

Role of platelets in allergic airway inflammation

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Increasing evidence suggests an important role for platelets and their products (eg, platelet factor 4, β -thromboglobulin, RANTES, thromboxane, or serotonin) in the pathogenesis of allergic diseases. A variety of changes in platelet function have been observed in patients with asthma, such as alterations in platelet secretion, expression of surface molecules, aggregation, and adhesion. Moreover, platelets have been found to actively contribute to most of the characteristic features of asthma, including bronchial hyperresponsiveness, bronchoconstriction, airway inflammation, and airway remodeling. This review brings together the current available data from both experimental and clinical studies that have investigated the role of platelets in allergic airway inflammation and asthma. It is anticipated that a better understanding of the role of platelets in the pathogenesis of asthma might lead to novel promising therapeutic approaches in the treatment of allergic airway diseases. (*J Allergy Clin Immunol* 2015;135:1416-23.)

Key words: Platelets, asthma pathophysiology, leukocyte recruitment, dendritic cells, T_H2 sensitization, airway remodeling

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Platelets are anucleated blood elements that are involved in hemostasis and thrombosis. However, it has also been recognized for some time that platelets can have a range of other functions that are relevant to the pathogenesis of allergic airway diseases, such as asthma. Intriguingly, it is now understood that platelets can act as inflammatory cells and contribute to host defense against infection, performing many functions normally associated with leukocytes. For example, platelets can undergo phagocytosis¹ and chemotaxis^{2,3} and release a wide variety of inflammatory mediators that coordinate inflammatory cell activation (release potent defensive products that are active against pathogens). They can also be activated by certain inflammatory mediators themselves.⁴⁻⁶ It is now clear that

Abbreviations used

ADP:	Adenosine diphosphate
BHR:	Bronchial hyperresponsiveness
CD40L:	CD40 ligand
DC:	Dendritic cell
5-HT:	5-Hydroxytryptamine
TLR:	Toll-like receptor

platelets are a necessary component of host defense exemplified by recent observations that platelet depletion leads to a reduced ability of animals to resist infections by microorganisms.^{1,7,8} This involvement of platelets is probably through an ability to optimize the recruitment of leukocytes into tissues because platelet depletion has now been reported to lead to a reduction of leukocytes infiltrating a number of tissues (including the lung) in response to both nonallergic (eg, bacterial LPS and zymosan³) and allergic stimuli.² Given that many inflammatory diseases are the result of inappropriate activation of pathways normally reserved for host defense, it is plausible that inappropriate platelet activation might also contribute to the pathogenesis of inflammatory diseases.

In the present review we provide evidence for platelet activation in patients with asthma and other allergic diseases, including recent evidence of platelet involvement during sensitization to allergens, along with our current understanding of the mechanisms whereby platelets can contribute to many of the features of allergic airways disease, including acute bronchoconstriction, leukocyte infiltration, airway remodeling, and bronchial hyperresponsiveness (BHR; summarized in Fig 1).⁹

EVIDENCE FOR PLATELET ACTIVATION IN PATIENTS WITH ALLERGIC INFLAMMATION AND ASTHMA

Of particular relevance to allergic inflammation is the observation that platelets express ***IgE receptors*** on their surfaces.¹⁰ Human platelets have been shown to express both high-affinity¹⁰ and low-affinity¹¹ IgE receptors, although the level of expression of these receptors is variable and they are not found on all platelets from a given donor. Nonetheless, the expression of IgE receptors on platelet surfaces is clearly important for optimal host defense against parasitic infections because, based on the seminal work of Capron and Joseph¹¹ in the 1980s, we know that platelets expressing IgE receptors play an essential role in killing certain types of parasites. Therefore it is not surprising that platelets from patients with allergic disease can also respond to common airborne allergens, such as those derived from house dust mite.^{2,12}

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Terms in boldface and italics are defined in the glossary on page 1417.

However, it is important to recognize that allergen-induced platelet activation is distinct from the classical aggregation and secretion induced by thrombotic stimuli.^{10,13} Moreover, platelets obtained from allergic donors undergo chemotaxis *in vitro* when exposed to the allergen to which the patient is sensitized. This direct activation of platelets to migrate in response to allergens might help explain the observation that platelets have been found extravascularly in the lung tissue of allergic mice at early time points after allergen exposure.² These observations of platelet migration into the lung are consistent with other reports that platelets have been found extravascularly in other settings, such as in synovial fluid of patients with arthritis.^{14,15}

Consistent with the presence of IgE receptors on platelets, platelet activation has been demonstrated to accompany allergen exposure in patients with allergic asthma. Thus allergen challenge is associated with a mild peripheral thrombocytopenia, presumably as a result of localized pulmonary recruitment¹⁶ and the presence of platelet leukocyte complexes in the circulation.^{12,17} Platelet activation has also been observed in patients with asthma, measured as an increase in the levels of a number of platelet-derived mediators, such as platelet factor 4, β -thromboglobulin (β -TG), RANTES, and thromboxane in peripheral blood or in bronchoalveolar lavage fluid, respectively.^{13,18,19} It has long been known that platelets isolated from peripheral blood of patients with allergic disease have also been shown to have abnormal platelet aggregatory responses *in vitro* that correlate with IgE levels.^{20,21} Such platelets are thought to represent an “exhausted platelet” phenotype as a result of the platelets having been continuously activated *in vivo*.^{20,21} This altered platelet behavior manifests itself in allergic patients as a mild hemostatic defect associated with prolonged bleeding time.^{20,21} Thus despite platelet activation in response to allergen exposure, the incidence of cardiovascular events in patients with asthma is no greater than in the general population, and indeed, there is some suggestion that they actually have less calcification of their arteries,^{22,23} suggesting a specific dichotomy in platelet activation and function in allergic inflammation distinct from that involved in hemostasis and thrombosis (see below). It has also been reported that patients with allergic asthma have a shortened platelet survival time that can be corrected after

treatment with glucocorticosteroids,^{12,13} and an increase in the number of *megakaryocytes* found in the pulmonary circulation has also been demonstrated at autopsy of patients who have died of status asthmaticus.²⁴

PLATELETS AND BRONCHOSPASM

One of the earliest observations that platelets might play a role in asthma was the ability of this cell to release *spasmogens* for airway smooth muscle⁵ and that depletion of platelets from experimental animals led to the abolition of bronchospasm induced by substances that could activate platelets.^{25,26} Indeed, more than 3 decades ago, platelet depletion was observed to inhibit allergen-induced bronchoconstriction in the rabbit,²⁶ observations subsequently confirmed²⁷ and extended to the bronchoconstriction induced by mediators known to be released by platelets such as platelet-activating factor.²⁵ It is now evident that platelets can release a number of spasmogens that can cause constriction of human airway smooth muscle,^{5,12} including *5-hydroxytryptamine (5-HT)*, which has been demonstrated to induce bronchoconstriction in asthmatic patients.²⁸ This is of interest because animals deficient in one of the enzymes involved in the synthesis of 5-HT have reduced allergen responses in the airways because of decreased platelet sequestration and subsequent release in the lungs.²⁹ Furthermore, we have recently demonstrated that platelet depletion can reduce the ability of bradykinin and capsaicin to induce bronchoconstriction in guinea pigs but is not able to influence bronchoconstriction induced by the direct-acting spasmogens methacholine and histamine.¹³

PLATELETS AND BHR

Platelet depletion has also been reported to reduce allergen-induced BHR in allergic rabbits²⁷ associated with a reduction in eosinophil infiltration into the lung. Recently, Lommatzsch et al³⁰ have suggested that platelets can cause airways obstruction and BHR through an effect involving nerve growth factor, and others have suggested the release of “platelet-derived hyperreactivity factor”^{13,30} might contribute to BHR. Interestingly, the platelet-derived mediator platelet

GLOSSARY

CD11B/CD18: Also known as complement receptor type 3 (CR3), which binds iC3. Deficiency of CD18 in patients with type I leukocyte adhesion deficiency results in a lack of β_2 -integrin adhesion molecules.

CD40 LIGAND: A cell-surface molecule found on CD4⁺ T lymphocytes. CD40 ligand interactions with CD40 are important for germinal center formation and terminal differentiation of B lymphocytes. CD40 ligand mutations are responsible for X-linked hyper-IgM syndrome.

5-HYDROXYTRYPTAMINE (5-HT): Also known as serotonin. Tryptophan is the precursor of serotonin. There are more than a dozen serotonin receptors. Ondansetron and sumatriptan bind to 5-HT receptors. Melatonin is synthesized from serotonin in the pineal gland. Most serotonin is found outside the central nervous system. Serotonin helps regulate cardiovascular function, bowel motility, and bladder control.

IgE RECEPTORS: The high-affinity receptor, known as Fc ϵ RI, has higher binding affinity for IgE than any other Fc receptor for its ligand. Fc ϵ RI is mainly expressed on mast cells and basophils. Fc ϵ RII, also known as

CD23, is related to C-type lectins and has a constitutive form (CD23a) and a form induced by IL-4 (CD23b). Fc ϵ RII mediates endocytosis of IgE-coated particles.

LEUKOTRIENES: Lipids derived from arachidonic acid through the lipoxygenase pathway. Leukotriene (LT) C₄ is degraded into LTD₄ and LTE₄. Cysteinyl leukotrienes are powerful bronchoconstrictors. Cysteinyl leukotrienes also have the ability to affect blood vessels, mucociliary clearance, and eosinophilic inflammation.

MEGAKARYOCYTES: A cell derived from hematopoietic stem cells that reside primarily in the bone marrow. Mature megakaryocytes release roughly 2×10^{11} platelets into the bloodstream per day in healthy adults.

SPASMOGENS: A substance that produces smooth muscle contractions. Examples include histamine, serotonin, and bradykinin. Airway smooth muscle in asthmatic patients has been shown to have increased shortening velocity and maximal shortening.

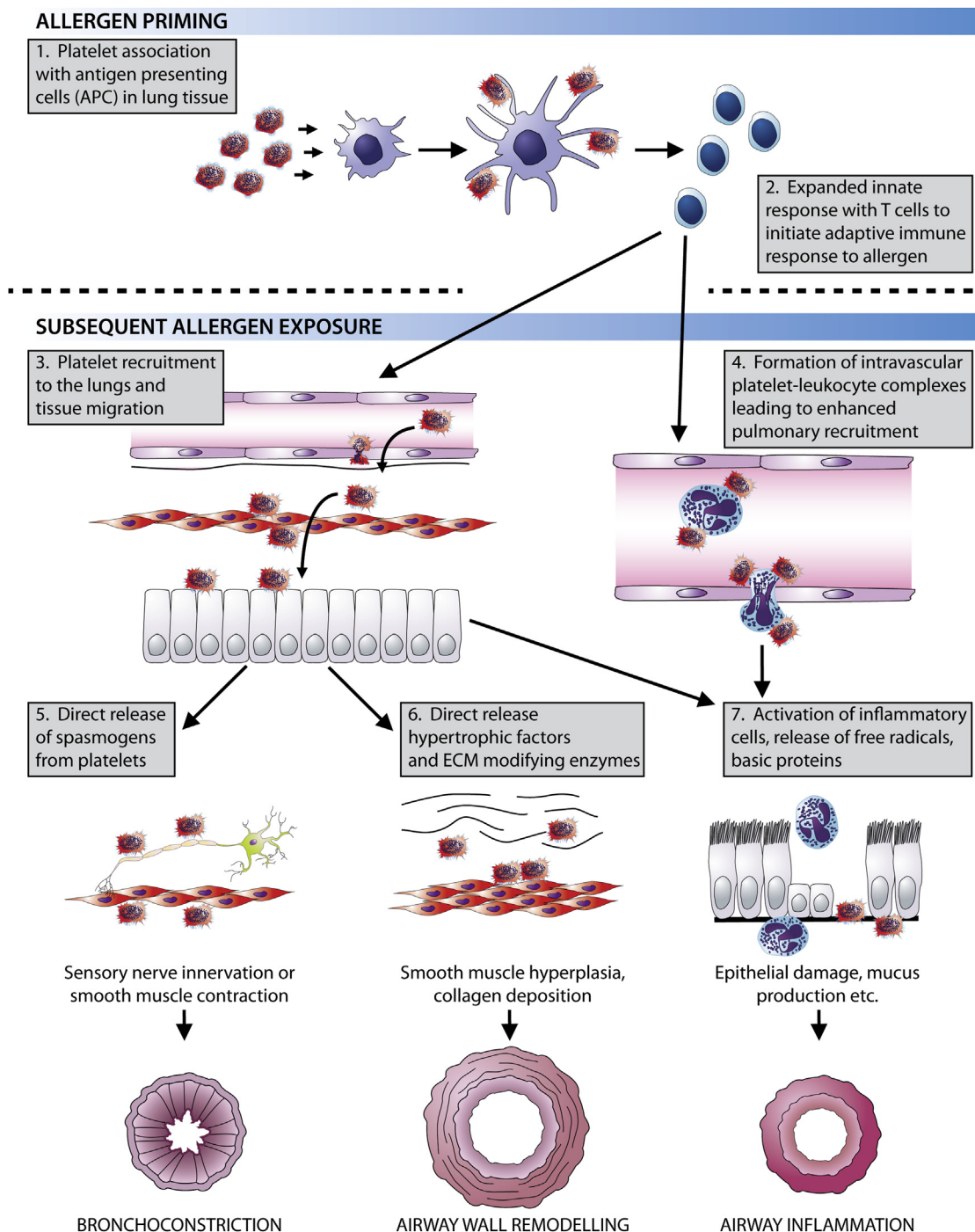


FIG 1. A direct role for platelets in asthma pathogenesis. There is evidence for platelet participation in both innate immune surveillance and the adaptive response to allergen and inflammatory events that occur after subsequent secondary exposure. *ECM*, Extracellular matrix. Adapted from Page and Pitchford.⁹

factor 4 has been shown to induce BHR in experimental animals, which could also contribute to the effects seen with platelet depletion on BHR in allergic animals.³¹ The ability of platelets to influence airway responsiveness (ie, a direct action of platelet-derived mediators on smooth muscle or through activation of airway nerves) suggests the migration of platelets into the tissue microenvironment around sensory nerve endings, airway smooth muscle, or both, which is supported by platelets

being found in close apposition to airway smooth muscle after exposure to the inflammatory mediator platelet-activating factor and after allergen exposure in sensitized mice.^{2,27} Indeed, platelets have been shown to have chemotactic ability toward various chemotactic substances, including allergen, N-formyl-L-methionyl-L-leucyl-L-phenylalanine and stromal cell-derived factor 1 α ,^{2,3} and have been found extravascularly in close apposition to airway smooth muscle in experimental

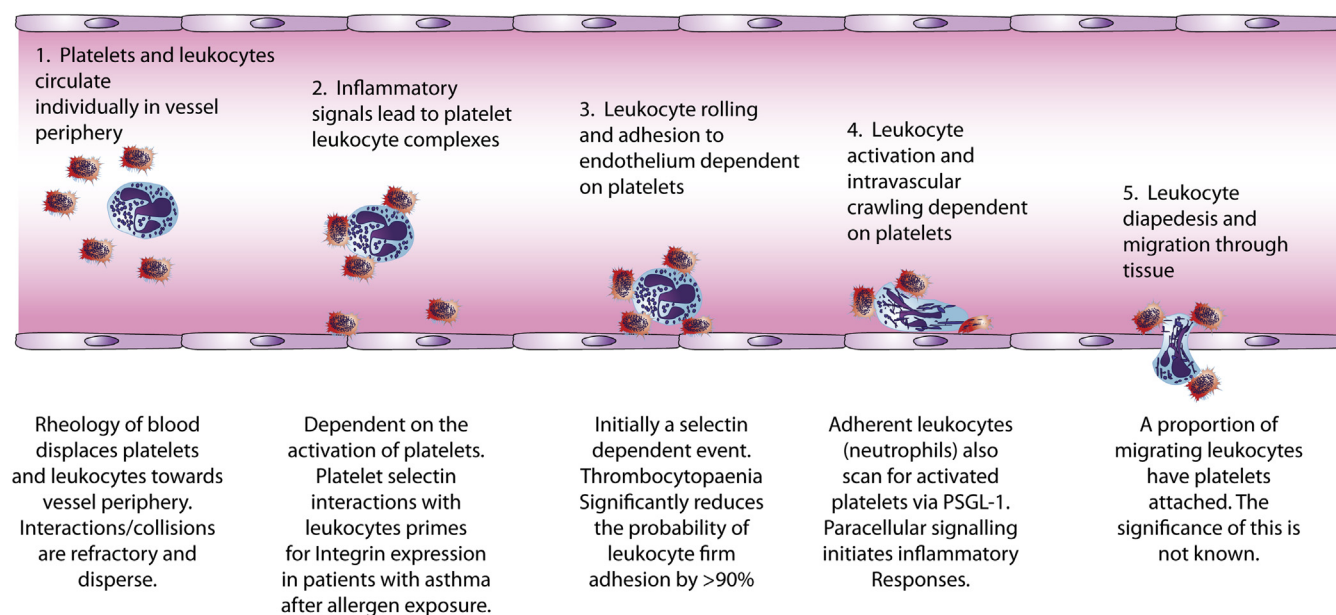


FIG 2. A multistage role for platelets during the leukocyte adhesion cascade. There is evidence for platelet participation in leukocyte recruitment at different stages of leukocyte activation and recruitment. Many of these processes have been observed in the context of allergic airways inflammation, either from blood samples taken from patients with asthma or *in vivo* experimentation. *PSGL-1*, P-selectin glycoprotein ligand 1.

animals in response to certain inflammatory mediators.¹² We have demonstrated that platelet depletion can reduce airway smooth muscle thickening (as well as reduced collagen deposition) after chronic allergen challenge of mice.³² Furthermore, we have recently reported platelets attached to airway smooth muscle in biopsy specimens obtained from allergic asthmatic patients (Kanabar et al, unpublished data), where, through the release of mitogens, such as platelet-derived growth factor and *leukotrienes*,^{4,33} others have shown platelets might be contributing to the remodeling that is a feature of patients with allergic asthma.³⁴ Platelets have also been reported on the damaged epithelial surface in lung biopsy specimens obtained from patients with symptomatic asthma.^{12,35}

One of the pathophysiologic consequences of airway remodeling is thought to be increased BHR, another characteristic phenotype of asthmatic patients. Therefore it is of considerable interest that a number of studies have reported that platelet depletion can reduce allergen-induced bronchoconstriction and allergen-induced BHR.^{27,36} However, the precise mechanism or mechanisms of this platelet-dependent BHR are not fully understood.

PULMONARY LEUKOCYTE RECRUITMENT IS PLATELET DEPENDENT

It is particularly interesting that there is now a growing body of evidence that platelets play an essential role in the recruitment of a range of inflammatory cells into a number of tissues, including the lung. Specifically, platelet depletion has been shown to reduce the allergen-induced infiltration of eosinophils, lymphocytes, monocytes/macrophages, and neutrophils into the lungs of rodents.^{27,36-38} In this regard circulating platelet-leukocyte complexes occur at far greater frequency after both spontaneous

asthma attacks in a biphasic manner or after allergen challenge in the clinic (Fig 2).^{13,17,37} The possible significance of these platelet-leukocyte complexes is presumably to act as a “priming” step to promote efficient leukocyte adhesion to the vascular endothelium because leukocytes attached to platelets display more of the α M subunit (CD11b) of the α M β 2 integrin macrophage 1 antigen (*CD11b/CD18*) than circulating leukocytes not attached to platelets.^{13,37,38} Furthermore, platelets have also been found associated with eosinophils isolated from peripheral blood of allergic donors.^{17,39} Thus, the expression or activity of the α 4 β 1 integrin very late antigen 4 (CD49d/CD29) on eosinophil surfaces is higher on eosinophils associated with P-selectin-bound platelets compared with that seen on eosinophils with no bound platelets or low-level bound P-selectin,^{13,39} and it has been demonstrated that platelets can increase the adherence of eosinophils to the vascular endothelium *in vitro*, a prerequisite step in the recruitment of eosinophils into tissues *in vivo*.^{3,40} The importance of these P-selectin-dependent phenomena have been demonstrated through the experimental use of blocking antibodies to P-selectin or P-selectin-deficient mice.^{4,41,42} We have also specifically demonstrated that this importance of P-selectin in pulmonary leukocyte recruitment is due to expression on activated platelets (rather than or as well as endothelial cells).⁴³

It is also now appreciated that neutrophils play an important part in more severe asthma and indeed that neutrophilic asthma might be a distinct phenotype.⁴⁴ Therefore in this context it is also of interest that platelet depletion has also been demonstrated to reduce the recruitment of neutrophils into various tissues, including the lung, in response to certain inflammatory stimuli, such as experimental allergen (ovalbumin) and LPS.^{43,45} The ability of platelets to interact with neutrophils has recently been shown to be dependent on the polarization of neutrophils and

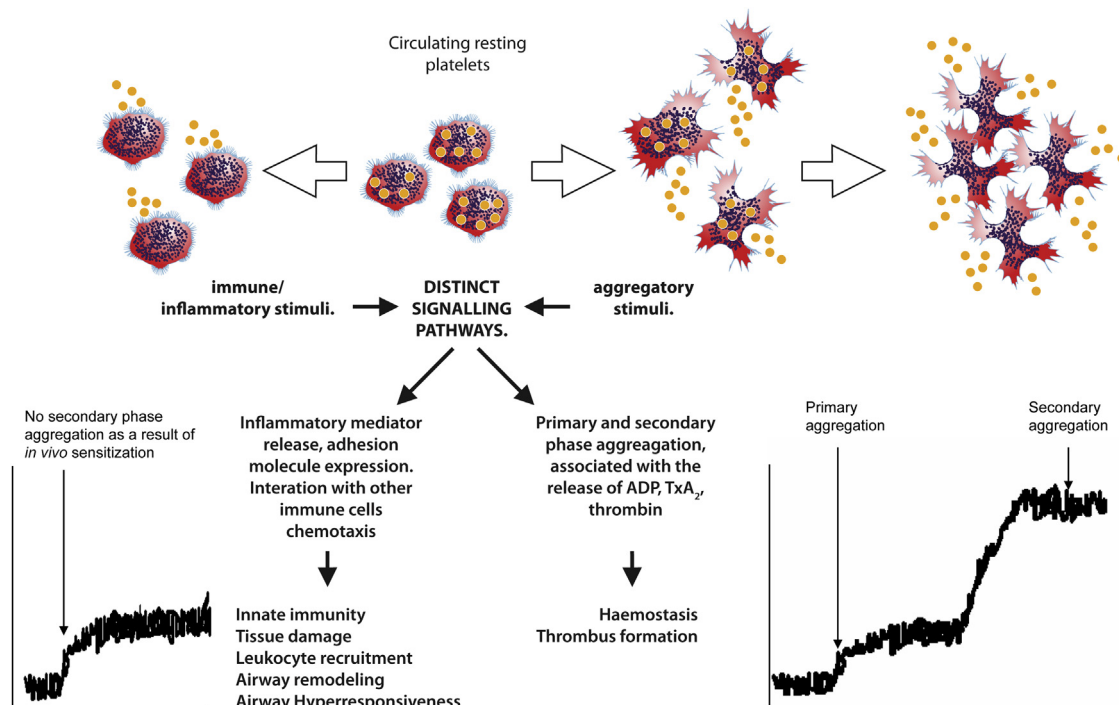


FIG 3. Dichotomy of platelet function (with respect to asthma and allergic inflammation). Depending on the type of stimulus involved, platelets can become activated by prothrombotic (*white arrow pointing right*) or proinflammatory (*white arrow pointing left*) mediators. Therefore the type of stimulus in turn dictates the ultimate function of the platelet. Intravascular platelet activation occurs during inflammatory reactions, whereas platelets from the same patients are refractory to a variety of stimuli *ex vivo*, possibly resulting from platelet “exhaustion.” Thus the secondary phase of aggregation disappears. Adapted from Page and Pitchford.⁶⁶

the presence of P-selectin glycoprotein ligand 1 at the poles, which recognizes P-selectin on platelets, suggesting that adherent neutrophils also required contact with platelets to initiate inflammatory events once recruited.⁴⁶ The formation of platelet/neutrophil complexes is sometimes a prerequisite for the ability of platelets to cooperate with neutrophils in the formation of novel inflammatory mediators.⁴⁵ Thus, it is plausible that the interactions between platelets and subsets of leukocytes, notably neutrophils, that are adhered to the endothelium are not restricted to early events of the adhesion cascade (eg, capture and rolling; Fig 2). Furthermore, the presence of platelets on a proportion of migrated leukocytes within the lungs of sensitized mice exposed to allergen (ovalbumin) suggests there might be a continuum of platelet-leukocyte interactions extravascularly in response to inflammation and injury, the significance of which is not known (Fig 2).

More recently, platelets have also been reported to modulate the activation of dendritic cells (DCs) in the lungs of allergic animals, demonstrating a potential extravascular interaction between platelets and immune cells involved in allergen presentation and sensitization.²⁹

PLATELET INTERACTIONS WITH IMMUNE CELLS AND EFFECTS ON ALLERGEN SENSITIZATION

One implication of the ability of platelets to migrate into tissue and interact with immune cells, such as DCs, macrophages, and T cells, is the possibility of platelets acting as a “bridge” between

the innate and adaptive immune responses.^{4,47} Indeed, recent investigations have demonstrated that platelets express the TLR1 through TLR4 and TLR6 through TLR9 and that at least TLR2, TLR4, and TLR9 are functional receptors in platelets facilitating antigen recognition and innate responses.^{6,48}

Thus platelets can recognize antigens broadly shared by pathogens but distinguishable from host molecules, which are collectively referred to as pathogen-associated molecule patterns, and in the case of TLR2, TLR4, and TLR9, platelets might recognize diacylated and triacylated lipopeptides, LPS, and bacterial or viral DNA, respectively.⁴⁸ One functional example of this is when platelets activated by LPS through TLR4 receptors promote neutrophil extracellular trap formation in response to bacteria.^{49,50} The role of TLRs in asthma and allergy is reviewed elsewhere,⁵¹ yet the significance of TLRs expressed on platelets in the processes leading to sensitization to allergens remains unknown.

Furthermore, platelets display functional receptors for numerous immunoglobulins, such as FcγRI, FcγRII, FcγRIII, FcεRI, FcεRII, and FcαRI/CD89, strongly indicating that platelets can play an important role in the development of the adaptive immune response.^{10,46} As described above, the functionality of platelet FcεRI and FcεRII (CD23) from allergic patients and platelet responses to allergen *in vivo* has been clearly demonstrated, and it is interesting to note that the specificity of these responses has been shown given that patients allergic to *Dermatophagoides pteronyssinus* (Der p 1) and exposed to synthetic peptides derived from the allergen Der p 1 were shown

to have activated platelets. This was a process mediated by IgE because these peptides did not stimulate platelets from healthy subjects or patients not allergic to Der p 1.⁵² Furthermore, we have shown platelet chemotaxis to allergens to which patients with asthma are specifically sensitized and not to unrelated allergens.²

The propagation of the immune response requires cell contact through receptor-ligand pairs between antigen-presenting and effector cells of the adaptive immune response, such as T cells. CD40 and *CD40 ligand* (CD40L) represent one such pair that is central in promoting these interactions.⁴ Both CD40 and CD40L have been identified on activated platelets.^{48,53} Interestingly, production of allergen-specific IgE and BHR is suppressed in allergen-sensitized mice deficient in either CD40 or CD40L,⁵⁴ and platelet CD40L has been described in other situations as an important link between the innate and adaptive immune responses to induce DC maturation, such as the expression of CD80 and CD83 and immunoglobulin class-switching.^{4,48,55} Platelet CD40L has recently been reported to be involved in the promotion of allergic airways inflammation by polarizing T_H2 responses after allergen exposure.³⁶

It is now accepted that DCs are essential in asthma pathogenesis, where myeloid DCs become involved very early in the disease by inducing sensitization to inhaled antigen.^{51,56,57} Increasingly, myeloid DCs are viewed as essential proinflammatory cells that induce effector cytokine production in primed T_H2 cells during airway inflammation.⁵⁶⁻⁵⁸ Therefore it is of interest that platelet-derived 5-HT is important for DC activation during allergic airways inflammation,^{29,59,60} and purines, such as adenosine diphosphate (ADP) and ATP, that can activate platelets also trigger and maintain asthmatic airway inflammation by activating DCs.⁶¹⁻⁶⁴ Intriguingly, we have previously demonstrated the presence of platelets residing extravascularly in the lungs of mice before allergen challenge, but the vast majority of these platelets were attached to a resident undefined mononuclear cell population and increased further during sensitization to allergen yet the relevance of this occurrence was not established.² We hypothesized that these platelets were a distinct population that had migrated to the lungs through signals unrelated to secondary exposure to allergen, which drives platelet migration into the lungs along with recruited leukocytes,^{2,3} and that these platelets might be involved in initial sensitization to allergen.

DICHOTOMY OF PLATELET ACTIVATION AND FUTURE PERSPECTIVES FOR ANTI-INFLAMMATORY DRUGS BASED ON INHIBITION OF PLATELET FUNCTION

Some years ago, Page⁶⁵ suggested the concept of a dichotomy of platelet activation with distinct mechanisms contributing to the activation of platelets in inflammation from that recognized to underpin platelet activation in hemostasis and thrombosis. This hypothesis was based on the observation that triggers for platelet activation in inflammation were distinct from those triggering platelet aggregation and that these inflammatory stimuli did not lead to platelet aggregation (Fig 3).⁶⁶ Rather, inflammatory stimuli (including allergen in platelets obtained from allergic donors) led to other types of platelet activation, such as production of oxygen free radicals, RANTES, and 5-HT^{4,10,67}; the formation of platelet/leukocyte complexes³⁷; or platelet

chemotaxis.^{2,3} Furthermore, drugs that could inhibit this platelet activation induced by inflammatory stimuli or allergen, such as the antiallergic agents nedocromil sodium, disodium cromoglycate, and cetirizine, did not inhibit platelet aggregation.^{68,69} We have very recently confirmed this hypothesis by demonstrating that when using ADP as a stimulus for platelet activation, there are distinct signaling mechanisms involved in platelet aggregation and platelet chemotaxis (Amison, unpublished observation).³⁸ In particular, we have demonstrated that P2Y₁ receptors for ADP, signaling through RhoA, are required for the activation of platelets by inflammatory stimuli, whereas P2Y₁₂ receptors that are required for platelet aggregation have no such effects.³⁸ This would explain why recent clinical studies with the P2Y₁₂ receptor antagonist prasugrel did not show any benefit in patients with allergic asthma.⁷⁰ We have also demonstrated that the Rac-GEF proteins P-Rex and Vav, which are essential for leukocyte (neutrophil) motility, are also present in platelets and central to the signaling required for platelet-dependent leukocyte recruitment.⁷¹ These observations open up the distinct possibility of targeting such signaling mechanisms as novel approaches for the development of anti-inflammatory drugs.

In conclusion, platelets should no longer be considered just cell fragments that contribute to plugging of injured blood vessels but as inflammatory cells in their own right that play a key role in the recruitment and activation of inflammatory cells in diseases such as asthma. Additionally, the ability of these cells to undergo chemotaxis and to migrate into the extravascular spaces suggests they have other important roles outside of blood vessels to combat pathogens or repair and remodel tissue after tissue injury. These observations coupled with the recent work showing platelets play a critical role in sensitization to allergens means that this is a cell that needs to be investigated more thoroughly as a major player in the pathogenesis of allergic airways disease.

FUTURE DIRECTION FOR PLATELET RESEARCH

The increasing number of observations suggesting a dichotomy of platelet activation between the signaling involved in thrombosis and hemostasis and the activation during inflammatory responses has considerable implications. If this dichotomy can be more fully understood, it opens up the real possibility of finding new anti-inflammatory and antiallergic drugs that target these novel pathways of platelet activation. Such observations also raise the distinct possibility of finding novel biomarkers for assessing allergic inflammatory diseases given the undoubted importance of platelets in leukocyte recruitment.

REFERENCES

1. Youssefian T, Drouin A, Masse JM, Guichard J, Cramer EM. Host defense role of platelets: engulfment of HIV and *Staphylococcus aureus* occurs in a specific subcellular compartment and is enhanced by platelet activation. *Blood* 2002; 99:4021-9.
2. Pitchford SC, Momi S, Baglioni S, Casali L, Giannini S, Rossi R, et al. Allergen induces the migration of platelets to lung tissue in allergic asthma. *Am J Respir Crit Care Med* 2008;177:604-12.
3. Rossaint J, Zarbock A. Platelets in leukocyte recruitment and function. *Cardiovasc Res* 2015 [Epub ahead of print].
4. Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. *Blood* 2014;123:2759-67.
5. Herd CM, Page CP. Pulmonary immune cells in health and disease: platelets. *Eur Respir J* 1994;7:1145-60.
6. Hamzeh-Cognasse H, Damien P, Chabert A, Pozzetto B, Cognasse F, Garraud O. Platelets and infections—complex interactions with bacteria. *Front Immunol* 2015;6:82.

7. Yeaman MR. Platelets: at the nexus of antimicrobial defence. *Nat Rev Microbiol* 2014;12:426-37.
8. de Stoppelaar SF, van 't Veer C, Claushuis TA, Albersen BJ, Roelofs JJ, van der Poll T. Thrombocytopenia impairs host defense in gram-negative pneumonia-derived sepsis in mice. *Blood* 2014;124:3781-90.
9. Page C, Pitchford S. Platelets coming of age: implications for our understanding of allergic inflammation. *Am J Respir Crit Care Med* 2013;187:459-60.
10. Joseph M, Gounni AS, Kusnier JP, Vormg H, Sarfati M, Kinet JP, et al. Expression and functions of the high-affinity IgE receptor on human platelets and megakaryocyte precursors. *Eur J Immunol* 1997;27:2212-8.
11. Capron M, Joseph M. The low affinity receptor for IgE on eosinophils and platelets. *Monogr Allergy* 1991;29:63-75.
12. Kornrup KN, Page CP. The role of platelets in the pathophysiology of asthma. *Platelets* 2007;18:319-28.
13. Page C, Pitchford S. Platelets and allergic inflammation. *Clin Exp Allergy* 2014;44:901-13.
14. Endresen GK. Evidence for activation of platelets in the synovial fluid from patients with rheumatoid arthritis. *Rheumatol Int* 1989;9:19-24.
15. Boillard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* 2010;327:580-3.
16. Kowal K, Pampuch A, Kowal-Bielecka O, DuBuske LM, Bodzenta-Lukaszyk A. Platelet activation in allergic asthma patients during allergen challenge with *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2006;36:426-32.
17. Johansson MW, Han ST, Gunderson KA, Busse WW, Jarjour NN, Mosher DF. Platelet activation, P-selectin, and eosinophil beta1-integrin activation in asthma. *Am J Respir Crit Care Med* 2012;185:498-507.
18. Averill FJ, Hubbard WC, Proud D, Gleich GJ, Liu MC. Platelet activation in the lung after antigen challenge in a model of allergic asthma. *Am Rev Respir Dis* 1992;145:571-6.
19. Knauer KA, Lichtenstein LM, Adkinson NF Jr, Fish JE. Platelet activation during antigen-induced airway reactions in asthmatic subjects. *N Engl J Med* 1981;304:1404-7.
20. Ind PW. Platelet and clotting abnormalities in asthma. *Clin Exp Allergy* 1991;21:395-8.
21. de Boer JD, Majoor CJ, van 't Veer C, Bel EH, van der Poll T. Asthma and coagulation. *Blood* 2012;119:3236-44.
22. Lasser EC, Berry C, Kortman K. Diminished atherosclerotic arterial calcifications in asthma. A possible role for elevated endogenous heparin-like material. *Allergy* 1987;42:549-52.
23. Cazzola M, Segreti A, Calzetta L, Rogliani P. Comorbidities of asthma: current knowledge and future research needs. *Curr Opin Pulm Med* 2013;19:36-41.
24. Slater D, Martin J, Trowbridge A. The platelet in asthma. *Lancet* 1985;1:110.
25. Coyle AJ, Spina D, Page CP. PAF-induced bronchial hyperresponsiveness in the rabbit: contribution of platelets and airway smooth muscle. *Br J Pharmacol* 1990;101:31-8.
26. Halonen M, Palmer JD, Lohman IC, McManus LM, Pinckard RN. Differential effects of platelet depletion on the physiologic alterations of IgE anaphylaxis and acetyl glyceryl ether phosphorylcholine infusion in the rabbit. *Am Rev Respir Dis* 1981;124:416-21.
27. Coyle AJ, Page CP, Atkinson L, Flanagan R, Metzger WJ. The requirement for platelets in allergen-induced late asthmatic airway obstruction. Eosinophil infiltration and heightened airway responsiveness in allergic rabbits. *Am Rev Respir Dis* 1990;142:587-93.
28. Lechin F, van der Dijks B, Orozco B, Jara H, Rada I, Lechin ME, et al. Neuropharmacologic treatment of bronchial asthma with the antidepressant tianeptine: a double-blind, crossover placebo-controlled study. *Clin Pharmacol Ther* 1998;64:223-32.
29. Durk T, Duerschmied D, Muller T, Grimm M, Reuter S, Vieira RP, et al. Production of serotonin by tryptophan hydroxylase 1 and release via platelets contribute to allergic airway inflammation. *Am J Respir Crit Care Med* 2013;187:476-85.
30. Lommatzsch M, Lindner Y, Edner A, Bratke K, Kuepper M, Virchow JC. Adverse effects of salmeterol in asthma: a neuronal perspective. *Thorax* 2009;64:763-9.
31. Coyle AJ, Uchida D, Ackerman SJ, Mitzner W, Irvin CG. Role of cationic proteins in the airway. Hyperresponsiveness due to airway inflammation. *Am J Respir Crit Care Med* 1994;150(suppl):S63-71.
32. Pitchford SC, Roffo-Vasquez Y, Sousa A, Momi S, Gesele P, Spina D, et al. Platelets are necessary for airway wall remodeling in a murine model of chronic allergic inflammation. *Blood* 2004;103:639-47.
33. Svensson Holm AC, Grenegard M, Ollinger K, Lindstrom EG. Inhibition of 12-lipoxygenase reduces platelet activation and prevents their mitogenic function. *Platelets* 2014;25:111-7.
34. Vignola AM, Kips J, Bousquet J. Tissue remodeling as a feature of persistent asthma. *J Allergy Clin Immunol* 2000;105:1041-53.
35. Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989;140:1745-53.
36. Tian J, Zhu T, Liu J, Guo Z, Cao X. Platelets promote allergic asthma through the expression of CD154. *Cell Mol Immunol* 2014 [Epub ahead of print].
37. Pitchford SC, Yano H, Lever R, Roffo-Vasquez Y, Ciferri S, Rose MJ, et al. Platelets are essential for leukocyte recruitment in allergic inflammation. *J Allergy Clin Immunol* 2003;112:109-18.
38. Amison RT, Momi S, Morris A, Manni G, Keir S, Gesele P, et al. RhoA signaling through platelet P2Y(1) receptor controls leukocyte recruitment in allergic mice. *J Allergy Clin Immunol* 2015;135:528-38.
39. Johansson MW. Activation states of blood eosinophils in asthma. *Clin Exp Allergy* 2014;44:482-98.
40. Ulfman LH, Joosten DP, van Aalst CW, Lammers JW, van de Graaf EA, Koenderman L, et al. Platelets promote eosinophil adhesion of patients with asthma to endothelium under flow conditions. *Am J Respir Cell Mol Biol* 2003;28:512-9.
41. Broide DH, Sullivan S, Gifford T, Sriramam P. Inhibition of pulmonary eosinophilia in P-selectin- and ICAM-1-deficient mice. *Am J Respir Cell Mol Biol* 1998;18:218-25.
42. Lukacs NW, John A, Berlin A, Bullard DC, Knibbs R, Stoolman LM. E- and P-selectins are essential for the development of cockroach allergen-induced airway responses. *J Immunol* 2002;169:2120-5.
43. Pitchford SC, Momi S, Giannini S, Casali L, Spina D, Page CP, et al. Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. *Blood* 2005;105:2074-81.
44. Fajt ML, Wenzel SE. Asthma phenotypes and the use of biologic medications in asthma and allergic disease: the next steps toward personalized care. *J Allergy Clin Immunol* 2015;135:299-311.
45. Page C, Pitchford S. Neutrophil and platelet complexes and their relevance to neutrophil recruitment and activation. *Int Immunopharmacol* 2013;17:1176-84.
46. Sreeramkumar V, Adrover JM, Ballesteros I, Cuartero MI, Rossaint J, Bilbao I, et al. Neutrophils scan for activated platelets to initiate inflammation. *Science* 2014;346:1234-8.
47. von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. *Circ Res* 2007;100:27-40.
48. Cognasse F, Nguyen KA, Damien P, McNicol A, Pozzetto B, Hamzeh-Cognasse H, et al. The inflammatory role of platelets via their TLRs and Siglec receptors. *Front Immunol* 2015;6:83.
49. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* 2007;13:463-9.
50. Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J Clin Invest* 2012;122:2661-71.
51. Lambrecht BN, Hammad H. The immunology of asthma. *Nat Immunol* 2015;16:45-56.
52. Cardot E, Pestel J, Callebaut I, Lassalle P, Tscipoulos A, Gras-Masse H, et al. Specific activation of platelets from patients allergic to *Dermatophagoides pteronyssinus* by synthetic peptides derived from the allergen Der p 1. *Int Arch Allergy Immunol* 1992;98:127-34.
53. von Hundelshausen P, Petersen F, Brandt E. Platelet-derived chemokines in vascular biology. *Thromb Haemost* 2007;97:704-13.
54. Mehlich PD, van de Rijn M, Brewer JP, Kisselgof AB, Geha RS, Oettgen HC, et al. CD40L, but not CD40, is required for allergen-induced bronchial hyperresponsiveness in mice. *Am J Respir Cell Mol Biol* 2000;23:646-51.
55. Sprague DL, Elzey BD, Crist SA, Waldschmidt TJ, Jensen RJ, Ratliff TL. Platelet-mediated modulation of adaptive immunity: unique delivery of CD154 signal by platelet-derived membrane vesicles. *Blood* 2008;111:5028-36.
56. van Rijt LS, Vos N, Willart M, Kleijman A, Coyle AJ, Hoogsteden HC, et al. Essential role of dendritic cell CD80/CD86 costimulation in the induction, but not reactivation, of TH2 effector responses in a mouse model of asthma. *J Allergy Clin Immunol* 2004;114:166-73.
57. Lambrecht BN, Hammad H. Lung dendritic cells in respiratory viral infection and asthma: from protection to immunopathology. *Annu Rev Immunol* 2012;30:243-70.
58. Lambrecht BN, Hammad H. The role of dendritic and epithelial cells as master regulators of allergic airway inflammation. *Lancet* 2010;376:835-43.
59. Idzko M, Panther E, Stratz C, Muller T, Bayer H, Zissel G, et al. The serotonergic receptors of human dendritic cells: identification and coupling to cytokine release. *J Immunol* 2004;172:6011-9.

60. Muller T, Durk T, Blumenthal B, Grimm M, Cicko S, Panther E, et al. 5-hydroxytryptamine modulates migration, cytokine and chemokine release and T-cell priming capacity of dendritic cells in vitro and in vivo. *PLoS One* 2009; 4:e6453.
61. Idzko M, Hammad H, van Nimwegen M, Kool M, Willart MA, Muskens F, et al. Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat Med* 2007;13:913-9.
62. Muller T, Vieira RP, Grimm M, Durk T, Cicko S, Zeiser R, et al. A potential role for P2X7R in allergic airway inflammation in mice and humans. *Am J Respir Cell Mol Biol* 2011;44:456-64.
63. Muller T, Robaye B, Vieira RP, Ferrari D, Grimm M, Jakob T, et al. The purinergic receptor P2Y2 receptor mediates chemotaxis of dendritic cells and eosinophils in allergic lung inflammation. *Allergy* 2010;65:1545-53.
64. Idzko M, Ferrari D, Eltzschig HK. Nucleotide signalling during inflammation. *Nature* 2014;509:310-7.
65. Page CP. The involvement of platelets in non-thrombotic processes. *Trends Pharmacol Sci* 1988;9:66-71.
66. Page CP, Pitchford SC. Platelets. In: Laurent G, Shapiro S, editors. *Encyclopedia of respiratory medicine*. Amsterdam: Elsevier; 2006. pp. 347-51.
67. Klouche M, Klinger MH, Kuhnel W, Wilhelm D. Endocytosis, storage, and release of IgE by human platelets: differences in patients with type I allergy and nonatopic subjects. *J Allergy Clin Immunol* 1997;100:235-41.
68. Tunon-de-Lara JM, Rio P, Marthan R, Vuillemin L, Ducassou D, Taytard A. The effect of sodium cromoglycate on platelets: an in vivo and in vitro approach. *J Allergy Clin Immunol* 1992;89:994-1000.
69. Joseph M, Tsicopoulos A, Tonnel AB, Capron A. Modulation by nedocromil sodium of immunologic and nonimmunologic activation of monocytes, macrophages, and platelets. *J Allergy Clin Immunol* 1993;92:165-70.
70. Lussana F, Di Marco F, Terraneo S, Parati M, Razzari C, Scavone M, et al. Effect of prasugrel in patients with asthma: results of PRINA, a randomized, double-blind, placebo-controlled, cross-over study. *J Thromb Haemost* 2015;13:136-41.
71. Pan D, Amison RT, Riffo-Vasquez Y, Spina D, Cleary SJ, Wakelam MJ, et al. P-Rex and Vav Rac-GEFs in platelets control leukocyte recruitment to sites of inflammation. *Blood* 2015;125:1146-58.