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# REVIEW

# Novel uses for anti-platelet agents as anti-inflammatory drugs

SC Pitchford

*Leukocyte Biology Section, National Heart and Lung Institute, Imperial College, London, UK*

An alteration in the character and function of platelets is manifested in patients with inflammatory diseases, and these alterations have been dissociated from the well-characterized involvement of platelets in thrombosis and haemostasis. Recent evidence reveals platelet activation is sometimes critical in the development of inflammation. The mechanisms by which platelets participate in inflammation are diverse, and offer numerous opportunities for future drug intervention. There is now acceptance that platelets act as innate inflammatory cells in immune responses, with roles as sentinel cells undergoing surveillance, responding to microbial invasion, orchestrating leukocyte recruitment, and migrating through tissue, causing damage and influencing repair processes in chronic disease. Some of these processes are targeted by drugs that are being developed to target platelet participation in atherosclerosis. The actions of platelets therefore influence the pathogenesis of diverse inflammatory diseases in various body compartments, encompassing parasitic and bacterial infection, allergic inflammation (especially asthma and rhinitis), and non-atopic inflammatory conditions, for example, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis (RA), inflammatory bowel disease (IBD) and atherosclerosis. This review will first discuss the evidence for platelet activation in these various inflammatory diseases, and secondly discuss the mechanisms by which this pathogenesis occurs and the various anti-platelet agents which have been developed to combat platelet activation in atherosclerosis and their potential future use for the treatment of other inflammatory diseases.
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**Keywords:** platelets; inflammation; $P2Y_1$; $P2Y_{12}$; P-selectin; GPIIb/IIIa; CD40; S1P; PPAR

**Abbreviations:** 5-HT, 5-hydroxytryptamine; ADP, adenosine di-phosphate; ATP, adenosine tri-phosphate; APC, antigen-presenting cell; ApoE, apolipoprotein E; $\beta$-TG, $\beta$-thromboglobulin; COPD, chronic obstructive pulmonary disease; Der p1, *Dermatophagoides pteronyssinus*; E-selectin, endothelial selectin; GP, glycoprotein; Ig, immunoglobulin; IBD, inflammatory bowel disease; CD11b, integrin $\alpha_M$; CD18, integrin $\beta_2$; ICAM-2, intercellular adhesion molecule-2; IL, interleukin; JAM, junctional adhesion molecule; LFA-1, CD11a/CD18, $\alpha_L\beta_2$ integrin, leukocyte functional antigen-1; Mac 1, CD11b/CD18, $\alpha_M\beta_2$ integrin, macrophage-1 integrin; MMP, matrix metalloproteinase; MCP-1 (CCL-2), monocyte chemoattractant protein-1; MCP-3 (CCL7), monocyte chemoattractant protein-3; MDC (CCL22), monocyte-derived chemokine; MIP-1$\alpha$, CCL3, macrophage inflammatory protein-1 alpha; NSAIDs, non-steroidal anti-inflammatory drugs; PPAR, peroxisome proliferator-activated receptor; PECAM-1, platelet endothelial cell adhesion molecule-1; PDGF, platelet-derived growth factor; PF-4, platelet-factor 4; P-selectin (CD62P), platelet selectin; PSGL-1 (CD154), P-selectin glycoprotein ligand-1; PAF, platelet-activating factor; TARC (CCL17), thymus and activation-regulated cytokine; $TXA_2$, thromboxane; RANTES (CCL5), regulated upon activation normally T-cell expressed and secreted; ROS, reactive oxygen species; RA, rheumatoid arthritis; SNAP-23, synaptosomal-associated proteins; S1P, sphingosine-1-phosphate; SFKs, Src-family tyrosine kinases; SDF-1 (CXCL12), stromal cell-derived factor-1; TGF$\beta$, transforming growth factor-$\beta$; TNF$\alpha$, tumour necrosis factor-$\alpha$; VAMP, vesicle-associated membrane protein; VEGF, vascular endothelial growth factor; VLA-4, $\alpha_4\beta_1$ integrin, very late antigen-4 integrin

## Platelet activation in asthma and rhinitis

A participation of platelets in the pathogenesis of asthma and rhinitis has been documented for a number of years. Primarily, platelet activation occurs during antigen-induced airway reactions in asthmatic patients. An altered functionality is manifested as heightened platelet activation *in vivo*, while platelets from the same allergic patients are found to be refractory to a variety of stimuli *ex vivo*, possibly resulting from platelet 'exhaustion', due to the inability of platelets to replenish many released mediators that require *de novo* synthesis because platelets lack a nucleus (Harker *et al.*, 1980;

Correspondence: Dr SC Pitchford, Leukocyte Biology Section, National Heart and Lung Institute, Imperial College, 3rd Floor, Sir Alexander Fleming Building, London SW7 2AZ, UK.
E-mail: s.pitchford@imperial.ac.uk
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Pareti *et al*., 1980). Platelet 'exhaustion' has been reported as an inability of noradrenaline and adenosine di-phosphate (ADP) to induce full aggregation of platelets, with no second-phase aggregation, an occurrence that has been correlated with increased serum immunoglobulin E (IgE) in asthmatic patients (Maccia *et al*., 1977; Palma-Carlos *et al*., 1991). However, full aggregation of platelets *in vitro* returns in the same patients when studies are repeated outside of the allergy (pollen) season (Maccia *et al*., 1977).

This alteration in platelet function has been associated with bronchial hyperresponsiveness that accompanies nocturnal asthma (Gresele *et al*., 1993). The phenomenon of platelet activation in response to allergen-induced anaphylaxis has been shown to be beyond the control of agents that stimulate cyclic adenosine monophosphate and metabolites of the arachidonate pathway. Platelet responses to allergen are thus different to platelet responses to normal aggregatory stimuli. Indeed, while non-steroidal anti-inflammatory drugs (NSAIDs) block platelet aggregation, platelet–leukocyte interactions are not blocked by NSAIDs and represent a mechanism of platelet activation during inflammation that is distinct from platelet aggregation (Storey *et al*., 2002; Li *et al*., 2003).

Atopy is also accompanied by a prolonged bleeding time, increased platelet mass and volume, and decreased platelet survival. This accelerated platelet consumption correlates to a shortened time taken to regenerate the platelet population. These phenomena can be corrected by treatment of asthmatic patients with glucocorticoids, or platelets treated with di-sodium chromoglycate *in vitro* before re-infusion, although these anti-inflammatory drugs have no known direct affects on platelet activation (Taytard *et al*., 1985; Tunon-De-Lara *et al*., 1992).

Large numbers of pulmonary megakaryocytes (precursors to platelets) and platelets have been obtained at autopsy from patients who have died from status asthmaticus. In addition, platelets are localized to various tissue compartments in the lung parenchyma of biopsies taken from asthmatic patients (Metzger *et al*., 1987; Jeffery *et al*., 1989) and this event is accompanied with bone marrow karyopoiesis and thrombopoiesis (Slater *et al*., 1985). This is perhaps the result of localized platelet recruitment and activation within lungs, since circulating venous platelet numbers have been shown to fall during both early- and late-phase responses to allergen (Kowal *et al*., 2006). Platelet–platelet, and platelet–leukocyte aggregates have also been detected in patients with spontaneous asthma attacks (Gresele *et al*., 1993). This occurs in a biphasic manner following allergen challenge and results in an increase in the expression of CD11b, an activation marker on the surface of leukocytes (Pitchford *et al*., 2003).

Raised levels of platelet-derived mediators such as the chemokines – $\beta$-thromboglobulin ($\beta$-TG) and platelet-factor 4 (PF-4) – are observed in plasma and broncho-alveolar lavage fluid of atopic individuals compared to normal individuals during allergen exposure (Slater *et al*., 1985; Gresele *et al*., 1993), while an increase in serum CD40 ligand (CD40L) of platelet origin has also been reported recently (Kowal *et al*., 2006a). Other platelet-derived mediators have also been observed in atopic patients after allergen provocation, including regulated upon activation normally T-cell expressed and secreted (RANTES, CCL5), platelet selectin (P-selectin), 5-hydroxytryptamine (5-HT), adenosine, histamine, platelet-derived growth factor (PDGF), platelet-activating factor (PAF), the *de novo* production of arachidonic acid metabolites including prostaglandin $E_2$ and thromboxane ($TXA_2$), platelet-specific lipoxygenase products including hydroyeicosatetraenoic acid, lysosomal enzymes such as matrix metalloproteinases (MMPs) and mediators sequestered from the circulation (for example, IgE) (reviewed in Pitchford and Page, 2002).

Production of antigen-specific IgE in response to allergen provocation is integral to atopic diseases. Interestingly, IgE binds to between 20 and 30% of platelets from normal individuals, this binding affinity rises up to the binding of 50% of platelets from patients with allergies (Maccia *et al*., 1977; Joseph *et al*., 1986). Platelets from atopic individuals are characterized by a much greater IgE content stored in $\alpha$-granules compared to non-atopics, which correlates to serum IgE levels from atopic patients. Stimulation of platelets from atopic patients resulted in the release of 65% of stored IgE levels by PAF stimulation but not by platelet mediators involved in aggregation, for example thrombin and ADP (Klouche *et al*., 1997).

## Platelet activation in chronic obstructive pulmonary disease

The involvement of platelets in chronic obstructive pulmonary disease (COPD) is less well researched than the involvement of platelets in asthma. However, the occurrence of platelet hyperreactivity has been demonstrated in *ex vivo* studies where platelets had an increased sensitivity to various agonists, and elevated levels of plasma $\beta$-TG and soluble P-selectin of platelet origin have been reported (Cordova *et al*., 1985; Ferroni *et al*., 2000). These reports reflect the occurrence of *in vivo* platelet activation as measured by increased synthesis of $TxA_2$ in patients with COPD, and the administration of a $TxA_2$ antagonist was beneficial in improving respiratory distress in patients with chronic pulmonary emphysema (Davi *et al*., 1997).

## Platelet activation in rheumatoid arthritis

Clinical studies have demonstrated that activation of circulating platelets occurs in patients with rheumatoid arthritis (RA) (Endresen, 1989; Joseph *et al*., 2001), and platelets have been observed in the synovial fliud of patients with RA (Farr *et al*., 1984; Endresen, 1989; Endresen and Forre, 1992). Interestingly, heterotypic platelet–monocyte and platelet–neutrophil complexes occur in the circulating blood of patients with RA (Endresen and Forre, 1992; Bunescu *et al*., 2004), and in common with other inflammatory conditions, these interactions may contribute to leukocyte activation and recruitment to the synovium.

## Platelet activation in inflammatory bowel disease

Patients suffering from exacerbations of Crohn's disease and ulcerative colitis have an increase in circulating platelet

numbers (Morowitz *et al*., 1968). This is often associated with a reduced platelet lifespan and reduction in mean platelet volume (Webberley *et al*., 1993; Jaremo and Sandberg-Gertzen, 1996). Furthermore, platelets from inflammatory bowel disease (IBD) patients are more sensitive to platelet agonists *in vitro* (van Wersch *et al*., 1990), while the platelet-specific chemokines PF-4 and $\beta$-TG are detected in plasma, revealing activation *in vivo* (Collins *et al*., 1994; Vrij *et al*., 2000). A role for platelets in mediating leukocyte recruitment to the inflamed colon is likely since platelet P-selectin and RANTES are also detected (Fagerstam *et al*., 2000), and this is localized to the intestinal microcirculation (Collins *et al*., 1997). Recent evidence suggests that increased circulating levels of soluble CD40L are of platelet origin in IBD patients (Danese *et al*., 2003a). Furthermore, platelets mediate leukocyte recruitment via CD40–CD40L interactions in patients with IBD and in a murine model of colonic inflammation induced by dextran sodium sulphate (Danese *et al*., 2003b; Vowinkel *et al*., 2007). Interestingly platelet activation may also be involved in chronic inflammatory events occurring in IBD as CD40–CD40L interactions have been shown to be necessary for angiogenesis in a murine model of IBD (Danese *et al*., 2007).

## Platelet activation in atherosclerosis

Inflammatory processes are a recognized feature of atherosclerotic lesions, eventually causing plaque rupture. The link between immune system activation and cardiovascular disease has been demonstrated through the involvement of inflammatory cytokines (Ross, 1999). In particular, activated endothelium attracts the adherence and accumulation of monocytes and CD4 and CD8T cells (Hansson *et al*., 1989; Hansson and Libby, 1996). In addition to this, evidence is accumulating to suggest that chemokines play a central role in the development of atherosclerotic plaques, with stromal cell-derived factor-1 (SDF-1, CXCL-12), monocyte chemoattractant protein-1 (MCP-1, CCL-2), RANTES, interleukin-8 (IL-8) and eotaxin observed in atherosclerotic plaques (Wilcox *et al*., 1994; Abi-Younes *et al*., 2000; Haley *et al*., 2000). This leads to macrophage infiltration into fatty streaks where the production of cytokines such as tumour necrosis factor-$\alpha$ (TNF$\alpha$), IL-1, transforming growth factor-$\beta$ (TGF$\beta$), proteolytic enzymes and growth factors secreted by immune cells precede plaque destabilization and rupture.

Platelet adhesion and thrombus formation is a ubiquitous feature in the initiation and generation of atherosclerotic lesions. However, interactions between platelets and inflammatory cells take place during atherosclerosis and this stimulation of an inflammatory response within the atherosclerotic plaque may trigger acute coronary events via reactive oxygen species (ROS) production and MMP secretion (Poubelle and Borgeat, 2002). Substantial clinical evidence demonstrates activation of circulating platelets in diseases with a substantial inflammatory component acting on the vasculature, for example, acute coronary syndromes such as myocardial infarction and unstable angina (Sarma *et al*., 2002) and atherosclerosis (Massberg *et al*., 2002). These studies suggest a participation of platelets in the inflammatory responses as well as the recognized events leading to thrombus formation. Platelet binding to leukocytes occurs during acute coronary events, and these heterotypic aggregates are formed as a result of activation by inflammatory mediators (Arber *et al*., 1991; Ott *et al*., 1996) and are largely bound via P-selectin/P-selectin glycoprotein ligand-1 (PSGL-1) interactions (Sarma *et al*., 2002). It is worth noting that an increased expression of CD40L occurs on the surface of platelets in acute coronary syndromes (Garlichs *et al*., 2001), although the significance of this is not yet known, it reveals another mechanism whereby platelets may further stimulate the inflammatory response during atherosclerosis.

## Inflammatory mechanisms affected by platelets

Various mechanisms common to many diseases have been documented whereby platelets modulate the inflammatory response. Such mechanisms include intravascular 'priming' of leukocytes for efficient recruitment to tissue, chronic inflammatory events leading to tissue remodelling and regeneration, release of platelet-derived mediators that cause tissue damage directly, and the involvement of platelets linking the innate and adaptive immune responses (Figure 1).

## Leukocyte recruitment and activation: influence of platelets

Circulating platelet–leukocyte complexes are a feature of a wide cross-section of inflammatory diseases, and it is believed that this phenomenon 'primes' resting circulating leukocytes for efficient recruitment to inflamed tissue. For example, studies with un-separated leukocyte populations reveal a significant increase in platelet–leukocyte complexes in allergic mice and in human asthmatics (Pitchford *et al*., 2003, 2005). Similar processes occur in patients with COPD (Ferroni *et al*., 2000), atherosclerosis (Arber *et al*., 1991; Ott *et al*., 1996; Neumann *et al*., 1997; Sarma *et al*., 2002; Huo *et al*., 2003) and RA (Joseph *et al*., 2001; Bunescu *et al*., 2004). In this regard, experimental models of disease have provided evidence for a requirement of platelets in pulmonary eosinophil and lymphocyte recruitment in rabbits, guinea-pigs and mice in models of allergic inflammation (Lellouch-Tubiana *et al*., 1988; Coyle *et al*., 1990; Pitchford *et al*., 2003, 2005); and neutrophil and monocyte recruitment in atherosclerosis (Arber *et al*., 1991; Neumann *et al*., 1997; Hayward *et al*., 1999) and RA (Schmitt-Sody *et al*., 2005). This phenomenon requires intact platelets expressing mediators on the cell surface, and in common with the occurrence of leukocyte recruitment in inflammatory diseases, platelet P-selectin is of particular importance (Diacovo *et al*., 1996a, b; Schober *et al*., 2002; Huo *et al*., 2003; Pitchford *et al*., 2005). With regard to asthma, this mechanism has been confirmed by various *in vitro* studies, revealing eosinophil attachment to inflamed endothelium is greatly enhanced in the presence of platelets taken from asthmatic patients, and P-selectin expressed by platelets is responsible for platelet–eosinophil interactions in particular (Jawien *et al*., 2002; Ulfman *et al*.,

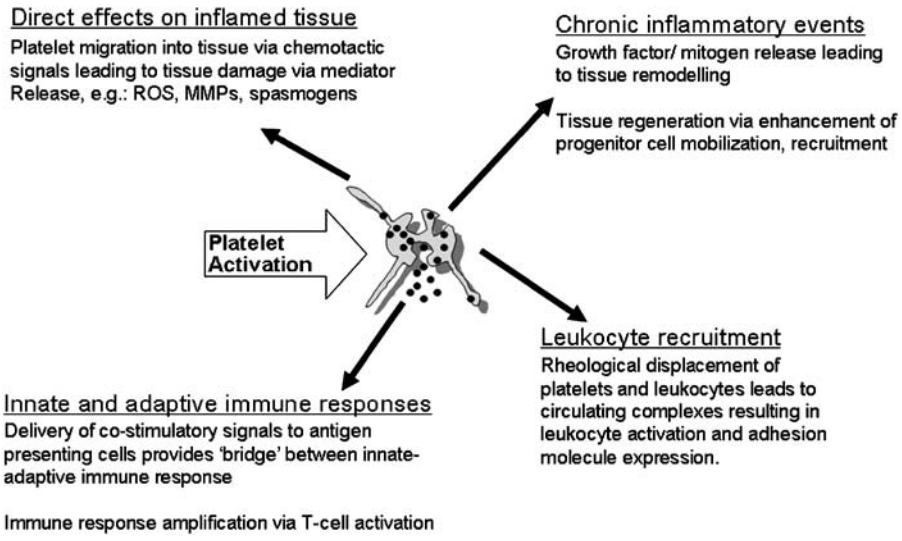


**Figure 1** Platelet participation in inflammation.

2003). Circulating leukocytes attached to platelets display significant increases in CD11b and very late antigen-4 integrin (VLA-4) expression, compared to leukocytes not attached to platelets, and circulating platelet–leukocyte complexes in non-inflamed animals (Pitchford *et al.*, 2005). Thus, platelets have the ability to activate leukocytes at the level of contact-dependent signalling and prime them for endothelial attachment. We and others have shown that the occurrence of platelet–leukocyte complexes is abolished by the administration of antibodies to P-selectin and its counter ligand PSGL-1, demonstrating the importance of platelet P-selectin on this mechanism (Mayadas *et al.*, 1993; Katayama *et al.*, 2000; Pitchford *et al.*, 2005).

Selectin-mediated rolling is thus an essential step towards firm cell–cell adhesion directed by $\beta_2$-integrins. This can result in the absence of exogenous stimuli (Yeo *et al.*, 1994) and is supported by CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac 1) expression induced by P-selectin–PSGL-1 interactions, as PSGL-1 functions as a signalling molecule (Evangelista *et al.*, 1996; Blanks *et al.*, 1998; Konstantopoulos *et al.*, 1998). Engagement of PSGL-1 by P-selectin results in tyrosine phosphorylation of a 110 kDa protein (Evangelista *et al.*, 1999) and activation of mitogen-activated protein kinase (Hidari *et al.*, 1997) in leukocytes complexed to platelets (Evangelista *et al.*, 1999). However, P110 tyrosine phosphorylation also requires integrin–counterligand interactions on the surface of platelets, resulting eventually in the complete adhesion (Evangelista *et al.*, 1999). Recent evidence reveals the importance of Src-family tyrosine kinases (SFKs) in stabilizing CD11b/CD18 interactions with platelets (Evangelista *et al.*, 2007). A principal $\beta_2$-integrin present on activated platelets is intercellular adhesion molecule-2 (ICAM-2) (Diacovo *et al.*, 1994). Platelet-derived ICAM-2 mediates lymphocyte–platelet adhesion via CD11a/CD18; and ICAM-2 may also contribute to neutrophil rolling and firm arrest, mediated by CD11b/CD18 under flow conditions (Kuijper *et al.*, 1998). Glycoprotein-Ib$\alpha$ (GPIb$\alpha$) has also been identified as a ligand for CD11b/CD18 (Simon *et al.*, 2000), and leukocyte engagement of platelet GPIb$\alpha$ via CD11b/CD18 has been shown to be critical for leukocyte accumulation in a mouse femoral artery injury model (Wang *et al.*, 2005).

Several other immunoglobulin-type receptors have also been described on platelets, including platelet endothelial cell adhesion molecule-1 (PECAM-1), endothelial cell selective adhesion molecule, junctional adhesion molecule-(JAM)-1 and -3, which are localized around tight junctions of endothelium and epithelium and modulate barrier function around the cleft of adjacent cells (Ozaki *et al.*, 1999). The physiological function of JAMs on platelets remains unclear; however, it is plausible that these receptors play a part in platelet adhesion to the sub-endothelium (Nasdala *et al.*, 2002). JAM-1 has been described as a counter-receptor for CD11a/CD18 (Ostermann *et al.*, 2002). Moreover, JAM-3, another novel counter receptor for CD11b/CD18 facilitates platelet–leukocyte interactions, and together with GPIb$\alpha$, appears to be the predominant counter-receptor for CD11b/CD18 (Santoso *et al.*, 2002). Analysis of different blood cell populations indicates that JAM-3 is exclusively expressed on platelets (Santoso *et al.*, 2002). Recent evidence suggests inhibition of JAM-3 and PECAM-1 completely inhibited neutrophil trans-endothelial migration *in vitro* and soluble JAM-3 administration significantly reduced neutrophil emigration in a murine model of peritonitis (Chavakis *et al.*, 2004). Therefore, JAMs may modulate the final process of platelet–leukocyte transmigration via the most apical regions of the inflamed endothelium.

Interestingly, and perhaps because of the diverse array of ligands by which stable platelet–leukocyte interactions occur, other cellular events unrelated to the tethering of leukocytes to endothelium have been reported. These include inflammatory gene activation. For example, P-selectin bound to antigen-primed $CD4^+$ T cells differentially modulates the production of pro-inflammatory cytokines (Damle *et al.*, 1992), and thus T-cell activation may be facilitated via adhesion with activated platelets. Furthermore, CD11b/CD18 can modulate NF-$\kappa$B activity via IL-1 receptor signalling pathway (Shi *et al.*, 2001). Biochemical events may also occur, since SFK activation via CD11b/CD18

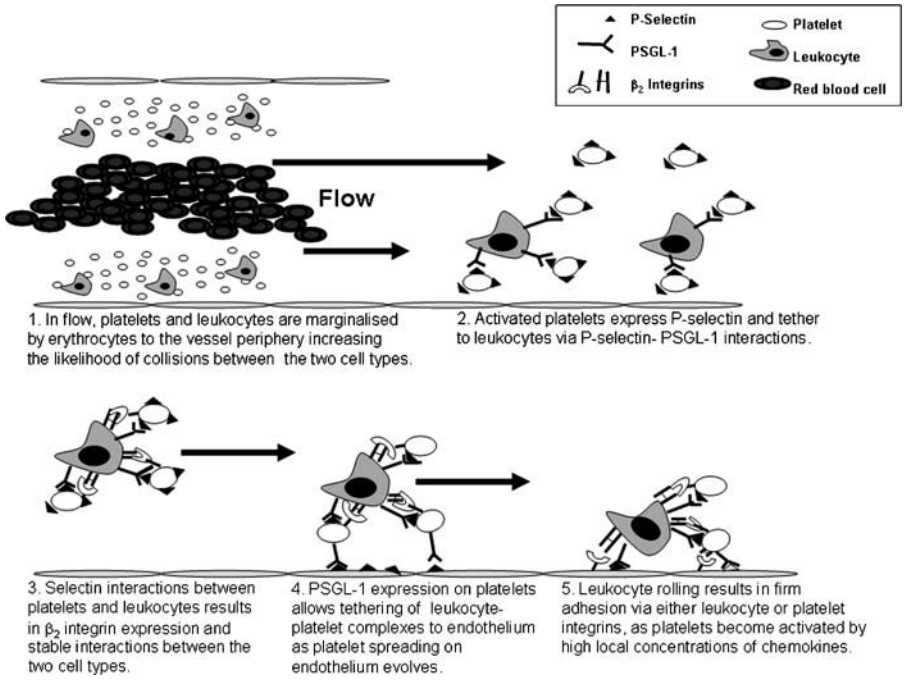


**Figure 2** Enhancement of leukocyte trafficking by platelet tethering.

activation results in respiratory burst and oxygen-free radical release, contributing to tissue damage (Lowell *et al*., 1996). Lastly, evidence supports a direct role for platelet P-selectin in phagocytosis by neutrophils, which is a recognized CD11b/CD18-dependent function (Cooper *et al*., 1994).

Platelet–leukocyte complexes form as a result of the rheological displacement of blood cells. This 'traps' leukocytes into an environment rich in platelets towards the vessel periphery, greatly enhancing the possibility of collisions between platelets and leukocytes (Figure 2). However, unless platelets become activated by inflammatory stimuli, the result of these collisions on circulating platelet–leukocyte complex formation appears to be unresponsive in terms of adhesion to the vascular endothelium, as recognized by the presence of circulating platelet–leukocyte complexes found in control subjects (Pitchford *et al*., 2003, 2005). It would appear that platelets require additional inflammatory stimuli above that which is required for stable interactions between platelets and leukocytes before firm adhesion to the endothelium takes place.

It is feasible that platelet activation and formation of platelet–leukocyte complexes is directed by a number of chemokines, since SDF-1, monocyte-derived chemokine (MDC, CCL22), thymus and activation-regulated cytokine (TARC, CCL17) and fractalkine can activate platelets in the presence of ADP (Abi-Younes *et al*., 2000, 2001; Kowalska *et al*., 2000; Schafer *et al*., 2004) via their receptors CXCR4, CCR1, CCR3, CCR4 and CX3CR1 (Clemetson *et al*., 2000; Schafer *et al*., 2004). Platelet activation by chemokines in the presence of low levels of ADP (insufficient alone to cause aggregation) is a very rapid process, resulting in near maximal activation within 5 s of stimulation. The rapidity of this response is highlighted by chemokine stimulation of platelet adhesion under flow, and exposed P-selectin to the platelet surface (Gear *et al*., 2001), which is temporally similar to leukocyte adhesion under flow (Alon and Feigelson, 2002). Thus, arterial blood flow concentrates platelets close to the endothelium, drawing platelets into the vicinity of higher local concentrations of chemokines released from endothelial cells. This signalling may result in the upregulation of selectins and integrins on the surface of platelets (Gear *et al*., 2001), enabling platelets to bind and activate circulating leukocytes. A relationship between platelet P-selectin expression and platelet–leukocyte endothelial arrest after chemokine activation has since been revealed in several studies (Schober *et al*., 2002; Huo *et al*., 2003; Von Hundelshausen *et al*., 2005).

## Platelets and tissue remodelling events

One consequence of persistent, chronic inflammation is alteration to tissue structure and function. In atherosclerosis, this may result in neo-intima formation (Ross, 1999), while in asthma, chronic inflammation may contribute to changes in airway architecture observed in this disease, referred to as airway remodelling (Vignola *et al*., 2000). Some of these processes may be independent of the separate requirement of platelets for leukocyte recruitment, since platelets may release a number of mitogens and enzymes that may contribute to tissue remodelling directly. As an example, airway remodelling occurs in experimental models where leukocyte recruitment has been inhibited by glucocorticosteroid administration, but not in animals depleted of platelets (Pitchford *et al*., 2004a). Thus, platelets may directly affect chronic inflammatory events that lead to smooth muscle proliferation, angiogenesis, myofibroblast proliferation and fibrosis (Tutluoglu *et al*., 2005; Zernecke *et al*.,

2005). Mechanisms that drive tissue remodelling are not fully understood. However, the recruitment and proliferation of circulating stem and progenitor cell populations, for example mesenchymal stem cells, endothelial progenitor cells and fibrocytes, have been reported (Schmidt *et al*., 2003; Zernecke *et al*., 2005; Jin *et al*., 2006; Massberg *et al*., 2006). While it is equally feasible that resident structural cells partake in tissue remodelling, there is an increasing body of evidence from studies of diseases with remodelling phenomena which suggest these processes may be greatly enhanced by the participation of progenitor cells, as they become recruited and undergo *in situ* proliferation and differentiation according to the micro-environment (Schmidt *et al*., 2003; Zernecke *et al*., 2005; Jin *et al*., 2006; Massberg *et al*., 2006). Interestingly, platelet-derived SDF-1$\alpha$ has been shown to be necessary for 'hermangioblast' mobilization from the bone marrow (Jin *et al*., 2006), while platelet P-selectin has been shown to be required for their recruitment to inflamed endothelium in models of atherosclerosis (Massberg *et al*., 2006). Furthermore, smooth muscle progenitor cells have been shown to require chemokine presentation by platelets for efficient recruitment (Zernecke *et al*., 2005), and platelets are also required for re-epithelialization of damaged corneal tissue (Li *et al*., 2006), with similar mechanisms occurring in airway wall remodelling in asthma. Thus platelets may directly participate in the tissue regenerative responses that occur as a result of progenitor cell mobilization from the bone marrow.

Platelets may also directly contribute to a favourable microenvironment for wound repair since platelets contain cellular mitogens such as PDGF, epidermal growth factor, insulin-like growth factor, TGF$\beta$ and vascular endothelial growth factor (VEGF) among other growth factors (Rendu and Brohard-Bohn, 2002). Interestingly, the major product of arachidonic acid metabolism in platelets, $TxA_2$, is known to induce the proliferation of smooth muscle cells and also endothelial cell migration and angiogenesis (Dorn, 1997; Daniel *et al*., 1999). PDGF affects human, rat and rabbit smooth muscle mitogenesis (Hirst *et al*., 1992). PDGF also acts as a potent chemoattractant for fibroblasts and has been implicated in pulmonary fibrosis (Bonner *et al*., 1998). TGF$\beta$ increases smooth muscle cell mitogenesis in culture, and it has also been suggested to increase airway obstruction by participating in sub-epithelial fibrosis via its chemotactic properties for fibroblasts and neutrophils (Okona-Mensah *et al*., 1998). VEGF is necessary for angiogenesis during vascular remodelling of ischaemic tissues and also contributes to increases in airflow resistance in obstructive lung disease (Lee *et al*., 2004). Angiogenesis is also a feature of airway remodelling in asthmatic individuals. The formation of new vessels within the lung parenchyma is inversely correlated to airway calibre and airways hyperresponsiveness (Hoshino *et al*., 2001).

Platelets may themselves directly alter the composition of the extracellular matrix. Within lysosomes, platelets contain a number of enzymes, termed MMPs, These enzymes are believed to disrupt the composition and integrity of cell membranes by degrading GPs, glycolipids and glycosaminoglycans (Ciferri *et al*., 2000; Falcinelli *et al*., 2005). The outcome of this is thought to induce the diapedesis of leukocytes, as well as release membrane-bound growth factors for wound repair (Corry *et al*., 2002). The implications of these actions are profound in the progression of disease as they may facilitate inflammatory cell diapedesis and stimulate tissue remodelling.

## Platelet involvement in antigen recognition

Platelets may also interact with immuno-modulatory cells, or platelets may become directly activated by immunoglobulins such as antigen-specific IgE in allergic inflammation. The actions discussed below strongly indicate that platelets serve an important role in the development of adaptive immunity.

Platelets secrete, or express, a number of factors that have been shown to activate T-lymphocytes, for example the chemokines: RANTES, monocyte chemoattractant protein-3 (MCP-3, CCL7) and macrophage inflammatory protein-1$\alpha$ (MIP-1$\alpha$, CCL3) may be released by platelets when in contact with T-lymphocytes via CD40–CD40L interactions (Sallusto *et al*., 1998). CD40–CD40L interactions can induce many cell-mediated inflammatory and immune responses. The release of such mediators can amplify the immune response to antigen by inducing further activation of T-lymphocytes (Danese *et al*., 2004). CD40L has been identified on activated platelets (Henn *et al*., 1998), and is functionally active, mediating IgM–IgG isotype switching, a crucial event in humoral immunity (Elzey *et al*., 2003). CD40L can also lead to the activation of endothelial cells to have a pro-inflammatory phenotype (Danese *et al*., 2004). Indeed, stimulation of endothelial cells by platelets expressing CD40L significantly contributes to inflammatory cell recruitment in atherosclerosis (Buchner *et al*., 2003). Interestingly, the production of allergen-specific IgE and airway hyperresponsiveness are suppressed in allergen-sensitized mice deficient in either CD40 or CD40L (Mehlhop *et al*., 2000). Such interactions may also be important in linking innate responses to that of an adaptive immune response involving platelets, since platelets activated by thrombin induce the activation and maturation of primary bone marrow dendritic cells (Czapiga *et al*., 2004). This process has been shown to be dependent on platelets delivering co-stimulatory signals via CD40L–CD40 expressed by antigen-presenting cells (APCs; Czapiga *et al*., 2004). Stimulation via this pathway leads to IL-12 production by APCs, and the surface expression of CD80 and CD83, and as such platelets may provide a bridge between tissue trauma and acquired immunity (Czapiga *et al*., 2004). It has also been demonstrated that platelets are able to undergo chemotaxis to formyl-Met-Leu-Phe (Czapiga *et al*., 2005), and are found proximal to dendritic cells in various tissue compartments (Pitchford *et al*., 2006). This contact may interfere with dendritic cell differentiation and cytokine production (Kissel *et al*., 2006).

Production of antigen-specific IgE in response to allergen provocation is a fundamental hallmark of atopic diseases (Burrows *et al*., 1989; Hamelmann *et al*., 1999). The cross-linking of antigen to IgE on the surface of mast cells is believed to provide the stimulus for mast cell degranulation in early-phase allergic reactions, an event that precipitates a

cascade of inflammatory events in response to allergen (Martin *et al*., 1989, 1993; Oshiba *et al*., 1996). Patients allergic to *Dermatophagoides pteronyssinus* (Der p1) and exposed to synthetic peptides derived from the allergen Der p1 were shown to have activated platelets. This was a process mediated by IgE, that did not stimulate platelets from healthy subjects or non-Der p1 allergic patients, illustrating the specific activation of platelets to allergic stimuli (Cardot *et al*., 1992).

Platelets contain both the high- ($10^{-9}$ M) and low-affinity ($10^{-7}$ M) receptors for IgE (FcεRI and FcεRII/CD23, respectively) on the surface membrane (Joseph *et al*., 1986, 1997; Cines *et al*., 1986; Hasegawa *et al*., 1999). However, it is apparent that only a few platelets express both FcεRI and FcεRII simultaneously (Joseph *et al*., 1997), and these may represent a subset of platelets that react in a dichotic manner to inflammatory stimuli compared to 'normal' platelets. The involvement of platelets in allergic inflammation may well represent inappropriate actions of platelets commonly displayed in IgE-mediated immunity against helminth and protozoan parasitic infections (Joseph *et al*., 1983, 1985; Momi *et al*., 2000). Platelet activation via FcεRI has been shown to induce the release of 5-HT, ROS and RANTES, demonstrating that platelets may play an important role in the progression of allergic inflammation via IgE-dependent mechanisms (Joseph *et al*., 1986; Klouche *et al*., 1997). It has since been shown that platelets accumulate in the lungs and de-granulate following antigen challenge in sensitized mice, preceding histamine release from mast cells, and platelets may therefore participate towards anaphylaxis directly in response to IgE (Yoshida *et al*., 2002). Platelets from asthmatic patients and allergic mice have been observed to undergo chemotaxis in response to allergen exposure, via platelet-bound, antigen-specific IgE, and this *in vitro* phenomenon is reciprocated *in vivo* as platelets migrate through lung tissue in response to allergen exposure towards the airway wall (as the focus of allergen exposure) (Zhang *et al*., 1993; Pitchford *et al*., 2004b).

The process of platelet activation by IgE has been demonstrated to be inhibited by drugs used for the treatment of atopic asthma and allergies, such as nedocromil sodium, disodium cromoglycate and cetirizine (Thorel *et al*., 1988; Tsicopoulos *et al*., 1988; De Vos *et al*., 1989; Joseph *et al*., 1989, 1993; Tunon-De-Lara *et al*., 1992). IgE stimulation of platelets represents a non-thrombotic pathway by which platelets can be specifically activated by allergen, and thus directly contribute to the inflammatory responses observed in allergy.

## Anti-platelet drugs that modulate inflammation

Some anti-platelet drugs are in use clinically with actions that are known to affect the inflammatory pathways in which platelets are involved, for example purinergic receptor antagonists. An example is clopidogrel, which is used in the treatment of thrombosis, and has beneficial effects on atherosclerosis. A new generation of $P2Y_1$ and $P2Y_{12}$ antagonists has since been developed, and it will be interesting to observe how their anti-inflammatory properties translate to diseases other than atherosclerosis. P-selectin antagonists, on the other hand, have been developed for their anti-inflammatory properties, and the translation from atherosclerosis to other inflammatory diseases has been more forthcoming, while studies of drugs that anatagonise the actions of pleiotropic mediators released by platelets have also shown efficacy in animal models of inflammation. In the future, drug development may focus on the recent increase in understanding of platelet-dependent mechanisms that control certain inflammatory pathways, and also exploit differences in platelet activation in thrombosis compared to inflammation.

## Purinergic receptor antagonists

Three purinergic receptors are expressed on the surface of platelets. The $P2X_1$ cation channel is activated by adenosine tri-phosphate (ATP), while two G protein-coupled receptors – $P2Y_1$ and $P2Y_{12}$ – are both activated by ADP (Kunapuli, 1998). All three receptors have a role in platelet activation and aggregation. However, differences in the activation kinetics of these last two receptors opens distinct possibilities for the use of antagonists to these receptors as anti-inflammatory compounds.

Activation of $P2Y_1$ coupled to Gαq leads to $Ca^{2+}$ release, resulting in platelet shape change and a transient aggregation to ADP. While activation of platelets to ADP via $P2Y_1$ is of low potency, it is a requisite step towards further activation of platelets by ADP and collagen. However, $P2Y_1$ does not significantly contribute to the platelet aggregation by other agonists. Selective $P2Y_1$ antagonists have been developed and include MRS2179, MRS2500 and MRS2279, which mimic ATP. Activation of $P2Y_{12}$ coupled to $G_{i2}$ results in full-platelet aggregation and irreversible clot formation *in vivo*. Activation of platelets via $P2Y_{12}$ amplifies aggregation initiated by $P2Y_1$; however, it is also necessary for complete aggregation induced by other platelet agonists, for example collagen, thrombin, $TXA_2$, adrenaline and 5-HT. $P2Y_{12}$ is the target of established inhibitors clopidogrel, ticlopidine and prasugrel; and newer antagonists such as AR-C69931X, AR-C66096MX, AZD6140 and C1330-7. Despite differences in the individual contribution of $P2Y_1$ and $P2Y_{12}$ activation on platelet aggregation, co-activation is necessary for full ADP-induced aggregation since antagonism of either receptor results in a decrease in the aggregatory response (Hechler *et al*., 1998; Jin and Kunapuli, 1998; Cattaneo, 2005).

Evidence now suggests that purinergic receptors are important for platelet-mediated inflammation and offer a new opportunity for suppression. Both $P2Y_1$ and $P2Y_{12}$ activation leads to the expression of platelet P-selectin and the formation of platelet–leukocyte complexes (Leon *et al*., 2003, 2004). The activation of platelets via chemokines and low levels of primary agonists such as ADP have been shown to be dependent on the purinergic $P2Y_1$ receptor rather than the $P2Y_{12}$ receptor (Suttitanamongkol and Gear, 2001), which is believed to have a greater role in sustained thrombus formation (Leon *et al*., 2003; Nylander *et al*., 2003; Mazzucato *et al*., 2004). Furthermore, $P2Y_1$ is involved in platelet–monocyte complex formation when platelets are

stimulated by lysophosphatidic acid (Haseruck *et al*., 2004). Although the phenomenon of platelet–leukocyte complex formation and adhesion has not been tested after platelet activation via chemokines, ADP signalling through $P2Y_1$ may contribute to the initial stages of platelet activation in an inflammatory setting. This may resemble a 'bottleneck' in the directing of leukocyte migration into inflamed tissue via the orchestration of chemokines. Thus, selective inhibition of the $P2Y_1$ in inflammatory diseases may be beneficial because antagonism by MRS2179- and $P2Y_1$-deficient mice leads to only a moderate prolongation of bleeding time (Leon *et al*., 1999; Fabre *et al*., 1999; Baurand *et al*., 2001). This could be advantageous as an anti-inflammatory drug as effects on the normal function of platelets in haemostasis may not be compromised.

Reported inhibition of both the $P2Y_1$ and $P2Y_{12}$ receptors leads to a decrease in inflammatory parameters *in vivo*. The administration of MRS2179 leads to a suppression of pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation (Pitchford and Page, 2006), while $P2Y_1$ and apolipoprotein E (ApoE) double 'knockout' mice have a significant reduction in atherosclerotic plaque size (Gachet, 2006). Recently, a role for platelets has been reported for leukocyte recruitment in a murine model of chronic contact hypersensitivity, where clopidogrel administration reduced cell infiltration into skin tissue and the production of chemokines: MIP-1$\alpha$, RANTES and TARC (Tamagawa-Mineoka *et al*., 2007). Specific $P2Y_1$ antagonism reduces platelet P-selectin expression and the occurrence of platelet–leukocyte complexes (Storey *et al*., 2000; Leon *et al*., 2003), which may represent a mechanism by which $P2Y_1$ antagonism has efficacy in reducing the extent of inflammation *in vivo*. Furthermore, the administration of clopidogrel also reduces platelet P-selectin expression, decreased platelet–PMN adhesion and platelet-dependent ROS production in neutrophils (Evangelista *et al*., 2005), findings reciprocated with thienopyridine and AR-C69931MX (Storey *et al*., 2002a, b; Leon *et al*., 2003), but not aspirin (Storey *et al*., 2002a).

Clinically, the effectiveness of clopidogrel has been compared to aspirin in the 'Clopidogrel Versus Aspirin in Patients at Risk of Ischaemic Events' trial (CAPRIE study) in patients with atherosclerotic disease (CAPRIE Steering Committee, 1996). An 8.7% relative risk reduction was demonstrated with the use of clopidogrel compared to aspirin in the occurrence of vascular death, ishaemic stroke or myocardial infarction. This beneficial effect was multiplied in high-risk patients (Bhatt *et al*., 2001) and in patients suffering from diabetes (Bhatt *et al*., 2002). Thus, the anti-inflammatory effects may account for the increased efficacy with the use of the $P2Y_{12}$ antagonist compared to aspirin. Indeed, clopidogrel withdrawal results in the re-emergence of inflammation in patients with diabetes and coronary artery disease (Angiolillo *et al*., 2006). Clopidogrel significantly reduces platelet-associated inflammatory markers in renal transplant patients with no clinical signs of atherosclerosis (Graff *et al*., 2005) and patients with stable coronary artery disease, for example P-selectin and CD40L release, platelet–leukocyte complexes and MMP9 release (Klinkhardt *et al*., 2002; Azar *et al*., 2006). This increased inflammatory activity of platelets in renal transplant patients may account for the high cardiovascular mortality rate as a result of the development of atherosclerotic lesions in such patients.

While the majority of reports illustrates the anti-inflammatory nature of P2Y receptor antagonism, some investigations suggest otherwise. In particular, clopidogrel administration has been shown to increase the expression of RANTES and MIP-1$\beta$ from peripheral blood mononuclear cells, and platelet P-selectin and CD63 (a marker of platelet lysosome release) expression remained unchanged in patients with coronary artery disease (Waehre *et al*., 2006). Since the group of patients studied had stabilized coronary heart disease, the baseline inflammation and platelet activation may have been lower than in other studies, thus making it difficult to accurately access the effect of clopidogrel, although a lack of efficacy on RANTES levels has been reported elsewhere (Bahrmann *et al*., 2002). Nevertheless, evidence overwhelmingly points to an efficacy of P2 antagonists on inhibition of platelet–leukocyte complex formation and platelet P-selectin expression, and it is necessary to investigate the effects of these drugs on inflammatory diseases other than atherosclerosis.

## P-selectin inhibition

The surface expression of P-selectin on activated platelets, the requirement of this adhesion molecule in the formation of platelet–leukocyte aggregates, the ensuing leukocyte activation and subsequent diapedesis have made P-selectin a potential anti-platelet target. Investigations have used a diverse array of compounds to inhibit P-selectin, from blocking antibodies, soluble protein ligands, oligosaccharides and small molecule antagonists.

Efforts have been made to engineer blocking antibodies for P-selectin or PSGL-1. In particular, the antibody RB40.34 has proven *in vivo* efficacy in models of ischaemia (Lehmberg *et al*., 2006). Furthermore, pulmonary eosinophil and lymphocyte recruitment were inhibited with the administration of RB40.34 in a murine model of allergic inflammation (Pitchford *et al*., 2005). The concept has been advanced towards the clinic with the production of a humanized antibody (mEP.SC7), which has been shown, to block binding of a leukaemia cell line to P- and endothelial-selectin (E-selectin), and has favourable pharmacokinetic properties when administered to Rhesus monkeys (He *et al*., 1998). Other attempts to block P-selectin have been made by targeting the counter ligand PSGL-1, either via the administration of antibodies or recombinant proteins. For example, intimal hyperplasia of the carotid artery was prevented with an anti-PSGL-1 immunoglobulin in a balloon injury model in pigs (Wang *et al*., 2001) and attenuated infarct size during ischaemia–reperfusion injury in dogs (Wang *et al*., 2002). These effects may be attributable to the ability of rPSGL-Ig to reduce leukocyte rolling and adhesion to acute inflamed endothelium (Eppihimer and Schaub, 2001; Theoret *et al*., 2001). Separate from cardiovascular disease, an rPSGL-1 antibody ameliorates cell accumulation, TNF$\alpha$ levels and joint severity in a murine model of RA (Sumariwalla, 2004). P-selectin-mediated cell adhesion has also been specifically

inhibited by phage display-derived peptide antagonists with high potency (Molenaar *et al*., 2002), which are reported to be most effective in tetrameric form.

Other P-selectin inhibitors include fucoidans extracted from brown seaweed. Fucoidans effectively inhibit leukocyte recruitment to inflamed peritoneum in rats (Preobrazhenskaya *et al*., 1997; Cumashi *et al*., 2007). *In vitro* evaluation showed inhibition of P-selectin-mediated neutrophil adhesion to platelets under flow conditions.

Synthetic low-molecular weight P-selectin antagonists have also been produced that mimic the carbohydrate moieties on the P-selectin counter ligands, being largely based on Sialyl LewisX. These have potent *in vivo* and *in vitro* activity. For example, oligosaccharides have been shown to inhibit eosinophil and neutrophil adhesion to immobilized platelets (Kim *et al*., 1998), and the monosaccharide dimer: bimosiamose (TBC1269) decreases reperfusion injury after myocardial infarction in rats (Onai *et al*., 2003). Although the activity of bimosiamose may also be attributable to antagonism of E-selectin, it has shown promising results during clinical development, having improved skin lesions in psoriasis patients and airway reactivity in mild asthmatics (Beeh *et al*., 2006; Friedrich *et al*., 2006). Interestingly, a synthetic pentasaccharide devoid of anti-coagulant properties and derived from fondaparinux (Frank *et al*., 2006) has recently been reported to reduce inflammation in a murine model of kidney ischaemia. Previous studies reveal this anti-inflammatory activity may be attributed to the ability of fondaparinux to inhibit P-selectin-dependent adhesion of U937 cells *in vitro* and a reduction in the recruitment of neutrophils to the peritoneum of thioglycolate-treated mice that is also dependent on platelet P-selectin (Frank *et al*., 2005). It must be stated, however, that other oligosaccharides, for example the pentasaccharide CY1503, that have been developed for reperfusion injury have not demonstrated clinical efficacy (Kaila and Thomas, 2002), but this has been attributed to poor bioavailability.

Lastly, small molecule inhibitors have been developed in the guise of quinoline salicylic acids. Having been evaluated for their ability to antagonise P-selectin, quinilone salicylic acid antagonists act by competing with the sialyl Lewisx moieties on P-selectin ligands (Kaila *et al*., 2007). Antagonism has been shown to be efficacious in a rat antigen-induced arthritis model of RA (Kaila *et al*., 2007). These perhaps offer an exciting subset of compounds that antagonise P-selectin with favourable pharmacodynamic profiles that may allow them to progress with efficacy through clinical trials.

## Antagonists of pleiotropic mediators released by platelets

Experimental data suggest that established anti-platelet agents (ridogrel) indicated in the treatment of other inflammatory diseases inhibit pathological processes with similarities to processes involved in the pathogenesis of asthma (Anderson *et al*., 2001). Inhibitors of $TXA_2$ are effective in inhibiting pulmonary leukocyte recruitment in murine models of allergic inflammation (Shi *et al*., 1998) and may act via the inhibition of $TXA_2$ synthase by platelets and antagonism of $TXA_2$ on effector cells. Indeed, these have been shown to be effective when used either alone or in combination for suppressing antigen-induced bronchoconstriction in guinea pigs (Yoshimi *et al*., 2001). Other drugs have been developed with dual leukotriene D4 and $TXA_2$ receptor antagonism with efficacy in experimental models of allergic inflammation (Yamada *et al*., 2003; Ishimura *et al*., 2006). However, the efficacy of $TXA_2$ synthase inhibitors and receptor antagonists does not spread to all inflammatory diseases where activated platelets are a component. $TXA_2$ release is a feature of Crohn's disease but ridogrel lacks efficacy (Carty *et al*., 2001), despite showing efficacy in experimental colitis models (Vilaseca *et al*., 1990).

Inhibitors of 5-HT, for example ketanserin (De Bie *et al*., 1998), are effective in inhibiting indices of allergic inflammation, acting on 5-$HT_2$ receptors. Indeed, studies reveal 5-HT originating from platelets is capable of altering the pathogenesis of asthma, since drug (tianeptine) induced 5-HT uptake by platelets has been shown to reduce the clinical severity of asthmatic patients (Lechin *et al*., 1998).

## GPIIb/IIIa integrin blockers: a cautionary note

The GP IIb/IIIa integrin is found on the platelet membrane and is the final common pathway in platelet aggregation. Intravenous antagonists of the GPIIb/IIIa integrin have significant clinical benefits in patients with acute coronary syndromes undergoing percutaneous coronary intervention (Bhatt and Topol, 2000; Topol *et al*., 2001). However, sub-therapeutic doses of GPIIb/IIIa antagonists especially orally active compounds have been shown to have deleterious outcomes on patients (Chew *et al*., 2001; Quinn *et al*., 2002). While this outcome may be the result of partial agonism at sub-therapeutic concentrations (Cox *et al*., 2000), leading to 'platelet escape' and thrombus formation, a pro-inflammatory profile of GPIIb/IIIa antagonists is apparent. *In vitro* studies reveal that both monoclonal antibodies and non-peptide inhibitors increase platelet P-selectin expression and platelet–leukocyte complexes (Caron *et al*., 2002; Klinkhardt *et al*., 2002), as well as the release of CD40L and tissue factor (Zhao *et al*., 2003) and may explain the negative clinical effects of GPIIb/IIIa antagonists. These studies also demonstrate that pathways leading to platelet aggregation are distinct to pathways leading to platelet activation of P-selectin expression and CD40L release. Thus, future drug design may be successful in inhibiting pro-inflammatory platelet activation but not platelet aggregation in inflammatory diseases where an inhibition of clotting could be deleterious.

## Future drug targets

Mediators and receptors that can be selectively targeted to inhibit platelet activation rather than platelet aggregation may be clinically relevant in the treatment of asthma, COPD, RA, IBD and atherosclerosis. Indeed, exploiting mediators involved in platelet activation that are disease specific would

obviously have advantages. Antagonists for specific chemokine receptors may be advantageous, for example the targeting of platelet CCR3 and CCR4 might be of benefit in treating asthmatics (Gonzalo *et al*., 1999; Sekiya *et al*., 2000), while the targeting of RANTES may be beneficial in the treatment of atherosclerosis since platelet-derived RANTES deposited on the surface of endothelial cells is necessary for monocyte accumulation (Von Hundelshausen *et al*., 2005). Indeed, RANTES receptor antagonists inhibit the infiltration of macrophages, and importantly, reduce neointima formation in ApoE-deficient mice (Schober *et al*., 2002; Huo *et al*., 2003).

CD40L is a principal mediator in inflammation, and the majority (>90%) of CD40L produced in the body is derived from platelets (Henn *et al*., 1998). CD40L is therefore a very attractive drug target for inhibiting platelet activation as it can induce an inflammatory cascade centred on the activation of endothelium and T cells (Buchner *et al*., 2003; Danese *et al*., 2004). Few reports exist of studies describing CD40 antagonism, although a monoclonal antibody (5D12) is currently being tested for the treatment of Crohn's disease (Kasran *et al*., 2005).

Another attractive target is sphingosine-1-phosphate (S1P), a lipid mediator stored in platelets, which are the major source of S1P in plasma (Yatomi *et al*., 2000). The mechanisms by which S1P modulate the pathogenesis of inflammation are ill defined but S1P acts via five specific receptors, $S1P_{1-5}$ (Chun *et al*., 2002). S1P can activate monocytes, endothelial cells, mast cells, eosinophils, smooth muscle cells and promote tissue recruitment of leukocytes (Roviezzo *et al*., 2004). Studies reveal an involvement for S1P in asthma, RA, IBD and atherosclerosis (Ammit *et al*., 2001; Kitano *et al*., 2006; Daniel *et al*., 2007; Nofer *et al*., 2007). Attempts have been made to produce antagonists to the S1P receptors, for example FTY720, and it has been demonstrated to ameliorate disease pathogenesis in models of colitis and atherosclerosis (Daniel *et al*., 2007; Nofer *et al*., 2007).

Peroxisome proliferator-activated receptors (PPARs) are another novel target for inhibiting platelet activation, acting as transcription factors for lipid and glucose metabolism. While being nuclear receptors, all three subtypes are expressed in platelets – $\alpha$, $\beta$ and $\gamma$ – despite platelets being anucleate. Selective agonists for all three receptors (fenofibrate: PPAR$\alpha$; GW0742 and L165041: PPAR$\beta$; and rosiglitazone: PPAR$\gamma$) are capable of inhibiting platelet aggregation (Ali *et al*., 2005). Recently, PPAR$\gamma$ agonists acting on platelets have been shown to have anti-inflammatory properties, inhibiting platelet release of CD40L and $TXA_2$ production (Akbiyik *et al*., 2004; Ray *et al*., 2006). The efficacy of PPAR agonists in inflammatory diseases needs to be thoroughly investigated.

A dichotomy of platelet function in thrombosis compared to inflammation is highlighted in the fact that only a subset of platelets displays receptors for IgE. This dichotomy in platelet function needs to be thoroughly researched and a better understanding of the molecular pathways by which inflammatory mediators activate platelets as opposed to aggregatory pathways may hold promise for future therapeutics. Similarly, the molecular mechanisms governing platelet granule and lysosome release are currently being investigated and may have potential. For example, the exocytotic pathway in platelets is unique as platelet-shape change leads to an organized rearrangement of secretory granules. Specific membrane proteins control the fusion of granules to the platelet membrane, and these include vesicle membrane proteins: vSNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) from the synaptobrevin/VAMP (vesicle-associated membrane protein) bind to complexes in the target membrane tSNAREs composed of syntaxins- and synaptosomal-associated proteins (for example, SNAP-23). Dense core and $\alpha$-granule secretion is mediated by the tSNARES: SNAP-23 and syntaxin 2 (Chen *et al*., 2000; Feng *et al*., 2002). However, differences are apparent in the requirement of different VAMPs for dense and $\alpha$-granule secretion (Polgar *et al*., 2002). Exploitation of these differences and differences that may be apparent compared to other secretory cells could, therefore, lead to selective inhibitors of platelet granule release of pro-inflammatory mediators and adhesion molecules.

## Concluding remarks

Despite recent advances revealing the phenomenon of platelet participation in inflammation, detailed investigations of platelet-dependent mechanisms of disease pathogenesis are in their infancy. It is therefore not surprising that as yet no specific therapy has been developed for the treatment of inflammatory diseases based on inhibiting platelet function. Clearly, the mechanisms that differentiate the necessary role of platelets in haemostasis to that of platelet activation in an inflammatory setting need to be distinguished to allow the emergence of targets for novel safe therapies. Nevertheless, the efficacy of platelet inhibition in the suppression of inflammation in disease models; suggests that potential drug targets directed at inhibiting platelet function may provide alternative and powerful treatments for inflammatory diseases in the future.

## Conflict of interest

The author states no conflict of interest.

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