

REVIEW

Platelets and allergic inflammation

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Summary

Irrefutable clinical evidence demonstrates the activation of platelets in allergic diseases, including asthma, allergic rhinitis, and eczema. Indeed, experimental models of allergic disease have now shown that platelets play a fundamental role in the tissue recruitment of leucocytes following exposure to allergens. Furthermore, the extravascular presence of platelets in lungs of patients with asthma, and in animal models of allergic lung inflammation suggests that platelets may also contribute directly to allergic inflammation, through alterations in lung function, or by modulating processes involved in airway wall remodelling. Despite significant platelet activation in patients with allergic diseases, it is of note that these patients have been described as having a mild haemostatic defect, rather than an increased incidence of thrombosis. This suggests a dichotomy exists in platelet activation during inflammation compared to haemostasis, and that hitherto undiscovered platelet activation pathways might be exploited to create novel anti-inflammatory therapies without affecting the critical function of platelets in haemostasis.

Background

Platelets have a well-established role in thrombosis and haemostasis, but there is now considerable evidence accumulating that this cell type can also play an important role in a number of inflammatory diseases, including allergic inflammation and asthma [1]. Platelet abnormalities in patients with allergy have been reported in the literature for more than 40 years [2]. It was the seminal observation by Benveniste et al. [3] in 1972 that leucocyte-dependent histamine release from platelets involved IgE activation that led to the discovery of the lipid mediator platelet activating factor (PAF). However, whilst it has been recognised that PAF is a potent inflammatory mediator capable of mimicking many aspects of the allergic condition such as inducing eosinophilia [4], the failure of several PAF antagonists in patients with asthma [5] suggests that any platelet involvement in this disease is not dependent solely on PAF. As we will now review, it is becoming increasingly apparent that the involvement of platelets in allergic inflammation and asthma is complex. There is increasing insight as to how these simple anucleated cells play an important role in everything from being a source of inflammatory mediators *per se* to being critical for optimal recruitment of leucocytes into tissues.

In 1978, Gallagher and colleagues observed that platelets isolated from the peripheral blood of allergic patients during the allergy season often showed reduced secondary wave platelet aggregation when they were assessed for their responsiveness to platelet activators *ex vivo* [6]. Others have confirmed this apparent lack of responsiveness of platelets to aggregatory stimuli, and that storage of mediators within platelets was diminished in patients with asthma [7, 8]. It was thus suggested that platelets were behaving in an 'exhausted' manner, by which platelets had been activated to inflammatory stimuli *in vivo* to release mediators, and were subsequently refractory to further subsequent activation (aggregation) *ex vivo* [6–10]. Since this observation, there have been many other reports of altered platelet behaviour in patients with allergic disease ranging from altered arachidonic acid metabolism to greater turnover of intra-cellular signalling pathways [11, 12]. Indeed, these platelet abnormalities may well account for the observation that patients with allergic asthma have a mild haemostatic defect and delayed thrombin generation detected using template bleeding times [13, 14]. Whether this 'exhausted platelet' syndrome seen in allergic patients does indeed represent continuous platelet activation *in vivo* is not yet known, but it is of interest that allergic patients undergoing allergen

provocation have a mild thrombocytopaenia, variously reported to be 10–25% occurring within minutes after allergen exposure and continuing for several hours afterwards [15–17]. Furthermore, an alteration in the lifespan of platelets has been reported in stable atopic asthmatic patients, decreasing from 8.9 days to 4.7 days, suggestive of continual platelet consumption as a result of chronic activation [18]. It was also reported that treatment with corticosteroids improved platelet turnover, thus reversing the observed shortening of platelet lifespan in atopic asthmatic patients [19]. In contradiction, other groups have reported no differences in platelet lifespan between healthy individuals and patients with asthma, or pulmonary platelet sequestration after allergen challenge, suggesting the mechanisms governing platelet production, transit and lifespan in the context of inflammatory disease are complex [20, 21]. Other potential indicators of changes to the rate of platelet production or consumption, for example, changes mean platelet volume (MPV) and mass, have been reported, which may correlate with changes in platelet function or activation [11, 13].

A recent review has described in extensive detail a hypothesis that platelets and the lungs have an intimate relationship, in that the lungs are reservoirs for megakaryocytes and a possible site of platelet production [22]. This circumstance may provide a pool of platelets that slowly transit through the lungs to help maintain vascular integrity of the alveolar capillaries or become responsive to inflammatory or repair signals [22]. Whilst there is controversy as to the anatomical site of platelet production, mathematical models support the concept that megakaryocytes egress from the bone marrow and get trapped in the capillary network of the lungs to create the observed population characteristics of MPV and mass [23, 24]. Indeed, Aschoff identified pulmonary megakaryocytes in humans in 1893, and whilst we do not know whether the dynamics of this postulated process are changed in patients with asthma, it could have implications for platelet volume, mass, and perhaps platelet behaviour [25, 26]. It is therefore interesting that megakaryocytes have been reported in lungs obtained at autopsy of patients who have died from *status asthmaticus* and alterations in their reactivity may influence disease progression [26, 27].

Increased numbers of platelet/leucocyte aggregates also occur in peripheral blood, suggesting that platelet activation is a result of allergic exposure in such patients [28, 29]. This is further supported by a number of observations showing increased markers of platelet activation (e.g. regulated upon transcription normally T-cell expressed and excreted, RANTES; platelet factor-4, PF-4; beta-thromboglobulin, β -TG) in the peripheral blood of patients with asthma [8, 15, 30, 31]. In particular, PF-4 is found to be significantly higher in patients

with severe asthma compared with non-severe asthma, indicating that the degree of platelet activation might increase with increased disease severity [29]. A possible consequence of platelet activation that results in peripheral thrombocytopaenia is the localised recruitment of platelets to the lungs. Indeed, platelet markers have been measured in bronchoalveolar lavage (BAL) fluid [32, 33], along with observations of platelets being found extravascularly in the airways [32, 33].

Platelets are a rich source of spasmogens [34, 35], mediators able to induce leucocyte activation and recruitment [36, 37], as well as substances able to contribute to remodelling and repair of tissues after injury making them well placed to contribute to some of the important features of asthma (Fig. 1). Furthermore, it is now recognised that platelets from allergic donors can express both the high and the low affinity IgE receptors on their surface and that exposure to appropriate antigens can lead to the generation of inflammatory mediators such as free radical species, serotonin/5-hydroxytryptamine (5-HT or serotonin) and RANTES [38–41]. Interestingly, activation of the high affinity IgE receptor can cause platelets to undergo chemotaxis [42]. Furthermore, it has long been recognised that platelets contain high concentrations of serotonin in their dense granules and several clinical observations have found altered levels of this vasoactive amine in patients with asthma. Tryptophan hydroxylase (THP) – 1 is a critical enzyme for the biosynthesis of serotonin outside of the CNS, and very recently, Dürk et al. [43] have utilised mice genetically deficient in THP-1 and mast cell deficient mice to demonstrate that platelets, rather than mast cells, are the main source of serotonin released during an allergic inflammatory response. Unlike mice, human mast cells are not a major source of serotonin and so it is of even more interest that Dürk et al. [43] have found elevated serotonin levels in BAL fluid following segmental allergen challenge of allergic asthmatics, as their results suggest that the serotonin comes from activated platelets. These results corroborate several earlier clinical observations reporting platelet activation accompanying allergic asthmatic responses [8, 15, 28, 29]. It is of particular interest that platelets have now been found extravascularly in a number of inflammatory situations [44], including in the lungs of allergic mice [42] and in the lungs of patients with asthma [32], and so it is intriguing to know whether the elevated serotonin found in the BAL fluid of allergic asthmatics is derived from circulating platelets and/or platelets that have undergone diapedesis into lung tissue. Either way the results presented by Dürk et al. are of further interest as they have reported that allergic mice deficient in THP-1 or treated with an inhibitor of THP-1, PCPA, exhibit reduced leucocyte infiltration into the lung and inhibition of the bronchial hyperresponsiveness (BHR) that

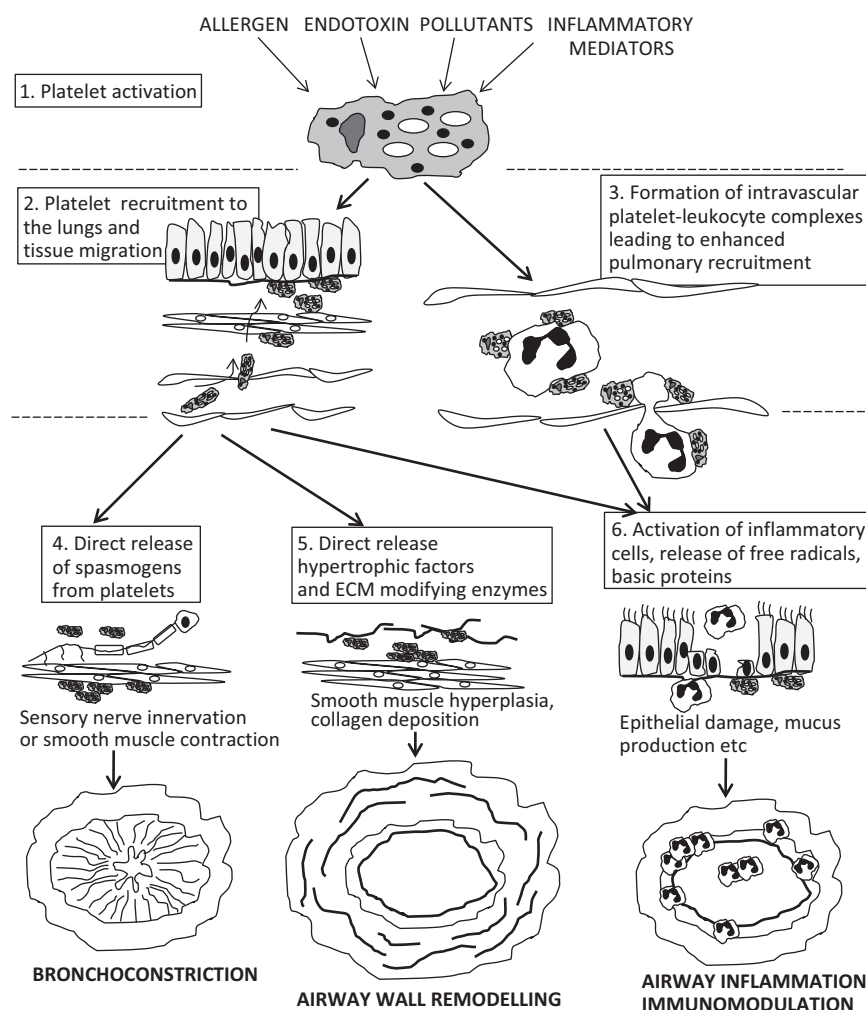


Fig. 1. A direct role for platelets in the pathogenesis of asthma. This picture was originally published in Page and Pitchford [137].

normally accompanies allergen challenge, suggesting that the platelet-derived serotonin is playing a central role in allergic inflammation in the lung. Given that there are some encouraging early clinical observations in patients with asthma administered either drugs affecting serotonin uptake [45] or antagonising 5-HT₂ receptors [46], it would seem timely to consider larger trials of such agents in patients with allergic airways disease. Such observations extend the growing body of the literature that platelets play a central role in both allergic [36] and non-allergic [47] leucocyte recruitment into the lung, as well as a critical role in other manifestations of allergic asthma such as airway remodelling [48]. Additionally, aspirin-exacerbated respiratory disease (AERD) is an asthma-like syndrome associated with excessive production of cysteinyl leukotrienes (cysLTs) and eosinophilic infiltration of the respiratory tissue, where platelet activation has also recently been described [49]. The mechanisms by which platelets influence these processes are documented below and provide a number of potential

targets for novel therapeutic interventions to treat asthma and allergic airway inflammation.

Platelet dependent leucocyte recruitment

Investigation of the mechanics of blood flow (haemorheology) has uncovered interesting phenomena that have fundamental implications relating to how platelets are required to initiate the 'adhesion cascade' that results in leucocyte recruitment during inflammation associated with allergic diseases such as asthma, allergic rhinitis and eczema. Laminar flow in blood vessels is influenced by wall friction, causing a shearing motion that results in a parabolic velocity profile of the fluid (Hagen-Poiseuille Law). Because blood is a non-Newtonian fluid, the viscosity of blood decreases with increasing shear rate. Thus, red blood cells (rbcs) actually aggregate to form reversible rouleaux (due to the discoid shape and large surface area of rbcs, they have a propensity to form stacks) under conditions of low shear and inwardly

migrate (due to their relative deformability compared with platelets and leucocytes) into the vessel core [50–53]. The dependency of blood viscosity on blood vessel diameter (blood viscosity decreasing in tubes of decreasing diameter or proximity to vessel periphery) is known as the Fåhræus-Lindqvist Effect [54]. The consequence of which is an axial redistribution of the blood elements (the related Fåhræus Effect), with rbc rouleaux moving towards the centre of the vessel, and as a consequence, the density of platelets and leucocytes rapidly increasing around the vessel periphery [55]. The rheological phenomenon of shear rate-dependent viscosity for blood therefore occurs in a region of the vessel where the velocity gradient is highest (i.e. around the vessel wall). Thus, cells travelling at different velocities along adjacent streamlines, and under certain conditions (i.e. during an inflammatory response), lead to the probability of increased cellular collisions. This peripheral zone therefore 'traps' leucocytes into an environment rich in platelets, thus greatly enhancing the possibility of collisions between platelets and leucocytes [50–53]. This may lead to the tethering of platelets to leucocytes to form rosettes through P-selectin recognition steps, regulating subsequent integrin expression, and to accelerate the action of firm adhesion to endothelium as platelet-bound leucocytes enter the capillary network. High-resolution video microscopy has been used to reveal the existence of membrane tethers involving P-selectin/PSGL-1 bonds that regulate leucocyte rolling on platelets and P-selectin, with changes in tether length and lifetime dependent on increasing shear force [56]. The biomechanics of activated platelet complex formation with leucocytes reveals that the high tensile strength of P-selectin/PSGL-1 binding enables P-selectin-dependent tethering at high shear rates, whereas integrin activation may mediate

platelet-leucocyte complex formation at low shear rates [57]. Nevertheless, un-stimulated leucocytes (that constitutively express PSGL-1/L-selectin) may also bind to activated platelets in an integrin-independent manner, suggesting that purely selectin-dependent cell adhesion is possible to create platelet-leucocyte complexes [57].

These rheological events occur in the circulation of patients with asthma upon allergen challenge. The association of platelets with eosinophils was reported in 1992, and more recently, staining of mixed leucocyte cytopspins from whole blood revealed 5–25% eosinophils attached to platelets from patients with mild or moderate asthma [58, 59]. Circulating platelet-leucocyte complexes occur at far greater frequency after both spontaneous asthma attacks in a biphasic manner, or after allergen challenge in the clinic (Fig. 2), [8, 28]. The possible significance of these platelet-leucocyte complexes is to act as a 'priming' step for further leucocyte adhesion, because leucocytes attached to platelets display more of the α M subunit (CD11b) of the α M β 2 integrin MAC-1 (CD11b/CD18), than circulating leucocytes not attached to platelets [28, 36]. It is therefore of considerable interest that recent research has correlated platelet activation with eosinophil inflammation in patients with asthma [60]. Furthermore, α 4 β 1-integrin very late antigen-4 (VLA-4), but not α M β 2 integrin MAC-1 expression, on a proportion of eosinophils correlated with eosinophil-bound platelets expressing P-selectin, after whole-lung antigen challenge of subjects with non-severe asthma, leading to circulating eosinophils bearing platelet-P-selectin with activated β 1-integrin disappearing from the circulation, presumably because these complexes are then sequestered in the lungs [29, 59]. Thus, the expression or activity of VLA-4 on the surface of eosinophils is

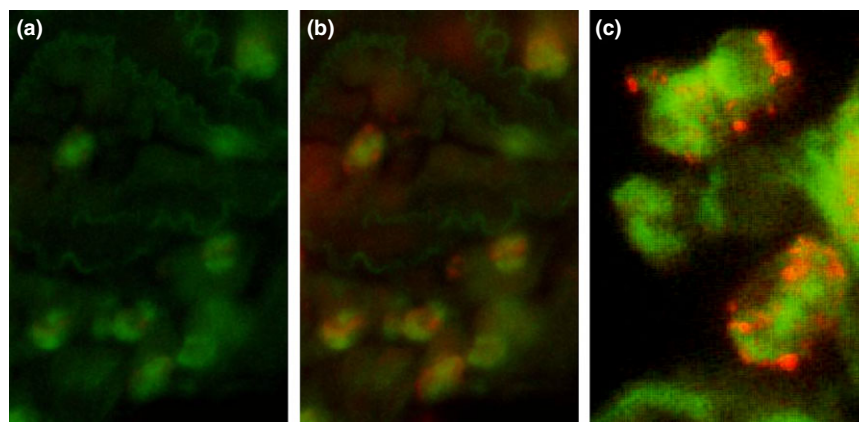


Fig. 2. Identification of platelet-eosinophil complexes taken from allergen-sensitised mice after allergen exposure. Lung samples taken from allergen-challenged mice were stained with rat anti-MBP for eosinophils (green fluorescence) and goat anti-CD41 for platelets (red fluorescence). (a) Image with green fluorescence filter only, (b) red and green fluorescence filters combined, showing eosinophils complexed with platelets, and (c) individual platelets (red) attached to eosinophils (green). This figure was originally published in *Blood* 2005 [36]; Copyright © 2005 by The American Society of Hematology.

higher on eosinophils associated with P-selectin bound platelets compared with eosinophils with no bound platelets or low level bound P-selectin [59, 61]. Recently, platelet-leucocyte (identified as eosinophils, neutrophils and monocytes) complexes have also been observed in AERD [49]. The mechanistic significance of these platelet-leucocyte interactions in allergic inflammation is that such activity has been shown to lead to increased eosinophil adhesion to the vascular endothelium via a platelet-P-selectin-dependent mechanism *in vitro* [62, 63]. Furthermore, these actions are not restricted to platelet/eosinophil interactions, because it is noted that P-selectin or platelets activate integrins on lymphocytes, monocytes and neutrophils *in vitro*, and therefore, platelet adherence to these distinct leucocyte types might also influence their tissue recruitment as a generalised mechanism [37, 49, 64–67].

The importance of platelets in pulmonary leucocyte recruitment after allergen challenge has now been extensively reported using *in vivo* models of platelet depletion. Platelet depletion, via both immunological and non-immunological methods, provides strong evidence for a requirement of platelets in pulmonary eosinophil and lymphocyte recruitment in rabbits, guinea pigs and mice [28, 36, 68, 69], and effector T cells in murine models of contact hypersensitivity [70, 71]. This process required intact platelets, because the re-infusion of lysed platelet products was insufficient to restore leucocyte recruitment, whereas the re-infusion of intact activated platelets expressing selectins on the cell surface restored leucocyte recruitment [36, 71]. With similarities to human patients undergoing allergen challenge, circulating leucocytes attached to platelets display significant increases in CD11b and VLA-4 adhesion molecule expression in mice sensitised to allergen, compared with leucocytes not attached to platelets, and circulating platelet-leucocyte complexes in non-inflamed animals [28, 36]. Activation of leucocytes at the level of contact-dependent signalling with platelets therefore induces the expression of integrins, presumably for firm adhesion. This has been confirmed by the experimental use of blocking antibodies to P-selectin, which results in the suppression of platelet-leucocyte complexes, integrin expression and subsequent tissue recruitment in models of asthma and chronic contact hypersensitivity [36, 69–76]. Indeed, the targeting of P-selectin or PSGL-1 as a novel therapeutic option has recently progressed through phase II clinical trials in patients with asthma using bimosiamose, a pan-selectin antagonist (but with more selectivity against P-selectin compared with E- and L-selectins). The administration of bimosiamose was effective at inhibiting the late-phase response after whole-lung antigen challenge, suggesting that the rationale for suppressing selectin-mediated cell interactions is clinically viable as a therapeutic strategy [77, 78].

It is of interest that platelet attachment to leucocytes does not only help determine the recruitment of leucocytes, but also synthetic processes in leucocytes which has implications for asthma, and in particular AERD which is characterised by overproduction of leukotrienes [49]. The transcellular metabolism of arachidonic acid allows platelets to enhance the formation and conversion of leucocyte-derived leukotrienes (LT)_{C₄}, LTD₄ and LTE₄. Hydroxyeicosatetraenoic acid (12-HETE) is produced by a platelet-specific enzyme (12-lipoxygenase) and is taken up by neutrophils to produce 12-,20-diHETE, a chemo-attractant that neutrophils are unable to produce in isolation [79–82]. 12-HETE also stimulates neutrophil 5-lipoxygenase and thus increases leukotriene production [83]. Platelet-P-selectin can regulate the trans-cellular metabolism of arachidonic acid metabolites within leucocytes [84], which contribute to more than 50% of LTC₄ synthase activity in patients with AERD [49]. It has also been reported that biochemical differences exist in the ability of platelets and neutrophils to contribute to trans-cellular metabolism of arachidonic acid if taken from healthy donors compared with patients with disease. For example, platelets from allergic donors increase neutrophil LTB₄ levels to a greater extent than that of platelets from healthy donors [49, 85].

Nevertheless, many other studies also reveal that other mediators released, or expressed, by platelets can modulate leucocyte recruitment, and some of these may be in selectin-independent manner [86]. Examples are the platelet-specific chemokines platelet factor 4 (PF-4, CXCL4) and β -thromboglobulins (β -TG, CXCL-7), RANTES (CCL5) and pleiotropic mediators such as leukotrienes, serotonin and sphingolipids [37, 87–89]. Thus, whilst there is a requirement for platelets expressing adhesion molecules on the surface, this must also be sequential to the release or expression of other platelet-derived factors in the regulation of leucocyte recruitment. From a physiological perspective, it is certainly not understood why the process of leucocyte activation and adhesion to post-capillary venules is so inefficient and should actually require platelet activation to optimally 'switch on' and recruit leucocytes. Perhaps the rapidity of platelet activation to danger signals combined with a larger surface area/volume ratio to express a critical density of adhesion molecules compared with larger leucocytes results in an evolutionary requirement for platelets in the rheological processes that determine the leucocyte adhesion cascade.

Platelet motility and migration into lung tissue

Current perceptions concerning the setting of platelet function in inflammation are heavily influenced by the clear intra-vascular role of platelets in haemostasis and thrombosis. Thus, dogma suggests that the participation

of platelets in inflammation is also confined to intravascular events and therefore indirect and dependent on their ability to influence leucocyte recruitment. However, accumulating evidence details a novel function of platelets in being able to respond to chemotactic signals and migrate through inflamed tissue (Fig. 3). Platelets have been observed to undergo diapedesis in sections of lung obtained from patients with asthma and lungs (including BAL fluid) from allergen-sensitised and exposed mice, rabbits and guinea pigs [32, 33, 42, 65, 90]. Using quantitative histology, we have recently reported that the migration of platelets into lung tissue and the localisation of platelets around the airway wall in allergen-sensitised and exposed mice were via an IgE-mediated process and that platelets from such mice could also undergo chemotaxis to the sensitising allergen *in vitro* [42]. Interestingly, the migratory response to allergen *in vivo* commenced before initial leucocyte recruitment and migration, whilst at later time points, around 50% of platelets quantified were not complexed to leucocytes [42]. Furthermore, there were instances where platelet migration occurred in the absence of leucocytes [42] (Fig. 3). Thus, whilst the extravascular presence of a proportion of platelets can be attributed to platelet complexes and subsequent dissociation from leucocytes, there appears to be a significant population of platelets that migrate independently of leucocyte contact. The rapidity of this response highlights that platelet activation by allergen is direct and independent

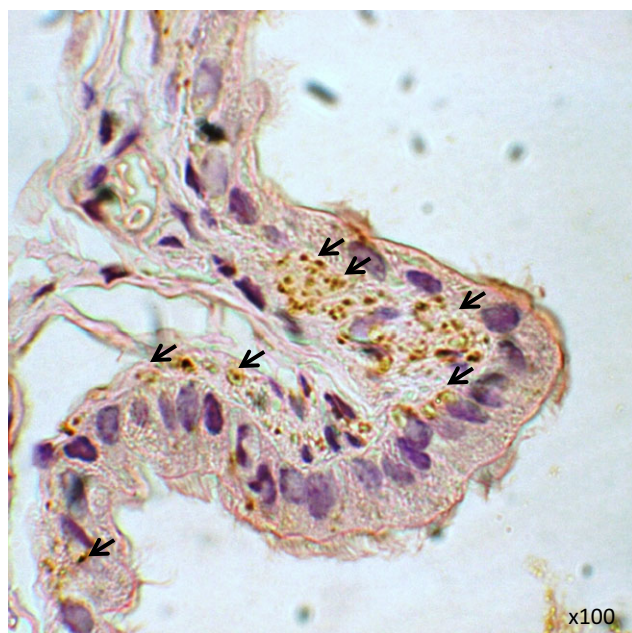


Fig. 3. Histological analysis of lungs taken from ovalbumin (OVA)-sensitised mice after allergen inhalation. Section was stained for the platelet-specific antigen CD41 (integrin α IIb). Platelets (brown) can be observed throughout the airway wall.

of activation of other cell types, for example mast cells [91]. It is interesting to note therefore that there is accumulating evidence of platelet migration into tissues in other inflammatory diseases, such as into the synovial fluid of patients with rheumatoid arthritis and transmigration across the vascular wall after long periods of ischaemia [92, 93]. The control of this platelet migration has not been assessed, although platelets express a number of different chemokine receptors (CCR1, CCR3, CCR4 and CXCR4) which are functional, because the ligands stromal cell-derived factor-1 (SDF-1 α), monocyte-derived chemokine (MDC) and thymus cell and regulated chemokine (TARC) can activate platelets [94–96]. Platelets have also been shown to undergo chemotaxis to f-MLP and SDF-1 α and can therefore be considered to be motile cells [93, 94]. Therefore, there is the potential that platelet migration through lung tissue and localisation to specific resident cells/structures is as a highly regulated process, as it is for leucocytes. Whilst the significance of this platelet migration into lung tissue has not yet been fully characterised, we now set out below how platelets might directly influence the development of BHR, bronchospasm, tissue damage and chronic inflammation leading to airway wall remodelling.

Platelets and bronchospasm/BHR

The observation that platelets migrate into the lung tissue of patients with asthma, and into the lungs of animals experimentally, opens the possibility that platelets may contribute directly to alterations in lung function in patients with asthma. For example, platelet depletion in allergen-sensitised rabbits and guinea pigs abolishes bronchoconstriction and anaphylaxis induced by inhaled spasmogens or allergens, respectively [64, 65]. There is now some understanding of the pathways and platelet mediators involved in these processes, with the investigation of the effects of intravenous administration of platelet agonists on bronchospasm and platelet accumulation in the lung [34, 35, 65, 97]. We have recently observed that platelet depletion will inhibit bronchospasm induced by 'indirect spasmogens' such as capsaicin and bradykinin, whilst not inhibiting direct acting spasmogens such as histamine and methacholine [98, 99] suggestive that platelet-derived mediators contribute to airway obstruction under certain circumstances. Furthermore, the inhibition of the release of bronchoactive agents from platelets abrogated the resulting BHR in animal models, confirming that platelet-derived mediators might also contribute to airway irritability [34, 35]. Indeed, a direct platelet participation in allergy, independent of leucocyte responses, was highlighted by the intradermal injection of supernatants from activated human platelets (but not leucocytes)

inducing delayed, sustained inflammatory responses in the skin of patients with atopic dermatitis [100]. These direct effects on tissue suggest that platelets are very capable of inducing sustained inflammation. Human platelets are capable of synthesising and releasing a number of bronchoactive mediators, for example histamine, serotonin, thromboxane (TXA₂), adenosine, 12-HETE and cytotoxic compounds within their granules, and those generated from platelet membranes that are capable of inducing tissue damage, such as reactive oxygen species (ROS), cationic proteins (PCPs, e.g. PF-4), and platelet basic proteins (PBPs, e.g. neutrophil-activating peptide 2, NAP-2; connective tissue-activating peptide 3, CTAP-III; thrombospondin-1 TC-1; thrombospondin-2, TC-2) which also have bactericidal, fungicidal or anti-parasite activity [38, 101–104]. Yet it is not comprehensively understood which of these mediators interact, and with what (e.g. sensory nerves, airway smooth muscle and epithelium) to elicit bronchospasm and BHR [101].

Platelets and chronic inflammation/lung remodelling

One consequence of persistent, chronic inflammation is alteration to tissue structure and function. In bronchial asthma, chronic inflammation may contribute to changes in airway architecture referred to as 'airway remodelling'. The observation that platelets migrate into the lung tissue of patients with asthma, and experimentally in animals [32, 33, 42, 69, 90], suggests that platelets might contribute directly to changes in the airway architecture by releasing factors that control the synthetic phenotype of airway epithelium, fibroblasts and airway smooth muscle. Indeed, in murine models of chronic allergic inflammation, the depletion of platelets led to a more comprehensive suppression of remodelling features (smooth muscle hyperplasia, subepithelial fibrosis, collagen deposition, epithelial hyperplasia) compared with the chronic treatment with glucocorticosteroids, suggesting platelet involvement in remodelling process might in some instances be independent of leucocyte-associated inflammation [46]. The chronicity of platelet activation has been highlighted in studies where platelet activation has been shown to persist some time after the late asthmatic response has occurred in asthmatic patients [15], even though documented increases in platelet-leucocyte interactions within the circulation have returned to basal levels at 24 h post-allergen exposure [28], implicating platelets in chronic inflammatory events and airway remodelling.

Platelets are a rich reservoir of mitogens and enzymes, and therefore, platelets may indeed contribute to a very favourable environment that induces synthetic responses in airway structural cells. A list of platelet mitogens includes platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming

growth factor- β (TGF- β), vascular endothelial growth factor (VEGF) and the major product of arachidonic acid metabolism in platelets, TXA₂, which are all known to have proliferative actions on structural cells found in the airways [105, 106], whilst platelet-derived enzymes (e.g. matrix metalloproteinases, β -hexosaminidases and heparanases) are released following allergen challenge of asthmatic patients and following ozone challenge in guinea pigs which may alter the composition of the extracellular matrix [107, 108]. Disruption of the composition and integrity of cell membranes by degradation of glycoproteins, glycolipids and glycosaminoglycans may also release membrane-bound growth factors for wound repair [109]. Recent evidence shows that platelet membranes are required to induce synthetic responses in airway smooth muscle cells [110], perhaps via the trans-metabolism of platelet-derived arachidonic acid by smooth muscle 5-lipoxygenase to produce leukotrienes and the production of reactive oxygen species via NADPH oxidase [111, 112]. The effect of platelets on the synthetic responses of resident structural tissue of the airways might also be supplemented by the ability of platelets to influence the survival, recruitment, proliferation and differentiation of circulating structural cells and stem cells involved in (inappropriate) tissue regeneration [113]. It can be surmised therefore that platelets are likely to influence lung regeneration and inappropriate remodelling of the airways after injury, although the interplay between platelets and different structural cells is likely to be extremely complex and involve a plethora of mediators at various levels.

Coagulation pathways and asthma

There is evidence of activation of the coagulation cascade in asthma. Several studies have consistently found markers for coagulation in the airways of subjects with asthma, and also after experimental rhinovirus infection, used to model an asthma exacerbation [114–121]. Experimentally, lung fibrinolysis via the installation of tissue plasminogen activator (tPA) reversed the increase in BHR in allergen-challenged mice [122]. Further, allergen-induced BHR could be mimicked in separate experiments where mice were exposed to nebulised fibrin [122]. The significance of fibrin deposition in the airways is that it may induce airway closure, leading to an increase in peripheral resistance, a situation that may be confounded by the ability of fibrin to inactivate surfactant [122]. Fibrin deposition and factors of the coagulation cascade might also contribute to other remodelling phenomena [120, 123–125]. Thus, it is not yet known how the coagulation cascade affects lung function. It is tempting to reason that the extravascular presence of platelets within the airways might influence

these pathways. However, such events should not be confused with 'classical' platelet aggregation and haemostasis, given that a majority of studies suggest patients with asthma have a mild haemostatic defect, a reduced risk of myocardial infarction, or at best a weak association with pulmonary embolism [13, 14, 126, 127]. Interestingly, recently, it was reported that patients with severe asthma might have a heightened risk of pulmonary embolism, yet it is also difficult to dissociate this risk from high inhaled or oral corticosteroid use [128, 129]. Given the lack of disturbance to haemostasis within the time frame of acute exposure to allergen, despite the immediacy with which platelet activation occurs, it therefore seems unlikely that platelet activation by allergic or inflammatory triggers *per se* is involved in reported risk of pulmonary embolism.

Different activation pathways – implications for drug development

The significance of platelet activation has therefore been revealed both clinically and in experimental models of inflammatory diseases, where platelets have been

demonstrated to be critical in leucocyte recruitment, tissue damage and chronic inflammatory events. Importantly, despite evidence of platelet activation in inflammatory events in general, and atopy in particular, there is an evident incongruence that there is an associated mild haemostatic defect rather than increased thrombosis in patients with asthma [13–15]. Furthermore, established anti-platelet drugs that specifically target platelet activation (aggregation) during haemostasis and thrombosis have no reported effect on alleviating patients with asthma, despite their wide usage. On the other side, drugs such as disodium cromoglycate (DSCG) that have long been known to be clinically effective in the treatment of patients with allergic disease have been shown to inhibit some of the inflammatory actions of platelets both *in vitro* and *ex vivo* without any concomitant effect on platelet aggregation [130–133].

This dichotomy in platelet function suggests that distinct platelet activation pathways exist that control platelet aggregation, in contrast to platelet activation induced by inflammatory mediators that leads to increased motility, platelet-leucocyte conjugates and release of inflammatory mediators (Fig. 4). Whilst

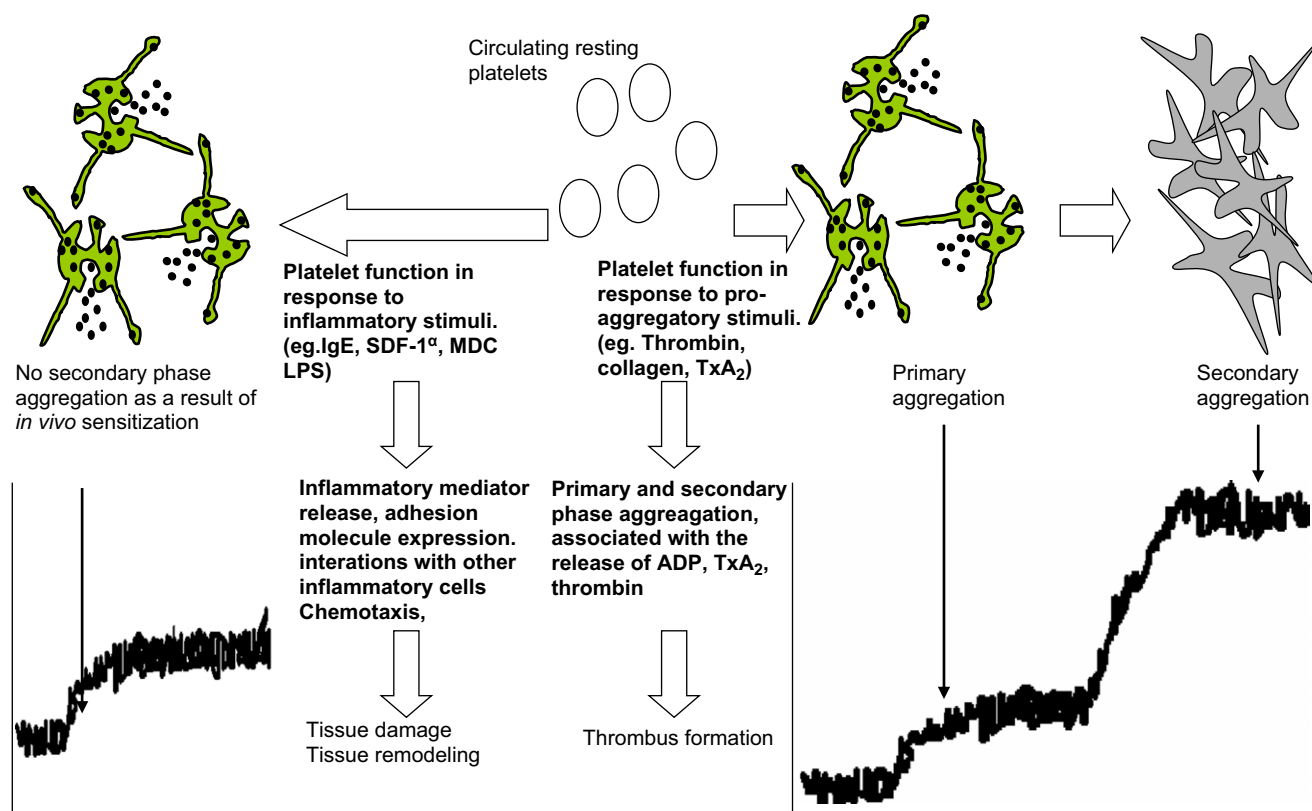


Fig. 4. Dichotomy of platelet function. Depending on the type of stimulus involved, platelets may become activated by prothrombotic mediators (arrows pointing right) or proinflammatory mediators (arrow pointing left). The type of stimulus in turn therefore dictates the ultimate function of the platelet. Intra-vascular platelet activation occurs during inflammation reactions whilst 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 [138]).

research into these inflammatory-dependent activation pathways is in their infancy, further elucidation of these pathways might provide avenues for the development of novel drugs to treat inflammation and asthma. For example, the inflammatory actions of platelets share a similarity to the function of leucocytes, the cell signalling pathways of which are better understood [134–136].

Summary

The increasingly complex web of cellular interactions that contributes to allergic inflammation must now

include the humble platelet and our improved understanding of this cell may well hold the key to finding improved treatments for this common condition. It is now clear that the ability of platelets to act as inflammatory cells involves distinct activation and signalling pathways than those involved in haemostasis and thrombosis that should provide exciting opportunities for identifying new drug targets for the development of novel anti-inflammatory drugs in the future.

Conflict of interest

The authors declare no conflict of interest.

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