Platelets, inflammation and tissue regeneration

Alan T. Nurden

Centre de Référence des Pathologies Plaquettaires, Plateforme Technologique et d'Innovation Biomédicale, Hôpital Xavier Arnozan, Pessac, France

Summary

Blood platelets have long been recognised to bring about primary haemostasis with deficiencies in platelet production and function manifesting in bleeding while upregulated function favourises arterial thrombosis. Yet increasing evidence indicates that platelets fulfil a much wider role in health and disease. First, they store and release a wide range of biologically active substances including the panoply of growth factors, chemokines and cytokines released from α-granules. Membrane budding gives rise to microparticles (MPs), another active participant within the blood stream. Platelets are essential for the innate immune response and combat infection (viruses, bacteria, microorganisms). They help maintain and modulate inflammation and are a major source of pro-inflammatory molecules (e.g. P-selectin, tissue factor, CD40L, metalloproteinases). As well as promoting coagulation, they are active in fibrinolysis; wound healing, angiogenesis and bone formation as well as in maternal tissue and foetal vascular remodelling. Activated platelets and MPs intervene in the propagation of major diseases. They are major players in atherosclerosis and related diseases, pathologies of the central nervous system (Alzheimers disease, multiple sclerosis), cancer and tumour growth. They participate in other tissue-related acquired pathologies such as skin diseases and allergy, rheumatoid arthritis, liver disease; while, paradoxically, autologous platelet-rich plasma and platelet releasate are being used as an aid to promote tissue repair and cellular growth. The above mentioned roles of platelets are now discussed.

Keywords

Platelets, immunity, inflammation, wound healing, angiogenesis, acquired diseases with platelet involvement

Correspondence to:
Alan T. Nurden
CRPP/PTIB
Hôpital Xavier Arnozan
33600 Pessac, France
Tel.: +33 5 57 10 28 51, Fax: +33 5 57 10 28 64
E-mail: Alan.Nurden@cnrshl.u-bordeaux2.fr

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Introduction

By their capacity to adhere to exposed subendothelium in a flow-dependent manner, to aggregate and to facilitate thrombin generation, platelets form the haemostatic plug and in so doing promote fibrin generation and blood clotting. In this way they stop blood loss. Yet, it is now clear that platelets have other important biological roles and act as circulating sensors in health and disease (1, 2). Platelets aid in recruiting leukocytes and progenitor cells to sites of vascular injury and inflammation. They induce changes in cell permeability and promote chemotaxis and cell proliferation, essential steps in tissue repair. The object of this review is to describe recent advances in our understanding of the mechanisms through which platelets and their secreted products intervene in inflammation and tissue regeneration. In so doing, I will highlight how (a) platelets are a source of active metabolites and proteins, (b) promote heterotypic cell interactions, (c) provide a surface together with cell-derived microparticles that promotes coagulation and protease activation and (d) exert an active role in sepsis and fighting infection (including promoting the innate immune response) (►Fig. 1). Platelets either secrete proteins into the milieu or present them on the activated platelet surface. As well as discussing the role of secreted products, I will discuss examples of pathological states such as atherosclerosis, Alzheimer's disease and cancer where activated platelets have a known role.

Platelets as a source of biologically active metabolites

One way by which platelets respond to stimulation is by secreting newly synthesised soluble metabolites. Thromboxane A_2 (TXA_2), a strong amplifier of platelet activation and a powerful vasoconstrictor, is also involved in the injury-induced vascular proliferative response. Sphingosine 1-phosphate, a novel active metabolite able to stimulate mitogenesis, is liberated from activated platelets during clotting. It stimulates fibronectin matrix assembly through a Rho-dependent signalling pathway (3). This sphingolipid can also induce tissue factor (TF) expression on endothelial cells and promote osteoclast differentiation (4, 5). Lysophosphatidic acid (LPA) induces endothelial cell migration, a process regulated by extracellular matrix molecules (6). Platelet-derived LPA was shown to support the progression of osteolytic bone metastases in breast cancer

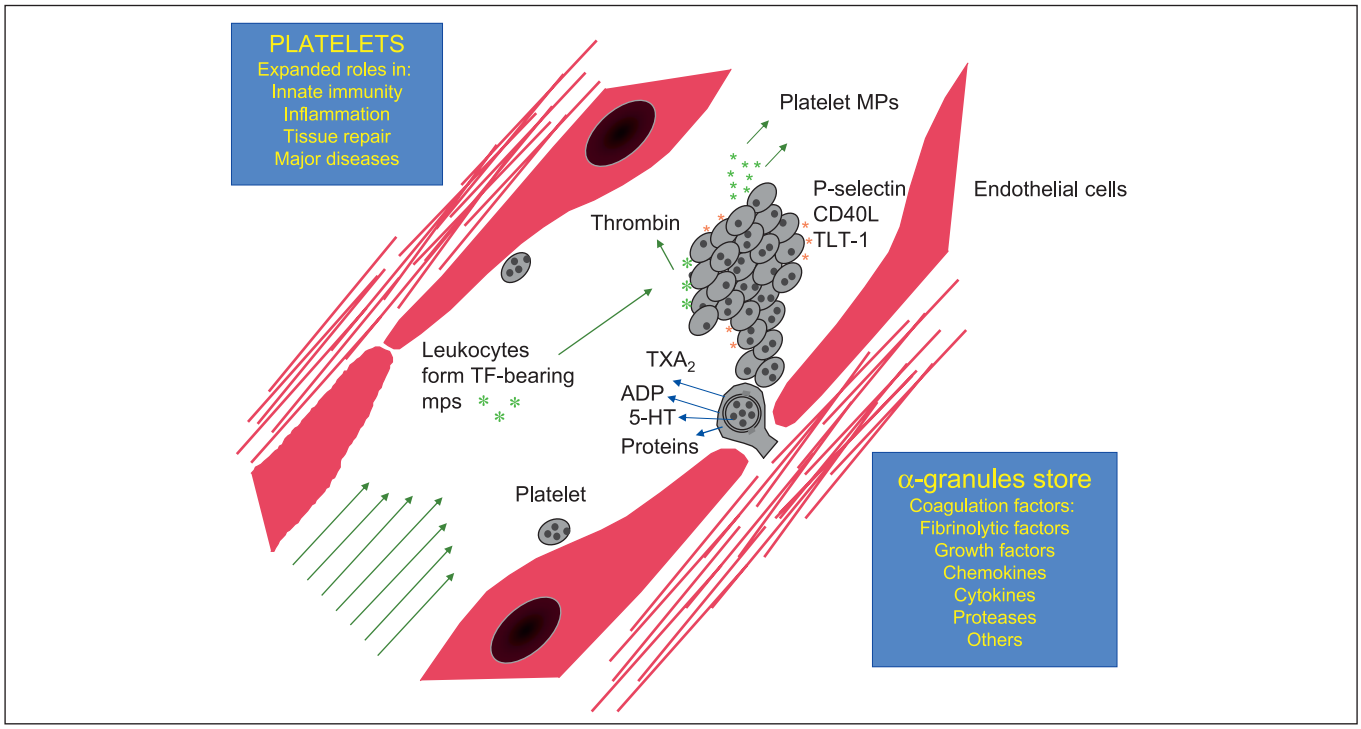

Figure 1: Cartoon showing a platelet aggregate formed at a site of vessel injury and illustrating the release of active metabolites (e.g. thromboxane A2, TXA_2), dense granule constituents (e.g. ADP, 5-HT [serotonin]) and α-granule proteins. Some of the major categories of secreted proteins are given in the panel, a comprehensive list is given in Table 1. Surface expression of α-granule membrane associated proteins (e.g. P-selectin, CD40L) and release of microparticles (MPs) is shown while tissue factor (TF) accumulates in the platelet membrane. All of these processes are involved in the new roles for platelets in innate immunity, inflammation, tissue repair as well as in the pathogenesis of major diseases.

where tumour cells induced LPA release from platelets and in turn the tumour cells responded by proliferating (7). Platelet-activating factor (PAF) is another platelet-derived bioactive lipid; acting in cooperation with P-selectin, it can play a role in mediating leukocyte arrest; a process intriguingly enhanced in platelets showing signs of apoptosis (8). PAF is able to induce the migration of endothelial and other tissue cells. PAF and leukotriene B4 may act as toll-like receptor (TLR) ligands to induce polymorphonuclear leukocyte migration (9). The endocannabinoid, 2-arachidonyl glycerol is another lipid mediator secreted by platelets (10).

Secretion from platelet granules

Platelets contain several types of secretory organelle with dense granules, α-granules and lysosomes being the most important. Morphologically distinct, the granules contain storage pools of active substances that are released following adhesion to collagen or other matrix components or in a dose-dependent response to soluble agonists such as ADP or thrombin. The most abundant source of proteins is the α-granule. Initial proteomic strategies for the analysis of the platelet secretome using multidimensional protein identification technologies suggested that > 300 proteins are released, some being well characterised; whilst many are not (11).

Dense granules

Release of substances from dense granules is by exocytosis, a process that requires Rab proteins and complex secretory machinery (reviewed in [12]). Among the released constituents are ADP/ATP, inorganic polyphosphate, pyrophosphate, serotonin and Ca^{2+}. Released ADP is an essential co-factor of platelet aggregation acting through the Gq-coupled $P2Y_1$ and Gi-coupled $P2Y_{12}$ receptors and is essential for primary haemostasis (13). ATP, on the other hand, acts through the ligand-gated $P2X_1$ receptor and is an important co-factor both in collagen-induced platelet aggregation and in epinephrine-mediated potentiation of the response to low dose thrombin (14). Purinergic signalling can influence vascular tone at sites of thrombus formation (15). Calcium is a central regulator of wound healing and is essential for fibrin formation, whilst polyphosphates are a recently recognised regulatory element of the coagulation and fibrinolytic systems reacting, amongst others, with factor XII (Hagemann factor) (16, 17). Serotonin not only mediates vasoconstriction and capillary permeability but also plays a role in liver regeneration as seen after hepatectomy in mice (18). Yet, in a mouse model of chronic hepatitis, serotonin released from activated platelets promoted sinusoidal microcirculatory failure and delays in the entry of CD8+ T cells helping virus persistence in the liver and aggravating the virus-induced pathology (19).

α-granules

The α-granules are the major storage organelles for secreted proteins (►Table 1). Their biogenesis occurs in megakaryocytes (MKs), begining with the budding of small vesicles containing neo-synthesised protein cargo from the trans-Golgi network and continuing through multivesicular bodies (MVBs) with orchestrated trafficking and fusion involving coat proteins (e.g. clathrin, COPII), adaptor proteins (e.g. AP-1, -2 and -3), fusion machinery (e.g. soluble NSF attachment protein receptors [SNARES]) and monomeric GTPases (e.g. Rabs) (data reviewed in 20). Preformed α-granules are transported from the MK body on microtubule tracks within the pro-platelets; the granules are captured by the developing platelets prior to their release into the circulation (21). The above processes are accompanied by clathrin-mediated endocytosis of surface receptor-bound plasma proteins (e.g. fibrinogen [Fg] bound to αIIbβ3) while pinocytosis also occurs (e.g. albumin, IgG). In MKs, both types of vesicle converge to the MVBs; therefore, the latter represent a developmental stage in α-granule maturation. Clathrin-dependent endocytosis of Fg continues throughout the platelet lifespan. In ►Table 1, the proteins are grouped where possible in terms of their functional properties. Many of the proteins are discussed below; P-selectin, CD40 ligand (CD40L), TF and metalloproteinases (MMPs) are given more prominence and feature later in the text.

Adhesive proteins

Abundant in quantity are Fg, fibronectin (Fn), vitronectin (Vn) and thrombospondin-1 (TSP-1). In part, they attach to internal pools of membrane receptors during secretion and participate in aggregation by supplementing the adhesive proteins in plasma that bind to activated surface receptors and bring about thrombus formation under flow (22). Elevated local concentrations of adhesive proteins may have other roles; for example, Fg can act as a mitogen while Fn potentiates the action of platelet-derived growth factor (PDGF) (data reviewed in [2]). Part of TSP is retained after thrombin-induced secretion, and forms mixed high density domains with Fg on the platelet surface (23). An excellent way of studying protein storage in α-granules of MKs or platelets is by immunofluorescence (IF) and confocal microscopy. ►Figure 2 illustrates the organisation of several α-granule proteins and compares their punctate IF-labelling with that of membrane glycoproteins Ib and αIIbβ3 and α-tubulin concentrated within subproximal

Table 1: Platelet α-granule contents and their functional categories.

Adhesive proteins	VWF + pro-peptide, Fg, Fn, Vn, TSP-1, TSP-2, laminin-8 (also α5-laminin subunit),	Cell contact interactions, haemostasis and clotting, extracellular matrix constituents
Clotting factors and their inhibitors	Factor V/Va, factor XI, multimerin, protein S, high-molecular-weight kininogen, protease nexin-1 and -2, TF pathway inhibitor (TFPI)*, protein C inhibitor, gas6**	Thrombin production and clotting. Cell proliferation
Fibrinolytic factors and their inhibitors	Plasminogen, PAI-1, u-PA, α2-antiplasmin, histidine-rich glycoprotein, thrombin-activatable fibrinolysis inhibitor (TAFI,) α2-macroglobulin	Plasmin production and vascular modelling
Proteases and anti-proteases	MMP-1, -2, -4, -9, ADAMTS13, ADAMS10, ADAMS17 (TACE), TIMPs 1–4, platelet inhibitor of FIX, C1 inhibitor, α1-antitrypsin	Angiogenesis, vascular modelling, regulation of coagulation, regulation of cellular behaviour
Growth and mitogenic factors	PDGF (A, B and C), EGF, IGF-1, VEGF (A and C), bFGF (FGF-2), HGF, BMP-2, -4, -6, CTGF, SCUBE1, IGFBP3	Chemotaxis, cell proliferation and differentiation, angiogenesis
Chemokines, cytokines and others	TGF-β1 and -β2, IL-1, RANTES (CCL5), IL-8 (CXCL8), MIP-1α (CCL3), MIP-2 (CXCL2), LIX (CXCL6) GRO-α (CXCL1), ENA-78 (CXCL5), SDF-1α (CXCL12), MCP-1 (CCL2), MCP-3 (CCL7), PF4 (CXCL4), pro-platelet basic protein (PBP), β-TG, NAP-2, connective-tissue-activating peptide III T(CXCL7), thymus and activation-regulated chemokine (TARC, CCL17), angiopoietin-1, High mobility group box 1 (HMGB1), IL-6sR, endostatin*, osteonectin*, bone sialoprotein, dickkopf-1, osteoprotegerin	Regulation of angiogenesis, chemotaxis, vascular modelling, cellular interactions, bone formation
Anti-microbial proteins	Thrombocidins and kinocidins	Bactericidal and fungicidal properties
Others	Chondroitin 4-sulfate, albumin, immunoglobulins G and M, amyloid β-protein precurser, disabled-2, complement factor H, bile salt-dependent lipase (BSDL), semaphorin 3A, PrP^C	Various
Membrane glycoproteins	αIIbβ3, αvβ3, GPIb, PECAM-1, most plasma membrane constituents, receptors for primary agonists, P-selectin, TLT-1, semaphorin 4D, CD63, CD40L, TF,* LIGHT, TNF-related apoptosis inducing ligand (TRAIL), FasL, furin, GLUT3, cellubrevin, SANP23, syntaxin-2, clathrin	Platelet aggregation and adhesion, endocytosis of proteins, secretion, inflammation, thrombin generation, platelet-leukocyte and platelet-vascular cell interactions

This list of proteins is as complete as possible but does not include the results of proteomic analyses (11, 95, 253). The abbreviations for many proteins are written in full in the text, space does not allow the addition of references for each protein. * The presence of TF is controversial; ** Gas6 is present in mouse but not in human platelets.

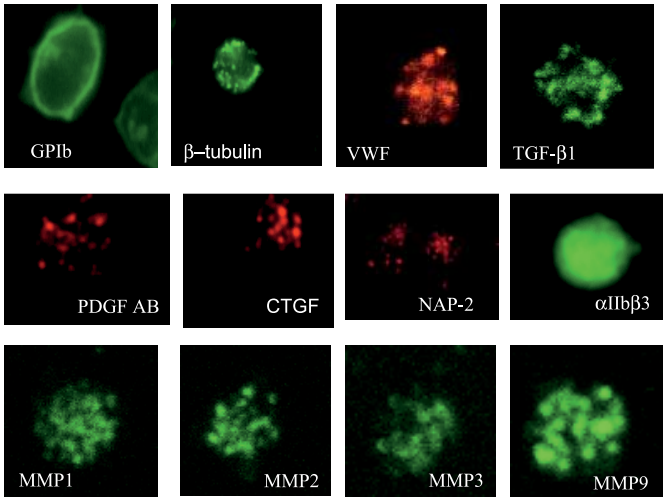

Figure 2: Immunofluorescence location of selected proteins in resting platelets. Studies were performed on platelets that were fixed with 1% paraformaldehyde (PFA) and permeabilised with 0.1% Triton X-100 prior to being incubated with commercial protein-specific mouse or rabbit antibodies followed by a second incubation with species-specific secondary antibodies labelled with Alexa-Fluor 488 (green) or Alexa-Fluor 568 (red) to mouse or rabbit IgG. Note the essential surface-membrane labelling of GPIb while αIIbβ3 labelling (performed with abciximab) extended to internal integrin pools. Slides were examined in a Zeiss Axioplan 2 microscope (Carl Zeiss, Le Pecq, France). Further methodological details are to be found in Villeneuve et al. (155). The α-tubulin is localised to the submembrane microtubule coils while the punctate IF labelling of the other proteins is synonymous with an internal organisation probably in α-granule pools. The abbreviations are given in the text.

membrane microtubular coils. Von Willebrand factor (VWF) is synthesised in MKs and IF studies suggest that VWF and Fg are found in different α-granule subpopulations (24). Other antigen pairs that fail to co-localise include vascular endothelial growth factor (VEGF) and endostatin, bFGF (FGF-2) (basic fibroblast growth factor-2) and TSP-1 (25) (the organisation of selected protein pairs is illustrated later in ▶Fig. 3). The hypothesis that pro- and anti-antigenic proteins are stored separately is supported by their differential release via activation of the PAR-1 and PAR-4 receptors by thrombin (26).

Growth factors, chemokines and cytokines

Platelets not only release growth factors (▶Table 1 and section on Angiogenesis), they are also a rich source of chemokines and cytokines (27). The most abundant is platelet factor 4 (PF4, CXCL4) a positively charged protein that binds to glycosaminoglycans. Not only does PF4 have a role in haemostasis/thrombosis, it is a chemotactic protein for monocytes and neutrophils, has immunoregulatory activity and is involved in a variety of pathologic states including septic shock (28). The structurally related platelet basic protein (PBP) serves as a precursor to active products such as β-thromboglobulin (CXCL7) and NAP-2 (neutrophil activating protein-2), a strong neutrophil chemoattractant (reviewed in [20]). NAP-2 has been implicated in repair cell homing to vascular lesions. Secreted RANTES (regulated upon activation, normal T cell expressed, and secreted/chemokine (C-C motif) ligand 5 [CCL5]) interacts with the endothelial surface in the presence of the cytokine interleukin 1β (IL-1β) and acts as a cell-associated signal for adhesion of monocytes (29). Interestingly, an interaction between platelet-derived RANTES and MCP-1, and MCP-4 may represent an inflammatory link between platelet and monocyte activation (30). Globally, released chemokines enhance recruitment of haematopoietic cells to the vascular wall, participating in vessel repair and regeneration after vascular injury. Cytokines are also present; as well as IL-1β; platelets express IL-6, IL-8. Human platelets activated by thrombin or PAF synthesise and release IL-1β in both microvesicles and soluble form and in sufficient quantitites to induce inflammatory gene expression in endothelial cells (1, 31).

Membrane receptors

The platelet α-granule membrane contains many of the functional receptors of the platelet surface including GPIb and αIIbβ3, which participate not only in thrombus formation but also in attaching platelets to other cells as part of inflammatory and immune interactions (32, 33). Absent from the unstimulated platelet surface, P-selectin mediates the interaction between activated platelets, leukocytes, immune cells, and endothelial cells. These interactions often involve multiple receptors with P-selectin on activated platelets or endothelial cells able to bind to P-selectin glycoprotein ligand (PSGL-1), Mac-1 and GPIbα amongst others. Selectins intervene at an early stage of leukocyte rolling and the principal leukocyte ligand for P-selectin is PSGL-1. This initial interaction leads to integrin activation and to the arrest of the cells. In parallel, the release of cytokines and chemokines influences the platelet-leukocyte interactions (e.g. PF4, RANTES, GRO-α [growth-related oncogene-α]) (34). These lead to up-regulation of certain leukocyte transcription factors, and the production of more cytokines and chemokines. Firm adhesion is mediated via the binding of Mac-1 to platelet GPIb and/or to Fg bound to αIIbβ3 on the platelet surface (reviewed in [32, 33]). Another platelet-expressed molecule that may participate in such interactions is JAM-C (junctional adhesion molecule-C), a surface glycoprotein of the immunoglobulin superfamily. JAM-C can interact homophilically, participating in platelet accumulation at injured sites as well as reacting with leukocytes or dendritic cells by binding to Mac-1 and contributing to vascular inflammation and atherosclerosis (35).

Lysosomes

These typically contain proteases (e.g. cathepsins, elastases) and other enymes (phosphatases, glycosidases) responsible for protein and matrix degradation and often favoured by an acidic environment (2, 20). These enzymes and membrane markers of lysosomes such as CD63 are not discussed in this review.

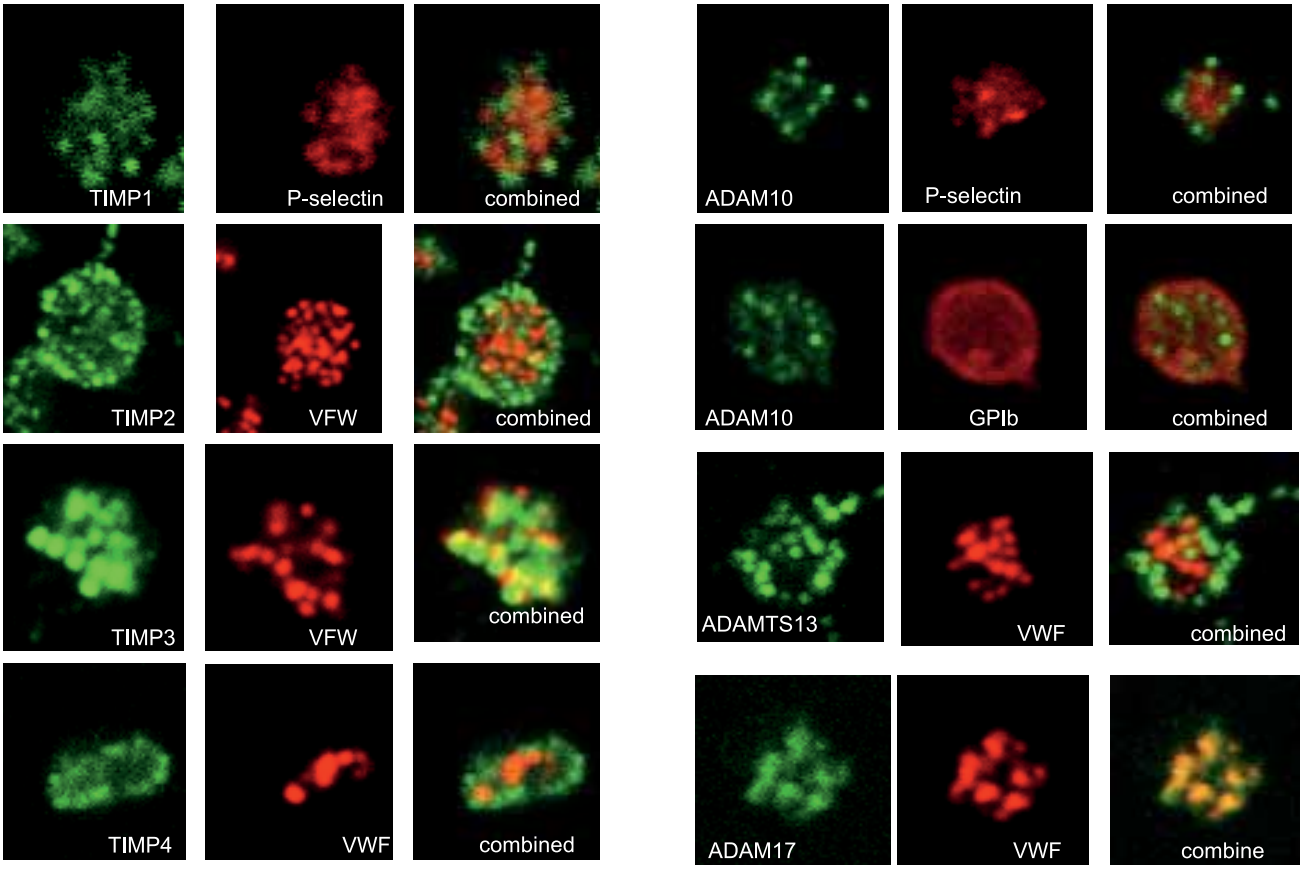

Figure 3: Confocal microscopy comparing the location of secretable proteins in resting platelets. Fixed and permeabilised platelets were prepared as in the legend to Figure 2. In two-colour microscopy, paired primary antibodies were from different species. Commercial protein-specific mouse or rabbit antibodies were used at predetermined dilutions followed by a second incubation with species-specific secondary antibodies labelled with Alexa-Fluor 488 (green) or Alexa-Fluor 568 (red) to mouse or rabbit IgG. Samples were visualised using a Leica SP5 confocal microscope using a 63X objective and Leica Microsystem LAS AF software (Leica Microsystèmes SAS, Rueil Malmaison, France). Further methodological details are to be found in Villeneuve et al. (155). In A) the localisation of TIMPs 1–4 is compared with VWF, appreciable co-localisation to α-granules was only found for TIMP-3. Note the presence of TIMP-2 within pseudopods. In B) the localisation of ADAM10, ADAM17 and ADAMTS13 is compared to VWF, α-granule localisation was only observed for ADAM17.

Mechanisms of platelet secretion

Secretion necessitates the transfer of granule contents to the exterior of the cell. This occurs when the granule membrane fuses with intracellular membrane systems that connect with the surface or the plasma membrane itself (36). This involves two categories of SNARE proteins: SNARES are glycoproteins that are incorporated in the membranes of secretory organelles (vesicular or v-SNARES) or are present on the target membrane (t-SNARES). The association between the two types of SNARE brings about membrane fusion. Platelets express multiple proteins that enter into the SNARE category, including VAMPS (vehicle-associated membrane proteins) (VAMP-2, -3, -7 and -8) while platelet t-SNARES include syntaxins 2, 4, 7 and 11 and SNAP-23 (synaptosomal-associated protein-23), -25 and -29 (data reviewed in 20). VAMP-8 is the dominant v-SNARE involved in α-granule release, and syntaxins 2 and 4 are also involved (37, 38). SNAP-23 and syntaxin-2 are involved in dense granule secretion. The Sec1/Munc (SM) complex acts as a clamp to regulate the function of SNARES (20). Rab27b regulates number and secretion of platelet dense granules (39).

Release of platelet microparticles

As well as providing a surface for thrombin generation, activated platelets show the characteristics of apoptotic cells with phosphatidylserine (PS) exposure and microparticle release. These processes occur by two distinct mechanisms (40). The first is Bak/Bax- and caspase-dependent but independent of platelet activation and extracellular calcium; in contrast, the second mechanism involves platelet activation and is calcium-dependent. A recent proteomic and functional characterisation of platelet microparticles has shown that they can be separated into different classes according to their size; and that they differ significantly with regard to their con-

tent of membrane receptors, adhesion molecules, chemokines, growth factors and protease inhibitors (41). Microparticles are biologically active; they are membrane templates that assemble pro-coagulant, fibrinolytic and proteolytic factors. They also carry surface receptors and biologically active proteins (chemokines, cytokines) and bioactive lipids. They differ from exosomes that are liberated from within the cell and originate in α-granules and MVBs of platelets. As well as platelets, other blood cells, endothelial cells and cells of neurovascular origin give rise to microparticles. Individual sub-populations can be quantified by targetting intrinsic cell-specific membrane glycoproteins (for a review see 42). Microparticles participate actively in ischaemic stroke, metastasis and tumour development as well as playing a role in inflammatory and neurogenerative disease (42–45).

Protein synthesis by platelets

Although platelets are anucleate, they have long been known to retain a limited capacity to synthesise proteins. Weyrich et al. showed that megakaryocytes and platelets possessed a spliceosome and were able to effect signal-dependent pre-mRNA splicing (46). This was first shown for IL-1β, where, after integrin engagement and receptor activation, platelets were able to excise introns from IL-1β pre-mRNA. The mature message was then translated into protein. A similar biosynthetic pathway in activated platelets was next shown for TF (47). Pre-mRNA splicing was controlled by Cdc2-like kinase and followed by increased TF expression, pro-coagulant activity, and accelerated formation of clots. Platelet-dependent protein synthesis requires prolonged stimulation, such as is found within clots, while the mechanism of protein release to the environment has yet to be defined.

Platelets, host defence and the immune response

Bacteria

Innate immunity is crucial for host survival during infection. Platelets have an early role in immune surveillance and act as sentinal cells. As reviewed by Yeaman (48), they react with and engulf bacteria and viruses, release anti-microbial and anti-fungal proteins, produce reactive oxygen species and play a role in modulating production of inflammatory cytokines. TLRs are perhaps the most important receptors of the innate immune system; often functioning as heterodimers, they recognise conserved structures of microorganisms including LPS as well as lipoproteins and other bacterial wall constituents. Platelets are said to possess TLR1, 2, 4, 6 and 9 (49, 50). TLR4 plays a major role in platelet adhesion to neutrophils, and in platelet enhancement of pro-inflammatory cytokine production. In severe sepsis, platelets can trigger not only the release of TNF-α and IL-6 but also the formation of neutrophil extracellular traps (NETs) consisting of DNA and nuclear proteins (51, 52). The NETs, whose function is to trap and clear pathogens from the circulation, are primarily formed in the liver sinusoids and in the pulmonary circulation. LPS-activated platelets bind to neutrophils already accumulated in the lungs or liver and promote NET formation; TLR4-deficient platelets are inactive. Sepsis is a complex syndrome whose pathophysiology involves inflammation, coagulation, apoptosis and altered endothelial permeability. Thrombocytopenia linked to platelet consumption can be severe in sepsis, and is linked to the rate of morbidity (53). Mortality in sepsis is reduced by the therapeutic use of activated protein C (APC) that dampens the excessive or insufficiently controlled host response (53). Platelet released products, including PF4, while enhancing the generation of APC *in situ* by the thrombin-thrombomodulin complex, inhibit APC anti-coagulant activity thereby targeting APC towards a protective function (54). Platelets accumulate at sites enriched in microbial stimuli such as N-formyl-methionyl-leucyl-phenylalanine or complement proteins C3a and C5a (48).

Stimulation via TLR2 resulted in P-selectin-dependent formation of platelet-neutrophil aggregates through the activation of PI3K/Akt as well as ERK1/2 and p38 signalling pathways (55). Engagement of TLRs may also promote the synthesis and release of reactive oxygen species. A recent study of the mechanism of TLR2 stimulation showed dependence on $P2X_1$-dependent Ca^{2+}-mobilisation, production of TXA_2 and ADP receptor activation (56). Interestingly, TLR2, which recognises a broad range of ligands, works in coordination with CD36 (abundant on platelets and macrophages), with the ectodomain of CD36 also reacting with negatively charged diacylglycerol ligands (released by bacteria) (57). Other innate receptors such as the triggering receptor expressed on myeloid cells (TREM), modulate the innate response either by amplifying or dampening TLR-induced signals. Platelets and megakaryocytes express TREM-like transcript-1 (TLT-1) that, like P-selectin, is mostly located in the α-granule membrane of resting platelets and only found on the platelet surface after platelet activation (58, 59). A soluble form of TLT-1 (sTLT-1) is released into plasma through the action of an unidentified MMP; its concentration is increased in sepsis and its presence correlated with disseminated intravascular coagulation (DIC). Significantly sTLT-1 can bind to Fg supporting a role in platelet aggregation and protection from haemorrhage. In addition, it may modulate neutrophil activation. Platelets also express a ligand for TREM-1 independently of their activation state and its interaction with TREM-1 on polymorphonuclear leukocytes (PMNs) enhanced LPS-induced respiratory burst and IL-8 production (60).

Viruses

Platelets actively participate in combatting viruses. Phagocytosis will result in virus removal from the circulation, but it also provides a means for their dissemination. For example, HIV viruses captured by platelets are found in endocytic vesicles and also in the

lumen of the surface-connected canalicular system where they were in contact with secreted proteins (61). Virus-IgG complexes are captured indirectly via FcγRIIA, while viruses themselves bind to a variety of surface receptors. These include integrins that recognise RGD-containing peptide sequences in viruses, complement (CD3) receptor type II, GPVI, coxackie-adenovirus receptor, CLEC-2 and DC-SIGN (61). CLEC-2 and DC-SIGN are important platelet receptors mediating the capture and transfer of HIV-1 virus (61, 62). Hantaviruses, associated with fever and both renal and cardiopulmonary syndromes, cause thrombocytopenia by directing platelets to infected endothelial cells to which the viruses bind via β3 integrins (63). Platelets activated by viruses can be captured in the liver with a special role played by Kuppfer cells while they can also be phagocytosed by spleen macrophages. Thrombocytopenia is a common event in viral infections, particularly in children.

Microorganisms and microbicidal proteins

Platelets play conflicting roles in the pathophysiology of malaria. For example, rapid activation of endothelial cells enables *Plasmodium falciparum* sequestration via CD36-dependent bridging of infected erythrocytes to platelet-decorated ultra-large von Willebrand factor strings (64). This pro-adhesive state is lost after the digestion of the VWF multimers by ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin motifs 13). Platelets are also able to kill malarial parasites and mediate survival to infection (65). One mechanism involves the preferential binding of activated platelets to infected red blood cells by a mechanism that was dependent on ADP (acting through $P2Y_1$) and TXA_2 and inhibited by aspirin. Binding of the platelets blocked development of the parasites. Platelets store a variety of proteins that can inactivate or kill pathogens; these are referred to as microbicidal proteins and kinocidins (2, 20, 48). Kinocidins refer to cytokines that are microbicidal; these include CXCL4, thymosin-β4, the derivatives of CXCL7 (PBP, CTAPIII-connective tissue activating peptide-III, NAP-2), MIP-1 (macrophage inflammatory protein-1) and RANTES. Onsite proteolysis plays a major role in creating microbicidal activity; for example, truncation of CTAP-III and NAP-2 at their c-terminus generates thrombocidins-1 and –2 (66). Among the secondary functions of kinocidins is the formation of chemotactic gradients for leukocytes and promoting phagocytosis (48). In a mouse model, platelets protected hosts by clearing lymphocytic choriomeningitis virus infection by acting with cytotoxic T lymphocytes (67). Activation of platelets by the fungus *Aspergillus fumigatus* leads to a role for platelets in Asperigillosis, an invasive mold infection associated with inflammation and thrombosis (68). This fungus potently induced P-selectin expression and the release of CD40L, RANTES and Dickkopf homolog1 in platelets. One consequence is an enhanced release of IL-8 from THP-1 monocytes and adherent monocytes.

Role of complement

The complement system consists of a series of proteases and inhibitors that are activated in cascade-like fashion during host defence. Platelets, together with platelet-derived MPs, focus complement to sites of vascular injury and inflammation (69). They express C1qRp/CD93 and α2β1 both of which bind C1q. Evidence points to P-selectin as a receptor for C3b, thereby localising the latter to inflamed sites of vascular injury (70). However, platelets also contain regulators of the alternative complement pathway including C1 inhibitor or complement factor H (CFH) so the net influence of platelets on complement activity may depend on local conditions. Interestingly, while secreted from platelets, CFH had a cytoplasmic location and was not localised to α-granules while it can bind to surface-expressed proteins such as αIIbβ3 or even TSP-1 (71). The arthus reaction is defined as a local antibody-mediated hypersensitivity reaction in which antibody–antigen complexes that fix complement are deposited in the walls of small vessels causing acute inflammation with an infiltration of neutrophils. Platelets were shown to control leukocyte recruitment in a murine model of cutaneous arthus reaction (72). Interaction of *Staphylococcus aureus* with platelets is part of a virulent mechanism in the induction of endocarditis, a process mediated by soluble fibrin and supported by TSP-1 (73). Secreted staphylococcus superantigen-like 5 (SSL5) also activates platelets and promotes their binding to the vascular bed under flow (74). Direct interaction of iron-regulated surface determinants IsdB of *Staphyloccus aureus* with αIIbβ3 has also been described (75).

Immune response

Platelet CD40L is a major player in the inflammatory response which will be addressed later (see section on CD40L). It is pertinent though to underline how the CD40L/CD40 interaction is essential for the adaptive immune response (76). In this regard platelets or platelet-derived microparticles bearing CD40L activate antigen-presenting cells, modulate dendritic cell activation, enhance T-cell responses, induce B-cell production of IgG antibodies, and enhance germinal centre formation in cooperation with T cells (76–79). In so doing, CD40L is responsible for B-cell isotype switching. CD40L can be involved at the onset of several autoimmune and inflammatory diseases including systemic lupus erythematosus (SLE), diabetes and atherosclerotic disease (80).

Platelets in inflammation

Inflammation and blood coagulation are intimately linked; with inflammation promoting the development of cardiovascular disease and tipping the haemostatic balance towards thrombosis and DIC (81, 82). In its extreme, infection may lead not only to widespread DIC and extensive microvascular thrombosis but also pro-

fuse bleeding and multiple organ failure. Inflammation will also promote bleeding when the platelet number is low. Thus, when thrombocytopenic mice were subjected to models of dermatitis, stroke or endotoxin-induced lung inflammation, there was loss of vascular integrity and massive bleeding at the inflamed site, mostly involving venules (83). At the molecular level, inflammation-induced activation of the coagulation system, with IL-6 and TNF-α as major players, involves (i) TF-mediated thrombin generation, (ii) an imbalance or shutting down of the normal physiologic anticoagulant mechanisms (TF pathway inhibitor, antithrombin and protein C systems), and (iii) inhibition of the fibrinolytic system by elevation of PAI-1 (plasminogen activator inhibitor-1). Fibrin deposition is an important part of the defence mechanism against invading organisms (see previous section). As well as platelets, extra- and intravascular cells can all contribute to fibrin network formation, with fibrin structure varying with respect to changes in cell type-dependent pro-coagulant activity and TF expression (84). TF-induced coagulation is a hallmark of the systemic inflammatory response in bacterial infections and viral haemorrhagic fevers. The presence of inflammatory cytokines causes dense fibrin networks that resist fibrinolysis. Other proteins can also be key players. In acute stroke, the inflammatory marker, VWF, an adhesion molecule for platelets, can play an important role in the infarction. Significantly, new evidence suggests that use of recombinant ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin motif-13) reduces infarct volume and improves cerebral outcome without bleeding complications (85).

As we have seen, bacteraemia, septic and endotoxic shock are major causes of multi-organ failure and death. They are caused by a rapid and excessive host inflammatory response to the invading microorganisms and their products. Bacterial cell-wall constituents such as endotoxins/lipopolysaccharides induce a systemic inflammatory response by activation of TLRs (86). In endotoxinaemia, this leads to the excessive production of inflammatory cytokines and enzymes. Elements of the complement and plasminogen activator systems have been involved in the pathogenesis of endotoxinaemia; mice deficient in MMP-9 have altered resistance to LPS-induced toxicity whereas mice deficient in protease inhibitors are more susceptible to LPS shock (data reviewed in 87). Platelets and their released products do not act alone; they form part of an intricate network of blood-borne pro- and anti-inflammatory factors. For example, dendritic cell PAR1 and sphingosine 1-phosphate receptor3 signalling in the lymphatic compartment couples coagulation and inflammation in such situations (88). C-reactive protein (CRP) is an acute phase plasma protein that circulates as a pentamer (pCRP). Activated platelets mediate the dissociation of pCRP into its monomeric form, a process that involves newly expressed lysophosphatidylcholine (89). Having pro-inflammatory effects within atherosclerotic plaques, mCRP induces enhanced monocyte chemotaxis, generation of reactive oxygen species, and enhanced monocyte adhesion.

Recruitment of inflammatory cells at sites of vessel injury drives the development of atherosclerosis. In brief, platelets participate in plaque formation and progression. They also act to localise, amplify and sustain coagulation and inflammatory responses after plaque rupture (for reviews of plaque development see 90, 91). The type 2 scavenger receptor CD36 was one of the first membrane glycoproteins to be identified on platelets and is expressed on other cell types including endothelial cells and macrophages. CD36 is a multiligand pattern recognition receptor (among the ligands that bind is TSP-1). CD36 also recognises oxidised low-density lipoprotein (LDL), diacylglycerides expressed on microbial pathogens, apoptotic cells and cell-derived microparticles (92). Genetic deletion of CD36 protects mice from thrombosis. Other scavenger receptors on platelets include scavenger receptor BI (SR-BI), oxidised LDL receptor-1 (LOX-1) and CD68 (348). SR-BI also binds oxidised lipoproteins and is found on endothelial cells and hepatocytes where it directs selective transport of cholesteryl esters from high-density lipoprotein (HDL) in the liver. SR-BI in the liver controls plasma HDL levels and SR-BI deficient mice display a profound dyslipoproteinaemia. Non-marrow-derived SR-BI deficiency and the dyslipidaemia associated with it lead to platelet hyperreactivity linked to increased platelet cholesterol content. In contrast, platelet-specific deficiency of SR-BI is associated with resistance to hyperreactivity induced by elevated platelet cholesterol content and protects mice from a pro-thrombotic phenotype in dyslipidaemia (93). Alpha(1)-acid glycoprotein (AGP) is an acute phase protein that contributes to inflammatory processes. AGP directly induced platelet shape change involving the Rho/Rho kinase signalling pathway in a process that was inhibited by NO (94).

Specific platelet-born molecules involved in non-haemostatic platelet responses

In a recent study, O'Connor et al. (95) studied the effect of platelet releasate on THP-1 monocyte migration in a transwell Boyden chamber. The releasate was then fractionated by ion exchange chromatography, samples subjected to SDS-PAGE followed by trypsin digestion of selected bands and analysis of the proteins present by tandem mass spectrometry. Underlining the complexity of the platelet secretome, some 315 proteins were identified. Of these 32 were present in the fraction that stimulated monocyte migration. Proteins that were not present in any other fraction included tissue inhibitor of metalloproteinase-1 (TIMP-1), vimentin and pigment epithelium-derived growth factor (PEDF). PEDF, an inhibitor of angiogenesis, and another protein in this fraction, involucrin (a scaffolding protein in stratified epithelium) are novel platelet proteins. Faced with this complexity I have decided to concentrate on a series of well-characterised molecules in the next part of this review.

P-selectin

P-selectin is perhaps the most cited of the biologically active molecules that appear on the platelet surface after activation and secretion (96, 97). Specifically localised to the membranes of α-granules

and dense bodies of resting platelets (▶ Fig. 3), it has a glycosylated N-terminal lectin domain followed by an epidermal growth factor motif, consensus repeats, a transmembrane domain and a short cytoplasmic tail. Lectin and epidermal growth factor domains mediate binding to PSGL-1 on leukocytes, an interaction crucial for leukocyte rolling on the injured vessel surface (see section on α-granules; [33, 34]). P-selectin on platelets or endothelial cells has a key role in inflammation and inflammation-related diseases; for example, P-selectin deficiency is protective against atherosclerosis (see section on atherosclerosis; [98]). Soluble P-selectin (sP-selectin) is derived either from alternative mRNA splicing or from proteolytic processing of the membrane-bound form (see section on metalloproteinases); its level in blood represents a measure of platelet and/or endothelial cell activation (99). It promotes leukocyte-derived microparticle production, the activation of leukocyte β2-integrins and the deposition of leukocytes on fibrinogen or a platelet monolayer in a shear- and PSGL-1-dependent manner. Genetically modified mice with elevated circulating amounts of sP-selectin in plasma showed abnormalities that included: (i) higher blood-brain barrier permeability, (ii) a more aggressive social behaviour, (iii) larger infarcts in a stroke model and (iv) increased atherosclerotic macrophage-rich lesions on an *apoE-/-* genetic background (100). Elevated sP-selectin levels were associated with enhanced generation of TF-expressing microparticles leading to shorter plasma clotting time and a pro-coagulant phenotype with facilitated fibrin generation (101). The results obtained for the mice with elevated circulating sP-selectin levels were mimicked in pro-coagulant mice (Factor V Leiden) and led to intensified thrombosis in a mouse stroke model (102). Therefore, sP-selectin joins with other risk factors for atherosclerosis such as homocysteine and apoE deficiency whilst also altering blood-brain barrier permeability and exacerbating stroke. Increased sP-selectin is associated with increased plasma LDL in hypercholesterolaemic subjects (103).

CD40L

CD40 ligand (CD40L, CD154), a 33kDa intrinsic membrane glycoprotein and a member of the TNF ligand superfamily, is a modulator of humoral and cellular immunity as well as being pro-inflammatory; it provides a link between the immune system and atherosclerosis and thrombosis (104). Although activated CD4+ T cells are a much-quoted source, megakaryocytes synthetise CD40L and platelets carry the bulk of it in blood (105, 106). Like P-selectin and TLT-1, much of CD40L appears localised to granule membranes. On platelet activation, it is translocated to the platelet surface where it is cleaved by metalloproteinases with a primary role for MMP-2 that acts by associating with αIIbβ3 (107). The result is the release of the bulk of the extracellular domain into the plasma as sCD40L. Both membrane-bound and sCD40L classically mediate their effects by binding to a cognate receptor CD40, whose expression is widespread and includes B cells, neutrophils, monocytes, macrophages, platelets, dendritic cells, endothelial cells, fibroblasts, keratinocytes and smooth muscle cells (104, 108). CD40 expression is itself modulated by a variety of soluble mediators (IL-3, IL-4) and inflammatory cytokines such as **interferon-γ**, TNF-α and IL-1. CD40 ligation is usually mediated by trimeric forms of CD40L (or sCD40L) and is followed by receptor internalisation and association with TNRF-associated factors, the latter recruit intracellular kinases and initiate signalling; the end result according to cell type is a cascade of events resulting in cytokine production, cell proliferation, cell adhesion and programmed cell death (108, 109).

The role of CD40L in atherogenesis and thrombosis is beginning to be understood. The interaction of CD40L with CD40 on endothelial and other vascular cells leads to the up-regulation of adhesion molecules (e.g. E-selectin, vascular cell adhesion molecule 1 [VCAM-1] and inter-cellular adhesion molecule 1 [ICAM-1]), pro-inflammatory chemokines (e.g. IL-6, IL-8, MCP-1 [monocyte chemoattractant protein-1], RANTES), and MMPs (MT1-MMP, MMP-1, –2, –3 and –9) as well as increasing TF expression (reviewed in 104, 108, 109). The changes favour monocyte and neutrophil recruitment to the developing plaque as well as matrix re-organisation. In contrast, thrombomodulin expression is decreased facilitating thrombin generation while the production of the vasoactive peptide apelin by endothelial cells is also down-regulated (110). Macrophages are stimulated to release many inflammatory molecules by CD40L, while newly recruited smooth muscle cells and finally fibroblasts contribute to the evolution of the atherosclerotic lesion and eventually to plaque instability. Through its possession of an RGD sequence, CD40L can bind to αIIbβ3 and enhance platelet activation and stabilise thrombi (111, 112). Significantly CD40L-/- mice show unstable arterial thrombi and delayed vessel occlusion. Surface exposure of CD40L on platelets within a thrombus promotes matrix degradation by endothelial cells (113). This involves (i) increased expression of urokinase-type plasminogen activator receptor and MTI-MMP, (ii) secretion of urokinase-type plasminogen activator, tissue-type plasminogen activator and MMP-1, with (iii) induced proteolytic activity via MMP-2 and MMP-9. Platelet-endothelial cell contacts participate in these effects. In parallel, the interaction of CD40L with endothelial cells can result in a diminished synthesis of NO and increased oxidative stress (114). CD40L can also directly influence vascular cells, for example, sCD40L induces human coronary artery smooth muscle cell proliferation and migration, effects blocked by antibodies against MMP-9 (115).

Other effects of CD40L in atherosclerosis include: (i) changes in dendritic cell maturation and T cell infiltration, (ii) changes in the vascular redox state and endothelial cell relaxation, (iii) release of adipokines and (iv) overexpression of adhesive molecules favouring leukocyte recruitment. Some of these effects can be reproduced by sCD40L that induces the migration of Mac-1 expressing inflammatory cells (108). Studies on CD40L -/- mice identified TNF-receptor associated factor 6 (TRAF6) signalling, downstream of CD40, as a key regulator of neointima formation and vascular remodelling (116). Interestingly, TRAF6 is also implicated in TLR signalling. Increased leukocyte recruitment may be facilitated by CD40L promotion of Mac-1 expression on neutrophils facilitating

formation of platelet-leukocyte aggregates and increased leukocyte oxidative activity (117). Although the CD40L-CD40 dyad is multifunctional, recent evidence has shown that CD40L can also interact directly with integrin receptors α5β1 and Mac-1 as well as αIIbβ3. In fact, trimeric sCD40L can form a molecular bridge between two different receptors (104, 118, 119). This considerably broadens the horizons for the biological activity of CD40L and its soluble form. For example, CD40L promoted Mac-1-dependent adhesion and migration of inflammatory cells as well as myeloperoxidase release *in vitro* (118). The activation of these pathways may contribute to pro-inflammatory and pro-thrombotic responses, increased cytokine production, and endothelial cell dysfunction.

High circulating levels of sCD40L are predictive of increased risk in patients with hypercholesterolaemia, diabetes, stroke or acute coronary syndromes. In a specific example, one study claimed that they were predictive of ischaemic stroke and myocardial infarction in patients with non-valvular atrial fibrillation (120). Atrial fibrillation has been linked to redox state, inflammation, and ischaemia. Ongoing platelet activation is thought to be responsible for the sCD40L release. Increased plasma CD40L levels have been found to be associated with inflammation and increased coagulability in many other situations. One example is orthopaedic surgery where in total knee and total hip arthroplasty there is an increased risk of post-surgery venous thromboembolism (121). Increased levels of sCD40L are also seen during transfusion-related acute lung injury (122). Levels of sCD40L accumulate during platelet storage and may prime human polymorphonuclear leukocytes (PMNs), which can promote PMN-mediated cytoxicity of endothelial cells in the lung. The extent of the damage will depend on the capacity of the leukocytes to roll and establish firm adhesions on inflamed tissue. The adhesion will be enhanced by the presence of ultralarge VWF multimers; their cleavage by the metalloprotease, ADAMTS13 can control the extent of the leukocyte (and platelet) interaction with the vessel wall and also of neutrophil extravasation (123). In this context, ADAMTS13 acts as a link between thrombosis and inflammation with ADAMTS13 deficiency being both pro-inflammatory and pro-thrombotic. The balance between VWF and ADAMTS13 may be a biomarker in systemic inflammation. Severe hereditary or acquired ADAMTS13 deficiency causes thrombotic thrombocytopenic purpura (TTP) (124).

Plasma sCD40L levels as well as those of other α-granule proteins such as PF4 were increased several fold in patients with IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, an X-linked and often fatal human disease linked to mutations in forkhead box protein 3 (Foxp3). This disease is reproduced in the spontaneous mouse mutant *scurfy* (125). IPEX disease is associated with thrombocytopenia, inadequate platelet function and bleeding in about 50% of cases (126). One possibility is that feedback messages provided by high levels of secreted α-granule proteins (such as PF4) inhibit megakaryocyte development (127). These high levels may also play a role in the severe atopic dermatitis that affects IPEX syndrome patients. Increased levels of sCD40L were seen in patients with metabolic syndrome and were accompanied by a lower plasma concentration of the hormonal protein, adiponectin (128). In fact, adiponectin was described as having an anti-inflammatory role through the modulation of the CD40/CD40L system. Increased levels of sCD40L were subsequently confirmed for 312 patients with metabolic syndrome when increased levels of high sensitivity C-reactive protein were also described (129). A systemic inflammatory response is characteristic of the metabolic syndrome.

Tissue factor

TF is the pivotal initiator of inflammation-induced thrombin generation. A 45 kDa transmembrane glycoprotein, TF is constitutively expressed in tissues that only come into contact with blood after disruption of vessel integrity. On exposure to blood, TF primarily binds FVIIa and the TF-VIIa complex catalyses the conversion of FX to FXa and this leads to thrombin generation (109). Platelets can participate in TF dissemination by taking up TF-enriched microvesicles liberated by other cells (e.g. monocytes) or, more controversially can synthesise it directly on strong activation through their possession of a spliceosome (47, 130). Activated platelets provide a suitable catalytic surface with exposed PS and accelerate thrombin generation. PS-expressing platelets are also called coated platelets and have high surface levels of proteins including factor V, fibrinogen, fibronectin and VWF (131). TF together with Factor VIIa can also directly stimulate cells by cleaving seven transmembrane domain receptors of the PAR family on blood (mononuclear cells, platelets) and vascular cells (endothelial cells, fibroblasts, smooth muscle cells). PAR receptors are linked into G-protein-coupled receptors and their stimulation in nucleated cells can lead to the production of cytokines and growth factors as well as reactive oxygen species and cell adhesion molecules. Studies using PAR-4 deficient mice showed a markedly lower inflammatory response to soluble TF despite the fact that binding of TF to PAR-2 can result in the up-regulation of inflammatory rersponses in macrophages and can affect neutrophil infiltration and TNF-α production ([132], reviewed in [109]). Interestingly, PAR-4 inhibition protects from multiorgan failure in a murine model of systemic inflammation while in mice genetically predisposed to a severe protein C deficiency acute inflammation is exacerbated; use of recombinant human aPC extends survival of the mice after LPS challenge (133). Bidirectional interplay between blood cells facilitates TF synthesis and the development of procoagulant activity. Thus, P-selectin and CD40L induce TF expression in monocytes (134). TF-bearing microparticles arise from lipid rafts in monocytes and fuse with activated platelets to provide a membrane surface that supports coagulation (135). In a two-way process, the expresion of TF and cytokines by monocytes is markedly stimulated by the presence of activated platelets and granulocytes. Blocking TF activity completely stopped inflammation-induced thrombin generation in models of experimental endotoxaemia or bacteraemia (136). Inflammatory cells (e.g. macrophages, smooth muscle cells, cardiomyocytes) in atherosclerotic plaques produce TF and this becomes exposed on plaque rupture

(reviewed in 109, 137). The expression of TF is primed by cell exposure to pro-inflammatory factors in the plaque such as IL-6, PDGF and MCP-1.

An enigma is the presence in platelets of tissue factor pathway inhibitor-1 (TFPI-1) the main natural inhibitor of the TF/FVIIa complex; TFPI-1 is synthesised by megakaryocytes, stored in platelets and expressed on the activated platelet surface following dual agonist activation (138). It is released from activated platelets both on microparticles and as a soluble form.

Metalloproteinases and their inhibitors

Platelets are a rich source of MMPs, multidomain enzymes containing a zinc ion coordinated by three histidine residues in their active site. MMPs are stored in cells as latent enzymes in which a cysteine sulfhydryl group in the amino terminal pro-peptide interacts with the zinc ion and blocks the active site. Their transformation into active enzymes requires the removal by proteolysis of the pro-peptide. MMPs have key roles in embryonic development, cell migration, wound healing, and tissue remodelling as well as in pathological processes such as tumour development, atherosclerosis and plaque destabilisation (139). They can also mediate haematopoietic stem cell mobilisation (140).

MMPs mediate the release of biologically active fragments of membrane proteins and modify cytokines and chemokines, acting as fine regulators of acute and chronic inflammatory and ischaemic processes. Their inactivation involves TIMPs and α2-macroglobulin, abundantly present in body fluids. MMPs are divided into subclasses based on their structure and on their substrate specificity (collagenases, gelatinases, stromelysins and membrane-type MMPs). MMP-2 and MMP-9 were the first to be identified in platelets; they cleave denatured collagen (gelatin), laminin, and collagen type VI, a major constituent of basement membranes as well as differentially regulating smooth muscle cell migration and cell-mediated collagen matrix formation (141–145). MMP-2 was claimed to principally have a cytosolic location, yet was externalised during platelet activation becoming membrane bound as part of the aggregation mechanism (145). Secreted proMMP-9 is converted into its active form by cleavage through soluble proteases such as MMP-3 and plasmin. In contrast, proMMP-2 is activated on the cell surface (including platelet surface) by a mechanism involving complex formation with TIMP-2 and MTI-MMP (143). MMP-1 when activated on platelets can cleave PAR-1 and activate incoming platelets or adjacent cells (146). As shown in ▶Figure 2, the bulk of stored MMPs in platelets is intracellular with IF detection in favour of an α-granule localisation.

MMPs share a zinc-binding consensus sequence with the ADAMs and ADAMTs enzymes. ADAMs are multidomain MMPs that are anchored in cell membranes; prominent in platelets are ADAM10 and ADAM17 (also known as α-secretase or TACE [TNF-α converting enzyme]). With ADAMTS13, their localisation is mostly intracellular in resting platelets, but whereas the punctate IF seen for ADAM17 mostly co-localised with VWF, that seen for ADAM10 and ADAMTS13 did not (▶Fig. 3). There is clear heterogeneity in the organisation of these proteins in platelets. ADAMS10 and ADAMS17 act as sheddases and are responsible for the calcium-dependent cleavage of functional membrane receptors such as GPIb, GPV, P-selectin and GPVI, while ADAMTS13 is responsible for cleaving high molecular weight VWF multimers (123, 147–149). How the enzymes find their substrates on the platelet surface remains an enigma. The relationship between MMP protein and activity levels is an important factor in inflammation. Many stimuli can stimulate or enhance MMP gene expression in diverse cell types; these include TGF-β1, interleukin-1β and TNF-α. One specific example is the up-regulation of MMP activity in HMSCs by inflammatory cytokines coming from platelets and or other cells, facilitating their migration to areas of tissue damage and inflammation. A novel protein able to stimulate HMSC migration is Wnt3a that is also secreted from platelets (150, 151).

Again we are faced with the enigma that platelets both store and release MMPs and their inhibitors. TIMP-1 and TIMP-2 were first shown to be present in MKs and platelets and their release contributed much to the amounts in plasma (152). Both TIMP-1 and TIMP-2 stimulate the proliferation of bone marrow fibroblasts and together with PDGF and TGF-β1 they are speculated to have a role in the development of bone marrow fibrosis. TIMP-4 is also present and was identified as the major platelet inhibitor of platelet MMPs; it was released on platelet activation and had a regulatory role in platelet aggregation (153). TIMPs have affinities for MMPs in the picomolar range and, in general have broad specificities. They also possess other biological functions (154). My laboratory has recently localised TIMPs 1–4 in platelets by confocal microscopy (155) (▶Fig. 3). While confirming their rapid release from platelets by thrombin, only the punctate IF for TIMP-3 co-localised to VWF-containing α-granules; furthermore, there was no co-localisation of the TIMPs, neither one with the other nor with the MMPs. This suggested a novel and so far unexplored mechanism for storage of biologically active proteins in platelets, perhaps in endosomes. Globally, the role of MMPs in inflammation may depend on the relative local balance of MMP and of TIMP activities.

Platelets and fibrinolysis

Platelets store and release proteins involved in fibrinolysis; they include not only plasminogen and urokinase plasminogen activator (u-PA) but also PAI-1, α2-antiplasmin, PNA-1 and PNA-2 (▶Table 1) (2, 20). Overall, the balance between the expression of plasminogen activators (+ve) and their inhibitors (-ve) regulates fibrin deposition and this balance can be changed by platelet accumulation during inflammation; pivotal regulators of PAI-1 expression are TNF-α and Il-1β. Fibrinolytic proteins have pleiotropic functions in addition to their primary role in fibrinolysis. For example, PAI-1 intervenes in wound healing, atherosclerosis, metabolic disturbances such as obesity and insulin resistance, tumour angiogenesis, chronic stress, bone remodelling, asthma, rheumatoid arthritis, fibrosis, glomerulonephritis and sepsis (156,

157). PAI-1 is a serpin that inhibits both plasmin and thrombin while PNA-1, recently localised to α-granules, also inhibits plasmin, u-PA and tissue plasminogen activator (t-PA). Interestingly, the functional activity of PNA-1 is potentiated by glycosaminoglycans, also stored in α-granules; PNA-1 is a more powerful inhibitor of thrombin than antithrombin or PAI-1 although probably both proteins act in concert. Studies on PNA-1 -/- mice showed that PNA-1 was a negative regulator of both mouse platelet aggregation and thrombus formation under flow acting by delaying the initial phase of platelet activation (158). Both PAI-1 and u-PA are recognised regulators of cell adhesion and migration mechanisms through their capacity to bind to integrins, with vitronectin another ligand for u-PA as it is for PAI-1 (159, 160). Significantly, PAI-1 deficiency protects against atherosclerosis (161). Fibrinolysis and pericellular proteolysis by plasmin depend on molecular co-assembly of plasminogen and its activator on cell, fibrin, or matrix surfaces. The cell surface need not be the same cell, for example Glu-plasminogen bound to either of the above surfaces can be transformed into plasmin by uPA expressed on monocytes or even cell-derived microparticles; this is a potentially important new pathway in inflammation (162).

Wound healing, angiogenesis and bone formation

It is useful to recapitulate that activated platelets release many proteins favouring wound healing and promoting angiogenesis. These include PDGF A, B and C, insulin-like growth factor-1 (IGF-1), VEGF (essentially VEGF-A) and connective tissue growth factor (CTGF) as well as a multitude of chemokines and cytokines (reviewed in [2, 20]) (▶Table 1). Other pro-angiogenic mediators from platelets include angiopoietin, SDF-1 (CXCL12), MMP-1, MMP-2 and MMP-9 ([20]; see preceeding sections). Essential for both wound healing and bone growth, angiogenesis is not only a vital process for normal development, it is also implicated in disease and, in particular, cancer ([163, 164]; see also following section on cancer). CD40L may be one of the many platelet-derived factors that stimulate angiogenesis by promoting endothelial cell proliferation and migration (165). The CD40L/CD40 interaction can up-regulate VEGF, FGF and PAF synthesis by endothelial cells (reviewed in [104]). VEGF, PDGF, IGF-1, FGF, hepatocyte growth factor (HGF) and epithelial growth factor (EGF) are considered pro-angiogenic. In platelets, the A and B isoforms of PDGF predominate, although PDGF-C is also present (166). The growth factors promote vessel wall permeability and recruitment, maturation and proliferation of blood and vascular cells with their precursors often interacting with cell surface glycosaminoglycans (167). Platelets furnish an initial and rapid supply at sites of vessel injury or at inflamed sites where platelets accumulate through haemostatic mechanisms. VEGF and FGF-2 exert trophic effects on endothelial cells and with PDGF promote sprouting of new vessels (168). VEGF or TGF-β1 inhibition resulted in a significant increase in leukocyte rolling on endothelial cells (169). This was associated with a specific increase in endothelial cell P-selectin expression, an increase in soluble levels of VCAM-1 and E-selectin, and impaired peripheral vasodilatation. So, curiously, VEGF and TGF-β may also be considered as housekeepers in maintaining the endothelium in a non-activated state. In contradiction, VEGF has also been said to be pro-inflammatory and to stimulate the adhesion of leukocytes to endothelial cells, a function suppressed by HGF (170).

But as well as activators of angiogenesis, platelets also store and secrete established inhibitors of vessel growth, possibly in a cellular compartment distinct from the pro-angiogenic proteins (24, 25). For example, the cytokine, TGF-β1, is abundant in platelets and plays an important role in regulating the immune response, cell proliferation, wound healing, and tissue fibrosis. It is said to be anti-angiogenic although it promotes the synthesis of matrix proteins (171). TGF-β1 also recruits inflammatory cells to the wound area. TSP-1, another anti-angiogenic protein (164), participates in shear-dependent activation of latent TGF-β1 by a mechanism that involves thiol-disulphide exchange (172). TGF-β1 is stored in α-granules non-covalently bound to latency-associated peptide (LAP), which, in turn, is disulphide bonded to latent TGF-β binding protein-1 (LTBP-1). GARP (glycoprotein A repetitions predominant, LRRC32), a leucine-rich repeat membrane GP (with structural similarities to GPIb and GPV), is essential for the tethering of latent TGF-β to platelets and also to FOXP3+ regulatory T cells (173). TSP-1 inhibits endothelial cell proliferation and stimulates endothelial cell apoptosis (174). It also facilitates vascular smooth muscle cell responses to PDGF (175). PF4, another abundant α-granule protein, interferes with the binding of VEGF and other growth factors to cells (28, 176). Other anti-angiogenic proteins stored and released from platelets include angiostatin, endostatin and TIMP-1 and -4 (154, 164, 177). As discussed earlier, evidence has been obtained that pro- and anti-angiogenic proteins may be stored in different subpopulations of α-granules and indeed be released by different mechanisms by selective engagement of the thrombin receptors PAR-1 and PAR-4 (26). In this context, ADP another potential platelet activator in the tumour cell environment, is a much more potent mediator of VEGF release from platelets than it is for endostatin (178). This suggests that ADP favours angiogenesis via its selective ability to induce VEGF release from platelets. ADP also promotes release of CTGF, another pro-angiogenic protein (179). Yet, activated platelets also provide a functional microenvironment for the anti-angiogenic fragment of histidine-rich glycoprotein (HRG) (180). This fragment, consisting largely of the histidine- and proline-rich domains, was enriched in the vessel wall in tissue from cancer patients. HRG is released from activated platelets and shows Zn^{2+}-dependent binding to heparan sulfate on endothelial cells. It is a possible negative regulator of angiogensis.

Both IL-1 and MIP-1α influence osteoclastogenesis and have been localised to α-granules by immunogold labelling and electron microscopy (181–183). Bone morphogenetic proteins (BMPs)-2, -4 and -6 are synthesised by megakaryocytes and released by platelets and are essential components of bone formation (184). IGF-1 and a modulating bone protein, IGFBP-3, are captured by platelets by en-

docytosis; IGF-1 directly stimulates bone matrix formation and replication of osteoblasts and their precursors (185).

Maternal tissue and foetal vascular remodelling

In early pregnancy, human chorionic gonadotrophin stimulates the corpus luteum to produce progesterone, which in turn maintains human embryo implantation in the uterus. Accumulating evidence suggests that circulating blood cells also play an important role in embryo implantation (186). Platelets provide chemokines that promote extravillous trophoblast invasion to reconstruct the maternal endometrial artery and to induce vascularisation during corpus luteum formation. The ductus arteriosus is a fetal shunt vessel between the pulmonary artery and the aorta that closes promptly after birth. Intravital microscopy was used to show in a neonatal mouse model that platelets are recruited to the luminal aspect of the ductus arteriosus during closure where some 12 h after birth they promote both thrombotic sealing of the constricted vessel and support luminal remodelling (187). Both GPVI and αIIbβ3 play a role in the occlusion while mice deficient in the nuclear factor, erythroid-derived-2 (encoded by *Nfe2*), with a defect in platelet biogenesis and platelet number also failed to occlude in 70% of animals. Mice with continued ductus arteriosus patency showed pulmonary vascular modelling and sclerosis consistent with chronic pulmonary hypertension. Platelets play an essential role in separating the blood and lymphatic vasculatures during embryonic angiogenesis. Mice deficient in the homeodomain transcription factor Meis1 completely lack megakaryocytes/platelets; their embryos fail to separate lymphoid/blood vasculature. The association of platelets with vascular endothelium at zones of contact between lymphatic sacs and veins confirmed a direct role of platelets in the separation of the two vasculatures (188). Platelets accumulate through their binding to podoplanin, a highly glycosylated component of the lymphatic endothelium and are activated via CLEC-2-SLP-76 signalling (189, 190). Podoplanin -/- embryos develop a non-separation phenotype characterised by a blood-filled lymphatic network. Interestingly, the same phenotype was seen with kindlin-3 mice where αIIbβ3 activation is defective. It is thought that while the aggregated platelets form a plug, the release of platelet constituents such as PDGF and TGF-β attract mural cells and promote tissue reconstruction.

Activated platelets and platelet-released microparticles in acquired disorders

Atherosclerosis and related diseases

These pathologies including myocardial infarction and stroke are often said to represent chronic inflammatory diseases (191). The activation of foamy macrophages, the local release of cytokines and chemokines, and the activation of MMPs may all involve platelets in addition to their essential participation in arterial thrombosis. Vascular remodelling is central to (re)stenosis, plaque rupture and aneurysm and is dependent on MMPs. Among the roles that MMPs may play is to promote smooth muscle cell migration, enhance macrophage infiltration and to bring about degradation of the elastic lamina and cardiac rupture (192–194; see section on metalloproteinases). High plasma concentrations of cholesterol may have direct effects on platelets. Studies on HDL receptor, SB-BI deficient mice with high plasma unesterified-to-total cholesterol levels and abnormally large HDL particles show how these factors contribute to atherosclerotic and coronary heart disease (93). HDL particles also induce a moderate thrombocytopenia (due to accelerated platelet clearance) and defective ADP-induced platelet aggregation that can increase bleeding risk (195). The effect of cholesterol on platelet function is clearly complex for cholesterol loading has also been shown to increase sensitivity to ADP and epinephrine while lipid-lowering therapy can lead to a reduced platelet response to agonists (196). These functional effects are probably related to physical changes in lipid rafts in the platelet membrane.

Injuries to the vessel wall lead to platelet deposition and activation, leukocyte recruitment and altered arterial structure. Platelet-induced chronic inflammation is a key step in atherosclerosis and promotes plaque development, intimal hyperplasia and restenosis. The importance of P-selectin is underlined by studies on P-selectin-deficient mice that are protected from atherosclerotic disease ([197]; also see section on P-selectin). Src family kinases also contribute to the signalling required for integrin activation and stable adhesion of leukocytes to adherent platelets (198). Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E while the αIIbβ3 integrin and GPVI are essential for atheroprogression and cerebral ischaemia (199, 200). Interestingly, the αIIbβ3 integrin, previously thought to be exclusive to platelets and cells of the megakaryocyte lineage, is now known to be expressed by mast cells and to play an important part in fibrinogen-associated chronic inflammation and innate immune responses (201). This included cytokine production by mast cells in response to *Staphylococcus aureus* with fibrinogen-binding capacity. Expression of pro-coagulant activity by inflammatory cells (in particular TF) will result in thrombin generation and a platelet-rich thrombus, an essential element of arterial thrombosis and a process facilitated by high shear forces (109). But this is a two-way process for coagulation factors (e.g. thrombin) or anticoagulant proteins (e.g. APC) can activate receptors on blood or vascular cells and affect cytokine production or inflammatory cell apoptosis. In an apparent contradiction, inflammation can also favour bleeding, particularly when the platelet count is low (83). The participation of activated platelets and microparticles in vessel wall occlusion in myocardial infarction and stroke has largely been reviewed elsewhere (202, 203).

Diseases of the nervous system

Multiple sclerosis (MS) is a major inflammatory disorder of likely autoimmune origins with the formation of sclerotic plaques in the central nervous system. The disease results in the destruction of oligodendrocytes, cells that are responsible for the formation of the myelin sheath. T cells and autoreactive lymphocytes attack the myelin sheath, recognising it as foreign. The loss of the myelin sheath leads to a disruption of neuronal function. As reviewed by Horstman et al. (45), there is evidence for chronic platelet activation and CD40L release in MS suggesting that platelets may contribute to the evolution of the disease. MMP-9 and chemokines play roles in MS; while mice knockout for both MMP-2 and MMP-9 were completely resistant to the development of experimental autoimmune encephalomyelitis – an animal model of brain inflammation (204). Inhibition of MMPs can also influence MS development similarly to gene knockdown (205).

Alzheimers disease, the most common form of dementia, has a complex origin with inflammation, the deposition of β-amyloid into cerebral plaques and the loss of neurons as primary pathological features (206). In this disease, a deterioration of memory is linked to the accumulation of misfolded proteins in the ageing brain. Self-aggregating β-amyloid peptides arise through the proteolysis of amyloid precursor protein. Neurofibrillary tangles with deposition of tau protein are another feature. As the disease advances, synaptic dysfunction, depletion of neurotrophin and neurotransmitters, mitochondria dysfunction, inflammation and vascular defects are among the many changes seen. Activated platelets play an important pro-inflammatory role, as well as participating in the proteolytic processing of amyloid precursor protein into β-amyloid peptide by ADAMS17 and in the deposition of these proteins; platelets are a carrier for them both (207, 208). Recently, increased fibrin deposition in the brain in a mouse model of Alzheimer disease was shown to be associated with increased blood-brain barrier permeability (209).

Among the players in central nervous system disorders are peroxisome proliferative-activated receptors (PPARs) (210). Platelets are a rich source of PPARs and they are released from activated platelets or are present on microparticles and have been said to play a role in CD40L release. Platelets may also intervene in the housekeeping of nervous tissue. For example, brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, promotes neuronal survival and increases synaptic efficacy in the mammalian brain and the central nervous system (211). BDNF is stored in platelet α-granules and secreted after stimulation by physiologic agonists and by shear stress (212). Like serotonin, BDNF binds to platelets and is endocytosed by them; it is not synthesised by megakaryocytes. It is likely that release of BDNF at sites of tissue damage will facilitate the repair of peripheral nerves. The granule population containing BDNF is not known.

Cancer

Differential changes in platelet VEGF, TSP-1, SDF-1 and CXCL4 have been shown in patients with metastatic cancer (213). Patients with newly diagnosed metastatic disease had increased platelet counts and a higher level of activated platelets. Whereas the level of VEGF in the platelets was increased, that of SDF-1, PF4 and TSP was decreased. While platelets may be specifically scavenging VEGF, these results suggest either ongoing differential release and/or changes in megakaryopoiesis in cancer. Overall, the role of platelets and granule secretion in maintaining vascular homeostasis in tumours is unclear. Tumours are associated with inflammation, a cause of haemorrhage in thrombocytopenia. Thrombocytopenia-induced haemorrhage appears to require leukocyte recruitment for it did not occur in mice deficient in β2 or β3 integrins where stroma-infiltrating leukocytes were reduced in numbers, suggesting that platelets counteract tumour-associated inflammation (83).

The long-held notion that platelets participate in tumour metastasis has largely been substantiated by studies on animal models including experiments on the effect of platelet depletion or the blockade of surface receptors (214). For example, a decrease in lung metastases after antibody-induced thrombocytopenia in a mouse tumour transplant model fed the hypothesis that platelet-tumour cell interactions helped cancer cells spread within the body (214). Thrombocytosis in malignancy is seen in a variety of cancers being linked to the positive influence of tumour-derived cytokines on megakaryocytopoiesis (215). Embolisation of platelet-tumour cell aggregates may be one way in which cancer is disseminated. Platelets, by bonding to tumour cells, may also protect them from the host immune defence systems via release of TGF-β1 that is able to down-regulate the *NKDG2D* gene thereby inhibiting natural killer cell anti-tumour reactivity (216). Platelet-tumour cell interactions and their contact with exposed subendothelial components involve key receptors such as GPIb, αIIbβ3, GPVI and exposed P-selectin (217–220). Platelet interaction with tumour cells favours the production and release of biologically active substances. Platelet-derived lysophosphatidic acid is one such factor (221). MMPs and other enzymes secreted by platelets can directly degrade the basement membrane, an important step in metastase dissemination. By providing a pro-coagulant surface, platelets and derived microparticles help to augment cancer-related coagulation. Malignancy is a recognised hypercoagulable state and thrombin promotes tumourigenesis and angiogenesis by enhancing VEGF expression and exerting pro-migratory and chemotactic effects (215). Microparticles released from activated platelets can directly promote metastasis and favour angiogenesis (222).

Platelets are the major serum source of VEGF and its promotion of blood vessel formation is a key step in tumour development. Platelets are also major sources of the anti-angiogenesis factors such as PF4, TSP-1 and endostatin ([164, 223]; see section on angiogenesis). Platelets selectively accumulate angiogenesis regulators, in particular the pro-angiogenesis factors VEGF and angiopoietin-1 allowing them also to be released locally (reviewed in [20, 224]). In a murine model, platelets have been shown to prefer-

entially store tumour-derived proteins during prostate cancer growth (225). As a result, platelet-associated PF4 and TSP-1, but not their plasma counterparts, have been suggested as potential biomarkers of early tumour presence (226, 227). Interestingly, while levels of VEGF, PDGF, PF4 and TSP in normal platelets vastly exceeded those of plasma, the concentration of endostatin in platelets and plasma were equivalent – arguing against a selective uptake of endostatin and a major anti-angiogenic role for endostatin (228). Despite these advances, the precise mechanisms for the involvement of platelets in tumour metastasis remain to be elucidated and agents that block surface receptors of platelets have yet to find their way into clinic. TSP-1 is up-regulated in platelets of tumour-bearing mice as a consequence of both increased numbers of MKs and of an augmented expression of TSP-1 mRNA (227). It was speculated that the production and delivery of TSP-1 by platelets is a critical host response to suppress tumour growth through inhibiting tumour angiogenesis. Mice lacking TSP-1 developed tumours 4-6 days earlier in an experimental model. MKs from TSP-/- mice (but not platelets), somewhat surprisingly endocytosed recombinant TSP-1. The newly recognised platelet receptor CLEC-2 might also be involved in haematogenous tumour metastasis as the receptor mediates tumour cell-induced platelet activation and aggregate formation possibly through binding to podoplanin (a type I transmembrane sialomucin-like glycoprotein up-regulated in many tumours) (229).

Other disease states

I will now briefly review other disease states where activated platelets may have a pathological role.

Skin diseases and allergy

A role of P-selectin expressing platelets has been established in several inflammatory skin diseases including atopic dermatitis and asthma (reviewed in [230, 231]). The activated platelets intervene in late-phase reactions following IgE-mediated hypersensitivity and promote platelet-leukocyte interactions. They also secrete cytokines that attract leukocytes to skin. In so doing they stimulate keratinocytes, leukocytes and endothelial cells while inducing the trafficking of leukocytes to skin tissue, inhibiting monocyte apoptosis, inducing fibrosis, promoting itchiness and regulating inflammation. In psoriasis, spontaneous platelet aggregation, shortened platelet survival and increased levels of plasma markers of platelet activation are features (232). Platelet release products and their phagocytosis activators play a role in enhancing blood neutrophilic function including phagocytosis and oxidative activity (233). Many of these processes depend on cell-cell interactions mediated by membrane glycoprotein receptors. A good example is the inflammatory role of platelets in cystic fibrosis, a role linked to the dysfunction of a chloride channel regulated by the cystic fibrosis gene (231).

Pulmonary disorders

Among other disorders associated with platelet activation and platelet-leukocyte interactions is transfusion related acute lung injury, a process in which neutrophils interact with platelets and lung endothelium (reviewed in 234). Supernatants from stored platelets can cause lung inflammation and coagulopathy with a role for platelet-released lysophosphatidylcholines in cell activation and neutrophil priming (235). In this context, it is worthy to note that IgG-complexes surprisingly induce little P-selectin expression on platelets but induce the release of similar amounts of sCD40L and RANTES to thrombin (236). MMPs have been implicated in the pathogenesis of chronic obstructive pulmonary disease (237) but have protective roles in asthma (238). In particular, both MMP-2 and MMP-9 are essential for the movement of inflammatory cells into the airway lumen thereby preventing lethal asphyxia. This is achieved through their ability to enhance and position chemotactic factors through cleavage. Other situations where MMPs can be active pathogenic agents include lung fibrosis (reviewed in [87]). Platelets support pulmonary recruitment of neutrophils in abdominal sepsis, playing a key role in regulating infiltration of neutrophils and oedema formation in the lung via up-regulation of Mac-1 and enhancing their capacity to bind platelets (239).

Crohns disease

It has been known for a long time that activated platelets circulate in Crohn's disease or ulcerative cholitis. Patients with Crohn's disease have an increased risk of thromboembolism and hyperactive platelets with an increased content of CD40L. This was associated with a selective increase in the platelet MMP-9 content (240). Inhibition of MMP-9 activity diminished sCD40L release, sugesting that this metalloprotease was responsible for the cleavage. TREM-1 expressed by macrophages may also have role in inflammatory bowel disease (241).

Rheumatoid arthritis

In early studies, Tchetverikov et al. (242) compared protein and activity levels of MMP-1 (a collagenase) and MMP-3 (a stromelysin) with TIMP-1 levels in synovial fluid of patients with knee joint injury, primary osteoarthritis and inflammatory arthritis. Patients with joint injury had increased levels of proMMP-1 and proMMP-3 in synovial fluid and an increase in activated MMPs. In particular, MMP-3 activity was increased in inflammatory arthritis along with levels of degradation products of cartilage such as aggrecan. Increased levels of activated MMPs, as shown by increased levels of MMP-α2-macroglobulin complexes, is also a feature of rheumatoid arthritis (243). Rheumatoid arthritis is characterised by autoimmune-induced inflammation of the joints and degradation of joint cartilage. In severe cases it results in severe bone erosion. Relationships between platelet activation markers including CD40L and rheumatoid arthritis have been shown in many studies. In this context, an important recent finding is the demonstration that platelets amplify inflammation in rheumatoid

arthritis via collagen-dependent microparticle production (244). Platelet-derived microparticles were identified in large numbers in joint fluid from patients with rheumatoid arthritis and other forms of inflammatory arthritis but not in patients with osteoarthritis. The microparticles were pro-inflammatory, and brought about cytokine responses (and in particular IL-8 release) from synovial fibroblasts via IL-1α and-1β. Platelet activation through GPVI-mediated platelet-collagen interactions was a primary cause of microparticle release and may occur in fenestrations of subsynovial capillaries. The platelet-derived microparticles were also abundant on synovial fluid leukocytes although microparticles derived from leukocytes themselves were much less frequent. Platelet depletion in a mouse model showed that platelets were indeed essential for inflammatory arthritis development.

Liver disease

Interplay between platelets and the microcirculation in the liver may be vital for normal liver function. I have already discussed how platelets release factors such as serotonin that promote liver regeneration (see section on dense granules). Although platelet function and coagulation are often perturbed in liver disease, many of the effects appear secondary to the disease (245). Some examples of a pathological effect do exist; for example, platelet-dependent accumulation of leukocytes in sinusoids mediates hepatocellular damage in bile-duct ligation-induced cholestasis while PF4 is a platelet-derived mediator of experimental liver fibrosis (246, 247). Interestingly, a protective role has recently been shown for platelet-derived sCD40L in hepatic steatosis (248). In brief, platelets modulated the cellular unfolded protein response through sCD40L release. Mice lacking CD40L rapidly developed severe hepatic steatosis when fed an oil-rich diet.

Alloantibody-mediated transplant rejection

In this situation, antibodies target the endothelial cell major histocompatibility complex (MHC)-1 within the transplanted tissue. This involves antibody-mediated endothelial cell activation and leukocyte trafficking in targeted tissues. Antibody-antigen complexes can activate platelets via Fc receptors. Complement activation after the deposition of microthrombi accelerates the endothelial cell damage and facilitates further platelet recruitment (249, 250). Platelet interactions with dendritic cells can contribute to the vasculopathy. Platelet secretion of chemokines recruits helper and cytotoxic T cells and these stimulate more platelets through CD40-CD154 interactions. P-selectin-PSGL-1 interactions also come into play.

Conclusions

I have shown that release of biologically active proteins from platelets is part of the natural healing process and of tissue regeneration. I have also highlighted how an excess of platelet reactivity in the wrong place and at the wrong time can have detrimental effects. This review is designed to highlight the expanded role of platelets in non-haemostatic events. It is not comprehensive, some diseases have been little mentioned (e.g. diabetes) and the cardiovascular roles of platelets and their implication in both arterial and venous thrombosis deliberately under-discussed. I would hope that the concepts raised in this review will open the door to new and so far largely unexplored uses of anti-platelet drugs (anti-integrin, anti P2Y and anti-PAR receptors) that should now be tested in the context of slowing down cancer growth and diffusion and in the progression of Alzheimers disease. Furthermore, the use of such drugs in a cardiovascular disease context may well be enhanced if combined with anti-inflammatory agents.

After vascular injury, platelet-rich clots form a scaffold for healing. Remarkably, this process has only recently been recognised as having potential therapeutic uses, namely the programmed release of growth factors and cytokines from platelets promoting wound healing and tissue regeneration. Autologous activated platelets retained in fibrin matrices are a source of molecular signals that control cell growth and cell differentiation through the release of a diverse panoply of proteins and other biologically active molecules. Often applied as an autologous platelet-rich plasma (PRP)-derived fibrin clot or as injectable platelet releasates, the use of PRP is now considered to enhance the natural healing process. Dental implant surgery and bone regeneration, orthopaedic surgery, muscle and tendon repair, skin ulcers, hole repair in eye surgery, and cardiac surgery are among the situations where applied autologous platelets are being used as a rapid, inexpensive and safe method to accelerate healing (reviewed in 2). This approach is also being extensively applied to the repair of sports injuries (251). A recent report describing how the addition of mesenchymal stem cells to the scaffold of platelet-rich plasma is beneficial for the reduction of the consolidation period in mandibular distraction osteogenesis perhaps shows the way forward (252).

Yet, a major enigma remains to be resolved. Platelets have now been shown to store and release such a wide range of biologically active proteins that the question as to why such a wide diversity of compounds is required needs to be asked. Not only this, time and again in this review I have shown how platelets stock and release activators and inhibitors of each biological pathway; angiogenesis, coagulation and fibrinolysis being just three examples. Why should this be? Perhaps local conditions determine which wins, if so the regulation of the non-haemostatic roles of the platelet is indeed finely tuned.

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Conflict of interest

None declared.

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