Eur Respir J, 1994, 7, 1145–1160
DOI: 10.1183/09031936.94.07061145
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European Respiratory Journal
ISSN 0903 - 1936

SERIES 'PULMONARY IMMUNE CELLS'
Edited by U. Costabel and C. Kroegel

Pulmonary immune cells in health and disease: platelets

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Pulmonary immune cells in health and disease: platelets. C.M. Herd, C.P. Page. ©ERS Journals Ltd 1994.

ABSTRACT: The platelet has traditionally been associated with disorders of the cardiovascular system; a well-recognized cell type actively involved in the maintenance of haemostasis and the initiation of repair following tissue injury.

It has been accepted that the primary function of platelets is their adhesion to the endothelium or to other components at sites of the injured vessel wall in the initiation of haemostasis. However, it has been suggested that the fundamental physiological role of the platelet within the mammalian circulation is in the defence of the host against invasion by foreign organisms. Studies from several groups suggest an important role of the platelet in allergic processes and immunological mechanisms.

In this review, we have summarized the origin, physiology, activation and function of the platelet, in addition to both experimental and clinical evidence implicating the involvement of this cell type in certain human lung diseases.
Eur Respir J., 1994, 7, 1145–1160.

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Keywords: Asthma
inflammation
platelet

Received: January 17 1994
Accepted after revision March 16 1994

Despite being devoid of a nucleus, platelets possess many of the features of classical inflammatory cells, such as polymorphonuclear leucocytes. They can undergo chemotaxis [1, 2]; phagocytose foreign particles [3]; contain and release various adhesive proteins; activate complement; interact with parasites, viruses and bacteria; alter vascular tone; enhance vascular permeability; and take up, store and metabolize various vasoactive substances [4]. Furthermore, experiments have indicated that platelets have the capacity to release mediators with potent inflammatory or anaphylactic properties, such as the ether-linked phospholipid platelet-activating factor (PAF), or the platelet-specific protein platelet factor 4 (PF_4), and so far unidentified factors able to induce histamine release from basophils.

Origin

Platelets are small, anucleate blood elements, and under normal conditions constitute a small fraction of the circulating cells; the platelet count in healthy human blood ranging from $1.3–4.0\times10^5$ platelets·μl^{-1}. Classically, they were thought to be derived from megakaryocytes in the bone marrow by the process of fragmentation [5], although this theory has been challenged on various grounds. It has been suggested that megakaryocytes travel to the lung vasculature from the bone marrow where they physically become fragmented following impact with the extensive capillary network [6].

Membrane receptors and surface markers

The plasma membrane represents the site of platelet interactions with the external environment and is ultimately involved in the control or generation of the many specialized functional properties of the cell. The platelet surface is a typical bilayer membrane composed of protein, lipids (predominantly phospholipids) and carbohydrate. Platelet surface glycoproteins are essential to platelet functions, they play a primary role in the adhesion of platelets to exposed subendothelial matrix proteins, interaction with ligands such as collagen and thrombin, and exposure of fibrinogen receptors to facilitate aggregation (reviewed in [7, 8]). Several glycoproteins of the integrin superfamily of adhesion receptors are present on the cell membrane. These molecules share a common noncovalent dimeric structure and are involved in the attachment of platelets to adhesive molecules (*e.g.* fibrinogen, fibronectin). Platelet membrane integrin molecules include the collagen receptor, the glycoprotein Ia-IIa complex (very late activation antigen (VLA)-2) ($\alpha_2\beta$), the fibronectin receptor, Gp Ic-IIa complex (VLA-5) ($\alpha_5\beta$), the laminin receptor, Gp Ic'-IIa complex (VLA-6) ($\alpha_6\beta$), von Willebrand factor receptor Gp Ib-IX complex and a vitronectin receptor $\alpha_v\beta_3$ (reviewed in [9]). Platelets (and endothelial cells) express a membrane adhesion protein of the selectin family, granule membrane protein (GMP-140) (also known as platelet-activation-dependent granule external membrane (PADGEM) or CD62), following degranulation. The amino-terminal extracellular portion of this molecule contains a lectin domain, which permits the interaction of platelets with

leucocytes [9]. Loss of cell surface glycoproteins appears to be a primary mechanism of platelet senescence *in vivo* [10].

Platelets possess a glycoprotein receptor for the 3rd component of complement (C3b) which resembles that located on mononuclear cells [11], and Fc receptors both for immunoglobulin G and E (IgG and IgE) antibodies [12, 13].

IgE receptor

The demonstration that platelet membranes possess IgE receptors [13, 14] has given credence to the platelet as an inflammatory cell involved in allergic processes. The identification of a specific IgE receptor on platelets came from the demonstration of cytotoxic functions by platelets from patients infected with the helminth *Schistosoma mansoni* [15]. Studies have indicated that human platelets can bind IgE *in vitro* and that the cross-linking of surface-bound IgE with anti-IgE or the specific antigens induces platelet activation and secretion. A specific receptor for the Fc fragment of IgE, the Fc epsilon receptor type II (FcεRII), which has been demonstrated on the platelet membrane, is of low affinity (10^{-7} M) compared with that found on mast cell or basophil surfaces, (Fc epsilon receptor type I (FcεRI)) (10^{-9} M) [13], but of comparable affinity to the IgE receptor located on other inflammatory cell types, such as alveolar macrophages and eosinophils [16]. The FcεRII is associated with the Gp IIb-IIIa fibrinogen receptor on the platelet membrane [16]. Only a small number of platelets from normal individuals (20–30%) bind IgE; however, more than 50% of the platelets from patients with aspirin-induced asthma, allergic patients and patients with parasitic diseases bind IgE [13, 15, 17].

A number of platelet receptors for ligands of biological or pharmacological significance have been identified. In recent years the major excitatory (including α_2-adrenoceptor, adenosine diphosphate (ADP), serotonin (5-HT_2), platelet activating factor (PAF), thromboxane A_2 (TXA_2), vasopressin, thrombin) and inhibitory (including β_2-receptors, adenosine, prostaglandin D_2 (PGD_2), prostaglandin I_2 (PGI_2)) surface membrane receptors of the human platelet have been characterized (reviewed in [7]).

Physiology

Beneath the cell membrane a bundle of microtubules travels the entire circumference of the cell. In the resting state this band is a flexible cytoskeleton exerting tension outward, maintaining both the normal morphometry of the unstimulated platelet and geographical integrity of the organelles. The alterations in platelet shape induced by cell activation is achieved predominantly by the circumferential band of microtubules (the major protein being tubulin) [18] and abundant cytosolic actin microfilaments [19]. In close configuration with the microtubule band is a microfilament matrix, which provides contractile force for the secretion of cell constituents during the platelet release reaction. Two membrane systems weave throughout the cell interior, effectively increasing the platelet surface area. The open canalicular system, a random series of invaginations of the plasma membrane, contains channels which are continuous with the extracellular space, which facilitate secretion from the amine- and protein-storage granules during the release reaction, and hence serve as a conduit through which endogenous substances pass to the cell exterior. The canaliculi also provide ready access to the interior of the platelet for plasma proteins and other substances [20]. The dense tubular system, derived from megakaryocyte endoplasmic reticulum, is associated with the circumferential microtubule band. This system is implicated as a major site of calcium sequestration, used for the initiation of platelet activation processes.

The most numerous organelles held within the platelet cytoplasm are the platelet granules. Dense granules contain ADP and adenosine triphosphate (ATP), 5-HT and Ca^{2+}. The more numerous alpha granules store vasoactive components, which have either been synthesized by the megakaryocyte or taken up from the circulation. In addition, they contain a variety of proteins, some platelet specific, which include adhesive proteins, the "antiheparinoid" PF_4, platelet-derived growth factor (PDGF), β-thromboglobulin (β-TG), transforming growth factor-β (TGF-β), fibrinogen, and clotting factors V and VIII (von Willebrand's factor)

Platelets are capable of only limited protein synthesis. Mitochondria are few in number, but contribute significantly to energy metabolism of the cell by providing ATP for the cytoplasmic metabolic pool. Lysosomes, glycogen granules and peroxisomes are randomly distributed throughout the cytoplasm.

The platelet lifespan has been estimated at 8–12 days, using a variety of radioisotopic labelling techniques [21, 22]. Destruction of effete platelets is accomplished by macrophages of the reticuloendothelial system in the spleen, liver and bone marrow.

Mediators

Platelets are a rich source of a wide range of biologically active materials that are capable of inducing or augmenting certain inflammatory responses (table 1). Such materials have been shown to be both preformed mediators stored in either the dense or α granules and newly formed mediators resulting from the perturbation of membrane phospholipids. These substances may be released from the cell following activation.

5-HT, stored in large amounts in human platelets, may contribute to the inflammatory response *via* its vasoconstrictor properties and capacity to increase vascular permeability [23]. 5-HT has also been shown to stimulate fibroblast growth [24]. Adenosine, which can be formed from the nucleotides stored and released by platelets, may play a role in bronchoconstriction [25]; and receptors for adenosine have been shown to be upregulated in allergic rabbits compared with normal rabbits [26].

Table 1. – Platelet-derived inflammatory mediators

Mediator	Effect
5-HT	Pro-aggregation
	Vasoconstriction
	Fibroblast proliferation
Adenosine	Bronchoconstriction
Histamine	Pro-aggregation
	Bronchoconstriction
PDHRF	Bronchoconstriction
	Airway hyperresponsiveness
Cationic proteins	Increased vascular permeability
	Chemotaxis
	Tissue damage
PF_4	Increased expression of Fc-IgG and Fc-IgE
	Chemotaxis
	Airway hyperresponsiveness
PDGF	Vasoconstriction
	Chemotaxis
	Smooth muscle proliferation
TGF-β	Chemotaxis
	Fibroblast proliferation
RANTES	Chemotaxis
TxA_2+	Pro-aggregation
	Vasoconstriction
	Bronchoconstriction
$PGF_{2\alpha}$	Vasoconstriction
PGE_2	Vasodilatation
12-HETE	Chemotaxis
PAF	Pro-aggregation
	Vasoconstriction
	Chemotaxis
	Bronchoconstriction
	Airway hyperresponsiveness
NO	Anti-aggregation

5-HT: serotonin; PDHRF: platelet-derived histamine-releasing factor; PF_4: platelet factor 4; PDGF: platelet-derived growth factor; TGF-β: tumour growth factor-β; TxA_2: thromboxane A_2; $PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$; PGE_2: prostaglandin E_2; 12-HETE: 12-hydroxyeicosatetraenoic acid; PAF: platelet-activating factor; IGE: immunoglobulin E; IgG: immunoglobulin G; NO: nitric oxide.

Human platelets contain, and are capable of synthesizing, histamine [27, 28], and of taking up the preformed amine with an energy-dependent process. Histamine release from human and guinea-pig blood has recently been demonstrated during aggregation *in vivo* [29]. Exogenous histamine has been shown to dose-dependently enhance platelet aggregation induced by a variety of stimuli through a C^{2+}-dependent, H_1 receptor driven process [30]. Histamine, which is released during platelet aggregation, potentiates the effect induced by proaggregatory stimuli [31, 32], which may lead to a positive feedback effect on thrombogenesis and on vascular inflammation. Human platelets have been shown to stimulate the release of histamine from mast cells and basophils through IgE-dependent mechanisms [33]. Thrombin, PAF and collagen can liberate this histamine-releasing substance from platelets [33, 34]. In addition to causing eosinophil chemotaxis, platelet-derived histamine-releasing factor (PDHRF) has been shown to induce both early- and late-onset airway obstruction as well as airway hyperresponsiveness in experimental animals [35].

Platelets contain cationic proteins, which can increase vascular permeability (possibly by their action on mast cells) [36, 37], in addition to a cationic protein that cleaves the 5th component of complement to form a factor which is chemotactic for leucocytes [38].

PF_4, a platelet-specific protein released following stimulation, possesses many properties that suggest a role in allergy and inflammation. It has been demonstrated to increase the expression of Fc-IgG and Fc-IgE receptors [39]. PF_4 stimulates basophils to release histamine [40], and has been shown not only to be chemotactic for polymorphonuclear leucocytes, monocytes and fibroblasts [41], but also for eosinophils [39]. The ability of PF_4 to activate eosinophils is of interest because it has been suggested that they contribute to the tissue damage observed in asthma which may be associated with airway hyperresponsiveness [42, 43]. PF_4 has recently been shown to increase airway responsiveness to inhaled methacholine in rats [44].

The interesting finding that the immune response suppressed by lymphoma cells in mice could be restored by the injection of mouse serum [45], suggested an active role of platelets in this phenomenon. It was subsequently shown that the substance that reversed the immunosuppression was PF_4 [46, 47]. The reversal of immunosuppression has been demonstrated *in vitro* using cultured mouse spleen cells [48]. The ability of PF_4 to reverse this immunosuppression does not appear to be related to its ability to bind heparin [49], but may be a function of its serine protease activity [47].

PDGF is generally believed to be the principal mitogen that stimulates cell division when vessel integrity has been compromised and platelet activation has occurred [50]. PDGF may also act as a mediator of inflammation and repair by affecting vascular tone (vasoconstriction) [51], exerting chemotactic effects towards monocytes and neutrophils [52], and by activating monocytes [53] and neutrophils [50]. Smooth muscle cells and fibroblasts are strongly attracted to low concentrations of PDGF [54–56], suggesting that these cells may migrate to injured sites where subsequent mitogenic stimulation furthers repair processes [50]. Similarly, TGF-β has been shown to be chemotactic for neutrophils and fibroblasts [57]. PDGF released at sites of continuous vessel wall injury has been suggested to contribute to the vascular smooth muscle thickening which characterizes cardiovascular diseases such as atherosclerosis [58]. Similarly, bronchial smooth muscle hypertrophy is a feature of the asthmatic lung at autopsy [59, 60], and it is possible that continuous platelet activation, recruitment and extravascular diapedesis into the airways, with consequent release of mitogens, could contribute to this feature of asthma. The role of platelet activation in the induction of myofibroblast proliferation and bronchial smooth muscle thickening characteristic of asthma as yet remains to be fully elucidated, although PDGF has been reported to act as a mitogen for airway smooth muscle cells in culture [61].

Recent findings that the cytokine RANTES (a member of the interleukin-8 (IL-8) supergene family), released

upon appropriate stimulation from platelets, is a potent chemoattractant for both monocytes [62] and eosinophils [63], serves as additional evidence for the contribution of platelets to the inflammatory response.

Upon cell stimulation and activation, products of the metabolism of membrane arachidonic acid are synthesized and liberated. TxA_2 is a potent vasoconstrictor and bronchial smooth muscle spasmogen [64]. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a vasoconstrictor, whereas prostaglandin E_2 (PGE_2) is a vasodilator and inducer/modulator of pain and fever. 12-hydroxyeicosatetraenoic acid (12-HETE), synthesized by the platelet specific enzyme 12-lipoxygenase on release of arachidonic acid [65], exerts chemotactic activity towards eosinophils [66].

Platelets have been shown to co-operate with leucocytes to produce chemotactic factors which the cells are unable to synthesize in isolation. Platelet 12-HETE can be metabolized by unstimulated neutrophils to yield 12,20-diHETE, a unique product which cannot by synthesized by either cell alone [65, 67, 68]. Furthermore, in the presence of activated platelets, leucocytes can produce increased amounts of leukotrienes because 12-hydroperoxyeicosatetraenoic acid (12-HPETE), produced by platelets, can stimulate the activity of leucocyte 5-lipoxygenase [69]. Neutrophils can also utilize arachidonic acid from stimulated platelets for the synthesis of 5-HETE and leukotriene B_4 (LTB_4) [70], a mediator with a wide proinflammatory profile [71]. PAF can also stimulate the synthesis of LTB_4 from these cells [72]. Conversely, platelets may produce leukotriene C_4 (LTC_4) from leukotriene A_4 (LTA_4) synthesized by leucocytes *via* glutathione-S-transferase [73], a powerful bronchial smooth muscle constrictor and proposed mediator of allergic asthma (reviewed in [74]). Both neutrophils and platelets can release PAF in modest amounts in response to appropriate activation stimuli [75, 76]. However, the presence of a small number of platelets in a suspension of neutrophils results in the generation of significantly increased amounts of PAF, far in excess of that predicated from the individual cell types [77]. Platelet aggregation is observed when mixtures of leucocytes and platelets are stimulated with leucocyte-specific agonists, a response inhibited by PAF antagonists [78]. PAF is an extremely potent inflammatory agent and has been implicated as a mediator of inflammation and asthma (reviewed in [79]).

Neutrophils have been shown to release a factor capable of activating platelets (neutrophilin) [80]. Platelet activation is also potentiated by neutrophils through the production of hydrogen peroxide and oxygen free radicals [81]. Furthermore, nitric oxide, produced from either vascular endothelial cells, circulating neutrophils or platelets themselves, makes a major contribution to the control of platelet and neutrophil aggregation and disaggregation *in vivo* [82].

Activation and function

Platelets play a central role in the prevention of excessive blood loss. Intact blood vessels are lined by haemostatically inert endothelial cells and, as a consequence, subendothelial structures do not normally come into contact with flowing blood. Vascular injury (either spontaneous or traumatic interruption of vascular continuity) is the stimulus required to initiate a series of complex and interdependent reactions. Platelet surfaces will adhere to the exposed collagen fibres, which occurs through the process of activation of several membrane glycoproteins of the integrin superfamily of adhesion receptors, as discussed previously. Following platelet activation, the fibrinogen receptor Gp IIb-IIIa becomes exposed, to which binds circulating von Willebrand's factor and fibrinogen, allowing platelet-to-platelet interactions. In addition, induction of the membrane adhesion protein of the selectin family, GMP-140 (PADGEM) permits the interaction of platelets with leucocytes [9]. Under shear forces within the arterial circulation, the Gp Ib-IX surface receptor complex is activated, which then interacts with von Willebrand's factor to facilitate the adherence of platelets to the vessel wall. The cells change shape from discoid to a more spherical form, a process mediated by the contractile microtubular system, characterized morphologically by the extension of short and long dendritic pseudopodia [83]. A secretory process ensues, whereby substances stored in platelet granules are extruded from the platelet, *i.e.* the platelet release reaction. ADP discharged from the dense granules and TxA_2 generated by the activation of platelet membrane phospholipase A_2, influence the recruitment of additional circulating platelets to clump on those already adhered to the injured site. If the flow conditions are sufficiently disturbed, platelet aggregates form on the vessel wall and serve as a focus for the acceleration of coagulation reactions *via* platelet factor 3. Contact of blood with the subendothelium and release of the tissue factor (thromboplastin) from the damaged vessels initiates a cascade of proteolytic reactions in the intrinsic coagulation pathway, culminating in the formation of thrombin. The newly formed thrombin acts synergistically with ADP and TxA_2 to promote further aggregation of platelets, to form an enlarging platelet mass as the haemostatic plug [84]. Thrombin converts fibrinogen, present in plasma and released from platelets, into fibrin monomers, which polymerize to stabilize and reinforce the platelet plug. The fibrin meshwork contains platelets and some red and white blood cells. Platelet contractile proteins, thrombosthenin and actomyosin, are stimulated by thrombin and clot retraction is initiated [84]. Subsequently, plasmin is cleaved from its plasminogen precursor and, by its lytic action on fibrin, causes the slow dissolution of the clot

Evidence exists for the involvement of platelets in nonallergic defence mechanisms, such as the removal of bacterial infections. It has long been known that platelets play a role in a number of bacterial diseases, and the phenomenon of adhesion between blood cellular elements and bacteria or other foreign particles has been known since early this century [85, 86]. Phagocytosis of foreign particles by platelets may represent one of the mechanisms that the platelet employs to remove bacterial invasion. Platelets are capable of adsorption and phagocytosis due to characteristics of their membrane system and inner structure. The ability of platelets to perform phagocytosis

has been observed with yeast, colloidal SiO_2, barium sulphate, ferritin and latex particles [85, 86]. Foreign particles are captured immediately or rapidly after they enter the bloodstream by the clumping together of platelets, which engulf these particles and/or phagocytose them. The clumping of platelets can be induced through the mechanism of ADP liberation from the platelets. These mixed thrombi are then eliminated by embolization into the microcirculation of different organs and liberated into the tissues at perivascular sites. Alternatively, mixed thrombi may ultimately migrate into lymph channels. Platelet aggregation can be induced following infection with various bacterial pathogens [86], which can become sequestered in clumps of platelets [87]. As a result of the subsequent platelet release reaction (and possibly also as a result of the production by the aggregated platelets of chemotactic metabolites of arachidonate), the platelet-bacterial aggregates become chemotactic for polymorphonuclear leucocytes and for monocytes. Platelets release bactericidal products, such as β-lysin [88, 89], known to have direct bacteriocidal activity against a range of organisms, including Bacillus, Clostridium, Micrococcus and Lactobacillus. Even though it is not known precisely how bacteria activate platelets, certain products of Gram-negative bacteria, such as endotoxin (lipopolysaccharide), can activate platelets directly and this can be manifested *in vivo* as thrombocytopenia [90], and platelet sequestration into various organs such as the lung, liver and spleen [91–94]. It has been suggested that by aggregating around invading bacteria, platelets may aid the clearance of the pathogens from the circulation, thus reducing the risk of septicaemia.

The physiological relevance of the platelet IgE receptor may be associated with a mechanism for aiding the removal of parasitic infections, as platelets have been shown to participate as effector cells in defence against helminth parasites [15, 95]. This proposed role of the platelet IgE receptor is reinforced by the observation that the passive transfer of platelets bearing IgE receptor towards schistosomes to naive rats can protect these animals from parasitic challenge [15]. The platelet IgE receptor appears not to be associated in any way with the formation of aggregates, but with the ability of platelets to mount a reaginic antibody-dependent cytotoxic response against helminth parasites, such as *Shistosoma mansoni* through oxidative killing (as demonstrated *in vivo* by chemiluminescence) [16]. Activation of the IgE receptor by exposure of sensitized platelets to an appropriate antigen has been shown to result in the production of cytotoxic free radicals [96, 97] in sufficient concentrations to kill parasites [98]. Platelets from *Schistosoma mansoni* infected patients or rats expressed direct anti-parasitic killing properties *in vitro* which has been in part attributed to the IgE-mediated release of cytotoxic free radicals [97]. The interaction of platelets with parasites may result in cytotoxic effects on schistosomal and filarial parasites through IgE-mediated mechanisms [4, 15]. The capacity of platelets to induce cytotoxicity is comparable with that observed with natural killer cells. Both these cytotoxicities can be inhibited by scavengers of activated oxygen species, although the exact biochemical mechanism underlying this phenomenon remains to be determined [99].

It appears that a distinction may exist between the mechanism of platelet activation resulting in the generation of free radicals and that resulting in granule release. The latter represents classical aggregation, an event normally associated with the contribution of platelets to haemostasis and thrombosis [79]. Platelets that release free radicals do not aggregate and platelet aggregation itself will inhibit any subsequent free radical release [100]. This type of activation can be elicited by a range of stimuli thought to be involved in the inflammatory response, including C-reactive protein [95, 101], substance P [102], the complement-derived peptides C3b and C5b-C9 [103], the eosinophil-specific major basic protein (MBP) [104], and the cytokines, interferon-gamma (IFN-γ) [105] and tumour necrosis factor-α [102]. Anti-allergic compounds, such as disodium cromoglycate [106] and nedocromil sodium [107], inhibit IgE-dependent release of free radicals from platelets, yet these drugs are ineffective against classical platelet aggregation [108]. Furthermore, the therapeutic efficacy of certain anti-parasite drugs, such as diethyl-carbamazine, may to some extent be related to their ability to generate free radicals from platelets [99].

It has been shown that a suppressive lymphokine released by activated mononuclear cells can inhibit the production of cytotoxic free radicals by IgE-coated platelets [109]. This lymphokine has been termed "platelet activity suppressive lymphokine" (PASL), a heat stable molecule of molecular weight 15,000–20,000 and a product of a T-lymphocyte subpopulation bearing the CD8+ antigen [109]. Furthermore, CD4+/CD8- lymphocytes have been observed to release factors, including IFN-γ, which can induce cytotoxic activity in normal platelets [110].

Human lung disease

Pulmonary embolism

Platelets are an important component of emboli found within the pulmonary circulation. Pulmonary embolism may occur as a complication of venous thrombosis or in response to injury, sepsis or with pathologies associated with the initiation of disseminated thrombosis [111]. Circulating platelet aggregates, with the potential to embolize in the lungs, have been demonstrated in man [112]. The findings of pulmonary platelet sequestration and thrombocytopenia associated with the adult respiratory distress syndrome (ARDS) have implicated a role for the platelet in the development of pulmonary insufficiency and oedema.

Studies have indicated that platelet aggregation and release may influence the pulmonary pressor response, not only by mechanical obstruction but as the result of the release of vasoactive materials [113–115]. Platelet release products have been shown to induce increased airway resistance [113, 115] and vascular permeability changes [36, 116–118]. Increased permeability associated

with the development of thrombocytopenia and pulmonary sequestration of platelet aggregates has been demonstrated [113, 115]. Furthermore, platelets may contribute to the maintenance of vascular endothelial integrity and have been shown to act as a permeability barrier [119, 120].

Acute lung injury in patients is generally associated with the development of thrombocytopenia [121, 122]. In addition, microemboli containing platelets have been observed histologically [123]. A wide variety of experimental animal models of acute pulmonary dysfunction are associated with thrombocytopenia and/or pulmonary platelet sequestration (reviewed in [111]). Platelet accumulation in the pulmonary vasculature has been demonstrated by external scintigraphy following the injection of radiolabelled platelets, and histologically in biopsied lung tissue and lung tissue removed at autopsy [122, 123]. In addition, platelet survival time is decreased in these patients and platelet turnover is increased [122].

Various experimental models of pulmonary embolism have been developed (reviewed in [111]). VAAGE and co-workers [113, 115, 124–128] reported a series of investigations concerning the effects of experimentally-induced platelet aggregation on pulmonary function. These studies showed that the airway and vascular constriction was dependent on the presence of platelets and a stimulus which would induce platelet release in addition to aggregation [125, 128].

Numerous techniques have been developed for the investigation of platelet function *in vivo*. A non-invasive technique for the continuous monitoring of platelets in the circulation of anaesthetised animals was described by PAGE *et al*. [129], whereby platelets are radiolabelled and externally monitored using scintillation detectors. The intravenous administration of a platelet agonist causes the formation of aggregates which become trapped in the microvasculature of the pulmonary circulation, which is detected as an increase in radioactive counts by a detector placed over the thoracic region of the animal. Using this system, most doses of platelet aggregatory stimuli produce reversible accumulation, except when the coagulation cascade is simultaneously activated to elicit clot formation (as assessed by [^{125}I]fibrinogen accumulation), as observed following high dose intravenous thrombin administration [130]. Spontaneous disaggregation of platelet aggregates within the pulmonary vasculature suggests that there is an endogenous mechanism for limiting platelet aggregation *in vivo*, although it is unlikely to be secondary to the generation of PGI_2 as the disaggregation produced is not altered in animals treated with nonsteroidal anti-inflammatory drugs (NSAIDs) [131]. However, endothelial-derived relaxant factor (EDRF) has been postulated to limit the extent of platelet aggregation induced by ADP [131, 132] and collagen [132] and is a substance released by endothelial cells in response to thrombotic stimuli [133]. As nitric oxide (NO) accounts for the biological activities of EDRF, the release of NO by pulmonary endothelial cells may, therefore, contribute to disaggregation, a suggestion confirmed by the use of endogenous inhibitors of NO generation [82]. Furthermore, local fibrinolytic activity may result in disaggregation [134], and recent evidence suggests that there is a synergistic interaction between NO donors and fibrinolytic drugs *in vivo* in controlling platelet accumulation [135].

Malignancy

Platelet activation is a feature both of malignant disease [136] and experimental malignancy (the injection of tumour cell suspensions into laboratory animals) [137]. In addition, injection of tumour cell suspensions known to metastasize into the lungs of rats and mice rendered thrombocytopenic, results in a decrease in the number of metastatic lung colonies found in those animals [138]. This type of observation has led to the suggestion that platelets have a role in the dissemination of malignant tumours [137]. It remains plausible that, just as platelets isolate and clear bacteria from the circulation as a physiological defence mechanism, the facilitation of the removal of tumour cells by platelets may accelerate a pathological process [137]. Several experimental and clinical studies have suggested that antiplatelet drugs may influence the metastatic pattern of tumour spread [138], suggesting that platelets may be a legitimate target for future drugs used in the control of tumours. Furthermore, PDGF has a high degree of sequence homology with one of the main oncogenes implicated in the induction of certain types of tumour [139]. Subcutaneous administration of TGF-β induces a granulation process analogous to that observed during wound repair, suggesting the involvement of this factor in the process [140]. Similarly, the release of TGF-β following platelet activation could be associated with diseases characterized by abnormal cell growth.

Asthma

Asthma is characterized clinically by hyperresponsiveness of tracheobronchial smooth muscle to various spasmogens, resulting in the widespread narrowing of the airways. In recent years it has been recognized that asthma is a chronic inflammatory disease associated pathologically with eosinophil infiltration and damaged airway epithelium. These underlying inflammatory events are considered important in the development of the enhanced airway responsiveness observed in asthmatic individuals. Airway inflammation is a complex event triggered by inflammatory stimuli interacting with primary effector cells resident in the airway, of which numerous cell types have been implicated. Release of inflammatory mediators from these cells may recruit and activate other effector cells, thus augmenting the inflammatory process. Evidence now exists in support of a primary role of the platelet in the pathogenesis of bronchial asthma. Platelets can participate in allergic asthma by acting as inflammatory cells, by releasing spasmogens and/or by interacting with other inflammatory cell types.

The phospholipid PAF has been proposed as a mediator of asthma as it can reproduce many of the characteristic features of the disease, including bronchospasm, mucus hypersecretion, increased vascular permeability and

increased airway responsiveness, both in experimental animals and man (reviewed in [79]). PAF may provide the link between platelet activation and allergic asthma [141], as evidence suggests that the ability of PAF to induce airway hyperresponsiveness and eosinophil infiltration may involve the activation of platelets [142, 143]. PAF is released from a number of inflammatory cells in the lung, including alveolar macrophages, eosinophils and neutrophils. Human alveolar macrophages [144, 145] and eosinophils [146], are rich sources of PAF, and are capable of releasing large amounts in response to activation by IgE-dependent mechanisms. These cell types are present in the airways of asthmatics and are activated following antigen provocation [147, 148]. Eosinophils obtained from hypereosinophilic patients (including asthmatics) have a much enhanced capacity to generate PAF [146]. In addition to these inflammatory cells [149–151], platelets [76, 149, 152] and vascular endothelial cells [153] have been shown to release PAF, all of which may play a role in the pathophysiology of asthma. Furthermore, isolated lungs from sensitized guinea-pigs have been shown to release PAF when challenged with antigen [154].

Animal evidence

Platelets have been observed to undergo diapedesis into the extravascular tissue of the lungs of guinea-pigs following antigen challenge or challenge with PAF [155]. The extravasated platelets have been observed in close proximity to bronchial smooth muscle and to infiltrating eosinophils. However, treatment of experimental animals with other platelet agonists such as ADP, whilst inducing platelet aggregation in the pulmonary vasculature, does not elicit extravascular diapedesis of platelets and eosinophils [155], suggesting a possible link between extravascular platelets and eosinophils. Platelets have also been reported in bronchoalveolar lavage (BAL) fluid obtained from allergic rabbits undergoing late-onset airways obstruction following antigen challenge [147]. Further evidence that platelets are involved in experimental allergic responses is the detection of markers of platelet activation, such as PF_4, in the plasma following antigen challenge in sensitized rabbits [156].

In several animal species, the intravenous injection of selected platelet agonists induces thrombocytopenia associated with bronchospasm [113, 157, 158]. This also occurs in sensitized animals challenged with specific antigen, which appears to be a platelet-dependent phenomenon, since platelet depletion protects against the lethal consequences of the antigen provocation [159, 160]. In isolated human bronchus, PAF only induces airway smooth muscle contraction in the presence of platelets. Furthermore, the intravenous administration of PAF into guinea-pigs induces bronchospasm associated with the accumulation of platelets in the lung [161, 162], and the bronchospasm is platelet-dependent, since platelet depletion abolishes the response [161]. Under these circumstances, platelet aggregates have been located histologically [159, 163], and by the use of radiolabelled platelets [162], within the pulmonary vasculature. It has been suggested that this bronchoconstrictor response is reflex in origin; however, peak changes in lung function largely (>90%) precede detectable accumulation of ^{111}In-labelled platelets in the pulmonary vasculature [162]. Furthermore, several classes of drugs, including the anti-asthma drugs ketotifen and theophylline, inhibit the platelet release reaction *in vitro* and platelet-dependent bronchospasm *in vivo*, but do not affect platelet accumulation within the pulmonary vasculature [164]. These observations indicate that platelet-derived mediators contribute to the bronchospasm as well as, or instead of, physical obstruction of pulmonary vessels by platelet aggregates. The dissociation of platelet release and aggregation *in vivo*, with the use of an experimental technique for the continuous monitoring of platelets within the pulmonary circulation [129], led to the development of a hypothesis that platelet activation plays a central role in the pathogenesis of asthma [165]. Furthermore, the pharmacological inhibition of the platelet release reaction [166, 167] or TXA_2 production [168] can suppress the bronchospasm, suggesting that the response is related to the release of bronchoactive agents from the platelets rather than the retention of platelet aggregates *per se*.

Inhalation of allergen by an appropriately sensitized individual may induce a delayed airway obstruction (referred to as a late asthmatic reaction), which may be associated with increased airway responsiveness [169]. The late-onset response to antigen challenge in IgE-sensitized rabbits is inhibited by prior treatment with a selective antiplatelet antiserum [170]. This phenomenon may be attributable to an interaction between platelets and eosinophils as the antigen-induced pulmonary eosinophil infiltration is inhibited in thrombocytopenic animals [170].

In the guinea-pig and rabbit, PAF-induced airway hyperresponsiveness is platelet-dependent, since it can be inhibited by rendering animals selectively thrombocytopenic by the intravenous administration of a specific lytic antiplatelet antiserum [143, 171]. Activation of platelets by PAF differs from activation by other agonists, since ADP, collagen, thrombin or the TxA_2 mimetic U46619, in amounts sufficient to cause comparable pulmonary platelet accumulation *in vivo*, do not induce airway hyperresponsiveness [172, 173]. Therefore, as with the bronchoconstrictor response, the actual pulmonary retention of platelets is not responsible for induction of airway hyperresponsiveness, thus implicating some other property of this cell type.

A factor released from platelets has been reported to induce airway hyperreactivity (platelet-derived hyperreactivity factor (PDHF)) [174]. The intravenous injection of PAF into thrombocytopenic guinea-pigs does not induce an acute bronchoconstrictor response nor enhanced airway responsiveness. However, in platelet-depleted guinea-pigs, the supernatant obtained from non-platelet-depleted guinea-pig platelet-rich plasma (PRP) incubated with PAF, induced airway hyperresponsiveness [174]. The generation of PDHF was inhibited by prior incubation of PRP with the stable prostacyclin-mimetic, iloprost. The secretion or formation of this mediator of

hyperresponsiveness appears to be PAF-specific, as neither platelet disruption nor activation of platelets with ADP induced its production. The chemical nature of this material remains, as yet, unidentified. Ketotifen and prednisolone have been shown to inhibit the airway hyperresponsiveness induced by PAF-stimulated platelet supernatants, whereas cromoglycate and aminophylline were without effect [175]. Similarly, when ketotifen or prednisolone were incubated with PRP prior to the addition of PAF, the injection of supernatants into thrombocytopenic guinea-pigs resulted in reduced airway hyperresponsiveness [175]. In addition, human platelet-derived histamine-releasing factor (PDHRF) has been shown to induce airway hyperresponsiveness as well as selective pulmonary eosinophil infiltration in allergic rabbits [35, 176].

Eosinophils and their products, such as major basic protein, have been implicated in the pathogenesis of asthma [42]. Platelet depletion has been shown to reduce PAF and antigen-induced eosinophil infiltration into the lungs of normal and allergic animals, respectively [142, 143, 170], suggesting a central role for platelets in the induction of eosinophil accumulation, which both facilitates the removal of parasitic infection and contributes to the airway hyperresponsiveness observed in asthma. These experimental observations may be of clinical relevance where thromboembolic diseases are often associated with the hypereosinophilic syndrome and patients with eosinophilia have coagulation abnormalities [177]. The mechanism by which platelets attract eosinophils into the lung may be *via* the release of the platelet-derived protein PF_4 which, as discussed earlier in this review, is released upon platelet activation and can exert a powerful chemotactic effect on human eosinophils [39]. Treatment of allergic rabbits with an anti-rabbit platelet antiserum inhibits the ability of antigen to induce late-onset airways obstruction, airway hyperresponsiveness and the associated infiltration of eosinophils recovered in BAL fluid 24 h following antigen challenge [170]. The PAF antagonist BN 52021 has been shown to inhibit the late-onset response and subsequent increase in airway responsiveness in allergic rabbits [178, 179], as well as the eosinophil influx and airway hyperresponsiveness in sensitized guinea-pigs [180, 181] following antigen exposure. These findings suggest that antigen-induced release of PAF may play a role in the platelet activation necessary to initiate the eosinophil infiltration into the airways which, in turn, contributes to airway hyperresponsiveness.

Thrombin activation, as evidenced by the presence of fibrinopeptide A, has been described in early and late phase allergic responses [182], and may, therefore, activate platelets during allergen-induced responses. However, the precise involvement of platelets and the mechanism(s) by which these blood elements affect inflammatory responses is yet to be fully elucidated.

Further evidence in favour of the platelet as an important effector cell in asthma has been provided by *in vitro* studies, in which platelets potentiate mucous glycoprotein release from tracheal submucosal glands induced by PAF [183].

Clinical evidence

A number of clinical studies have now revealed that platelet activation is a feature of diseases where there is activation of the allergic response, although such diseases are not normally associated with thrombosis [184–194]. In certain clinical [195] and experimental [196] conditions where there is known to be excessive platelet activation in the circulation, platelets become partially refractory to subsequent stimulation *in vitro*. In particular, the second phase of platelet aggregation *in vivo* is often unresponsive to physiological stimuli. A number of studies have reported that platelets from asthmatics behave abnormally *in vitro*, lacking the second wave of aggregation [17, 197–199] or defective release of platelet 5-HT, PF_4 [17] and platelet nucleotides [200] following stimulation with platelet agonists. These *in vitro* abnormalities are suggestive of overstimulation *in vivo* [201, 202] (table 2).

In asthmatic patients the uptake of 5-HT by platelets

Table 2. – *In vitro* and *in vivo* platelet abnormalities in asthma

In vitro	*In vivo*
Abnormal aggregation to ADP	Thrombocytopenia
Abnormal aggregation to adrenaline	Circulating platelet aggregates
Abnormal aggregation to collagen	Increased plasma levels of 5-HT, PF_4 and β-TG
	Increased levels of PF_4 and β-TG in BAL fluid
Reduced release of 5-HT and PF_4	Increased urinary excretion of TxB_2 metabolites
Reduced release of platelet nucleotides	
	Abnormal megakaryocytes present in lung tissue at autopsy
	Reduced platelet survival time
Reduced uptake of 5-HT	Reduced platelet regeneration time
	Increased bleeding time
	Increased platelet size
	Increased platelet mass
Elevated resting levels of cytoplasmic Ca^{2+}	Accumulation of platelets in lung microvasculature
Elevated IP_3 production	Increased platelet numbers in BAL fluid

ADP: adenosine diphosphate; 5-HT: serotonin; PF_4: platelet factor 4; β-TG; β-thromboglobulin; BAL: bronchoalveolar lavage; TxB_2: thromboxane B_2; IP_3: inositol-triphosphate.

has been shown to be attenuated, possibly due to previous exposure of platelets to an increased concentration of this amine [203]. Increased plasma levels of 5-HT have been reported in asthmatics [204], as well as elevated resting levels of cytoplasmic Ca^{2+} and inositol-triphosphate (IP_3) production [205], findings suggestive of *in vivo* platelet stimulation.

Thrombocytopenia was first reported to accompany asthmatic attacks in 1955 [184]. This observation of platelet activation *in vivo* during provoked or spontaneous asthmatic attacks has also been shown by the detection of circulating platelet aggregates [186, 192], or the morphological characterization of activated platelets in the circulation [187]. Furthermore, a number of studies have demonstrated the release of two platelet-specific proteins, PF_4 and β-TG, into the circulation associated with bronchoconstriction induced by antigen or exercise [185, 186, 188, 189, 192, 206]. The release of these markers is indicative of *in vivo* platelet activation and, in the study of KNAUER *et al*. [185], the increased plasma levels of platelet-derived markers occurred in parallel with the bronchoconstriction induced by antigen provocation of allergic asthmatics. Release of PF_4 and β-TG was not observed following comparable bronchoconstriction induced by methacholine, suggesting that the platelet-derived markers were released as a consequence of the allergic reaction rather than of the bronchoconstriction.

Evidence of platelet activation has been reported in plasma obtained *ex vivo* during exacerbations of nocturnal asthma [207, 208], which has recently been shown to correspond with airway hyperresponsiveness [208]. In another recent study, PF_4 and β-TG have been demonstrated in BAL fluid from allergic asthmatics following antigen challenge [209]. Platelet products were significantly elevated during the late inflammatory response to antigen and were significantly correlated with elevations in markers of airway permeability (albumin), eosinophil granule proteins (eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO)) and inflammatory prostanoids (PGE_2 and $PGF_{2\alpha}$). Furthermore, TxA_2 release has been shown to accompany the exposure of allergic asthmatics to inhaled antigen, by measurement of urinary excretion of TxB_2 metabolites [210].

Release of platelet-derived factors, such as PF_4, β-TG and TxB_2, and altered *in vivo* platelet aggregatory responses have not been consistently observed [211–215]. Furthermore in some studies, pulmonary platelet sequestration was not found to follow antigen challenge in asthmatic volunteers [215, 216]. However, numerous other clinical observations support the concept that platelets may be involved in this disorder. In lung tissue removed at autopsy from patients dying from status asthmaticus, abnormal megakaryocytes have been reported to be present in abundance [193, 217], suggestive of a potential abnormality in this system. Platelet survival time in atopic asthmatics is severely shortened, a finding suggestive of continuous cell activation [191]. Shortened platelet regeneration time, an index of *in vivo* platelet activation associated with accelerated platelet consumption (*i.e.* increased platelet turnover) [218], has been reported in asthmatics undergoing acute asthma attacks [192], and increased bleeding time has been observed in a group of atopic asthmatics [190]. In addition, altered responsiveness of platelets from allergic patients has been observed by numerous investigators (reviewed in [192]), the incidence being greatest in patients presenting with high serum IgE titres [17]. Furthermore, platelet size [219], platelet count and platelet mass [190] have been found to be increased in asthmatics.

Platelets have been reported to accumulate in the microvasculature of the lung in patients undergoing bronchial provocation with allergen [148], and have also been detected by electron microscopy in BAL fluid obtained from allergic asthmatics undergoing late-onset airways obstruction following antigen provocation [147]. In this clinical situation, the extravascular platelets were observed in close association with other inflammatory cells, such as the eosinophil [147]. In addition, platelets have been observed undergoing diapedesis in sections biopsied from asthmatics (see [220]). Subepithelial extravasation of platelets together with fibrinous material has been observed at sites of denuded epithelium in bronchial biopsies from symptomatic asthmatics [221]. A recent study reports that platelets from asthmatic subjects migrate *in vitro* in response to antigen, possibly by interaction with platelet-bound antigen-specific IgE [2].

The fate of platelets in the circulation of asthmatics is unknown, although overt trapping in the pulmonary vasculature is not a feature of either stable asthmatics or those undergoing bronchoconstriction [192].

Aspirin-induced asthma

Platelets isolated from patients with aspirin-induced asthma exhibit an abnormal response to aspirin *in vitro* compared with normal individuals or allergic non-aspirin-sensitive asthmatics, generating cytotoxic mediators and oxygen-derived free radicals in the presence of acetyl salicylic acid (ASA; aspirin) or various NSAIDs, such as indomethacin [98]. Basophils from ASA-sensitive patients do not release histamine, and monocytes do not express cytotoxic properties or any burst of chemiluminescence in the presence of aspirin or other NSAIDs. Evidence does not support a role of IgE in this response, since serum from patients was unable to passively sensitize platelets removed from healthy volunteers to NSAIDs, as well as the absence of an inhibitory effect of polyclonal or monoclonal antibodies against the FcεRII. It has been suggested that the abnormal response of platelets from ASA-sensitive asthmatics may reside in the involvement of endogenous prostaglandin H_2 (PGH_2) in the control of synthesis and/or biological effect of platelet lipoxygenase products [222].

It has previously been shown that sodium cromoglycate and nedocromil sodium could modulate *in vivo* platelet responsiveness to aspirin in ASA-sensitive asthmatics [223], with nedocromil sodium being approximately 500 times more potent in inhibiting the response. Similarly, inhalation of nedocromil sodium by ASA-sensitive asthmatics resulted in a dramatic inhibition of platelet responsiveness to aspirin (platelet cytotoxicity) when examined *ex vivo* [224]. Since the platelet is the

only cell so far shown to respond to aspirin or other NSAIDs in ASA-sensitive asthmatics, these findings provide further evidence for a major role of the platelet in this form of bronchial asthma.

Therapeutic perspectives

Animal studies have shown that several selective, but structurally unrelated PAF antagonists inhibit various aspects of asthma pathophysiology, including antigen-induced bronchoconstriction, late phase response, airway hyperresponsiveness, oedema formation, mucus hypersecretion and pulmonary eosinophil infiltration (reviewed in [225]). As yet, there are few reported clinical studies of PAF antagonists in humans. Pretreatment with BN 52063 has been shown to attenuate the response to PAF in the skin of normal subjects [226], and to antigen-induced cutaneous responses in atopic subjects [227]. BN 52063 has also been shown to reduce the bronchoconstrictor response to inhaled PAF in normal volunteers [228], whereas WEB 2086 [229] and UK-74,505 [230] completely abolished the response. Furthermore, BN 52063 [231] and BN 52021 [232] have been shown to inhibit the immediate bronchoconstrictor response to inhaled allergen. Recent findings with UK-74,505, the most potent PAF antagonist yet studied in man [233], confirm preliminary reports of WEB 2086 [234] and MK-287 [235], which have shown no effect on the early or late response to inhaled allergen in mild atopic asthmatics or on the subsequent airway hyperresponsiveness.

The lack of effect of these PAF antagonists against allergen challenge in man, despite achieving plasma levels capable of inhibiting *ex vivo* platelet aggregation induced by PAF, may be due to a number of reasons. Firstly, PAF may not be as important a mediator in asthma as previously thought. Secondly, PAF released *in vivo* is a family of related compounds, whereas PAF antagonists have been developed as antagonists to PAF C_{16}. It is possible, therefore, that other PAF homologues may be of biological significance. Thirdly, current PAF antagonists have not been designed to penetrate cells and, thus, may not interact with intracellular receptors. As the bulk of PAF appears to be retained intracellularly in a variety of cell types [236], PAF antagonists may need to be able to enter cells or PAF synthesis may need to be inhibited, rather than its extracellular effects antagonized [237].

Treatment of atopic asthmatic individuals with anti-asthma drugs, such as glucocorticoids and ketotifen, has been shown to correct abnormal platelet survival [194]. A recent study reports that in asthmatic subjects the anti-allergy drug nedocromil sodium inhibits platelet activation induced by PAF *ex vivo* [238]. Therefore, the efficacy of these drugs may reside in their ability to restore normal platelet behaviour.

Conclusion

Evidence exists to implicate an active role for the platelet in primary defence mechanisms, such as antibody-dependent cytotoxicity. Inappropriate activation of this system in allergic patients may contribute to eosinophil infiltration and subsequent damage to the host tissue resulting in the heightened airway responsiveness characteristic of bronchial asthma.

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