bjh review

Plasma microparticles and vascular disorders

Susan F Lynch[1,2] and Christopher A Ludlam[1,2]

[1]College of Medicine and Veterinary Medicine, University of Edinburgh, and [2]Department of Clinical and Laboratory Haematology, Royal Infirmary of Edinburgh, Edinburgh, UK

Summary

Microparticles are circulating, phospholipid rich, submicron particles released from the membranes of endothelial cells, platelets, leucocytes and erythrocytes. Investigation into their biological activity has revealed diverse actions in coagulation, cell signalling and cellular interactions. These actions are mediated through their phospholipid rich surfaces and the expression of cell surface molecules which reflect their cell of origin and its state of activation.

Microparticle numbers are reported to be elevated in a number of conditions where vascular dysfunction and inflammation are important pathophysiological mechanisms, for example coronary artery disease or thrombotic microangiopathies. Currently, there are a variety of different methods used for the quantitation of circulating microparticles; however with standardisation their assessment may prove to be of clinical value, reflecting the state of the vasculature. Knowledge of the functional properties of microparticles will contribute to our understanding of the mechanisms underlying vascular dysfunction and prothrombotic states.

Keywords: microparticles, endothelial function, coagulation, cellular interactions, vascular disorders.

There has been a resurgence of interest in circulating microparticles from endothelial cells, platelets and leucocytes because of their newly recognised diverse physiological and pathological functions. Microparticles are plasma particles of <1 μm diameter that are formed by the exocytic budding of cell membranes. During their formation the symmetry of the plasma membrane lipid bilayer is altered, resulting in the exposure of a surface that is rich in negatively charged phospholipids. In addition, the microparticles bear antigens expressed on the surface of the cells from which they originate. It is this anionic phospholipid surface that can bind coagulation factors, and the expression of functional molecules such as tissue factor (TF) or selectins that mediate the biological actions of microparticles. Furthermore, elevated levels of microparticles have been found in a number of conditions associated with vascular dysfunction, thrombosis and inflammation.

This review will address the formation and biological activity of microparticles; the methods used in their isolation and identification; and their role in prothrombotic disorders including antiphospholipid syndrome, the thrombotic microangiopathies and cardiovascular disease.

Formation and biological functions of microparticles

Formation and composition of microparticles

Microparticles are released from the surface of cells following cell activation or apoptosis by triggers including chemical stimuli, such as cytokines, thrombin and endotoxin, or physical stimuli, such as shear stress or hypoxia (VanWijk *et al*, 2003). Following cell activation, microparticle formation is dependent on a rise in the cytosolic calcium concentration with consequent activation of calpain and protein kinases and phosphatase inhibition. These changes result in cytoskeletal reorganisation, membrane blebbing and the formation of microparticles (Wiedmer & Sims, 1991; Yano *et al*, 1994; Miyazaki *et al*, 1996). Microparticles have also been shown to be released during apoptosis induced *in vitro* by growth factor deprivation or complement proteins (Hamilton *et al*, 1990; Jimenez *et al*, 2003a).

Platelet glycoprotein (GP) receptors can also be involved in platelet microparticle (PMP) formation. For example, the GPIb receptor mediates adhesion to von Willebrand Factor (VWF) and, under shear stress, stretching of the platelet membrane occurs followed by separation of areas of tethered membrane and the production of microparticles (Reininger *et al*, 2006). P-selectin levels also correlate with PMP levels in mice and *in vitro*, P-selectin immunoglobulin can induce microparticle formation in human blood. This effect is abolished by blocking antibodies to the counter-receptor P-selectin glycoprotein ligand-1 (PSGL) (Hrachovinova *et al*, 2003).

Correspondence: Dr Susan Lynch, Department of Clinical and Laboratory Haematology, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh EH16 4SA, UK.
E-mail: slynch1@staffmail.ed.ac.uk

First published online 26 February 2007
doi:10.1111/j.1365-2141.2007.06514.x

Like their parent cells, microparticle membranes contain phospholipids and protein antigens. The process of apoptosis and the intracellular calcium rise that often follows cell activation both cause alteration of the normal lipid bilayer of the plasma membrane. Specifically, there is exposure of the internal negatively charged phospholipids to the external surface (Zwaal & Schroit, 1997). These membrane phospholipids, in particular phosphatidylserine, can bind to coagulation factors and promote the formation and activity of tenase and prothrombinase complexes. Consequently, the microparticles formed during cell activation or apoptosis have surfaces rich in negatively charged phospholipids that can promote procoagulant activity. The protein composition of microparticles reflects that of the cell membrane from which they are released. This includes constitutively expressed antigens, which allow the identification of the cellular origin of the microparticle. Additionally, they may bear antigens, including functional molecules, which have been induced on the parent cell by the activating or apoptotic triggers, leading to microparticle release (Combes *et al*, 1999; Jimenez *et al*, 2001).

It is likely that both the cell origin and the nature of the trigger influence the number and phenotype of the microparticles released and, consequently, their pathophysiological effects. This was demonstrated by culturing endothelial cells from brain, kidney and coronary arteries and exposing them to apoptotic or activating stimuli (Jimenez *et al*, 2003a). The endothelial microparticles (EMP) released following apoptotic stimuli had higher levels of surface Annexin V binding to phosphatidylserine, and of constitutive endothelial cell markers such as CD31 (Platelet Endothelial Cell Adhesion Molecule, PECAM). In contrast, EMP induced by activation with tumour necrosis factor a (TNF-α) expressed higher levels of inducible antigens, such as CD62E (E-selectin), which were also increased on the parent endothelial cells. Additionally, microvascular endothelial cells released significantly more microparticles overall compared with the macrovascular coronary artery endothelium. The formation of phenotypically heterogeneous microparticles is represented in Fig 1A.

Coagulation

Microparticles are likely to support coagulation in a number of different ways. As discussed above, the phospholipid properties of microparticles permit them to bind coagulation factors and promote the formation and activity of coagulation enzyme complexes, a role which has traditionally been thought to be provided by activated platelets. Microparticles expressing TF can also be identified in some circumstances, thus providing a suitable environment to both initiate and support coagulation. In addition to their support of the fluid phase of coagulation, microparticles also have a role in the recruitment of cells to developing thrombi. Furthermore, under certain conditions they can also exhibit anticoagulant properties dependent on their origin and the stimulus to release. Accordingly, microparticles may contribute to the complex regulation of the balance between an anti- or prothrombotic vasculature. Understanding the influence of individual factors on the predominant effect of microparticles in any given situation will require further investigation.

Microparticles have been demonstrated to support coagulation via both factor VII (FVII)/TF dependent and independent pathways. Using a thrombin generation assay to study the procoagulant potential of microparticles Pereira *et al* (2006), reported that platelet-free plasma from patients with antiphospholipid syndrome had an increased endogenous thrombin potential compared with healthy controls. This effect was dependent on the presence of PMP and correlated with microparticle numbers (Pereira *et al*, 2006). Combes *et al* (1999) found that TNF-α stimulation of cultured human umbilical vein endothelial cells (HUVEC) resulted in an increase in the release of EMP expressing surface TF. The addition of increasing concentrations of these EMP to a coagulation assay shortened the plasma clotting time compared with EMP from unstimulated HUVEC. The effect was not seen in FVII deficient plasma, showing the procoagulant activity of the EMP to be FVII/TF dependent in this situation. In contrast, Berckmans *et al* (2001) identified circulating microparticles in healthy volunteers which supported low-grade thrombin generation, but this activity was not blocked by TF or FVII blocking antibodies.

Microparticles can also contribute to the development of platelet and fibrin rich thrombi at sites of vascular injury, through the recruitment of cells and the accumulation of TF. This has been demonstrated in mouse models where fluorescently labelled microparticles accumulated in areas of developing thrombus (Hrachovinova *et al*, 2003). Monocyte microparticles have been found to express both PSGL-1 and TF (Falati *et al*, 2003; del Conde *et al*, 2005). The binding of these monocyte microparticles to P-selectin on activated endothelial cells or activated platelets within the developing thrombus, may therefore be expected to promote TF accumulation and localised thrombin generation. del Conde *et al* (2005) demonstrated that these microparticles bound and fused with activated platelets via PSGL-1 with a resultant increase in the platelet TF-FVIIa activity. In mice lacking P-selectin/PSGL-1 or in the presence of blocking antibodies, platelet thrombi with minimal TF and fibrin were formed (Falati *et al*, 2003).

A subset of EMP bearing VWF, have been identified in the plasma of patients with thrombotic thrombocytopenic purpura (TTP). Their interaction with platelets and effect on ristocetin-induced platelet aggregation was further investigated using flow cytometry (Jy *et al*, 2005). Platelets were incubated with normal plasma, VWF/Factor VIII concentrate (Humate P) or TNF-α-induced microvascular EMP. In the absence of ristocetin, very few platelet aggregates were seen with either normal plasma, Humate P or EMP. In the presence of ristocetin >95% platelet aggregation occurred. This aggregation could be blocked by antibodies to the platelet receptor for VWF, GPIb (CD42b), or by the removal of EMP by microfiltration. Further, they found that the addition of

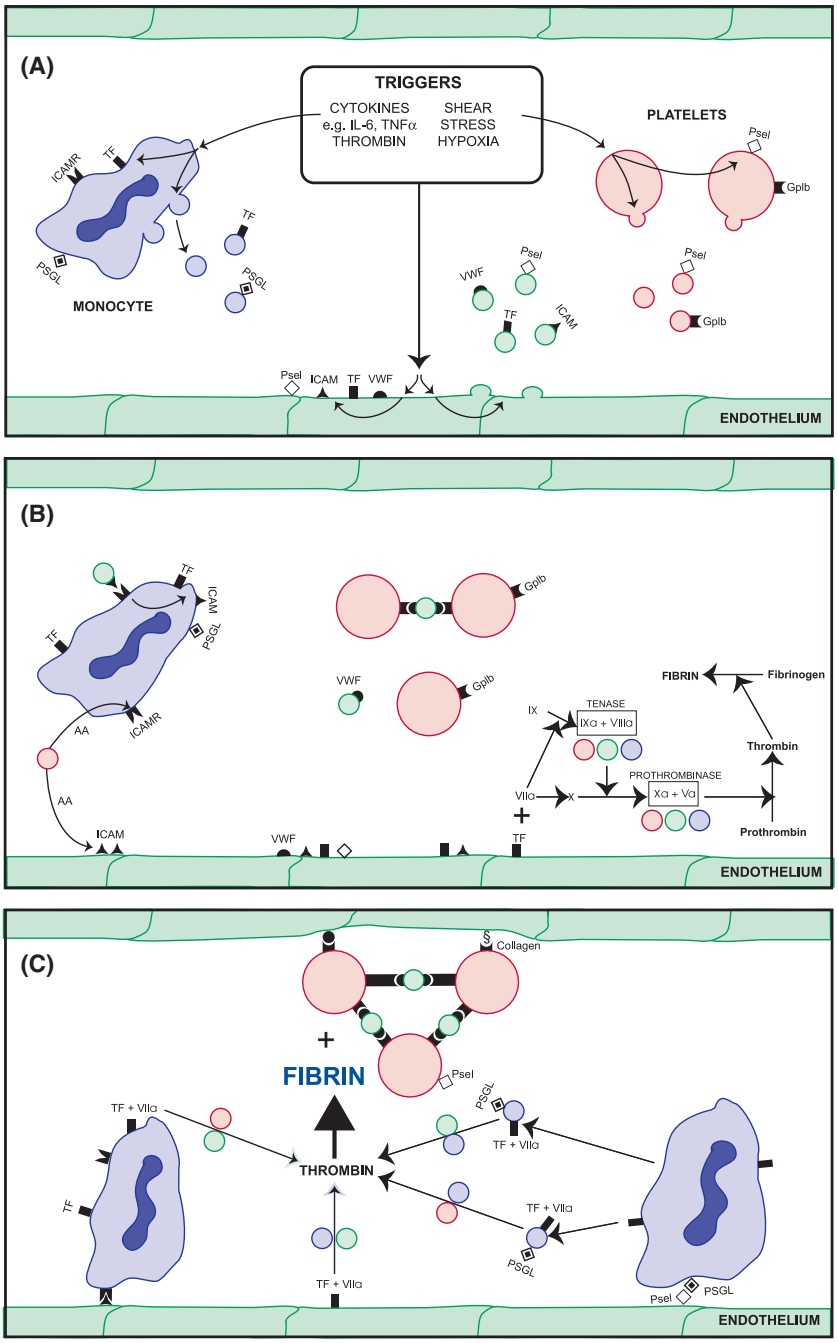

Fig 1. (A) Formation of microparticles. (B) Functions of microparticles in cellular interactions and coagulation. (C) Postulated role of microparticles in the development of thrombus. GPIb, glycoprotein Ib; ICAM, intercellular adhesion molecule; ICAMR, ICAM receptor; Psel, P-selectin; PSGL, P-selectin glycoprotein ligand; TF, tissue factor; VWF, von Willebrand Factor; AA, Arachidonic acid.

EMP to severe VWD plasma could restore ristocetin-induced platelet aggregation and was synergistic with the effect of Humate P.

As might be expected from the known properties of endothelial cells, EMP with anticoagulant activity can also be formed. Increased expression of tissue factor pathway inhibitor (TFPI) by EMP was reported in patients following acute myocardial infarction (AMI). Furthermore, this could be shown to inhibit the TF activity of the EMP (Steppich *et al*, 2005). The effect of activated protein C (APC), which has both anticoagulant and anti-inflammatory properties, on endothelial cells and EMP formation has also been studied (Perez-Casal *et al*, 2005). Cultured endothelial cells exposed to APC released EMP with membrane-bound endothelial protein C receptor (EPCR). APC bound to this full length EPCR was shown to retain its anticoagulant activity in reducing thrombin forma-

tion. In contrast, the binding of circulating APC to soluble EPCR cleaved from endothelial cell membranes by metalloproteinases, inhibited APC anticoagulant activity (Liaw *et al*, 2000).

Endothelial function

Endothelial microparticles have been shown to reflect endothelial activity, being released following activating or injurious external stimuli, and to themselves induce changes in endothelial function.

Endothelial dysfunction is a common feature of many vascular disorders including atherosclerosis, diabetes, antiphospholipid syndrome, TTP and sickle cell disease, where it is likely to have an important pathogenic role. Elevated levels of microparticles have been reported in all of these disorders (see later). Correlation between EMP levels and other serum markers of endothelial dysfunction including thrombomodulin (TM) and endothelial adhesion molecules has also been found (Ogura *et al*, 2004; Koga *et al*, 2005; Nomura *et al*, 2005). Soluble intercellular adhesion molecule (sICAM), which has been found to be related to endothelial dysfunction and coronary artery disease (CAD), was measured concurrently with EMP in patients with diabetes mellitus (DM) (Koga *et al*, 2005). Levels of both EMP and sICAM were elevated compared with non-diabetic controls and were greater in DM patients with CAD than those without CAD.

Endothelial microparticles were also assessed in patients following stem cell transplantation and rose in parallel with levels of vascular cell adhesion molecule-1 (VCAM-1) and E-selectin. In addition to the potential use of total EMP levels as a surrogate marker of endothelial disturbance, the phenotypic profile of EMP may help to discriminate between endothelial activation and apoptosis. For example, the ratio of $CD62^+$ (E-selectin) EMP to $CD31^+$ (PECAM) EMP has been shown *in vitro* to be high in activation, low in apoptosis (Jimenez *et al*, 2003a).

The effects of microparticles on vascular function have also been addressed. Endothelium-derived nitric oxide (NO) is the major mediator of acetylcholine-induced vasorelaxation of rat aorta *in vitro*. Exposure of rat aorta to EMP obtained from cultured endothelial cells, resulted in impaired acetylcholine-induced relaxation and reduced NO production (Brodsky *et al*, 2004). The same effect was seen using circulating microparticles obtained from patients following myocardial infarction (MI) (Boulanger *et al*, 2001). This response was abolished by removal of the endothelium or by inhibition of NO synthetase. The effect was not seen with non-ischaemia induced microparticles or the microparticle supernatant. Of note, this effect was seen with MI-induced microparticles at three times lower concentrations than the non-ischaemic microparticles, suggesting qualitatively different biological activity (Boulanger *et al*, 2001).

Amabile *et al* (2005) studied microparticle levels in patients with end-stage renal failure (ESRF) and compared them with *in vivo* measurements of vascular dysfunction. They found a strong correlation between EMP levels and reduced flow-mediated brachial artery dilation and increased indices of arterial stiffening. *In vitro*, microparticles from patients with ESRF, but not healthy controls or microparticle supernatant, caused impaired endothelial dependent vasorelaxation in rat aorta and reduced NO release. These effects correlated strongly with EMP levels and could be induced by purified EMP alone. Circulating EMP may therefore contribute to the vascular changes seen in ESRF through inhibition of the endothelial NO pathway.

A similar study of EMP in relation to *in vivo* indices of endothelial function has been performed in CAD (Koga *et al*, 2005). EMP levels correlated with the presence of coronary artery lesions in diabetic patients undergoing angiography. In a subset of these patients, *in vivo* measurements of coronary artery function were also made. An inverse correlation was found between EMP numbers and coronary artery blood flow and diameter change in response to the infusion of acetylcholine, which induces endothelium dependent vasodilatation. No such correlation was seen with endothelium independent vasodilatation induced by isosorbide dinitrate.

Cellular interactions

Microparticles bear antigens of their cell of origin and can transfer these surface molecules to other cell types. In doing so they may alter the biological activity of the recipient cells. Additionally, the binding of microparticle surface antigens to their specific counter-receptor may induce intracellular signalling pathways.

In a study of the effects of EMP on cultured monocytic THP-1 cells, EMP were produced by *in vitro* stimulation of HUVEC with TNF-α and heterogeneously expressed a number of adhesive receptors, including PECAM-1, ICAM-1, VCAM-1, E-selectin and $\alpha v\beta 3$ integrin. Following incubation with these EMP, cultured monocytes were found to express these endothelial antigens at the cell surface (Sabatier *et al*, 2002a). The exact mechanism of association was not elucidated but the results suggested that it was likely to involve receptor binding rather than membrane fusion. In addition, the co-incubated monocytes showed increased levels of TF mRNA and increased TF-dependent procoagulant activity. This effect was significantly inhibited by the addition of blocking antibodies to ICAM-1 and its counter-receptor, suggesting that the effect is partly dependent on the interaction of EMP and monocyte adhesion molecules.

A key feature in atherosclerosis is monocyte adhesion to endothelial cells followed by subendothelial transmigration. Cytokines, such as interleukin (IL)-1β and TNF-α, can affect this process by inducing the synthesis or upregulation of leucocyte–endothelial adhesion molecules. The *in vitro* stimulation of both monocytes and endothelial cells by high shear stress-induced PMP resulted in significantly increased production of IL-8, IL-1β and TNF-α (Nomura *et al*, 2001). Furthermore, treatment of endothelial cells and monocytes

with PMP prior to co-incubation was reported to modulate monocyte-endothelial cell interactions, by increasing the expression of adhesion molecules on both cell types (Barry *et al*, 1998; Nomura *et al*, 2001). PMP have also been shown *in vitro*, to increase platelet aggregation and to induce endothelial cell expression of cyclo-oxygenase-2 and production of prostaglandin I_2 (Barry *et al*, 1997). These effects could be replicated by arachidonic acid isolated from the PMP lipids (Barry *et al*, 1997; Barry *et al*, 1998). This suggests a mechanism whereby microparticles modulate cell function by the transcellular delivery of bioactive substances.

The production of platelet, endothelial and leucocyte microparticles can be increased by inflammatory conditions (Joop *et al*, 2001; Daniel *et al*, 2006). Microparticles from healthy volunteers formed by *in vivo* stimulation with a chemotactic peptide were able to induce IL-6 and monocyte chemoattractant protein-1 (MCP-1) release and TF expression by endothelial cells *in vitro*. There was an associated increase in the procoagulant activity of the endothelial cells, which was TF-dependent. This effect appeared to be mediated by leucocyte microparticles as it was largely unchanged by platelet blocking antibodies and could not be replicated by thrombin-induced PMP (Mesri & Altieri, 1999). Similarly, the addition of neutrophils to cultured endothelial cells induced the release of IL-6 and IL-8, an effect which could be replicated by cell-free supernatant or purified microparticles, but not microparticle-free supernatant (Mesri & Altieri, 1998). PMP from activated platelets can also mediate leucocyte–leucocyte interactions *in vitro* via binding of P-selectin to its ligand PSGL-1 on leucocytes (Forlow *et al*, 2000). These attachments can then lead to increased accumulation of leucocytes on a P-selectin surface, for example, activated endothelium at sites of vascular injury. Figure 1B illustrates some of the known cellular interactions of microparticles.

Such interactions as outlined above may provide novel mechanisms of crosstalk between the cellular elements of the coagulation and inflammatory systems, the importance of which is increasingly recognised. Microparticles may therefore contribute to the increased risk of thrombosis in systemic inflammatory diseases where increased numbers of microparticles have been identified, or in localised inflammatory environments, such as atherosclerotic lesions where activated monocytes, endothelial cells and platelets are co-localised. A schematic portrayal of the postulated role of microparticles in the development of a localised thrombus is shown in Fig 1.

Laboratory assessment of microparticles

'The beginning of wisdom is a definition of terms', Socrates

The definition of microparticles and the methods used in their isolation and quantitation varies between research groups and there is a need to appreciate the different methodology used in individual studies. A forum addressing this problem, with contributions from a number of groups studying microparticles, was recently published (Biro *et al*, 2004; Dignat-George *et al*, 2004a; Hugel *et al*, 2004; Jimenez *et al*, 2004; Jy *et al*, 2004; Nomura, 2004; Shet *et al*, 2004). A consensus definition of microparticles was proposed, as plasma particles of less than 1 μm in diameter, bearing surface antigens of their cell of origin. Some groups use an additional criterion of Annexin V binding as evidence of the phosphatidylserine rich surface; however, not all microparticles otherwise defined meet this criterion (Shet *et al*, 2003).

Isolation, identification and quantitation

Microparticles can be directly quantitated in platelet-poor plasma (PPP), obtained by serial centrifugation of citrated whole blood. Alternatively, washed microparticles can be isolated from the PPP by ultracentrifugation before resuspension and analysis.

Flow cytometry techniques are the most widely used method for identification and quantitation of plasma microparticles. The PPP or microparticle suspensions are labelled with fluorescently conjugated monoclonal antibodies. Annexin V binding can be used to confirm the phospholipid properties of the microparticles. Antibodies to specific surface antigens expressed on the cells of origin are used to identify the subtype of microparticle, for example anti-CD42 (GPIb) for identification of PMP or anti-glycophorin A for erythrocyte microparticles.

Flow cytometry also allows the criterion of size to be applied to microparticle analysis, by assessment of their forward light scatter. The identification of events of a specified size is most accurately done using calibration beads of known diameter for comparison. Alternatively, some groups have identified microparticles as those particles of a size less than the platelet population; however, this is a less standardised method, being subject to biological variation. Absolute quantitation of microparticles can be achieved using commercially produced counting beads of known concentration, which are added to the samples themselves, or used to calculate the volume of sample analysed over a standard collection time.

Solid phase capture assays can also be employed. These isolate and immobilise the microparticles in platelet-free plasma, using Annexin V monoclonal antibodies to bind to their phospholipid surfaces and/or antibodies to specific cell surface antigens. This method of quantification exploits the functional properties of the microparticle phospholipids by using an assay of prothrombinase activity and expresses quantity in phosphatidylserine equivalents. A disadvantage of this technique is that it cannot directly assess the size of the microparticles although the sample can be filtered to remove particles of greater than 1 μm before analysis. Also the functional activity of different populations of microparticles may not be directly proportional to their absolute numbers.

In attempt to develop easier methods for microparticle detection, commercial enzyme-linked immunosorbent assays for microparticle quantitation have also been investigated.

These have used combinations of antibodies to platelet antigens to allow PMP capture and detection in PPP (Osumi *et al*, 2001). Good correlation with measurement by standard flow cytometry methods in samples of *in vitro* induced PMP was demonstrated and the assay was subsequently used for the detection of PMP *in vivo* in patients with acute coronary syndromes (Nomura *et al*, 2003).

The absolute numbers of microparticles detected in patients and control subjects varies widely between studies. For example, values of circulating EMP from 10 to 6119×10^6 EMP/l have been reported. This is likely to be due, in part, to methodological differences. Firstly, variation in the isolation techniques may be a factor and there has been no direct comparison of the two main methods used. Secondly, a variety of cell-specific antibodies have been used and the specificity chosen is likely to influence the results. For example CD42a and CD62P (P-selectin) are both platelet-specific antigens but CD42a is present on all platelets while CD62P is found only on activated platelets. Table I presents a selection of the antibodies specificities that have been used in the *in vivo* studies of microparticles.

Table I. Specificities of monoclonal antibodies used in the identification of microparticles.

Subtype	Antigen	Comments	References
Endothelial	CD31 (CD42−)	PECAM-1	1–7
	CD31 (CD41−)		8
	CD62E	E-selectin	1–4, 9
	CD144	VE Cadherin	8–14
	CD51/$a_v b_3$	Vitronectin receptor	1, 2, 7, 15–18
	CD146	MelCAM	6, 11, 19
	CD105	Endoglin	13, 14
	CD54	ICAM-1	13, 14
Platelet	CD42a	GPIX	20, 21
	CD42b	GPIb	5, 6, 22, 23
	CD42	GPIbIX	7
	CD41	GPIIbIIIa	8, 10, 11, 16, 18
	CD61	GPIIIa	9, 19, 22, 24
	CD62P	P-selectin - activation	20, 23
Monocyte	CD14	Endotoxin receptor	9, 10, 16, 20, 21, 23
Erythrocyte	CD235	Glycophorin A	8, 9, 22

References: (1) Jimenez *et al* (2001), (2) Jimenez *et al* (2003b)), (3) Gonzalez-Quintero *et al* (2003), (4) Gonzalez-Quintero *et al* (2004), (5) Chirinos *et al* (2005), (6) Mallat *et al* (2000), (7) Bernal-Mizrachi *et al* (2003), (8) Amabile *et al* (2005), (9) VanWijk et al (2002a), (10) Shet *et al* (2003), (11) Faure *et al* (2006), (12) Koga *et al* (2005), (13) Simak *et al* (2004), (14) Simak *et al* (2006), (15) Combes *et al* (1999), (16) Sabatier *et al* (2002b)), (17) Dignat-George *et al* (2004b)), (18) Bretelle *et al* (2003), (19) Pereira *et al* (2006),(20) Nomura *et al* (2005), (21) Ogata *et al* (2006), (22) Hugel *et al* (1999), (23) Villmow *et al* (2002), (24) Harlow *et al* (2002).

Thirdly, although a criteria of <1 μm has been suggested for microparticle definition, in practice there is variation in the size criteria used, from 0·8 μm to 1·5 μm or 'smaller than platelets'. Platelets are reported to be 2–3 μm in diameter, and so it is likely that the populations of PMP and small platelets may form a continuum. It remains to be established whether there are biologically significant differences in the epidemiology and activity of the two populations, although studies have demonstrated that PMP numbers are not directly related to the whole blood platelet count (Jy *et al*, 1992). Despite the variation in absolute numbers, studies that have assessed the relative proportions of microparticle subtypes in plasma showed fairly consistent results. In general, PMP are found to be the most abundant with leucocyte, endothelial and erythrocyte microparticles accounting for the remainder (Combes *et al*, 1999; Berckmans *et al*, 2001; Daniel *et al*, 2006) although this profile may be altered in some disease states.

Microparticles in vascular disorders

The second part of this review considers the evidence for the role of microparticles in disease, in relation to our current knowledge of the underlying disease processes, and with an emphasis on disorders relevant to the haematologist. The results of the studies that have been included in this review are summarised in Table II and III. Microparticles have been studied in conditions including cardiovascular disease and diabetes, thrombotic disorders (e.g. TTP), inflammatory states (e.g. Crohn's disease) and multiple sclerosis. Many of these conditions share common pathophysiological mechanisms of vascular dysfunction and a prothrombotic state.

Systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS)

It is now widely held that the antiphospholipid antibodies (aPL) found in APS play an active role in the pathogenesis of the disorder, perhaps through the alteration of vascular endothelial function to induce a prothrombotic state. These antibodies are directed against plasma proteins, including β_2GP_1 and prothrombin, bound to anionic phospholipids that are found abundantly on activated platelets, apoptotic cells and microparticles.

In a preliminary study, it was reported that EMP levels were significantly higher in patients with lupus anticoagulant (LA) compared to healthy controls. SLE patients without LA had levels similar to controls (Combes *et al*, 1999). This association between the presence of LA and elevated EMP levels was confirmed in a later study of a similar patient group (Dignat-George *et al*, 2004b). It was also found that EMP levels were elevated both in patients with APS and in patients with SLE and aPL but no thrombosis, compared with healthy controls. In contrast, EMP levels were not elevated in patients with SLE but without aPL, or in patients with thrombosis but no aPL

Table II. Microparticle levels in prothrombotic disorders compared with healthy controls.

Disease	Study	TMP	PMP	EMP	LMP	RMP
SLE + aPL/LA	Combes *et al* (1999)			↑		
	Dignat-George *et al* (2004b))			↑		
	Pereira *et al* (2006)	↑	↑	↑	↑	
APS	Dignat-George *et al* (2004b))			↑		
Acute TTP	Jimenez *et al* (2003b))			↑		
Pre-eclampsia	Harlow *et al* (2002)		↓			
	VanWijk *et al* (2002a)	=	=	=	↑	=
	Bretelle *et al* (2003)	=	↓	=		
	Gonzalez-Quintero *et al* (2003, 2004		=	↑		
SCD	Shet *et al* (2003)	↑	=	=	↑	↑
PNH	Hugel *et al* (1999)		↑			=
	Simak *et al* (2004)		↑/=	↑	=	=
MPD	Villmow *et al* (2002)		↑			

TMP, total microparticles; PMP, platelet microparticles; EMP, endothelial microparticles; LMP, leucocyte microparticles; RMP, red cell microparticles; ↑, statistically significant increase; =, no difference; ↓, statistically significant reduction; SLE, systemic lupus erythematosus; aPL, antiphospholipid antibodies; LA, lupus antocoagulant; APS, antiphospholipid syndrome; TTP, thrombotic thrombocytopenic purpura; SCD, sickle cell disease; PNH, paroxysmal nocturnal haemoglobinuria; MPD, myeloproliferative disease.

Table III. Microparticle levels in cardiovascular disorders compared with healthy controls.

Disease	Study	TMP	PMP	EMP	LMP	RMP
VTE	Chirinos *et al* (2005)		(ns↑)	↑		
Type I DM	Sabatier *et al* (2002b))	↑	↑	↑		
Type II DM	Sabatier *et al* (2002b))	↑	=	=		
DM retinopathy	Ogata *et al* (2006)		↑		↑	
DM + CAD	Bernal-Mizrachi *et al* (2004)			↑		
ACS	Bernal-Mizrachi *et al* (2003)		↑	↑		
	Mallat *et al* (2000)	↑	=	↑	=	
Acute CVA	Simak *et al* (2006)		(ns↑)	↑	=	=
CRF	Amabile *et al* (2005)	↑	↑	↑		↑
	Faure *et al* (2006)		↑	↑	=	
Hypertension	Preston *et al* (2003)		↑	↑		

TMP, total microparticles; PMP, platelet microparticles; EMP, endothelial microparticles; LMP, leucocyte microparticles; RMP, red cell microparticles; ↑, statistically significant increase; (ns↑), non-significant increase; =, no difference; VTE, venous thromboembolism; DM, diabetes mellitus; CAD, cardiovascular disease; ACS, acute coronary syndrome; CVA, cerebrovascular accident; CRF, chronic renal failure.

(Dignat-George *et al*, 2004b). A further elevation in EMP was seen among those patients with a history of thrombotic complications in the study by Combes *et al* (1999) however this effect was not confirmed in the later study (Dignat-George *et al*, 2004b). Pereira *et al* (2006) also reported elevated microparticle levels, which were mainly of platelet origin, in patients with SLE. However, they found no association with the presence or absence of aPL or the presence of active disease (Pereira *et al*, 2006).

Two of these studies also addressed the functional capacity of microparticles. Pereira *et al* (2006) reported that the endogenous thrombin potential measured in platelet-free plasma, was elevated in patients compared to controls and correlated with the numbers of PMP. Dignat-George *et al* (2004b)) investigated the capacity of plasma from these patients to induce microparticle release from cultured HUVEC. Plasma from patients with APS or SLE (with or without aPL), induced a 4-fold increase in EMP compared to medium alone, which was significantly higher than that induced by healthy control plasma. They also measured the procoagulant potential of the induced microparticles using the plasma clotting time. Only the EMP stimulated by APS plasma significantly shortened the clotting time compared to healthy controls, despite a similar increase in EMP numbers by plasma from patients with SLE.

Overall, these studies support a procoagulant role for microparticles in the pathogenesis of APS. There were differences in the associations found between microparticle levels and the presence or absence of aPL or clinical thrombotic events. This may be a consequence of methodological differences in the preparation of the microparticles and the antibodies used in their identification and enumeration

(Table I). However, given the varied clinical spectrum of these disorders, it is possible that the qualitative and quantitative differences seen in microparticle formation may result from different immunologic stimuli, and that this may influence the disease phenotype.

TTP and other microangiopathies

Microvascular endothelial injury triggering the formation of platelet-rich thrombi, is thought to be of primary importance in the pathogenesis of TTP and related disorders.

Jimenez and colleagues studied the effect of plasma from patients with acute TTP on cultured brain and renal microvascular endothelial cell lines (Jimenez *et al*, 2001). A 5- to 6-fold increase in EMP generation was seen with TTP plasma compared to control, with a proportional increase in their procoagulant activity measured by the Russell Viper Venom Time. The phenotype of the EMP generated by TTP plasma was similar to that of EMP induced by culture with activating rather than apoptotic stimuli (Jimenez *et al*, 2003a), with an increased ratio of $CD62E^+$ to $CD31^+$ EMP. In addition, more than 60% of the $CD62E^+$ EMP co-expressed VWF (Jimenez *et al*, 2003b). Multimeric analysis of the EMP-associated VWF showed it to be in the form of ultra large VWF (ULVWF). The group then went on to demonstrate that these EMP strongly induced platelet aggregation, in the presence of ristocetin, by a VWF-dependent mechanism. The EMP-induced platelet aggregates were also found to be significantly more stable than those induced by normal plasma or Humate P.

In relation to the clinical states of the patients, they reported elevated numbers of EMP in the plasma of patients with acute TTP compared to normal controls or those in remission. The EMP phenotype in the patients also reflected that found *in vitro*, with an increased ratio of $CD62E^+$ to $CD31^+$ EMP. The level of co-expression of VWF on the $CD62E^+$ EMP was five times that of the normal controls. These findings support endothelial activation in TTP, in contrast to previous studies that have suggested that endothelial apoptosis is the dominant feature (Laurence *et al*, 1996; Mitra *et al*, 1997). Furthermore, these studies suggest a pathophysiological role for microvascular EMP in TTP through the expression of ULVWF and the induction and stabilisation of platelet aggregates.

Recently Nomura *et al* (2005) investigated the levels of microparticles in patients following allogeneic stem cell transplantation (SCT) where transplant-related complications include vascular disorders, such as veno-occlusive disease, pulmonary vasculopathy and thrombotic microangiopathy (TMA). Although only one of the 21 patients studied developed TMA/TTP, a continuous rise was seen in platelet, endothelial and monocyte derived microparticles in all patients, for up to 4 weeks following transplantation. This paralleled a rise in soluble endothelial markers including V-CAM and E-selectin. A previous study showed no increase in cellular microparticles during the conditioning period (Inbal *et al*, 2004). The endothelial dysfunction may therefore relate to the immunological effects of the transplant or to the immunosuppressive drugs used. Alternatively, it may reflect the infective and inflammatory complications commonly encountered following transplant. Further studies in a larger patient group may be useful to examine of the role of microparticles as a potential biomarker for the development of vascular complications after SCT.

Pregnancy and pre-eclampsia

During normal pregnancy there are multiple changes in the vasculature and the balance of haemostasis shifts towards a procoagulant state. Markers of coagulation activation are elevated in the pre-eclamptic state compared to normal pregnancy and uteroplacental thrombosis is thought to be important in some causes of recurrent pregnancy loss. Thus, vascular dysfunction and haemostatic imbalance are likely to have a role in both these pregnancy complications.

In otherwise healthy women with recurrent pregnancy loss, total microparticle levels measured in the non-pregnant state were elevated (>2 standard deviations above mean of controls) in a greater proportion compared to parous women (12/96 vs. 2/90) (Carp *et al*, 2004). Endogenous annexin V has a high affinity for phospholipids and is highly expressed on the surface of syncytiotrophoblasts. This may provide a mechanism whereby circulating microparticles are recruited to the site of placental implantation and could exert procoagulant or proinflammatory effects.

As might be anticipated, microparticle levels are elevated in normal pregnancy; however, reported differences between normal pregnancies and pre-eclampsia have been inconsistent. Unexpectedly, two studies found reduced PMP levels in pre-eclampsia (Harlow *et al*, 2002; Bretelle *et al*, 2003) despite equivalent circulating platelet counts and increased platelet activation, as measured by the expression of P-selectin. PMP have been shown to bind to fibrin and it is possible that this reduction in PMP may reflect their consumption in the fibrin deposits found in pathological placental beds. Despite the reduction in microparticle numbers, Bretelle *et al* (2003) found no change in their total procoagulant activity using a prothrombinase assay, suggesting a qualitative change in their function. In contrast, VanWijk *et al* (2002a) found no difference in total microparticle numbers between the two groups, with PMP accounting for the majority. However, they noted an elevation in the subpopulation of granulocyte microparticles in pre-eclamptic patients and numbers correlated with the degree of hypertension.

Gonzalez-Quintero *et al* (2003, 2004) also found PMP numbers to be equivalent but found elevated levels of EMP in pre-eclampsia compared with either normal pregnancy or gestational hypertension. The levels of EMP correlated with both the degree of hypertension and proteinuria. This is in keeping with other evidence for endothelial dysfunction in pre-eclampsia. Endoglin, the receptor for transforming growth factor (TGFβ), is upregulated on placental vascular

endothelium in pre-eclampsia and shed into the plasma. It has been implicated in the pathogenesis in animal models and a recent study suggested that elevated soluble endoglin levels may be useful as an early predictor for the development of pre-eclampsia (Levine *et al*, 2006). It remains to be seen whether relative or absolute changes in microparticle levels may similarly be useful as predictors of vascular pregnancy complications. Functional studies of the effects of plasma from pre-eclamptic women on vascular function *in vitro* showed a reduction in bradykinin-mediated relaxation of myometrial arteries by isolated microparticles, although the effects of whole plasma showed conflicting results (VanWijk *et al*, 2002b).

Sickle cell disease

The vaso-occlusive episodes of sickle cell disease were previously thought to be secondary to vessel occlusion by sickled erythrocytes. More recent evidence suggests that other factors are important, particularly microvascular endothelial activation and endothelial–erythrocyte adhesion. In addition, sickle cell disease is a procoagulant state, as evidenced by elevated levels of *in vivo* markers of coagulation and fibrinolysis, including prothrombin fragments (PF1 + 2), thrombin–antithrombin complexes (TAT) and D-dimer (Switzer *et al*, 2006).

Erythrocyte microparticle levels are significantly increased in sickle cell patients compared with controls and account for the majority of circulating microparticles. However, endothelial and monocyte microparticles are also increased compared to healthy controls both in the steady state and greater still in vaso-occlusive crises, supporting the theory of endothelial activation (Shet *et al*, 2003). A proportion of these endothelial and monocyte microparticles express TF and shorten the plasma clotting time, an effect partially inhibited by anti-TF antibodies. The total levels of microparticles and the TF positive subset also correlate with D-dimer, TAT and prothrombin fragment measurements. Thus, microparticles contribute to the procoagulant state seen in sickle cell disease. Their potential role in mediating erythrocyte–endothelial interactions via the expression of adhesion molecules warrants further study.

Paroxysmal nocturnal haemoglobinuria (PNH)

Elevated levels of microparticles have been reported in the prothrombotic disorder PNH (Hugel *et al*, 1999; Simak *et al*, 2004). Hugel *et al* (1999) found that PMP accounted for the majority and that erythrocyte microparticles were infrequent. They did not assess the samples for endothelial or leucocyte subtypes. The procoagulant potential of the microparticles was confirmed using a prothrombinase assay (Hugel *et al*, 1999). In contrast, Simak *et al* (2004) found that the greatest proportion of microparticles was of erythrocyte origin and that this was not significantly different from normal controls. EMP were elevated in the group of PNH patients as a whole, but some individual patients also showed increased numbers of PMP (Simak *et al*, 2004). The contrasting findings between these studies, once again may be due to methodological differences. The inter-individual variation however, is more likely to reflect disease status or the presence of comorbid conditions.

Myeloproliferative disorders

Platelet microparticle levels have been reported to be elevated above controls in polycythaemia vera, primary thrombocythaemia and myelofibrosis, concomitant with elevated markers of platelet activation (Villmow *et al*, 2002). The pathogenesis of the predominantly thrombotic complications of these disorders is uncertain but is likely to be multifactorial. Previous studies have identified leucocyte activation and elevated serum markers of endothelial disturbance and a procoagulant state. This suggests that further investigation of endothelial and leucocyte microparticles and their procoagulant potential may prove rewarding.

Cardiovascular disease and venous thrombosis

Endothelial dysfunction, vascular inflammation and a prothrombotic state arise in patients with CAD, the vascular complications of diabetes, hypertension, cerebrovascular disease and venous thrombosis (VTE). As might be expected from the pathophysiologic processes involved, elevated microparticles have been reported in all of these diseases.

Endothelial microparticles and PMP were measured in 25 patients with deep vein thrombosis or pulmonary embolism, compared with healthy controls (Chirinos *et al*, 2005). EMP levels were markedly elevated in patients with VTE compared to controls. PMP were not elevated despite higher levels of platelet expression of the activation marker P-selectin. Increased leucocyte expression of the activation marker CD11b and EMP-monocyte conjugates in the VTE patients was also seen. The observed elevation of EMP reflects the state of endothelial activation in VTE. EMP may also contribute to thrombus development by localising the inflammatory effects of leucocytes at sites of endothelial injury and themselves providing a source of TF and a catalytic phospholipid surface. Persistent D-dimer elevation following a period of anticoagulation for VTE is predictive of recurrence and may reflect ongoing hypercoagulability. Similar studies of microparticles in this setting may also be useful to provide further evidence about the ongoing state of endothelial activation in these patients.

Microparticles are elevated in diabetic patients, however studies have found differences in the microparticle profile in relation to disease type and the presence or absence of complications. Sabatier *et al* (2002b)) reported that in type I diabetes, the procoagulant potential of microparticles, as measured by a prothrombinase assay, was elevated and correlated with degree of glycaemic control. In contrast they

found that although total numbers of microparticles were elevated in type II diabetes, there was no associated increase in their procoagulant potential. Levels of PMP and monocyte microparticles have been shown to correlate with the extent of diabetic retinopathy, which is associated with microvascular damage (Ogata *et al*, 2006).

In a prospective observational study of 217 diabetic patients referred for investigative angiography, elevated EMP levels were predictive for the presence of coronary artery lesions, odds ratio 3·5 (1·8–6·9). Further, it was a more significant independent risk factor than length of diabetic disease, lipid levels or the presence of hypertension (Koga *et al*, 2005). Interestingly, elevated EMP levels were predictive in identifying a subpopulation of diabetic patients without typical anginal symptoms who had angiographic evidence of CAD. In a similar study, EMP levels were 2·5 times higher in the presence of high-risk coronary lesions compared to low risk, however EMP values in very severe stenosis (>45%) did not differ from normal (Bernal-Mizrachi *et al*, 2004). This is analogous to the association of elevated CRP with high risk lesions but relatively lower concentrations in the presence of near total occlusion. It has been suggested that this may reflect reduced blood flow in the stenosed vessel or, more likely, less acute inflammation in the vascular tree. Correlating levels of EMP with *in vivo* measurement of indices of coronary endothelial dysfunction, Koga *et al* (2005) found that EMP levels inversely correlated with coronary blood flow in response to acetylcholine stimulation of endothelium dependent vasodilatation.

Procoagulant microparticles, particularly EMP, are elevated in patients with acute coronary syndromes compared to patients with stable anginal symptoms or normal controls (Mallat *et al*, 2000; Bernal-Mizrachi *et al*, 2003). This reflects the degree of acute vascular injury and inflammation at the time of measurement. Steppich *et al* (2005) reported that in acute MI, microparticles may also have an anticoagulant function through expression of TFPI and reduction of TF-dependent thrombin generation which may help limit thrombus formation.

Circulating EMP are also elevated in acute ischaemic stroke (cerebrovascular accident; CVA) (Simak *et al*, 2006). Simak and colleagues compared EMP subtypes in patients with mild or moderate-severe acute CVA. EMP expressing PPS were elevated in all acute CVA compared with controls. All EMP subtypes studied were endoglin positive and all were elevated in the moderate-severe group compared with control patients. Endoglin expression is associated with endothelial apoptosis and is upregulated on cultured endothelial cells under hypoxic conditions. The greater elevation of endoglin positive EMP in the moderate-severe CVA group may therefore reflect the severity of endothelial injury. Notably, the patient samples were collected at an average of 37 h following hospital admission suggesting an ongoing procoagulant state.

In chronic renal failure (CRF), patients who are at increased risk of accelerated cardiovascular disease have evidence of endothelial dysfunction. In keeping with this, elevated levels of EMP have been found in CRF (Amabile *et al*, 2005; Faure *et al*, 2006), however no difference was identified in EMP levels between those with or without a history of vascular disease. Assessment of *in vivo* measures of endothelial dysfunction in end-stage renal failure (ESRF) shows a strong correlation with levels of EMP (Amabile *et al*, 2005). Further, EMP from these patients also impaired endothelial-dependent vasorelaxation *in vitro* in rat aorta and reduced NO release (see earlier). The study of microparticles may also provide evidence for the causes of endothelial dysfunction in CRF. Culture of HUVEC with uraemic toxins induced EMP formation, supporting a direct effect of uraemia on endothelial function (Faure *et al*, 2006). Hypertension is another important factor in renal disease and the associated shear stress is a trigger for microparticle formation. In severe hypertension, in the absence of CRF, both EMP and PMP are elevated and correlate with systolic pressure (Preston *et al*, 2003). Together these results suggest that microparticles induced, for example, by hypertension or uraemia, may contribute to the progression of vascular changes in ESRF perhaps through inhibition of the endothelial NO pathway or induction of a microvascular procoagulant state.

Conclusion

Microparticles are phenotypically and functionally heterogeneous, possibly even more so than their parent cells. At the observational level they may prove useful as circulating biomarkers of endothelial dysfunction and prothrombotic state, both in disease and to monitor the effects of treatment, such as statins in vascular diseases. In order for microparticle measurement to be clinically useful however, standardisation of sampling and analysis methods would be required. An important question is whether microparticles are more than simply a reflection of the pathophysiological state of the vasculature. The recognition of their diverse biological actions and intercellular signalling capabilities suggests they have a role as functional messengers; they may mediate global vascular changes in response to localised vascular injury, or crosstalk between the inflammatory and coagulation systems. Understanding the complex balance of their positive and negative effects on coagulation and inflammation, within a systemic model, will be critical; but in doing so it may reveal their potential as targets for therapeutic intervention, for example to promote haemostasis or prevent thrombosis.

Acknowledgements

We thank Dr David Stirling and Dr Caroline Duncan for their constructive review of the manuscript.

References

Amabile, N., Guerin, A.P., Leroyer, A., Mallat, Z., Nguyen, C., Boddaert, J., London, G.M., Tedgui, A. & Boulanger, C.M. (2005) Circulating endothelial microparticles are associated with vascular

dysfunction in patients with end-stage renal failure. *Journal of the American Society of Nephrology*, **16,** 3381–3388.

Barry, O.P., Pratico, D., Lawson, J.A. & FitzGerald, G.A. (1997) Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. *Journal of Clinical Investigation*, **99,** 2118–2127.

Barry, O.P., Pratico, D., Savani, R.C. & FitzGerald, G.A. (1998) Modulation of monocyte-endothelial cell interactions by platelet microparticles. *Journal of Clinical Investigation*, **102,** 136–144.

Berckmans, R.J., Neiuwland, R., Boing, A.N., Romijn, F.P., Hack, C.E. & Sturk, A. (2001) Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. *Thrombosis and Haemostasis*, **85,** 639–646.

Bernal-Mizrachi, L., Jy, W., Jimenez, J.J., Pastor, J., Mauro, L.M., Horstman, L.L., de Marchena, E. & Ahn, Y.S. (2003) High levels of circulating endothelial microparticles in patients with acute coronary syndromes. *American Heart Journal*, **145,** 962–970.

Bernal-Mizrachi, L., Jy, W., Fierro, C., Macdonough, R., Velazques, H.A., Purow, J., Jimenez, J.J., Horstman, L.L., Ferreira, A., de Marchena, E. & Ahn, Y.S. (2004) Endothelial microparticles correlate with high-risk angiographic lesions in acute coronary syndromes. *International Journal of Cardiology*, **97,** 439–446.

Biro, E., Nieuwland, R. & Sturk, A. (2004) Measuring circulating cell-derived microparticles. *Journal of Thrombosis and Haemostasis*, **2,** 1843–1844.

Boulanger, C.M., Scoazec, A., Ebrahimian, T., Henry, P., Mathieu, E., Tedgui, A. & Mallat, Z. (2001) Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. *Circulation*, **104,** 2649–2652.

Bretelle, F., Sabatier, F., Desprez, D., Camoin, L., Grunebaum, L., Combes, V., D'Ercole, C. & Dignat-George, F. (2003) Circulating microparticles: a marker of procoagulant state in normal pregnancy and pregnancy complicated by preeclampsia or intrauterine growth restriction. *Thrombosis and Haemostasis*, **89,** 486–492.

Brodsky, S.V., Zhang, F., Nasjletti, A. & Goligorsky, M.S. (2004) Endothelium-derived microparticles impair endothelial function in vitro. *American Journal of Physiology. Heart and Circulatory Physiology*, **286,** H1910–H1915.

Carp, H., Dardik, R., Lubetsky, A., Salomon, O., Eskaraev, R., Rosenthal, E. & Inbal, A. (2004) Prevalence of circulating procoagulant microparticles in women with recurrent miscarriage: a case-controlled study. *Human Reproduction*, **19,** 191–195.

Chirinos, J.A., Heresi, G.A., Velasquez, H., Jy, W., Jimenez, J.J., Ahn, E., Horstman, L.L., Soriano, A.O., Zambrano, J.P. & Ahn, Y.S. (2005) Elevation of endothelial microparticles, platelets, and leukocyte activation in patients with venous thromboembolism. *Journal of the American College of Cardiology*, **45,** 1467–1471.

Combes, V., Simon, A.C., Grau, G.E., Arnoux, D., Camoin, L., Sabatier, F., Mutin, M., Sanmarco, M., Sampol, J. & Dignat-George, F. (1999) In vitro generation of endothelial microparticles and possible prothrombotic activity in patients with lupus anticoagulant. *Journal of Clinical Investigation*, **104,** 93–102.

del Conde, I., Shrimpton, C.N., Thiagarajan, P. & Lopez, J.A. (2005) Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. *Blood*, **106,** 1604–1611.

Daniel, L., Fakhouri, F., Joly, D., Mouthon, L., Nusbaum, P., Grunfeld, J.P., Schifferli, J., Guillevin, L., Lesavre, P. & Halbwachs-Mecarelli, L. (2006) Increase of circulating neutrophil and platelet microparticles during acute vasculitis and hemodialysis. *Kidney International*, **69,** 1416–1423.

Dignat-George, F., Sabatier, F., Camoin-Jau, L. & Sampol, J. (2004a) Measuring circulating cell-derived microparticles. *Journal of Thrombosis and Haemostasis*, **2,** 1844–1845.

Dignat-George, F., Camoin-Jau, L., Sabatier, F., Arnoux, D., Anfosso, F., Bardin, N., Veit, V., Combes, V., Gentile, S., Moal, V., Sanmarco, M. & Sampol, J. (2004b) Endothelial microparticles: a potential contribution to the thrombotic complications of the antiphospholipid syndrome. *Thrombosis and Haemostasis*, **91,** 667–673.

Falati, S., Liu, Q., Gross, P., Merrill-Skoloff, G., Chou, J., Vandendries, E., Celi, A., Croce, K., Furie, B.C. & Furie, B. (2003) Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. *The Journal of Experimental Medicine*, **197,** 1585–1598.

Faure, V., Dou, L., Sabatier, F., Cerini, C., Sampol, J., Berland, Y., Brunet, P. & Dignat-George, F. (2006) Elevation of circulating endothelial microparticles in patients with chronic renal failure. *Journal of Thrombosis and Haemostasis*, **4,** 566–573.

Forlow, S.B., McEver, R.P. & Nollert, M.U. (2000) Leukocyte-leukocyte interactions mediated by platelet microparticles under flow. *Blood*, **95,** 1317–1323.

Gonzalez-Quintero, V.H., Jimenez, J.J., Jy, W., Mauro, L.M., Hortman, L., O'Sullivan, M.J. & Ahn, Y. (2003) Elevated plasma endothelial microparticles in preeclampsia. *American Journal of Obstetrics and Gynecology*, **189,** 589–593.

Gonzalez-Quintero, V.H., Smarkusky, L.P., Jimenez, J.J., Mauro, L.M., Jy, W., Hortsman, L.L., O'Sullivan, M.J. & Ahn, Y.S. (2004) Elevated plasma endothelial microparticles: preeclampsia versus gestational hypertension. *American Journal of Obstetrics and Gynecology*, **191,** 1418–1424.

Hamilton, K.K., Hattori, R., Esmon, C.T. & Sims, P.J. (1990) Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. *The Journal Of Biological Chemistry*, **265,** 3809–3814.

Harlow, F.H., Brown, M.A., Brighton, T.A., Smith, S.L., Trickett, A.E., Kwan, Y.L. & Davis, G.K. (2002) Platelet activation in the hypertensive disorders of pregnancy. *American Journal of Obstetrics and Gynecology*, **187,** 688–695.

Hrachovinova, I., Cambien, B., Hafezi-Moghadam, A., Kappelmayer, J., Camphausen, R.T., Widom, A., Xia, L., Kazazian, H.H., Schaub, R.G., McEver, R.P. & Wagner, D.D. (2003) Interaction of P-selectin and PSGL-1 generates microparticles that correct hemostasis in a mouse model of hemophilia A. *Nature Medicine*, **9,** 1020–1025.

Hugel, B., Socie, G., Vu, T., Toti, F., Gluckman, E., Freyssinet, J.M. & Scrobohaci, M.L. (1999) Elevated levels of circulating procoagulant microparticles in patients with paroxysmal nocturnal hemoglobinuria and aplastic anemia. *Blood*, **93,** 3451–3456.

Hugel, B., Zobairi, F. & Freyssinet, J.M. (2004) Measuring circulating cell-derived microparticles. *Journal of Thrombosis and Haemostasis*, **2,** 1846–1847.

Inbal, A., Lubetsky, A., Shimoni, A., Dardik, R., Sela, B.A., Eskaraev, R., Levi, I., Tov, N.S. & Nagler, A. (2004) Assessment of the coagulation profile in hemato-oncological patients receiving ATG-based conditioning treatment for allogeneic stem cell transplantation. *Bone Marrow Transplantation*, **34,** 459–463.

Jimenez, J.J., Jy, W., Mauro, L.M., Horstman, L.L. & Ahn, Y.S. (2001) Elevated endothelial microparticles in thrombotic

thrombocytopenic purpura: findings from brain and renal microvascular cell culture and patients with active disease. *British Journal of Haematology*, **112,** 81–90.

Jimenez, J.J., Jy, W., Mauro, L.M., Soderland, C., Horstman, L.L. & Ahn, Y.S. (2003a) Endothelial cells release phenotypically and quantitatively distinct microparticles in activation and apoptosis. *Thrombosis Research*, **109,** 175–180.

Jimenez, J.J., Jy, W., Mauro, L.M., Horstman, L.L., Soderland, C. & Ahn, Y.S. (2003b) Endothelial microparticles released in thrombotic thrombocytopenic purpura express von Willebrand factor and markers of endothelial activation. *British Journal of Haematology*, **123,** 896–902.

Jimenez, J.J., Jy, W., Horstman, L.L. & Ahn, Y.S. (2004) Measuring circulating cell-derived microparticles. *Journal of Thrombosis and Haemostasis*, **2,** 1850–1851.

Joop, K., Berckmans, R.J., Nieuwland, R., Berkhout, J., Romijn, F.P., Hack, C.E. & Sturk, A. (2001) Microparticles from patients with multiple organ dysfunction syndrome and sepsis support coagulation through multiple mechanisms. *Thrombosis and Haemostasis*, **85,** 810–820.

Jy, W., Horstman, L.L., Arce, M. & Ahn, Y.S. (1992) Clinical significance of platelet microparticles in autoimmune thrombocytopenias. *Journal of Laboratory and Clinical Medicine*, **119,** 334–345.

Jy, W., Horstman, L.L., Jimenez, J.J. & Ahn, Y.S. (2004) Measuring circulating cell-derived microparticles. *Journal of Thrombosis and Haemostasis*, **2,** 1842–1843.

Jy, W., Jimenez, J.J., Mauro, L.M., Horstman, L.L., Cheng, P., Ahn, E.R., Bidot, C.J. & Ahn, Y.S. (2005) Endothelial microparticles induce formation of platelet aggregates via a von Willebrand factor/ristocetin dependent pathway, rendering them resistant to dissociation. *Journal of Thrombosis and Haemostasis*, **3,** 1301–1308.

Koga, H., Sugiyama, S., Kugiyama, K., Watanabe, K., Fukushima, H., Tanaka, T., Sakamoto, T., Yoshimura, M., Jinnouchi, H. & Ogawa, H. (2005) Elevated levels of VE-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease. *Journal of the American College of Cardiology*, **45,** 1622–1630.

Laurence, J., Mitra, D., Steiner, M., Staiano-Coico, L. & Jaffe, E. (1996) Plasma from patients with idiopathic and human immunodeficiency virus-associated thrombotic thrombocytopenic purpura induces apoptosis in microvascular endothelial cells. *Blood*, **87,** 3245–3254.

Levine, R.J., Lam, C., Qian, C., Yu, K.F., Maynard, S.E., Sachs, B.P., Sibai, B.M., Epstein, F.H., Romero, R., Thadhani, R., Karumanchi, S.A. & the CPEP Study Group (2006) Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *The New England Journal of Medicine*, **355,** 992–1005.

Liaw, P.C.Y., Neuenschwander, P.F., Smirnov, M.D. & Esmon, C.T. (2000) Mechanisms by which soluble endothelial cell protein C receptor modulates protein C and activated protein C function. *Journal of Biological Chemistry*, **275,** 5447–5452.

Mallat, Z., Benamer, H., Hugel, B., Benessiano, J., Steg, P.G., Freyssinet, J.M. & Tedgui, A. (2000) Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation*, **101,** 841–843.

Mesri, M. & Altieri, D.C. (1998) Endothelial cell activation by leukocyte microparticles. *The Journal of Immunology*, **161,** 4382–4387.

Mesri, M. & Altieri, D.C. (1999) Leukocyte microparticles stimulate endothelial cell cytokine release and tissue factor induction in a JNK1 signaling pathway. *Journal of Biological Chemistry*, **274,** 23111–23118.

Mitra, D., Jaffe, E.A., Weksler, B., Hajjar, K.A., Soderland, C. & Laurence, J. (1997) Thrombotic thrombocytopenic purpura and sporadic hemolytic-uremic syndrome plasmas induce apoptosis in restricted lineages of human microvascular endothelial cells. *Blood*, **89,** 1224–1234.

Miyazaki, Y., Nomura, S., Miyake, T., Kagawa, H., Kitada, C., Taniguchi, H., Komiyama, Y., Fujimura, Y., Ikeda, Y. & Fukuhara, S. (1996) High shear stress can initiate both platelet aggregation and shedding of procoagulant containing microparticles. *Blood*, **88,** 3456–3464.

Nomura, S. (2004) Measuring circulating cell-derived microparticles. *Journal of Thrombosis and Haemostasis*, **2,** 1847–1848.

Nomura, S., Tandon, N.N., Nakamura, T., Cone, J., Fukuhara, S. & Kambayashi, J. (2001) High-shear-stress-induced activation of platelets and microparticles enhances expression of cell adhesion molecules in THP-1 and endothelial cells. *Atherosclerosis*, **158,** 277–287.

Nomura, S., Uehata, S., Saito, S., Osumi, K., Ozeki, Y. & Kimura, Y. (2003) Enzyme immunoassay detection of platelet-derived microparticles and RANTES in acute coronary syndrome. *Thrombosis and Haemostasis*, **89,** 506–512.

Nomura, S., Ishii, K., Kanazawa, S., Inami, N., Uoshima, N., Ishida, H., Yoshihara, T., Kitayama, H. & Hayashi, K. (2005) Significance of elevation in cell-derived microparticles after allogeneic stem cell transplantation: transient elevation of platelet-drived microparticles in TMA//TTP. *Bone Marrow Transplantation*, **36,** 921–922.

Ogata, N., Nomura, S., Shouzu, A., Imaizumi, M., Arichi, M. & Matsumura, M. (2006) Elevation of monocyte-derived microparticles in patients with diabetic retinopathy. *Diabetes Research and Clinical Practice*, **73,** 241–248.

Ogura, H., Tanaka, H., Koh, T., Fujita, K., Fujimi, S., Nakamori, Y., Hosotsubo, H., Kuwagata, Y., Shimazu, T. & Sugimoto, H. (2004) Enhanced production of endothelial microparticles with increased binding to leukocytes in patients with severe systemic inflammatory response syndrome. *Journal of Trauma*, **56,** 823–830.

Osumi, K., Ozeki, Y., Saito, S., Nagamura, Y., Ito, H., Kimura, Y., Ogura, H. & Nomura, S. (2001) Development and assessment of enzyme immunoassay for platelet-derived microparticles. *Thrombosis and Haemostasis*, **85,** 326–330.

Pereira, J., Alfaro, G., Goycoolea, M., Quiroga, T., Ocqueteau, M., Massardo, L., Perez, C., Saez, C., Panes, O., Matus, V. & Mezzano, D. (2006) Circulating platelet-derived microparticles in systemic lupus erythematosus. Association with increased thrombin generation and procoagulant state. *Thrombosis and Haemostasis*, **95,** 94–99.

Perez-Casal, M., Downey, C., Fukudome, K., Marx, G. & Toh, C.H. (2005) Activated protein C induces the release of microparticle-associated endothelial protein C receptor. *Blood*, **105,** 1515–1522.

Preston, R.A., Jy, W., Jimenez, J.J., Mauro, L.M., Horstman, L.L., Valle, M., Aime, G. & Ahn, Y.S. (2003) Effects of severe hypertension on endothelial and platelet microparticles. *Hypertension*, **41,** 211–217.

Reininger, A.J., Heijnen, H.F., Schumann, H., Specht, H.M., Schramm, W. & Ruggeri, Z.M. (2006) Mechanism of platelet adhesion to von Willebrand factor and microparticle formation under high shear stress. *Blood*, **107,** 3537–3545.

Sabatier, F., Roux, V., Anfosso, F., Camoin, L., Sampol, J. & Dignat-George, F. (2002a) Interaction of endothelial microparticles with monocytic cells in vitro induces tissue factor-dependent procoagulant activity. *Blood*, **99,** 3962–3970.

Sabatier, F., Darmon, P., Hugel, B., Combes, V., Sanmarco, M., Velut, J.G., Arnoux, D., Charpiot, P., Freyssinet, J.M., Oliver, C., Sampol, J. & Dignat-George, F. (2002b) Type 1 and type 2 diabetic patients display different patterns of cellular microparticles. *Diabetes*, **51,** 2840–2845.

Shet, A.S., Aras, O., Gupta, K., Hass, M.J., Rausch, D.J., Saba, N., Koopmeiners, L., Key, N.S. & Hebbel, R.P. (2003) Sickle blood contains tissue factor-positive microparticles derived from endothelial cells and monocytes. *Blood*, **102,** 2678–2683.

Shet, A.S., Key, N.S. & Hebbel, R.P. (2004) Measuring circulating cell-derived microparticles. *Journal of Thrombosis and Haemostasis*, **2,** 1848–1850.

Simak, J., Holada, K., Risitano, A.M., Zivny, J.H., Young, N.S. & Vostal, J.G. (2004) Elevated circulating endothelial membrane microparticles in paroxysmal nocturnal haemoglobinuria. *British Journal of Haematology*, **125,** 804–813.

Simak, J., Gelderman, M.P., Yu, H., Wright, V. & Baird, A.E. (2006) Circulating endothelial microparticles in acute ischemic stroke: a link to severity, lesion volume and outcome. *Journal of Thrombosis and Haemostasis*, **4,** 1296–1302.

Steppich, B., Mattisek, C., Sobczyk, D., Kastrati, A., Schomig, A. & Ott, I. (2005) Tissue factor pathway inhibitor on circulating microparticles in acute myocardial infarction. *Thrombosis and Haemostasis*, **93,** 35–39.

Switzer, J.A., Hess, D.C., Nichols, F.T. & Adams, R.J. (2006) Pathophysiology and treatment of stroke in sickle-cell disease: present and future. *The Lancet Neurology*, **5,** 501–512.

VanWijk, M.J., Nieuwland, R., Boer, K., van der Post, J.A.M., VanBavel, E. & Sturk, A. (2002a) Microparticle subpopulations are increased in preeclampsia: possible involvement in vascular dysfunction? *American Journal of Obstetrics and Gynecology*, **187,** 450–456.

VanWijk, M.J., Svedas, E., Boer, K., Nieuwland, R., VanBavel, E. & Kublickiene, K.R. (2002b) Isolated microparticles, but not whole plasma, from women with preeclampsia impair endothelium-dependent relaxation in isolated myometrial arteries from healthy pregnant women. *American Journal of Obstetrics and Gynecology*, **187,** 1686–1693.

VanWijk, M.J., VanBavel, E., Sturk, A. & Nieuwland, R. (2003) Microparticles in cardiovascular diseases. *Cardiovascular Research*, **59,** 277–287.

Villmow, T., Kemkes-Matthes, B. & Matzdorff, A.C. (2002) Markers of platelet activation and platelet-leukocyte interaction in patients with myeloproliferative syndromes. *Thrombosis Research*, **108,** 139–145.

Wiedmer, T. & Sims, P.J. (1991) Participation of protein kinases in complement C5b-9-induced shedding of platelet plasma membrane vesicles. *Blood*, **78,** 2880–2886.

Yano, Y., Kambayashi, J., Shiba, E., Sakon, M., Oiki, E., Fukuda, K., Kawasaki, T. & Mori, T. (1994) The role of protein phosphorylation and cytoskeletal reorganization in microparticle formation from the platelet plasma membrane. *The Biochemical Journal*, **299** (Pt 1), 303–308.

Zwaal, R.F.A. & Schroit, A.J. (1997) Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood*, **89,** 1121–1132.