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Shed membrane microparticles from circulating and vascular cells in regulating vascular function

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Martínez, M. Carmen, Angela Tesse, Fatiha Zobairi, and Ramaroson Andriantsitohaina. Shed membrane microparticles from circulating and vascular cells in regulating vascular function. Am J Physiol Heart Circ Physiol 288: H1004–H1009, 2005; doi:10.1152/ajpheart.00842.2004.—Inflammation has a pivotal role in the development of atherosclerosis and acute activation of the vascular wall with consecutive local thrombosis and altered vasomotion. This process is orchestrated by the interactions between inflammatory cells, such as platelets and T and B lymphocytes, and vascular cells, endothelial cells, and smooth muscle cells. When they are activated by an agonist, shear stress, or apoptosis, these cells release vesicles shed from the blebbing plasma membrane called microparticles. Microparticles harbor cell surface proteins and contain cytoplasmic components of the original cell. They exhibit negatively charged phospholipids, chiefly phosphatidylserine, at their surface, which accounts for their procoagulant character and proinflammatory properties, including alteration of vascular function. Elevated levels of circulating microparticles have been detected in pathological states associated with vascular dysfunction, including attenuation of endothelium-dependent vasodilatation and/or alteration of responsiveness of vascular smooth muscle to vasoconstrictor stimuli in conduit and resistance arteries. This review points out the characteristics of microparticles as well as the biological messages they can mediate. In particular, it summarizes the signaling cascades involved in microparticle-induced vascular dysfunction with special attention to the cellular origin of these vesicles (platelet, endothelial, and leukocytic), which may explain their differential consequences on vascular remodeling. The available information provides a rationale for the paracrine role of microparticles as vectors of transcellular exchange of message between circulating cells and cells from the vessel wall. This review focuses on the description of microparticle interactions with cells of the cardiovascular system and, in particular, on the regulation of vasomotion in relation to endothelial dysfunction and smooth muscle reactivity.

CHARACTERISTICS OF MICROPARTICLES: GENERATION, COMPOSITION, AND QUANTITY

During cell activation by agonists or physical or chemical stress, including apoptosis and subsequent increase of intracellular calcium concentration, modifications of the plasma membrane, such as phosphatidylserine externalization, and an increase in bleb formation take place (34). All cell types subjected to activation can virtually release the membrane fragments, called microparticles, generated from blebs. Microparticles are small vesicles (0.05–1 μm), and their composition reflects the state of the membrane of the originating cell. In general, microparticles derive from circulating cells, such as platelets, leukocytes, and erythrocytes, and cells that compose the vessel wall, mainly endothelial cells, macrophages, and smooth muscle cells. The importance of microparticles in physiology and pathophysiology in regulating coagulation and thrombosis was first demonstrated in the field of hemostasis (10), which is beyond the scope of this review.

There are many good reviews regarding the composition of microparticles, the membranes of which consist mainly of lipids such as phosphatidylserine and several proteins (33). Indeed, on the surface, microvesicles bear antigens characteristic of the cell from which they originated and carry other membrane and cytoplasmic constituents. For example, functional adhesion complexes such as glycoprotein IIb-IIIa and P-selectin are harbored by microparticles from platelets (12). Microparticles of endothelial origin carry CD31 or CD146, whereas CD4, CD3, or CD8 is present at the surface membrane of leukocytic microparticles (18, 19).

These properties of microparticles are important, because they participate in the mechanisms by which they mediate the signals and in the differential consequences on the cell types that are activated. Thus microparticles can be viewed as a new pathway that can be used by cells to exchange information in...
addition to the transduction linked to the activation of classical known receptors or transporters.

Microparticles are present in blood from healthy individuals and patients (4). Thus they probably play a physiological and/or pathophysiological role. A recent review advances the hypothesis that microparticles should play a part in development, angiogenesis, wound healing, and, more generally, tissue remodeling, in the form of positive or negative gradients of information delivered to neighboring cells (10). Microparticles have been studied in various disease states, in which their number, cellular source, and composition are altered. Evidence is emerging that microparticles play an important role in coagulation, inflammation, and vascular dysfunction (for a review, see Ref. 10). However, their effects on vascular functions are unclear, and only a few studies have addressed this point.

The majority of in vivo circulating microparticles derive from platelets compared with microparticles from other circulating or vascular cells. Under several pathological situations, the number of total microparticles as well as the proportion of their different origins can change (33). Thus, in diseases such as atherosclerosis, congestive heart failure, diabetes, preeclampsia, and cancer, the level of circulating microparticles is considerably enhanced. It is difficult to determine the number of circulating microparticles in these diseases, because the methods used to quantify them are different (protein amount, procoagulant ability of phosphatidylserine exposed on their surface, and number of microparticles determined by flow cytometry) and need to be standardized, but circulating microparticles are increased in patients with the above-mentioned diseases compared with healthy patients, regardless of the method employed.

The phenotype of circulating microparticles is also different in different pathological states, and detection of its cellular origin may serve as a predictor or marker of the diseases (Fig. 1). Indeed, platelet microparticles are enhanced in myocardial infarction (18), hypertension (24), diabetes (22), and cancer (16), whereas endothelial microparticles are the most abundant in acute coronary syndromes (18) and Type I diabetes mellitus (26). In diabetic patients, the number of microparticles of leukocyte origin is threefold higher than in healthy donors (26). In addition, human immunodeficiency virus (HIV)-infected patients show elevated levels of microparticles bearing CD4 antigen (1). Elevated levels of microparticles from granulocytes and lymphocytes have been reported in preeclampsia (31). Also, in severe trauma, circulating levels of microparticles generated from activated leukocytes and harboring adhesion markers were enhanced (11). Only two reports mentioned that microparticles can be released by smooth muscle cells (28, 6), but these data suggest a potential role of smooth muscle cell microparticles in atherosclerosis and thrombus formation. Because of the variety of microparticles, it is plausible that they may exert pleiotropic effects on the vascular wall. Moreover, depending on the microparticle composition, one can speculate that different subpopulations of microparticles (from platelets, leukocytes, etc.) may serve as vectors of exchange of specific message in regulating vascular function and dysfunction.

**LONG-RANGE SIGNALING OF MICROPARTICLES**

The physiological and pathophysiological roles of microparticles are unclear: they can be beneficial or deleterious, depending on situations. Whereas a body of data implicates microparticles generated from platelets in the initiation and amplification of the coagulation cascade and in thrombosis, the function of microparticles from other cells (leukocytes and endothelial cells) is uncertain, but without any doubt, it is linked to the molecules harbored at their surface or within their “cytoplasm.”

Recent data provide evidence that the microparticles, independent of their origin, can transfer biological information between cells, acting as veritable vectors of signal molecules. Even though microparticles can act on hematopoietic and circulating cells, most of the exchange of information from microparticles takes place at the level of the endothelium and contributes to the physiological and pathophysiological role of microparticles. Thus they can affect vasodilatation and anti-thrombotic and antiadhesive properties of the vascular wall. Also, microparticles may be involved in the regulation of vascular permeability and smooth muscle cell proliferation.

In addition to their role in the regulation of hemostasis and thrombosis, platelet microparticles evoke monocyte adhesion to endothelial cells by inducing adhesion molecule exposure, stimulate proliferation, survival, adhesion, and chemotaxis of hematopoietic cells and increase engraftment of hematopoietic stem cells (2, 13). Also, platelet microparticles induce angiogenesis in vitro (15), probably through activation of endothelial cells (3). Microparticles generated from endothelial cells bear molecules able to initiate coagulation, induce monocyte adhe-

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**Fig. 1.** Cellular origin of circulating microparticles in different pathological states. HIV, human immunodeficiency virus.
sion, activate neutrophils, and promote angiogenesis (29). The microparticles shed by leukocytes can stimulate endothelial cells (20), transfer tissue factor to platelets (25), and impair endothelium-dependent vasodilatation (19).

MICROPARTICLE EFFECTS ON THE VASCULAR SYSTEM

The field of microparticles and regulation of vascular tone is recent, and little information is available. However, the effects of microparticles can be summarized as follows: they can affect endothelial cell (5, 19) and smooth muscle cell (23, 30) responses and, hence, vasoreactivity as well as angiogenesis (15, 29). Endothelial responses can be acute, resulting from the release of several factors, or prolonged, implying changes in expression of genes involved in structural and functional regulation of the vascular wall. Indeed, the endothelium is a primary target for cardiovascular risk factor, in which the effects of microparticles may constitute an adaptive phenomenon or contribute to the aggravation of diseases. Little information is available regarding the effect of microparticles on the regulation of vascular tone via a direct action on smooth muscle. Nevertheless, the primary triggers that influence the regulatory function of the blood vessel by microparticles depend on their cellular origin independently of the process involved in their release. Two well-known cellular processes lead to the release of microparticles: cell activation and apoptosis (34). The mechanisms governing their formation are probably different, accounting for differences in terms of size and lipid and protein composition. Jimenez et al. (14) observed that surface markers of endothelial microparticles in in vitro activation are different from those in vitro apoptosis. It is difficult to discriminate between microparticles released by in vivo activated and apoptotic cells.

Here, in this article, we attempt to summarize the effects of microparticles on vascular function at the level of endothelial and smooth muscle cells depending on their cellular origin (Fig. 2). To the best of our knowledge, very few reports address the effect of microparticles on resistance arteries.

Microparticles generated from activated platelets stimulate platelets and endothelial cells through modifications of arachidonic acid metabolism and generation of thromboxane A2 (TxA2). Indeed, platelet microparticles induce expression of the proinflammatory inducible isoform of cyclooxygenase (COX-2) and the generation and release of prostacyclin (PGI2) (3). Recently, it has been shown that platelet microparticles enhance arachidonic acid-induced contractions in the aorta and methacholine-induced contractions in rabbit pulmonary arteries (23). These effects are inhibited by thromboxane receptor antagonists and thromboxane synthase inhibitors. This report emphasizes the ability of platelet microparticles to act as a cellular source of TxA2 and, thus, regulate vascular tone. Although there is no evidence of a link between platelet microparticles and the genesis of the inflammation, these results suggest that these microparticles may play a role in development of inflammatory diseases in vivo.

An increased number of circulating endothelial microparticles has been observed in several pathologies reflecting endothelial cell damage and dysfunction. In addition, endothelial microparticles alone can aggravate endothelial dysfunction. Indeed, microparticles generated from endothelial cells impair endothelium-dependent relaxation and nitric oxide (NO) production in the rat aorta (7). It has also been shown that the effect induced by endothelial microparticles is related to an increase in superoxide anion production (7), which might reduce the bioavailability of NO.

Few studies have shown the role of microparticles shed from smooth muscle cells. Schecter et al. (28) suggested that active extracellu lar tissue factor found in the injured arterial wall and atherosclerotic plaques derives, in part, from smooth muscle cell microparticles. In another study, it was shown that the ability of apoptotic smooth muscle cell-generated microparticles to enhance thrombus formation is reduced in the presence of functional tissue factor harbored at their surface (6). Thus tissue factor-rich microparticles from smooth muscle cells might have the main role in generation and maintenance of atherosclerotic plaques.

As described above, under several pathological situations, the level of circulating microparticles generated from leukocytes is strongly enhanced. In this context, it appears relevant to explore the effects of this type of microparticles on vascular function, inasmuch as contact between lymphocytes and endothelial cells is a prerequisite for the recruitment of immune cells from blood at the sites of inflammation. Lipopolysaccharide-stimulated monocytes release microparticles bearing tissue factor and active adhesion complexes, disseminating a

Fig. 2. Representative studies describing microparticle-evoked effects, depending on their cellular origin, in the vascular system. NO, nitric oxide; TxA2, thromboxane A2; PGI2, prostacyclin.
procoagulant potential (27). Also, microparticles shed from activated monocytes are a major secretory pathway for the rapid release of the proinflammatory cytokine interleukin-1β (17). Thus monocytic microparticles could participate in development of the inflammatory response.

It has been established that microparticles from freshly isolated leukocytes behave as inflammatory mediators and initiate signal transduction in human umbilical vein endothelial cells (20). Among the activated pathways, leukocyte microparticles stimulate the secretion of interleukin-6 in endothelial cells through the phosphorylation of JNK1 without the involvement of NF-κB or the ERK pathway (21).

In vitro apoptotic T lymphocyte-derived microparticles, at concentrations that can be reached in circulating blood under immunological dysfunction (e.g., HIV), impair endothelium-dependent relaxation in conductance and small resistance arteries in response to agonist and shear stress, respectively (19). Interestingly, microparticle treatment affects NO- and PGI2-dependent relaxation in conductance and small resistance arteries in response to vasoconstrictor agents in mouse aorta, in addition, the effects of microparticles generated in vitro from T lymphocytes are not mediated through their interaction with adhesion molecules such as leukocyte functional antigen-1. Furthermore, these effects are independent of Fas-Fas ligand interaction, because microparticles, lacking Fas or Fas ligand, generated after T lymphocyte activation and not apoptosis, evoke similar effects (i.e., reduction of endothelial NO synthase and overexpression of caveolin-1).

Also, T lymphocyte-derived microparticles can affect vascular contraction by acting directly on smooth muscle cells (Fig. 3) (30). These microparticles induce vascular hyporeactivity in response to vasoconstrictor agents in mouse aorta, in that they are reversed by NO synthase plus COX-2 inhibitors. The hyporeactivity induced by microparticles is associated with an increased production of NO and PGI2 occurring from upregulation of proinflammatory protein expression, inducible NO synthase, and COX-2. The mechanism involves an interaction of microparticles with smooth muscle cells through the Fas-Fas ligand pathway responsible for the activation of NF-κB, which in turn upregulates inducible NO synthase and COX-2 expression. These data provide a rationale to explain the paracrine role of microparticles as vectors of transcellular exchange of message in promoting vascular dysfunction during inflammatory diseases.

Another important action of microparticles in the vascular system is their ability to induce angiogenesis. It has been shown that platelet microparticles from healthy individuals promote proliferation, migration, and tube formation in cultured endothelial cells. The latter effects of microparticles are mediated by their lipid components, probably sphingosine 1-phosphate. The ability of platelet microparticles to induce angiogenesis is related to the activation of ERK and phosphoinoside 3-kinase pathways (15). Also, microparticles of endothelial origin can elicit angiogenesis, but the mechanisms by which they mediate their effects are different from those reported for platelet microparticles. Indeed, metalloproteinases harbored by endothelial microparticles regulate the focalized proteolytic activity essential for invasion during neovascular structure formation (29). Although these effects have been described in in vitro systems, one would expect that this effect of endothelial microparticles may contribute to neovascularization in in vivo situations.

**EFFECTS OF MICROPARTICLES ON INFLAMMATORY DISEASES**

Circulating microparticles, rich in endothelial and platelet surface markers, from patients with acute myocardial infarction cause severe endothelial dysfunction in rat aorta by affecting the endothelial NO transduction pathway but not endothelial NO synthase expression (5). Although the total number of circulating microparticles was unaltered in preeclampsia, the proportion of T lymphocytes and granulocyte microparticles is increased in preeclamptic women (31). Circulating microparticles from these patients abolish endothelium-dependent relaxation, in contrast to mi-
cromic particles from healthy pregnant women (32). However, the mechanisms responsible for diminished endothelium-dependent relaxation after microparticle treatment have not been identified.

In another study, lymphocyte-derived microparticles from diabetic patients or in vivo circulating microparticles from diabetic or HIV-infected patients impaired endothelial NO synthase expression to the same extent as in vitro T lymphocyte-generated microparticles (19). Although circulating microparticles from diabetic patients are heterogeneous, they contain an elevated proportion of microparticles derived from T cells, which may explain the results. These data and those obtained from microparticles generated during acute myocardial infarction (5) and preeclampsia (32) indicate that, independent of their origin, microparticles alter NO metabolism and can participate in development of cardiovascular diseases.

On the other hand, circulating microparticles from diabetic patients significantly reduce the contractile response to agonists through the interaction of Fas ligand from microparticles with Fas from the vessel wall, leading to intracellular signaling. Furthermore, microparticles shed by the apoptotic lymphocytes of the same diabetic patients are able to induce vascular hyporeactivity (30). Microparticles induce hyporeactivity even in vessels with functional endothelium (30), in which they produce endothelial dysfunction (19). Thus the overall effect of microparticles was to reduce the response to vasoconstrictor agents. Similar observations were reported in inflammatory disorders such as cirrhosis, portal hypertension (8), and sepsis (9), in which vascular hyporesponsiveness to a vasoconstrictor and reduced endothelium-dependent relaxation have been reported. These results emphasize the role of microparticles as vectors of transcellular exchange of message in promoting vascular dysfunction accompanying inflammatory diseases.

CONCLUSION

Several lines of data support the hypothesis that microparticles contribute to vascular homeostasis and the pathogenesis of cardiovascular diseases, including inflammation and vascular dysfunction, in addition to their well-known action on the coagulation process. With regard to the regulation of vascular tone, microparticles are involved in endothelial dysfunction in conductance and resistance arteries. The mechanisms depend on the cellular origin of the microparticles and may trigger differential consequences on vascular remodeling. The most important impact is probably on alteration of the shear stress-induced response, which is important in the processes leading to atherosclerosis. However, two crucial questions need to be fully addressed. First, is the pivotal role of microparticles a cause or a consequence of vascular diseases? Microparticles are able to induce vascular dysfunction, on the one hand, and can be formed and released on cell activation by inflammatory mediators such as cytokines (interleukin-1β or interleukin-6), on the other. Second, would the knowledge of microparticle composition and constituents for a better understanding of the mechanism driven by the phenotype of microparticles involved be helpful in the search for therapeutic agents? Nevertheless, microparticles can be considered vectors of transcellular exchange of message in regulating vascular function at the same level as mediators acting on different receptor-mediated events.

REFERENCES


