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Platelets in aspirin-exacerbated respiratory disease

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Abstract

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 pre-formed mediators of inflammation that are released upon activation, and are a rich source of newly-synthesized lipid mediators that alter vascular permeability and smooth muscle tone. Accordingly, the activity of platelets 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 asthma in general and in AERD in particular, and considers the potential therapeutic implications.

Keywords

Platelet; Samter's Triad; Aspirin-exacerbated respiratory disease; AERD; Asthma; Nasal polyp; Leukotriene; Thromboxane; Prostaglandin; Eosinophil

Introduction

Aspirin exacerbated respiratory disease (AERD) is an acquired syndrome that is irreversible and frequently debilitating, and usually presents with an onset in young adulthood. It is present in ~7% of all adults with asthma, is overrepresented in studies of severe asthma and refractory nasal polyposis, and it is estimated that there are approximately 1.2 million adults

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in the United States living with AERD.¹² The pathognomonic feature of the disease is a respiratory reaction that occurs upon ingestion of aspirin or any other drug that inhibits cyclooxygenase (COX)-1. These reactions classically involve bronchoconstriction, acute nasal congestion 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 C₄ synthase (LTC₄S) pathway. Urinary levels of leukotriene E₄ (LT)E₄, the stable metabolite of the cysLTs, are typically 3-4 fold higher in patients with AERD than the levels found in urine from aspirin tolerant asthmatic controls.³ Clinical reactions to COX-1 inhibitors are characterized by dramatic further increases in urinary LTE₄,³ 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 cysLT receptor (CysLT₁R) 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/LTC₄S 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 life span in the circulation of approximately 8-10 days before being removed from circulation by the spleen.⁵ Platelets play a critical role in the 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 may 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 (e.g., collagen, integrins, thrombin, other soluble mediators) to specific platelet receptors, and can result in shape change, aggregation, expression of adhesion receptors, release of cytoplasmic granule contents, and the generation of lipid mediators.⁶ For example, upon 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 1**), many of which are likely to be relevant in asthma and allergic inflammation. Several powerful bronchoconstricting agents, including histamine,

serotonin, thromboxane A₂ (TXA₂), and platelet-activating factor (PAF), are all released by activated platelets.¹⁰⁻¹³ Platelets may 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 (TARC/CCL17),^{14, 15} regulated on activation, normal T cell expressed and secreted (RANTES/CCL5),¹⁶ PAF,¹⁷ and platelet factor 4 (PF4/CXCL4).^{18, 19}

In addition to the production of eosinophil chemotactic factors, platelets also release granulocyte-macrophage colony-stimulating factor (GM-CSF), which delays eosinophil apoptosis and enhances eosinophil survival. The cytoplasm of resting platelets contains GM-CSF, which is then released upon activation, at concentrations high enough to prevent eosinophil apoptosis. A study in vitro 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 eosinophilic inflammation.²⁰

Nucleotides—Platelets store high concentrations of nucleotides (ATP, ADP) in their dense granules, which are released in response to activation.²¹ ADP plays a crucial autocrine/paracrine role in amplifying aggregation responses by acting at platelet-associated P2Y₁ and P2Y₁₂ receptors,²² the latter being the target of thienopyridine drugs. ATP can also serve as an endogenous danger signal to prime dendritic cells for Th2 responses, and to induce the release of interleukin-33, an alarmin-like cytokine that is a component of the type 2 innate immune response linked to the pathophysiology of asthma.²³ 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.²⁴ The major platelet-derived eicosanoids are 12-lipoxygenase-derived hydroperoxyeicosatetraenoic acid, and the COX-derived prostanoids TXA₂ and prostaglandin D₂ (PGD₂).²⁵ TXA₂ is a powerful, short-lived mediator that signals through T prostanoid (TP) receptors to amplify platelet activation in an autocrine manner,²⁶ and also facilitates leukocyte recruitment by inducing endothelial expression of adhesion receptors.²⁷ Platelet-derived PGD₂ mediates vasodilatation,²⁸ and also suppress aggregation responses²⁹ by signaling through DP1 receptors. PGD₂ also signals through chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2/DP2) as a major chemoattractant for eosinophils, basophils, Th2 cells³⁰ and innate type 2 helper cells.³¹ Both TXA₂ and PGD₂ act as bronchoconstrictors via TP receptors,³² 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 may also relate to the clinical reactions caused by ingestion of aspirin in patients with AERD (see below). The

lysophosphatidyl choline remaining in the platelet membrane after the liberation of arachidonic acid can then be converted to PAF,³³ a potent pro-inflammatory lipid mediator with both autocrine and paracrine effects. PAF has been implicated in asthma and anaphylaxis.^{34,35}

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 LTA₄.³⁶ In neutrophils, LTA₄ is preferentially hydrolyzed by LTA₄ hydrolase to form LTB₄, whereas in monocytes, mast cells, eosinophils, and basophils, it is conjugated to reduced glutathione by the terminal enzyme LTC₄S to form LTC₄, the parent cysLT,³⁷ which is exported out of the cell and converted into LTD₄ and then into the stable end-metabolite LTE₄. Platelets lack 5-LO, but express LTC₄S. Early in vitro experiments demonstrated that activated neutrophils generated LTA₄ in excess of their capacity to hydrolyze it to LTB₄. When platelets were added to activated neutrophils, the excess LTA₄ was converted to LTC₄, indicative of neutrophil-platelet transcellular metabolism.³⁸ Subsequently, platelet-dependent transcellular conversion of neutrophil-derived LTA₄ to LTC₄ was found to require cell-cell contact involving platelet-neutrophil adhesion through platelet P-selectin.³⁹ Since neutrophils are the most numerous 5-LO expressing cell type, adherent platelets, which are present in inflammatory disease states including AERD, thus have the potential to amplify cysLT generation. Interestingly, platelet LTC₄S is maintained in a partially inactive state with protein kinase C-dependent phosphorylation through basal TP receptor signaling. Upon administration of oral aspirin, TXA₂ production is suppressed, which then allows for increased platelet LTC₄S activity.⁴⁰ 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 LTA₄ to LTC₄, 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 (fMLP) display marked systemic increases in both LTB₄ and LTC₄, both of which were sharply reduced by platelet depletion.⁴¹ Thus, despite their lack of 5-LO, platelets may play a major role in controlling leukotriene generation through a reciprocal transcellular mechanism.

Platelet activation in asthma

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 and colleagues. In a protocol of aerosolized ragweed allergen inhalation challenge of subjects with atopic asthma, they demonstrated that an increase in plasma levels of PF4, used as a marker of platelet activation, occurred during antigen-induced early phase reactions and that it correlated with the induced fall in FEV₁. Within less than 10 minutes after allergen challenge the plasma levels of PF4 increased 2-4× in allergic asthmatic patients, but not in non-allergic healthy controls.⁴² A subsequent study demonstrated that the 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.⁴³ Additionally, the development of a late-asthmatic response in allergic asthma patients challenged with dust mite was associated with platelet activation, as prolonged elevation 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.⁴⁴ These studies, which each followed different time-points after allergen challenge, suggest that consequences of platelet activation may 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 biopsies of patients with symptomatic asthma, but not within the biopsies of asymptomatic asthmatics.⁴⁵ Further studies then indicated that platelet activation and release of platelet products may be involved in the mechanisms that underlie asthma exacerbations. Levels of β -thromboglobulin and PF4 were demonstrated to increase in the plasma after episodes of exercise-induced asthma, with the rise in plasma mediators preceding the fall in peak expiratory flow. This suggested a causative association between the release of platelet proteins and the onset of bronchoconstriction.⁴⁶ Changes in platelet activation, 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 subjects.⁴⁷ 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.⁴⁸ Thus, while 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 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 subjects during allergen-induced late asthmatic responses. CD11b and CD18 expression on the leukocytes with adherent platelets was upregulated in comparison to free leukocytes.⁴⁹ In patients with asthma, activated P-selectin-expressing platelets adhere onto circulating eosinophils in the blood and lead to increased CD11b and $\alpha 4\beta 1$ expression on those eosinophils. Additionally, during a whole-lung challenge of asthmatic subjects, 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.⁵⁰ 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.

The role of platelets in mouse models of airway inflammation

A series of murine studies by Pitchford and colleagues 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 levels of circulating platelet-leukocyte aggregates increase in sensitized mice after allergen challenge. If the mice were depleted of platelets prior to allergen challenge, the challenge-induced pulmonary infiltration of leukocytes was reduced and could then be restored by platelet infusion.⁴⁹ 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 in 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 CD11b and $\alpha 4\beta 1$.⁵¹ Additionally, the presence of platelets was necessary for the development of ovalbumin-induced airway remodeling and subepithelial fibrosis, as their studies showed that platelet depletion protected mice against airway remodeling.⁵² These observations strongly suggest the functional importance of platelet-adherent granulocytes identified in aforementioned studies of asthma and allergen challenge in humans.

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 Fc ϵ RI α , can be sensitized to allergen, and can then migrate toward the relevant allergen.⁵³ This may 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 humans with asthma, 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 may be functionally influenced by IgE in asthma.

Platelet idiosyncrasy in 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 anti-helminthic 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 controls, 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 the platelets with PGH₂, indicating that the aspirin-induced activation response was tonically inhibited by a COX product, though 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.⁵⁴ Two subsequent studies also suggested inherent differences in the in vitro stimulation responses of platelets from patients with AERD compared to those from aspirin-tolerant controls. Platelets from patients with AERD released nearly twice as much ATP when activated with PAF or collagen than did platelets from healthy controls⁴⁸ and PAF caused a significant increase in TXB₂ release only in platelets from patients with AERD.⁴⁹ 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 anti-platelet therapeutics may be appropriate for patients with AERD.

In vivo studies of platelet function in 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 AERD. We determined the frequencies of platelet-adherent leukocytes in the sinus tissue and blood of subjects with AERD and compared them to those found in the tissue and blood of aspirin-tolerant controls. Nasal polyps from subjects with AERD contained many extravascular platelets that colocalized with leukocytes (2-3 fold higher numbers and percentages in nasal polyps from subjects with AERD than in polyps from aspirin-tolerant controls). Additionally, the percentages of circulating neutrophils, eosinophils, and monocytes with adherent platelets were markedly higher in the blood of subjects with AERD than in aspirin-tolerant controls. Indeed, up to 80% of circulating eosinophils from subjects 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 $\beta 2$ integrins than did the platelet nonadherent subsets (and higher levels of $\beta 1$ integrins as well in the case of eosinophils), suggesting that platelets could prime these cells for adhesion and recruitment to the tissues.⁵⁵ Although these platelet-related increases in leukocyte receptor expression were seen in the blood of both aspirin-tolerant controls and patients with AERD, the dramatic difference in the total percentages of circulating leukocytes to which platelets adhere in AERD implies that the platelet-related effects on leukocyte activation may be especially relevant to tissue inflammation in AERD.

Because platelets express LTC₄S 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 AERD. Using a specific enzymatic activity assay, we found that the levels of LTC₄S activity in granulocyte fractions from the blood of subjects with AERD were several fold higher than the levels in granulocytes from aspirin tolerant asthmatic controls. By removing adherent platelets, we found that ~70% of the LTC₄S activity in the granulocytes from subjects with AERD was platelet-derived. Moreover, removal of the platelets sharply reduced the generation of LTC₄ by granulocytes stimulated with calcium ionophore. Finally, the levels of LTE₄ in the urine correlated strongly with the percentages of platelet-adherent eosinophils, neutrophils, and monocytes in the blood.⁵⁵ We also found that in both aspirin tolerant asthmatic and AERD subjects, adherent platelets

increased the overall activity of the 5-LO pathway in peripheral blood granulocytes, as the net quantities of all 5-LO pathway products generated by calcium ionophore-stimulated granulocytes were lower in the samples from which platelets had been removed.⁵⁵ Indeed, the percentages of adherent platelets on neutrophils from subjects with AERD correlated strongly with their capacity to generate LTB₄ in response to stimulation with the bacterial peptide fMLP.⁵⁶ Thus, platelet-leukocyte aggregates may make a substantial contribution both to the overproduction of cysLTs that is typical of AERD, and to the generation of biologically active LTB₄ and other 5-LO pathway products. As leukotriene overproduction contributes notably to both the chronic and the 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 prostaglandin E₂ synthase-1 (mPGES-1-null mice), the dominant enzyme responsible for converting COX-2-derived PGH₂ to PGE₂, develop exaggerated eosinophilic airway inflammation in response to inhalation of dust mite allergen. When subsequently challenged by inhalation with lysine-aspirin, mPGES-1 null-mice develop bronchoconstriction, accompanied by sharp increases in BAL fluid levels of cysLTs. Large numbers of platelet-adherent granulocytes were detected by 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 subjects with AERD. Antibody-mediated depletions of platelets and neutrophils protected mPGES-1-null mice from aspirin-induced bronchoconstriction, and prevented the 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 AERD, and suggest that deficiencies in the function of the inducible system for PGE₂ production may be a key determinant of the aberrant platelet function observed in the disease. TP receptor function is maintained homeostatically by PGE₂ and EP₂ receptor signaling, which may account for the critical role for this receptor in this model. The studies also suggest that TP receptor blockade may be therapeutically useful in AERD.⁵⁷

In addition to their capacity to facilitate cysLT generation, platelets in both mice⁵⁸ and humans⁵⁹ express CysLT₁R and type 2 cysLT receptor (CysLT₂R), suggesting that platelets may respond to cysLTs. Two different mouse models validate this hypothesis. In wild type mice that are sensitized with ovalbumin, the administration of LTE₄ by inhalation prior to ovalbumin challenge sharply increases the numbers of eosinophils in the BAL fluid, increases the numbers of goblet cells in the bronchial mucosa, and amplifies the expression of interleukin-13 in the lung. These responses to LTE₄ were absent in mice subjected to antibody-induced platelet depletions, or treated with the thienopyridine drug clopidogrel. Mice lacking P2Y₁₂ receptors (the target of clopidogrel) also lacked these responses to inhaled LTE₄, whereas mice lacking both CysLT₁R and CysLT₂R had responses similar to

the wild type controls. Although mouse platelets failed to activate in response to LTE₄, they exhibited strong activation responses to LTC₄, which depended entirely on CysLT₂R. Furthermore, inhalation of LTC₄ markedly amplified the eosinophilic response of sensitized mice to ovalbumin. As was the case for the LTE₄ effects, the effects of LTC₄ in this model were completely prevented by depletion of platelets, and depended on P2Y₁₂ receptors as well as on CysLT₂R. Thus, while these studies await confirmation in humans, they suggest that platelets are not only sources of cysLTs, but also responders to cysLTs, and may account for some of the potent proinflammatory effects of these mediators in AERD.^{58, 60} Moreover, the fact that the pulmonary inflammatory responses of mice to both LTC₄ and LTE₄ require P2Y₁₂ receptors suggests a potential role for P2Y₁₂ receptor antagonism in disease treatment.

Current anti-platelet clinical trials ongoing for AERD

Clinical trials exploring the potential for platelet-targeted therapies in AERD are in their early stages. Because P2Y₁₂ receptors were essential for the platelet-dependent proinflammatory effects of cysLTs in the mouse studies described above, and since thienopyridines reduce the formation of platelet-leukocyte aggregates,⁶¹ 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 P2Y₁₂ receptor and is commercially available for the management of patients with acute coronary syndrome, was designed and is ongoing to determine whether blocking P2Y₁₂ receptors attenuates the severity of reactions to aspirin in patients with AERD (NCT01597375). Though 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/day), which generally does not provide any symptom control in AERD, does not reduce platelet-leukocyte aggregation.⁶¹ 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.⁶²

As preclinical models suggested a role for TP receptors in 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. While these early studies are focused on the ability of platelet-targeted drugs to interfere with the physiologic responses to aspirin challenges, longer term studies will be necessary to determine whether platelets contribute to the chronic, persistent disease features such as respiratory tract eosinophilia, nasal polyposis, poorly controlled asthma, and baseline overproduction of the cysLTs. Based on preclinical models, all of these may involve some contribution from platelets and their mediators.

Summary

There is extensive data to support that many of the hallmarks of AERD, including cysLT overproduction, chronic asthma, and eosinophilic respiratory inflammation, may be linked to the proinflammatory actions of activated platelets. Whether or not circulating platelets in 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 PGE₂ synthetic system may be permissive for the high frequency formation of platelet-leukocyte aggregates, and may amplify the contribution(s) of platelets to airway inflammation. It is noteworthy that several defects in the synthesis of PGE₂ 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 AERD is sufficiently strong that several clinical trials investigating the therapeutic efficacy of platelet inhibitors are currently underway.

Abbreviations

AERD	Aspirin-exacerbated respiratory disease
COX	Cyclooxygenase
LT	Leukotriene
5-LO	5-lipoxygenase
LTC₄S	Leukotriene C ₄ synthase
cysLTs	Cysteinyl leukotrienes
PG	Prostaglandin
CysLT₁R	Type 1 cysLT receptor
CysLT₂R	Type 2 cysLT receptor
fMLP	Formyl peptide
TXA₂	Thromboxane A ₂
PAF	Platelet-activating factor
TARC	Thymus and activation-regulated chemokine
RANTES	Regulated on activation, normal T cell expressed and secreted
PF4	Platelet factor 4
GM-CSF	Granulocyte-macrophage colony-stimulating factor
TP	T prostanoid receptors
CRTH2	Chemoattractant receptor-homologous molecule expressed on Th2 cells
BAL	Bronchiolar lavage

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What do we know?

- AERD is a distinct chronic inflammatory syndrome characterized by the triad of asthma, eosinophilic nasal polyposis, and stereotypical respiratory reactions upon 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 cysLTs.
- Activated platelets can activate other immune cells and upon 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 upon 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 individuals with AERD, and these complexes contribute to the increased activation of the leukocytes and to the overproduction of cysLTs through transcellular leukotriene metabolism.

What is still unknown?

- Whether platelets in 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 in AERD.
- The therapeutic efficacy of platelet inhibitors in either treatment of the chronic inflammatory state or abrogation of the acute aspirin-induced reactions in AERD.

Table 1

Platelet mediators with actions relevant to asthma and AERD

Mediator	Localization/formation within platelets	Presumed role of mediator
Histamine ^{11, 63}	Dense granules	Bronchoconstriction, vasodilation, eosinophil chemotaxis, upregulation of eosinophil adhesion molecules
Serotonin ^{12, 64}	Dense granules	Bronchoconstriction, eosinophil chemotaxis
Nucleotides (ATP/ADP) ²¹	Dense granules	Amplification of platelet aggregation, priming of dendritic cells for Th2 responses
Thromboxane A ₂ ¹³	Newly synthesized	Bronchoconstriction, platelet activation
PAF ¹⁷	Newly synthesized	Bronchoconstriction, eosinophil chemotaxis, induction of TXA ₂ release from platelets, induction of LTC ₄ release from eosinophils, mast cell activator, systemic increase in urinary LTE ₄ upon inhalation of PAF
Leukotrienes ⁴¹	Newly synthesized	Bronchoconstriction
PGD ₂ ²⁵	Newly synthesized	Bronchoconstriction, vasodilation, activation/chemotaxis of eosinophils, basophils, Th2 cells, and innate lymphoid Type 2 cells
PF4/CXCL4 ¹⁸¹⁹	α-granules	Bronchoconstriction, eosinophil activation and adhesion, neutrophil activation and adhesion
RANTES/CCL5 ⁶⁵	α-granules	Eosinophil chemotaxis and activation
GM-CSF ²⁰	Not yet known	Delayed apoptosis of eosinophils and neutrophils
Arachidonic acid ²⁴	Released from membrane phospholipids via phospholipase A2 activation	Bronchoconstriction, production of downstream proinflammatory lipids