Circulating Microparticles

A Potential Prognostic Marker for Atherosclerotic Vascular Disease

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Although the presence of subcellular procoagulant particles in highly centrifuged plasma has been known for many years, it was not until 1967 that shed membrane procoagulant fragments from activated platelets were described by Wolf1 as “platelet dust.” Since then, numerous studies have reported the in vitro release of vesicles from activated or apoptotic cells and their presence in human plasma. Among the different membrane vesicles that cells could release, “microparticles” (MPs) usually refer to those >100 nm in diameter (Figure 1) and derived from the plasma membrane. Smaller vesicles (40 to 100 nm) originating from the endoplasmic membranes are described as “exosomes,” and larger particles (>1.5 μm) containing nuclear material are best known as “apoptotic bodies.”2–4 Both MPs and apoptotic bodies externalize the anionic phospholipid phosphatidylserine, which plays a crucial role in the activation of both coagulation and complement cascades and supports the binding and activity of secreted phospholipase A₂.

This brief review summarizes the mechanisms leading to the formation and release of MPs, as well as the different approaches to detect their cellular origin in human plasma. This article also discusses the significance of circulating MPs of endothelial origin as both a marker and a trigger of endothelial dysfunction and the possible prognostic value of plasma MPs to assess cardiovascular risk.

Mechanisms of MP Formation and Release

The current knowledge on MP formation derives mainly from experiments on isolated or cultured cells showing that both cell activation and cell apoptosis can lead to MP release. However, in vivo mechanisms involved in MP formation and shedding remain mostly unknown.

The initial step in MP formation requires plasma membrane budding, leading to the formation of membrane blebs. This step requires increases in intracellular calcium, changes in membrane lipid asymmetry, and cytoskeleton protein reorganization (Figure 2).3,5–7 The formation of membrane vesicles is also associated with the loss of plasma membrane asymmetry, a characteristic of quiescent cells. This leads to exposure of phosphatidylserine on the outer leaflet as a consequence of the calcium-dependent activation of scramblase and floppase/ABC1 and the inhibition of translocase/flippase activities (Figure 2).5,8,9 Phosphatidylserine exposure is not always followed by the release of MPs, which may be regulated by the level of intracellular calcium. Moreover, MPs shedding necessitates modifications in cell structural architecture involving disruption of cytoskeleton proteins organization (Figure 2). In platelets, MP release seems to be controlled by calcium-dependent activation of calpain, a cytosolic cysteine protease involved in rearrangement of cytoskeleton proteins and protein cleavage to activate various receptors and proenzymes.5

MP Formation and Release After Cell Activation

Platelets release MPs after activation by thrombin, ADP plus collagen, the complement complex C5b-9, the calcium ionophore A23187, and by high shear stress.10–13 Endothelial cells, monocytes, vascular smooth muscle cells, and hepatocytes can also release MPs after activation by bacterial lipopolysaccharides, inflammatory cytokines including tumor necrosis factor or interleukin-1, the complement complex C5b-9, aggregated low-density lipoproteins, or reactive oxygen species.14–17 Increase in intracellular calcium, most likely at the site of membrane vesicle formation, seems to be a critical step for MP release, but the role of cytoskeleton is not yet fully elucidated (Figure 2).18

Apoptosis-Induced MP Release

MPs released from apoptotic cells may be different in lipid and protein composition from membrane vesicles shed after cell activation and could possibly have different pathophysiological effects. Apoptosis is characterized by cell condensation and DNA fragmentation. Blebbing of the cellular membrane occurs rapidly after cells enter the apoptotic process. Bleb formation depends on actin cytoskeleton and actin–myosin contraction, which is regulated by caspase 3–induced Rho kinase I activation (Figure 2).6,7 Rho kinase activation is required for relocalization of DNA fragments from the nuclear region to membrane blebs, suggesting that MPs from apoptotic cells may contain nuclear material.7,10 Interestingly, statins inhibit MP release from cultured endothelial cells by altering the Rho kinase pathway.20

MP Composition

An important parameter that determines the biological effects of MPs is their protein and lipid composition, which may vary depending on the cell they originate from and the type of stimulus involved in their formation. For instance, the phospholipid composition of MPs isolated from synovial fluid of...
patients with rheumatoid arthritis differs from that of MPs isolated from the plasma of healthy subjects. In addition, the degree of phospholipid oxidation varies on the stimulus initiating the release of membrane vesicles. MP also expose proteins that are specific to the cell that they stem from and that can be used for the determination of their cellular origin using antibodies directed against these specific epitopes. Proteomic analyses have revealed that the spectrum of proteins found in MPs released in vitro from cultured cells is influenced in part by the type of stimulus used to trigger cell vesiculation. This different pattern of protein expression may be helpful in distinguishing within a subpopulation of circulating MPs those released after apoptotic stimuli from MPs resulting of cell activation.

Why Do Cells Release MPs?
The reason that cells shed MPs from their main body may be an attempt to reverse the apoptotic process by getting rid of unwanted signaling molecules like the proapoptotic caspase 3. The release of MPs would also allow cells to escape phagocytosis by removing quickly from the cell surface “eat-me-signals,” such as phosphatidylserine. Alternatively, membrane shedding could constitute a signaling entity to phagocytes and neighbor cells, because their interaction modulates inflammation, immune responses, and repair mechanisms. Actually, the release of membrane vesicles to signal to neighbor or remote target cells is not a specific property of eukaryotic cells. Bacteria also shed membrane “bubbles” containing toxins aimed at other bacterial strains or their host.

Detection and Measurement of MPs
Plasma MPs can be detected and their cellular origin characterized using capture assays or flow cytometry, 2 complementary approaches reviewed in a recent forum. A crucial point in circulating MP analysis concerns the preanalytical steps involved in blood sampling and platelet-free plasma preparation. Both methods of MP analysis rely on antibody detection of specific cellular markers and annexin V binding of phosphatidylserine. Although the general understanding is that MPs express phosphatidylserine, which is detected by annexin V labeling, some plasma MPs analyzed by flow cytometry express specific markers of their cellular origin but do not bind annexin V even in the presence of high calcium concentrations. For example, in patients with sickle cell disease, circulating endotheial MPs were either positive or negative for annexin V. Similar finding were observed for monocyte-derived MPs but not for platelet- or erythrocyte-derived MPs in these patients. We observed recently that very few circulating platelet-derived CD41+/H11001 MPs bind annexin V in some patients with end-stage renal failure, whereas most CD41+ MPs from other patients with the same disease were positive for annexin V (Figure 3). Taken all together, these observations suggest that if phosphatidylserine is exposed on the outer leaflet of these annexin V negative MPs, it may be unavailable for annexin V binding as
already engaged in some other molecular interactions. Indeed, phosphatidylserine binds with high affinity to lactadherin, a milk-fat globule glycoprotein found on plasma exosomes, or to proteins from the coagulation cascade, such as protein S, an antithrombotic protein cofactor to protein C. These protein–protein interactions are involved in the phagocytosis of apoptotic cells by macrophages. Alternatively, the lack of experimental evidence for phosphatidylserine exposure by membrane MPs expressing specific cellular markers may suggest that other pathways in addition to the loss of membrane asymmetry may be involved in their formation and release, but this remains to be demonstrated.

**Plasma Levels of MPs in Cardiovascular Diseases**

Circulating levels of MPs are augmented in most cardiovascular diseases when comparing a patient population with a matched group of healthy subjects (Table). Similar findings have been observed in patients with autoimmune and thrombotic diseases. The general consensus is that plasma levels of MPs reflect an equilibrium between their release and their removal by phagocytes. However, there is no information regarding the respective degree of involvement of each pathway in determining plasma levels of MPs in patients with cardiovascular diseases. Interestingly, red blood cell MPs, when injected intravenously to normal rats, are rapidly removed from the circulation by scavenger receptors on Kupffer cells.

The observation that circulating MPs are increased after acute myocardial infarction (Table) raises the question of whether these MPs might come from the ruptured plaque. This possibility is highly unlikely because of the different pattern of cellular origin between plaque and plasma MPs. In addition, although MPs are much more abundant in atherosclerotic plaques than in plasma and account for the procoagulant activity of the lipid core, at least a dozen large lesions (such as those found in human carotid arteries) would have to rupture simultaneously to fully account for circulating levels of MPs in these patients.

Circulating MPs are increased in patients with cardiovascular risk factors, such as hypertension, obesity, or cholesterol. Chronic treatment with antioxidants, such as vitamin C or carvedilol, decreases circulating endothelial MPs in patients with heart failure. In addition, in vitro experiments have indicated that statins impair endothelial MPs formation, whereas platelet MP release is not affected by aspirin, but reduced by ticlopidine, abciximab, or cilostazol.

**Circulating MPs: Bystander or Mediator?**

Most of the experimental evidence available so far indicates that MPs influence diverse biological functions. However, one should be cautious in interpreting data from studies with MPs generated in vitro or from cultured cells, because they may not be fully representative of those found in vivo. The pattern of proteins found on MPs (which differs between blood-borne tissue factor.” They are involved in the formation of tissue factor–platelet hybrids, a critical phenomenon in thrombus propagation, after transfer of tissue factor from leukocyte MPs to platelet membranes. This property may not be restricted to leukocyte-derived MPs, because the presence of tissue factor on platelet–erythrocyte- and hematopoietic cell–derived MPs leads also to thrombus propagation in vivo. In addition, similar observations were reported in vitro regarding endothelial MP-induced tissue factor expression and procoagulant activity in the monocyte cell line THP1.

In vivo, MPs are captured by thrombus-associated platelets through the interaction of MP P-selectin glycoprotein ligand (PSGL)-1 with platelet P-selectin, leading to a concentration of tissue factor, which initiates and accelerates blood coagulation and fibrin formation. The first hint that MPs may affect endothelial biology came from studies investigating the effects of MPs derived from in vitro activated platelets on cultured endothelial cells, demonstrating the transcellular delivery of MP arachidonic acid leading to increased expression of endothelial cyclooxygenase type 2. Platelet MP release is not affected by aspirin, but reduced by ticlopidine, abciximab, or cilostazol.

Platelet MPs also stimulate endothelial cells in vitro to release cytokines and express adhesion molecule. In addition, platelet MPs can directly interact with activated vascular endothelial cells by increasing leukocytes/monocytes arrest after transcellu-
lar delivery of the chemokine Regulated on Activation, Normal T Expressed and Secreted (RANTES).56

MPs also impair the release of NO from vascular endothelial cells. This was observed on isolated arteries exposed in vitro to circulating concentrations of MPs from patients with acute coronary syndromes, end-stage renal failure, or preeclampsia but not with MPs from healthy subjects.32,57,58 The endothelial dysfunction caused by circulating human MPs seems to be mediated by particles of endothelial origin and is associated with an impaired release of NO but no alteration in endothelial NO synthase expression (Figure 4).32,57 Similar results were reported for MPs released from cultured endothelial cells, smooth muscle cells, or from a lymphocyte cell line, although the molecular mechanisms may be different from those triggered by MPs isolated from human plasma.59–62 Other effects of MPs on vascular reactivity other than impairment of endothelial function have been reported as well. Platelet MPs generated ex vivo cause vascular contraction in vitro by acting as a source of thromboxane A2 for the vessel wall.63 In addition, impaired vascular reactivity because of increased expression of inducible NO synthase and cyclooxygenase-2 (COX2) in smooth muscle cells has been observed in the isolated mouse aorta after exposure to MPs derived from a T-cell line or from circulating MPs isolated from the plasma of diabetic patients.60 Further

Changes in Plasma Levels of Circulating MPs in Patients With Cardiovascular and Other Diseases

<table>
<thead>
<tr>
<th>Disease/Condition</th>
<th>Plasma Changes</th>
<th>MPs Cellular Origin</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute coronary syndrome</td>
<td>+</td>
<td>Endothelial, platelet MPs</td>
<td>76–78</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>+</td>
<td>Endothelial MPs</td>
<td>42</td>
</tr>
<tr>
<td>Heart transplantation</td>
<td>+</td>
<td>Endothelial MPs</td>
<td>79</td>
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<tr>
<td>Hypertension</td>
<td>+</td>
<td>Endothelial, platelet, monocyte MPs</td>
<td>39,80</td>
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<tr>
<td>Metabolic syndrome</td>
<td>+</td>
<td>TF+ MPs</td>
<td>40</td>
</tr>
<tr>
<td>Diabetes type 2</td>
<td>+</td>
<td>Platelet and total annexin V+ MPs</td>
<td>81–83</td>
</tr>
<tr>
<td>Diabetes type 1</td>
<td>+</td>
<td>Endothelial and platelet MPs</td>
<td>83</td>
</tr>
<tr>
<td>End-stage renal disease</td>
<td>+</td>
<td>Endothelial and other origins</td>
<td>32,73</td>
</tr>
<tr>
<td>Cardiopulmonary bypass</td>
<td>+</td>
<td>Multiple origin</td>
<td>84</td>
</tr>
<tr>
<td>Peripheral artery disease</td>
<td>+</td>
<td>CD63 + Platelet MP</td>
<td>77</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>+/-</td>
<td>T cell, granulocytes, endothelial/platelet</td>
<td>58,85,86</td>
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<tr>
<td>Pulmonary or venous embolism</td>
<td>+</td>
<td>Platelet, endothelial</td>
<td>87,88</td>
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<tr>
<td>Postprandial hypertriglyceridemia</td>
<td>+</td>
<td>Endothelial MPs</td>
<td>89</td>
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<tr>
<td>Lupus anticoagulant</td>
<td>+</td>
<td>Endothelial MPs</td>
<td>14</td>
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<tr>
<td>Multiple sclerosis</td>
<td>+</td>
<td>Endothelial and CD31+ MPs</td>
<td>90</td>
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<tr>
<td>Sepsis</td>
<td>+/-</td>
<td>Annexin V+, platelet, endothelial MPs</td>
<td>91,92</td>
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<tr>
<td>HIV-1 infection</td>
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<td>Lymphocyte-derived MPs</td>
<td>93</td>
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<tr>
<td>Crohn’s disease</td>
<td>+</td>
<td>Total annexin V+ MPs</td>
<td>94</td>
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<tr>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>+</td>
<td>Endothelial MPs</td>
<td>95</td>
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<tr>
<td>Sickle cell disease</td>
<td>+</td>
<td>Multiple origin</td>
<td>31</td>
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<tr>
<td>Thrombotic thrombocytopenic purpura</td>
<td>+</td>
<td>Endothelial MPs</td>
<td>96</td>
</tr>
</tbody>
</table>

+ and – indicate an increase or a decrease in plasma levels, respectively; TF, tissue factor.

Figure 4. Paracrine effect(s) of endothelial MPs predisposing to endothelial dysfunction in vivo.
details on the molecular mechanisms of MP effects on vascular reactivity can be found in Martinez et al. 64

Finally, endothelial, platelet, and tumor cell–derived MPs seem to be able to stimulate angiogenesis, an effect mediated by reactive oxygen species, metalloproteinases, growth factors such as vascular endothelial growth factor, or sphingomyelin. 65–69 Apoptotic bodies from endothelial cells (larger than MPs) could also contribute to tissue repair mechanisms by stimulating the differentiation of progenitor cells. 70

Circulating MPs as a Potential Marker for Cardiovascular Diseases
Circulating MPs, mainly those derived from endothelial cells, also express markers of cellular injury, such as the soluble markers intercellular adhesion molecule-1, vascular cell adhesion molecule, E-selectin, or von Willebrand factor. Actually, many of these markers are both true soluble molecules and MP-bound forms, indicating that expression of these hallmarks of cell injury on endothelial MPs could reflect the degree of endothelial dysfunction. 71

Recent experimental evidence suggests that plasma level of endothelial MPs is a specific marker of endothelial dysfunction in patients with cardiovascular diseases. Indeed, circulating levels of endothelial CD144+ (VE-cadherin) MPs inversely correlate with the amplitude of the flow-mediated dilatation in the brachial artery of patients with end-stage renal failure, whereas no correlation was observed for platelet or annexin V + MPs. 32 This relationship was independent of age and blood pressure. 32 Similar findings were observed in patients with acute coronary syndromes. 72 All together, these findings indicate that the more that circulating endothelial MPs are detected, the patient’s arteries are less likely to normally vasodilate in response to increases in flow rate or to acetylcholine. In addition, plasma levels of endothelial MPs correlate with indexes of arterial stiffness in hemodialyzed patients, suggesting that high plasma endothelial MPs also reflect alterations in arterial properties. 32 Although the question of whether circulating levels of endothelial MPs cause or result from endothelial dysfunction is still much debated, in vitro evidence indicates that the augmented plasma levels of endothelial MPs, as observed in patients with end-stage renal diseases, could result from endothelial injury or apoptosis. 32, 73 Cytokines, activated platelets, or oxidized low-density lipoprotein could play a role as well. However, the in vivo stimuli of endothelial vesculation in patients with cardiovascular diseases remain mostly unknown so far. Plasma endothelial MPs could originate from both circulating mature endothelial cells and endothelial cells lining the vessel wall. It is noteworthy that levels of circulating endothelial cells are also elevated in patients with cardiovascular diseases, as observed for endothelial MPs. 74 Alternatively, we cannot exclude the possibility that some circulating MPs originate from progenitor cells, because they share similar marker proteins.

Prognostic Potential of Circulating MPs
Preliminary data indicate that plasma levels of MPs could be of prognostic value for the occurrence of cardiovascular diseases. In a 6-month follow-up study, circulating annexin V + MPs appear as a robust predictor of the occurrence of secondary myocardial infarction or death in 500 patients with acute coronary syndromes. 75 Furthermore, circulating leukocyte-derived MPs, unlike platelet-derived MPs, predict subclinical atherosclerosis burden appreciated by plaque numbers in carotid arteries, abdominal aorta, and femoral arteries in >200 asymptomatic subjects. 41

Perspectives
Although the prognostic potential of circulating MPs is still in its infancy, the different studies mentioned above clearly demonstrate that their detection and quantification is an interesting and potentially valuable tool to appreciate cardiovascular risk of asymptomatic patients. Obviously, the response to this important question will first necessitate a standardized approach to MP detection and characterization. In addition, refinement in the detection of MP cellular origin will help shed light on the mechanisms leading to their release in the plasma and/or their clearance.

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References


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