Role of platelet adhesion in homeostasis and immunopathology

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Abstract

Various molecules expressed on the surface of platelets have been shown to mediate the protective or deleterious role of these cells in immuno-inflammatory mechanisms. Increasing evidence points to the involvement of the cell adhesion molecules, gpIIb-IIIa, P-selectin, CD31, LFA-1, and CD36 in the interaction between platelets and endothelial cells as well as other cell types. The possible role of these molecules in the ability of platelets to support endothelium and to protect against tumour necrosis factor mediated cytotoxicity or parasitic invasion are reviewed. The involvement of platelets as effectors of tissue damage in cerebral malaria, lipopolysaccharide induced pathology, and pulmonary fibrosis is also discussed. This has then been extended to include the intercellular mechanisms underpinning their pathogenic role in metastasis, transplant rejection, stroke, brain hypoxia, and related conditions. A better understanding of the complex regulation and hierarchical organisation of these various platelet adhesion molecules may prove useful in the development of new approaches to the treatment of such diseases.

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This review outlines the role of a number of key surface molecules expressed by platelets in various immuno-inflammatory mechanisms. As platelets exhibit a plethora of surface as well as intracellular molecules thought to be implicated in such functions, we found it necessary to restrict this overview to those considered most important in these cellular processes. We chose those involved in adhesion, without any attempt to establish a hierarchy of importance in pathogenic mechanisms (fig 1). Several other pathways of cell-cell communication between platelets and various other cell types, such as those implicated in haemostasis, have been reviewed elsewhere.¹

Much of the information currently available on phenomena related to platelet adhesion concerns platelet-endothelial cell interactions, platelet-platelet aggregation, and thrombus formation. Only recently, have the molecular mechanisms underlying the interaction between platelets and leucocytes become the topic of intense research. Descriptions of the

functions of adhesion molecules as regulators of leucocyte trafficking have provided a new stimulus for interesting investigations into the biology of leucocyte adhesion. Adhesion phenomena have also gained importance in broadening our understanding of tumour cell dissemination and extravasation. As platelets are abundant in the bloodstream and exhibit such marked adhesive properties, we consider that alterations in the expression of specific adhesion molecules may play an important part in mediating their protective or pathogenic roles in the body. With this in mind, we have collated data from both clinical and experimental studies on this aspect of platelet biology.

Surface molecules mediating platelet interactions with other cell types

GLYCOPROTEIN IIb-IIIa

Platelet anchorage and signalling is mainly regulated by the integrin gpIIb-IIIa (CD41a,b/CD61, α IIb- β 3, fibrinogen receptor).^{2,3} Blocking the gpIIb-IIIa receptor using monoclonal antibodies or small peptides such as Asp-Gly-Arg (RGD), which competes with the fibrinogen binding site, significantly inhibit activated platelet adhesion to activated endothelium.⁴ GpIIb-IIIa is also a central molecule in the mediation of platelet aggregation. Fibrinogen binding to gpIIb-IIIa plays a pivotal role in formation of platelet thrombi by providing molecular bridges spanning platelets and mediating intracellular signalling triggered by this interaction. Fibrinogen binding to gpIIb-IIIa ensues in response to external signals generated at the site of vascular injury. These external signals lead to a conformational change of the gpIIb-IIIa induced by inside-out signalling mediated by intraplatelet signal transducers. The structure and functions of gpIIb-IIIa have been reviewed recently.^{5,6} In the case of immune complex induced platelet aggregation, there is evidence for a close topographical and functional association of gpIIb-IIIa with Fc γ receptor type II.⁷

On stimulation, endothelial cells release von Willebrand factor (vWF) that mediates platelet adhesion and shear stress induced aggregation.⁸ Shear stress induced platelet aggregation is mediated by vWF binding to platelet membrane gpIb and IIb-IIIa. Activation of gpIIb-IIIa is initiated by the gpIb-IX complex whereby vWF first binds to platelet gpIb-IX, leading to the interaction of the ligand fibrinogen with gpIIb-IIIa, resulting in platelet spreading and aggregation. A tyrosine phosphorylated protein, pp125FAK, is involved in

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Figure 1 Main platelet surface molecules involved in adhesion. Roman numerals pertain to the platelet glycoprotein nomenclature. vWF, von Willebrand factor; vWF-R, von Willebrand factor receptor (CD24b/CD42a); lam-R, laminin receptor (VLA-6, CD49f/CD29) (the fibronectin receptor is not shown, it is identical to the lam-R, except that the α chain is CD49e); vn-R, vitronectin receptor (CD51/CD61, α V β 3); fn-R, fibrinogen receptor, gpIIb-IIIa, CD41/CD61, α IIb- β 3; coll-R, collagen receptor (CD49b/CD29); TSP, thrombospondin; PEMP-1, Plasmodium falciparum erythrocyte membrane protein 1, CD36, gpIV, TSP-receptor; CD31, platelet-endothelial cell adhesion molecule 1, PECAM-1, platelet gpIIa'; α V β 3, vitronectin receptor; GAG, glycosaminoglycans; PSGL-1, P-selectin glycoprotein ligand 1; ICAM-1, intercellular adhesion molecule 1, CD54; LFA-1, leucocyte function antigen 1, CD11a/CD18.

this signalling process.³ As a consequence of platelet activation, conformational changes and fibrinogen binding, gpIIb-IIIa becomes associated with the cytoskeleton. The cytoplasmic sequences of both gpIIb and gpIIIa directly bind to talin, a molecule connecting to the cytoskeleton.⁹

The expression of gpIIb-IIIa is unique to the megakaryocyte lineage. Besides its predominant role in platelet-platelet aggregation, it also allows their aggregation on to heterologous cells,¹⁰ notably on to polymorphonuclear (neutrophils) leucocytes.¹¹⁻¹² In addition, fibrinogen receptors have also been reported to be expressed on some tumour cells.¹³⁻¹⁴ However, other mechanisms are involved in interactions between platelets and various cell types, notably that of thrombospondin with its receptor,¹⁵ or with other binding partners such as CD36¹⁶ or the vitronectin receptor.¹⁵

The interactions between gpIIb-IIIa and its ligands can be abrogated using several types of inhibitor. Ligands for gpIIb-IIIa comprise fibrinogen, of which RGD is a recognition sequence, fibronectin, vWF, thrombospondin, and vitronectin.¹⁷ Several types of gpIIb-IIIa antagonists have been described and characterised both *in vitro* and *in vivo*. These include small peptides competing with recognition sequences (such as RGD), pentamidine,¹⁸ SKF 107260,¹⁹ DMP728,²⁰ SC-49992,²¹ Ro-43-8857,²² disintegrins, a family of snake venom derived proteins,²³ and disagregin.²⁴ Additionally, platelet adhesion to fibronectin can be inhibited by direct binding of heparin to the fibronectin surface.²⁵ GpIIb-IIIa can also be antagonised indirectly by interfering with the binding of vWF to gpIIb. VCL, a recombinant monomeric fragment of vWF, containing the gpIIb binding domain, has such properties.²⁶ Finally, it has also been demonstrated that platelet aggregation is inhibited by fibrinolysis.²⁷ Tissue-type plasminogen activator (tPA) induces thrombolysis in coronary arteries through the local generation of plas-

min. Plasmin also proteolyzes gpIIb and plasma vWF. Consequently, tPA inhibits platelet aggregation in response to pathological shear stress by altering the multimeric composition of vWF.

P-SELECTIN

P-selectin (CD62-P), originally described as PADGEM/GMP 140, is an α granule membrane protein that is expressed on the platelet surface on activation.²⁸⁻²⁹ Antibodies to P-selectin can inhibit platelet aggregation, suggesting an active role of P-selectin in platelet-platelet interactions.³⁰ In addition, P-selectin mediates platelet and leucocyte rolling on stimulated endothelium, as shown by the impairment of this phenomenon in mice genetically deficient for this molecule.³¹ This interaction requires endothelial rather than platelet P-selectin. As platelet rolling is independent of platelet activation, this suggests a constitutive expression of a P-selectin ligand on platelets. The P-selectin glycoprotein ligand-1 (PSGL-1)³² found on myeloid cells is also expressed on human platelets.³³ Furthermore, it has been shown that activated platelets can signal chemokine synthesis by monocytes via PSGL-1 on the latter cells.³⁴

P-selectin is also crucial in neutrophil adhesion to damaged endothelium under flow conditions.³⁵ The finding that neutrophils adhere predominantly to platelets attached to the extracellular matrix, and not directly to endothelial cells, supports the hypothesis that platelet adhesion to an injured vessel wall leads to P-selectin expression by platelets, thus mediating leucocyte co-localisation. Studies with specific antibodies showed that platelet dependent neutrophil adhesion was selectin mediated. Inhibition of P-selectin caused a marked inhibition of adhesion at high shear stress, whereas the role of leucocyte L-selectin was less pronounced. Such platelet mediated leucocyte delivery to endothelial cells has been documented using intravital microscopy.³⁶ It

has also been shown that granulocytes enhance tissue factor expression on monocytes via platelet interaction and a P-selectin dependent pathway.³⁷

Circulating lymphocytes gain access to lymph nodes because of their ability to initiate rolling along specialised high endothelial venules (HEVs). One mechanism of rolling involves L-selectin binding to peripheral node addressin (PNAd) on HEVs. Activated platelets are shown to bind to circulating lymphocytes and to mediate rolling in HEVs *in vivo* through another molecule, P-selectin, which also interacts with PNAd. *In vitro*, activated platelets enhanced tethering of lymphocytes to PNAd and sustained lymphocyte rolling, even in the absence of functional L-selectin. Thus, a platelet pathway operating through P-selectin provides a second mechanism for lymphocyte delivery to HEVs. More recently, the involvement of P-selectin in the immigration of Th1 cells into inflamed skin was demonstrated by *in vivo* blocking experiments³⁸ suggesting adhesion mechanisms able to distinguish between the two T helper subsets and mediating their differential trafficking.

As a further ligand, the highly glycosylated heat stable antigen (HSA) CD24, a cell surface molecule expressed by many cell types in the mouse, has been shown to mediate the binding of monocytic cells and neutrophils to P-selectin. Thus, in addition to PSGL-1, CD24 belongs to a group of monospecific P-selectin ligands on myeloid cells and is thereby able to link myeloid cells to endothelial cells and platelets.^{39 40}

PLATELET-ENDOTHELIAL CELL ADHESION MOLECULE 1 (PECAM-1, CD31)

CD31, a transmembrane glycoprotein of the immunoglobulin supergene family, is expressed on platelets as well as on endothelial cells, monocytes, neutrophils, and naive T lymphocytes. It is engaged in homotypic and heterotypic interactions, notably with $\alpha V\beta 3$ ⁴¹ and with glycosaminoglycans.⁴² CD31 mediates the binding of platelets to injured endothelial cells. Importantly, it has been shown that the platelet deposition occurs at sites of minor endothelial cell injury, where the endothelial cell layer was intact, and collagen or basal lamina were not exposed.⁴³ Monoclonal antibodies to CD31 protect against transendothelial migration of leucocytes, notably neutrophils, in models of acute peritoneal inflammation⁴⁴ and myocardial ischaemia-reperfusion injury.⁴⁵ Anti-CD31 monoclonal antibodies also protected against platelet deposition in a model of minor endothelial damage of mouse brain pial arterioles.⁴⁶

LEUCOCYTE FUNCTION ANTIGEN 1 (LFA-1)

In mice, the expression of LFA-1 on platelets has been shown by immunoprecipitation⁴⁷ and flow cytometry.⁴⁸ This platelet LFA-1 is thought to interact with ICAM-1 upregulated on endothelial cells in case of inflammation, such as in experimental cerebral malaria,⁴⁸ Schwartzman reaction⁴⁹ or pulmonary

fibrosis.^{50 51} The relevance of these findings in humans remains the subject of much debate. Monoclonal antibodies against the LFA-1 subunit of the leucocyte adherence complex, CD18, inhibited the binding of bone marrow megakaryocytes to HUVEC.⁵² The low and variable expression of LFA-1 (that can be increased upon exposure to thrombin) has been shown on human platelets.⁵³ However, anti-LFA-1 antibodies do not interfere with mononuclear leucocyte induced inhibition of platelet aggregation.⁵⁴ This is evidence against an involvement of platelet LFA-1 in the aggregation mechanism. Although there is no evidence for ICAM-1 expression on human platelets, it is notable that these cells express ICAM-2 and are able to bind T lymphocytes via this receptor.⁵⁵

CD36 (gpIV)

CD36 is expressed on platelets as well as on monocytes and endothelial cells.⁵⁶ The numerous functional aspects of this cell surface glycoprotein, as well as its various ligands, have been reviewed recently.⁵⁷ Blocking experiments with monoclonal antibodies indicated that CD36 mediates platelet aggregation⁵⁸ but not the adhesion of platelets to collagen and subendothelial matrix.⁵⁹ CD36 binds to thrombospondin, the role of which in platelet adhesion remains controversial. Both adhesive and anti-adhesive properties have been attributed to this molecule. In contrast to the extensive platelet spreading observed on fibronectin at all shear rates, platelet spreading on thrombospondin occurred only sporadically and at high shear rates. GpIa-IIa, gpIIb-IIIa, CD36, and the vitronectin receptor, which are all proposed platelet receptors for thrombospondin, are not solely responsible for platelet adhesion to thrombospondin. CD36 may thus play a dual role in adhesive processes *in vivo*: it may function in conjunction with other adhesive proteins to maintain optimal platelet adhesion at various shear rates; and it may serve as a modulator of cellular adhesive functions under specific microenvironmental conditions.⁶⁰ The mechanisms by which CD36 increases $\alpha V\beta 3$ function have been analysed. It involves the integrin associated protein (IAP, CD47), which serves as a receptor for the carboxy terminal cell binding domain of thrombospondin.⁶¹

The CD36-thrombospondin interaction has a further role in the stabilisation of platelet aggregates, leading to irreversible aggregation, via the formation of a macromolecular complex involving CD36, thrombospondin, gpIIb-IIIa, and fibrinogen, as well as in platelet-monocyte and platelet-melanoma cell adhesion.⁵⁷ CD36 and thrombospondin, together with the vitronectin receptor $\alpha V\beta 3$ integrin, have been identified as the adhesion molecules on the surface of macrophages implicated in the clearance of neutrophils undergoing programmed cell death.⁶² The efficient phagocytosis of apoptotic neutrophils is a crucial process for the resolution of inflammatory responses. A functional domain encompassing amino acids 155-183 on the CD36 molecule is directly

involved in the recognition and subsequent phagocytosis of apoptotic neutrophils.⁶³

Interestingly, cloning of a drosophila haemocyte-macrophage receptor, croquemort (catcher of death), has shown it to be a member of the CD36 superfamily. CD36 mediated phagocytosis supports the concept that phagocytosis evolved primarily as a cellular process for the removal of effete cells.⁶⁴ Along this line, it could be speculated that the presence of such a scavenger receptor, CD36, on platelets might allow the interaction between platelets and apoptotic or damaged endothelial cells.

OTHER SURFACE MOLECULES

Other platelet receptors, such as complement and Fc receptors,⁶⁵ α_2 -adrenergic receptors, receptors for ADP, thrombin, and various types of collagen, have been described and reviewed elsewhere.⁶⁶ Furthermore, platelet-endothelial cell adhesion is regulated by endothelial cell derived mediators, such as prostacyclin and endothelium derived relaxing factor (nitric oxide). In vitro, platelet prostacyclin receptor desensitisation causes a marked augmentation of platelet-endothelial cell adhesion.⁶⁷

The fibrinogen receptor gpIIb-IIIa-fibrinogen interactions and the binding of P-selectin to its diverse ligands may account primarily for the phenomenon of platelet aggregation. However, these molecules, together with CD31 and CD36, also play substantial roles in adhesion events involving platelets, leucocytes, endothelial cells, and other cell types.

The protective role(s) of platelets

Platelets play a protective role in a number of bacterial diseases, and their adhesion to bacteria or other foreign particles has long been recognised.⁶⁸ Apart from this, consistent with the observation that thrombocytopenia is associated with increased capillary fragility, an endothelial supporting function of intact platelets has been described.⁶⁹ This is thought to be due not merely to platelets adhering to the capillary wall but also to the platelets insinuating themselves into the junctions between endothelial cells or actually becoming incorporated into the endothelial cytoplasm. This fusion phenomenon has been given little attention and is discussed below in the context of vascular pathology. When radiolabelled platelets are transfused into thrombocytopenic animals, ultrastructural studies have revealed single platelets in close association with the endothelial membranes, and the radioactive label deposited in the capillaries of numerous organs. Labelled platelet poor plasma did not result in a similar labelling of the capillary endothelial layers.⁷⁰ The factors responsible for the capillary permeability of isolated perfused organs maintained for transplantation were investigated. It was found that capillary integrity was better preserved when the perfusate contained whole, viable platelets in addition to plasma proteins.⁷¹ The endothelial cell regeneration after arterial injury was stimulated by platelets, and this was dependent on attachment of the platelets to the area of injury as the

supernatant of aggregated platelets had no effect.⁷²

Platelets also appear to be capable of protecting cells from tumour necrosis factor (TNF) mediated cytotoxicity.⁷³ This was shown in L-929 fibrosarcoma, where the inhibitory effect depended on the concentration of platelets and was as high as 50%. The decrease in responsiveness to TNF reflected neither a degradation of TNF nor an inability of L-929 cells to bind TNF. Furthermore, addition of platelets one or two hours after TNF was still protective suggesting that platelets promoted hyporesponsiveness of L-929 cells to a post-binding effect of TNF. Platelet induced reduction of the TNF effect could be reproduced with platelet supernatants. A role for 12(S) hydroxyeicosatetraenoic acid (HETE) is likely because this metabolite reduced TNF induced cytotoxicity in a dose dependent manner.

These observations indicate that platelets can ameliorate cytokine induced cytotoxicity and suggest that this biological effect should be evaluated on cell types other than tumour cells. Conversely, platelets are able to kill tumour cell lines directly, such as the chronic myelogenous leukaemia cell K-562.⁷⁴ This, together with data discussed below, illustrates the complex and sometimes double faceted properties of platelets, depending on the experimental setting.

In the context of parasitic diseases, other functions of platelets have been described that are protective for the infected host. In vitro, human platelets have been shown to inhibit the growth of *Plasmodium falciparum*,⁷⁵ and neither gpIIb-IIIa nor CD36 seem to be involved in this effect.⁷⁶ Platelets also mediated cytotoxicity against *Toxoplasma gondii*.⁷⁷ In vivo, TNF stimulated platelets were able to protect rats against *Schistosoma mansoni* infection.⁷⁸ In this latter case, an important role for IgE and Fc ϵ RII expressed on platelets has been described.^{79, 80}

Pathogenic roles of platelets

CEREBRAL MALARIA

Thrombocytopenia is a frequent complication of malaria infection. Direct interactions between blood platelets and malaria parasites⁸¹ as well as immune mechanisms⁸²⁻⁸⁴ have been implicated in the pathogenesis of this condition. In addition, platelets can participate, notably via their surface CD36, in the phenomenon of parasitised erythrocyte sequestration, thus promoting the vascular occlusion characteristic of cerebral malaria.⁸⁵ Endothelial ICAM-1 mediates the initial, rolling-type interaction between endothelium and sequestered cells, while, via their CD36, platelets may be responsible for a tight adhesion of parasitised erythrocytes.⁸⁶ In vitro experiments indicate that the great majority of adherent parasitised cells form rolling rather than static attachments to endothelial cells and ICAM-1, whereas static attachments predominate for platelets, CD36, and TSP. Thus, the different receptors may have complementary roles in modulating adhesion in microvessels.

Investigations in murine models of cerebral malaria suggest a new effector mechanism of TNF induced endothelial cells lesion—the role for TNF in cerebral malaria (both human and murine) has been reviewed.⁸⁷ It consists of increased adhesion of platelets to the surface of endothelial cells and their subsequent fusion into the cytoplasm of endothelial cells. In the mouse system, platelets express LFA-1 on their surface, therefore, they were envisaged in the pathogenesis of cerebral malaria. Otherwise there was no clear explanation for the dramatic protective effect of anti-LFA-1 monoclonal antibody (MoAb), even when this MoAb was given shortly before death.^{88, 89} The following findings suggest that the platelet is a critical effector of the neurovascular injury in cerebral malaria.⁴⁸ First, investigation of brains from mice with cerebral malaria using electron microscopy revealed that during cerebral malaria platelets adhere to and probably damage brain endothelial cells. Interestingly, platelets have also been found in brain vessels in fatal cases of human cerebral malaria.⁹⁰ Second, radiolabelled platelet distribution studies indicated that platelets significantly sequestered in the brain and lung vasculature during cerebral malaria. Non-cerebral malaria was not associated with cerebral sequestration of platelets. Third, treatment in vivo with anti-LFA-1 MoAb selectively abrogated the cerebral sequestration of platelets; moreover, this correlated with prevention of the neurological syndrome. The α (CD11a) and, to a lesser extent, the β (CD18) chains of integrin LFA-1 were found to be expressed on mouse platelet membranes. Finally, animals with malaria rendered thrombocytopenic were significantly protected against cerebral malaria, further indicating that platelets are central to the pathogenesis of cerebral malaria.

Thus, a CD11a dependent interaction between platelets and endothelial cells appears pivotal to microvascular damage. Microvascular lesions of cerebral malaria would thus depend on platelet mediated, TNF induced endothelial cell damage (fig 2).⁹¹ These data suggest a novel mechanism of action for

anti-LFA-1 MoAb in the neurovascular complications of murine malaria and illustrate an unexpected role for platelets in vascular pathology.

The mechanism of platelet fusion with endothelial cells may be the same in physiological and pathological situations, with the main difference between these two conditions being the intensity of the phenomenon. Morphological data should orientate towards the precise mechanisms involved, which remain incompletely understood. In experimental cerebral malaria, cerebral capillaries and venules contain platelets in dense contact to the endothelium or fusing to damaged brain endothelial cells. Endothelial cells are damaged, interrupted, and show the presence of dense and α granules, suggesting the fusion between platelets and endothelium. Monocytes also adhere to brain endothelial cells but, unlike platelets, do not show any membrane fusion and the endothelium is unaffected. These morphological features of platelet-endothelium interactions are reminiscent of those reported in the context of the endothelium supporting role of platelets.⁶⁹⁻⁷¹ TNF induced microvascular injury might be caused by an exaggeration of the fusion phenomenon caused by ICAM-1 upregulation on endothelial membranes. Recently, a particular role for membrane bound TNF has been demonstrated in ICAM-1 upregulation, and thereby in cerebral malaria pathogenesis.⁹² Platelets carrying the LFA-1 molecule would then be bound in higher amounts on these endothelial surfaces, and cell fusion would occur at a higher rate (fig 2). In humans, other ligand-receptor pairs, implicating P-selectin or CD36, could participate in this phenomenon.

The involvement of platelets as effectors of vascular lesions may not be restricted to experimental pathology. In humans, cerebral malaria has been associated with accumulation of platelets in cerebral microvessels, although the nature of the platelet-endothelial interactions in this setting have not been investigated in detail.⁹⁰ Platelet trapping in lesions of brain vasculitis has also been reported in patients

Figure 2 Endothelial cell-platelet interactions in experimental cerebral malaria. pRBC, parasitised red blood cell; Th1, T helper 1 cell subset; memTNF, membrane form of TNF; MO, monocyte; T, T lymphocyte.

with systemic lupus erythematosus (SLE).⁹³ However, differences between human and murine platelets suggest that the involvement of platelets may be different in human from mouse pathology.

Thus, the role of platelets in the vascular pathology of cerebral malaria is an illustration of unexpected properties of TNF.⁹⁴ The way platelets interact with endothelial cells and modify the physiology of these cells represents a possibly important effector pathway of TNF induced vascular pathology. This can be further amplified by IL-1 expressed on the surface of activated platelets.⁹⁵ Indeed, platelet surface IL-1 may provide a mechanism for altering in an extremely localised and rapid manner the properties of IL-1 responsive cells with which platelets come in direct contact during processes of inflammation and vessel wall damage. This platelet IL-1 may also activate endothelial cells.⁹⁶

SEPTIC SHOCK, ARDS, AND LPS INDUCED PATHOLOGY

Platelet and neutrophil accumulation in the pulmonary microvasculature contributes to the pathogenesis of acute respiratory distress syndrome (ARDS), one of the complications of septic shock.⁹⁷ Pulmonary hypertension classically occurs during the first hours of ARDS and may be induced in normal, but not thrombocytopenic, dogs by intravenous injection of bacterial endotoxin (LPS).⁹⁸ Platelet aggregates were localised in both pulmonary and hepatic capillaries.^{99–101} The hepatic sequestration of platelets observed in these experiments may not result simply from the formation of intravascular microthrombi. Histological examination of the livers of endotoxaemic mice revealed the translocation of platelets from the blood stream to the sinusoidal and perisinusoidal (Disse) spaces between hepatocytes and endothelial cells.^{99–102} After a transient accumulation of platelets in pulmonary vasculature, LPS administration results in platelet sequestration in liver and spleen.¹⁰³ Interaction of activated platelets with other cell types, such as monocytes, neutrophils, and endothelial cells, may lead to production of mediators and subsequent tissue damage leading to septic shock.^{97–104–105}

That platelets could participate in the pathogenesis of LPS induced lesions was deduced from the various pieces of evidence that platelets can be effector cells of biological functions such as the lysis of tumour cells or of parasites. The involvement of platelets in LPS induced pathology had so far been viewed only as a consequence of vascular damage, by the role of platelets in haemostasis. Indeed, it is known that the localised Schwartzman reaction, consisting of haemorrhagic necrosis in the skin following sequential injections of LPS and TNF, is preceded by the sequestration of neutrophils and platelets along microvessels.¹⁰⁶ The role of platelets was further investigated in the local Schwartzman reaction as well as in the systemic toxicity of LPS in D-galactosamine sensitised animals.⁴⁹ In both experimental conditions, it was found that platelet depletion in vivo (by

either poly- or monoclonal antiplatelet antibody treatment) afforded significant protection. Furthermore, in the local reaction, platelets were found to be localised in the dermal venules before haemorrhage occurred. Anti-LFA-1 and anti-ICAM-1 MoAbs prevented both platelet localisation and haemorrhagic necrosis. Thus, at least in the local reaction, the combined effects of LPS and TNF result in a binding of platelets to microvascular endothelium via their β_2 integrins, which seems necessary for the development of the haemorrhagic necrosis.

Indirect evidence for a role of platelets in LPS or TNF pathology has been provided by experiments demonstrating the protective capacity of $\alpha(1)$ -acid glycoprotein.¹⁰⁷ This typical acute phase protein protects mice from lethal shock induced by TNF or endotoxin. The protection is observed both in normal and in galactosamine sensitised mice. Complete inhibition of all TNF induced metabolic changes was observed: fall in body temperature, release of liver transaminases, enhanced clotting time, and mortality. As $\alpha(1)$ -acid glycoprotein is known to inhibit platelet aggregation, its protective efficacy in this setting may suggest a pathogenic role of platelets.

Other illustrations of a role of platelets have been obtained in a sheep model of endotoxic shock.¹⁰⁸ Platelet sequestration in the lungs and liver of LPS challenged animals was more efficiently blocked by the dual cyclooxygenase and lipoxygenase inhibitor ketoprofen than by the classic cyclooxygenase inhibitor aspirin. In sheep sepsis, pentoxifylline, provided it was given before LPS, was also able to reduce pulmonary and hepatic platelet sequestration.¹⁰¹ In a model of porcine sepsis, platelets were found occluding the hepatic vasculature, and this as well as other ultrastructural changes were prevented by the dopaminergic and β_2 adrenergic receptor agonist dopexamine,¹⁰⁹ raising the possibility that this molecule, in addition to its systemic circulatory effects, can also modulate platelet–endothelial interactions.

The role of platelet membrane glycoproteins and platelet–leucocyte adhesion was studied in patients with sepsis and multiple organ failure.¹¹⁰ Flow cytometry was used to examine platelet membrane expression of various adhesion molecules on circulating platelets and the appearance of platelet specific antigen (gp IIb, CD41) on leucocytes as an index of platelet–leucocyte adhesion. The results were compared with severity of disease and according to outcome in patients. In septic patients fibrinogen receptor activity on platelets was significantly above normal values. When multiple organ failure was present, thrombospondin surface expression on circulating platelets also increased significantly. Concomitantly, platelet–leucocyte adhesion was increased in sepsis and decreased in patients with multiple organ failure. Significant lower numbers of circulating platelet–leucocyte aggregates occurred in non-survivors. Thus, sepsis is associated with increased surface expression of platelet adhesion molecules and increased occurrence of circulating platelet–leucocyte

Figure 3 Tumour cell-platelet interactions. 12(S)HETE, 12(S) hydroxyeicosatetraenoic acid. (See fig 1 for other abbreviations).

aggregates. The decrease in circulating platelet-leucocyte aggregates in multiple organ failure might result from enhanced peripheral sequestration. An increased platelet-leucocyte adhesion and sequestration might thus account for the development of multiple organ failure in the course of sepsis.

PULMONARY FIBROSIS

Piguet *et al*⁵⁰ originally described increased platelet accumulation in an experimental model of pulmonary fibrosis. In this context, treatment of mice with anti-LFA-1 MoAb prevents platelet accumulation as well as tissue damage. The arguments in favour of a role of platelets as effectors of these lesions include (a) increased amounts of platelets sequester in damaged organs; (b) their sequestration is prevented by *in vivo* treatment with anti-LFA-1 mAb; and (c) microvascular damage is prevented by *in vivo* treatment with antiplatelet antibodies. More recently, it has been shown that bombesin acts as an inhibitor of the development of pulmonary fibrosis, possibly by decreasing pulmonary platelet trapping.¹¹¹

METASTASIS PROMOTING EFFECT

Inhibition of integrin binding by RGD peptides inhibits metastasis, indicating an important role for $\beta 3$ integrins in tumour cell metastasis.¹¹² Conversely, measures leading to enhanced platelet aggregation provided by inflammatory stimuli or tumour cells correlated with a higher rate of metastasis.^{13 113} Many different mechanisms have been proposed by which platelets may promote tumour metastasis. These include stabilising tumour cell arrest in the vasculature, stimulation of tumour cell proliferation, promoting tumour cell extravasation by potentiating tumour cell induced endothelial cell retraction, and enhancing tumour cell interactions with the extracellular matrix.¹³

Enhanced endothelial cell retraction—a prerequisite for extravasation of most tumour cell types—has been proposed as one mechanism whereby platelets may facilitate metastasis. This was found to be mediated by 12(S)-HETE, a lipoxygenase metabolite of arachidonic acid, the biosynthesis of which was enhanced upon tumour cell-platelet-endothelial cell interaction.¹¹⁴ However, in the

absence of natural killer cells, equally strong metastasis was observed in thrombocytopenic and normal mice (Nieswandt *et al*, 1997, unpublished data). This demonstrated that platelets are not essential for tumour cell extravasation. The principal mechanism by which platelets promote metastasis appears to be tumour cell protection from natural killer cell lysis by covering their surface. Whether this protective effect was only by steric hindrance or platelet specific inhibition of other antitumour functions of natural killer cells is not clear. In the absence of natural killer cells virtually all inoculated tumour cells completed the metastatic cascade, irrespective of the presence or absence of platelets. This suggests that stabilisation of tumour cells in the vasculature and enhancement of their interactions with the subendothelial matrix may represent platelet effects of secondary importance.

A possible molecular link between aggregated platelets and the tumour cell surface may be provided by CD36-TSP interaction,^{15 16 57} gpIIb-IIIa,^{13 14} or P-selectin³⁹ on platelets and the respective binding partners on the different tumour cell types (fig 3). Several tumour cell lines have been reported to express CD36,¹¹⁵ as well as the $\alpha V\beta 3$ integrin.¹⁵ Under flow conditions *in vitro*, tumour cell attachment to extracellular matrix is crucially dependent on platelets and the interaction of platelet gpIIb-IIIa with tumour cell αV integrins.^{116 117} Other platelet factors involved in metastatic dissemination of tumour cells are chemoattractants.¹¹⁸ Further data supporting the hypothesis that platelets facilitate metastasis by enhancing extracellular matrix degradation are provided by the finding of platelet heparitinase¹¹⁹ and the observation that the increase of gelatinase secretion upon tumour cell-platelet interaction supports invasiveness.¹²⁰

TRANSPLANT REJECTION

Rejection of a transplanted organ is associated with occlusion of blood vessels and extensive damage to vascular walls. Platelet aggregates are largely responsible for this vascular occlusion and may also contribute to the vascular damage. When small blood vessels are occluded by platelet aggregates showing morphological signs of degranulation there will be vascular damage, whereas the endothelium remains intact in vessels containing platelets that have not degranulated. When platelets are pretreated to deplete their granules of 5-hydroxytryptamine, the extent of vascular injury in response to intravascular immune complex formation is reduced, and treatment with drugs that inhibit platelet aggregation and the release reaction prolongs the survival time of transplanted organs.¹²¹

More recently, it was shown that in a model of delayed xenograft rejection as seen in complement depleted rat recipients of guinea pig cardiac xenografts, a specific gpIIb-IIIa antagonist (SDZ GPI 562) significantly increased graft survival. Immunohistological studies showed little effect of this antagonist on platelet aggregation or activation and no effect on fibrin deposition. However, the combination

of high doses of gpIIb-IIIa antagonist and complement depletion by cobra venom factor resulted in a significant decrease in intragraft platelet aggregation, P-selectin expression, and leucocyte infiltration compared with decompensation alone. Thus, the diminution of intragraft platelet microthrombi formation and leucocyte infiltration suggests an important role for platelet dependent mechanisms in leucocyte recruitment during delayed xenograft rejection.¹²²

STROKE, BRAIN HYPOXIA, AND RELATED CONDITIONS

Pathophysiological cerebrovascular reactions including symptomatic angiospasm following subarachnoid haemorrhage, segmental occlusive constriction in atherosclerotic cerebral arteries, and constrictive vasomotion in microvessels, involve intravascular activation of blood cells leading to scattered microvessel plugging, increased vascular permeability, oedema formation, and cytotoxic actions of blood cell released agents on the underlying tissue. A variety of pathophysiological stimuli may trigger endothelial reorganisation with the expression of different prothrombotic factors and activation of platelets and leucocytes that, combined, leads to blood cell adhesion to the endothelial monolayer, aggregation as thrombi, and the formation of numerous spasmogenic substances.¹²³ Some drugs have been demonstrated to limit platelet and leucocyte activity and to protect the endothelial defence mechanisms, but an optimal therapeutic strategy has yet to be elaborated.

One of the molecules implicated in the trapping of platelets in brain microvessels is CD31; it has been demonstrated that a monoclonal antibody to this adhesion molecule delays platelet adhesion-aggregation at a site of minor endothelial injury.⁴⁵ The model used was injury of the endothelium of arterioles on the surface of the mouse brain by a helium-neon laser in the presence of intravascular Evans blue. The first recognisable platelet aggregate forming at the injured site, as assessed by intravital microscopy, showed that aggregation latency was significantly prolonged by anti-CD31 MoAb, while aggregation *ex vivo* was not affected. Thus, PECAM is an important modulator of platelet adhesion-aggregation at sites of minor endothelial damage in brain arterioles. The data are consistent with the hypothesis that PECAM sites on the endothelium are involved and may be exposed by the injury to promote adhesion-aggregation.

The same model was used to test the hypothesis that, once formed, platelet aggregates may injure underlying cerebrovascular endothelium.¹²⁴ Such injury could make the same site selectively attractive to the next wave of passing emboli or activated platelets. This vicious circle could account for repetitive, stereotypic symptoms in transient ischaemic attacks. The degree of platelet activation (rounded or degranulated forms) paralleled the degree of endothelial damage ascertained in electron micrographs. This association could represent either an effect of endothelium on

platelets or an effect of platelets on endothelium. Although the former alternative cannot be totally ruled out, the observations seem to fit the hypothesis that progressive endothelial damage can result from increasing activation and degranulation of overlying platelets.

Other models have shown that on cerebral ischaemia reperfusion, adhesion molecules such as P-selectin and ICAM-1 are upregulated on microvessels in the ischaemic zone, leading to leucocyte adhesion and activation, but the precise role of platelets in this setting remains to be investigated.¹²⁵

Conclusions

Relatively few studies have directly addressed the role of platelets in inflammation. During the past decade, adhesion molecules have been the focus of intense research, with much importance given to cell-cell interactions; however, their complex role in the activation pathways of haemostasis and inflammation require further study. Of particular interest will be investigations on the interplay of platelet surface molecules and leucocyte integrins or selectins and endothelial cell adhesion molecules.

With respect to the activation of cells, a major contribution was the finding that the pairing of adhesion molecules in cell-cell interactions facilitates the signalling into both cells to mediate, in part, cell activation. The signals can either be mediated via the adhesion molecules serving as membrane receptors in heterophilic or homophilic interactions or via the respective membrane associated ligands. Furthermore, soluble ligands can induce signalling via adhesion molecule-receptor aggregation or conformational changes after ligand binding. Such signalling, which leads to activation of cells, can also induce the release of biologically important mediators from platelets. As platelets are so numerous and degranulation is a rather rapid event this may represent a very effective biological response. Localisation by adhesion in some microvascular beds (such as at inflammatory sites) provides an ideal means of restricting the effect to the site of interest.

A role for platelets can be predicted in some types of hypersensitivity reactions, and notably immune complex mediated diseases. The involvement of platelets in the pathogenesis of these conditions should be revisited. Differences between animal (rabbit or mouse) and human platelets should also be considered. Based on physiological and pathological relevance *in vivo*, one should design experiments aiming at a better definition of the hierarchy of importance of various platelet adhesion molecules in selected biological effects. Exploiting such findings should be useful for developing new therapeutic approaches.

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