European Journal of Clinical Investigation (2004) **34**, 392–401

Review

Cellular microparticles: new players in the field of vascular disease?

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Abstract

Microparticles are small membrane vesicles that are released from cells upon activation or during apoptosis. Cellular microparticles in body fluids constitute a heterogeneous population, differing in cellular origin, numbers, size, antigenic composition and functional properties. Microparticles support coagulation by exposure of negatively charged phospholipids and sometimes tissue factor, the initiator of coagulation *in vivo*. Microparticles may transfer bioactive molecules to other cells or microparticles, thereby stimulating cells to produce cytokines, cell-adhesion molecules, growth factors and tissue factor, and modulate endothelial functions. Microparticles derived from various cells, most notably platelets but also leucocytes, lymphocytes, erythrocytes and endothelial cells, are present in the circulation of healthy subjects. Rare hereditary syndromes with disturbances in membrane vesiculation leading to a decreased numbers of microparticles clinically present with a bleeding tendency. In contrast, elevated numbers of microparticles are encountered in patients with a great variety of diseases with vascular involvement and hypercoagulability, including disseminated intravascular coagulation, acute coronary syndromes, peripheral arterial disease, diabetes mellitus and systemic inflammatory disease. Finally, microparticles are a major component of human atherosclerotic plaques.

In view of their functional properties, cell-derived microparticles may be an important intermediate in the cascade of cellular and plasmatic dysfunctions underlying the process of atherogenesis.

Keywords Apoptosis, atherogenesis, coagulation, inflammation, microparticles, vascular disease.
Eur J Clin Invest 2004; 34 (6): 392–401

Introduction

Already in the 1940s it was known that human plasma and serum contained a subcellular factor facilitating fibrin formation [1,2]. It was not until 1967 when, using electron microscopic techniques, Wolf demonstrated that this subcellular factor consisted of small vesicles ('microparticles'), which were called 'platelet dust'. These microparticles, showed procoagulant activity, comparable to that of intact platelets [3]. Their procoagulant activity was designated as platelet factor 3 (PF3) [4]. Subsequently, it was shown that (platelet-derived) microparticles (PMPs) were formed during the attachment of platelets to the vascular wall *in vitro* [5]. In recent years, the interest for microparticles has substantially increased, not only because of their procoagulant properties but also because of their putative role in inflammatory processes and their ability to directly affect endothelial functions (Fig. 1) [6–9]. Their suspected involvement in clinical disease was demonstrated for the first time in patients with idiopathic thrombocytopenic purpura (ITP) [10].

The majority of *in vivo* microparticles in blood is derived from platelets [11], whereas microparticles from erythrocytes, granulocytes, monocytes, lymphocytes and endothelial cells usually circulate at lower numbers. Interestingly, significant differences exist between microparticle fractions

Disclosure: The authors have had no financial interest with regard to the preparation of this paper.

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Received 7 January 2004; accepted 25 April 2004

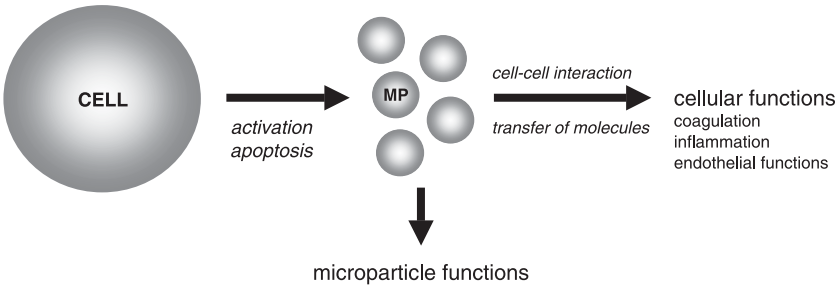

Figure 1 Formation and functional properties of cellular microparticles (MPs)

or subpopulations found in the circulation of healthy subjects [12,13] and those found in patients suffering from various diseases with increased thromboembolic risk or vascular damage, such as atherosclerotic vascular disease, sepsis, diabetes mellitus, severe hypertension and end-stage renal failure [14–22]. Also, microparticles constitute an important component of the human atherosclerotic plaque [23].

To summarize, microparticles are closely associated with the presence and the possible development of atherosclerotic and inflammatory vascular damage. In this review, we describe the structure, detection, pathogenesis and characteristics of microparticles. Finally, the possible clinical relevance of microparticles will be discussed in the context of various diseases.

Characterization of microparticles: size and composition

Platelet activation plays a key role in the development of arterial thrombosis resulting in major clinical syndromes, such as acute myocardial infarction. During platelets activation, vesiculation of parts of the plasma membrane occurs leading to the formation of PMP, the size of which typically ranges from 0·1 to 1·0 μm. Platelets and other cells are surrounded by a plasma membrane consisting of a phospholipid bilayer, containing phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC) and sphingomyeline (SM). In unstimulated cells, the distribution of these phospholipids within the bilayer is asymmetrical. The neutral (uncharged) phospholipids PC and SM are primarily located in the outer (exoplasmic) membrane leaflet, while the negatively charged PS and PE are present within the inner (cytoplasmic) leaflet. The asymmetrical distribution of phospholipids in the plasma membrane is actively maintained by various enzymes, such as the aminophospholipid-translocase or flippase [24]. During cell activation or apoptosis, the asymmetrical distribution of these phospholipids disappears. As a consequence, negatively charged phospholipids such as PS and PE become (surface) exposed. The intracellular mechanisms underlying the release of microparticles are as yet not fully understood, but they seem to be associated – among others – with the inducing stimulus leading to the actual vesiculation. It is now becoming apparent that the formation of microparticles is a highly regulated process: the phospholipid composition of PMPs shows characteristics from intracellular rather than from plasma membrane fractions and recent studies in endothelial cells showed that constitutively exposed proteins from these cells are hardly transmitted to endothelial-cell-derived microparticles [25,26].

Microparticles expose various antigens, notably those also exposed by their 'parent' cells, i.e. the cells from which they are released. For instance, PMPs expose glycoproteins (GP) Ib (CD42b), platelet-endothelium adhesion molecule-1 (PECAM-1; CD31) and the fibrinogen receptor, the integrin αIIbβ3 (GPIIb–IIIa). In addition, PMPs can expose activation markers such as P-selectin (CD62P). Similarly, microparticles from other cells can be characterized: examples are microparticles from erythrocytes that stain for glycophorine A, granulocytic microparticles for CD66, monocytic microparticles for CD14, lymphocytic microparticles for CD4 and CD8 and endothelial-cell-derived microparticles for CD31, CD34, CD51 (vitronectin), CD62E, and CD146 (MUC18, S-Endo-1) [13,14,17–22,26]. All these microparticles can also expose activation markers that are characteristic of their respective 'parent' cell.

Detection of microparticles

Flowcytometry

Microparticles can be detected by flowcytometry in blood samples or fractions there from, as well as in other body fluids such as synovial fluid [6,27]. Using labelled antibodies against cell-specific antigens and/or activation markers and annexin V, a protein that binds specifically to negatively charged phospholipids in the presence of calcium ions, microparticle fractions or subpopulations can be quantified and concurrently their cellular origin as well as their 'activation status' can be established. To correct for autofluorescence and binding of antibodies to Fc-receptors, microparticles are also stained with a (labelled) control antibody plus annexin V, but without calcium ions. Of each event detected by the flowcytometer, the size (forward scatter, FSC) and density (sidescatter, SSC) are determined electronically, as well as the fluorescence in various channels. Fluorescence reflects the amount of antibody bound and therefore is an estimate for the amount of antigen exposed on the membrane surface. Figure 2 illustrates the visualization of PMPs by flowcytometry.

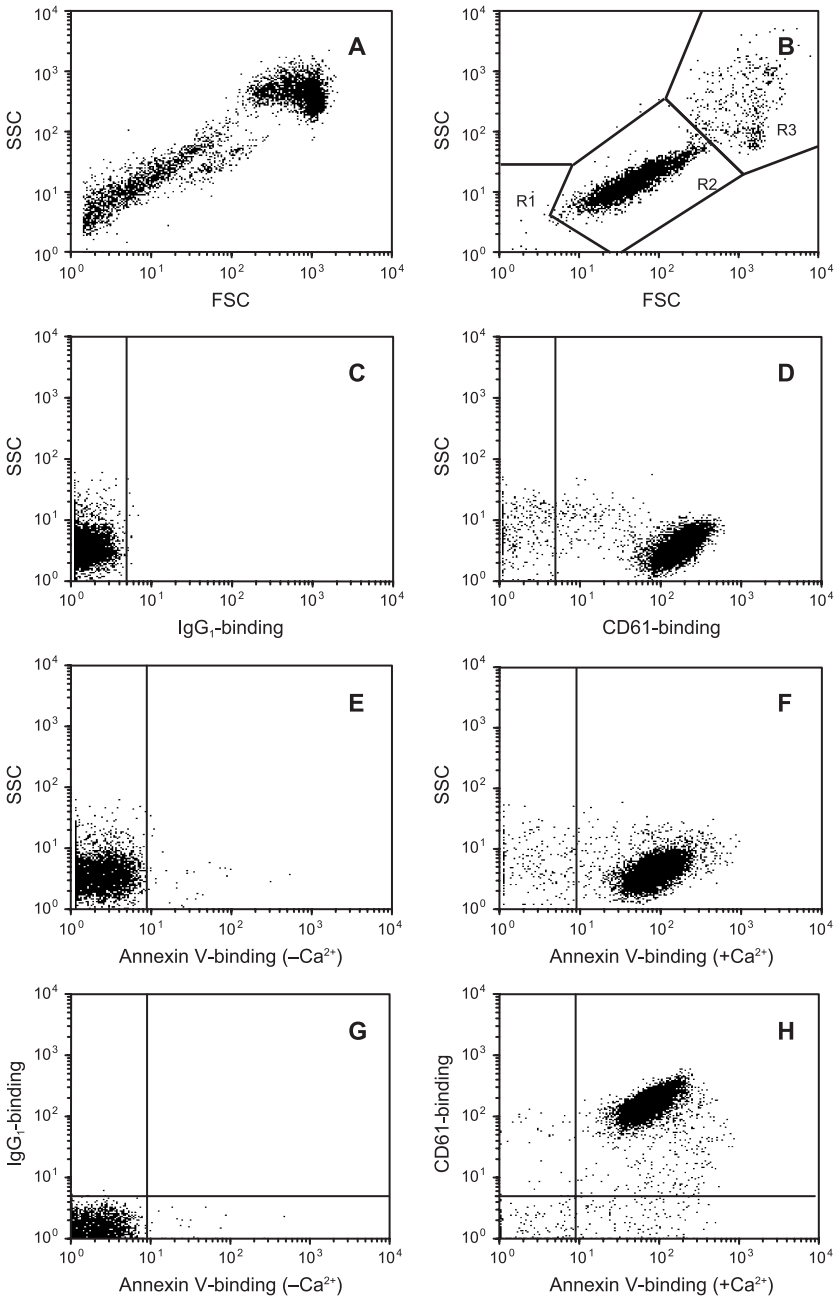

Figure 2 Use of flowcytometry for microparticle analysis. Flowcytometric analysis of whole blood by size (forward scatter, FSC) and density (side scatter, SSC) predominantly yields erythrocytes (visible in the upper right of panel A); events staining positive for (labelled) antibody-directed against platelet-antigen are platelets in region 2 (R2) (B), whereas R3 contains larger events such as complexes of platelets or platelet-derived microparticles, and R1 contains events smaller than platelets, the PMPs. In cell-free plasma, microparticles can be analyzed after additional centrifugation (C–H). (D) Microparticles are stained with an anti-GPIIIa (CD61) monoclonal antibody, as compared with a control antibody (C). Almost all events bind annexin V in the presence of calcium ions (F), but not in the absence of such ions (E). Double staining of microparticles with anti-CD61 plus annexin V in the presence of calcium ions allows visualization of PMPs exposing negatively charged phospholipids (H). As a control, microparticles are stained with annexin V in the absence of calcium ions and control IgG_1 antibody (G; control).

Electron microscopy

Figure 3 shows scanning electron microscopy images of unstimulated cultured human umbilical vein endothelial cells (HUVECS) and the formation of microparticles upon stimulation with interleukin-1α. The diameter of the vesicles released by stimulated HUVECS ranges from 0·1 to 1·0 μm.

Enzyme-linked immunosorbent assay

One of the most frequently used ELISAs to quantify cell-derived microparticles employs a plate coated with annexin V [7,28,29]. Upon addition of a (plasma) sample, microparticles present within this sample will bind to annexin V. After washing, a cell-specific antibody can be added to quantify numbers of cell-specific microparticles. Alternatively, after washing the procoagulant activity of the (bound) microparticles can be determined using a prothrombinase assay.

Mechanisms of microparticle formation: activation and apoptosis

It is generally accepted that all eucaryotic cells release

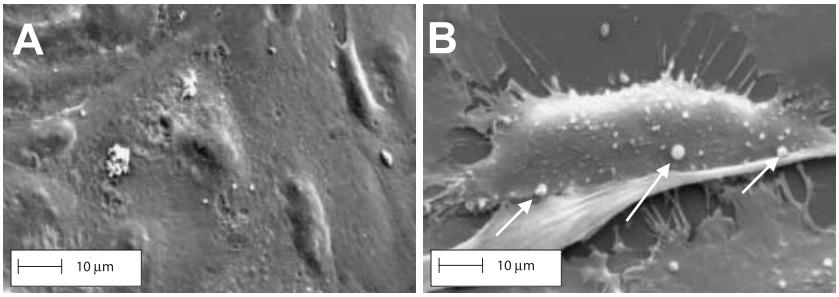

Figure 3 Scanning electron microscope images showing unstimulated cultured human umbilical vein endothelial cells (A) and the formation of microparticles after stimulation of the cells with interleukin-1α (B).

microparticles. Microparticle formation *in vitro* occurs whenever a stimulus is applied which induces either cell activation or apoptosis. To date, however, it is unclear whether the mechanisms underlying microparticle formation are identical during these two conditions are identical.

Cell activation

Platelets can be activated by different agonists that bind to specific receptors on the platelet membrane. Thus, stimuli such as thrombin, collagen and adenosine diphosphate (ADP) activate specific transmembrane receptors that transmit signals into the cell. These signals induce changes in second messenger concentrations which in turn modulate cellular responses [6,7]. Stimulation of platelets by these agents not only leads to platelet aggregation and secretion, but also results in membrane vesiculation and the release of microparticles. Alternatively, agents, such as calcium ionophores, trigger microparticle release by directly changing the intracellular concentrations of second messenger molecules. Platelet-derived microparticles are also formed during prolonged storage of platelets, or when platelets are exposed to high shear-stress conditions *in vitro* [5]. The latter conditions resemble those occurring *in vivo* at stenoses of the vascular tree.

Although the molecular mechanisms underlying microparticle formation are as yet unresolved, the increase in intracellular levels of calcium ions, resulting in the activation of enzymes such as calpain, play an important role [6,7]. Calpain degrades cytoskeletal proteins, and its inhibition partly prevents collagen- and thrombin-induced microparticle formation [30].

Apoptosis

Programmed cell-death or apoptosis is associated with the abolition of the phospholipid asymmetry of the plasma membrane and condensation of the nucleus, followed by DNA fragmentation and the release of apoptotic blebs or microparticles [8]. The intracellular enzyme family of caspases plays an important role in apoptosis [8]. The irreversible step in which procaspase 3 (CPP32) is converted into the active caspase-3 is regarded as fundamental in the apoptotic process. Caspase-3 activates rho-associated kinase (ROCK I) resulting in the release of apoptotic membrane vesicles, which can also contain DNA fragments [31].

Functional characteristics of microparticles

The most frequently described characteristic of both *in vitro* and *in vivo* microparticles is their procoagulant activity [7,13,16,32]. Recent observations, however, also suggest their involvement in inflammatory processes [27], in the transfer of bioactive molecules to other cells and microparticles [9] and the inhibition of endothelium-dependent vasodilatation [33,34]. Not all the properties of cellular microparticles should necessarily be regarded as noxious: specific microparticle subpopulations may even prevent vascular damage. Thus, *in vitro*-generated PMPs were shown to enhance the activation of protein C, thus facilitating the inhibition of coagulation factors Va and VIIa and preventing thrombin formation [35]. Other microparticle fractions were reported to induce cellular growth, chemotaxis, apoptosis and the outgrowth of transplanted haematopoetic stem cells [36,37].

The various functional characteristics of *in vitro*-generated microparticles as well as of those isolated from the circulation of various patient populations will be discussed (see also Table 1).

Microparticles and coagulation

Coagulation activation plays an essential role in the development of atherothrombosis. Subjects with a high risk of cardiovascular disease show various degrees of hypercoagulability. Coagulation activation requires plasmatic coagulation factors, calcium ions and a procoagulant membrane surface. An essential characteristic of such a suitable surface is the exposure of negatively charged phospholipids. As previously stated, the exposure of such phospholipids is one of the characteristics of microparticles. Coagulation factors bind, via their negative Gla-domains, to the negatively charged phospholipids in the presence of calcium ions, thus forming tenase- and prothrombinase-complexes. Platelet-derived microparticles expose more binding sites for factors Va, VIIIa, and IXa per unit of membrane surface area than activated platelets. Thus, at least *in vitro*, thrombin formation is supported more efficiently by microparticle membranes than by platelet membranes when corrected for unit surface area.

The procoagulant activity of microparticles can be quantified using the thrombin generation test [13,14]. In this assay, the conversion over time of a specific chromogenic substrate by thrombin is measured photospectrometrically. In this system, microparticles supply the procoagulant

Table 1 Characteristics of *in vitro*- and *in vivo*-generated microparticles

In vitro-generated platelet microparticles:
stimulate CD11b expression on leucocytes, leucocyte–leucocyte interactions, phagocytosis
induce CD11a/CD18 and CD11b/CD18 on monocytes, resulting in monocyte adhesion to endothelial cells
induce ICAM-1 exposure on endothelial cells, resulting in monocyte adhesion to endothelial cells
stimulate COX2-expression in monocytes and endothelial cells
stimulate thrombocyte aggregation, intracellular calcium flux, inositol phosphate formation
stimulate protein kinase C, mitogen-activated protein (MAP) kinases and stress (JNK) kinases
transcellular transfer of arachidonic acid, resulting in amplification and modulation of platelet activation
transfer of various cytokine- and chemokine-receptors to haematopoetic and malignant cells
transfer of CXCR4-receptors for HIV-1 virus to cells
enhance engraftment of transplanted bone marrow cells
enhance APC-catalysed inactivation of Factor Va
colocalize plasminogen-activator inhibitor-1 and vitronectin
In vivo-circulating platelet microparticles:
initiate and propagate coagulation/enhance thrombin formation
expose P-selectin
expose tissue factor
transfer tissue factor to other cells and cell-derived microparticles
In vitro-generated endothelial-cell microparticles:
induce monocyte adhesion to endothelial cells
activate neutrophils
initiate and propagate coagulation/enhance thrombin formation
expose matrix metalloproteinases-2 and -9, induce matrix degradation and angiogenesis
In vivo-circulating endothelial-cell microparticles:
inhibit endothelium-dependent vasodilation
initiate and propagate coagulation/enhance thrombin formation
are associated with type 1 diabetic microalbuminuria
In vitro-generated leucocyte microparticles:
expose tissue factor, transfer tissue factor to platelets and their microparticles
activate endothelial cells and stimulate the secretion of IL-6 via stress-associated signal routes (JNK1)
In vivo-circulating leucocyte microparticles:
are present in human atherosclerotic plaques, in close association with tissue factor
are associated with type 2 diabetic microvascular damage

surface and a possible initiator of coagulation, e.g. tissue factor, and plasma provides the necessary coagulation factors. By adding calcium ions, (activated) coagulation factors can bind to the (microparticle) membranes to initiate and/or facilitate coagulation. In this assay, the generation of thrombin is completely dependent on the presence of microparticles, and in their absence no coagulation occurs.

In vivo, coagulation is initiated by tissue factor, a transmembrane protein that binds factor VII(a) and catalyses its autoactivation. In turn, the tissue factor/factor VIIa complex directly activates factor X to factor Xa. Factor Xa, in the presence of its cofactor Va, forms the prothrombinase complex that converts factor II (prothrombin) into IIa (thrombin). Alternatively, the tissue factor/factor VIIa complex activates factor IX into factor IXa. Together with its cofactor, factor VIIIa, factor IXa forms the tenase complex that subsequently activates factor X into factor Xa. In this system, there is an important role for coagulation factor XI. Minute quantities of thrombin can activate factor XI into factor XIa. Subsequently, factor XIa activates factor IX into factor IXa, thereby enhancing the formation of thrombin.

In vitro, microparticles can both initiate and propagate coagulation [21,38,39]. However, the mechanisms by which *in vivo* microparticles support coagulation *ex vivo* were highly dependent on the clinical conditions. For instance, thrombin formation by microparticles from blood of a patient with meningococcal sepsis and diffuse intravascular coagulation (DIC) was completely inhibited by antibodies directed against either tissue factor or factor VII [17]. These antibodies also completely inhibited thrombin generation by microparticles from human pericardial blood, i.e. blood that collects in the pericardial cavity during coronary artery bypass grafting (CABG) [16]. In contrast, neither of these antibodies inhibited thrombin generation initiated by microparticles obtained from healthy subjects. Thrombin generation by these microparticles as well as thrombin generation by microparticles from patients with sepsis and multiorgan failure was mediated by factor XI and in some patients also by factor XII [13,18]. Only recently it was discovered that also tissue factor-independent mechanisms are able to initiate coagulation. One example is the binding of factor X to the monocytic protein Mac-1 (CD11b/CD18) and the subsequent activation of factor X into factor Xa by catepsin G [40]. Possibly, microparticles may also use similar tissue factor-independent mechanisms to initiate coagulation.

An important question is whether microparticles are procoagulant *in vivo*. This issue is not easily resolved, but several lines of evidence suggest that microparticle-mediated coagulation is indeed clinically relevant. First, microparticles from various patient populations support coagulation *in vitro* [13,16–18]. Second, the presence of highly procoagulant, tissue-factor-exposing microparticles in certain disease conditions coincided with strongly elevated levels of *in vivo* coagulation activation markers, such as prothrombin fragment F_{1+2} and thrombin-antithrombin complexes. Examples are microparticles from a patient with fulminant DIC and meningococcal septic shock, microparticles from pericardial cavity blood during CABG, and microparticles from synovial fluid from patients with rheumatoid arthritis [16,17,27]. Third, numerous studies demonstrated an association between elevated numbers of microparticles and the increased risk of thromboembolic complications [10,19,21,41]. Fourth, an increased bleeding tendency and decreased levels of circulating microparticles have been described in several rare syndromes [42–44]. Finally, direct infusion of artificial phospholipid vesicles in baboons caused severe DIC [45], and systemic administration of microparticles in rats resulted in thrombus formation [46]. Taken together, these data suggest that clinical presentation of systemic hypercoagulation may involve microparticles exposing coagulant tissue factor. Therefore, it may be of interest to develop drugs that interfere with the mechanisms

underlying the formation of these microparticles rather than symptomatic treatment of the hypercoagulable state as such.

Microparticles and inflammation

Like coagulation, inflammatory processes underlie the pathogenesis of atherothrombotic vascular disease [47]. Elevated plasma levels of acute-phase reactants and other markers of inflammation occur in various high-risk patient populations [14,39]. Microparticles can directly activate and stimulate cells to produce inflammatory substances mediators such as cytokines [48–50]. In addition, at least *in vitro* microparticles mediate intercellular interactions [50–52]. Finally, subpopulations of microparticles isolated from human plasma expose C1q, C3 and C4, strongly suggesting their direct involvement in activation of the complement system [53]. Currently, the relation between cellular microparticles and C-reactive protein (CRP) is studied. This acute-phase protein is known to bind to membranes and, in the membrane-bound form, may activate the classical pathway of the complement system, ultimately leading to vascular damage.

Table 1 lists the reported cell–microparticle and microparticle–microparticle interactions. At present, however, there is no direct evidence that microparticles are involved in inflammatory disease *in vivo*. Although elevated levels of microparticle subpopulations are present in the circulation of patients with inflammatory disease, both of infectious and autoimmune origin, a causal relationship between microparticles and inflammatory processes cannot readily be established, because cytokines trigger cells, thereby stimulating the release of microparticles, whereas microparticles trigger cells to produce and release cytokines [48]. Therefore, it is as yet unclear whether cellular microparticles are a cause or consequence of inflammatory processes and the associated vascular damage.

Microparticles and endothelial-cell functions

In vitro microparticles adhere to endothelial cells and subsequently stimulate these cells to produce cell-specific adhesion-cell molecules, cytokines and tissue factor [9]. Also, *in vivo* microparticles were found to influence endothelial functions *ex vivo*: microparticles from patients with acute coronary syndromes directly impaired endothelium-dependent vasodilatation in rat aorta-rings, presumably by inhibition of the nitric oxide (NO)-mediated signal transduction [33]. Also microparticles from women with preeclampsia impaired the endothelium-dependent vasodilatation [34]. Several studies also suggest a relationship between circulating microparticles and endothelial function. Patients with complicated diabetes mellitus, who were treated with a platelet aggregation inhibitor, lowered the numbers of circulating PMPs and decreased plasma concentrations of vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) [39]. Conversely, stimulation of endothelial cells *in vitro* by TNFα induced the formation of microparticles exposing adhesion-cell molecules, including ICAM-1, E-selectin, vitronectin-3 and platelet-endothelial cell-adhesion molecule-1 (PECAM-1). In patients with various systemic and autoimmune diseases elevated levels of microparticles originating from endothelial cells were found [54–56].

Microparticles and signal transduction, growth, angiogenesis and metastasis

Microparticles may expose adhesion-cell molecules, specifically adhere to, e.g. endothelial cells, and stimulate these cells to produce various intermediates, such as E-selectin and tissue factor [36,50,57]. The actual 'communication' between microparticles and cells may occur through transfer of bioactive molecules such as arachidonic acid. Thus, microparticles treated with secretory phospholipase A_2, an acute-phase reactant, contained elevated levels of lysophospholipids and arachidonic acid and such microparticles could activate endothelial cells by the transfer of this arachidonic acid [9,49,58].

In patients with type 2 diabetes we previously described elevated numbers of microparticles from platelets, granulocytes and lymphocytes that exposed tissue factor [14]. Both *in vitro* and *in vivo* studies demonstrated the presence of tissue factor-positive PMP subpopulations, which concurrently expose antigens originating from granulocytes, monocytes or lymphocytes, suggesting a possible transfer of tissue factor by, as well as to, these PMP.

Platelet-derived microparticles have the ability to transfer the CXCR4 receptor from CXCR4-positive to CXCR4-negative cells [59]. This receptor is mandatory for the HIV-1 virus to enter cells, suggesting a role for PMPs in the dissemination of HIV-1 particles. Also, microparticles transfer cytokine and chemokine receptors to haematopoetic, but also to malignant cells, by which mechanism these vesicles may modulate cellular activation, proliferation, survival, apoptosis and chemotaxis [60]. Adherence of PMPs to transplanted bone marrow cells stimulated their outgrowth, which may be regarded as a beneficial effect of the PMP. Conversely, in addition to activated platelets, PMPs are involved in paraneoplastic thromboembolic complications and metastasis. Only recently, matrix metalloproteinases-2 and -9, enzymes that play a role in matrix degradation and angiogenesis, were detected in microparticles of endothelial origin [61].

Taken together, cellular microparticles may be carriers of antigens and receptors, including tissue factor, E-selectin and VCAM-1, all of which were previously regarded as 'soluble' in plasma. Plasma concentrations of these substances are widely used as measures of endothelial dysfunction in human. By assessing the colocalization of these proteins with cell-specific antigens on microparticles and by measuring the plasma levels of these substances before and after centrifugation (i.e. after removal of the microparticle fraction), it becomes possible to determine the real cellular origin of these antigens, which currently are all ascribed to endothelial cells.

Clinical relevance of cellular microparticles

During the last 5 years a growing number of publications appeared reporting elevated numbers of microparticle subpopulations in association with various disease states as well as studies investigating the composition and functional characteristics of microparticles. To date, however, it is unclear whether microparticles are a cause or merely a consequence of metabolic and vascular disease.

Platelet-derived microparticles

The clinical relevance of PMPs may be illustrated by the rare hereditary Scott syndrome, a disease characterized by a bleeding tendency and a decreased formation of PMPs [42,62]. The diminished formation of PMPs is caused by a signal transduction defect which diminishes the transmembrane migration and exposure of PS. Castaman's disease and Glanzmann's thrombastenia are other rare syndromes in which an increased bleeding tendency is associated with a decreased release of PMPs [43,44]. Low numbers of circulating microparticles were found in patients with sepsis and these showed an inverse correlation with markers of *in vivo* coagulation [18]. Conversely, elevated numbers of PMPs were found in patients suffering from diseases associated with an increased risk of thromboembolic processes and vascular damage, including ITP [10], acute coronary syndromes [15,19], acute cerebrovascular disease [41], heparin-induced thrombopenia [38], peripheral arterial disease [63], complicated diabetes mellitus [39], severe hypertension [20], end-stage renal disease [21], multiple sclerosis [55] and malignancy [60]. In some studies the procoagulant activity of the *in vivo* microparticles, predominantly PMPs, was also demonstrated. In patients with uncomplicated type 2 diabetes mellitus, we found elevated numbers of tissue-factor-exposing PMPs [14]. Unexpectedly, this microparticle-associated tissue factor did not show procoagulant activity, possibly because of its presence in the 'encrypted' state, in which it binds anti-tissue factor antibody and factor VII/VIIa, but lacks procoagulant activity [64]. Previous studies showed that tissue-factor activity is critically dependent on the microenvironment within the membrane [65]. Therefore, it was hypothesized that microparticle-associated tissue factor from these patients may play a role in other processes, such as angiogenesis, growth and signal transduction.

Endothelial-cell microparticles

Increased numbers of microparticles from endothelial cells were reported in patients with acute coronary syndromes [19], confirming the pathophysiologic role of endothelial injury in acute coronary events [66]. Also, high circulating levels of endothelial-cell microparticles were reported in severe hypertension, thrombotic thrombocytopenic purpura, systemic lupus erythematosus and multiple sclerosis (Table 2). Decreased numbers of endothelial-cell microparticles were measured in subjects with sepsis and multiorgan failure. Some authors explain their occurrence by apoptosis whereas others regard these vesicles as a result of endothelial-cell activation. In a recent study, increased levels of endothelial-cell microparticles were associated with albuminuria in subjects with type 1 diabetes mellitus, but not in those with type 2 diabetes [22]. We found similar numbers of endothelial-cell-derived microparticles

Table 2 Circulating nonplatelet microparticles in diseases with vascular involvement

Cellular origin	Disorder	Microparticle numbers
Granulocytes	sepsis/multiorgan failure	↑
	type 2 diabetes mellitus*	↑
	preeclampsia	↑
Monocytes	atherosclerotic plaques	↑
	type 2 diabetes mellitus	↑
	lung cancer	↑
Endothelial cells	systemic lupus erythematosus	↑
	acute coronary syndromes	↑
	congestive heart failure	↑
	sepsis	↓
	thrombotic thrombocytopenic purpura	↑
	multiple sclerosis	↑
	type 1 diabetes mellitus	↑
	severe hypertension	↑
Lymphocytes	HIV	↑
	atherosclerotic plaques	↑
	type 2 diabetes mellitus*	↑
	preeclampsia	↑

*Proportion of granulocyte- and lymphocyte-derived microparticles that exposed tissue factor.

in patients with uncomplicated type 2 diabetes and in healthy controls [14]. To note, as in the studies published, different endothelial-cell markers were used in various study populations; the findings cannot be readily compared [13,14,19,22,26,54,56].

Granulocyte, monocyte and lymphocyte microparticles

Table 2 shows the occurrence of various nonplatelet microparticles in human disease. Elevated numbers of granulocyte-derived microparticles were reported in patients with meningococcal sepsis, in patients with multiorgan failure and in women with preeclampsia [17,18,34], suggesting that the occurrence of such microparticles is associated with infection and/or inflammation. High numbers of monocyte-derived microparticles have thus far only been reported in one patient suffering from meningococcal septic shock who developed severe DIC [17]. These microparticles exposed highly coagulant tissue factor. In patients with type 2 diabetes, monocyte-derived microparticles were associated with plasma E-selectin levels and the highest microparticle numbers were found in subjects with diabetic nephropathy [67]. Elevated levels of lymphocyte-derived microparticles (CD4+, CD8+) have been found in preeclamptic woman and in HIV-infected patients, suggesting increased apoposis of lymphocytes [34,38]. Finally, an interesting finding is the presence of tissue factor in the vicinity of monocyte and lymphocyte microparticles in human atherosclerotic plaques [23]. In summary, the occurrence of microparticles originating from white blood cell types is associated with inflammation, infection and possibly endothelial dysfunction and the development of atherothrombosis. The relative contribution of such microparticles to the development of the afore-mentioned pathologies, however, remains to be established.

Conclusions

Microparticles from various cell types – but predominantly from thrombocytes – occur in the human circulation. Elevated numbers of circulating microparticles are found in patients who suffer from diseases associated with an increased thromboembolic risk and vascular damage. Microparticles initiate and propagate coagulation by exposing negatively charged phospholipids on their membrane surface. In addition, under certain conditions, microparticles also expose tissue factor, the initiator of coagulation. The clinical relevance of the presence of microparticles in the circulation of healthy subjects is as yet unclear, but it may be regarded as a reflection of the dynamics between resting, activated and apoptotic cells. In addition, the numbers of circulating microparticles also reflect the result of their production and clearance. In vascular disease states it still remains to be elucidated whether microparticles are a cause or a consequence of the condition, as disease-related factors such as infectious agents, cytokines and metabolic disturbances are known to trigger microparticle formation. Still, it may be assumed that microparticles do contribute to the severity of disease, as they can disseminate procoagulant and proinflammatory activities throughout the body. Therefore, microparticles may be viewed as part of a cascade of reactions in response to a stimulus. This stimulus that led to their generation determines their numbers, size, biochemical composition and functional characteristics.

Although microparticle formation may be regarded as an adaptive process, such as e.g. the classical inflammatory response, an overshoot of this response, i.e. an excessive release of microparticles, may become harmful to the organism and as such unwanted. Conversely, defective microparticle formation, in particular of PMPs, may result in an increased bleeding tendency. Patients with a haemorrhagic trait owing to congenital or acquired forms of platelet abnormalities can be treated with plasma cryoprecipitate. The therapeutic efficacy of cryoprecipitates is in part attributed to their content of high concentrations of PMPs [68].

Various antiplatelet drugs, including the GPIIb/IIIa receptor antagonist abciximab [69] and the cAMP phosphodiesterase inhibitor cilostazol [39], offer therapeutic possibilities, as they reduce excessive PMP formation. Short-term administration of vitamin C at a high dose reduced the number of circulating endothelial-cell-derived microparticles in patients with congestive heart failure [70]. The possible beneficial effect of antioxidants was recently also demonstrated by an anecdotal observation, in which consumption of a flavinoid-rich cocoa beverage reduced circulating numbers of PMPs in healthy subjects [71].

Future research should provide insight into the factors that induce microparticle formation and the molecular mechanisms underlying the process of generation of these vesicles, i.e. activation and apoptosis. Collectively, the data obtained from these studies should provide answers to the question as to whether cellular microparticles play a causative role in the development of thromboembolic complications and vascular damage in humans.

Acknowledgements

The authors thank Mrs A.N. Böing (Laboratory of Experimental Clinical Chemistry, AMC, Amsterdam) and Dr J. van Marle (Department of Cell Biology and Histology, AMC), for the preparation of the scanning electron microscope images. M.E.T. is supported by a grant from the Dutch Diabetes Foundation (grant no. 2000·00·025).

References

1 Chargaff E, West R. The biological significance of the thromboplastic protein of blood. *J Biol Chem* 1946;**166**:189–97.

2 O'Brien JR. The platelet-like activity of serum. *Br J Haematol* 1955;**1**:223–8.

3 Wolf P. The nature and significance of platelet products in human plasma. *Br J Haematol* 1967;**13**:269–88.
4 Hardisty RM, Hutton RA. Platelet aggregation and the availability of platelet factor 3. *Br J Haematol* 1966;**12**:764–76.
5 Warren BA, Vales O. The release of vesicles from platelets following adhesion to vessel walls in vitro. *Br J Exp Pathol* 1972;**53**:206–15.
6 Horstman LL, Ahn YS. Platelet microparticles: a wide-angle perspective. *Crit Rev Oncol Hematol* 1999;**30**:111–42.
7 Nieuwland R, Sturk A. Platelet-derived microparticles. In: Michelson AD, editor. *Platelets.* London: Academic Press, Elsevier Science;2002.pp.255–65.
8 Freyssinet JM, Toti F, Hugel B, Gidon-Jeangirard C, Kunzelmann C, Martinez MC *et al.* Apoptosis in vascular disease. *Thromb Haemost* 1999;**82**:727–35.
9 Barry OP, FitzGerald GA. Mechanisms of cellular activation by platelet microparticles. *Thromb Haemost* 1999;**82**:794–800.
10 Kahn I, Zucker-Franklin D, Karpatkin S. Microthrombocytosis and platelet fragmentation associated with idiopathic/autoimmune thrombocytopenic purpura. *Br J Haematol* 1975;**31**:449–60.
11 George JN, Thoi LL, McManus LM, Reimann TA. Isolation of human platelet membrane microparticles from plasma and serum. *Blood* 1982;**60**:834–40.
12 George JN, Pickett EB, Saucerman S, McEver RP, Kuniki TJ, Kieffer N *et al.* Platelet surface proteins: studies on resting and activated platelets and platelet membrane microparticles in normal subjects, and observations in patients during adult respiratory distress syndrome and cardiac surgery. *J Clin Invest* 1986;**78**:340–8.
13 Berckmans RJ, Nieuwland R, Boing AN, Romijn FP, Hack CE, Sturk A. Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. *Thromb Haemost* 2001;**85**:639–47.
14 Diamant M, Nieuwland R, Berckmans RJ, Pablo RF, Smit JWA, Sturk A *et al.* Elevated numbers of tissue-factor exposing microparticles correlate with components of the metabolic syndrome in uncomplicated type 2 diabetes. *Circulation* 2002;**106**:2442–7.
15 Tate DA, Bode AP, Nichols TC, Dehmer GJ. Platelet activation detected by platelet-derived microparticles in coronary sinus blood from patients with unstable coronary syndromes. *Circulation* 1992;**86**:3193A.
16 Nieuwland R, Berckmans RJ, Rotteveel-Eijkman RC, Maquelin KN, Roozendaal KJ, Jansen PG *et al.* Cell-derived microparticles generated in patients during cardiopulmonary bypass are highly procoagulant. *Circulation* 1997;**96**:3534–41.
17 Nieuwland R, Berckmans RJ, McGregor S, Boing AN, Romijn FP, Westendorp RG *et al.* Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood* 2000;**95**:930–5.
18 Joop K, Berckmans RJ, Nieuwland R, Berkhout J, Romijn FP, Hack CE *et al.* Microparticles in patients with multi–organ dysfunction syndrome and sepsis support coagulation through multiple mechanisms. *Thromb Haemost* 2001;**85**:810–20.
19 Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM *et al.* Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation* 2000;**101**:841–3.
20 Preston RA, Jy W, Jimenez JJ, Mauro LM, Horstman LL, Valle M *et al.* Effects of severe hypertension on endothelial and platelet microparticles. *Hypertension* 2003;**41**:211–7.
21 Ando M, Iwata A, Ozeki Y, Tsuchiya K, Akiba T, Nihei H. Circulating platelet-derived microparticles with procoagulant activity may be a potential cause of thrombosis in uremic patients. *Kidney Int* 2002;**62**:1757–63.
22 Sabatier F, Darmon P, Hugel B, Combes V, Sanmarco M, Velut J-G *et al.* Type 1 and type 2 diabetic patients display different patterns of cellular microparticles. *Diabetes* 2002;**51**:2840–5.
23 Mallat Z, Hugel B, Ohan J, Leseche G, Freyssinet JM, Tedgui A. Shed membrane microparticles with procoagulant potential in human atherosclerotic plaques. *Circulation* 1999;**99**:348–53.
24 Sims PJ, Wiedmer T. Unraveling the mysteries of phospholipid scrambling. *Thromb Haemost* 2001;**86**:266–75.
25 Olas B, Lundell K, Holmsen H, Fukami MH. Biochemical properties of platelet microparticle membranes formed during exocytosis resemble organelles more than plasma membrane. *FEBS Lett* 2002;**525**:29–32.
26 Abid Hussein MN, Meesters EW, Osmanovic N, Romijn FP, Nieuwland R, Sturk A. Antigenic characterization of endothelial cell-derived microparticles and their detection ex vivo. *J Thromb Haemost* 2003;**1**:2434–43.
27 Berckmans RJ, Nieuwland R, Böing AN, Romijn FP, Kraan M, Tak PP *et al.* Microparticles from synovial fluid support coagulation exclusively via a factor VII-dependent mechanism. *Arthritis Rheum* 2002;**46**:2857–66.
28 Aupeix K, Hugel B, Martin T, Bischoff P, Lill H, Pasquali JL *et al.* The significance of shed membrane particles during programmed cell death in vitro, and in vivo, in HIV-1 infection. *J Clin Invest* 1997;**99**:1546–54.
29 Myamoto S, Marcinkiewicz C, Edmunds LH Jr, Niewiarowski S. Measurement of platelet microparticles during cardiopulmonary bypass by means of captured ELISA for GPIIb/IIIa. *Thromb Haemost* 1998;**80**:225–30.
30 Shcherbina A, Remold-O'Donnell E. Role of caspase in a subset of human platelet activation responses. *Blood* 1999;**93**:4222–31.
31 Coleman ML, Sahai EA, Yeo M, Bosch M, Dewar A, Olson MF. Membrane blebbing during apoptosis results form caspase-mediated activation of ROCK I. *Nat Cell Biol* 2001;**3**:339–45.
32 Giesen PLA, Rauch U, Bohrmann B, Kling D, Roque M, Fallon JT *et al.* Blood-borne tissue factor: another view of thrombosis. *Proc Natl Acad Sci USA* 1999;**96**:2311–5.
33 Boulanger CM, Scoazec A, Ebrahimian T, Henry P, Mathieu E, Tedgui A *et al.* Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. *Circulation* 2001;**104**:2649–52.
34 Van Wijk MJ, Svedas E, Boer K, Nieuwland R, VanBavel E, Kublickiene KR. Isolated microparticles, but not whole plasma, from women with preeclampsia impair endothelium-dependent relaxation in isolated myometrial arteries from normal pregnant women. *Am J Obstet Gynecol* 2002;**187**:1686–93.
35 Tans G, Rosing J, Christella M, Thomassen LGD, Heeb MJ, Zwaal RFA *et al.* Comparison of anticoagulant and procoagulant activities of stimulated platelets and platelet-derived microparticles. *Blood* 1991;**77**:2641–8.
36 Baj-Krzyworzeka M, Majka M, Pratico D, Ratajczak J, Vilaire G, Kijowski J *et al.* Platelet-derived microparticles stimulate proliferation, survival, adhesion, and chemotaxis of hematopoietic cells. *Exp Hematol* 2002;**30**:450–9.
37 Janowska-Wieczorek A, Majka M, Kijowski J, Baj-Krzyworzeka M, Reca R, Turner AR *et al.* Platelet-derived microparticles bind to hematopoietic stem/progenitor cells and enhance their engraftment. *Blood* 2001;**98**:3143–9.
38 Warkentin TE, Hayward CP, Boshkov LK, Santos AV, Sheppard JA, Bode AP *et al.* Sera from patients with

heparin-induced trombocytopenia generate platelet-derived microparticles with procoagulant activity: an explanation for the thrombotic complications of heparin-induced trombocytopenia. *Blood* 1994;**84**:3691–9.
39 Nomura S, Shouzu A, Omoto S, Hayakawa T, Kagawa H, Nishikawa M *et al.* Effect of cilostazol on soluble adhesion molecules and platelet-derived microparticles in patients with diabetes. *Thromb Haemost* 1998;**80**:388–92.
40 Schwarz M, Nordt T, Bode C, Peter K. The GPIIb/IIIa inhibitor abciximab (c7E3) inhibits the binding of various ligands to the leukocyte integrin Mac-1 (CD11b:/CD18, alpha Mbeta2). *Thromb Res* 2002;**107**:121–8.
41 Lee YJ, Horstman LL, Janania J, Reyes Y, Kelley RE, Ahn YS. Elevated platelet microparticles in transient ischemic attacks, lacunar infarcts, and multiinfarct dementias. *Thomb Res* 1993;**72**:295–304.
42 Toti F, Satta N, Fressinaud E, Meyer D, Freyssinet JM. Scott syndrome, characterized by impaired transmembrane migration of procoagulant phosphatidyl serine and hemorrhagic complications, is an inherited disorder. *Blood* 1996;**87**:1409–15.
43 Castaman G, Ye-Feng L, Battistin E, Rodeghiero F. Characterization of a novel bleeding disorder with isolated prolonged bleeding time and deficiency of platelet microvesicle generation. *Br J Haematol* 1997;**96**:458–63.
44 Gemmell CH, Sefton MV, Yeo EL. Platelet-derived microparticle formation involves glycoprotein IIb-IIIa. Inhibition by RGDS and a Glanzmann's thrombasthenia defect. *J Biol Chem* 1993;**268**:14586–9.
45 Taylor FB Jr, He SE, Chang AC, Box J, Ferrell G, Lee D *et al.* Infusion of phospholipid vesicles amplifies the local thrombotic response to TNF and anti-protein C into a consumptive response. *Thromb Haemost* 1996;**75**:578–84.
46 Biró E, Sturk-Maquelin K, Vogel GMT, Meuleman DG, Smit MJ, Hack CE *et al.* Human cell-derived microparticles promote thrombus formation in vivo in a tissue factor-dependent manner. *J Thromb Haemost* 2003;**1**:2561–8.
47 Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999;**340**:115–26.
48 Mesri M, Altieri DC. Leukocyte microparticles stimulate endothelial cell cytokine release and tissue factor induction in JNK1 signaling pathway. *J Biol Chem* 1999;**274**:23111–8.
49 Barry OP, Pratico D, Lawson JA, FitzGerald GA. Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. *J Clin Invest* 1997;**99**:2118–27.
50 Barry OP, Pratico D, Savani RC, FitzGerald GA. Modulation of monocyte–endothelial cell interactions by platelet microparticles. *J Clin Invest* 1998;**102**:136–44.
51 Forlow SB, McEver RP, Nollert MU. Leukocyte–leukocyte interactions mediated by platelet microparticles under flow. *Blood* 2000;**95**:1317–23.
52 Huber J, Vales A, Mitulovic G, Blumer M, Schmid R, Witztum JL *et al.* Oxidized membrane vesicles and blebs from apoptotic cells contain biologically activate oxidized phospholipids that induce monocyte–endothelial interactions. *Arterioscler Thromb Vasc Biol* 2002;**22**:101–7.
53 Nauta AJ, Trouw LA, Daha MR, Tijsma O, Nieuwland R, Schwaeble WJ *et al.* Direct binding of C1q to apoptotic cells and cell blebs induces complement activation. *Eur J Immunol* 2002;**32**:1726–36.
54 Jimenez JJ, Jy W, Mauro LM, Horstman LL, Ahn YS. Elevated endothelial microparticles in thrombotic thrombocytopenic purpura: findings from brain and renal microvascular cell culture and patients with active disease. *Br J Haematol* 2001;**112**:81–90.
55 Kolodny L, Ahn YS, Sheremata WA. Evidence of platelet activation in multiple sclerosis. *Ann Neurol* 1996;**40**:520.
56 Minagar A, Jy W, Jimenez JJ, Sheremata WA, Mauro LM, Mao WW *et al.* Elevated plasma endothelial microparticles in multiple sclerosis. *Neurology* 2001;**56**:1319–24.
57 Rauch U, Bonderman D, Bohrmann B, Badimon JJ, Himber J, Riederer MA. Transfer of tissue factor from leukocytes to platelets is mediated by CD15 and tissue factor. *Blood* 2000;**96**:170–5.
58 Fourcade O, Simon MF, Viode C, Rugani N, Leballe F, Ragab A *et al.* Secretory phospholipase A2 generates the novel lipid mediator lysophosphatidic acid in membrane microvesicles shed from activated cells. *Cell* 1995;**80**:919–27.
59 Rozmyslowicz T, Majka M, Kijowski J, Murphy SL, Conover DO, Poncz M *et al.* Platelet- and megakaryocyte-derived microparticles transfer CXCR4 receptro to CXCR4-null cells and make them susceptible to infection by X4-HIV. *AIDS* 2003;**17**:33–42.
60 Kim HK, Song KS, Park YS, Kang YH, Lee YJ, Lee KR *et al.* Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastatic predictor. *Eur J Cancer* 2003;**39**:184–91.
61 Taraboletti G, D'Ascenzo S, Borsotti P, Giavazzi R, Pavan A, Dolo V. Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. *Am J Pathol* 2002;**160**:673–80.
62 Weiss HJ, Vicic WJ, Lages BA, Roger J. Isolated deficiency of platelet procoagulant activity. *Am J Med* 1979;**67**:206–13.
63 Zeiger F, Stephan S, Hoheisel G, Pfeiffer D, Ruehlmann C, Koksch M. P-Selectin expression, platelet aggregates, and platelet-derived microparticle formation are increased in peripheral arterial disease. *Blood Coagul Fibrinolysis* 2000;**11**:723–8.
64 Bach RR. Mechanism of tissue factor activation on cells. *Blood Coagul Fibrinolysis* 1998;**9**:S37–S43.
65 Andree HA, Nemerson Y. Tissue factor: regulation of activity by flow and phospholipid surfaces. *Blood Coagul Fibrinolysis* 1995;**6**:189–97.
66 Forman MB, Puett DW, Virmani R. Endothelial and myocardial injury during ischemia and reperfusion: pathogenesis and therapeutic implications. *J Am Coll Cardiol* 1989;**13**:450–9.
67 Omoto S, Nomura S, Shouzu A, Nishikawa M, Fukuhara S, Iwasaka T. Detection of monocyte-derived microparticles in patients with type II diabetes mellitus. *Diabetologia* 2002;**45**:550–5.
68 George JN, Pickett EB, Heinz R. Platelet membrane microparticles in blood bank fresh frozen plasma and cryoprecipitate. *Blood* 1986;**68**:307–9.
69 Goto S, Tamura N, Li M, Handa M, Ikeda Y, Handa S *et al.* Different effects of various anti-GPIIb-IIIa agents on shear-induced platelet activation and expression of procoagulant activity. *J Thromb Haemost* 2003;**1**:2022–30.
70 Rössig L, Hoffmann J, Hugel B, Mallat Z, Haase A, Freyssinet JM *et al.* Vitamin C inhibits endothelial cell apoptosis in congestive heart failure. *Circulation* 2001;**104**:2182–7.
71 Rein D, Paglieroni TG, Wun T, Pearson DA, Schmitz HH, Gosselin R *et al.* Cocoa inhibits platelet activation and function. *Am J Clin Nutr* 2000;**72**:30–5.