Cell-Derived Microparticles in the Pathogenesis of Cardiovascular Disease
Friend or Foe?

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Abstract—Microparticles are ascribed important roles in coagulation, inflammation, and endothelial function. These processes are mandatory to safeguard the integrity of the organism, and their derangements contribute to the development of atherosclerosis and cardiovascular disease. More recently, the presumed solely harmful role of microparticles has been challenged because microparticles may also be involved in the maintenance and preservation of cellular homeostasis and in promoting defense mechanisms. Here, we summarize recent studies revealing these 2 faces of microparticles in cardiovascular disease. (***Arterioscler Thromb Vasc Biol.*** 2011;31:4-9.)

Key Words: atherosclerosis ■ coagulation ■ cardiovascular disease ■ microparticles

Cardiovascular disease (CVD) remains the leading cause of death in developed countries despite advances in treatment.[1] Although assessment and treatment of traditional cardiovascular risk factors, including hypertension, diabetes mellitus, and smoking, play a central role in primary and secondary disease prevention, even adequate treatment of these risk factors does not completely reduce the risk of CVD. Therefore, a quest for novel biomarkers or mediators is ongoing, which may help us to gain more insight into the mechanisms leading to the development of atherosclerosis, the underlying cause of CVD.[2] Cumulating evidence suggests that microparticles (ie, vesicles budded from the outer membrane of cells on their activation or as part of the apoptosis of the cell) and other microvesicles may provide such a mediator. Since their identification in the late 1960s, the scientific and clinical interest in cell-derived microparticles has increased substantially. This is not surprising because microparticles affect coagulation and inflammation, along with endothelial function, cellular survival, and intercellular communication.[3–5]

Most microparticles in blood are derived from platelets, whereas microparticles from erythrocytes, granulocytes, monocytes, lymphocytes, and endothelial cells are also present, albeit at much lower numbers. There are substantial differences between the fractions of microparticles or subpopulations in the blood of healthy subjects and those present in patients suffering from diseases with increased thromboembolic risk or vascular damage, such as atherosclerotic vascular disease, sepsis, diabetes, chronic severe hypertension, and preeclampsia.[3–5] Accordingly, in patients with acute myocardial infarction, elevated numbers of microparticles are present compared with healthy controls.[3,6,7] Moreover, subtypes of microparticles differ between patients with stable angina and those with acute coronary syndromes or myocardial infarction.[7,8] Earlier, it was demonstrated that microparticles from patients with myocardial infarction cause endothelial dysfunction and might contribute to the general vasomotor dysfunction observed after myocardial infarction.[9]

The clinical relevance of the presence of microparticles in the blood of healthy subjects is unclear but can be regarded as a reflection of the dynamics of their production by resting, activated, and apoptotic cells and their clearance. In vascular disease states, it remains to be elucidated whether microparticles are a cause or a consequence of the condition because disease-related factors, such as infectious agents, cytokines, and metabolic disturbances, are all known to affect the release of microparticles.[3–5] Still, it is likely that microparticles contribute to the severity of disease because they can disseminate procoagulant and proinflammatory activities. Therefore, microparticles may be viewed as part of a cascade of reactions in response to various stimuli. The stimulus that leads to their generation determines their numbers, size, biochemical composition, and functional characteristics. Microparticles are likely to be important and essential mediators of physiological and pathological conditions; in this review, we will focus on the clinical relevance of microparticles in CVD.

Microparticles: Friend or Foe?

All body fluids, ranging from blood and urine to lacrima and mother's milk, and human atherosclerotic plaques contain

cell-derived vesicles. Microparticles as outer membrane–derived vesicles are 1 type of vesicle, as are exosomes, which are extruded from intracellular multivesicular bodies once they fuse with the outer membrane. Different types of vesicles originating from a variety of cells are concurrently present in body fluids under physiological and pathological conditions. Most clinical studies thus far have focused on the presence of microparticles in blood (ie, their cellular origin, composition, and function in disease).[3–5]

Microparticles may not only have deleterious effects by promoting coagulation and inflammation or by modifying endothelial function, which all contribute to the development of CVD; but may also have beneficial effects. First, recent studies have shown that microparticles are efficient vectors that exchange biological information between cells (intercellular communication).[3–5] Second, the release of microparticles protects cells against the consequences of external stimuli or stress. Endothelial cells escape from complement-induced lysis by releasing microparticles carrying the lytic complement C5b-9 complex.[10] Similarly, the release of microparticles protects cells against an overshoot in (internal) cellular reactions triggered by external stressors. Regarding the latter, microparticles play a role in "cellular waste management" because they contain increased (compared with parent cell) concentrations of chemotherapeutics, oxidized phospholipids, or caspase 3.[3–5] Microparticles of viable non-apoptotic cells, including endothelial cells, contain caspase 3 in vitro and in vivo.[11–13] Endothelial cells accumulate caspase 3 and undergo apoptosis when the release of (caspase 3–containing) microparticles is inhibited,[13] indicating that the release of microparticles is part of a protective mechanism to prevent the intracellular accumulation of caspase 3 at dangerously high levels. Possibly, caspase 3 facilitates its own removal from cells by cleaving several kinases, including Rho-associated kinase I and p21-activated kinase, which become constitutively active on cleavage and promote membrane blebbing. On incubation of human endothelial cells with simvastatin at clinically relevant concentrations, the cells remain viable and seemingly unchanged; however, a marked increase in caspase 3–containing microparticles is observed, suggesting that an increased release of caspase 3–containing microparticles is needed to help the cells to remain healthy and viable.[14] The effects of statins on the release of microparticles may depend on the cell types studied; the experimental conditions, including the concentration and type of statin used; or the combined application of a statin with a proinflammatory or proapoptotic inducer. It is beyond the scope of this brief review to elaborate on this topic. In line with our results are the findings that in patients with subclinical or less occlusive atherosclerosis, more endothelial microparticles are present when compared with patients with established or symptomatic atherosclerosis,[8,15] suggesting that the ability of the endothelium to release microparticles depends on its integrity and viability. In other words, if the ability of the endothelium to release microparticles becomes impaired or inhibited, the integrity and viability of the cells may deteriorate.

Microparticles and Coagulation

The most described and reviewed characteristic of circulating and atherosclerotic plaque–derived cell-derived microparticles is their procoagulant phenotype. By exposing phosphatidylserine and sometimes tissue factor (TF), microparticles can initiate and propagate coagulation.[3–5] However, TF often seems to be exposed by circulating microparticles in a noncoagulant form, as shown in patients with type 2 diabetes.[16] This inactive or encrypted TF may become procoagulant after the capture of TF-bearing microparticles in the developing thrombus.[17] This is supported by the recent finding that those patients with acute ST-segment elevation myocardial infarction who had the highest plasma level of TF-exposing microparticles were characterized by a procoagulant phenotype, as reflected by increased plasma levels of thrombin-antithrombin complexes, and by an increased risk of fibrinolysis failure.[18] Furthermore, microparticles accumulate in human atherosclerotic plaques, and these microparticles have an increased ability to initiate TF-dependent coagulation compared with the plasma microparticles.[19]

The capture of TF-bearing microparticles in the developing thrombus, finally resulting in arterial occlusion, is thought to be mediated by P-selectin and/or CD36 exposed on activated platelets.[17,20] Important factors in deencryption of the TF procoagulant activity are protein disulfide isomerases (PDIs), believed to be released from platelets and endothelial cells. In addition, platelet microparticles can expose PDIs.[21] Recently, it was demonstrated that fibrin generation in vivo required PDI released from endothelial cells, whereas platelet-derived PDI contributed only to the total amount of thrombus-associated PDI (but was not required).[22]

The relationship between microparticles and coagulation and other processes is complex. For instance, under normal conditions, a continuous low-grade generation of thrombin is essential for activation of protein C, which, in turn, efficiently inhibits additional thrombin generation by inactivating coagulation factors Va and VIIIa. Previously, it was reported that plasma samples from healthy humans containing low levels of microparticles have relatively high concentrations of thrombin-antithrombin and prothrombin fragment $1+2$, suggesting that in these subjects insufficient activation of protein C may occur.[23–25] Under clinical conditions, in which elevated levels of microparticles can be present and in which microparticles may expose coagulant TF, this inverse correlation is no longer present; then, microparticles are more directly associated with in vivo coagulation activation.

Microparticles are thought to contribute to acute thrombus formation via various mechanisms; they also contribute to anticoagulant responses to modulate an adequate and physiological coagulation response. However, additional research is essential to better understand their contribution to coagulation in clinical conditions, including CVD.

Microparticles and Inflammation

Similar to coagulation, inflammatory processes contribute to the pathogenesis of atherothrombotic vascular disease.[26] Patients with elevated plasma levels of inflammation markers, including C-reactive protein, are at a high risk of developing CVD, including those with diabetes, metabolic syndrome,

and chronic systemic inflammatory or autoimmune disease (eg, rheumatoid arthritis).[26,27] Microparticles may contribute to inflammatory responses by various mechanisms.[3–5] For example, microparticles from leukocytes stimulate the expression of proinflammatory genes in endothelial cells, leading to the production of cytokines and leukocyte–endothelial cell adhesion molecules in vitro.[28] Furthermore, microparticles in plasma samples from high-risk patients expose complement components (C1q, C3, and C4) and several complement activator molecules (eg, C-reactive protein) in rheumatoid arthritis patients and IgG (but not C-reactive protein) in patients with myocardial infarction.[29,30]

But again, microparticles may also have a beneficial function in the inflammatory response. Microparticles from polymorphonuclear leukocytes contain the functionally active anti-inflammatory protein annexin 1, and annexin 1–containing microparticles inhibit the interaction between leukocytes and endothelial cells in vitro and in an animal model in vivo.[31] Therefore, in health, microparticles can affect both proinflammatory and anti-inflammatory processes, thus ensuring an appropriate inflammatory response. In diseases such as rheumatoid arthritis or atherosclerosis, their increased and prolonged presence, altered properties, and activities may become harmful and contribute to overall vascular deterioration.

Microparticles and Endothelial Functions

Under normal conditions, the multiple functional characteristics of the endothelium, in addition to anti-inflammation and anticoagulation, include regulation of vascular tone, vascular wall permeability, and cell growth; collectively, they protect the vascular system. An altered function of endothelial NO synthase, decreased bioavailability of NO, or both are fundamental abnormalities that can lead to the pathophysiological manifestations of endothelial dysfunction.[32] In vitro, microparticles from various cellular or disease origins or both induce endothelial dysfunction, especially by altering the balance between NO and reactive oxygen species (ROS) production and release.[33–35] For example, microparticles from T lymphocytes decrease NO production and increase oxidative stress in endothelial cells.[35] These effects are associated with a reduction of endothelial NO synthase activity, which depends on phosphatidylinositol-3-kinase (PI3K), extracellular signal–regulated kinase 1/2, and nuclear factor κB pathways. The increase of ROS production that is downregulated by the PI3K pathway involves xanthine oxidase and nuclear factor κB pathways. Also, exposure to microparticles results in increased caveolin-1 expression and decreased phosphorylation; these effects are independent of the PI3K and extracellular signal–regulated kinase 1/2 cascade, respectively.[35] In vivo, NO-associated vasodilatation after shear stress can be measured by ultrasonography of the brachial artery (flow-mediated dilatation) and is associated with the viability of the endothelium. Impaired flow-mediated dilatation has been associated with the presence of endothelial microparticles in various clinical conditions.[36,37]

In contrast to the harmful role of microparticles, they may actually improve endothelial dysfunction. T-cell microparticles carrying the morphogen sonic hedgehog induced NO production directly by the sonic hedgehog pathways (involving PI3K and protein kinase B [Akt]).[38] By using a model of ischemia/reperfusion in mice, these sonic hedgehog–presenting microparticles enhanced NO-mediated relaxation of mouse coronary arteries in vivo in response to acetylcholine, which was accompanied by an increase of NO production in tissues and blood even after ischemia/reperfusion. Moreover, microparticles, especially those of nonplatelet origin, released during sepsis (the clinical condition that ultimately challenges the endothelium) were protective against vascular hyporeactivity, which accounts for hypotension in patients with septic shock.[39] In agreement with these results, Soriano and coworkers[40] have shown that elevated levels of endothelial and platelet microparticles predict a more favorable outcome in severe sepsis in terms of mortality rate and organ dysfunction. Evidently, microparticles are able to restore endothelial injury through their dual ability to increase NO and reduce ROS. In summary, microparticles can have both detrimental and beneficial effects on endothelial functions, especially by altering the balance between NO and ROS production and release. It seems that these effects are dependent on the specific stimulus underlying the release of microparticles by their parent cells.

Finally, circulating and atherosclerotic plaque-derived microparticles can affect angiogenesis. One characteristic feature of vulnerable atherosclerotic plaques is an increased number of vasa vasorum. Whereas microparticles from platelets and endothelial cells were shown to promote and inhibit angiogenesis in vitro in initial studies, respectively, more recently, researchers showed that microparticles isolated from human atherosclerotic plaques promote endothelial cell proliferation in vitro and stimulate angiogenesis in vivo, respectively. Thus, microparticles may contribute to neovascularization of atherosclerotic plaques, thereby affecting the vulnerability of rupture.[41–43]

Microparticles and CVD

In the light of the previously described procoagulant and proinflammatory properties of microparticles, together with the association between elevated numbers of microparticles and clinical CVD, the prevailing view is that circulating microparticles are harmful, contributing to CVD and risk of CVD. However, as previously outlined, in addition to their potentially harmful effects, cell-derived microparticles may also be beneficial and protect against cellular and vascular damage. This is summarized in the Figure. Therefore, it is not surprising that both elevated and lower levels of circulating microparticles have been associated with (risk factors of) CVD. For example, cigarette smoking is a well-established risk factor for CVD and has been reported to lead to hemostatic, platelet, and endothelial abnormalities.[44] Although the numbers of platelet microparticles tended to be lower in young males compared with nonsmoking controls, levels of circulating endothelial microparticles were elevated on secondhand smoke exposure, and exposure of human monocytes to smoke increased the release of (TF-exposing) microparticles.[45–47] It is clear that smoking affects the release of microparticles; however, this effect is likely to be cell type, concentration, and duration dependent.
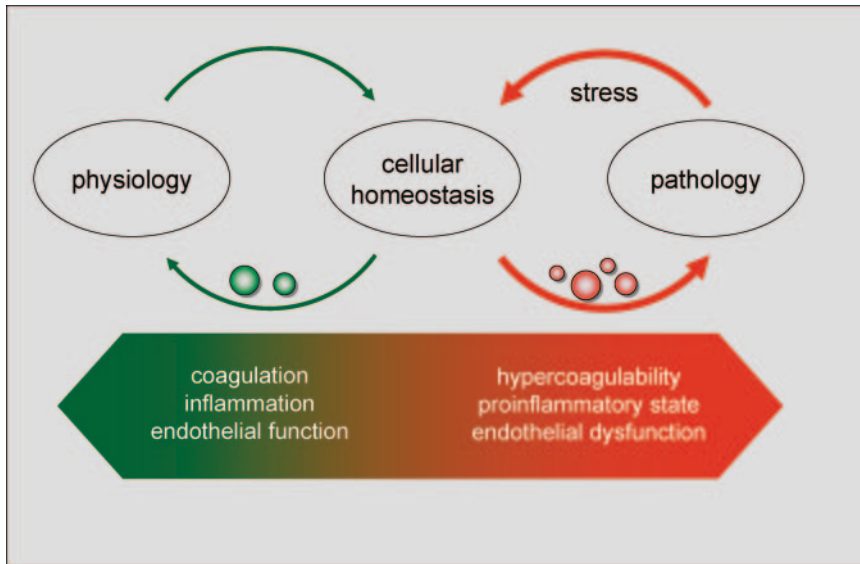
Figure. Proposed model describing the role of microparticles in cardiovascular health and disease. Cells release microparticles (left, green) to ensure homeostasis. These microparticles contribute to the regulation of physiological processes, including (anti)coagulation, inflammation, and regulation of endothelial functions. Under abnormal (stress) conditions, cells release microparticles that differ in numbers, composition, and function (right, red), thereby contributing to a procoagulant and proinflammatory phenotype, endothelial dysfunction, as part of the development of CVD.

Interestingly, elevated platelet microparticles were described in patients with both type 1 and 2 diabetes, hyperlipidemia, obesity/metabolic syndrome, and hypertension.[3,5] Thus, in plasma samples from patients with chronic severe hypertension compared with patients with mild hypertension and controls, more microparticles exposing platelet endothelial cell adhesion molecule-1 (PECAM-1) (CD31), but not glycoprotein Ib (CD42) (ie, microparticles presumably of endothelial or platelet origin or both), were found.[48,49] In these patients, elevated numbers of microparticles are likely to reflect the cellular stress of endothelial cells and platelets. In addition, microparticles from plasma of hypertensive patients with albuminuria attenuated proliferation and migration of endothelial progenitor cells and increased endothelial hydrogen peroxide production, cellular senescence, and apoptosis compared with microparticles from hypertensive patients with normoalbuminuria.[50] This suggests that the release of microparticles contributes to the progression of endothelial damage, which could then result in a vicious circle.

High numbers of circulating microparticles of different cellular origin have been associated with subclinical atherosclerosis, measured by carotid intima-media thickness and coronary artery calcifications, quantified by computed tomography, as previously described.[8,15,51–53] Recently, it was proposed that circulating microparticles are the major factor associated with carotid artery remodeling, with high numbers of circulating microparticles preventing compensatory remodeling in vessels with increased carotid intima-media thickness.[53] Moreover, T-cadherin–exposing endothelial microparticles were elevated in patients with subclinical atherosclerosis compared with healthy subjects and patients with established coronary artery disease, suggesting that the shedding of T-cadherin–positive microparticles represents a protective mechanism that can shift the balance in cellular stress response to the prosurvival signaling branches, which is only later followed by deleterious and apoptotic phases.[15] This indicates that the release of microparticles attenuates compensatory outward artery remodeling in patients with (subclinical) atherosclerosis. However, longitudinal, rather than the presently available cross-sectional, studies are required to definitively demonstrate the causative role of microparticles in arterial remodeling.

An important clinical question is whether microparticles are markers of CVD risk and whether they can be used as independent predictors of CVD outcome in relevant populations. Recently, the associations of the Framingham risk score and numbers of platelet-, leukocyte-, endothelial progenitor cell–, and endothelium-derived microparticles were reported.[52,54,55] From these 4 studies, only 1 included a prospective investigation, in which 488 patients with various CVD risk factors were observed for a mean of 36 months.[55] The addition of endothelial (CD144+) microparticles to the Framingham risk model improved the classification of risk and appeared as a significant and independent predictor of future CVD events in a high-risk population.[55] These results are promising and imply that more prospective studies are needed to further detail the prognostic value of cell-derived microparticles in individuals at high risk for CVD. The lack of large-scaled studies in which microparticles are measured routinely may be partly because of the fact that the measurement of microparticles is still complex and elaborate. The detection and characterization of (individual) microparticles and other vesicles remain difficult because of their small size and heterogeneity, which has led to confusing and sometimes conflicting results between laboratories.[56] In addition, the detection of rare microparticles (eg, endothelial microparticles) remains a real challenge. Although some laboratories have used antibodies against specific endothelial proteins, other investigators[56,57] have used non–endothelial-specific antibodies or combinations.

Conclusion

Because their presence and role in various diseases has been recognized, the interest in cell-derived microparticles, also as markers or mediators in the development of CVD, has grown substantially. During the past years, the role of microparticles in the development of CVD has changed from "platelet dust" or artifacts to novel and possibly essential elements in cellular

homeostasis and communication. Furthermore, the release of microparticles contributes to the activation of (intentionally protective) protein cascades, such as coagulation, inflammation, and endothelial dysfunction (Figure), which all facilitate atherogenesis. Therefore, microparticles may not only reflect the presence of CVD but also play a causative role in its development.

On the other hand, the release of microparticles can protect cells from dangerous or redundant products, compounds, or cellular waste that may accumulate in response to cell stress induced by, for example, CVD risk factors. Thus, microparticles can contribute to cellular homeostasis. Although the release of microparticles may be beneficial to the individual cell releasing them, this release can also have paracrine and even systemic effects, which affects the surrounding cells. Whether cell-derived microparticles are a cause, consequence, or both of CVD remains to be established. Large-scaled prospective studies, rather than the presently available cross-sectional studies, are mandatory to more definitively demonstrate the interrelation of changes in occurrence, phenotype, and function of microparticles and the incidence and progression of CVD in various populations.

Future Directions

Future research should focus on further characterization of cell-derived microparticles as opposed to exosomes and possibly other cell-derived vesicles in various diseases. Moreover, multidisciplinary research should focus on further refinement and validation of detection methods of microparticles. These methodological investigations may contribute to more uniformity and validation of microparticle quantification, detection, identification of their parent cell, and biochemical and functional characterization. With a multidisciplinary approach and the proper verification studies in relevant patient populations, microparticles may prove to be true biomarkers of disease state and progression.

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