Microparticles in endothelial cell and vascular homeostasis: are they really noxious?

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Endothelial damage and release of membrane microparticles are key steps in the pathogenesis of inflammation, in the broad acceptance of the term.[1,2] At the site of endothelium injury, secretion of pro-inflammatory cytokines and expression of cytoadhesins by endothelial cells are well-described features involved in the recruitment and diapedesis of inflammatory cells. The concomitant breakdown of the endothelial barrier enables infiltration of the vascular wall or perivascular space by cells or mediators capable of perpetuating the inflammatory response. Having long been considered as inert remnants of cell destruction or surrogate markers of cell death, microparticles shed by apoptotic or damaged cells are now known to behave as potent (dys)regulators of endothelial function. Interacting with proximal or distal cells, they contribute to a long-range transmission of biological information that may ultimately alter endothelial function itself.[2]

Indeed, both inflammation and thrombosis are orchestrated by interactions between circulating cells, platelets, vascular and smooth muscle cells and derived microparticles. Two distinct storage pools of microparticles may be involved in the pathophysiology of thrombosis: (i) circulating microparticles released from vascular and peripheral blood cells, and (ii) microparticles shed by apoptotic cells sequestered within the vascular wall and eventually exposed to the blood flow after plaque rupture. There is recent evidence that thrombosis can be triggered by tissue factor, the major initiator of the clotting cascade, disseminated by microparticles in peripheral blood. It has been consistently reported that deleterious circulating microparticles of various vascular origins can elicit an adverse response by endothelial cells, including pro-inflammatory and pro-thrombotic pathogenic amplification. Experimental and clinical data underscore the noxious causative and multiple roles of microparticles in inflammatory responses, modulation of vascular tone, vascular remodeling, angiogenesis or apoptosis assessed in various pathologies such as atherothrombosis, vascularitis, sepsis and anaphylaxis. For instance, in atherothrombosis, microparticles were reported to be involved in: (i) cytokine release and cytoadhesin expression by endothelium and leukocytes, (ii) monocyte arrest and plaque infiltration, (iii) smooth muscle cell proliferation, (iv) proteolysis, (v) neovessel formation, a source of intraplaque hemorrhage in advanced lesions, (vii) enhanced oxidative stress, and (viii) increased thrombogenicity.[2] On the other hand, since microparticles are thought to reflect a subtle balance between cell stimulation, proliferation, and death, it is conceivable that they act as signals for the maintenance of homeostasis in multicellular organisms. Challenging the linear model *from cell death to inexorable auto-amplification of tissue damage*, in this issue of the journal Pérez-Casal *et al.* propose another mechanism through which endothelial-derived microparticles harbouring endothelial protein C receptor (EPCR) can act as true cytoprotectors, thus contributing to endothelium homeostasis and barrier stabilization.[3]

Pro-inflammatory effects of microparticles on endothelial cells and the vascular wall

The effects of microparticles on the alterations of the pro-inflammatory endothelium phenotype have been documented by a number of investigations. The first demonstration that microparticles may affect the endothelial phenotype came from studies investigating the effects of platelet-derived microparticles on cultured endothelial cells. In a pioneering study, a transcellular microparticle-mediated delivery of arachidonic acid to endothelial cells and the concomitant expression of cyclooxygenase type 2 was demonstrated.[4] In addition, microparticles were shown to: (i) stimulate the release of pro-inflammatory endothelial cytokines, including interleukin (IL)-6, monocyte chemoattractant protein-1, and (ii) induce the expression of endothelial cytoadhesins (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 and E-selectin),[4,5] and (iii) induce the expression of appropriate counter-receptors at the leukocyte membrane surface. More recently, the transcellular delivery of the chemokine RANTES by platelet-derived microparticles was shown to promote monocyte arrest at the activated endothelial surface.[6] Altogether, these processes enable the recruitment and diapedesis of activated leukocytes. Microparticles were shown to favor endothelial activation and monocyte-endothelium interactions, the two initial steps of atherosclerotic plaque formation. Beyond their effect on vascular cells, circulating microparticles also contribute to an enhanced inflammatory vascular response through various mechanisms. Microparticles are providers of aminophospholipid substrates for secretory phospholipase A2 enabling the production of lysophosphatidic acid, a potent pro-inflammatory mediator and platelet agonist. Platelet-derived microparticles are able to bind, activate and aggregate neutrophils *in vitro*.[7] Monocyte-derived microparticles constitute a secretion pathway for IL-1β, an endothelial agonist.[8] When sequestered within the atheromatous plaque, microparticles disturb the inflammatory balance by exposing active tumor necrosis factor-alpha converting enzyme (TACE)/A Disintegrin And Metalloproteinase domain-17 (ADAM 17) that cleaves tumor necrosis factor (TNF) and its receptors.[9] Indeed, $TACE^{+}$-microparticles are able to stimulate the release of pro-inflammatory TNF-α by endothelial cells.

Anti-inflammatory effects of microparticles

The first hint suggesting an anti-inflammatory effect

of membrane vesicles was provided by the work of Gasser and Schifferli.[10] They demonstrated that neutrophil microvesicles have no pro-inflammatory activity on human macrophages as assessed by IL-8 and TNF-α release but increased the secretion of transforming growth factor β1, a potent inhibitor of macrophage activation. In addition such vesicles were able to block the inflammatory response of macrophages to lipopolysaccharide. Thus, neutrophils appear to release potent anti-inflammatory effectors, under the form of membrane vesicles, at the earliest stage of inflammation, already providing a drive to its resolution.[10] Recently, the anti-inflammatory effect of neutrophil-derived microparticles on endothelial cells was reported to follow a new pathway. When shed from adherent neutrophils, such microparticles bear annexin A1, an endogenous anti-inflammatory protein able to inhibit neutrophil adhesion to the endothelium.[11]

Prothrombotic potential of microparticles on endothelial cells

Various endothelial agonists such as cytokines, microorganisms, including *Chlamydia pneumoniae*, have been found to be able to induce membrane remodeling and exposure of procoagulant phospholipids, tissue factor expression and its release under a microparticle-bound form. On the one hand, the shedding of endothelial-derived microparticles (EMP) could be viewed beneficial as these microparticles contribute to the *sorting* of several deleterious pro-apoptotic factors such as caspase-3, thus preventing endothelial cell apoptosis and detachment.[12] On the other hand, in the vicinity of vascular damage, the procoagulant character of released microparticles could also alter the balance of hemostasis. Evidence of this hypothesis was first provided by Mesri and Altieri, who showed that circulating leukocyte microparticles are up-regulated by inflammatory stimulation *in vivo* and activate a stress signaling pathway in endothelial cells, leading to increased tissue factor activity.[5] Similarly, monocyte-derived microparticles up-regulated the expression of active tissue factor by endothelial cells. In parallel, microparticles induced a rapid expression of von Willebrand factor at the cell surface, enabling transient attachment of non-activated platelets to the endothelium. These two major cell responses appear to be under the control of reactive oxygen species (ROS) delivered by circulating microparticles.[13] $TACE^{+}$-microparticles from the plaque were shown to promote the shedding of EPCR from endothelial cells, constituting another mechanism by which they contribute to enhanced *in situ* thrombogenicity.[9] Indeed, the loss of EPCR from the endothelial cell surface would impede the binding of activated protein C (APC). Endothelial microparticles themselves are part of the amplification loop leading to enhanced *in situ* thrombogenicity.[14] Released upon aggression, they induce monocyte tissue factor expression and procoagulant activity.[15]

Altogether, these findings indicate an alternative mechanism of endothelial cell activation mediated by microparticles, contributing to enhanced thrombogenicity in injured vessels.

Anticoagulant potential of microparticles

Anionic phospholipids exposed by activated vascular cells or derived microparticles promote the assembly of both procoagulant and protein C anticoagulant enzyme complexes, the latter probably requiring approximately 10-fold higher phosphatidylserine concentrations. Depending on their cell origin, the expression of thrombomodulin, tissue factor pathway inhibitor, EPCR or protein S at the microparticle surface is another indication of their eventual involvement in anticoagulant pathways.[14] Strikingly, although microparticles are recognized as potent procoagulant mediators in the vasculature, especially when they harbor active tissue factor, EMP induced by APC interacting with functional EPCR were recently demonstrated to support efficient anticoagulant activities enabling factor Va inactivation at the microparticle surface. Both the active site of APC and protease activated receptor-1 (PAR-1) were found to be necessary to induce microparticle formation.[16] The reported evidence support the concept that shed EMP do not display a univocal prothrombotic potential, anticoagulant properties counteracting the thrombotic propensity when EMP release is induced by APC stimulation (see below).

Circulating microparticles and vascular dysfunction

Several studies provide evidence that circulating microparticles could be considered as a surrogate marker of vulnerable plaque or global vascular damage.[17,18] For instance, in newly menopausal women, procoagulant microparticle levels increase with the decline of endogenous estrogen concentration, and thus may be indicative of the cardiovascular risk at menopause.[19] Elevation of plasma EMP levels particularly reflects cellular injury and now appears as a marker of vascular dysfunction. As indirect proof, a linear correlation between aortic pulse wave velocity, a global indicator of arterial stiffness, or the Framingham risk score, and circulating EMP or endothelial progenitor cell-derived microparticle levels was recently described.[18] Circulating EMP correlate with endothelial dysfunction in patients with ischemic left ventricular dysfunction or within the coronary circulation. Because endothelial dysfunction and arterial stiffness are major determinants of cardiovascular risk, several trials have investigated the effects of circulating microparticles on vascular function. Platelet-derived microparticles are a source of thromboxane A2, a potent regulator of vascular tone, as shown in rabbit aorta. In addition, microparticles from apoptotic T lymphocytes impair endothelium-dependent relaxation through endothelial nitric oxide synthase down-regulation and caveolin-1 overexpression.[20] Endothelial dysfunction was also elicited by microparticles derived from apoptotic smooth muscle cells that diminished nitric oxide production in mouse aorta. The effect, mediated by endothelial β3 integrins, was sensitive to anti-GPIIbIIIa antagonists.[21]

Protective effects of circulating microparticles

Angiogenesis

The ability of various microparticles to promote angiogenesis provides a first illustration on how they could contribute to vascular repair or, at least, to the limitation of vascular damage. Although angiogenesis is considered

Figure 1. Possible beneficial effects of microparticles and recombinant human activated protein C on endothelial and vascular function during sepsis or heatstroke.

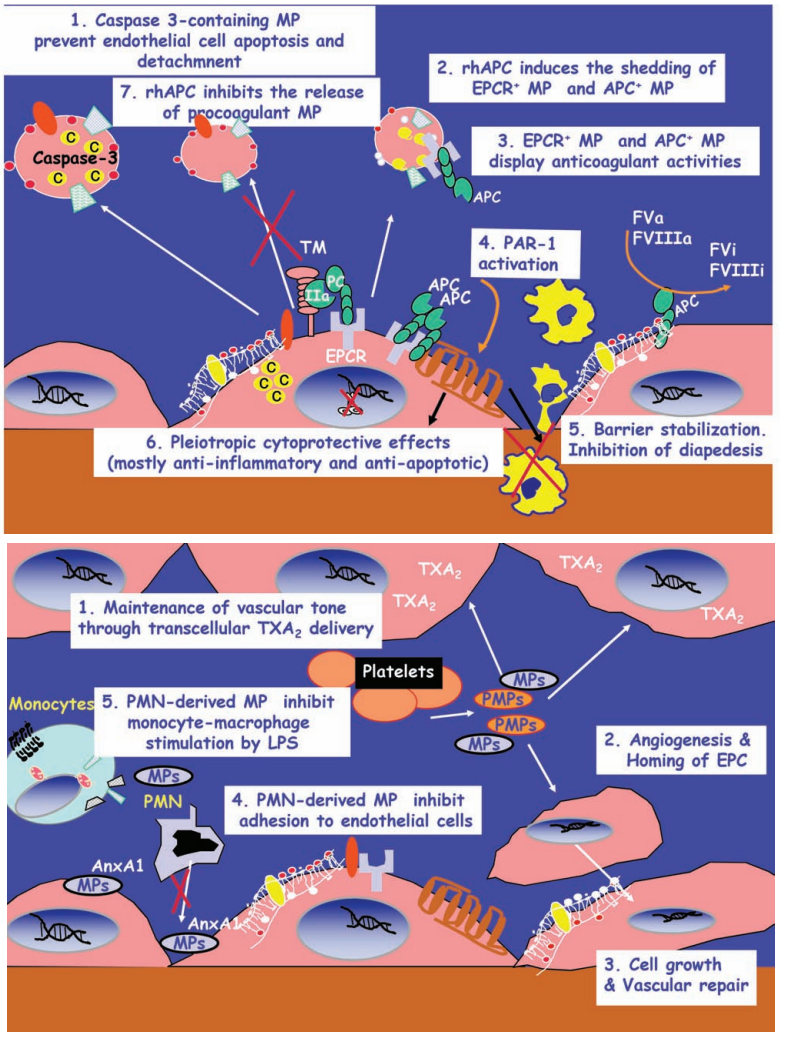

Figure 1A. Role of endothelial microparticles. Endothelial-derived microparticles contribute to the sorting of several pro-apoptotic factors such as caspase-3 thus preventing endothelial cell detachment and apoptosis (1). During sepsis, recombinant human activated protein C (rhAPC) induces the release of endothelial-derived microparticles (MP) harboring functional endothelial protein C receptor (EPCR) and activated protein C (APC) (2). Both APC+-MP and free APC inactivate factor Va (FVa) and factor VIIIa (FVIIIa) (3), protease activated receptor-1 (PAR-1) (4), participate in barrier stabilization (5), and display cytoprotective effects on endothelial cells through the reduction of apoptosis (6). The beneficial effect of rhAPC on coagulation includes the release of APC+-MP, able in turn to inhibit the release of procoagulant MP (7), as a result of diminished cell apoptosis (6).

Figure 1B. Role of circulating microparticles of other origins. During sepsis, elevated levels of platelet, endothelial and leukocytes derived microparticles occur. Microparticles (MP) isolated from patients with septic shock were demonstrated to prevent vascular hyporeactivity through thomboxane A2 (TXA_2) delivery enhancing the contraction of aorta from lipopolysaccharide (LPS)-treated mice (1). Platelet-derived microparticles (PMP) might contribute to the homing of endothelial progenitor cells (EPC), to angiogenesis and vascular repair (2, 3). In addition, Polymorphonuclear cell (PMN)-derived microparticles contain annexin 1 (AnxA1), an endogenous anti-inflammatory protein that is able to prevent cell adhesion to the endothelium (4). At the earliest stage of inflammation, PMN-derived microparticles down-regulate monocyte-macrophage activation (4).

deleterious in neoplasia or within the atheromatous plaque since it could promote intraplaque hemorrhage and rupture, it can be viewed beneficial through the delivery of additional blood supply within ischemic organs. Platelet adhesion receptors delivered by platelet-derived microparticles to hematopoietic stem cells would favor endothelial homing by promoting chemotaxis, cell adhesion, proliferation and survival. Indeed, in rats, locally injected platelet-derived microparticles improved revascularization of the ischemic myocardium in a growth factor-dependent mechanism.[22] More recently, microparticles isolated from atheromatous plaques were demonstrated to enhance vascular endothelial growth factor production by endothelial cells, endothelial proliferation and neovessel formation in a CD40L-dependent process.[23] Borne by platelet-derived microparticles, various regulators of angiogenesis, among which tissue factor, could also contribute to the signaling pathways by direct vectorization to target cells. Sphingomyelin and the cytoplasmic domain of tissue factor are two other pro-angiogenic components sorted in tumor-released microparticles. By contrast, other microparticle phospholipid components such as phosphatidylserine showed little effect on endothelial migration.

The nature of the effect of EMP on angiogenesis mostly appears related to their concentration. At physiological levels, EMP showed no pro-angiogenic property whereas pathological concentrations (100-fold enhancement) impaired angiogenesis and enhanced the rate of apoptosis.[24] The oxidative stress induced by EMP could be a key factor in the balance governing angiogenesis and apoptosis. In rodent or human endothelial cells, isolated EMP exhibiting the p22(phox) subunit of the NADPH oxidase are providers of superoxide anions. The *in vitro* demonstrated expression of several proteases, including metalloproteinases and urokinase plasminogen activator, by EMP, could contribute to basement membrane invasion by neovessels through matrix proteolysis *in vivo*.

Protective effects of microparticles on vascular hyporeactivity

Regarding critical conditions such as septic shock or trauma, microparticles were first considered conveyers of deleterious biological information for endothelial function, triggering blood coagulation, intravascular disseminated coagulopathy and death. Indeed, elevated lev-

els of platelet, granulocyte and endothelial-derived microparticles were measured in patients with septic shock, meningococcemia or traumatic brain injury.[25-27] The link between sepsis and increased coagulopathy was nicely illustrated by the work of Aras *et al.* in human endotoxemia. Volunteers receiving an infusion of endotoxin exhibited an early increase in tissue factor$^+$-microparticles and to a lesser extent of EMP.[28] Beneficial effects of microparticles in patients with septic shock were more recently suggested by Soriano *et al.*[29] In their study, lower levels of endothelial, platelet and leukocyte-derived microparticles were associated with higher mortality rates and organ dysfunction. In septic shock, vascular hyporeactivity together with tissue hypoperfusion and hypoxia account for severe hypotension enabling amplification of the shock. Microparticles may protect against vascular hyporeactivity by maintaining a tonic pressor response. This challenging hypothesis was supported by the demonstration that microparticles isolated from patients with septic shock were able to prevent vascular hyporeactivity through thromboxane A2 delivery, accounting for enhanced aortic contraction in lipopolysaccharide-treated mice.[25]

Cytoprotective effects of microparticles mediated by activated protein C

Over the past years, several studies have demonstrated that APC reduces mortality in endotoxemia, and provides neuroprotective effects in ischemic stroke. APC is a vitamin K-dependent serine protease that inactivates factor Va and factor VIIIa to down-regulate thrombin generation. In humans, the administration of recombinant human (rh) APC (Xigris®, Eli Lilly, Indianapolis, USA) reduces mortality in severe sepsis.[30] Interestingly, APC was also demonstrated to be able to generate anticoagulant endothelial or monocyte-derived microparticles that harbor EPCR (see above).[16] In this issue of the journal, Pérez-Casal *et al.* provide evidence that part of the cytoprotective effects of APC are mediated by microparticles harboring EPCR, therefore able to bind APC, termed APC$^+$-microparticles.[3] A variety of cytoprotective properties of APC, namely: (i) alteration of genes profiles, (ii) modulation of cytokine or cytoadhesin transcription levels, (iii) anti-apoptotic activity, and (iv) endothelial barrier stabilization, could be mimicked *in vitro* by APC$^+$-microparticles.[30]

Gene profiling of endothelial cells stimulated by APC$^+$-microparticles showed significant changes in anti-apoptotic and anti-inflammatory patterns. The relevance of these findings was confirmed in endothelial apoptosis models and permeability assays. From cDNA array analysis, the authors suggest that cytoptotective effects of APC$^+$-microparticles could be mediated by the upregulation of anti-apoptotic gene products such as Bcl-X_L, concomitant with the suppression of proapoptotic Bax expression.[30] Of particular interest, both free APC and APC$^+$-microparticles prevent endothelial cell apoptosis through EPCR-dependent PAR-1 activation, induction of sphingosine kinase-1 and the up-regulation of sphingosine-1-phosphate. Evidence was given that APC$^+$-microparticles are barrier protective, to a nearly similar extent to that of free APC.

Since the PAR-1-thrombin pathway mediates endothelial barrier destabilization, the question as to how PAR-1 tunes opposite biological effects when activated by two different plasma serine proteases deserves further clarification. Possible explanations are based on the following considerations: (i) APC effects could depend on the activation of Rac (protective), whereas thrombin induced a Rho (destabilizing) signaling, (ii) APC effects could involve direct or indirect interactions between EPCR and sphingosine 1-phosphate, a contributor to cytoskeleton stabilization.[30]

Altogether, these data underline the paradigm of microparticles as potent conveyors of APC exerting cytoprotective activities.[3] Although results obtained from *in vitro* models are difficult to translate/extrapolate to clinical settings, these observations shed new light on the pathophysiological relevance of microparticles as modulators of the pro- to anti-inflammatory balance. The detection of elevated circulating APC$^+$-microparticles levels measured by Pérez-Cazal *et al.* in patients with sepsis after treatment by rhAPC reinforces this hypothesis.[3]

Other confirmation was provided by Bouchama *et al.*, using a baboon heatstroke model.[31] They investigated whether the antithrombotic and cytoprotective effect of rhAPC could protect baboons from fatal heatstroke. Despite a lack of demonstrable antithrombotic effect, rhAPC provided cytoprotection and anti-inflammatory effects as demonstrated by the decreased levels of soluble thrombomodulin, IL-6 and procoagulant microparticles, presumably reflecting diminished cell apoptosis. One likely reason for the absence of an antithrombotic effect could be the relatively low dose regimen applied in the baboons (24 μg/kg/h) and the absence of a loading dose.[31] From a mechanistic point of view, this observation suggests that free or microparticle-conveyed APC displays cytoprotective effects at lower doses than those necessary to blunt the activation of coagulation. Hence, it is likely that during sepsis or heatstroke, the infusion of APC induces the release of anticoagulant microparticles harboring EPCR and APC whilst it diminishes the generation of procoagulant microparticles as a result of the inhibition of vascular cell apoptosis. When considering the hemostasis balance, the benefit of the switch towards anticoagulant properties could be hampered by adverse hemorrhagic effects. Of particular importance for pharmacological development, such a hypothesis is consistent with the view that the APC cytoprotective effect could, at least in part, be dissociated from its anticoagulant activity.[30]

In summary, microparticles should not be appreciated solely as noxious effectors contributing to endothelial dysfunction since a proportion may display cytoprotective biological activities preserving endothelial function and/or vascular integrity. Indeed, at the endothelial cell level, shed microparticles enable the sorting of deleterious effectors such as caspases but also provide specific cytoprotective effects through harbored EPCR and APC. Although a number of issues remain to be clarified regarding the pathways through which microparticles regulate or alter endothelial function, phenotypic and functional characterization of microparticles should be considered a key step in an attempt to understand their

role in endothelial and vascular homeostasis. However, the possibility of regulating pro and anticoagulant properties of circulating microparticles combined with potent cytoprotection through rhAPC infusion appears an appealing challenge in the treatment of complex vascular failure.[3,16]

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