Defining a role for platelets in allergic inflammation

S.C. Pitchford
Leukocyte Biology Section, National Heart and Lung Institute, Imperial College, London SW7 2AZ, U.K.

Abstract
There is now considerable evidence suggesting a role for platelets as inflammatory cells. These actions are distinct from their classically known actions performed during thrombosis and haemostasis, and include the expression of adhesion molecules and contact-dependent activation of leucocytes, the release of a plethora of inflammatory mediators, activation in cells of the adaptive immune response and the ability to migrate and undergo chemotaxis. Chronic asthma is a disease characterized by a mixed inflammatory cell pulmonary infiltrate, AHR (airways hyper-responsiveness) and tissue remodelling. Clinical data from patients suffering from asthma, allergic rhinitis and allergic dermatitis reveal changes in platelet behaviour and function during or after allergen exposure. Furthermore, mouse models of allergic inflammation demonstrate a role for intact platelets in eosinophil and lymphocyte recruitment to the lungs, a mechanism that is P-selectin (platelet selectin)-dependent. Models of chronic inflammation also reveal the participation of platelets in tissue remodelling events whereby platelet depletion was found to be more effective in suppressing airway remodelling processes than the administration of a glucocorticosteroid. This process of destruction and repair to the architecture of airway tissue is therefore perhaps enhanced by platelet activation. Recent evidence demonstrates that platelets can undergo chemotaxis and indicates an ability to migrate through inflamed tissue, where they localize to specific tissue sites. Indeed, platelets have been shown to become activated and recruited to various body compartments in direct response to allergen via IgE and this is suggestive of a link between the innate and adaptive immune responses. Thus these actions may lead to pathophysiological events that alter disease progression, since platelet depletion suppresses AHR in allergic rabbits. Further investigations into the role of platelets in inflammation may be beneficial in the search for future therapeutic targets in the treatment of asthma and allergy.

Introduction
Numerous studies have revealed an alteration in the character and function of platelets from patients with allergic inflammatory disease, e.g. asthma, allergic rhinitis and atopic dermatitis. These alterations have been dissociated from the well-characterized involvement of platelets in thrombosis and haemostasis. Mechanisms by which platelets participate in allergic inflammation are still being defined, but it appears that platelets act as innate inflammatory cells in immune responses, and may become activated upon allergen stimulus. Evidence suggests that platelet activation is instrumental in causing tissue damage during both acute and chronic inflammatory processes. This review will, first, discuss the evidence for platelet activation in atopic diseases and, secondly, introduce the various mechanisms by which platelets participate in allergy.

Evidence of platelet activation in allergic inflammation

Platelets in asthma and rhinitis
Inflammatory processes involving platelet activation in patients with asthma and rhinitis have been highlighted in recent experimental results from clinical studies and in vivo and in vitro experimentation. Platelet activation occurs during antigen-induced airway reactions in asthmatic subjects and this altered function is revealed as heightened platelet activation in vivo. Platelets from the same allergic patients are found to be refractory to a variety of stimuli ex vivo; in particular, noradrenalin and ADP are unable to induce full aggregation of platelets, with no second phase aggregation. This possibly results from platelet ‘exhaustion’, where platelets have been previously stimulated in vivo and are unable to replenish many released mediators that require de novo synthesis because platelets lack a nucleus [1,2]. Platelet exhaustion has been correlated with increased serum IgE in asthmatic patients [3,4] and, interestingly, full aggregation of platelets in vitro is restored in the same patients when studies are repeated outside of the allergy (pollen) season [3]. This alteration in platelet function has been associated with bronchial hyper-responsiveness

Key words: allergy, asthma, atopy, dermatitis, IgE, platelet
Abbreviations used: AHR, airways hyper-responsiveness; BALF, bronchoalveolar lavage fluid; CCL22, CC chemokine ligand 22; EGF, epidermal growth factor; FcεRI, high-affinity IgE receptor; 12-HETE, 12-hydroxyeicosatetraenoic acid; 5-HT, 5-hydroxytryptamine; IL, interleukin; MDC, macrophage-derived chemokine; NSAID, non-steroidal anti-inflammatory drug; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; PGE2, prostaglandin E2; RANTES, regulated upon activation, normal T-cell expressed and secreted; SDF-1, stromal cell-derived factor-1; TARC, thymus- and activation-related chemokine; TXA2, thromboxane A2;
Depending on the type of stimulus involved, platelets may become activated by prothrombotic mediators (arrows pointing right) or pro-inflammatory mediators (arrow pointing left). The type of stimulus in turn therefore dictates the ultimate function of the platelet. Intravascular platelet activation occurs during inflammation reactions, while 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 [63] © 2006 Elsevier.

that accompanies nocturnal asthma [5]. Platelet responses to allergen are thus different from platelet responses to normal aggregatory stimuli (Figure 1). This is typified by the fact that NSAIDs (non-steroidal anti-inflammatory drugs) block platelet aggregation; however, the formation of platelet–leucocyte complexes, which is a feature of platelet activation in inflammation, is not suppressed by NSAIDs and therefore represents a mechanism of platelet activation that is separate from the role of platelets in thrombosis [6].

Pulmonary platelets have been obtained at autopsy from patients who have died from status asthmaticus, an event accompanied with bone marrow karyopoiesis and thrombopoiesis [7]. Circulating platelet–leucocyte complexes have also been detected in patients with spontaneous asthma attacks [5], occurring in a biphasic manner, and also in atopic asthmatics following allergen challenge [8], resulting in an increase in the expression of activation markers on the surface of leucocytes [8] (see the section ‘Platelet involvement in inflammatory and immunological mechanisms’). In this regard, experimental models of asthma have provided evidence for a requirement of platelets in pulmonary eosinophil and lymphocyte recruitment into lung tissue in rabbits, guinea-pigs and mice, as well as a requirement for bronchial hyper-responsiveness to experimental allergen [8–11]. Furthermore, platelets have also been observed to undergo diapedesis in sections of lung from asthmatic patients, and lungs from allergen-sensitized and -challenged guinea-pigs, rabbits and mice [11–15], suggesting that platelets may also contribute to the pathogenesis of respiratory diseases via processes that are independent of leucocyte involvement (see the section ‘Platelet involvement in inflammatory and immunological mechanisms’).

Raised levels of platelet-derived mediators, such as the chemokines, β-thromboglobulin and PF-4 (platelet factor-4), are observed in plasma and BALF (bronchoalveolar lavage fluid) of atopic individuals compared with normal individuals during allergen exposure and are often used as markers of platelet activation in inflammation [5,16]. Other platelet-derived mediators have also been observed in atopic patients after allergen provocation, including RANTES (regulated upon activation, normal T-cell expressed and secreted), P-selectin, 5-HT (5-hydroxytryptamine), adenosine, histamine, PDGF (platelet-derived growth factor), PAF (platelet-activating factor), the de novo production of arachidonic acid metabolites, including PGE2 (prostaglandin E2) and TXA2 (thromboxane A2), platelet-specific lipoxygenase products, lysosomal enzymes and mediators sequestered from the circulation (e.g. IgE) (reviewed in [17]). Some of these mediators, when they have originated from platelets, have been proven to alter the pathogenesis of asthma [18,19].

**Platelets in allergic dermatitis**

Allergic dermatitis is a chronic inflammatory disease, where patients have increased levels of serum IgE and blood eosinophilia. While the involvement of platelets in allergic dermatitis
Platelet involvement in inflammatory and immunological mechanisms

The influence of platelets on leucocyte recruitment and activation

Circulating platelet–leucocyte complexes are a feature of atopy, a process that activates circulating leucocytes for efficient adhesion to inflamed endothelium and subsequent migration into tissue. Studies with un-separated leucocyte populations reveal a significant increase in platelet–leucocyte complexes in allergic mice and in human asthmatics [8,9]. Similar processes occur in a murine model of allergic dermatitis [22]. There is much evidence for a requirement of platelets in pulmonary eosinophil and lymphocyte recruitment in rabbits, guinea-pigs and mice in allergic inflammation [8–11], and effector T-cells in murine models of contact hypersensitivity [22,23]. Leucocyte recruitment in these models requires intact platelets expressing P-selectin on the cell surface [9,23], enhancing leucocyte attachment to the endothelium [22,24,25]. Circulating leucocytes attached to platelets display significant increases in CD11b (integrin αM) and VLA-4 (very late antigen-4) adhesion molecule expression in mice sensitized to allergen, compared with leucocytes not attached to platelets, and circulating platelet–leucocyte complexes in non-inflamed animals [9]. This activation of leucocytes at the level of contact-dependent signalling therefore induces the expression of integrins 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 and subsequent tissue recruitment in models of asthma and chronic contact hypersensitivity [9,23,26].

Platelet chemotaxis and migration through tissue

Recent evidence suggests that platelets are recruited to the lungs immediately following allergen exposure in an experimental animal model [27]. When allergen is administered intravenously, platelet accumulation is an event that precedes histamine release from mast cells [27], suggesting that allergen may directly activate platelets, and platelets were shown to remain in the lung for long periods [27], perhaps migrating through lung tissue. Indeed, platelets have also been observed to undergo diapedesis in sections of lung from asthmatic patients [12], and lungs (including BALF) from allergen-sensitized and -challenged mice, rabbits and guinea-pigs [11,13,15], suggesting that platelets are also contributing to the pathogenesis of respiratory diseases via processes that are independent of leucocyte involvement. In particular, platelets have been found localized to areas of bronchial smooth muscle, underneath the epithelium and areas of eosinophil infiltration [12]. The actions of allergen stimulus have been shown to be selective, since other platelet agonists such as high doses of ADP that are capable of inducing platelet aggregation in the pulmonary vasculature do not induce the diapedesis of platelets [11,28,29].

It is feasible that platelet recruitment to sites of inflammation is directed by a number of chemokines, since SDF-1 (stromal cell-derived factor-1), CXCL12 (CXC chemokine ligand 12), MDC (macrophage-derived chemokine), CCL22 (CC chemokine ligand 22) and TARC (CCL17) can activate platelets via their receptors CXCR4, CCR3 and CCR4, although no migratory response of platelets to these chemokines has been demonstrated in vivo [30–33]. SDF-1, MDC and TARC are present in tissue compartments or plasma of patients with asthma and dermatitis [21,34–36], their expression (MDC and TARC) being induced by the Th2 cytokines IL-4 (interleukin-4) and IL-13. MDC and TARC also have relevance in inducing AHR (airways hyper-responsiveness) and leucocyte recruitment experimentally [34–36]. Indeed, platelet-induced leucocyte recruitment to sites of allergic skin inflammation can be blocked by neutralization of RANTES or TARC [21].

Direct pathological effects of platelets on inflamed tissue

Studies have reported the effects of platelets on lung function in experimental models of asthma. The intravenous administration of platelet agonists induces bronchospasm and an accumulation of platelets in the lung [11,28,29]. Groups have evaluated the effect of platelet depletion in rabbits and guinea-pigs, which results in the abolition of the acute bronchoconstriction induced by inhaled allergen, while platelet depletion has been shown to protect against anaphylaxis in allergic guinea-pigs and rabbits [10,11]. Platelet-derived mediators probably contribute to the development of AHR since the inhibition of bronchoactive agents released by platelets abrogates the resulting AHR in such models [18,19]. Furthermore, the intradermal injection of supernatants from activated human platelets, but not leucocytes, induced delayed, sustained responses in the skin of patients with atopic dermatitis [37]. These direct effects on tissue, therefore, suggest that the platelet is very capable of inducing sustained inflammation.

Human platelets are capable of synthesizing and releasing histamine [38], and platelets may also induce the release of histamine from mast cells and basophils through IgE-dependent mechanisms, resulting in bronchoconstriction [39]. Other pleiotropic inflammatory mediators released by activated platelets, e.g. 5-HT, adenosine, PF-4 and
PDHRF (platelet-derived histamine-releasing factor), can cause bronchoconstriction (reviewed in [17]) in experimental models of allergic inflammation.

Other substances released by platelets in allergic reactions are those of arachidonic acid and phospholipid metabolism, produced de novo upon activation by a wide variety of inflammatory stimuli. For example, TXA₂, PGE₂ and 12-HETE (12-hydroxyeicosatetraenoic acid) have diverse inflammatory actions such as vasoconstriction (TXA₂), vasodilation (PGE₂), naïve T-cell priming (PGE₂), chemotaxis (12-HETE) and synergistic production of cysteinyl leukotrienes by leucocytes via 5-lipoxygenase (12-HETE) which are potent inflammatory mediators inducing bronchospasm, mucus hypersecretion, chemotactic activity and an increase in vascular permeability (reviewed in [17]).

PAF, another phospholipid mediator, is often generated along with arachidonic acid metabolites and is now known to be produced de novo by a number of inflammatory cells including platelets via the activation of phospholipase A₂. Many actions of PAF have since been uncovered that are of relevance to allergic inflammation [40], including induction of eosinophilia and platelet accumulation to the lungs, causing platelet extravasation in close proximity to areas rich in airway smooth muscle, and the induction of AHR.

Platelets and chronic inflammation

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’ [41]. Indeed, 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 [16], even though documented increases in platelet–leucocyte interactions within the circulation have returned to basal levels at 24 h post-allergen exposure [8], implicating platelets in chronic inflammatory events and airway remodelling [14,42]. Platelets may release a number of mitogens and enzymes that may contribute to airway remodelling directly, affecting bronchial smooth muscle growth, myofibroblast proliferation, subepithelial fibrosis, as well as altering the composition of the extracellular matrix after increased thrombopoeisis [42]. This response to injury is perhaps the release of chemotactic factors for circulating structural cells [43]. Platelets may indeed contribute to such a favourable microenvironment since platelets contain a number of cellular mitogens such as PDGF, EGF (epidermal growth factor), TGF-β (transforming growth factor-β) and VEGF (vascular endothelial growth factor) that have known proliferative actions on structural cells of the airways (reviewed in [17,44]). Interestingly, the major product of arachidonic acid metabolism in platelets, TXA₂, is known to induce the proliferation of smooth muscle cells and also endothelial cell migration and angiogenesis [45,46].

Platelets also contain a number of enzymes, e.g. matrix metalloproteinases, β-hexosaminidases and heparanases, which are released when platelets become activated [47,48]. Increased levels of these enzymes have been observed in the BALF following allergen challenge of asthmatic subjects and following ozone challenge in guinea-pigs [49], and these may alter the composition of the extracellular matrix. Disruption of the composition and integrity of cell membranes by degrading glycoproteins, glycolipids and glycosaminoglycans may also release membrane-bound growth factors for wound repair [50].

Platelet activation by IgE

Production of antigen-specific IgE in response to allergen provocation is a fundamental hallmark of atopic diseases [51,52]. The cross-linking of antigen to IgE on the surface of mast cells is believed to provide the stimulus for mast cell degranulation in early-phase allergic reactions, an event that precipitates a cascade of inflammatory events in response to allergen [53,54]. Patients allergic to Dermatophagoides pteronyssinus and exposed to synthetic peptides derived from the allergen Der p1 were shown to have activated platelets. This was a process mediated by IgE, which did not stimulate platelets from healthy subjects or non-Der p1-allergic patients, illustrating the specific activation of platelets to allergic stimuli [55].

Platelets contain both the FcεRI (high-affinity IgE receptor) and FcεRII (low-affinity IgE receptor) on the surface membrane [56,57]. The involvement of platelets in IgE-mediated responses in atopic disease may represent inappropriate and excessive response to allergen since platelet activation has been reported in IgE-mediated immunity against helminth and protozoan parasitic infections [58–60]. The activation of platelets via the FcεRI with monoclonal IgE resulted in platelet cytotoxicity to Schistosoma mansoni larvae [61], and the stimulation of platelets via FcεRI has been shown to induce the release of 5-HT and RANTES, demonstrating that platelets may play an important role in the progression of allergic inflammation via IgE-dependent mechanisms [56,62]. Platelets from allergic patients also produce free-radical oxygen species in response to IgE stimulation via specific allergens or antibodies [56]. Platelets recruitment and degranulation into the lungs following antigen challenge in sensitized mice occurs before histamine release from mast cells, and therefore platelets may participate in anaphylaxis directly in response to IgE [27]. IgE stimulation of platelets represents a non-thrombotic pathway by which platelets can be specifically activated by allergen and thus directly contribute to inflammatory responses observed in allergy.

Concluding remarks

A clear dichotomy exists in platelet function, with an inflammatory activity clearly distinguishable from the processes observed during thrombosis and haemostasis. It is now very clear that platelets possess a formidable array of machinery that allows them to play an active role in primary defence mechanisms, contributing to the evolution of innate immunity into acquired immune responses. Atopic diseases
such as asthma and allergic dermatitis appear to inappropriately activate mechanisms by which platelets subsequently contribute to tissue pathogenesis. There are thus novel possibilities by which the pathogenesis of allergic inflammation can be better understood and perhaps influenced to a greater and better degree than current therapies allow.

References