**Brief Review**

**Procoagulant Microparticles
Disrupting the Vascular Homeostasis Equation?**

Olivier Morel, Florence Toti, Bénédicte Hugel, Babé Bakouboula, Laurence Camoin-Jau, Françoise Dignat-George, Jean-Marie Freyssinet

**Abstract**—Apoptosis and vascular cell activation are main contributors to the release of procoagulant microparticles (MPs), deleterious partners in atherothrombosis. Elevated levels of circulating platelet, monocyte, or endothelial-derived MPs are associated with most of the cardiovascular risk factors and appear indicative of poor clinical outcome. In addition to being a valuable hallmark of vascular cell damage, MPs are at the crossroad of atherothrombosis processes by exerting direct effects on vascular or blood cells. Under pathological circumstances, circulating MPs would support cellular cross-talk leading to vascular inflammation and tissue remodeling, endothelial dysfunction, leukocyte adhesion, and stimulation. Exposed membrane phosphatidylserine and functional tissue factor (TF) are 2 procoagulant entities conveyed by circulating MPs. At sites of vascular injury, P-selectin exposure by activated endothelial cells or platelets leads to the rapid recruitment of MPs bearing the P-selectin glycoprotein ligand-1 and blood-borne TF, thereby triggering coagulation. Within the atherosclerotic plaque, sequestered MPs constitute the main reservoir of TF activity, promoting coagulation after plaque erosion or rupture. Lesion-bound MPs, eventually harboring proteolytic and angiogenic effectors are additional actors in plaque vulnerability. Pharmacological strategies aimed at modulating the release of procoagulant MPs appear a promising therapeutic approach of both thrombotic processes and bleeding disorders. (Arterioscler Thromb Vasc Biol. 2006;26:2594-2604.)

**Key Words:** microvesicles ■ phosphatidylserine ■ tissue factor

**Membrane Remodeling and Vesiculation**

In the plasma membrane, the transverse distribution of aminophospholipids is controlled by specific transporters governing their inward (flip) or outward (flop) translocation. In the resting membrane, flippase activity is prominent, aminophospholipids, chiefly phosphatidylserine (PhtdSer) and phosphatidylethanolamine, being sequestered in the inner leaflet. After stimulation, there is a swift egress of aminophospholipids to the outer leaflet, with flippase activity being reduced while overwhelming floppase activity leads to transient mass imbalance of the exoplasmic leaflet at the expense of the cytoplasmic one. Membrane budding is ultimately resolved into the release of MPs that expose procoagulant PhtdSer, the proportion of which in the outer leaflet may vary. MPs are usually referred to as 0.1- to 1-μm membrane fragments exposing PhtdSer. Other vesicles released from intracellular pools of vesicles (eg, exosomes) or not exposing PhtdSer are beyond the scope of this review. Cytoskeleton integrity and calcium entry are other features involved in aminophospholipid transbilayer fluxes. Whether...
“flip-flop” is mandatory for the generation of procoagulant MPs is suggested from the phenotype of Scott syndrome, a rare bleeding disorder in which PhtdSer exposure and plasma membrane shedding are deficient.6

The Hemostatic Balance at the MP Surface and Causes of Disruption

MPs: Effectors of the Coagulation System

With respect to activated platelets, circulating MPs provide an additional procoagulant phospholipid surface for the assembly of the characteristic enzymes complexes of the blood coagulation cascade. Their catalytic properties rely on a procoagulant anionic aminophospholipid, PhtdSer, translocated to the exoplasmic leaflet after membrane remodeling. Once accessible to circulating blood factors, PhtdSer enables local concentrations necessary to achieve the kinetics requisite for optimal thrombin generation and efficient hemostasis. Indeed, shielding PhtdSer-rich surfaces decreases the catalytic efficiency of both tenase and prothrombinase complexes by ≈200- and 1000-fold respectively.7 Additionally, PhtdSer considerably enhances the procoagulant activity of TF, the main cellular initiator of blood coagulation.

Circulating MPs in the Modulation of the Hemostatic Balance

Because of their plasma membrane reactivity, platelets constitute the main source of circulating procoagulant MPs under many pathophysiological situations,8 with leukocyte, erythrocyte, or endothelial lineages being other providers.1,2 MPs harbor functional membrane or cytoplasmic effectors (selectins, GPIIbIIIa, GPIb, von Willebrand factor, arachidonic acid, thromboxane A2, etc.) able to promote prothrombotic responses. When bearing appropriate counterligands, MPs can transfer their procoagulant potential to target cells. For instance, platelet-derived MPs (PMPs) can bind to soluble and immobilized fibrinogen, thus delivering procoagulant entities to the thrombus via the formation of aggregates.9 In vitro, interaction between endothelial MPs and monocytes promotes TF mRNA expression and TF-dependent procoagulant activity.10

At the MP surface, the presence of thrombomodulin, TF pathway inhibitor (TFPI)11 or protein C12 is indicative of possible MP contribution to anticoagulant pathways. Nevertheless, after lipopolysaccharide-treatment promoting TF expression, thrombomodulin activity at monocytes and derived-MP surfaces is overwhelmed by TF and prothrombinase activities.13 During fibrinolysis for myocardial infarction, a reduced TFPI expression at the MP surface was associated to TF-driven coagulation.11 Reactive oxygen species, highly expressed in cell-MP aggregates, may contribute to TFPI inactivation and to the de-encryption of TF activity.13 When expressed at the MP surface, TF activity would thus prevail as a result of insufficient anticoagulant counterbalance. Another mechanism worth considering in the thrombo-resistance at the endothelium and monocyte MP surfaces involves activated protein C pathway.14 Activated protein C has been reported to promote the shedding of endothelial and monocyte MPs through protease activated receptor-1 and endothelial protein C receptor-dependent mechanisms. Such MPs harbor functional endothelial protein C receptor, protected from metalloproteinase cleavage, and display anticoagulant ability toward factor Va inactivation.14

Circulating MPs and Blood-Borne TF

The long-standing dogma that TF is a constitutive protein expressed in minute amounts, switching the procoagulant properties of the endothelium toward the initiation of a clotting TF-driven process when upregulated, was recently challenged by the description of a reservoir of circulating TF spread by MPs.15 Termed blood-borne TF, it can be trapped within the developing thrombus through CD15, CD18, and TF-dependent interactions.15 The cellular origin of blood-borne TF is still debated, most likely varying with the pathophysiologic context. Under resting conditions, blood-borne TF was mainly harbored by PMPs,8 whereas monocyte-derived MPs could be expected important providers after stimulation by lipopolysaccharide.16 The absence of TF mRNA in megakaryocytes raises the question of TF transfer to platelets. Multiple fusions and exchanges between monocyte, endothelial, and platelet plasma or MP membranes have been considered.17–19 Polynuclear leukocytes constitute another controversial source of blood-borne TF evidenced by MP labeling, which does not exclude fusion or phagocytosis events between cell lineages.20,21 The contribution of endothelial-derived MPs (EMP)s) to blood-borne TF appears limited in the absence of stimulation, its importance being evoked under circumstances of drastic endothelial activation.10,22,23 Leukocyte-derived MPs and EMPs were also demonstrated to induce TF expression by monocytes endothelial cells,10,24,25

The debate remains as to whether TF exposed at the MP surface is a major factor for MP thrombogenicity,26,27 TF encryption/de-encryption being a probable crucial step. A circulating alternatively spliced form of TF was proposed to account for thrombogenicity.28 However, its clinical relevance remains to be established since representing only a small proportion of active blood-borne TF.29

Microparticle–Selectin Interactions in the Growing Thrombus

During the past decade, selectins, MPs, and TF have merged into a determining triad in thrombosis.30,31 P-selectin, an adhesion molecule expressed at platelet and endothelial cell surfaces, is necessary for TF accumulation and leukocyte incorporation into the thrombus after endothelial injury.32 The interactions between P-selectin glycoprotein ligand-1 (PSGL-1) and platelet P-selectin were proved necessary to concentrate TF activity at the thrombus edge. The accumulation of hematopoietic cell-derived TF in the developing thrombus correlates with the kinetics of MPs accumulation before the leukocyte–thrombus interaction.33 In a murine model of hemophilia A, soluble P-selectin (sP-selectin) was demonstrated to promote the shedding of leukocyte-derived TF− MPs, most likely from monocytes, that correct hemostasis.34 MPs plasma levels increase with age, except in PSGL-1−null mice, indicating the contribution of the P-selectin/PSGL-1 pathway. Moreover, in mice engineered to express a
several-fold elevation in sP-selectin plasma concentrations, unusual procoagulant potency and elevated proportions of circulating leukocyte-derived MPs harboring TF were evidenced.35,36 Other mechanisms triggered by P-selectin could contribute to increased thrombotic propensity. P-selectin was shown to favor the transfer of TF sorted from raft into monocyte-derived MPs and delivered as a functional entity to platelets.37 P-selectin also promotes PhtdSer exposure by monocytes and TF expression.7,38

MPs and Stabilization of the Thrombus: Role in Stasis

TF+-MPs recruited through P-selectin interactions may stabilize the thrombus by inducing fibrin formation.39 Because P-selectin–PSGL-1 interactions usually mediate unstable rolling, additional cytoadhesins such as MAC-1, a β integrin on leukocyte TF+-MPs, could contribute to thrombus stabilization.40 EMPs exposing unusually large von Willebrand multimers would promote the formation of platelet aggregates with increased stability.40

The contribution of blood-borne TF to the thrombotic process could be crucial in ischemia or stasis. Stasis would limit the dilution of recruited MPs, blood-borne TF becoming determinant even at sites of limited injury. In engineered mice presenting high levels of leukocyte-derived MPs and submitted to ischemia, P-selectin is upregulated at the endothelium surface suggesting increased MPs recruitment leading to the formation of larger thrombi observed in deep vein.41,42 In patients with venous thromboembolism, a marked activation of endothelium, platelets and leukocytes could be demonstrated through combined measurements of EMPs (harboring TF+-MPs, could contribute to thrombus stabilization.40 EMPs exposing unusually large von Willebrand multimers would promote the formation of platelet aggregates with increased stability.40

The contribution of blood-borne TF to the thrombotic process could be crucial in ischemia or stasis. Stasis would limit the dilution of recruited MPs, blood-borne TF becoming determinant even at sites of limited injury. In engineered mice presenting high levels of leukocyte-derived MPs and submitted to ischemia, P-selectin is upregulated at the endothelium surface suggesting increased MPs recruitment leading to the formation of larger thrombi observed in deep vein.41,42 In patients with venous thromboembolism, a marked activation of endothelium, platelets and leukocytes could be demonstrated through combined measurements of EMPs (harboring TF+-MPs, could contribute to thrombus stabilization.40 EMPs exposing unusually large von Willebrand multimers would promote the formation of platelet aggregates with increased stability.40

Procoagulant MPs in Immune-Mediated Thrombosis

In “immune-mediated acquired thrombophilic disorders” such as the antiphospholipid syndrome and heparin-induced thrombocytopenia, auto-antibodies could contribute to the release of procoagulant MPs. Elevated levels of EMPs are a common characteristic of antiphospholipid syndrome, solely detected in patients with systemic lupus erythematosus presenting anti-phospholipid antibodies.23 EMPs were correlated with the lupus anticoagulant that is strongly associated with thrombotic propensity. Treatment of cultured endothelial cells by plasma samples from antiphospholipid syndrome patients promotes EMPs release, pointing at a possible anti-phospholipid antibodies effect. In contrast, PMPs elevation also reported in systemic lupus erythematosus patients is not related to anti-phospholipid antibodies occurrence or disease evolution.44

Heparin-induced thrombocytopenia is a common cause of drug-related immune-mediated thrombocytopenia known to favor thrombotic diathesis. Heparin-induced thrombocytopenia patients develop antibodies against circulating heparin-platelet factor-4 (PF4) complexes, leading to platelets cross-linking and activation. Released PMPs expose GPIb, GPIIbIIIa, P-selectin, and thrombospondin and were found highly thrombogenic with the potency to trigger the activation of the coagulation system.45 PMPs would additionally bind to the subendothelial matrix, thus promoting the recruitment of additional platelet complexes and the constitution of a PMPs reservoir.46

Procoagulant MPs in Atherothrombosis

Source of MPs Harboring TF Within the Plaque

In acute coronary syndromes, TF triggers the formation of intracoronary thrombi following endothelial injury. The acellular lipid-rich core of an atherosclerotic plaque represents its most thrombogenic part,47 with enhanced TF activity being directly supported by TF+-MP exposure of PhtdSer.4 Apoptotic macrophages constitute the main source of membrane-bound TF.4,48 Smooth muscle cells (SMCs) may also contribute to TF+-MPs accumulation in the lipid core. In vitro, under settings of minimal apoptosis, human SMC release TF+-MPs, whereas TF expression is upregulated by both native or aggregated low-density lipoprotein (LDL), the engagement of the latter with LDL receptor-related protein leading to the expression of cellular TF activity and TF+-MPs release.50 During Fas-mediated SMC apoptosis, TF activity and PhtdSer exposure are highly enhanced, shifting cell and MP membranes toward a procoagulant state.51

Regulation of MP Clearance Within the Plaque

Apoptotic cells and MPs are cleared by phagocytes in order to prevent (tissue) inflammatory responses. This dogma of a clean death was challenged by the demonstration of Fas-mediated activation of several inflammatory genes during apoptosis. Little is known on the clearance of apoptotic bodies within the plaque. Phagocytosis could be blunted or saturated by oxidized LDL, an abundant pro-atherogenic component of LDL cholesterol, able to interfere in the recognition of MPs phosphatidylcholine moities by macrophage scavenger receptors. Indeed, continuous infusion of lysophosphatidylcholine moities was shown to impair apoptotic clearance in mice.52 In turn, apoptotic bodies could promote macrophage apoptosis and enhanced MPs shedding.53 Apoptotic cells recruited to atherosclerotic plaques thus represent a reservoir of highly thrombogenic material, including derived MPs, made accessible to the blood stream in case of spontaneous or mechanical disruption.51

Possible Contribution of Lesion-Bound MPs to Plaque Vulnerability

Several mechanisms involving MPs from the plaque could account for instability, as suggested by in vitro data. MP would mediate the recruitment of inflammatory cells within the plaque. Endothelial-derived MPs released on VEGF or FGF2 stimulation harbor functional matrix metalloproteinases possibly favoring fibrous cap proteolysis.54 In the course of plaque remodeling, MPs of various origin could modulate angiogenesis, a key determinant of plaque vulnerability.55 PMPs, possibly incorporated within the plaque after local engulfment of a thrombus could promote angiogenesis,56 whereas EMPs could enhance oxidative stress leading to apoptosis.57 In addition, PMPs are providers of active NADPH oxidase involved in reactive oxygen species
-mediated endothelial or SMC apoptosis. Furthermore, apoptotic lymphocyte-derived MPs could impede endothelial nitric oxide (NO) synthesis by downregulating NO synthase expression, leading to endothelial apoptosis.

Respective Contribution of Vessel Wall TF and Blood-borne TF to the Growing Thrombus

After erosion or rupture, plaque-bound TF was long considered sufficient to initiate thrombus formation. While certainly accurate at a microscopic level, the role assigned to this sequestered form of TF is not physically realistic at a macroscopic scale considering the rapid growth of a thrombus. The diffusion of procoagulant intermediates would be completed within hours and even more, owing to obstruction by adherent platelets and fibrin deposition on the damaged surface. To decipher the respective contribution of circulating or sequestered TF-MPs in thrombus development, reciprocal bone marrow transplants were performed between wild-type and engineered mice expressing minimal TF. Results suggest that whereas arterial vessel-wall TF is involved in the initiation of platelet activation, blood-borne TF spread by MPs mediates thrombus propagation. Once vessel wall TF is covered by a layer of fibrin and platelets, the plaque might become impermeable to circulating clotting factors. Thus, blood-borne TF would prevail in puncture wounds or venous thrombosis, its contribution being less effective in the presence of high amounts of plaque TF.

The MP Reservoir Contributes to the Amplification of the Thrombotic Response

Because TF-MPs are the main thrombogenic components of the atherosclerotic plaque, spontaneous or mechanical disruption during percutaneous coronary intervention (PCI) could lead to procoagulant MPs release. The exposure of the subendothelium could in turn enhance platelet activation and MP shedding. PCI therefore constitutes a relevant model to understand the contribution of MPs to thrombotic processes. A peak of circulating PMPs was reported 8 hours after PCI, occurring in parallel with the fall of the platelet count. Ex vivo, plaque disruption by scraping led to the release of TF-MPs. Indeed, intracoronary samples retrieved after angioplasty from distal protection devices exhibited TF-MPs. Because circulating white blood cells are unable to upregulate TF before 96 hours after PCI, membrane-bound TF would mainly originate from the ruptured plaque. Indeed, we could evidence a significant raise of procoagulant MPs across the tightest lesions assessed by intravascular ultrasound (unpublished data). Sequestration of TF-MPs within the myocardial microvasculature could contribute to the no-reflow phenomenon. Active membrane-bound TF shed from dissected plaques is elevated in patients presenting profound impairment of myocardial perfusion. Likewise, perfusion of the porcine coronary beds with membrane-bound TF induced no-reflow and positive TF staining in the microvasculature obstructed by fibrin thrombi. In addition to their procoagulant properties, MPs could contribute to the drastic reduction of myocardial perfusion through multiple and intricate pathways: (1) impairment of coronary flow through NO synthase pathway; (2) enhancement of inflammatory response and oxidative stress; and (3) recruitment of leukocytes through PMP rolling. Other amplification loops might be triggered during atherothrombosis. PMPs release has previously been reported under a variety of conditions including collagen or thrombin stimulation, shear or oxidative stress conditions, elevated plasminogen activator inhibitor-1 (PAI-1), P-selectin, or CD40L plasma concentrations.

MPs in the Development of Atherothrombosis

Several studies point at MPs of various origins as effectors of vascular wall inflammation. MPs upregulate cytokine expression in monocytes and endothelium, promote leukocyte–leukocyte aggregation and recruitment through P-selectin. In turn, leukocyte-derived MPs would stimulate inter leukin (IL)-6 and MCP-1 endothelial release and TF expression through a JNK1 signaling pathway. Under conditions of oxidative stress, endothelial-derived MPs contain oxidized phospholipids that promote monocyte–endothelial interactions. MPs are able to upregulate cytoadhesion expression in monocytes and endothelial cells through the delivery of arachidonic acid, thus reinforcing cell adherence. As providers of proinflammatory IL-1β, MPs also contribute to endothelial inflammation. At high shear stress, MPs GPIb and P-selectin–dependent rolling would enable delivery of RANTES, a CC chemokine, to the inflamed endothelium and favor monocyte adhesion. Interestingly, MPs would thus contribute to the atherosclerotic plaque development through P-selectin or RANTES mediation in cells of arterial or microvascular origin but might be ineffective in the inflamed venous bed, as reported in human umbilical vein endothelial cells (HUVECs). Most of the experiments aimed at examining the effects of circulating MPs using in vitro models were carefully performed, with relevant controls. In some circumstances, it cannot be excluded that contamination with pyrogenic material may have led to overestimation of their deleterious role.

Circulating MPs and Vascular Dysfunction

Because endothelial dysfunction and arterial stiffness are major determinants of the cardiovascular risk, several trials investigated the effects of circulating MPs on the vascular function (Table 1). MPs shed under pathological situations exhibit multiple abilities in the dysregulation of vascular tone, whereas MPs from healthy volunteers were ineffective on normal isolated vessels. Indeed, MPs are a source of thromboxane A2, a potent regulator of vascular tone, as shown in rabbit aorta. In addition, MPs from apoptotic T lymphocytes impair endothelium-dependent relaxation, independently of CD11a-CD18 or Fas–FasL pathways. Impairment was linked to endothelial nitric oxide synthetic (eNOS) downregulation and caveolin-1 overexpression. Endothelial dysfunction was also targeted by MPs from rat FasL-mediated apoptotic SMCs that diminished NO production. The effect, mediated by endothelial β3 integrins, was sensitive to abciximab or epifibatide. EMPs from rat renal microvasculature alter endothelium-dependent relaxation and NO production in the aorta, an effect related to superoxide anion production. In patients
with end-stage renal failure, EMPs levels were found correlated with the loss of flow-mediated dilation and increased aortic pulse wave velocity, whereas PMPs were not correlated with vascular dysfunction. EMPs, possibly released on uremic toxins accumulation, were confirmed as reducers of NO release.\textsuperscript{75,82} In patients with coronary artery disease, circulating EMPs appeared a reliable parameter in the identification of high-risk patients.\textsuperscript{79} Endothelial dysfunction or injury but also as effectors able to amplify a pre-existing vascular dysfunction. Conversely, in inflammatory diseases, circulating MPs of lymphocytic origin could promote ex vivo vascular hyporeactivity through the release of vasodilatory toxins accumulation, were confirmed as reducers of NO release.\textsuperscript{75,82} In patients with coronary artery disease, circulating EMPs appeared a reliable parameter in the identification of high-risk patients.\textsuperscript{79} Endothelial dysfunction or injury but also as effectors able to amplify a pre-existing vascular dysfunction. Conversely, in inflammatory diseases, circulating MPs of lymphocytic origin could promote ex vivo vascular hyporeactivity through the release of vasodilatory.

### Angiogenesis and MPs

Whereas angiogenesis can be considered beneficial in providing an alternate supply of blood flow within the ischemic myocardium, it was demonstrated noxious for plaque stability.\textsuperscript{55} PMPs were shown to induce angiogenesis, in vitro and in vivo.\textsuperscript{56,86} Platelet adhesion receptors, eventually delivered by PMPs to hematopoietic stem cells, would favor endothelial homing by promoting chemotaxis, cell adhesion, proliferation, and survival.\textsuperscript{87,88} In rats, locally injected PMPs improved revascularization of the ischemic myocardium, in a VEGF-, PDGF-, or bFGF-dependent process.\textsuperscript{86} Consistent with a VEGF-mediated effect, extracellular signal regulated kinases, PI3-kinase, and Src are involved in the signaling of PMPs-mediated angiogenic response. Borne by PMPs, various regulators of angiogenesis, among which TF could also contribute to the signaling pathway by direct vectorization from MPs to the target cell. Sphingomyelin, a pro-angiogenic lipid component, is sorted in tumor-released MPs. By contrast, other constituent phospholipids of MPs such as PhtdSer showed little effect on endothelial migration.\textsuperscript{89}

MPs cellular origin, concentration, and sequential recruitment might determine the magnitude of the angiogenic signal (Table 2). For instance, MPs isolated from HUVECs and applied at low concentrations promoted angiogenesis, whereas high concentrations produced an opposite response.\textsuperscript{54} At physiological concentrations, EMPs showed no pro-angiogenic effect whereas pathological concentrations (\(\approx 100\)-fold enhancement) impaired angiogenesis and enhanced the apoptotic rate.\textsuperscript{57} In human and rodent endothelial cells, the oxidative stress induced by EMPs appeared as a key actor in the balance governing angiogenesis and apoptosis.\textsuperscript{57,78} Future pharmacological strategies aimed at modulating circulating EMP levels or composition could prove useful in pathological issues where abnormalities of neovascularization prevail.

### Table 1. Effects of Microparticles on Vascular Tone

<table>
<thead>
<tr>
<th>Cellular Origin of MPs</th>
<th>Inducer or Pathological Issue</th>
<th>Vascular Effect</th>
<th>Mechanisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>Smooth muscle cells</td>
<td>Enhancement of arachidonic acid-induced contraction (aorta); enhancement of metacholine–choline contraction (pulmonary arteries)</td>
<td>Transfer of thromboxane A2</td>
<td>80</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td>Fas-mediated apoptosis</td>
<td>Impairment of endothelium-dependent relaxation</td>
<td>Uncharacterized</td>
<td>79</td>
</tr>
<tr>
<td>T-lymphocytes</td>
<td>Diabetes</td>
<td>Impairment of endothelium-dependent relaxation (conductance and small resistance arteries)</td>
<td>Decreased endothelial expression of NO synthase (eNOS), COX-1 unaffected; CD11a/CD18, Fas/FasL pathway not involved</td>
<td>59</td>
</tr>
<tr>
<td>Endothelial, platelets, erythrocytes</td>
<td>End-stage renal failure</td>
<td>Impairment of endothelium-dependent relaxation</td>
<td>Decreased cGMP production; effects appeared mainly mediated by EMPs</td>
<td>75</td>
</tr>
<tr>
<td>T-lymphocytes, granulocytes</td>
<td>Myocardial infarction</td>
<td>Endothelial dysfunction</td>
<td>Decreased endothelial expression of NO synthase (eNOS), COX-1 unaffected; CD11a/CD18, Fas/FasL pathway not involved</td>
<td>76</td>
</tr>
<tr>
<td>T-lymphocytes</td>
<td>Actinomycin and diabetes</td>
<td>Vascular hyporeactivity in response to vasoconstrictors agents (mouse aorta)</td>
<td>Production of NO and PGI2; upregulation of iNOS and COX-2; NFkB-dependent Fas/FasL mediation</td>
<td>85</td>
</tr>
</tbody>
</table>
TABLE 2. Effects of Microparticles on Angiogenesis and Vascular Remodeling

<table>
<thead>
<tr>
<th>Cellular Origin of MPs</th>
<th>Inducer or Pathological Issue</th>
<th>Vascular Effect</th>
<th>Mechanisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet</td>
<td>Thrombin-released PMPs (healthy donor)</td>
<td>Neovascularization in the ischemic myocardium</td>
<td>VEGF, PDGF, bFGF</td>
<td>86</td>
</tr>
<tr>
<td>Platelet</td>
<td>Thrombin-released PMPs (healthy donor)</td>
<td>Angiogenesis</td>
<td>ERK, phosphoinositide 3-kinase pathways</td>
<td>89</td>
</tr>
<tr>
<td>Thrombin-released PMPs</td>
<td>Serum deprivation</td>
<td>Recruitment of hematopoietic stem cells</td>
<td></td>
<td>87, 88</td>
</tr>
<tr>
<td>Endothelium</td>
<td>Sepsis</td>
<td>Endothelial and SMC apoptosis</td>
<td>Oxidative stress, NADPH oxidase</td>
<td>58</td>
</tr>
<tr>
<td>Endothelium</td>
<td>Serum deprivation</td>
<td>Angiogenesis induction (low-dose); angiogenesis inhibition (high-dose)</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>Endothelium</td>
<td>Serum deprivation</td>
<td>No effect on angiogenesis (physiological concentrations); angiogenesis impairment (pathophysiologic concentrations)</td>
<td>Oxidative stress</td>
<td>57</td>
</tr>
<tr>
<td>Tumor cells</td>
<td>VEGF, FGF, serum deprivation</td>
<td>Proteolysis, basement membrane invasion</td>
<td>MMP</td>
<td>54</td>
</tr>
</tbody>
</table>

Circulating MPs: Effectors in the Tuning of Thrombotic Propensity Associated With Cardiovascular Risk

At each stage of their life cycle, vascular cells are subjected to a variety of stimulations leading to MPs release. In vascular dysfunction, quantitative or qualitative variation of circulating MPs can therefore be expected to tune the biological signal they disseminate. Although MP may play a very important role in several pathophysiologic conditions, some reservations should be made, particularly in in vitro studies using endothelial cell culture, which probably does not reflect what is actually taking place on the endothelium of the whole vessel with its media sublayer. Furthermore, the endothelial response may vary with the vascular territory or cell lineage.

Healthy Individuals

In the peripheral blood of healthy subjects, low levels of MPs are detected. A typical Western meal is nevertheless able to increase blood-borne TF and circulating levels of EMPs, the latter being tightly correlated with triglyceride levels. Mean levels of circulating MPs appear stable in adulthood, whereas a 2-fold elevation is characteristic of childhood. This observation does not weaken MPs as reliable markers of the thrombotic risk but rather testifies to massive cell and tissue remodeling in child development. In young healthy men, subclinical inflammation assessed by IL-6 plasma levels was associated to circulating CD31-RMPs as a possible result of increased endothelial apoptosis.

Diabetes Mellitus

In diabetes, a wide panel of blood or vascular cells, including platelets, endothelial cells, monocytes, and islets of Langerhans, release MPs. Shedding is triggered by a variety of cytokines or stimuli, such as tumor necrosis factor-α, IL-1β, soluble CD40L, advanced glycation end products, oxidative stress, and hyperglycemia. MPs phenotype and procoagulant potential may vary according to the type of diabetes or glycemic control. Indeed, high procoagulant activity borne by circulating MPs was detected in patients with poorly controlled HbA1c levels. EMPs and PMPs appear particularly elevated in type 1 patients, the highest EMPs levels being detected in patients with microvascular complications. In type 2 diabetes, high levels of monocyte- or platelet-derived MPs were reported indicative of nephropathy and retinopathy. During acute myocardial infarction, the raise in platelet-, endothelial-, and monocyte-derived MPs contributes to enhanced thrombogenicity, regardless of the diabetes type.

Hypertension

Platelets and endothelial cells become activated during hypertension and are possible actors in the development of a thrombotic response through the release of procoagulant MPs. High levels of circulating MPs, from platelet, monocyte, or endothelial lineages, were observed in patients with hypertension, EMP being features of both systolic and diastolic pressures. Apart from neurohormonal stimulation, physiological (230 sec⁻¹) or supra physiological shear rates (1500 to 10 800 sec⁻¹) would per se promote platelet activation and the release of MPs able to induce a proadhesive phenotype on target endothelial cells. Altogether, MPs could be viewed pathogenic effectors of organ injury in severe hypertension.

Dyslipidemia

Altered MPs patterns have been reported in patients with hyperlipidemia. On oxidation or aggregation, LDL were demonstrated potent inducers of membrane blebbing. Differences in plasma selectins, platelet-, monocyte-, and/or endothelial-derived MPs were pointed at in hyperlipemic patients, the highest values being observed in those combining 3 major cardiovascular risk factors (hypertension, diabetes mellitus, hyperlipemia).

MPs: A Marker of Vascular Damage

In asymptomatic subjects, leukocyte-derived MPs were recently demonstrated an independent marker of preclinical carotid atherosclerosis, still informative after adjustment for Framingham risk, waist circumference, high-sensitivity C-reactive protein. First results in myocardial infarction were recently confirmed in a larger multicentric international study reporting procoagulant MPs of better value in mortality prognosis than usual biological markers. It
is therefore tempting to consider circulating MPs relevant indicators of the overall vascular status in the assessment of the individual atherothrombotic risk.

**Pharmacological Modulation of Circulating MPs**

The noxious potential of some categories of MPs offers new pharmacological perspectives. Several therapies known to be beneficial in cardiovascular disorders were reported to reduce both MPs concentration and procoagulant activity (Table 3). These observations support the hypothesis that part of the beneficial effect is linked to decreased MPs pathogenicity, at quantitative or qualitative level.

**Statins**

Several observations suggest that MPs can be targeted by statin treatment. In vitro, fluvastatin was reported to inhibit EMPs shedding, partly through Rho small GTPases, key regulators in cytoskeleton remodeling. In vivo, GPIIbIIIa sorting was diminished in PMPs from patients with type 2 diabetes mellitus treated by pravastatin. Circulating monocyte-derived MPs were reduced in hypertensive patients treated by a combination of losartan and simvastatin.

**GPIIbIIIa Antagonists and Other Anti-platelet Treatments**

Various anti-platelet treatments, such as GPIIbIIIa antagonists and thienopyridines, lower circulating PMP levels. Within a given pharmacological class, the potency may vary. For instance, the specific inhibition of PMPs release by abciximab was reported higher than by eptifibatide, whereas the reduction of shear stress-induced aggregation was found similar. Specific inhibition of P-selectin proteolytic shedding at high shear stress or sensitivity to steric hindrance because of abciximab binding were suggested as possible mechanisms. The pharmacological reduction of circulating PMPs was associated to diminished leukocyte, PMPs, and reduced release of soluble cytoadhesins vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, suggesting a weakened transcellular activation.

**Other Candidates in the Pharmacological Control of MP Release**

Several signaling pathways are associated with membrane shedding and are therefore possible targets for a pharmacological control. Oxidative stress, cytokines, and neurohormonal stimulation (angiotensin II, catecholamines) promote MPs release. A more ubiquitous target would be any specific or occasional PhtdSer membrane translocator such as ABCA1, modulating membrane remodeling, and subsequent blebbing and MPs shedding.

**MPs in the Treatment of Bleeding Disorders**

In bleeding disorders, the procoagulant potential spread by MPs could be of therapeutic benefit. In hemophilic mice, bleeding correction was achieved by procoagulant leukocyte-derived MPs generated through soluble P-selectin infusion. In hemophiliacs, part of the procoagulant potential of recombinant FVIIa could rely on the endogenous generation of

### Table 3. Pharmacological Modulation of Circulating Levels of Microparticles According to the Clinical Background

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cellular Origin of MPs</th>
<th>Effect on MP Level</th>
<th>Pathological Context</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-platelet Ticlopidine</td>
<td>Platelets, monocytes</td>
<td>Diabetes, dyslipidemia</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>Platelets</td>
<td>Peripheral vascular disease, diabetes</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>Abciximab</td>
<td>Platelets, leukocytes</td>
<td>Heart failure</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>Anti-oxidants Vitamin C</td>
<td>Platelets, endothelium</td>
<td>Heart failure, myocardial infarction</td>
<td>67, 122</td>
<td></td>
</tr>
<tr>
<td>Statins Simvastatine (with losartan)</td>
<td>Monocytes</td>
<td>Hyperlipemia + diabetes mellitus</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>Pravastatin</td>
<td>Platelets (GPIIa)</td>
<td>Type 2 diabetes</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Fibrates Bezafibrate</td>
<td>Platelets</td>
<td>Hyperlipemia</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Beta-blockers Carvedilol</td>
<td>Endothelium</td>
<td>Heart failure</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>AT II receptor Losartan</td>
<td>Monocytes</td>
<td>Hypertension, diabetes mellitus</td>
<td>103, 113</td>
<td></td>
</tr>
<tr>
<td>inhibitors Valsartan</td>
<td>Monocytes, endothelium</td>
<td>Diabetes mellitus (ineffective in patients without diabetes)</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>Eprosartan</td>
<td>Platelets</td>
<td>Hypertension</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Ca2+ channel Efonidipine</td>
<td>Platelets, monocytes</td>
<td>Hypertension, diabetes mellitus</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>inhibitors Nifedipine</td>
<td>Platelets, monocytes</td>
<td>Hypertension, diabetes mellitus</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Benidipine</td>
<td>Platelets, endothelium, monocytes</td>
<td>Hypertension, diabetes mellitus</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>Serotonin antagonist Sarpagrelate hydrochloride</td>
<td>Platelets</td>
<td>Type 2 diabetes</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Digitalis Digoxin</td>
<td>Platelets, endothelium</td>
<td>Atrial fibrillation</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>Prostaglandins Iloprost (with nitric oxide gas)</td>
<td>Platelets</td>
<td>Cardiopulmonary bypass</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Anti-TNF antibody Infliximab</td>
<td>uncharacterized</td>
<td>Bowel inflammatory disease</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>rF VIIa</td>
<td>Platelets</td>
<td>Hemophilia</td>
<td>119</td>
<td></td>
</tr>
</tbody>
</table>
procoagulant MPs.\textsuperscript{119} Uprogulation of endogenous reservoirs of procoagulant MPs could also provide new insights in the treatment of acquired (thrombocytopenia) or congenital bleeding disorders.

**Conclusion**

MPs constitute a reservoir of bioactive vascular effectors involved in thrombotic responses, vascular wall inflammation, and remodeling. Circulating procoagulant MPs appear relevant indicators of the overall vascular status enabling the assessment of the individual atherothrombotic risk. The pharmacological control of MPs release can be viewed the next promising challenge in the restoration of vascular homeostasis.

**Acknowledgments**

We are indebted to Janet M. Thompson for careful reading of the manuscript.

**Sources of Funding**

This work was partly supported by a fellowship from OPAL-Atherothrombose awarded to O.M., by a fellowship from the Fondation pour la Recherche Medicale awarded to B. Bakouboula, and institutional grants from the Institut National de la Sante et de la Recherche Medicale, the Universite Paris-Sud 11, The Universite du Mediterranee, and the Agence Nationale pour la Recherche (ANR-05-PCOD-24-01).

**Disclosures**

None.

**References**


Procoagulant Microparticles: Disrupting the Vascular Homeostasis Equation?
Olivier Morel, Florence Toti, Bénédicte Hugel, Babé Bakouboula, Laurence Camoin-Jau, Françoise Dignat-George and Jean-Marie Freyssinet

Arterioscler Thromb Vasc Biol. 2006;26:2594-2604; originally published online September 21, 2006;
doi: 10.1161/01.ATV.0000246775.14471.26
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/26/12/2594

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/