Leukocyte-Derived Microparticles in Vascular Homeostasis

Anne Angelillo-Scherrer

Abstract: Leukocyte-derived microparticles (LMPs) may originate from neutrophils, monocytes/macrophages, and lymphocytes. They express markers from their parental cells and harbor membrane and cytoplasmic proteins as well as bioactive lipids implicated in a variety of mechanisms, maintaining or disrupting vascular homeostasis. When they carry tissue factor or coagulation inhibitors, they participate in hemostasis and pathological thrombosis. Both proinflammatory and anti-inflammatory processes can be affected by LMPs, thus ensuring an appropriate inflammatory response. LMPs also play a dual role in the endothelium by either improving the endothelial function or inducing an endothelial dysfunction. LMPs are implicated in all stages of atherosclerosis. They circulate at a high level in the bloodstream of patients with high atherothrombotic risk, such as smokers, diabetics, and subjects with obstructive sleep apnea, where their prolonged contact with the vessel wall may contribute to its overall deterioration. Numbering microparticles, including LMPs, might be useful in predicting cardiovascular events. LMPs modify the endothelial function and promote the recruitment of inflammatory cells in the vascular wall, necessary processes for the progression of the atherosclerotic lesion. In addition, LMPs favor the neovascularization within the vulnerable plaque and, in the ruptured plaque, they take part in coagulation and platelet activation. Finally, LMPs participate in angiogenesis. They might represent a novel therapeutic tool to reset the angiogenic switch in pathologies with altered angiogenesis. Additional studies are needed to further investigate the role of LMPs in cardiovascular diseases. However, large-scale studies are currently difficult to set up because microparticle measurement still requires elaborate techniques which lack standardization. (Circ Res. 2012;110:356-369.)

Key Words: microparticle ■ leukocyte ■ vascular homeostasis ■ cardiovascular disease

Microparticles (MPs) are released by budding from the cell membrane surface, a process called “ectocytosis,”1,2 which can occur either spontaneously or in response to various stimuli. They are characterized by their size (diameter: 0.1–1 μm), the presence of negatively charged phospholipids at their surface, and an antigenic profile pointing to their cellular origin.2–7 Various body fluids including blood,4,6,8 urine,9 pleural fluid,10 ascites,11 synovial fluid,12 and saliva13 contain MPs. In addition, MPs may derive from the atherosclerotic plaque.

Circulating MPs in blood originate from different cells such as red blood cells, leukocytes, platelets, and endothelial...
cells (ECs). Their blood level results from the balance between their rate of release from the cell surface and their clearance from the circulation. Studies of mouse models suggest that clearance of circulating MPs occurs in the spleen. In healthy subjects, more than 80% of circulating MPs are produced by the bone marrow, where they mature in response to cytokines at an extremely high rate to supply the baseline needs of circulating cells, surviving only few hours in the peripheral blood. Neutrophils migrate from the circulating blood to sites of tissue inflammation in response to chemotactic cytokines at an extremely high rate to supply the baseline needs of circulating cells, surviving only few hours in the peripheral blood. Neutrophils migrate from the circulating blood to sites of tissue inflammation in response to chemotactic signals and thereby promote inflammation. When activated, neutrophils degranulate during a process called exocytosis and release MPs from the cell surface by ectocytosis. Moreover, they are vectors of biological information exchange between cells, a process called intercellular communication (reviewed in Mause and Weber).

Changes in the number and cellular origin of MPs, and in the composition of their population in circulating blood, may be due to some physiological or pathological conditions, including cardiovascular diseases. Ectocytosis can also take place within tissues, resulting in localized accumulation of MPs. MPs are not only markers of cell activation or damage; they interfere with major pathophysiological processes such as hemostasis, inflammation, cell survival and apoptosis, endothelial function, vascular remodeling, and angiogenesis. Moreover, they are vectors of biological information exchange between cells, a process called intercellular communication (reviewed in Mause and Weber).

This review will focus on the implication of leukocyte-derived MPs (LMPs) in vascular homeostasis. Special attention will be made to their clinical relevance in cardiovascular diseases (CVD).

### Leukocyte-Derived Microparticles and the Cell From Which They Have Originated

LMPs may originate from neutrophils, monocytes and B- and T-lymphocytes. They carry markers from their parental cells (Table 1) and harbor membrane and cytoplasmic proteins as well as bioactive lipids implicated in a variety of fundamental processes (Table 2 and Figure 1).

### Neutrophils and Neutrophil-Derived MPs

Neutrophils represent the most abundant population of the leukocytes circulating in human blood (40% to 75%). They are produced by the bone marrow, where they mature in response to a variety of cytokines at an extremely high rate to supply the baseline needs of circulating cells, surviving only few hours in the peripheral blood. Neutrophils migrate from the circulating blood to sites of tissue inflammation in response to chemotactic signals and thereby promote inflammation. When activated, neutrophils degranulate during a process called exocytosis and release MPs from the cell surface by ectocytosis.

Neutrophil-derived MPs (NMPs) expose phosphatidylserine on their outer membrane leaflet, activate the classic pathway of complement, and fix C4 and C3 fragments. Opsonized NMPs bind in turn to red blood cells via the complement receptor 1. This might play a role in their sequestration and clearance and might affect their biological activities. NMPs behave as inflammatory mediators in activating ECs and L-selectin expression on their surface might be essential for their adhesion.

In contrast, they also exert anti-inflammatory effects on macrophages and, when carrying annexin A1, inhibit the interaction between neutrophils and ECs. NMPs contain αvβ3 (macrophage antigen-1, Mac-1) in a functionally active conformation which can mediate their interaction with resting platelets. As a consequence, platelets become activated, increase P-selectin expression, and perpetuate thrombus formation. In addition, Mac-1+ NMPs interact with urokinase, plasminogen and metalloproteinase-2 and -5, suggesting a role in fibrinolysis and tissue remodeling.

### Table 1. Markers for Leukocyte-Derived Microparticles

<table>
<thead>
<tr>
<th>Cellular Origin of Microparticles</th>
<th>Marker</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte</td>
<td>CD45*</td>
<td>74, 153, 167, 168</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>CD15</td>
<td>168, 169</td>
</tr>
<tr>
<td></td>
<td>CD64</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>CD66b</td>
<td>74, 116, 167</td>
</tr>
<tr>
<td></td>
<td>CD66e</td>
<td>170</td>
</tr>
<tr>
<td>Monocyte</td>
<td>CD14</td>
<td>63, 74, 116, 167, 168, 170, 171</td>
</tr>
<tr>
<td></td>
<td>CD11a</td>
<td>126, 172</td>
</tr>
<tr>
<td></td>
<td>CD18</td>
<td>49</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>CD2</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>CD3</td>
<td>63, 65</td>
</tr>
<tr>
<td></td>
<td>CD4</td>
<td>74, 116, 167, 170</td>
</tr>
<tr>
<td></td>
<td>CD8</td>
<td>74, 167, 170</td>
</tr>
<tr>
<td></td>
<td>CD19</td>
<td>63, 168</td>
</tr>
<tr>
<td></td>
<td>CD20</td>
<td>74, 167, 170</td>
</tr>
</tbody>
</table>

*Studies on the coexpression of CD45 and other leukocyte antigens on microparticles reveal that CD45 is not expressed on 100% of leukocyte-derived microparticles.
dase or proteinase 3 might deliver focused antimicrobial activity and NMPs conveying elastase, metalloproteinase-9 or proteinase 3 might promote local tissue destruction. The number of NMPs augments during strenuous physical exercise probably reflecting granulocyte activation.

Table 2. Membrane and Cytoplasmic Proteins as Well as Bioactive Lipids Harbored by Leukocyte-Derived Microparticles and Their Implication in a Variety of Fundamental Processes

<table>
<thead>
<tr>
<th>Proteins/Lipids</th>
<th>Cellular Origin</th>
<th>Processes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>αβ₂ (Mac-1, CD11b/CD18)</td>
<td>Neutrophil</td>
<td>Platelet activation (via GPIbα); interaction with urokinase, plasminogen, and metalloprotease-2 and -5, suggesting a role in fibrinolysis and tissue remodeling</td>
<td>35–38</td>
</tr>
<tr>
<td>Annexin A1</td>
<td>Neutrophil</td>
<td>Inhibition of the interaction neutrophil-endothelial cell; confers anti-inflammatory properties to NMPs</td>
<td>33, 34</td>
</tr>
<tr>
<td>Annexin III</td>
<td>Neutrophil</td>
<td>Might play a role in ecotocytosis</td>
<td>27</td>
</tr>
<tr>
<td>C4, C3 fragments, CD35/CR1</td>
<td>Neutrophil</td>
<td>NMPs activate the classic pathway of complement and fix C4 and C3 fragments; the opsonized ecotosomes then bind to red blood cells via CD35/CR1</td>
<td>29</td>
</tr>
<tr>
<td>CCR5</td>
<td>PBMN</td>
<td>Transfer of CCR5 to PBMN, monocyte, T-lymphocyte; HIV infection</td>
<td>173</td>
</tr>
<tr>
<td>CD15</td>
<td>Monocyte</td>
<td>Binding to P-selectin on activated platelets; transfer of tissue factor to platelets</td>
<td>51</td>
</tr>
<tr>
<td>CD40 ligand</td>
<td>Macrophage from atherosclerotic plaque</td>
<td>Stimulation of endothelial cell proliferation after CD40 ligation; promotion of angiogenesis; lymphocyte activation within atherosclerotic lesions</td>
<td>59</td>
</tr>
<tr>
<td>Endothelial cell protein C receptor</td>
<td>Monocyte</td>
<td>Activation of anticoagulant protein C by the thrombin-thrombomodulin complex</td>
<td>46, 47</td>
</tr>
<tr>
<td>Fas ligand</td>
<td>T-lymphocyte</td>
<td>Interaction with smooth muscle cells through the Fas-Fas ligand pathway; NFκB activation, upregulation of NO synthase, and COX2 expression</td>
<td>66</td>
</tr>
<tr>
<td>HLA class I and II</td>
<td>Macrophage from atherosclerotic plaque</td>
<td>Lymphocyte activation within atherosclerotic lesions</td>
<td>60</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Macrophage from atherosclerotic plaque</td>
<td>Transfer of ICAM-1 to endothelial cells to recruit inflammatory cells</td>
<td>120</td>
</tr>
<tr>
<td>L-selectin</td>
<td>Neutrophil</td>
<td>Might be essential for NMP adhesion</td>
<td>27</td>
</tr>
<tr>
<td>P-selectin glycoprotein ligand-1</td>
<td>Monocyte</td>
<td>Docking onto activated platelets and endothelial cells by binding to P-selectin, leading to accumulation of TF and thereby to fibrin deposition</td>
<td>36, 57, 58</td>
</tr>
<tr>
<td>Sonic hedgehog</td>
<td>T-lymphocyte</td>
<td>Repair of endothelial injury; angiogenesis; induction of NO production</td>
<td>67, 84, 161</td>
</tr>
<tr>
<td>Tissue factor pathway inhibitor</td>
<td>Monocyte</td>
<td>Procoagulant activity; transfer of tissue factor to activated platelet for coagulation initiation</td>
<td>36, 45</td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>Monocyte</td>
<td>Anticoagulant activity</td>
<td>45</td>
</tr>
<tr>
<td>Activated protein C</td>
<td>Monocyte</td>
<td>Anticoagulant activity</td>
<td>45</td>
</tr>
<tr>
<td>Caspase 1</td>
<td>Monocyte</td>
<td>Transfer of caspase 1 to smooth muscle cells; delivery of a cell death message</td>
<td>61</td>
</tr>
<tr>
<td>Elastase</td>
<td>Neutrophil</td>
<td>Focused antimicrobial activity; matrix degradation (local tissue destruction)</td>
<td>27</td>
</tr>
<tr>
<td>Metalloproteinase-9</td>
<td>Neutrophil</td>
<td>Matrix degradation (local tissue destruction)</td>
<td>27</td>
</tr>
<tr>
<td>Myeloperoxydase</td>
<td>Neutrophil</td>
<td>Focused antimicrobial activity</td>
<td>27</td>
</tr>
<tr>
<td>Proteinase 3</td>
<td>Neutrophil</td>
<td>Focused antimicrobial activity; matrix degradation (local tissue destruction)</td>
<td>27</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>Neutrophil</td>
<td>Mediation of immunosuppressive/anti-inflammatory functions; signal for phagocytosis</td>
<td>27, 32</td>
</tr>
</tbody>
</table>

Mac1 indicates macrophage antigen-1; GPIbα, glycoprotein Ibα; NMP, neutrophil-derived microparticle; CR1, complement receptor 1; CCR5, c-c chemokine receptor type 5; PBMN, peripheral blood mononuclear cell; NFκB, nuclear factor kappa B; NO, nitric oxide; COX2, cyclooxygenase 2; HLA, human leukocyte antigen; and ICAM-1, intercellular adhesion molecule-1.

**Monocytes and Monocyte-Derived MPs**

Monocytes arise in the bone marrow from progenitor cells and enter the circulation via chemokine receptor 2, where they circulate for a few days, representing 2–8% of blood leukocytes. When circulating in blood, they may participate in the immune...
response or extravasate and accumulate in reservoir sites where they are eliminated through efferocytosis in absence of differentiation signal. Experimental studies suggest that progenitor cells circulate in blood and differentiate in extramedullar tissues. Moreover, macrophages, which are highly heterogeneous cells, can arise in tissues without a monocyte intermediate (reviewed by Swirski). For instance, the arterial wall contains numerous resident macrophages. Monocytes are important for all stages of atherosclerosis. However, the role of monocyte subsets in various stages of the disease, the mechanism of their recruitment and migration, their expression profiles, and their interaction with other...
cells within the atherosclerotic plaque are not completely known.44

Monocyte-derived MPs (MonoMPs) purified from monocyte suspension display coexisting tissue factor (TF), and activated protein C (APC) and thrombomodulin anticoagulant activity at their surface45 (Figure 2). MonoMPs also express the endothelial cell protein C receptor (EPCR), thereby promoting the activation of anticoagulant protein C by the thrombin-thrombomodulin complex46,47 (Figure 2). After exposure to endotoxin, there is an early increase in MonoMPs associating TF procoagulant activity.16 In this condition, TF predominates on MonoMPs over thrombomodulin.45 MP:s derived from endotoxin-stimulated monocytes also express TF pathway inhibitor (TFPI).48 TF+/MonoMPs constitute the second largest pool of thrombogenic MPs after platelet-derived MP:s (PMPs).49–51

TF+ MonoMPs can interact with neutrophils,52 transferring to them a procoagulant activity. Indeed, neutrophils are capable of fusion with MPs derived from other cell types.53–56 MonoMPs from THP-1 cells express CD15 which mediates binding to P-selectin on activated platelets.51 MonoMPs are captured by activated platelets within thrombi by a P-selectin/P-selectin glycoprotein 1 (PSGL-1)-dependent mechanism that leads to accumulation of TF and ultimately enhances fibrin deposition.57,58 They are also enriched in TF and PSGL-1, which would allow docking onto activated platelets and ECs by binding to P-selectin.36

MonoMPs increase endothelial thrombogenicity and apoptosis. They also induce tubule formation, which may indicate their angiogenic effect.49

MP:s derived from macrophages located within the atherosclerotic plaque express CD40 ligand (CD40L) and stimulate EC proliferation after CD40 ligation. They also promote lymphocyte activation and angiogenesis within the plaque.59 Lymphocyte activation is also favored by the expression of HLA I and II by plaque MonoMPs.60 MonoMPs transfer caspase 1, a cell death message to smooth muscle cells.61

Lymphocytes and Lymphocyte-Derived Microparticles
Pluripotent stem cells from the bone marrow give rise to lymphoid progenitor cells that, in turn, give origin to B-, T-, and NK/T-lymphocytes. T-lymphocyte progenitors migrate to the thymus, where selection and maturation processes occur. Mature T-lymphocytes then leave the thymus to enter the peripheral blood. B-lymphocyte progenitors differentiate in B-lymphocytes in the bone marrow and enter the peripheral blood when they are mature, and then migrate to the secondary lymphoid organs. NK/T-lymphocytes appear to originate from the same precursor as conventional T-lymphocytes and also differentiate in the thymus.62 Lymphocytes represent 25–40% of blood leukocytes.

B-lymphocyte-derived MP:s are elevated in the circulation of patients with autoimmunity63 together with LMP:s of other origin (MonoMPs and T-lymphocyte-derived MP:s, TMP:s). TMP:s can activate monocytes to produce cytokines.64 TMP:s bind monocytes but not T-lymphocytes. Interaction of TMP:s with monocytes has been inhibited by high-density lipoproteins (HDL). HDL may inhibit cytokine production in human monocytes by interfering with the binding of activating factors at the surface of stimulated T-lymphocytes to receptors at the surface of monocytes.65 TMP:s carrying Fas ligand (FasL) on their surface interact with smooth muscle cells through the Fas-FasL pathway, activating nuclear factor kappa B (NFκB) and upregulating nitric oxide (NO) synthase and cyclooxygenase-2 expression.66 TMP:s carrying sonic hedgehog (Shh) promote angiogenesis and induce NO production.57,66 Activated T-lymphocytes generate TMP:s that induce degranulation and cytokine release from human mast cells. TMP:s convey mast cell-activating factors similar to cells from which they originate.69

Leukocyte-Derived Microparticles in Inflammation
LMP:s can affect both proinflammatory and anti-inflammatory processes, thus ensuring an appropriate inflammatory response.
LMPs in Proinflammatory Processes
LMPs stimulate the expression of proinflammatory genes in ECs, leading to the production of cytokines and leukocyte-endothelial cell adhesion molecules in vitro.31

Endotoxin, a Toll-like receptor 4 ligand, or inosine-cytosine polynucleotide, a Toll-like receptor 3 ligand, promote the release of MPs from murine macrophages, which correlates with NO production.70 NO plays a central role in organ dysfunction associated with sepsis.71 The increase of endogenous NO production is caused by enhanced expression of the inducible form of NO synthase, at least in part in the vessel wall.72

Volunteers exposed to endotoxin exhibit an early increase in circulating TF+ MPs, which are mainly CD14+ MonoMPs.16 This provokes a procoagulant state that is intensified by the exhaustion and/or dampened activation of the 2 natural anticoagulants, antithrombin, and APC. Septic patients display higher circulating level of MPs from nonactivated and activated platelets, ECs, and activated leukocytes (CD62L+) MPs may constitute a link between inflammation and thrombosis observed in sepsis.73 Patients with meningococcal sepsis have elevated circulating TF+ MPs. The plasma of a patient with a fulminating disease course and severe disseminated intravascular coagulation contained TF+ MonoMPs (CD14+), which promoted very high thrombin generation.74 Similarly, elevated TF+ MonoMPs were found in the bloodstream of primates with Ebola fever.75 These data indicate a potential role for TF+ MonoMPs in disseminated intravascular coagulation associated with severe infections.

These observations were completed by experimental data where mice received intravenous injection of MPs previously isolated from patients with septic shock. Infused MPs in mice exert different effects on target tissues with regard to the expression of proinflammatory proteins related to nitrative stresses. Thus, MPs appear to play a role in organ dysfunction observed in sepsis.76

Endothelium-derived microparticles (EMP)s and MonoMPs upregulate podocyte production of proinflammatory mediators, but only MonoMPs upregulate podocyte secretion of vascular endothelial growth factor (VEGF), a regulator of glomerular permeability. Thus, MPs including LMPs alter endocytic functions of podocytes and induce secretion of proinflammatory cytokines, potentially leading to glomerular inflammation and the development of proteinuria.77

LMPs in Anti-Inflammatory Processes
LMPs may also play an anti-inflammatory role. NMPs contain the functionally active anti-inflammatory protein annexin 1. Annexin 1– containing NMPs inhibit the interaction between leukocytes and endothelial cells in vitro and in an animal model in vivo.54 This effect could potentially dampen neutrophil recruitment in the late phase of the innate response.

MPs of nonplatelet origin released during sepsis confer a protection against vascular hyporeactivity, which accounts for hypotension in patients with septic shock.73 Furthermore, patients with severe sepsis and lower levels of PMPs, EMPs, and LMPs display a higher mortality rate and a more prominent organ dysfunction,78 in association with excessive NO production.

APC induces the formation and release of EPCR+ EMPs and MonoMPs.47 A recent study has shown that treatment of septic patients with recombinant APC increases CD13+ EPCR+ MPs. CD13 is a marker for ECs but also for leukocytes of myeloid origin. CD13+ MPs were higher in patients treated with recombinant APC who survived than in those who did not survive. APC has antiapoptotic and anti-inflammatory effects through the activation of the endothelial protease-activated receptor 1. These effects could be disseminated by circulating MPs even at distal vascular sites.79

Leukocyte-Derived Microparticles and Vascular Function
MPs from various cellular origins, including LMPs, induce endothelial dysfunction, especially by altering the balance between NO and reactive oxygen species production and release.3,31,80,81 TMPs induce endothelial dysfunction in both conductance and resistance arteries by alteration of NO and prostacyclin pathways. Endothelial NO synthase and caveolin-1 expression are regulated by these TMPs.82 TMPs decrease NO production and increase oxidative stress in ECs.80 These effects are associated with a reduction of endothelial NO synthase activity, which depends on phosphatidylinositol-3-kinase (PI3K), extracellular signal–regulated kinase ½ (ERK1/2), and NfκB pathways. The increase of reactive oxygen species production, downregulated by the PI3K pathway, involves xanthine oxidase and NFκB pathways. In addition, exposure to TMPs results in increased caveolin-1 expression as previously described,82 but decreases its phosphorylation; these effects are independent of PI3K and ERK1/2.80 Thus, NO bioavailability is reduced during TMPs treatment and can account for their capacity to promote endothelial dysfunction. The in vivo role of TMPs was investigated in mice. TMPs injected in them decreased acetylcholine-evoked endothelial relaxation of precontracted aorta.80

In sharp contrast, TMPs carrying the morphogen Shh induces NO production directly by the Shh pathway which involves PI3K and Akt.67 Hedgehog proteins are diffusible morphogens that can be membrane anchored. They play an essential role during development and, in adults, they participate in cell differentiation, proliferation and angiogenesis.83 TMPsShh+ correct endothelial injury by promoting the release of NO from ECs.57 In a model of ischemia/reperfusion in mice, TMPsShh+ enhance NO-mediated relaxation of mouse coronary arteries in response to acetylcholine. This is accompanied by an increase of NO production in tissues and blood after ischemia/reperfusion.84

In human subjects, NO-associated vasodilation after shear stress can be assessed by ultrasonography of the brachial artery by measuring flow-mediated dilation. Impaired flow-mediated dilation has been associated with the presence of EMPs in various clinical conditions.85–87 However, the level of LMPs was not measured in these studies.
Leukocyte-Derived Microparticles and Traditional Cardiovascular Risk Factors

Several conditions involved in the development of atherosclerosis increase in vitro the release of MPs from cells of the peripheral blood and/or the vascular wall.

Hypertension

Most studies have focused on EMPs as possible biomarkers of endothelial dysfunction in patients with hypertension and some of them also provide information on PMPs and endothelial progenitor cell–derived MPs.98–90 However, reports on circulating LMPs in hypertensive patients are scarce. In one study, PMPs and CD14+ MonoMPs were found higher in hypertensive patients than in controls.91 After administration of efondipine to hypertensive patients, PMPs and CD14+ MonoMPs decreased significantly only in those with diabetes.92 In another study, diabetic and hypertensive patients displayed higher levels of PMPs that correlate with EMPs and MonoMPs.93 Thus, elevated MPs might promote the development of atherosclerotic complications in hypertensive patients. Because calcium channel blockers administration to hypertensive patients with diabetes reduces circulating levels of MPs, this treatment may prevent the development of cardiovascular complications caused by circulating MPs.

Smoking

Cigarette smoking was reported to lead to hemostatic, platelet, and endothelial abnormalities.93 It increases TF expression on peripheral monocytes94 and cultured mouse alveolar macrophages95 and in atherosclerotic lesions.96 Consequently, smokers have higher TF plasma concentrations than non-smokers, and smoking 2 cigarettes in a row further augments their TF level.96

The number of annexin V+ MPs isolated from plasma is lower in smokers than in nonsmokers,97 and circulating PMPs level tends to be lower in young male smokers.98 Because nicotine is known to inhibit apoptosis, THP-1 cells were treated with nicotine. MP release was reduced in presence of nicotine.97 In sharp contrast, level of EMPs (CD31+; CD144+CD62E+) was found to be elevated on secondhand smoke exposure yielding cotinine levels commonly observed in passive smokers.99 Exposure of THP-1 cells to aqueous solutions of cigarette smoke increases the release of (TF-exposing) MPs in a process that requires nicotine.97 In sharp contrast, exposure of THP-1 cells to aqueous solutions of cigarette smoke increases the release of (TF-exposing) MPs in a process that requires nicotine.97

Diabetes Mellitus and Hyperlipidemia

High levels of MPs originating from different cell types are found in the circulation of diabetic patients. However, differences were observed in the presence or in the absence of MPs and in the MP profile in relation to disease type.101 A comparable total number of MPs was found both in asymptomatic patients with well controlled and uncomplicated type 2 diabetes and in control subjects. However, the patients display a higher proportion of TF+ PMPs and TMPs expose neutrophil markers. The number of MPs does not correlate with markers of coagulation activation. This suggests that TF on MP might be involved in other processes than coagulation, for example, transcellular signaling and angiogenesis. Levels of PMPs and MonoMPs have been shown to correlate with diabetic complications or the extent of diabetic complications.102,103–109 Losartan, an angiotensin II receptor blocker, inhibits MonoMPs generation, suggesting that angiotensin II is implicated in vascular changes occurring in type 2 diabetes. Thus, angiotensin II receptor blocker associated with a statin might be valuable as antiatherosclerotic therapy in these patients.105

In patients with uncomplicated type 2 diabetes, consumption of high-fat meals results in dysmetabolic changes and subsequent endothelial stress and injury, thereby contributing to atherogenesis and CVD risk. CD144+ EMPs circulate in patients with uncomplicated type 2 diabetes and are associated with postprandial metabolic derangements and impaired flow-mediated dilation.97 CD144+/annexin V+ EMPs are higher in patients than in control subjects and rise after consumption of meals. In healthy males, after 2 consecutive high-fat meals, mild elevations in plasma glucose and triglycerides were paralleled by impaired flow-mediated dilation, increased markers of oxidative stress and total circulating MPs. These findings may have consequences for subjects with post prandial dysmetabolism, including those with type 2 diabetes.110 A single high-fat meal leads to a significant elevation of plasma EMP (CD31+/CD41−; CD144+/CD62E+) levels in healthy, normolipemic subjects and correlate with post prandial elevation of serum triglycerides. LMPs (CD31+/CD45+) are negligible. Thus, EMPs may be an indirect marker of endothelial dysfunction in injury induced by postprandial triglyceride-rich lipoproteins.111 However, whether EMPs play a role in promoting atherogenesis is unknown.

Leukocyte-Derived Microparticles and Atherosclerosis

Atherosclerosis is a chronic inflammatory disease that involves EC permeability, accumulation of low-density lipoproteins (LDL) in the intima, followed by the diapedesis of leukocytes and formation of foam cells, migration and proliferation of smooth muscle cells, production of connective tissue, and neovascularization. Ultimately, the erosion of the plaque or its rupture occurs, resulting in thrombosis and arterial occlusion.112–114 ECs express an increased amount of adhesion molecules.115 Cytokines and chemokines are also secreted in excess by activated cells of the vessel. Consequently, monocytes and lymphocytes are recruited in the intima of the vessel where they accumulate.

MPs isolated from atherosclerotic lesions are highly thrombogenic and originate from multiple cells, including macrophages, lymphocytes, red blood cells, smooth muscle cells, and ECs but not from platelets.116,117 About 55% of them are LMPs, released from apoptotic cells located within the atherosclerotic plaque. LMPs from the plaque have an increased ability to initiate TF-dependent coagulation compared with the plasma MPs.118 The capture of TF+ LMPs in
the developing thrombus is thought to be mediated by P-selectin and/or CD36 exposed on activated platelets.\textsuperscript{118,119} MonoMPs transfer intercellular adhesion molecule 1 (ICAM-1) to ECs to recruit additional monocytes into the plaque. This process may participate in plaque progression.\textsuperscript{120} MonoMPs may contribute to macrophages and foam cells apoptosis,\textsuperscript{121–123} which is associated with the release of MonoMPs, further increasing plaque MonoMP accumulation. Consequently, more monocytes are recruited and more macrophages and foam cells undergo apoptosis.

The normal intima lacks vasa vasorum, whereas the outer media and the adventitia contain blood vessels. When atherosclerosis progress, the intima thickens and neointima formation within the intima occurs. Thus, one characteristic feature of the vulnerable atherosclerotic plaque is an increased number of vasa vasorum. CD40L\textsuperscript{+} MonoMPs promote EC proliferation and stimulate angiogenesis. They may therefore contribute to the remodeling of atherosclerotic plaques, thereby increasing their vulnerability.\textsuperscript{124,125}

Circulating EMPs and LMPs are increased in patients with high atherothrombotic risk. These MPs induce endothelial dysfunction. Circulating CD11a\textsuperscript{+} LMPs are elevated in symptom-free subjects without a history of CVD but with ultrasound evidence of subclinical atherosclerosis.\textsuperscript{126} These subjects have moderate to high Framingham risk, metabolic syndrome, elevated C-reactive protein, or 2- to 3-site disease detected by high-resolution ultrasound.\textsuperscript{127}

Thus, circulating LMPs appear to be independently associated with subclinical atherosclerosis burden, an association that is not found in PMPs and EMPs. In contrast, other studies demonstrate an association of an increased level of PMPs and/or PMPs with coronary artery disease (CAD) and myocardial infarction\textsuperscript{128–130} and of high EMPs in patients with CAD and most of the traditional risk factors.\textsuperscript{126} This might be explained by the fact that symptom-free subjects may have less endothelial injury and apoptosis than those with clinically advanced arterial disease, or in the use of different techniques for MP numbering.

### Leukocyte-Derived Microparticles and Cardiovascular Diseases

MPs are released in the bloodstream of patients with CVD. In case of atherosclerosis, MPs stemmed from endothelial and inflammatory cells, and platelets are augmented\textsuperscript{17,90,108,111,117,129–138} Endothelial dysfunction that may constitute an independent predictor of adverse events in CAD\textsuperscript{139,140} can be assessed by the numbering of CD31/\textsuperscript{+} annexin V\textsuperscript{+} MPs in plasma.\textsuperscript{128,137,141} CD31/annexin V\textsuperscript{+} MPs might derive from 3 different cell types (ECs, activated platelets, and leukocytes).\textsuperscript{157}

In a prospective study of 200 patients, the prognostic value of circulating CD31/\textsuperscript{+} annexin V\textsuperscript{+} MP levels was evaluated for the occurrence of cardiovascular events. The authors conclude that the level of CD31/\textsuperscript{+} annexin V\textsuperscript{+} MPs constitutes an independent predictor of cardiovascular events in stable CAD patients and may be used for risk stratification.\textsuperscript{137}

The association between the Framingham risk score and the numbers of PMPs, LMPs, EPCMPs, and EMPs was evaluated.\textsuperscript{126,142,143} From these studies, only 1 was prospective, including 488 patients with various CVD risk factors who were observed for a mean of 36 months.\textsuperscript{142}

These reports are promising. However, additional prospective studies are needed to further detail the prognostic value of LMPs in individuals at high risk for CVD. The lack of large-scaled studies might be explained by the fact that MP numbering is still technically demanding and thereby difficult to perform in routine laboratories. Moreover, because measurement procedure is not standardized, multicenter studies are complicated to organize.

#### Leukocyte-Derived Microparticles and Venous Thromboembolism

There has been recent interest in the role of MPs in thrombosis.\textsuperscript{144,145} The measurement of MPs has been proposed as a possible diagnostic/prognostic marker for venous thromboembolism.\textsuperscript{146} LMPs increase thrombus production in animal models.\textsuperscript{147} Patients with arterial or venous thrombosis have higher circulating levels of MPs as compared with control subjects.\textsuperscript{148} Circulating PMPs and CD45\textsuperscript{+} LMPs are elevated in carriers of Factor V Leiden\textsuperscript{149} (see also the recent review of Owens and Mackman\textsuperscript{21}).

#### Leukocyte-Derived Microparticles and Obstructive Sleep Apnea

Obstructive sleep apnea (OSA) is characterized by recurrent episodes of partial or complete obstruction of the upper airway during sleep, resulting in hypopneas or apneas, responsible for repeated falls in oxygen saturation.\textsuperscript{150} OSA is independently associated with death from cardiovascular diseases, including myocardial infarction and stroke.\textsuperscript{150,151} Because myocardial infarction and stroke are complications of atherosclerosis, investigators tried to establish links between OSA and atherosclerosis during the last decade (reviewed in Drager et al\textsuperscript{150}). They found that OSA may accelerate atherosclerosis by aggravating main atherogenic risk factors such a hypertension, diabetes, and dyslipidemia. The role of leukocytes was highlighted in EC injury in OSA with decreased apoptosis and increased expression of L-selectin adhesion molecule.\textsuperscript{152}

An increase in LMPs has recently been described in OSA.\textsuperscript{153–155} Patients with minimally symptomatic OSA were compared with control subjects matched for sex, age, body mass index, waist-to-hip ratio, and cardiovascular comorbidities. Higher levels of CD31/\textsuperscript{+} CD41\textsuperscript{+} PMPs and CD45\textsuperscript{+} MPs were observed in OSA patients, whereas CD31/\textsuperscript{+} CD41\textsuperscript{−} EMPS levels were comparable in both groups.\textsuperscript{153} Patients with OSA and marked nocturnal desaturations display higher circulating levels of CD66b\textsuperscript{+} NMPs and MPs from activated leukocytes (CD62L\textsuperscript{+}).\textsuperscript{154} Moreover, a positive correlation between morning CD62L\textsuperscript{+} MPs and the severity of sleep disordered breathing was demonstrated.\textsuperscript{155} The correlation between evening-to-morning change in CD62L\textsuperscript{+} MPs and an apnea-hypopnea index of ≥5 events per hour in otherwise healthy patients provides evidence in support of a direct link between sleep-disordered breathing and LMP release.\textsuperscript{155} Experimental data in the mouse support that CD62L\textsuperscript{+} MPs induce endothelial dysfunction in OSA. When CD62\textsuperscript{+} MPs from patients with OSA-induced noctur-
nal desaturation are injected intravenously into mice, they reduce endothelial-NO release independently of oxidative stress, and increase endothelial adhesion molecule expression.154 Thus, nocturnal release of LMPs could be involved in the early morning alteration of endothelial function in OSA, as reported by Izhaki et al156 and thereby may contribute to the increase of cardiovascular events after the awakening.

Two recent studies found no difference in EMP levels between OSA and non-OSA subjects.153,157 In contrast, Yun et al158 observed higher EMP (CD31+/CD42−; CD31+/annexin V+; CD62E+) levels were in OSA than in non-OSA subjects. They also demonstrated that the EMP level correlates with the apnea-hypopnea index and that treatment reduces the level of CD62E+ EMPs. The level of LMPs was not measured in this study. Elevated circulating EMP levels in subjects with OSA but no other comorbidities are also found in another study.159 In children with OSA, increased concentrations of MPs (EMPs, EPCMPs, CD11b+ LMPs, and CD42a+ PMPs) appear to be severity dependent. In these patients, PMPs were associated with vascular dysfunction.160

These data suggest a link between circulating LMPs, EMPs, and PMPs and OSA, and a role for MPs in OSA-associated endothelial dysfunction. Additional studies, including randomized controlled trials including vascular reactivity assessment are mandatory to establish this.

### Leukocyte-Derived Microparticles and Angiogenesis

LMPs can affect angiogenesis by inducing changes in ECs, either by increasing the production of proangiogenic factors or decreasing the production of antiangiogenic factors (see also the recent review of Martinez and Andriantsitohaina19). Accumulation of MPs within the atherosclerotic plaque represent an endogenous signal for atherosclerotic plaque neovascularization and vulnerability.59 Indeed, the density of intraplaque neovessels favors intraplaque hemorrhage and augments the risk of rupture.162,163 Recently, CD40L+ MPs isolated from endarterectomy specimens, which are mainly of macrophage origin (93%), were found to stimulate endothelial proliferation and promote neovessel formation after CD40 ligation.59 VEGF and PI3K/Akt may participate in the endothelial proliferation effect of plaque MPs through ligation of CD40.59

TMPs generated in vitro from human apoptotic/stimulated lymphocytes express the morphogen Shh (TMPs<sub>Shh</sub>+) on their surface and induce cell differentiation.68 The morphogen Shh pathway is critical for normal growth. TMPs<sub>Shh</sub>+ induce the formation of capillary-like structures in an in vitro model using human ECs by increasing the expression of proangiogenic factors and adhesion molecules. Silencing of the Shh receptor or the Shh signaling reverses these effects.163 In vivo treatment of hind limb ischemic mice with TMPs<sub>Shh</sub>+ enhances the neovascularization, as reflected by the improvement of both blood flow and number of capillaries in the ischemic leg when compared with the nonischemic leg.84 TMPs<sub>Shh</sub>+ treatment promotes an increase of Shh expression in ischemic skeletal muscle, an effect prevented by a Shh inhibitor, and an increase in NO production as a consequence of the activation of endothelial NO synthase. Moreover, TMPs<sub>Shh</sub>+ treatment increases the expression of several proangiogenic factors. These studies suggest a potential contribution of TMPs<sub>Shh</sub>+ to reparative neovascularization after ischemic injury.84 MPs<sub>Shh</sub>− may constitute an autologous therapy for pathologies with altered angiogenesis by resetting the angiogenic switch and thereby creating a natural bypass around vessels.164

MPs<sub>Shh</sub>− suppress angiogenesis and inhibit both EC survival and proliferation through the increase of reactive oxygen species generation. They act through the downregulation of VEGF receptor-2 and extracellular signal-regulated kinase pathways.165 Moreover, MPs<sub>Shh</sub>− from apoptotic lymphocytes may constitute promising antiangiogenic agents for the treatment of lung carcinomas.166

### Conclusion

LMPs are bioactive particles implicated in vascular homeostasis. They participate in numerous processes including thrombosis, inflammation, endothelial function, atherosclerosis, and angiogenesis. They circulate at a high level in the bloodstream of patients with increased atherothrombotic risk or CVD. Recent prospective studies indicate that numbering MPs, including LMPs, might be useful in predicting cardiovascular events. However, the interpretation of these data and studies remains difficult and is sometimes even hazardous because of the lack of standardization of the definition and methods of analysis. In addition, LMPs may display various properties depending on the stimuli used for their in vitro generation and the cell from which they stem from, for example, primary cell or immortalized cell line. Published data also result from analysis performed on LMPs isolated from body fluids or tissues. These LMPs from different sources may carry identical lineage cell surface antigen but might be biologically different. Indeed, extrapolation of data obtained in vitro with LMP derived from immortalized cell line to LMP generated in vivo should be performed cautiously.

Large-scale studies are needed to further evaluate the prognostic value of MPs. However, such studies are currently problematic to set up because MP measurement is still technically demanding and, in addition, as mentioned above, lacks standardization.

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### Disclosures

None.

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