADP)[17]	Dense granules	Amplification of platelet aggregation, priming of dendritic cells for T_H2 responses
TXA_2[12]	Newly synthesized	Bronchoconstriction, platelet activation
PAF[13]	Newly synthesized	Bronchoconstriction, eosinophil chemotaxis, induction of TXA_2 release from platelets, induction of LTC_4 release from eosinophils, mast cell activation systemic increase in urinary LTE_4 levels on inhalation of PAF
Leukotrienes[20]	Newly synthesized	Bronchoconstriction
PGD_2[19]	Newly synthesized	Bronchoconstriction, vasodilation, activation/chemotaxis of eosinophils, basophils, T_H2 cells, and innate lymphoid type 2 cells
PF4/CXCL4[14,15]	α-Granules	Bronchoconstriction, eosinophil activation and adhesion, neutrophil activation and adhesion
RANTES/CCL5[23]	α-Granules	Eosinophil chemotaxis and activation
GM-CSF[16]	Not yet known	Delayed apoptosis of eosinophils and neutrophils
Arachidonic acid[18]	Released from membrane phospholipids through phospholipase A_2 activation	Bronchoconstriction, production of downstream proinflammatory lipids

through T prostanoid (TP) receptors to amplify platelet activation in an autocrine manner[30] and also facilitates leukocyte recruitment by inducing endothelial expression of adhesion receptors.[31] Platelet-derived PGD_2 mediates vasodilatation[32] and also suppresses aggregation responses[33] by signaling through DP1 receptors. PGD_2 also signals through chemoattractant receptor-homologous molecule expressed on T_H2 cells (DP2) as a major chemoattractant for eosinophils, basophils, T_H2 cells,[34] and innate type 2 helper cells.[35] Both TXA_2 and PGD_2 act as bronchoconstrictors through TP receptors,[36] acting through a neurally mediated cholinergic pathway.

Because platelets lack nuclei, they cannot express COX-2, an inducible COX isoform, and rely exclusively on constitutively expressed COX-1 to generate these prostanoids. This accounts for the ability of aspirin, which at low doses is a powerful inhibitor of COX-1 but a weak inhibitor of COX-2, to suppress platelet-derived prostanoids. Suppression of these prostanoids likely accounts for some of the anti-inflammatory and cardioprotective effects of low-dose aspirin and might also relate to the clinical reactions caused by ingestion of aspirin in patients with AERD (see below). The lysophosphatidylcholine remaining in the platelet membrane after the liberation of arachidonic acid can then be converted to PAF,[37] a potent proinflammatory lipid mediator with both autocrine and paracrine effects. PAF has been implicated in asthma and anaphylaxis.[38,39]

In addition to their capacity to generate eicosanoids themselves, platelets can also modulate the generation of eicosanoids, specifically leukotrienes, by other cells. Inflammatory leukocytes (neutrophils, monocytes, eosinophils, mast cells, and basophils) can oxidize arachidonic acid through 5-LO to form the unstable intermediate leukotriene LTA_4.[40] In neutrophils LTA_4 is preferentially hydrolyzed by LTA_4 hydrolase to form LTB_4, whereas in monocytes, mast cells, eosinophils, and basophils, it is conjugated to reduced glutathione by the terminal enzyme LTC_4S to form LTC_4, the parent cysLT,[41] which is exported out of the cell and converted into LTD_4 and then into the stable end-metabolite LTE_4. Platelets lack 5-LO but express LTC_4S. Early *in vitro* experiments demonstrated that activated neutrophils generated LTA_4 in excess of their capacity to hydrolyze it to LTB_4. When platelets were added to activated neutrophils, the excess LTA_4 was converted to LTC_4, which is indicative of neutrophil-platelet transcellular metabolism.[42] Subsequently, platelet-dependent transcellular conversion of neutrophil-derived LTA_4 to LTC_4 was found to require cell-cell contact involving platelet-neutrophil adhesion through platelet P-selectin.[43] Because neutrophils are the most numerous 5-LO–expressing cell type, adherent platelets, which are present in inflammatory disease states, including AERD, have the potential to amplify cysLT generation. Interestingly, platelet LTC_4S is maintained in a partially inactive state with protein kinase C–dependent phosphorylation through basal TP receptor signaling. On administration of oral aspirin, TXA_2 production is suppressed, which then allows for increased platelet LTC_4S activity.[44] Whether this uncoupling contributes to the overproduction of cysLTs that is characteristic of AERD is unknown (see below).

In addition to their capacity to convert LTA_4 to LTC_4, activated platelets can contribute to 5-LO activity in neighboring cells by providing abundant free arachidonic acid. Rabbits challenged intravenously with the bacterial tripeptide formyl peptide display marked systemic increases in levels of both LTB_4 and LTC_4, both of which were sharply reduced by platelet depletion.[20] Thus despite their lack of 5-LO, platelets might play a major role in controlling leukotriene generation through a reciprocal transcellular mechanism.

PLATELET ACTIVATION IN ASTHMATIC PATIENTS

Platelet activation during allergen challenge

Several studies have demonstrated platelet activation in antigen challenge models of allergic asthma. Perhaps the first demonstration of a potential role of platelets in the pathophysiology of asthma was in 1981 by Knauer et al.[45] In a protocol of aerosolized ragweed allergen inhalation challenge of patients with atopic asthma, they demonstrated that an increase in plasma levels of PF4, which was used as a marker of platelet activation, occurred during antigen-induced early-phase reactions and that it correlated with the induced decrease in FEV_1. Within less than 10 minutes after allergen challenge, plasma levels of PF4 increased 2 to 4 times over baseline levels in allergic asthmatic patients but not in nonallergic healthy control subjects.[45] A subsequent study

demonstrated that bronchoalveolar lavage (BAL) levels of PF4 and β-thromboglobulin increased by 10-fold at 19 hours after subsegmental airway challenge with ragweed allergen, indicating activation of platelets within the microvasculature of the lung.[46] Additionally, development of a late asthmatic response in allergic asthmatic patients challenged with dust mite was associated with platelet activation because a prolonged increase in levels of platelet activation markers and decreased blood platelet counts were seen in patients with late asthmatic responses to the dust mite challenge, even at 24 hours after challenge.[47] These studies, which each followed different time points after allergen challenge, suggest that consequences of platelet activation might contribute to both early- and late-phase allergic reactions.

Platelet activation during asthma exacerbations

In 1989, it was shown that platelets could be found aggregated together with electron-dense fibrous material at the airway luminal edge within the bronchial biopsy specimens of patients with symptomatic asthma but not within the biopsy specimens of patients with asymptomatic asthma.[48] Further studies then indicated that platelet activation and release of platelet products might be involved in the mechanisms that underlie asthma exacerbations. β-Thromboglobulin and PF4 levels were demonstrated to increase in plasma after episodes of exercise-induced asthma, with the increase in plasma mediators preceding the decrease in peak expiratory flow. This suggested a causative association between the release of platelet proteins and the onset of bronchoconstriction.[49] Changes in platelet activation, as assessed by plasma levels of β-thromboglobulin and PF4, were also studied over 24 hours during exacerbations of nocturnal asthma and were related to the diurnal changes in peak expiratory flow. Platelet activation was highest when the peak expiratory flow rate was at its lowest in those asthmatic patients.[50] Additionally, ATP release from thrombin-stimulated platelets and plasma levels of β-thromboglobulin and PF4 were measured in 15 asthmatic patients during both asymptomatic and symptomatic periods. These parameters of platelet activation were all significantly increased during the periods of symptomatic asthma.[51] Thus although none of these studies established causality, they do indicate that platelet activation is a consistent factor associated with spontaneous asthma exacerbations. Moreover, the extent of activation correlates with the severity of airflow obstruction. It is possible that activation of platelets plays a prominent role in the development of asthma signs and symptoms.

Platelet-leukocyte aggregates

The P-selectin–dependent adherence of activated platelets onto circulating leukocytes primes those leukocytes for the subsequent adhesion and transmigration into the lung tissue. Circulating levels of these platelet-leukocyte aggregates have been found to increase in the blood of allergic asthmatic patients during allergen-induced late asthmatic responses. CD11b and CD18 expression on the leukocytes with adherent platelets was upregulated in comparison with that on free leukocytes.[52] In asthmatic patients, activated P-selectin–expressing platelets adhere to circulating eosinophils in the blood and lead to increased CD11b and α4β1 expression on those eosinophils. Additionally, during a whole-lung challenge of asthmatic patients, a model of asthma exacerbation known to cause platelet activation, eosinophils with adherent platelets and activated CD11b disappeared quickly from the circulation and appeared to enter the lungs. This indicates that platelet activation and binding of activated platelets to eosinophils allows for quick recruitment of those eosinophils into inflamed tissues.[53] It has become increasingly clear that through this P-selectin–dependent adherence mechanism, platelets participate in facilitating the recruitment of inflammatory cells into the lung and in the development of airways inflammation.

ROLE OF PLATELETS IN MOUSE MODELS OF AIRWAY INFLAMMATION

A series of murine studies by Pitchford et al[52] highlight potential effector functions of platelets in airway inflammation and allergic asthma and document the role of adherent platelets in priming leukocytes for adhesion. Using a mouse model of airway inflammation induced by ovalbumin sensitization and challenge, they showed that numbers of circulating platelet-leukocyte aggregates increase in sensitized mice after allergen challenge. If the mice were depleted of platelets before allergen challenge, the challenge-induced pulmonary infiltration of leukocytes was reduced and could then be restored by platelet infusion.[52] These findings suggested that platelet contributions to cell recruitment occur at the level of the circulation and that platelets are required for leukocyte recruitment to the lung during allergic pulmonary inflammation. The formation of platelet-leukocyte complexes depended on the expression of platelet P-selectin, which allowed circulating platelets to bind to and stimulate leukocytes for endothelial attachment by inducing leukocyte expression of CD11b and α4β1.[54] Additionally, the presence of platelets was necessary for the development of ovalbumin-induced airway remodeling and subepithelial fibrosis because their studies showed that platelet depletion protected mice against airway remodeling.[55] These observations strongly suggest the functional importance of the platelet-adherent granulocytes identified in the aforementioned studies of asthma and allergen challenge in human subjects.

In addition to the capacity for platelets to facilitate granulocyte adhesiveness and recruitment, free platelets also migrate to the allergen-challenged mouse lung. Mouse platelets express FcεRIα, can be sensitized to allergen, and can then migrate toward the relevant allergen.[56] This might allow platelets to participate in the development of allergic pulmonary inflammation by migrating directly to the asthmatic lung. Although no studies have directly demonstrated IgE receptors on platelets in asthmatic patients, platelets isolated from helminth-infected human subjects display IgE binding and IgE-associated cytotoxic activity to helminths. Thus platelets are among the effector cells that might be functionally influenced by IgE in asthmatic patients.

PLATELET IDIOSYNCRASY IN PATIENTS WITH AERD: *IN VITRO* STUDIES

The initial evidence for a "platelet-intrinsic" component of AERD arose from studies focused on the potential role for platelets in antihelminthic immunity. These early studies demonstrated that *in vitro* aspirin stimulation of platelets from donors with AERD, but not of platelets from aspirin-tolerant asthmatic or nonasthmatic control subjects, elicited chemiluminescence (indicative of an oxidative burst) and caused the release of cytotoxic activity against the larvae of *Schistosoma mansoni*. The chemiluminescent response and release of cytotoxic activity

were both eliminated by the treatment of platelets with PGH_2, indicating that the aspirin-induced activation response was tonically inhibited by a COX product, although which downstream COX product is most relevant was not inferred from these studies. The aberrant *in vitro* response to aspirin stimulation was eliminated when platelets from the same AERD donors were studied again after a therapeutic desensitization procedure.[57] Two subsequent studies also suggested inherent differences in the *in vitro* stimulation responses of platelets from patients with AERD compared with those from aspirin-tolerant control subjects. Platelets from patients with AERD released nearly twice as much ATP when activated with PAF or collagen than did platelets from healthy control subjects,[51] and PAF caused a significant increase in TXB_2 release only in platelets from patients with AERD.[52] The molecular basis underlying these findings has not yet been identified. However, differences in platelet biochemistry seem likely, and these studies imply that aberrant platelet functions could be a relevant feature of AERD and suggest that trials examining the use of targeted antiplatelet therapeutics might be appropriate for patients with AERD.

IN VIVO STUDIES OF PLATELET FUNCTION IN PATIENTS WITH AERD

The accumulation of large numbers of eosinophils in the respiratory tissues of patients with AERD is a histologic hallmark of the disease. Because mouse models and human studies had suggested that adherent platelets could amplify eosinophil tissue recruitment and survival, we undertook studies of platelet-leukocyte interactions in patients with AERD. We determined the frequencies of platelet-adherent leukocytes in the sinus tissue and blood of patients with AERD and compared them with those found in the tissue and blood of aspirin-tolerant control subjects. Nasal polyps from patients with AERD contained many extravascular platelets that colocalized with leukocytes (2- to 3-fold higher numbers and percentages in nasal polyps from patients with AERD than in polyps from aspirin-tolerant control subjects). Additionally, the percentages of circulating neutrophils, eosinophils, and monocytes with adherent platelets were markedly higher in the blood of patients with AERD than in aspirin-tolerant control subjects. Indeed, up to 80% of circulating eosinophils from patients with AERD had adherent platelets based on their cytofluorographic staining for CD61, a platelet marker. Platelet-adherent subsets of eosinophils, neutrophils, and monocytes had higher expression of β_2-integrins than did the platelet nonadherent subsets (and higher levels of β_1-integrins as well in the case of eosinophils), suggesting that platelets could prime these cells for adhesion and recruitment to tissue.[58] Although these platelet-related increases in leukocyte receptor expression were seen in the blood of both aspirin-tolerant control subjects and patients with AERD, the dramatic difference in the total percentages of circulating leukocytes to which platelets adhere in patients with AERD implies that the platelet-related effects on leukocyte activation might be especially relevant to tissue inflammation in patients with AERD.

Because platelets express LTC_4S and *ex vivo* studies had supported their capacity to amplify cysLT production, we sought to determine whether adherent platelets might contribute to the overproduction of cysLTs seen in patients with AERD. Using a specific enzymatic activity assay, we found that levels of LTC_4S activity in granulocyte fractions from the blood of patients with AERD were several-fold higher than levels in granulocytes from aspirin-tolerant asthmatic control subjects. By removing adherent platelets, we found that approximately 70% of the LTC_4S activity in the granulocytes from patients with AERD was platelet derived. Moreover, removal of the platelets sharply reduced LTC_4 generation by granulocytes stimulated with calcium ionophore. Finally, LTE_4 levels in urine correlated strongly with the percentages of platelet-adherent eosinophils, neutrophils, and monocytes in blood.[58] We also found that in both aspirin-tolerant asthmatic subjects and patients with AERD, adherent platelets increased the overall activity of the 5-LO pathway in peripheral blood granulocytes because the net quantities of all 5-LO pathway products generated by calcium ionophore–stimulated granulocytes were lower in samples from which platelets had been removed.[58] Indeed, the percentages of adherent platelets on neutrophils from patients with AERD correlated strongly with their capacity to generate LTB_4 in response to stimulation with the bacterial peptide formyl peptide.[59] Thus platelet-leukocyte aggregates might make a substantial contribution both to the overproduction of cysLTs that is typical of AERD and to the generation of biologically active LTB_4 and other 5-LO pathway products. Because leukotriene overproduction contributes notably to both the chronic and aspirin-induced symptoms of AERD, therapies aimed at reducing the numbers of these platelet-leukocyte aggregates could provide suitable options for the treatment of AERD.

PLATELET-DEPENDENT EFFECTOR MECHANISMS IN MOUSE AERD MODELS

A recently developed mouse model of AERD strongly supports the pathogenic role of platelets. Mice lacking microsomal PGE_2 synthase-1 (mPGES-1–null mice), the dominant enzyme responsible for converting COX-2–derived PGH_2 to PGE_2, have exaggerated eosinophilic airway inflammation in response to inhalation of dust mite allergen. When subsequently challenged by means of inhalation with lysine-aspirin, mPGES-1–null mice experience bronchoconstriction, accompanied by sharp increases in BAL fluid cysLT levels. Large numbers of platelet-adherent granulocytes were detected by means of cytofluorographic analysis in the blood of mPGES-1–null mice, and the lungs of these mice contained abundant extravasated platelets and platelet-granulocyte complexes, recapitulating the findings in nasal polyps from patients with AERD. Antibody-mediated depletion of platelets and neutrophils protected mPGES-1–null mice from aspirin-induced bronchoconstriction and prevented increases in BAL fluid levels of cysLTs induced by lysine-aspirin challenge. Remarkably, administration of a TP receptor antagonist or genetic deletion of TP receptors completely blocked the reaction to lysine-aspirin while sharply decreasing the numbers of eosinophils in the lungs. These studies strongly support a causative role for platelet-adherent granulocytes in driving the overproduction of cysLTs in patients with AERD and suggest that deficiencies in the function of the inducible system for PGE_2 production might be a key determinant of the aberrant platelet function observed in the disease. TP receptor function is maintained homeostatically by PGE_2 and EP_2 receptor signaling, which might account for the critical role for this receptor in this model. The studies also suggest that TP receptor blockade might be therapeutically useful in patients with AERD.[60]

In addition to their capacity to facilitate cysLT generation, platelets in both mice[61] and human subjects[62] express $CysLT_1R$ and type 2 cysteinyl leukotriene receptor ($CysLT_2R$), suggesting

that platelets might respond to cysLTs. Two different mouse models validate this hypothesis. In wild-type mice sensitized with ovalbumin, the administration of LTE_4 by means of inhalation before ovalbumin challenge sharply increases the numbers of eosinophils in BAL fluid, increases the numbers of goblet cells in bronchial mucosa, and amplifies the expression of IL-13 in the lung. These responses to LTE_4 were absent in mice subjected to antibody-induced platelet depletion or treated with the thienopyridine drug clopidogrel. Mice lacking $P2Y_{12}$ receptors (the target of clopidogrel) also lacked these responses to inhaled LTE_4, whereas mice lacking both $CysLT_1R$ and $CysLT_2R$ had responses similar to those of wild-type control mice. Although mouse platelets did not activate in response to LTE_4, they exhibited strong activation responses to LTC_4, which depended entirely on $CysLT_2R$. Furthermore, inhalation of LTC_4 markedly amplified the eosinophilic response of sensitized mice to ovalbumin. As was the case for the LTE_4 effects, the effects of LTC_4 in this model were completely prevented by depletion of platelets and depended on $P2Y_{12}$ receptors, as well as on $CysLT_2R$. Thus although these studies await confirmation in human subjects, they suggest that platelets are not only sources of cysLTs but also responders to cysLTs and might account for some of the potent proinflammatory effects of these mediators in patients with AERD.[61,63] Moreover, the fact that the pulmonary inflammatory responses of mice to both LTC_4 and LTE_4 require $P2Y_{12}$ receptors suggests a potential role for $P2Y_{12}$ receptor antagonism in disease treatment.

CURRENT ANTIPLATELET CLINICAL TRIALS ONGOING FOR AERD

Clinical trials exploring the potential for platelet-targeted therapies in patients with AERD are in their early stages. Because $P2Y_{12}$ receptors were essential for the platelet-dependent proinflammatory effects of cysLTs in the mouse studies described above and because thienopyridines reduce the formation of platelet-leukocyte aggregates,[64] we speculated that these antiplatelet therapies could be efficacious as treatments for AERD. Therefore a double-blind, placebo-controlled clinical trial of prasugrel, a thienopyridine that inhibits the $P2Y_{12}$ receptor and is commercially available for the management of patients with acute coronary syndrome, was designed and is ongoing to determine whether blocking $P2Y_{12}$ receptors attenuates the severity of reactions to aspirin in patients with AERD (NCT01597375). Although the effect of high-dose daily aspirin therapy on the rates of platelet-leukocyte aggregate formation has not yet been fully studied, it is known that low-dose aspirin (100 mg/d), which generally does not provide any symptom control in patients with AERD, does not reduce platelet-leukocyte aggregation.[64] A recent proof-of-concept placebo controlled trial of prasugrel for the treatment of aspirin-tolerant asthma revealed a significant effect on platelet aggregation and a modest effect on airway reactivity to mannitol.[65]

Because preclinical models suggested a role for TP receptors in patients with AERD, an ongoing trial (NCT02216357) aims to determine the safety and efficacy of ifetroban, an investigational new drug that is an orally active TP receptor antagonist, in patients with AERD. Although these early studies are focused on the ability of platelet-targeted drugs to interfere with the physiologic responses to aspirin challenge, longer-term studies will be necessary to determine whether platelets contribute to the features of chronic persistent disease, such as respiratory tract eosinophilia, nasal polyposis, poorly controlled asthma, and baseline overproduction of cysLTs. Based on preclinical models, all of these might involve some contribution from platelets and their mediators.

SUMMARY

There are extensive data to support that many of the hallmarks of AERD, including cysLT overproduction, chronic asthma, and eosinophilic respiratory inflammation, might be linked to the proinflammatory actions of activated platelets. Whether circulating platelets in patients with AERD possess an intrinsic defect that contributes to the underlying cause of the disease has yet to be determined. Mouse studies suggest that deficits in the PGE_2 synthetic system might be permissive for the high-frequency formation of platelet-leukocyte aggregates and could amplify the contribution or contributions of platelets to airway inflammation. It is noteworthy that several defects in the synthesis of PGE_2 or the functions of its receptors have been identified in cells and tissues of patients with AERD. Regardless of whether platelet aberrancy is primary or secondary, the evidence suggesting an effector role for platelets in patients with AERD is sufficiently strong that several clinical trials investigating the therapeutic efficacy of platelet inhibitors are currently underway.

What do we know?

- **AERD is a distinct chronic inflammatory syndrome characterized by the triad of asthma, eosinophilic nasal polyposis, and stereotypical respiratory reactions on ingestion of aspirin or COX-1 inhibitors.**
- **Overproduction of cysLTs is a biochemical hallmark of AERD, and the clinical reactions to COX-1 involve dramatic further increases in urinary cysLT levels.**
- **Activated platelets can activate other immune cells and, on adhesion to granulocytes, can facilitate granulocyte recruitment into the tissues.**
- **Platelets produce many potent mediators of inflammation that are relevant to asthma, including chemokines and lipids, which are released on activation.**
- **Platelet activation and platelet granule products play a role in symptomatic asthma exacerbations.**
- **There are high numbers of platelet-adherent granulocytes in the peripheral blood and nasal polyp tissue of patients with AERD, and these complexes contribute to the increased activation of leukocytes and to the overproduction of cysLTs through transcellular leukotriene metabolism.**

What is still unknown?

- **Whether platelets in patients with AERD have an intrinsic defect that contributes to the underlying pathophysiology of the disease**
- **The role of platelets in the acute aspirin-induced respiratory reactions seen in patients with AERD**
- **The therapeutic efficacy of platelet inhibitors in either treatment of the chronic inflammatory state or abrogation of the acute aspirin-induced reactions in patients with AERD**

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