Platelets and Airway Diseases

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Abstract

The role of platelets as inflammatory cells is now well established. Given the peculiar characteristics of the lung circulation, with a broad capillary bed, platelets are especially involved with the physiology of the lungs and play a key role in a number of inflammatory lung disorders. The platelet precursors, megakaryocytes, are detected in the lung microcirculation; moreover platelets with their endothelium-protective and vascular reparative activities contribute to the lung capillary blood barrier integrity. Given the function of the lungs as first wall against pathogen invasion, platelets participate in immune defence of the normal lung. On the other hand, platelets may turn into effectors of the inflammatory reaction of the lungs to allergens, to infectious agents, to chemical agents and may contribute strongly to the perpetuation of chronic inflammatory reactions, largely by their ability to interact with other inflammatory cells and the endothelium. In this chapter we provide an overview of the role of platelets in several inflammatory lung disorders discussing the pathophysiologic bases of platelet involvement in these conditions and the experimental and clinical evidence for a role of platelets in lung diseases.

Platelets have a well-established role in haemostasis and thrombosis, but there is now considerable evidence that they also play an important role in a number of inflammatory diseases (see Ware and Post 2017), including respiratory diseases such as asthma (Pitchford and Page, Clin Exp Allergy 36: 399–401, 2006) and chronic obstructive pulmonary disease (COPD) (Ferroni et al. J Investig Med 48:21–27, 2000). There is a relevant amount of information on the involvement of platelets in allergic inflammatory diseases, and platelet abnormalities in patients with allergy have been reported for more than 40 years following the seminal observation by Benveniste et al. in 1972 that leukocyte-dependent histamine release from platelets involved IgE-mediated activation (Benveniste et al. J Exp Med. 136:1356–77, 1972). This work led to the discovery of the lipid mediator platelet-activating factor (PAF) capable of inducing eosinophilia (Arnoux et al. Am Rev Resp Dis 137: 855–860, 1988). It is becoming increasingly apparent that platelets are central to inflammatory diseases of the airways, being a source of inflammatory mediators (see Table 1) and spasmogens per se and being critical for the recruitment of leukocytes into tissues.

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Platelets in Asthma

Pathophysiologic Observations: Altered Platelet Functionality

> – Platelets accumulate into the lungs upon allergen exposure.
> – Platelet activation by allergen via an IgE-mediated process reveals an important causal involvement of platelets in the inflammatory response.
> – Studies in animals reveal platelets are necessary for pulmonary leukocyte recruitment, chronic inflammatory events leading to airway remodelling, and the release of spasmogens to cause bronchoconstriction. Emerging reports suggest platelets are involved in initial allergen processing with antigen-presenting cells.
> – Many of these processes can be attributable to a direct effect of platelets, in their capacity to migrate through lung tissue and be present in the localised environment.

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, and it was suggested that they behaved like 'exhausted' platelets (Gallagher et al. 1978). Since this early 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 (Audera et al. 1988; Block et al. 1990). 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 the template bleeding time (Szczeklik et al. 1986). This 'exhausted platelet' syndrome may be the consequence of continuous platelet activation in vivo and is compatible with the observation that allergic patients undergoing allergen provocation have a mild thrombocytopenia (Kowal et al. 2006; Sullivan et al. 2000; Maestrelli et al. 1990) with increased numbers of platelet–leukocyte aggregates in their peripheral blood. Such observations clearly support the conclusion that platelet activation accompanies allergic exposure in such patients (Gresele et al. 1982, 1993; Pitchford et al. 2003; Sladek et al. 1990). This may explain recent observations suggesting that patients with asthma often have co-morbidities, including cardiovascular disease (Cazzola et al. 2013) and an increased risk of pulmonary embolism (Majoor et al. 2013), particularly patients with more severe asthma possibly associated with use of glucocorticosteroids (Varas-

Lorenzo et al. 2007). This hypothesis is further supported by a growing number of clinical observations showing increased markers of platelet activation in allergic asthmatics. These include RANTES (CCLS), platelet factor 4 (PF4), CXCL4 and β-thromboglobulin (β-TG, CXCL7) in the peripheral blood of patients with asthma (Maccia et al. 1977; Kowal et al. 2006; Yasuba et al. 1991; Knauer et al. 1981), as well the demonstration of increases in some of these platelet markers in bronchoalveolar lavage (BAL) fluid obtained from such patients (Jeffery et al. 1989; Metzger et al. 1987) (Table 1). Furthermore, there are other observations demonstrating extravascular platelets in the airways of both experimental animals (Pitchford et al. 2008) and in patients with asthma (Jeffery et al. 1989; Metzger et al. 1987) (see Petito et al. 2017).

In the context of respiratory diseases, it is of interest that platelets have long been recognised as a rich source of spasmogens (Arnoux et al. 1988; Yoshimi et al. 2001) that can lead to both contraction of airway smooth muscle in vitro (Inoue and Kannan 1989) and bronchoconstriction in vivo (Robertson and Page 1987; Yoshimi et al. 2001). Indeed, we have recently reported that a range of inflammatory mediators, exemplified by bradykinin, elicit airway obstruction in vivo that is platelet dependent, building on earlier observations of platelet involvement in airway obstruction (Keir et al. 2015; Coyle et al. 1993). However, perhaps of greater interest for respiratory diseases are the increasing numbers of observations demonstrating a crucial role for platelets in the recruitment of leukocytes into various tissues, including the lung (Pitchford et al. 2003, 2005; Kornerup et al. 2010). Platelet-derived mediators are known to be able to induce leukocyte activation and recruitment (Page and Pitchford 2013), as well as the release of substances that contribute to the 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 asthma patients express both the high- and the low-affinity IgE receptors on their surface and that exposure to sensitising antigens can lead to the generation of inflammatory mediators, such as oxygen free radical species, 5-HT and RANTES (Joseph et al. 1983, 1997; Hasegawa et al. 2001; Klouche et al. 1997). Interestingly, activation of the high-affinity IgE receptor can cause platelets to undergo chemotaxis (Pitchford et al. 2008), confirming other observations that suggest that platelets behave like primitive leukocytes and demonstrate directional movement in response to certain stimuli (Czapiga et al. 2005; Kraemer et al. 2010), stimuli that tend to be distinct from those that elicit platelet aggregation (Abi-Younes et al. 2001; Kowalska et al. 2000; Clemetson et al. 2000).

Platelets have long been recognised to contain high concentrations of 5-hydroxytryptamine (5-HT or serotonin) in their dense granules, and several clinical observations

Table 1 Principal platelet-derived inflammatory mediators and their involvement in airway diseases

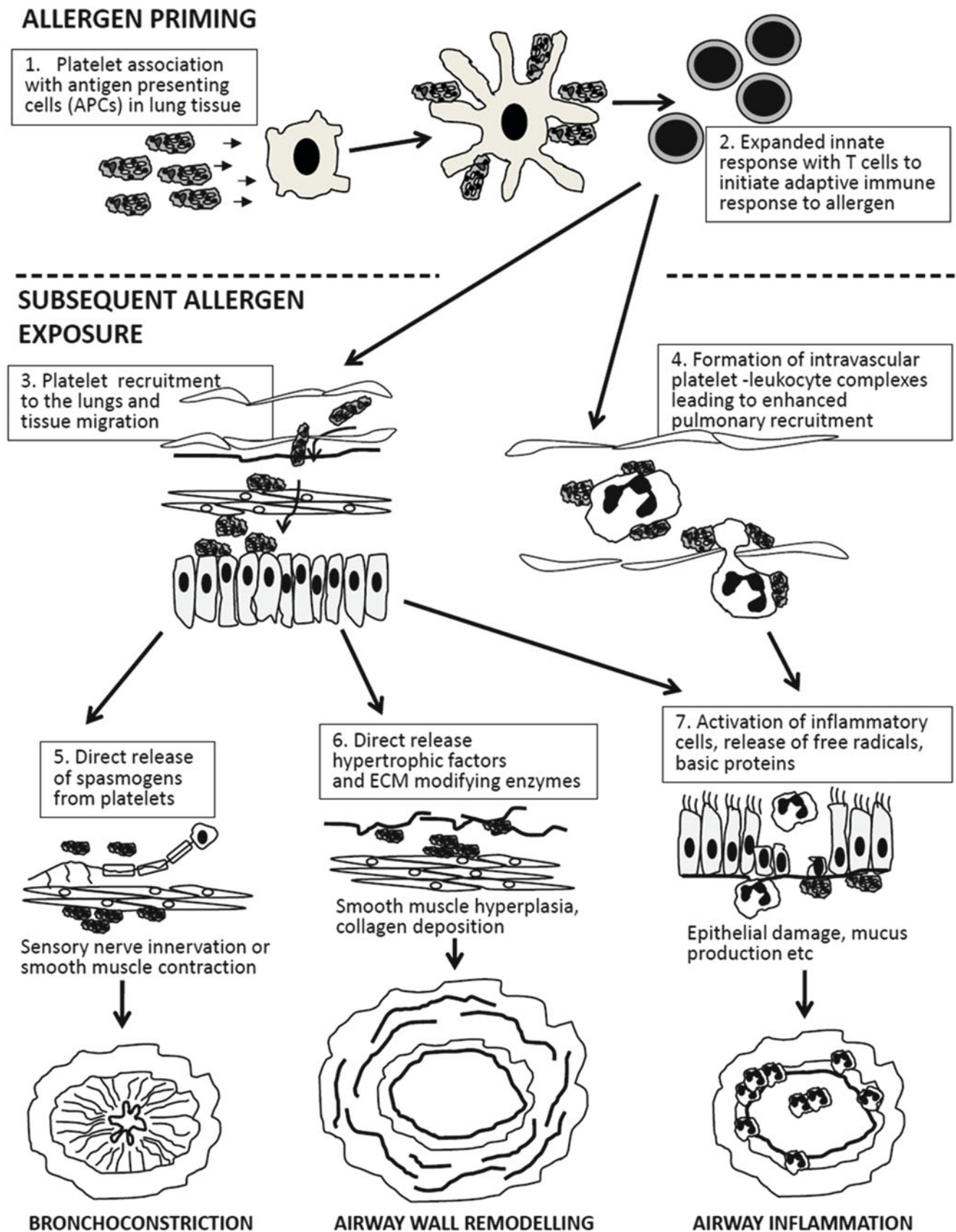| Mediator	Action
$\alpha v\beta 3$	NF-κB activation and induction of inflammatory cell responses (cell adhesion and transmigration into the lung)
CD40L	TNF superfamily: antigen-presenting cell activation, B-cell responses, endothelial cell activation
P-selectin	Selectin: leukocyte adhesion, complement activation
IL-1β	Cytokine: acute phase response, leukocyte and endothelial activation
TXA2	Eicosanoid: T-cell differentiation, monocyte activation, smooth muscle proliferation
Nitric Oxide (NO)	Reactive oxygen species: anti-inflammatory and antithrombotic
ADP	Platelet, leukocyte, endothelial cell activation
TNFα	Modulate air wall remodelling
LTB4	Modulate leukocyte recruitment
CXCL-7/β-TG	Modulate leukocyte recruitment, bronchospasm
CCL5/RANTES	Monocyte adhesion on the endothelium and recruitment
CXCL4/PF-4	Chemokine: monocyte, neutrophil and T-cell recruitment, Th differentiation, bronchospasm
TGFβ	Cytokine: cell proliferation, T-cell differentiation, B-cell and macrophage phenotype regulation
PDGF	Growth factor: cell growth and differentiation, monocyte/macrophage differentiation
VWF	Platelet adhesion, PMN extravasation
SDF-1	Chemokine: T cell, monocyte and PMN chemotaxis
VEGF	Growth factor: angiogenesis, adhesion molecule expression
5-HT	Dendritic cell activation, bronchospasm

have shown altered levels of this vasoactive amine in patients with asthma (Maccia et al. 1977; Malmgren et al. 1982; Ring et al. 1980). Furthermore, some studies have shown a beneficial effect of the 5-HT antagonist ketanserin in asthma (Cazzola et al. 1990, 1992). Tryptophan hydroxylase (THP)-1 is a critical enzyme for the biosynthesis of 5-HT outside of the central nervous system (CNS), and it is of interest that Durk et al. have utilised mice genetically deficient in THP-1, as well as mast cell-deficient mice, to demonstrate that platelets, rather than mast cells, are the main source of 5-HT released during an allergic inflammatory response (Dürk et al. 2013). Unlike mice, human mast cells are not a major source of 5-HT and so it is of even more interest that Idzko and colleagues have found elevated 5-HT levels in BAL fluid following segmental bronchial allergen challenge in allergic asthmatics, as their results suggest that the 5-HT found in the lung likely comes from activated platelets (Dürk et al. 2013). These results provide further support to other clinical observations reporting platelet activation in patients with allergic asthma (Maccia et al. 1977; Kowal et al. 2006; Pitchford et al. 2003; Johansson et al. 2012). It is of particular interest that platelets have now been found extravascularly in a number of inflammatory situations (Feng et al. 1998), including in the lungs of experimental animals (Pitchford et al. 2008) and patients with asthma (Jeffery et al. 1989). It becomes intriguing to know whether the elevated 5-HT found in BAL of allergic asthmatics is derived from circulating platelets and/or from platelets that have undergone diapedesis into lung tissue. Either way the results presented by Durk 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 leukocyte infiltration into the lung and inhibition of the bronchial hyperresponsiveness (BHR) that normally accompanies allergen challenge, suggesting that the platelet-derived 5-HT 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 drugs either affecting 5-HT uptake (Lechin et al. 1998) or antagonising 5-HT2 receptors (Lima et al. 2007), it would seem timely to consider large trials of such agents in patients with allergic airways disease.

Studies in Experimental Animal Models: Leukocyte Recruitment

There is now a growing body of literature that suggests that platelets play a central role in both allergic (Pitchford et al. 2003, 2005) and non-allergic (Kornerup et al. 2010) leukocyte recruitment into the lung, as well as a critical role in other manifestations of allergic asthma such as airway remodelling (Pitchford et al. 2004). The mechanisms by which platelets influence these processes are discussed below and open up the possibility of identifying a number of potential new targets for the treatment of inflammatory diseases such as asthma and COPD.

Investigation of the mechanics of blood flow (haemorheology) has uncovered interesting phenomena that have fundamental implications related to how platelets are required to initiate the 'adhesion cascade' that results in leukocyte recruitment into tissues associated with inflammatory airway diseases. In laminar flow in blood vessels, a shearing motion occurs due to the influence of wall friction that results in a

ALLERGEN PRIMING

1. Platelet association with antigen presenting cells (APCs) in lung tissue

2. Expanded innate response with T cells to initiate adaptive immune response to allergen

SUBSEQUENT ALLERGEN EXPOSURE

3. Platelet recruitment to the lungs and tissue migration

4. Formation of intravascular platelet-leukocyte complexes leading to enhanced pulmonary recruitment

5. Direct release of spasmogens from platelets

Sensory nerve innervation or smooth muscle contraction

BRONCHOCONSTRICTION

6. Direct release hypertrophic factors and ECM modifying enzymes

Smooth muscle hyperplasia, collagen deposition

AIRWAY WALL REMODELLING

7. Activation of inflammatory cells, release of free radicals, basic proteins

Epithelial damage, mucus production etc

AIRWAY INFLAMMATION

Fig. 1 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. This picture was originally published in Idzko, Pitchford, and Page J Allergy Clin Immunol (2015) 135: 1416-1423

parabolic velocity profile of the fluid (Hagan–Poiseuille Law); because blood is a non-Newtonian fluid, the viscosity of blood decreases with increasing shear rate and thus red blood cells actually aggregate to form reversible rouleaux under conditions of low shear and move inwardly (due to their relative deformability compared to platelets and leukocytes) into the inner part of the vessel (Goldsmith and Spain 1984a, b; Goldsmith et al. 1981, 1999). This phenomenon is known as the Fahraeus effect, the consequence of which is a redistribution of the blood elements, with the density of platelets and leukocytes increasing around the vessel periphery. The region of the vessel where the velocity gradient is highest is around the vessel wall; thus cells travelling at different velocities along adjacent streamlines have increased probability of cellular collisions. This peripheral zone therefore 'traps' leukocytes into an environment rich in platelets, greatly enhancing collisions between platelets and leukocytes (Goldsmith and Spain 1984a, b; Goldsmith et al. 1981, 1999) and leading to the tethering of platelets to leukocytes to form rosettes through P-selectin recognition steps, to the subsequent upregulation of integrin expression and to the firm adhesion to endothelium as platelet-bound leukocytes enter the capillary network. High-resolution video microscopy has revealed the existence of membrane tethers involving P-selectin/PSGL-1 bonds that regulate leukocyte rolling on platelets and P-selectin with changes in tether length and lifetime dependent on increasing shear force (Schmidtke and Diamond 2000). The biomechanics of the complex formation of activated platelets with leukocytes reveals that the high tensile strength of P-selectin/PSGL-1 interactions enables P-selectin-dependent tethering at high shear rates, whereas integrin activation may mediate platelet–leukocyte complex formation at low shear rates. Nevertheless, unstimulated leukocytes (which constitutively express PSGL-1/L-selectin) may also bind to activated platelets in an integrin-independent manner, suggesting that cell adhesion that is not purely selectin dependent may create platelet–leukocyte complexes (Xiao et al. 2006).

These events occur in the circulation of patients with asthma upon allergen challenge. Thus, circulating platelet–leukocyte complexes are found in allergic asthmatic patients after both spontaneous asthma attacks and after allergen challenge, in a biphasic manner (Gresele et al. 1993; Pitchford et al. 2003). The possible significance of these platelet–leukocyte complexes is to act as a 'priming' step for further leukocyte adhesion, since leukocytes attached to platelets display enhanced expression of the β1 integrin MAC-1 (Pitchford et al. 2003, 2005; Johansson et al. 2012). It is therefore of considerable interest that recent research has correlated platelet activation with eosinophil inflammation in patients with asthma (Benton et al. 2010). Furthermore, in patients with asthma, β1 integrin expression on eosinophils correlated with eosinophil-bound platelets expressing P-selectin and antigen challenge leads to the

disappearance from the circulation of eosinophils bearing platelet P-selectin, presumably because these complexes are sequestered in the lungs (Johansson et al. 2012). The mechanistic significance of these platelet/eosinophil interactions is that this leads to increased eosinophil adhesion to the vascular endothelium via a platelet-P-selectin-dependent mechanism (Jawień et al. 2002; Ulfman et al. 2003).

The importance of platelets in pulmonary leukocyte recruitment after allergen challenge has now been extensively proven using in vivo models of platelet depletion. In fact platelet depletion, either via immunological or non-immunological methods, strongly reduces pulmonary eosinophil and lymphocyte recruitment in rabbits, guinea pigs and mice (Pitchford et al. 2003, 2005; Coyle et al. 1990; Lellouch-Tubiana et al. 1988) and the recruitment of effector T cells in murine models of contact hypersensitivity (Ludwig et al. 2004; Tamagawa-Mineoka et al. 2007). This process required intact platelets, since the reinfusion of lysed platelet products was insufficient to restore leukocyte recruitment, whereas the reinfusion of intact activated platelets expressing selectins on the cell surface restored leukocyte recruitment (Pitchford et al. 2005; Tamagawa-Mineoka et al. 2007). With similarities to allergic asthmatic patients undergoing allergen challenge, mice sensitised to allergen have circulating leukocytes attached to platelets displaying significantly increased CD11b (integrin αM) and VLA-4 (very late antigen-4) in comparison with leukocytes not attached to platelets (Pitchford et al. 2003, 2005). Contact with platelets therefore induces activation of leukocytes, thus enhancing the expression of integrins, presumably for firm adhesion. This has been confirmed by the experimental use of antibodies blocking P-selectin which results in the suppression of platelet–leukocyte complexes, integrin expression and subsequent tissue recruitment in models of asthma and chronic contact hypersensitivity (Pitchford et al. 2005; Ludwig et al. 2004; Tamagawa-Mineoka et al. 2007; Symon et al. 1999; Mayadas et al. 1993; Lukacs et al. 2002; De Sanctis et al. 1997; Broide et al. 1998). Indeed, the targeting of P-selectin or PSGL-1 as a novel therapeutic option is currently progressing in phase II clinical trials in patients with asthma (Bedard and Kaila 2010).

Nevertheless, many other studies also reveal that other mediators released, or expressed, by platelets can modulate leukocyte recruitment. Examples are the platelet-specific chemokines PF-4 (CXCL4), β-TG (CXCL-7) and RANTES (CCL5) and pleiotropic mediators such as leukotrienes, 5-HT and sphingolipids (Page and Pitchford 2013; Brandt et al. 2000; Duerschmied et al. 2013; Florey and Haskard 2009). Thus, whilst there is a requirement for platelets expressing adhesion molecules on their surface for leukocyte recruitment, this must also be sequential to the release or expression of other platelet-derived factors. However, it is not yet understood from a physiological perspective why

the process of leukocyte activation and adhesion to post-capillary venules is so inefficient and actually requires platelet activation. Perhaps the rapidity of platelet activation to danger signals combined with a higher surface area/volume ratio, allowing a critical density of adhesion molecules to be expressed compared to larger leukocytes, has resulted in an evolutionary requirement for platelets in the rheological processes that trigger the leukocyte adhesion cascade.

Pathophysiologic Observations: The Extravascular Presence of Platelets

Current perceptions concerning platelet function in inflammation are heavily influenced by the knowledge of the intravascular 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 that it is indirect and totally dependent on the ability of platelets to influence leukocyte recruitment. However, accumulating evidence details a novel function of platelets in being able to respond to chemotactic signals and migrate extravascularly through inflamed tissue (see Petito et al. 2017) (Fig. 2).

Platelets have been observed to undergo diapedesis in sections of the lungs obtained from patients with asthma and the lungs (and BAL fluid) from allergen-sensitised and allergen-exposed mice, rabbits and guinea pigs (Jeffery et al. 1989; Metzger et al. 1987; Pitchford et al. 2008; Lellouch-Tubiana et al. 1988; Beasley et al. 1989). 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 allergen-exposed mice was an IgE-mediated process and that platelets from such mice could also undergo chemotaxis to the sensitising allergen in vitro (Pitchford et al. 2008). Interestingly, the migratory response of platelets in the lungs in vivo commenced before significant leukocyte recruitment, whilst at later time points, when leukocytes had also entered lung tissue, around 50 % of platelets were still not complexed to leukocytes (Pitchford et al. 2008). The rapidity of this response highlights that platelet activation by allergen is direct and independent of activation of other cells, like mast cells (Yoshida et al. 2002). It is interesting to note that evidence is accumulating of platelet migration into tissues in other inflammatory diseases, such as into the synovial fluid in patients with rheumatoid arthritis, and transmigration across the vascular wall after long periods of ischaemia (see Ware and Post 2017) (Boilard et al. 2010; Kraemer et al. 2010). The mechanisms controlling this platelet function have not been elucidated, but platelets express a number of different chemokine receptors (CCR1, CCR3, CCR4 and CXCR4) which are functional,
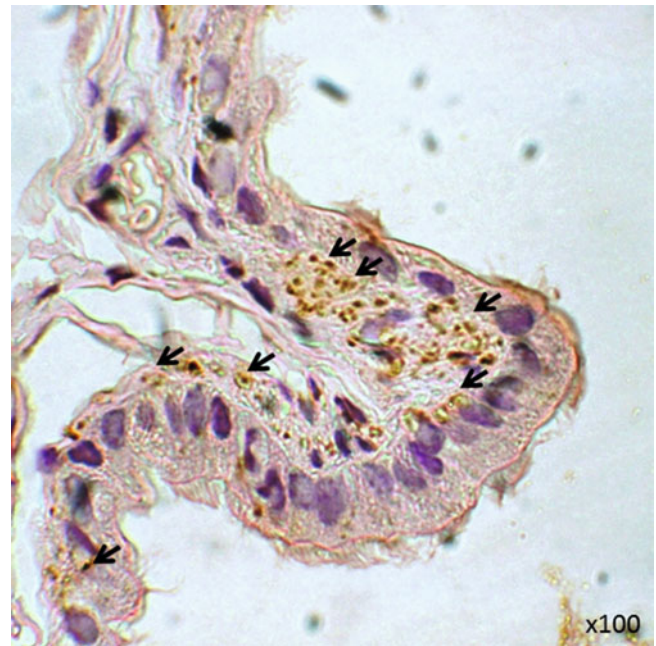
Fig. 2 Histological analysis of the lungs taken from ovalbumin (OVA)-sensitised mice after allergen inhalation. Section was stained for the platelet-specific antigen CD41 (integrin αIIb). Platelets (*brown*) can be observed throughout the airway wall. Image originally published in Page & Pitchford (2014) Clin Exp Allergy 44: 901–913

since the ligands SDF-1 α, MDC and TARC can activate platelets (Abi-Younes et al. 2001; Kowalska et al. 2000; Clemetson et al. 2000). Platelets have also been shown to undergo chemotaxis to f-MLP and SDF-1α and can therefore be considered as motile cells (Czapiga et al. 2005; Kraemer et al. 2010). Therefore, platelets can migrate through lung tissue and localise to specific resident cells/structures in a highly regulated process, as it is for leukocytes. Whilst the significance of platelet migration into lung tissue has not yet been fully characterised, platelets may directly influence the development of sensitisation towards allergen, BHR, bronchospasm, tissue damage and chronic inflammation leading to airway wall remodelling.

Studies in Experimental Animal Models: Involvement with Antigen-Presenting Cells and Contact with Allergen

Platelets express both the high-affinity (FcεRI) and low-affinity (CD23) receptors for IgE, as well as functional receptors for other immunoglobulins. Platelets can be activated by specific allergen via IgE-dependent processes, as revealed when platelets are taken from patients allergic to *Dermatophagoides pteronyssinus* (Der p1) and exposed to synthetic peptides derived from the allergen Der p1 ex vivo (Cardot et al. 1992). We have shown that platelets from

patients with asthma will undergo chemotaxis specifically towards the known allergen (rather than allergens to which individual patients are not allergic too) (Pitchford et al. 2008). The implications of direct platelet activation and motility by allergen are not yet known, but it is interesting to note that platelets activate dendritic cells (DCs) in the airways (Dürk et al. 2013). Both CD40 and CD40L have been identified on activated platelets and might be responsible for this cellular interaction (Semple et al. 2011; Henn et al. 1998). Platelet CD40L has been described in other situations as an important link between the innate and adaptive immune response to induce DC maturation, for example, the expression of CD80 and CD83, and immunoglobulin class switching (Elzey et al. 2003; Czapiga et al. 2004; Sprague et al. 2008). Platelet CD40L has recently been reported to be involved in the promotion of allergic airway inflammation by polarising Th2 responses after allergen exposure (Tian et al. 2015). Despite this evidence, it remains to be understood whether platelets are involved in the initial allergen sensitisation process per se.

Studies in Experimental Animal Models: Lung Function

The observation that platelets migrate into the lung tissue of patients with asthma, and into the lungs of allergen-challenged sensitised animals, 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 (Coyle et al. 1990; Lellouch-Tubiana et al. 1988). There is now some understanding of the pathways and platelet mediators involved in these processes from the observations on the effects of intravenous platelet agonists on bronchospasm and platelet accumulation in the lung (Arnoux et al. 1988; Yoshimi et al. 2001; Lellouch-Tubiana et al. 1988; Robertson and Page 1987). We have recently observed that platelet depletion inhibits bronchospasm induced by 'indirect spasmogens', such as capsaicin and bradykinin, whilst it does not inhibit direct-acting spasmogens, such as histamine and methacholine (Keir et al. 2015), suggesting 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 changes in airway obstruction, confirming that platelet-derived mediators might also contribute to airway tone (Arnoux et al. 1988; Yoshimi et al. 2001). Indeed, a direct participation of platelets in allergy, independent of leukocyte responses, was highlighted by the intradermal injection of supernatants from activated human platelets (but not leukocytes) inducing delayed, sustained inflammatory responses in the skin of patients with atopic dermatitis (Matsuda et al. 1997). These effects on tissue suggest that platelets are very capable of directly inducing sustained inflammation. Human platelets synthesise and release a number of bronchoactive mediators, for example, histamine, 5-HT, TXA2, adenosine and 12-hydroxyeicosatetraenoic acid (12-HETE), contain cytotoxic compounds within their granules and generate substances capable of inducing tissue damage, such as reactive oxygen species (ROS), cationic proteins (PCPs), platelet basic proteins (PBPs) and matrix metalloproteinases (Saxena et al. 1989; Knauer et al. 1984; Busti et al. 2010). Yet it is not understood how these mediators interact, and with what (e.g. sensory nerves, airway smooth muscle, epithelium), to elicit bronchospasm and BHR.

Studies in Experimental Animal Models: Chronic Inflammation and Lung Remodelling

One consequence of persistent, chronic inflammation is the alteration of tissue structure and function. In bronchial asthma, chronic inflammation contributes to changes in airway architecture referred to as 'airway remodelling'. The observation that platelets migrate into lung tissue of patients with asthma and in experimental animals (Jeffery et al. 1989; Metzger et al. 1987; Pitchford et al. 2008; Lellouch-Tubiana et al. 1988; Beasley et al. 1989) suggests that platelets may contribute directly to changes in the airway architecture by releasing factors that control the synthetic phenotype of the airway epithelium and of fibroblasts and airway smooth muscle cells. Indeed, in murine models of chronic allergic inflammation, the depletion of platelets led to a comprehensive suppression of lung remodelling (smooth muscle hyperplasia, subepithelial fibrosis, collagen deposition, epithelial hyperplasia), an effect that chronic treatment with glucocorticosteroids did not attain, suggesting that platelet involvement in tissue remodelling may in some instances be independent of leukocyte-associated inflammation (Pitchford et al. 2004). In patients with asthma, platelet activation was shown to persist for some time after the late asthmatic response had occurred (Kowal et al. 2006), even though the increases in circulating platelet–leukocyte aggregates returned to basal levels 24 h after allergen exposure (Pitchford et al. 2003), thus implicating platelets in chronic inflammatory events and airway remodelling.

Platelets are a rich reservoir of mitogens and enzymes and therefore may contribute to generate an environment that induces synthetic responses in structural airway cells. Platelet mitogens include PDGF, EGF (epidermal growth factor), TGF-β (transforming growth factor-β), VEGF (vascular endothelial growth factor) and the major product of arachidonic acid metabolism in platelets, TXA2, which all have proliferative activity on structural cells of the airways (Pitchford and Page 2002; Rendu and Bohard-Bohn 2002).

Platelet-derived enzymes include matrix metallopro-teinases, β-hexosaminidases and heparanases that are released following allergen challenge in asthmatic patients and following ozone challenge in guinea pigs and may alter the composition of the extracellular matrix (Falcinelli et al. 2005; Kelly et al. 2000). 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 (McKenzie 2007). Recent evidence shows that plate-let membranes are required to induce synthetic responses in airway smooth muscle cells (Svensson Holm et al. 2011), and this function was controlled by platelet 5-lipoxygenase, 12-lipoxygenase and the production of reactive oxygen spe-cies (Svensson Holm et al. 2008, 2014). These effects may be supplemented by the ability of platelets to influence the survival, recruitment, proliferation and differentiation of circulating stem cells involved in (inappropriate) tissue regeneration (Stellos et al. 2010). It can be surmised there-fore that platelets may influence lung regeneration as well as inappropriate remodelling of the airways after injury, although the interplay between platelets and different struc-tural cells is likely to be extremely complex and involve a plethora of mediators.

Platelets in Infectious Pulmonary Disease

– Platelets have a broad repertoire of receptor molecules that enable them to sense invading pathogens and infection-induced inflammation.
– Consequently, platelets exert direct antimicrobial mechanisms and initiate an intense crosstalk with other cells of the innate and adaptive immunity, including neutrophils, monocyte/macrophages, dendritic cells and B and T lymphocytes, favouring antimicrobial activity.

In mammalians, platelets have retained key structures and functions of immune effector cells which are integral to antimicrobial host defence (Morrell et al. 2014). In the bloodstream they act as quiescent 'sentinels' of tissue injury and microbial threat. Platelets exert activities that span from acute inflammation to adaptive immune responses that con-fer to them an important role as immune regulators during sepsis (Semple et al. 2011). Platelets store antimicrobial peptides, such as β-defensins, thrombocidins, PF-4 (CXCL4), RANTES (CCL5), connective tissue-activating peptide-3, platelet basic protein, thymosin β-4, fibrinopep-tide B and fibrinopeptide A, and contribute to the innate immune system (Tang et al. 2002; Kraemer et al. 2011). Inflammatory lung diseases and other pulmonary disorders cause accumulation of platelets in the lungs, and tissue injury and systemic conditions such as sepsis can activate platelets in the circulation and sequester them in the pulmo-nary vascular bed (Fig. 3).

Pathophysiologic Observations

Platelets participate in inflammatory and immune responses using a variety of molecular mechanisms, including the release of chemokines (β-TG, PF4, ENA-78, Gro-α, RANTES, SDF1α and others), cytokines (HMBG1) and growth factors (PDGF, VEGF, EGF, FGF, TGFbeta, and so on), and the synthesis of lipid mediators, like thrombox-ane A2 and PAF, but contribute also to the maintenance of the endothelial barrier. Key differences exist between the characteristics of endothelium in the pulmonary circulation and the systemic vasculature. Notably, ICAM-1 and integrin $\alpha_v\beta_3$ are highly expressed by the pulmonary circulation as compared to other vascular beads (Panes et al. 1995; Singh et al. 2000). It is thus possible that platelet retention in the lungs is mediated by the constitutive expression of these two adhesion molecules by pulmonary endothelial cells. Activa-tion of platelets, which is a requirement for their firm adhe-sion to endothelial cells, results in a conformational change
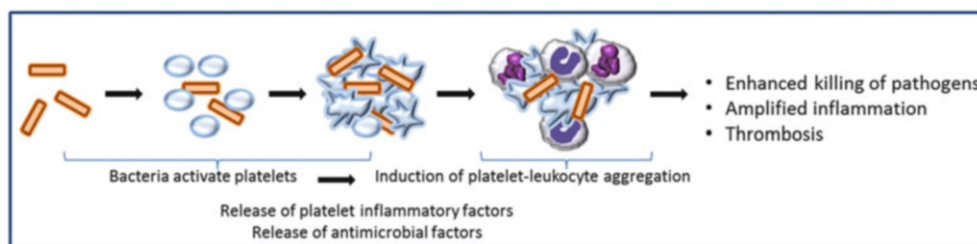
Fig. 3 Direct interaction of bacteria with platelets can lead to aggre-gation, release of antimicrobial factors and pro-inflammatory molecules and formation of platelet–leukocyte aggregates. Under some conditions, bacterial toxins of several classes can trigger these responses of human and murine platelets. Evidence suggests that these activities mediate immobilisation and killing of bacteria and other pathogens. In addition, however, each can contribute to local thrombo-sis and inflammation

in platelet $\alpha_{IIb}\beta_3$ that increases its affinity for fibronectin, which can then act as a bridge to endothelial cell $\alpha_v\beta_3$ in the low shear stress circulation of the pulmonary vascular bed (Goldenberg and Kuebler 2015). Moreover, platelets can induce inflammatory responses by expressing CD40L which is recognised by surface CD40 on endothelial cells which will then secrete adhesion molecules and TF (Freedman 2003). In addition, activated platelets display P-selectin which mediates their adhesion to PMN by binding to PSGL-1, favouring tethering and rolling on the endothelium, thereby guiding neutrophils to transmigrate (Weyrich et al. 2009) (Fig. 4).

Platelets express a ligand for triggering receptor expressed on myeloid cells (TREM)-1, a surface receptor that plays important roles in innate and adaptive immunity expressed by neutrophils and monocytes. Platelets bound to endothelium support the engagement of neutrophil TREM-1 and result in increased expression of pro-inflammatory chemokines and cytokines, thus amplifying the inflammatory response (Haselmayer et al. 2007). Platelets additionally function as a threshold switch for the release of neutrophil extracellular traps (NETs), web-like structures of DNA that can trap and kill microbes in the vasculature, by neutrophils (Brinkmann et al. 2004) (Rondina et al. 2017; Pryzdial et al. 2017; Foote et al. 2017). This occurs primarily in small vessels, like the liver sinusoids and pulmonary capillaries, where NETs, and particularly the histone components, are responsible for death of lung epithelial and endothelial cells, often with consequent tissue damage and organ dysfunction (Saffarzadeh et al. 2012).

Platelets also express Toll-like receptors (TLRs), a highly conserved family of pattern recognition receptors expressed from flies to mammals. TLRs bind pathogen-associated molecules that are broadly expressed by many infectious organisms. LPS is the most studied TLR ligand, and others include unmethylated cytosine guanine dinucleotide, double-stranded RNA and lipoproteins. Platelets express low levels of TLR2 (Aslam et al. 2006), TLR4 and TLR9 (Cognasse et al. 2005). In addition, additional TLRs and other secondary signalling molecules can become expressed following platelet activation. For example, high concentrations of thrombin induce the expression of TLR9 by platelets. TLR2 and TLR4 are functional in inflammatory responses such as sepsis, e.g. TLR4 triggers the expression of IL-6 and cyclooxygenase 2 (COX-2) in response to LPS (Scott and Owens 2008). LPS has been shown to induce thrombocytopenia and platelet accumulation in the lungs of wild-type but not TLR4-deficient mice. Moreover, LPS stimulation of platelet TLR4 induces platelet binding to and activation of adherent neutrophils. Indeed, stimulation of platelet TLR2 by synthetic bacterial lipopeptide directly activates the platelet's thrombotic and inflammatory response through the phosphoinositide 3-kinase (PI3-K) signalling pathway (Blair et al. 2009). In sepsis, neutrophils and
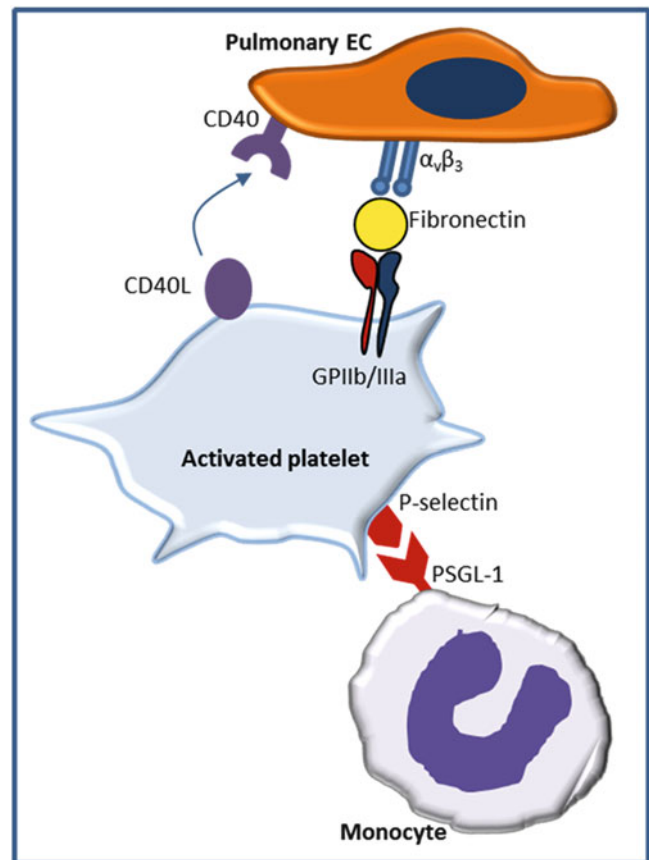
Fig. 4 Interactions between platelets and pulmonary ECs and monocytes in pulmonary infectious disease

platelets co-localise in several organs. Upon stimulation via TLR4, platelets induce neutrophil activation and the formation of NETs that display proteolytic activity, leading to the killing of microbes (Clark et al. 2007). Furthermore, platelets directly kill bacteria by releasing microbicidal peptides or by phagocyticing bacteria (Semple et al. 2011; Kraemer et al. 2011; Krijgsveld et al. 2000). Exposure of platelets to Gram-negative bacteria induces platelet aggregation, activation, P-selectin expression and formation of platelet/neutrophil aggregates via TLR2 (Beaulieu et al. 2011). Moreover, incubation of megakaryocytes with TLR2 leads to the generation of platelets with an enhanced pro-inflammatory gene and protein expression profile (Aslam et al. 2006).

In addition to their roles in innate immunity, platelets act as mediators between innate and adaptive immune response cells (Aslam et al. 2006; Elzey et al. 2003, 2005; Semple et al. 2011). Thus, platelets serve as early effectors of dendritic cells (DCs) activation in tissue injury through the release of CD40L and IL-1β and DCs are essential to link innate and adaptive immunity (Medzhitov and Janeway 2000; Bancherau and Steinman 1998). Platelet-mediated leukocyte adhesion to endothelium may also enhance lymphocyte trafficking during adaptive immunity and host

defence (Diacovo et al. 1996; von Hundelshausen and Weber 2007).

Studies in Experimental Animal Models

Platelets have a key role in immune surveillance by monitoring the surface of active macrophages (Wong et al. 2013). Platelets survey liver Kupffer cells through transient interactions via their GPIb with VWF constitutively expressed on Kupffer cells. When Kupffer cells capture blood-borne *S. aureus* or *Bacillus cereus*, they upregulate their VWF and the normally transient interaction with platelets becomes a sustained adhesion that actively encases the bacteria for eradication within the first minute of encounter. GPIb-deficient mice are significantly more susceptible to Kupffer cell damage and mortality in infections generated by the above-mentioned bacteria, ostensibly due to inefficient detection of Kupffer cell infection by platelets (Wong et al. 2013). Therefore, the targeting of infected macrophages by platelets seems to be an early and key host defence step to facilitate bacterial clearance and protection against disseminated infection (see Slaba and Kubes 2017).

The depletion of platelets in mice submitted to Klebsiella-induced pneumosepsis enhanced mortality in a way proportional to the extent of platelet depletion, by increasing the release of pro-inflammatory cytokines. Although Klebsiella sepsis did not directly enhance the expression of P-selectin on circulating platelets, the infection did increase the responsiveness of platelets to thrombin receptor stimulation (De Stoppelaar et al. 2014). In accordance, lipopolysaccharide (LPS) has been described to act as a platelet 'primer' (Gresele et al. 2008); in fact LPS induces platelet hypersensitivity to subthreshold concentrations of platelet agonists (Zhang et al. 2009).

During severe influenza A virus infection in mice, platelets massively infiltrated bronchoalveolar lavage fluid and were detected in ultrathin cryosections of the lungs staining positive for virus antihaemagglutinin, indicating that platelets actively incorporate influenza virus (Mazur et al. 2007).

Platelets in Cystic Fibrosis

> – Platelets from cystic fibrosis (CF) patients have increased reactivity.

> – A positive feedback loop of inflammation and platelet activation, which in turn lead to more inflammation and platelet activation, may exacerbate lung disease in patients with CF.

Cystic fibrosis (CF) is a multi-organ disease which affects the pancreas, the gastrointestinal tract, the male reproductive system and especially the respiratory tract. It is a lethal genetic disorder caused by a mutation in the gene, located in the long arm of chromosome 7, coding for the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride ion channel belonging to the superfamily of the ATP-binding cassette (ABC) proteins (Riordan et al. 1989). Chronic lung inflammation, due to an impairment of mucociliary clearance and to the consequent bacterial colonisation, represents the main cause of mortality and morbidity in CF patients.

Pathophysiologic Observations

CF patients have an increase in circulating activated platelets and platelet reactivity, as determined by enhanced monocyte–platelet aggregates, neutrophil/platelet aggregates and platelet surface P-selectin (Sturm et al. 2010; O'Sullivan et al. 2005). Moreover, elevated concentrations of sCD40L and TXA2 metabolites, which correlate with decreased lung function, further implicate platelets in the pathophysiology of this lethal disorder (Ciabattoni et al. 2000; Falco et al. 2004).

CFTR was found to be expressed by neutrophils and platelets, where it is localised in the cytosol, cell membranes and α-granules, and when blocked a markedly decreased thrombin-induced AKT phosphorylation and an upregulation of TRAP-stimulated p38MAPK phosphorylation were observed (Mattoscio et al. 2010). p38 MAPK activation has been associated with platelet secretion of pro-inflammatory mediators; thus the CFTR-dependent increase in p38 MAPK phosphorylation is consistent with a pro-inflammatory profile of CF platelets.

Another abnormality reported in CF patients is the reduction of plasma levels of vitamin E, which may increase fatty acid oxidation and thus the production of isoprostanes, which in turn activate platelets (Ciabattoni et al. 2000). Moreover, reduced nitric oxide (NO) generation was also observed in patients with CF and found to be a negative prognostic index associated with bacterial colonisation of the lung and sustained inflammation (Balfour-Lynn et al. 1996; Grasemann and Ratjen 2012). Endothelial dysfunction may also contribute to reduce NO generation in CF

(Poore et al. 2013), and the reduction of NO may contribute to platelet hyperreactivity. Platelets also produce NO upon eNOS activation that may act as a negative feedback mechanism to limit platelet activation and platelet recruitment to a growing thrombus (Cozzi et al. 2015; Momi et al. 2014; Freedman et al. 1997). Furthermore, the blockade of CFTR blunted NO generation by platelets suggesting this as another mechanism of the platelet hyperfunction in CF (Mattoscio et al. 2010).

Platelet 12-lipoxygenase is a key enzyme in the biosynthesis of lipoxins (Romano et al. 1993), arachidonic acid (AA) metabolites with potent anti-inflammatory properties. Lipoxin formation occurs in vivo during platelet–PMN interactions (Gangemi et al. 2003) and represents a main stop signal of inflammation (Serhan et al. 2008). Of interest, CF patients display a defect in the biosynthesis of lipoxin A4 (Karp et al. 2004), whereby mutated CFTR downregulates lipoxin formation by selective inhibition of platelet 12-lipoxygenase activity.

Patients with CF have increased plasma levels of ATP, due to the dysfunction of CFTR which acts as an ATP transporter, as well as a chloride channel, that can contribute to platelet activation (Lader et al. 2000). ATP may in fact act as a platelet agonist in vivo under special circumstances, like under high shear stress conditions, and may thus also contribute to the hyperreactivity of CF platelets (Birk et al. 2002).

Altogether, these data suggest an involvement of platelets in the sustained inflammatory response of CF originating from increased pro-inflammatory platelet-activating mediators and reduced counter-regulatory signals (Mattoscio et al. 2010; O'Sullivan et al. 2005).

Patients with CF show thrombocytosis, increased expression of P-selectin on circulating platelets (O'Sullivan et al. 2005), increased platelet reactivity to ADP and TRAP (Stead et al. 1987) and increased release of platelet-derived mediators (e.g. TNFα, CD40L, LTB4 and interleukins) (Shoki et al. 2013). Moreover, patients with CF display increased circulating platelet–leukocyte and platelet–monocyte complexes (O'Sullivan et al. 2005) and enhanced NET formation in airway fluids correlating with impaired obstructive lung function. These phenomena are blocked by the intra-airway delivery of small-molecule antagonists of CXCR2, with inhibition of NET formation and improved lung function (Marcos et al. 2010). In CF, increased platelet number correlated with decreased PaO2 (Gross and Luckey 1969), increased urinary thromboxane metabolite excretion with decreased FEV1 (Davì et al. 1995; Ciabattoni et al. 2000) and increased plasma sCD40L with decreased pulmonary function (Falco et al. 2004). Taken together these studies suggest a link between platelet activation and progressive impairment of lung function in CF. Whether platelet abnormalities are the cause or consequence of lung inflammation in CF is unclear. However, platelet dysfunction in CF could be the result of platelet abnormalities intrinsic to CF, of the accumulation of plasma factors unique to CF and/or of generalised inflammation.

Studies in Experimental Animal Models

Mice carrying the F508del mutation of CFTR developed more severe thrombocytopenia, higher levels of plasma TxB2 and PAF levels and enhanced neutrophilic lung infiltration when challenged with LPS relative to wild-type controls (Zhao et al. 2013). Blockade of PSGL-1 (P-selectin glycoprotein ligand-1) or of P-selectin, PAF-receptor antagonism or correction of mutated CFTR trafficking by KM11060 (an analogue of sildenafil, which restores a function of the F508del-mutated CFTR chloride channel) all significantly increased plasma lipoxin A4 levels, whilst the depletion of platelets significantly decreased plasma lipoxin A4 and enhanced LPS-induced lung inflammation (Wu et al. 2014).

Therefore, a growing body of evidence suggests that platelets play a very important role in CF and along with evidence of platelet involvement in other inflammatory lung diseases suggests that they may represent a potential therapeutic target in CF. However, much work remains to be done before anti-platelet therapy can be recommended for patients with CF.

Platelets in Chronic Obstructive Pulmonary Disease (COPD)

Platelet activation represents a mechanism that may contribute to the increased cardiovascular risk associated with stable COPD and with exacerbations.

Chronic obstructive pulmonary disease (COPD) is a respiratory disorder characterised by progressive lung destruction with airflow limitation associated with an airway and systemic inflammatory response (Rabe et al. 2007). Low-grade, chronic systemic inflammation in COPD is frequently complicated by acute atherothrombotic events, independent of the smoking history or other cardiovascular risk factors (Malerba et al. 2013), and coronary artery disease is one of the leading causes of death in COPD (Hansell et al. 2003). Moreover, COPD patients have an increased risk of ischaemic stroke (Truelsen et al. 2001) and venous thromboembolism, particularly during acute exacerbations (Tillie-Leblond et al. 2006). Although the mechanisms responsible for the association between COPD and atherothrombosis are still largely unknown, platelet activation is one of the proven links.

Pathophysiologic Observations

A link between hypoxia and platelet activation has been suggested since hypoxia facilitates the activation of cyclooxygenase-1 and thus thromboxane formation (Ponicke et al. 1987). More recently, a significantly enhanced urinary 11-dehydro TxB2 excretion, the urinary metabolite of platelet-released TxA2, was reported in patients with COPD, irrespective of the smoking status, that inversely correlated with arterial oxygen tension (Davì et al. 1997). Increased in vivo platelet activation in hypoxaemic COPD patients with secondary pulmonary hypertension was also detected by a significant increase in circulating platelet aggregates and plasma β-TG (Cordova et al. 1985), suggesting that activation of platelets in the pulmonary blood vessels may be associated with the induction of pulmonary hypertension.

Moreover, an elevation in arginase activity in platelets, which is associated with a reduction of NO production, has been described in COPD patients, and this may also contribute to enhanced platelet reactivity (Guzman-Grenfell et al. 2011). Increased levels of circulating soluble P-selectin, most likely of platelet origin, have also been observed in COPD patients (Ferroni et al. 2000), and platelet P-selectin is crucial for the formation of platelet–leukocyte aggregates (Totani et al. 2016). It is therefore conceivable that neutrophil recruitment to the lungs, a feature of COPD patients, is favoured by interactions with activated platelets. Platelet–leukocyte interactions mediate the process of NETosis (see Evangelista et al. 2017) and NETs are a major contributor to chronic inflammation and lung tissue damage in COPD (Grabcanovic-Musija et al. 2015). NETs were found in COPD patients and are associated with other markers of activated innate immune response, including the expression of the pro-inflammatory cytokines IL-1β and CXCL8 and of the inflammasome component NLRP3. Consequently NETs may contribute, through a positive feedback and the stimulation of neutrophilic chemokines and cytokines, to the persistent airway neutrophilia observed in COPD (Wright et al. 2016). Further research is required to elucidate the participation of platelets in NETosis and the exact role that NETs are playing in COPD. Furthermore, the use of anti-platelet therapy in patients has been shown to improve survival in patients with oxygen-dependent COPD (Ekstrom et al. 2013).

## Platelets in Acute Lung Injury and Acute Respiratory Distress Syndrome

> – Platelet/neutrophil interactions may be major pathogenic contributors in ALI.

> – The interaction between platelets and neutrophils is mediated by platelet P-selectin and neutrophil PSGL-1.
> – Platelets contribute to NET formation that involves the expulsion of nuclear material embedded with histones, by neutrophils in ALI/ARDS. Histones were found in the bronchoalveolar lavage fluid in ARDS; in a transfusion-related acute lung injury model in mice, depletion of platelets was protective.

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are life-threatening diseases occurring in critically ill patients. They are manifestations of an inflammatory response of the lung to direct and indirect insults and are characterised by severe hypoxaemia, hypercapnia, diffuse inflammatory lung infiltration and impairment of pulmonary compliance (Ragaller and Richter 2010). In particular, ALI is defined as an acute lung disease with bilateral pulmonary infiltrates in a chest radiogram consistent with the presence of oedema, and no evidence of pulmonary hypertension (Pepe et al. 1982). ALI is an important complication of sepsis.

Pathophysiologic Observations

Growing evidence suggests that platelets play a role in the pathogenesis of ALI (Zarbock et al. 2006). Intrapulmonary triggers, like acid aspiration and pneumonia, and systemic inflammatory stimuli activate the lung microvascular endothelium with upregulation of adhesion molecules, including ICAM-1 (Basit et al. 2006), release and presentation of chemokines (Belperio et al. 2006) and increase of lipid mediators (Zarbock et al. 2006; Kiefmann et al. 2004). During endotoxaemia platelets roll on and adhere to pulmonary capillary endothelial cells, as shown by intravital and electron microscopy (Kiefmann et al. 2006; Zarbock and Ley 2009), and release chemokines and lipid mediators that activate leukocytes and endothelial cells (Zarbock et al. 2006) (Fig. 5). Platelet microparticles (PMPs), small (50 nm to 1 μm) circulating cell-derived vesicles that break off from intact platelets upon activation and contain a variety of enzymes and proteins as well as mRNAs (McVey et al. 2012) (see Cointe et al. 2017), may play a role in ALI by promoting the inflammatory response of the lungs through the activation of neutrophil cell surface proteins (e.g. IL-8, MAC-1, PECAM-1, etc.) (Reutershan and Ley 2004; Bastarache et al. 2009; Shang and Yao 2014) and the production of cytokines and inflammatory mediators by endothelial cells (Eickmeier et al. 2013). A recent study showed that PMPs carry membrane-bound sCD40L, promote PMN-mediated HUVEC damage and may affect the
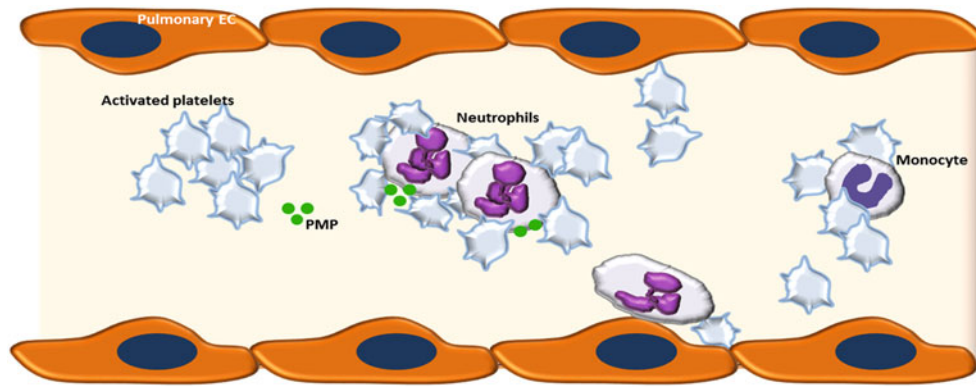
Fig. 5 Platelet–platelet, platelet–endothelial, platelet–PMN and platelet–endothelial cell–PMN interactions occur in pulmonary arterioles, capillaries and venules. These events can mediate acute lung injury. Different molecular interactions mediate adhesion of activated platelets to PMNs including P-selectin/PSGL-1, integrin MAC-1/integrin αIIbβ3 or ICAM-2, integrin MAC-1/GPIbα or JAM-3; ICAM-2/αLβ2 (LFA-1). Platelets also interact with monocytes under these conditions

development of ALI (Xie et al. 2015). Moreover, PMPs from LPS-stimulated platelets induce VCAM-1 production by cultured human endothelial cells (Levy and Serhan 2014).

Neutrophils and platelets are both key players in the pathophysiology of ALI. Thus, in a transfusion-related acute lung injury model in mice, depletion of either neutrophils or platelets was protective (Looney et al. 2009).

NETs are the product of a neutrophil death process that involves the expulsion of nuclear material embedded with histones, proteases, antimicrobial proteins and peptides. Histones have been found in the BAL fluid in acute respiratory distress syndrome (ARDS) (Bosmann et al. 2013). Platelets contribute to NET formation by neutrophils (see Slaba and Kubes 2017). Using an in vitro flow chamber system, it was shown that platelet-bound TLR4 detected TLR4 ligands in blood, activated neutrophils and facilitated the formation of NETs in the bloodstream. Moreover, using in vivo imaging in mice, it appeared that the lung capillaries where platelets bound neutrophils were hot spots for bacterial trapping and that the removal of platelets impaired bacterial clearance (Clark et al. 2007). Interestingly LPS, even at high concentrations, is not able to induce the formation of NETs in the absence of platelets, suggesting that platelets are not just facilitators in this process but essential mediators. On the other hand, the histone/DNA complexes of NETs activate platelets that in turn further promote NETosis, thrombosis and coagulation (Semeraro et al. 2011). Finally, extracellular histones, known to act as strong drivers of inflammation and tissue damage, activate TLRs resulting in pro-inflammatory cytokine production contributing to ALI development (Ward and Grailer 2014). All these observations point to an involvement of platelets in ARDS. One SNP within the LRRC16A gene (rs7766874), associated with variations in the platelet count, was involved in the pathophysiology of

ARDS in a large series of 629 ARDS cases and 1,026 at-risk control subjects. However, it remained unclear whether the reduced platelet counts in patients with the LRRC16A SNP affected ARDS risk by a reduced platelet production, increased platelet activation/consumption or platelet sequestration in lung tissues (Wei et al. 2015).

Finally, another evidence has demonstrated that activated coagulation and impaired fibrinolysis are associated with ALI. During ALI, the coagulation system is activated, producing fibrin deposition in the lung (Idell et al. 1989) (Fig. 6). The newly produced thrombin, on the one hand, activates protease-activated receptors (PARs) on endothelial cells, inducing an inflammatory response with upregulation of cytokines as well as thrombin formation, whilst, on the other hand, it also activates platelets by binding to platelet PAR1 and 4 (Coughlin 2005). Thrombin also induces the conversion of fibrinogen to fibrin that, together with activated platelets, can induce the formation of microvascular thrombosis.

Studies in Animal Models

Inhibition of platelet/neutrophil aggregate formation resulted in reduced neutrophil recruitment, increased survival and lower levels of hypoxia in LPS-induced ALI in mice (Zarbock et al. 2006). In a ventilator-induced lung injury model in rats, increased levels of VWF were found on freshly isolated lung endothelial cells. The increased expression of VWF was inhibited by platelet removal from the lung perfusion and by a P-selectin-blocking antibody, and it was also drastically reduced in the lungs of mice perfused with blood from P-selectin knockout animals. These findings indicate that in ventilation-induced stress, platelets transfer VWF to endothelial cells and that platelet P-selectin plays a critical role in this
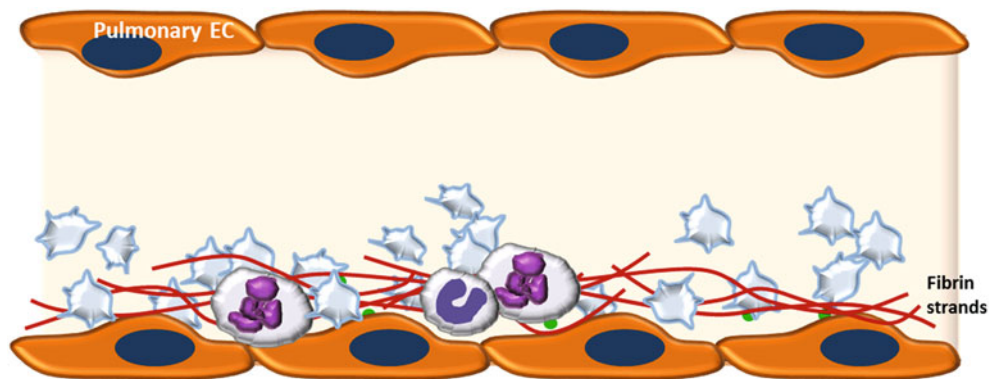
Fig. 6 Platelets and PMNs accumulate in macro- and microvessels in the lungs of patients with ALI/ARDS. Sequestration of platelets and PMNs is frequently associated with fibrin deposition and endothelial injury

transfer (Yiming et al. 2008). These data imply that during mechanical ventilation-induced lung injury, platelets deliver leukocyte-binding proteins to endothelial cells promoting leukocyte recruitment, thus playing a key role in generating a pro-inflammatory milieu.

Platelet depletion markedly reduced lung neutrophil infiltration in a murine model of lung injury induced by aerosolised LPS (Grommes et al. 2012). In the same model, antagonism of the CCL5-CXCL4 interaction reduced lung oedema, neutrophil infiltration and tissue damage, suggesting an important and causative role for platelet-derived chemokines in the development of lung injury (Grommes et al. 2012).

Moreover, in a mouse model of transfusion-associated lung injury (TRALI), platelet inhibition by aspirin or by a glycoprotein IIb/IIIa inhibitor reduced the degree of lung injury and the formation of NETs (Caudrillier et al. 2012). Therefore, platelet inhibition could reduce lung injury in ARDS by reducing the formation of NETs.

Take-Home Messages

A role for platelets in lung disorders has been revealed by studies in experimental animal models and in patients with different forms of acute and chronic lung injury with an inflammatory component, with platelets being critical for leukocyte recruitment, tissue damage and lung dysfunction. The complex web of cellular interactions that contribute to lung tissue damage, increasingly unravelled, includes now the humble platelet, and the improved understanding of the central pathophysiologic role of this cell may hold the key to improved treatments for these common conditions. It is becoming clear that the ability of

platelets to act as inflammatory cells involves activation and signalling pathways distinct from those involved in haemostasis and thrombosis. This may provide new exciting opportunities for identifying new drug targets for the treatment of inflammatory lung disorders.

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