

EDITORIAL

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Platelet activation in asthma: integral to the inflammatory response

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Platelet activation has been documented in a wide variety of inflammatory diseases, and especially allergic asthma for a number of years, with changes in platelet behaviour and function being demonstrated. Clinical evidence has been supplemented with experimental *in vivo* and *in vitro* data that suggests platelet activation is not an artefact of inflammation but rather is both an integral and necessary component of the inflammatory response.

Primarily, the presence of intravascular platelet aggregates within bronchial biopsy specimens of asthmatic patients, along with the appearance of platelets in the extra-vascular compartment and on the surface of damaged epithelium demonstrate the ability of platelets to localize to the lungs during an asthma attack [1, 2]. Furthermore, early studies revealed the release of platelet-specific granular products are increased during symptomatic periods of asthma and after bronchial provocation. This indicates that intra-vascular platelet activation occurs, as measured by the release of platelet-specific chemokines from granular compartments, for example: platelet factor-4 (PF-4) and β -thromboglobulin (β -TG) [3].

Platelet activation can occur early (within 10 min) after the onset of the early asthmatic response (EAR) to allergen, as measured by β -TG and PF-4 levels [4], and platelet activation was inversely correlated to a fall in forced expiratory volume in 1 s, although it is interesting to note some previous studies conflict with this timing [5]. However, the realization that basal levels of these mediators are raised in some asthmatic study populations [5] and not others [4, 6, 7] suggests that platelets taken from patients who are experiencing a prolonged asthmatic period may become 'exhausted', containing fewer preformed mediators because of chronic *in vivo* stimulation and platelets

may therefore respond poorly from patients undergoing laboratory tests. In these cases platelet activation is probably better studied through the use of *in vitro* techniques where the phenomenon of platelet 'exhaustion' reveals platelets to be refractory to aggregatory stimuli. Nevertheless, despite these varying reports of the onset of platelet activation after allergen exposure, platelet activation has been reported to accompany nocturnal asthma, and this correlated to maximal increases in bronchial hyper-reactivity, clearly demonstrating platelet activation can result from a spontaneous asthma attack [8].

Intriguingly, evidence has also revealed platelet-derived mediators in lavage fluid at later time points after allergen challenge (19 h) [6]. No extension had been made to determine whether platelet activation coincided with an inflammatory response, although clinical evidence of platelet involvement in the late asthmatic response (LAR) has since been confirmed with the incidence of increased circulating platelet-leucocyte complexes in asthmatic patients 6 h after allergen challenge [9]. Thrombocytopenia also accompanies the LAR [10], with the assumption that platelets accumulated to the lungs. This phenomenon also persisted along with airway inflammation after airway obstruction returned to normal levels (24 h), although no analysis was made to specifically investigate platelet activation in such circumstances [10].

Although no studies have yet proven whether platelet activation in allergic asthmatics can be directly attributed to the inflammatory response to allergen, *in vitro* studies do certainly reveal that a direct pathway of platelet activation by relevant stimulators exists. Human platelets contain both the high- and low-affinity receptors for IgE (Fc ϵ RI and Fc ϵ RII/CD23, respectively) on their membrane [11–13] and it is interesting that a larger proportion of platelets from allergic asthmatics, as compared with healthy controls, express the high-affinity IgE receptor on their surface [14]. The activation of platelets taken from asthmatic patients via IgE results in the release of inflammatory mediators revealing that a non-thrombotic

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Table 1. Temporal analysis of possible events involving platelet activation in asthma

EAR	LAR	Chronic inflammation
Platelet recruitment to lungs and chemotaxis	Enhanced leucocyte recruitment by circulating platelets	Effects on airway remodelling, release of growth factors, cytokines
Delivery of co-stimulatory signals to APCs, e.g. airway dendritic cells	Immune response amplification via T cell stimulation	
Release of spasmogens		

APCs, antigen presenting cells; EAR, early asthmatic response; LAR, late asthmatic response.

pathway for platelet activation exists [11–13]. Moreover, platelets taken from patients allergic to *Dermatophagoides pteronyssinus* and exposed to synthetic peptides derived from the allergen were activated by a process mediated by IgE, which did not stimulate platelets from healthy subjects or non Der p1 allergic patients, illustrating that a pathway of specific platelet activation in response to a sensitizing allergic stimulus exists [15]. Together, these *in vitro* studies suggest a participation of platelets in allergic inflammatory responses separate to recognized events leading to thrombus formation.

As yet, no clinical studies have investigated the dynamics of platelet activation in response to allergen comparing the EAR and LAR in relation to inflammatory cell recruitment. Such studies might help to unravel various possible mechanisms by which platelets participate in allergic inflammation. In this current issue of *Clinical and Experimental Allergy*, Kowal et al. [16] have attempted to investigate the temporal characteristics of intravascular platelet activation in asthmatic patients in response to allergen exposure. Measuring the platelet-specific chemokines: β -TG, and PF-4; and also soluble P-selectin as granule release products of platelet activation, distinct patterns in platelet activation have been revealed. Comparing single responders (SR) to dual responders (DR; to allergen challenge) all three markers were significantly increased in the blood during the EAR in both SR and DR groups. Intriguingly, although levels of β -TG peaked during the EAR, sP-selectin and PF-4 levels peaked during the LAR, and were significantly greater in DR compared with SR. This event correlated with the occurrence of eosinophilia, raised IgE levels and a decrease in circulating platelets in SR and DR (peaking during the LAR in DR), and was inversely correlated to changes in lung function. This is the first study that addresses associations between platelet activation within the circulation and the development of allergic inflammation during the LAR in humans.

Experimental models of allergic inflammation have revealed a requirement for platelets in pulmonary leucocyte recruitment in guinea-pigs, rabbits, and mice [9, 17, 18], and this is dependent on platelet P-selectin expression [19]. Thus, peak sP-selectin expression, coinciding with a trough in circulating platelet and eosinophil numbers in DR in the LAR in the present study [16] reflects an ability of platelets to bind to eosinophils [20]. Platelets

form heterogeneous platelet–leucocyte complexes in the circulation after allergen challenge [9, 19] and may therefore facilitate eosinophil tethering to endothelium [21]. Thus, intravascular platelet activation is not an artefact of the inflammatory response to allergen, but represents a rheological event that vastly improves the efficiency of leucocyte rolling on endothelium.

Platelet activation also occurred in both SR and DR groups within minutes of the onset of EAR [16]. P-selectin expression after platelet activation is a rapid event, and P-selectin is shed from the surface of platelets within minutes of activation, yet peak β -TG levels in the present study preceded peak sP-selectin expression, suggesting platelet activation was achieved over several temporally distinct stages. Whilst platelet activation occurred within the circulation, the actions of platelets need not be confined to events taking place in the circulation. Platelets may be involved in other physiological processes that are independent of, or precede the interplay between platelet activation and inflammatory cell recruitment during the LAR (activated platelets were observed in SR, who have no LAR). Recent evidence suggests platelets are recruited to the lungs immediately following allergen exposure in an experimental animal model [22]. When allergen is administered intravenously, platelet accumulation is an event that precedes histamine release from mast cells [22]. If this is also true following allergen exposure in asthmatic individuals it possibly leads to other distinct events whereby platelets contribute to the inflammatory response. This has recently been highlighted in the ability of platelets to undergo chemotaxis [23] (which suggests they may migrate through tissue); their ability to deliver co-stimulatory signals to antigen presentation cells [24], and an ability to communicate with T cells [25]. Furthermore, other studies suggest platelet activation may even persist some time after the LAR has occurred [10], even though documented increases in platelet–leucocyte interactions within the circulation have returned to basal levels at 24 h post-allergen exposure [9], implicating platelets in chronic inflammatory events: for example the onset of airway remodelling [26, 27] (see Table 1).

Future studies are now required to elucidate the mechanisms by which intravascular activation of platelets occurs in response to allergen. It is not known whether platelets from allergic asthmatic subjects become 'primed'

by expressing a distinct population of receptors enabling them to readily react to stimuli. Extensive research may in future reveal these differences in the phenotype of platelets. Furthermore, it is necessary to determine other possible actions by which platelets contribute to both the early and late phase response, and also the effects platelets may have on chronic inflammation. It is therefore necessary to not confine future studies to the intra-vascular activation of platelets, but also platelets that have localized to the lungs.

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