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Microparticles and Exosomes: Are They Part of Important Pathways in Sepsis Pathophysiology?

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1. Introduction

Microparticles are a heterogeneous population of small membrane-coated vesicles released by several cell lines upon activation or apoptosis. Microparticles generation seems to be a well regulated process, although these vesicles are highly variable in size, composition and function. Despite being previously considered inert debris without specific function, recent data demonstrated important pathophysiological mechanisms orchestrated by microparticles in vascular diseases associated with endothelial dysfunction. These vesicles have been implicated, among others, in the pathogenesis of thrombosis, inflammation, atherosclerosis and vascular cell proliferation. In addition to microparticles, activation of the endocytic-lisosomal cellular system of circulating cells induce the release of smaller vesicles denominated exosomes that can also participate in vascular derangement. This role of microparticles and exosomes in mediating vascular dysfunction suggests that they may represent novel pathways in short or long-distance paracrine transcellular signaling in vascular environment. The mechanisms involved in the origin of microparticles and exosomes, their composition and participation in the pathogenesis of sepsis will be discussed in this review.

2. Origin of microparticles

Circulating cells in vascular environment as well as endothelial cells after activation or apoptosis are capable of releasing membranous fragments (vesicles), of size varying from 100 nm to 1000 nm (Fig. (**1**)). These vesicles present, on their surfaces, at least some of the antigenic markers of the parent cell (Azevedo et al., 2007). The first description of these vesicles was made in 1967, with the reports of a "platelet dust" (platelet membrane fragments) in human plasma (Wolf et al., 1967). After a more precise characterization on their origin, composition and function, these vesicles were called microparticles (or microvesicles) and there is now increasing evidence for their role in transcellular communication in microvascular environment. However, the precise functions of these fragments and their interaction with cells in vasculature remain incomplete.

The release of microparticles has been described in several circumstances in normal physiology as well as in disease states. In health, it has been reported that 80% of

circulating microparticles express membrane antigens that suggest a platelet origin. These vesicles have also been implicated to play a role in inflammation, coagulation and diseases associated with impairment of vascular function, e.g. atherosclerosis, diabetes and hypertension (Tushuizen et al., 2011). Usually, microparticles release is the result of cell activation or apoptosis, although it is not known whether these events lead to the formation of similar microparticles, in terms of size, lipid and protein composition and pathophysiological effects. Microparticles release is an integral part of the membrane-remodeling process in which the asymmetric distribution of constitutive phospholipids between the two leaflets of the cell membrane is lost, with released microparticles exposing phosphatidylserine in the outer membrane, which acts as a template for the prothrombinase complex assembly and for their role in coagulation activation (Zwaal & Schroit, 1997).

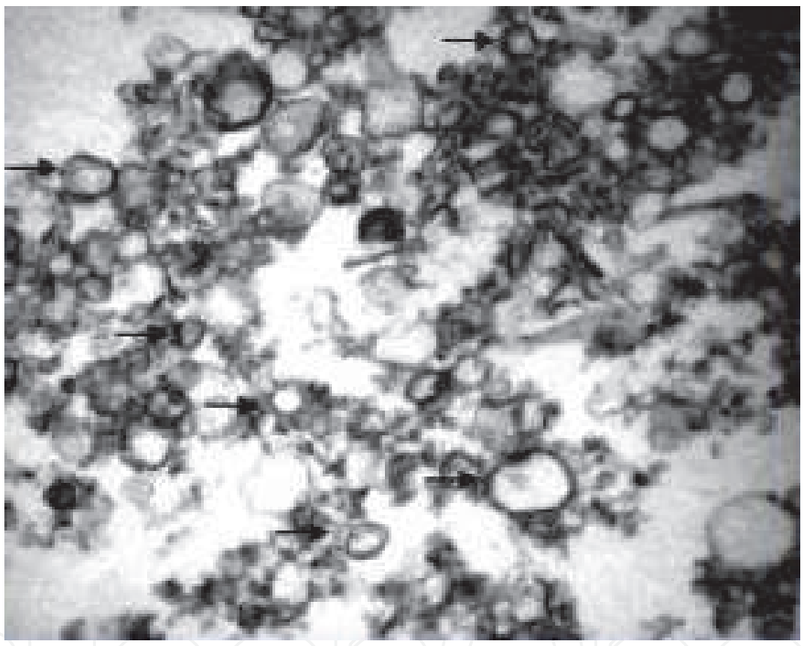

Fig. 1. Microparticles (arrows) isolated from plasma of a patient 24 hours after percutaneous coronary angioplasty. Electron micrography depicts a heterogeneous population of vesicles ranging in size from 80 to 200 nm (original magnification X39000). Adapted from Azevedo et al., 2007 with permission.

It is important to note, in first place, that circulating microparticles are a population of vesicles from different cell types and from different cellular compartment origins. As already discussed, the most common mechanism of microparticle release is cell activation or apoptosis, which induces plasma membrane budding, leading to the formation of membrane blebs. However, apoptosis can also induce the formation of apoptotic bodies, which are sometimes considered as members of microparticles' family. Apoptotic bodies are cell fragments many times larger in diameter and volume than microparticles that are

consequence of cell fragmentation in the final stages of apoptosis, in contrast with microparticles, that are released in the early moments of programmed cell death. These apoptotic bodies also expose phosphatidylserine in the outer membrane, but unlike microparticles, have poor prothrombotic properties. Probably, the role of phosphatidylserine in these corpuscles is to recruit phagocytic cells to the site of apoptotic death (Janiszewski et al, 2004).

Other type of vesicle released after cell activation that can sometimes overlap microparticle function is the exosome. Dissimilar to microparticles that are fragments of cell membranes, exosomes are vesicles produced in the endocytic-lysosomal system of several cell lines. Exosomes are smaller than microparticles (usually less than 0.1 μm), have different surface antigens and poor clotting capacity (Azevedo et al, 2007). The role of exosomes in sepsis will be discussed in more details below.

Virtually all cell types subjected to activation can release microparticles after blebbing of plasma membrane. The most common cell types associated with microparticle release are platelets, endothelial cells, neutrophils, smooth muscle cells, monocytes and T lymphocytes. (Azevedo et al, 2007). Their ubiquity has suggested a more general role for microparticles in cellular regulation, possibly with functions reminiscent of their original cell.

3. Composition of microparticles

The membrane of microparticles, which is derived from the parental cell plasma membrane, consists primarily of lipids and proteins, in variable amounts. The precise content of lipids and proteins is dependent on the cell they originate from and the type of stimulus involved in their formation.

Microparticles are surrounded by a phospholipid bilayer. During the budding process, the normal phospholipid asymmetry of the membrane is lost, with microparticles exposing negatively charged phospholipids such as phosphatidylserine (PS) and phosphatidylethanolamine (PE) in their outer membrane leaflet. Exposure of PS plays a role in the in vivo effects of microparticles, since PS is an efficient site for coagulation factor activation. Analysis of components of microparticles from blood of healthy donors indicates that phosphatidylcholine represents more than 60% of their lipid content (Weerheim et al, 2002). Other lipids present in minor concentrations are sphingomyelin, PS and PE. Although these microparticles are in the vast majority derived from platelets (~75%), their phospholipid composition is different from parental cell.

Protein composition in the surface of microparticles is dependent of parental cell type. These surface antigens are specific for the cell they originate from and can help to identify the origin of microparticle. However, microparticle can differ in the expression of cell surface molecules from their parental cells. This is particularly important when microparticles express molecules upregulated or translocated by cell activation or apoptosis. For example, IL-1 alpha-activated cultured endothelial cells can release microparticles displaying E-selectin and endothelial cell adhesion molecule 1 in significantly higher concentrations than resting cultivated endothelial cells (Abid Hussein et al, 2003). There are also data depicting different protein composition of microparticles in response to different agonists and with the same parental cell stimulated with the same agonist (Hughes et al, 2000). Taken together, these differences indicate that shedding seems to be a well-regulated process that originates unique microparticle characteristics depending on the cell source, stimulus, scenario and pathophysiological conditions.

More recent data demonstrate that, besides proteins and lipid composition on the surface of microparticles, the inner portion of these vesicles also contain several enzymes and genetic material capable of interacting and producing effects on the target cell (Meziani et al, 2010). Subunits of enzymes with superoxide producing activity like NADPH oxidase have been identified on microparticles originated from platelets (Janiszewski et al, 2004). In addition, microparticles and exosomes from cultured cells and normal individuals have been demonstrated to contain mRNA and microRNA, which suggests that these vesicles may play a role on the cell to cell transfer of genetic contents (Hunter et al, 2008 and Valadi et al, 2007).

4. Mechanisms of microparticle release

Platelets release microparticles after activation by thrombin, ADP plus collagen, calcium ionophore A23187 and high shear stress. Endothelial cells, monocytes and vascular smooth cells can release microparticles after activation by bacterial lipopolysaccharide, inflammatory cytokines, complement complex C5b-9 or reactive oxygen species (Boulanger et al, 2006).

The mechanisms governing plasma membrane shedding and consequent microparticle release are only partially understood. Usually, the shedding starts within minutes after addition of an agonist, by a calcium-dependent process that can be blocked by calcium chelators. One of the possible molecules governing this process is calpain μ, which is a calcium-dependent cytosolic-protease that cleaves talin and α-actin. Inhibition of calpain by calpeptin or calcium chelators prevents the release of microparticles (Azevedo et al, 2007). However, molecules other than calpain may be involved in calcium-dependent microparticles release, since blockade by calpeptin did not induce an inhibition of microparticle release to the same extent as EGTA, suggesting a role for other calcium dependent processes. Cytosolic calcium increase may also activate kinases and inhibit phosphatases, which together with calpain activation, are responsible for cytoskeleton disruption. Membrane skeleton disruption is the result of several mechanisms, such as myosin light-chain phosphorylation mediated by myosin light-chain kinase (MLCK) upon activation or Rho-associated kinase I (ROCK-I), in apoptosis. Phosphorilation of myosin light chains (MLC) stimulates the contractile activity of myosin, with myosin ATPase activation creating movement between actin and myosin filaments (Azevedo et al, 2007). This movement may tensionate plasma membrane and cause detachment of the cytoskeleton from the membrane, with the formation of blebs and the subsequent release of microparticles. However, the precise interaction between cell membrane and cytoskeleton, which permits microparticle formation, is still unknown. Recent data has implicated ROCK-II (an isoform of ROCK-I) in thrombin-induced microparticle release from endothelial cells. These new data recently incorporated indicates that the knowledge on mechanisms inducing cytoskeleton rearrangement during bleb formation is still scarce.

Another important feature in microparticle formation is the loss of phospholipid asymmetry of membranes after cell activation. Usually, PS and PE are specifically segregated in the inner leaflet, whereas phosphatidylcholine and sphingomyelin are enriched in the external one. This distribution is controlled by three enzymes: an inward-directed pump, a flippase (aminophospholipid translocase), specific for PS and PE; an outward-directed pump referred to as "floppase"; and a lipid scramblase, promoting unspecific bidirectional

redistribution across the bilayer. The increase in calcium content after cell activation may lead to collapse of the membrane asymmetry by stimulating scramblase and floppase activities and concomitantly inhibiting the flippase. The increase in PS exposure in the outer leaflet that follows microparticle formation enhances coagulative properties and facilitates removal of apoptotic bodies by phagocytic cells. PS also binds annexin-5, which has been used in several studies for microparticle quantification (Azevedo et al, 2007).

Whether microparticles release is the result of cell membrane shedding, the release of exosomes from the parental cell is mainly orchestrated by the endocytic-lisossomal system. Endocytosis is a range of processes performed by the cell in order to internalize specific regions of the plasma membrane as well as small amounts of extracellular fluid. In this process, intracellular compartments of endocytic pathway called multivesicular bodies (MVB) composed of numerous vesicles are able to fuse with the plasma membrane, releasing these vesicles abroad. After incorporation into the cell, the absorbed material is accumulated in endosomes, which are major sites of entry for the captured molecules. The endosomes then become MVB, which are characterized by being more spherical, had lower intra-luminal pH and a different protein distribution. It is unclear the mechanism by which endosomes become MVB. In MVB, the presence of vesicular bodies inside is better characterized and once formed, these structures are destined for several processes: they can serve as storage sites; They can direct proteins to be degraded through their fusion with lysosomes (organelles that constitute, together with the MVB, the major site of protein and lipid degradation in the cell); Or they can fuse with the plasma membrane, thereby releasing their vesicles (exosomes) into the extracellular medium (Azevedo et al, 2007).

5. Microparticles in inflammatory conditions and sepsis

There is now emerging evidence that microparticles and exosomes participate actively in regulation of vascular function in several healthy and disease states. Microparticles, regardless their cell origin, can transfer biological information between cells, therefore acting as vectors of signaling molecules. Most of the exchange of information from microparticles takes place at the level of endothelium and contributes to their (patho)physiological role. Microvesicles (microparticles and exosomes) have been reported to be part of the disease mechanisms in several conditions, such as inflammation, thrombosis and vascular dysfunction, all elements that are reported to be extremely involved in the pathogenesis of sepsis.

It is now demonstrated from in vitro and in vivo studies that microparticles may play a role in inflammatory conditions, since they display a variety of proinflammatory activities. Microparticles from endothelial cells, platelets and leukocytes can promote adhesion and rolling of leukocytes, contain proinflammatory cytokines and trigger the release of microparticles from several cell types in vitro (Huber at el, 2002, Forlow et al, 2000). In addition, oxidized phospholipids from endothelial microparticles released by oxidative stress may cause monocyte adherence to endothelial cells and neutrophil activation (Huber et al, 2002). A recent study demonstrated also that microparticles isolated from septic shock patients injected into rats induce the expression of inducible nitric oxide synthase, nuclear factor kappa B and cyclooxygenase-2 in the lungs and hearts of these animals (Mastronardi et al, 2011).

Besides endothelial microparticles, other vesicles released from different cell sources may have a role in mediating cellular interactions in vascular milieu. Platelet-derived microparticles, for instance, can enhance the binding of neutrophils to other neutrophils under flow conditions. This effect seems to be mediated by an interaction between P-selectin on microparticles and P-selectin-glycoprotein ligand 1 on neutrophils, since the blockade of these surface molecules can reduce this binding (Forlow et al, 2000). Microparticles derived from platelets can also stimulate monocyte-endothelial interactions, by delivering arachidonic acid to endothelial cells, which induces the upregulation of expression of cellular adhesion molecules (ICAM-1) on endothelium and CD11a/CD18 and CD11b/CD18 on monocytes (Barry et al, 1998).

Platelet-derived microparticles, as well as vesicles from other cell lines, can contribute to inflammation by stimulating the production of several cytokines. Microparticles derived from leukocytes can increase the production of IL-6, monocyte chemotactic protein 1 (MCP-1) and Tissue Factor (TF) in endothelial cells (Mesri & Altieri, 1999). Platelet-derived microparticles have been associated with increased production of IL-8, IL-1beta and TNF-alpha by a monocytic cell line (THP-1) and endothelial cells in high shear stress conditions (Nomura et al, 2004). In leukocytes, endotoxin stimulation induced the shedding of microvesicles containing platelet-activating factor (PAF), a known inducer of inflammatory response (Watanabe et al, 2003).

Evidence that microparticles participate in the genesis of inflammatory diseases is supported by studies that depicted increased number of microparticles in inflammatory conditions in vivo. Meningococcal sepsis, for instance, is associated with increased levels of microparticles released mainly from granulocytes and platelets (Nieuwland et al, 2000). These vesicles are highly procoagulant, which demonstrates the correlation among inflammation and coagulation in the pathogenesis of several vascular diseases. In patients with sepsis and multiple organ dysfunction syndrome, Joop et al found increased number of microparticles released from granulocytes, and diminished levels of microparticles derived from platelets and erythrocytes were also found. Trauma patients have also increased levels of leukocyte microparticles with enhanced expression of adhesion molecules on days 2 to 5 after injury (Fujimi et al, 2003). In addition, in sepsis, circulating levels of endothelial and platelet microparticles were negatively correlated with unfavorable outcomes during multiple organ dysfunction syndrome (Soriano et al, 2005).

Sepsis has also been associated with significant endothelial dysfunction. Many studies have isolated microparticles from blood of patients with disease states marked by vascular dysfunction, and these vesicles were associated with this impairment in isolated arteries (Martin et al, 2004, Tesse et al 2005). Microparticles released from T-lymphocytes are capable of impairing endothelial function after 12 or 24 hours of incubation, also decreasing eNOS expression and increasing caveolin-1 expression of endothelial cells in culture (Martin et al, 2004). Another investigation reported impairment of vascular function with microparticles released from an apoptotic T cell line in a mechanism associated with transcription factor NF-κB production and proinflammatory protein upregulation (Tesse et al, 2005). Microparticles originated from endothelial cells in culture induce superoxide production by aortic rings associated with impairment of acethylcoline-induced vasorelaxation. These microvesicles also inhibit NO production by aortic rings and display p22(phox), a subunit of superoxide-producing enzyme NADPH

oxidase, thus demonstrating an important role for oxidative stress in vascular dysfunction (Brodsky et al, 2004). However, there is still a lot to be discovered on the mechanisms of microparticles induced endothelial dysfunction.

Another hallmark of sepsis is activation of coagulation. The most known property of microparticles is their ability to induce coagulation activation with subsequent thrombosis of vascular beds. There is substantial in vitro evidence of the involvement of microparticles in activation of coagulation system (Muller et al, 2003). In vivo studies depicting increased concentrations of microparticles in diseases associated with coagulation activation corroborate the in vitro data (Nieuwland et al, 2000).

Circulating microparticles provide an additional procoagulant phospholipid surface for the assembly of the clotting enzymes complexes that promote thrombin generation. The assembly of vitamin-K dependent tenase and prothrombinase complexes in microparticles is known as platelet factor 3 activity. Platelet microparticles also display activated factor V in their surface, which may contribute to activation of clotting. Endothelial microparticles, in turn, released after stimulation of endothelial cells in culture with plasminogen activator inhibitor-1 (PAI-1) become procoagulant with an accelerated thrombin production (Brodsky et al, 2002). This activation of coagulation that occurs after microparticle release culminates with the generation of thrombin, which consequently induces hemostasis or a prothrombotic state. The stimulation of clotting that follows microparticle release requires a tight control exerted by natural anticoagulant systems. Indeed, binding of protein S to microparticle surface has already been described, with subsequent binding of protein C and activated protein C.

A more direct mechanism relating microparticles and initiation of coagulation was described when TF was reported to be present in the surface of platelet-derived microvesicles (Muller et al, 2003). TF has also been described in microparticles derived from monocytes and smooth muscle cells after apoptosis (Azevedo et al, 2007). Moreover, microparticles are capable of inducing TF expression on monocytes (Sturk-Maquelin et al, 2003). Since TF mRNA has not been demonstrated in megakaryocytes, platelet-derived and microparticles-derived TF is likely to originate from other cell lines, incorporated in platelets by transcelular exchange (Scholz et al, 2002). Tissue factor production in microparticles has also been associated with inflammatory conditions such as meningococcal disease (Nieuwland et al, 2000), thus demonstrating the continuous interplay between inflammation and coagulation activation.

The evidence for microparticle contribution to coagulation in vivo is circumstantial. There are reports of increase in microparticle numbers in several diseases associated with hypercoagulation such as heparin induced thrombocytopenia and acute coronary syndromes. Moreover, several diseases related with hypercoagulation are associated with production of microparticles exposing TF, such as disseminated intravascular coagulation ((Nieuwland et al, 2000). This demonstrates a probable role of microparticles as contributors of vascular dysfunction in cardiovascular diseases and sepsis.

6. Exosomes are a special type of microparticles

Exosomes are frequently referred as a specialized category of microparticles with specific functions in immune response and protein sorting. They are released mainly from antigen presenting cells, although exosomes have been identified after platelet (Heijnen et al, 1999)

and mast cell (Denzer et al, 2000) activation and in body fluids, such as urine (Zhou et al, 2006) or bronchoalveolar lavage (Admyre et al, 2003). Dissimilar to microparticles, exosomes are more homogeneous in size (diameters ranging from 60 to 100 nm) and composition, and are enriched in tetraspaning proteins (Azevedo et al, 2007). They are also derived from endocytic-lisosomal cellular system, whereas microparticles are fragments of plasmatic membrane. Platelet-derived exosomes display PS in a much less extent than microparticles, thus they are poor coagulation activators (Heijnen et al, 1999). However, they exhibit major histocompatibility complex (MHC) class I or II molecules in their surface, which demonstrate their role in antigen presentation (Théry et al, 2002). Common filtration and centrifugation processes used to separate microparticles frequently cannot eliminate these small particles. Thus, some of the biologic effects of microparticles observed in the literature may be due to the presence of exosomes in the preparation. The major differences between microparticles and exosomes regarding origin and composition are described in table 1.

Property	Microparticles	Exosomes
Origin	Plasma Membrane	Endocytic-lisosomal system
Type of Generation	Regulated	Constitutive
Mechanism of Release	Shedding from plasma membrane	Exocytosis of MVB
Intracellular Storage	No	Yes
Protein Composition	Annexin 2, caspases	CD9, CD63, cytokines, Integrins
Lipid Composition	Cholesterol	Cheramides, cholesterol

Table 1. Main differences between microparticles and exosomes

The role of exosomes in sepsis remains deeply unexplored. One previous study from our laboratory identified in septic patients' plasma exosomes derived predominantly from platelets. These vesicles have been associated with vascular dysfunction of sepsis, due to their effects in inducing apoptosis of endothelial cells and vascular smooth muscle cells in culture, in a mechanism mediated by oxidative stress (Janiszewski et al, 2004; Gambim et al, 2007). They display components of NADPH oxidase in their membrane and are capable of production of reactive oxygen species *per se* (Janiszewski et al, 2004; Gambim et al, 2007). In addition, these vesicles may induce contractile dysfunction in isolated hearts as well as in isolated papillary muscle preparations. This dysfunction is enhanced by previous treatment of the animals with LPS and the mechanism associated is probably NO-mediated (Azevedo et al, 2007). Thus, in a condition associated with severe vascular dysfunction such as sepsis, exosomes may play a role in regulating cardiovascular function.

7. Conclusions

In this review we have assessed the current knowledge on microparticles formation, composition and function, as well as their role in sepsis. Accumulating data suggest that these microvesicles play a role in inflammation, thrombosis and vascular dysfunction, three pathways clearly involved in the pathogenesis of sepsis. Additional studies that clarify the composition of these vesicles as well as the underlying mechanisms involved in their effects

will probably help in the development of additional interventional strategies for prevention and treatment of sepsis.

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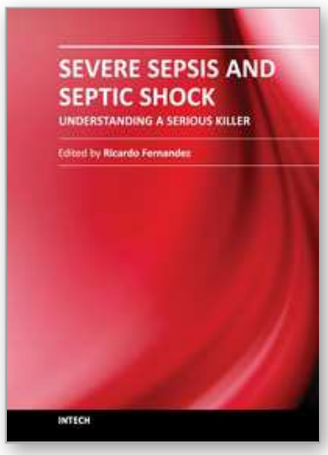

Severe Sepsis and Septic Shock - Understanding a Serious Killer
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Despite recent advances in the management of severe sepsis and septic shock, this condition continues to be the leading cause of death worldwide. Some experts usually consider sepsis as one of the most challenging syndromes because of its multiple presentations and the variety of its complications. Various investigators from all over the world got their chance in this book to provide important information regarding this deadly disease . We hope that the efforts of these investigators will result in a useful way to continue with intense work and interest for the care of our patients.

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