

Cellular microparticles: a disseminated storage pool of bioactive vascular effectors

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Purpose of review

Microparticles (MP) or microvesicles are fragments shed from the plasma membrane of stimulated or apoptotic cells. Having long been considered inert debris reflecting cellular activation or damage, MP are now acknowledged as cellular effectors involved in cell–cell crosstalk. This review focuses on procoagulant MP circulating in the vascular compartment, their role in hemostasis and thrombosis, and possible impact in vascular functions.

Recent findings

Microparticles can be viewed as a “storage pool” by themselves, disseminating blood-borne tissue factor activity and procoagulant phospholipids. Increasing evidences of integrated loops involving dynamic exchanges and transfer events through multiple MP-cell interactions are summarized.

Summary

Microparticles can be considered true targets in the pharmacological control of thrombosis. Another challenging issue is to take advantage of their procoagulant potential for the management of hemophilia.

Keywords

blood-borne tissue factor, P-selectin pathway, hemostasis, thrombosis and coagulation disorders

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Introduction

Microparticles (MP), also referred to as microvesicles or more rarely ectosomes, are submicron fragments shed from the plasma membrane of stimulated or apoptotic cells [1,2]. MP constitute relevant hallmarks of cellular activation or damage, whilst the cells they stem from remain sequestered in tissues or are promptly submitted to phagocytic clearance [3]. MP also act as transcellular effectors [1,2] (Table 1 through 5).

Detectable in the peripheral blood of normal individuals, MP are elevated in clinical situations where the thrombotic risk is increased [1]. Conversely, a defect in membrane shedding is associated with bleeding disorders occurring in Scott syndrome [4]. Procoagulant potency of MP is demonstrated in patients suffering from pathologic or drug-induced thrombocytopenia, protected from bleeding at higher circulating MP levels [5,6]. Under most pathophysiologic conditions, platelet-derived MP appear the main procoagulant circulating species, leukocytic or endothelial origins being less represented. Actual data are suggestive of a beneficial pharmacological control of circulating MP levels in thrombotic disorders [7,8], and more amazingly in the management of hemophilia [9••].

Functional characteristics of circulating microparticles

Blood MP provide an additional procoagulant phospholipid surface for the assembly of the clotting enzymes complexes promoting thrombin generation. Their catalytic property relies on a procoagulant anionic aminophospholipid, phosphatidylserine (PhtdSer), made accessible upon membrane remodeling and shedding processes occurring after cell stimulation [10], and on the possible presence of tissue factor (TF), the cellular initiator of coagulation expressed in stimulated monocytes and endothelial cells. PhtdSer is able to considerably enhance TF procoagulant activity [11]. MP carry or harbor pro-inflammatory lipids and membrane glycoproteins (selectins, adhesion molecules, CDs...) testifying to their cellular origin. MP are also thought to contain cytoplasmic components [1,2,10].

Thrombin in conjunction with collagen, is the physiologic platelet agonist for procoagulant PhtdSer exposure and consecutive membrane shedding [10]. In the blood

Table 1. Microparticles, chemotaxis, and adhesiveness

Cellular origin	Stimulus or pathology	Target cell	Molecular target/ phenotypic alteration	Cellular effects	Possible mechanisms of action	References
Platelets	From healthy volunteers	Endothelial cells (HUVEC)	ICAM-1 No effect on the expression of VCAM-1, P- or E-selectin	Enhancement of monocyte-endothelium adhesiveness	PKC, arachidonic acid	[55]
	Thrombin, storage	Neutrophils	CD11b expression,	Phagocytic activity		[88]
	High shear stress	Monocytic cells (THP-1)	Up-regulation of: CD11b, CD32, CD33*, IL-8, IL-1 β , TNF- α		*PSGL-1 dependent	[54]
	High shear stress	Endothelial cells	Up regulation of: CD54, CD63, IL-8, IL-1 β , IL-6			[54]
Endothelial cells	Hydroperoxide	Monocytes	Palmitoyl glycerol-Phosphorylcholine (POVPC)	Enhancement of monocyte-endothelium adhesiveness	Oxidized phospholipids	[89]
Monocytic cells	LPS	–	CD11a, CD18, CD14 expression	–		[90]
	P2X7 stimulation	–	secretion of active IL-1 β	–		[91]
Leukocytes	Inflammatory peptide (N-fMet-Leu-Phe)	Endothelial cells	IL-6, MCP-1 TF expression		Induction of JNK pathway. NF- κ B & ERK-1 unaffected	[57]
		Neoplastic epithelial cells	Increased binding to ICAM-1	Increased binding to endothelial cells	20–65 kDa protein phosphorylation	[92]
		Endothelial cells (HUVEC)	IL-6, IL-8 No effect on TNF- α , IL-1 β , PDGF			[56]
Polymorphonuclear leukocytes	LPS-induced neutrophil adhesion	Platelets		PAF synthesis PAF-containing MP		[93]

flow, platelets behave as true sensors responding to a variety of physiologic or pathogenic stimuli. Released MP presenting procoagulant potency can therefore be considered early and prime contributors to the integrated amplification loops of the hemostatic response. Depending on the nature of the vascular disorders, MP from different cell origins could also be involved.

Anionic phospholipids promote the assembly of both procoagulant and protein C anticoagulant enzyme complexes, the latter requiring ~10-fold higher PhtdSer concentrations. At the MP surface, it is reasonable to assume that the anticoagulant potential of activated protein C is probably overwhelmed when TF is also harbored (see below) [12].

Microparticles constitute a dynamic “storage pool” of bioactors

Released through multiple pathways (high shear stress, oxidative stress, inflammatory or procoagulant stimulation, apoptosis...), MP can interact with cells of the vascular compartment and promote stress signaling. They behave as vectors disseminating biologic information eventually delivered to cells exposing appropriate coun-

terreceptors for ligands they harbor. Cellular effects depend on their membrane and cytoplasmic composition, and of course on the nature of the target cell (Tables 1–5).

Microparticles constitute a dynamic circulating storage pool by themselves, able to induce vascular responses to pro-apoptotic, inflammatory, or thrombotic stimuli. Therefore, the clinical background associated with elevated MP levels [13–16] or accelerated clearance [17] should be taken into close consideration in deciphering their multiple effects. For instance, misleading quantification or phenotypes could result from accelerated degradation by secretory phospholipase A₂ [17], interactions with the vascular wall [18], or trapping in cell–cell aggregates or within the thrombus [19].

Additional complexity was recently observed as circulating MP may bear antigens from different cellular origin, pointing to multiple transcellular MP-mediated exchanges [20••,21]. Antigens specifically expressed during cell activation could prove useful in identifying the various pathways of MP release and discriminating underlying pathologies and associated damages. In cardio-

Table 2. Microparticles, coagulation, and thrombogenicity

Cellular origin	Stimulus or pathology	Target cell	Molecular target/ phenotypic alteration	Vascular or cellular effects	Possible mechanisms of action	References
Platelets	Thrombin, SFFLRN, A23187	Platelets	GP1Ib-IIIa	Platelets-MP aggregates	GP1Ib-IIIa mediated	[94]
	Collagen	Monocytes	TF transfer	MP binding to soluble and immobilized fibrinogen CD62P	P-selectin mediated	[21•]
	Collagen	Neutrophils, monocytes	TF exposure and de-encryption	Platelets-leukocytes aggregates	Upregulation of neutrophil adhesion molecules ROS generation	[20••,95]
Endothelial cells	Thrombotic Thrombocytopenic Purpura plasma	–	Membrane surfaces expression of vWF and E-selectin	–	–	[96]
Monocytes	LPS	–	–	TF-bearing MP CD 18-bearing MP	–	[12, 90]
	LPS	Platelets	Platelet P-selectin	TM-bearing MP Accumulation of TF in the thrombus	PSGL-1-bearing MP/platelets aggregates	[44••]
Leukocytes	Inflammatory peptide (N-fMet-Leu-Phe)	Endothelial cells	TF up-regulation	Cytokine release	JNK pathway NF- κ B, ERK-1 unaffected	[57]
	TNF- α	Platelets	CD15	TF transfer	CD15 & TF-dependent	[36]

vascular disorders, endothelial antigens CD31 and CD62E, respectively shed with MP during apoptotic or cytokine stimulation, could be of particular relevance [22,23•,24].

Circulating microparticles in the formation of arterial or venous thrombus

Incorporation of procoagulant MP into the growing thrombus and their main contribution in thrombus propagation is now demonstrated, although uncertainty remains on their composition and cellular sources with respect to thrombus localization [25].

Microparticle-borne tissue factor

Tissue factor exposure by cells of the vascular wall has been long considered mandatory in arterial thrombosis. Apoptotic smooth muscle cells and monocytes are the vessel-wall providers of TF-bearing MP released into the blood stream following atherothrombotic plaque disruption or erosion [26,27].

More recent data provide a new paradigm pointing to an additional circulating storage pool of TF associated with MP and constituting the main reservoir of blood-borne TF activity [20••,28,29•]. A proportion of MP-associated

Table 3. Microparticles and vasomotricity

Cellular origin	Stimulus or pathology	Target cell	Molecular/target/ phenotypic alteration	Cellular effects	Possible mechanisms of action	References
Platelets, lymphocytes, granulocytes	Pre-eclampsia	Myometrial arteries	–	Vasomotor dysfunction	Abolishment of bradykinin-mediated relaxation	[60]
Platelets	–	Rabbit pulmonary artery	–	Vasomotor dysfunction	Thromboxane A2	[62]
T lymphocytes	Actinomycin D, phytohemagglutinin, diabetes mellitus	Endothelial cells (HUVEC)	Downregulation of eNOS Upregulation of caveolin-1	Vasomotricity impairment	No involvement of CD11a/CD18 or Fas/FasL	[63]

Table 4. Microparticles, vascular remodeling and angiogenesis, and hematopoiesis

Cellular origin	Stimulus or pathology	Target cell	Molecular target/ phenotype alteration	Cellular effects	Possible mechanisms of action	References
Platelets	Thrombin, collagen A23187 ionophore	Smooth muscle cells		Mitogenesis	PDGF-independent	[67]
		Endothelial cells (HUVEC)		Proliferation, chemotaxis, tube formation	PT-sensitive G protein, PI ₃ -kinase pathway	[66••]
Endothelial cells	Serum, VEGF, FGF	Endothelial progenitor cells		Differentiation into PBMC	MP lipidic components	[66••]
		Hematopoietic cells		Adhesion, activation, proliferation, cell survival	MAP-kinase PI ₃ -K-AKT STAT protein	[69]
Endothelial cells	Serum, VEGF, FGF	HUVEC		Autocrine stimulation of invasion	MMP-2 & MMP-9-bearing MP MT-1-MMP release	[65]

TF triggers factor VII-mediated thrombin generation [30], truly soluble TF being ineffective in the absence of procoagulant phospholipids [31]. The thrombogenicity of human MP harboring TF was recently correlated with TF accessibility in a venous model [29•]. Although uncertainty remains on the origin and the eventual encryption of TF [32], the procoagulant character of circulating TF-bearing MP is further confirmed by studies in sickle cell disease. Such MP, of monocyte and endothelial origin, are increased during crisis, correlated with coagulation markers and were reported to shorten human plasma clotting time [33•].

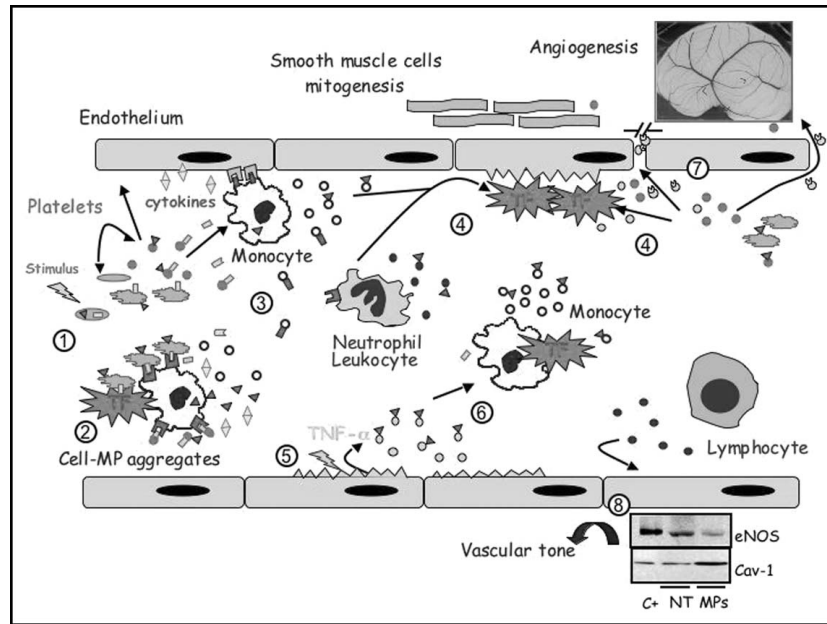
Amazingly, in the plasma from normal individuals, MP-associated TF mainly appears of platelet origin. Since TF mRNA could not be evidenced in megakaryocytes, platelet TF is likely to originate from other cell lineages, possibly after several transfer steps [20••,21,32•,34,35•]. Activated monocytic cells could be a circulating source of TF acquired by platelets through CD15- and TF-dependent interactions [36]. TF transfer through leukocyte-derived MP incorporating into the growing throm-

bus would thus make adherent platelets capable of triggering and propagating thrombosis [36]. Platelet-derived MP released in the presence of collagen seem also able to transfer TF to monocytes through P-selectin and PSLG-1 interactions [21]. Both observations emphasize the importance of the subendothelial matrix in concentrating circulating TF-bearing platelet-derived MP on target cells. Indeed, collagen was shown to promote the adhesion of platelet-derived MP to neutrophils and monocytes through P-selectin and also CD18. Inhibition of these interactions by specific antibodies led to a marked decrease in TF activity in blood cell suspensions, confirming MP pivotal role in the initiation of the coagulation cascade [20] (Tables 1 and 2).

Although the original cellular source of TF probably varies according to vascular disorders, interactions between cells and TF-harboring MP are determinant for the up-regulation of TF activity. This was confirmed by the study of reactive oxygen species (ROS) and cytokines secreted in platelet-leukocyte aggregates [37], where up-regulation of TF activity precisely takes place [20••].

Table 5. Microparticles, cell survival, and apoptosis

Cellular origin	Stimulus or pathology	Target cell	Molecular target/ phenotypic alteration	Cellular effects	Possible mechanisms of action	References
Cancer cell lines	Serum free conditioned medium	Cancer cell lines	Fas	Induction of apoptosis	FasL-bearing MP	[74]
			FasL	Inhibition of apoptosis	Fas-bearing MP	
Melanoma cells	Melanome	Lymphocytic lineage (Jurkat)	FasL	Induction of apoptosis via Fas/FasL pathway	FasL-bearing MP	[77]
Trophoblastic cells		T lymphocytes	FasL	Induction of apoptosis via Fas/FasL pathway ⇒ Immune privilege	FasL-bearing MP	[75]
Epithelial ovarian cancer cells	Supernatants	Jurkat T cells	FasL	Induction of apoptosis via Fas/FasL pathway	FasL-bearing MP	[76]

Figure 1. Microparticles in the vascular compartment: a disseminated storage pool of bioactive effectors

Platelet MP constitute most circulating MP, reflecting the high responsiveness of platelets to a variety of stimuli. In this respect, platelets can be considered as true sensors, while derived MP can propagate amplification of the stimulation through their ability to mediate transcellular exchanges of biologic information, leading to further generation of MP of other cellular origins.

- (1) Platelets constitute a circulating storage pool of blood-borne TF (triangle), likely originating from other cells after multiple transfer steps involving soluble P-selectin (grey rectangles), CD15 and TF.
- (2) Constitution of cells/MP aggregates involving platelets, leukocytes, and promoting TF activity (star shape) at the membrane surface. Truly soluble TF (triangle) is ineffective in the absence of procoagulant phospholipids.
- (3) Enhanced release and recruitment of MP relies on platelets/platelet-derived MP (dark circles) and monocytes/monocyte-derived MP (grey circles, bold circumference) interactions involving P-selectin and PSGL-1 (dark U shape). P-selectin stimulates TF synthesis and expression by monocytes.
- (4) Circulating MP of leukocytic and platelet origin are potent activators of endothelial cells, leading to enhanced cytoadhesions (grey U shape and dark rectangle) expression and cytokine release (diamond) followed by monocyte activation at the vicinity of endothelial activation site.
- (5) Cytokines upregulate TF expression by endothelial cells and promote MP release.
- (6) Endothelial-derived MP (grey circles) harbor functional TF and promote TF expression by monocytes.
- (7) Endothelial-derived MP play a pivotal role in vascular remodeling through exposed membrane metalloproteinases (MMP) (Pacman shape). Platelet-derived MP promote angiogenesis and mitogenesis of smooth muscle cells.
- (8) Circulating MP of lymphocytic origin may regulate vascular tone by modulating endothelial gene expression.

Additionally, in such aggregates, ROS may inactivate tissue factor pathway inhibitor [38] *via* neutrophil proteases and promote the expression of encrypted TF activity [39]. Interaction and fusion events, which typically occur between monocytes, platelets, and derived MP, would thus facilitate the generation of monocytes/monocyte-derived MP and platelets/platelet-derived MP hybrids leading to MP enriched in decrypted TF activity [40], in line with *in vivo* observations in acute coronary syndromes (ACS) and antiphospholipid syndrome (APS). In ACS, oxLDL, a potent source of ROS, together with platelet-derived MP and high shear stress induce platelet-MP aggregates thereby facilitating the release of TF-bearing monocyte-derived MP [41,42]. Another source of TF could be provided by endothelial-derived MP [23], also able to induce additional TF expression by monocytic cells [34].

Intravascular TF activity localized on injured endothelium is clearly dependent on neutrophil, platelet, and MP adhesion, leukocyte-derived MP being readily asso-

ciated with venous thrombosis [43•]. Colocalization of platelets and leukocytes on growing fibrin clot and the recently reported generation of TF-bearing MP induced by soluble P-selectin point to the central role of inflammation during thrombogenesis [44••].

Microparticle-selectins interactions in the developing thrombus

When exposed by cells, harbored by MP, or circulating as truly soluble form, selectins of leukocyte, platelet, or endothelial origin become potent contributors to thrombus initiation [45] and propagation, notably through sustained MP generation [9••,43•]. P-selectin, involved in leukocyte rolling, is upregulated during venous thrombosis and promotes vein wall inflammation. Additionally, E-selectin, expressed by activated endothelial cells and derived MP, reinforces the thrombotic response [45]. Indeed, in diabetes mellitus patients, known to develop accelerated atherosclerosis, both P- and E-selectins were found drastically elevated, as well as platelet-derived MP levels [46]. The prominent role of platelet-borne P-

selectin in MP recruitment, through interactions between P-selectin glycoprotein ligand-1 (PSGL-1) and TF-bearing MP, was recently demonstrated in a laser-induced arteriole endothelial injury model, showing their colocalization on the leading edge of the thrombus [44••]. Other groups have established the contribution of leukocytes [47] in venous thrombosis induced by collagen, TF-bearing MP of platelet origin playing an important part in the initiation and propagation of coagulation *in vitro* [20••]. Platelet and/or leukocyte-derived MP would thus concentrate TF activity above a threshold to allow blood coagulation to be triggered. Therefore, interactions between P-selectin and PSGL-1 may have a dual function, first in the generation of procoagulant MP [9••], second in the recruitment of MP to the growing thrombus [48••,49••] (Tables 1 and 2).

Amplification of platelet-derived microparticle release

During thrombosis, the extent of platelet membrane shedding is modulated by various cellular effectors. Truly soluble CD40 ligand (sCD40L), a glycoprotein released from several types of activated cells, including platelets, known to contribute to endothelial activation and thrombus stabilization, promotes the release of platelet MP [50]. Correlation between levels of sCD40L and procoagulant MP of endothelial or platelet origin [16,51] was evidenced in myocardial infarction, emphasizing the role of amplification loops involved in thrombus propagation, possibly enhanced by cytokines [51]. Augmented MP release upon collagen and thrombin binding to glycoprotein VI and $\alpha_{IIb}\beta_3$ [52,53] or by shear stress [54] was also reported.

Circulating microparticles induce vascular wall inflammation

Circulating MP of platelet or leukocytic origin modulate cellular interactions through the upregulation of cytokines and cytoadhesins in endothelial cells (IL-8, IL-1 β , and IL-6, CD54, ICAM-1, MCP-1) and monocytes (IL-8, IL-1 β , TNF- α , CD11b/CD18, CD11a/CD18) [54–57] (Table 1). MP account for enhanced inflammation [54] as noticed in sepsis [18]. In vascular inflammatory areas, where they are precisely highly recruited, platelet-derived MP could in turn promote the recruitment of more immune cells (monocytes, NK cells, T and B lymphocytes) [58].

Microparticles in vasomotricity and regulation of endothelial genes

A diminished production of endothelial relaxant factors, including nitric oxide (NO), was associated with atherothrombotic disease. By regulating the vascular tone, MP could be responsible for flow disturbance and thrombus formation [59,60] (Table 3). Indeed, during severe hypertension, endothelial and platelet-derived MP were correlated with blood pressure [61]. Platelet-derived MP

were recently demonstrated to be a source of thromboxane A₂ modulating the vascular tone in aorta or pulmonary artery [62]. When applied at concentrations relevant to pathologic situations, leukocyte-derived MP impair endothelial function, in both conductance and resistance arteries, through NO and prostacyclin pathways. By diminishing the expression of endothelial NO-synthase and enhancing that of caveolin-1, MP would reduce eNOs translocation into the cytosol and its subsequent activation [63]. Finally, at sites of endothelial injury, a defect in NO bioavailability, would lead to surviving factor deprivation and endothelial cell apoptosis.

Microparticles in angiogenesis and vascular remodeling

Various agonists of vascular origin known to contribute to angiogenesis and vascular remodeling (VEGF, fibroblast growth factor 2, PAI-1) induce the shedding of endothelial-derived MP [64], possibly carrying membrane metalloproteinases (MMP-2 and MMP-9) [65]. Such MP promote basement membrane invasion and would contribute to the regulation of focalized proteolytic activity at sites of endothelial injury in an autocrine loop [65]. Paracrine loops involving platelet-derived MP were recently evidenced *in vitro*, where they enhance endothelial progenitors differentiation towards mononuclear lineage, promote chemotaxis and tube formation by endothelial cells [66••], and smooth muscle cell mitogenesis [67] (Tables 4 and 5). Interestingly, platelet-derived MP, highly elevated in metastatic gastric cancer, may be key actors in the combined processes of angiogenesis and metastasis [68] (Table 4 and 5).

Other bioactive properties of microparticles

A better understanding of the mechanisms governing membrane shedding in normal and malignant cells would be useful for new therapeutic approaches. MP modulate the biologic functions of target cells through the transfer of cytoplasmic content or delivery of various lipids and new membrane receptors [69]. MP can promote the upregulation of membrane protein expression and the acquisition of new phenotypes by acceptor cells. In AIDS, functional CXCR4 transfer by MP of megakaryocytic lineage could contribute to virus spreading [70••]. Platelet-derived MP bound to hematopoietic progenitor cells enhance their engraftment through the upregulation of cytoadhesins [71,72]. *In vitro*, they were shown to stimulate proliferation, survival, adhesion, and chemotaxis of hematopoietic or bone cells [73]. Pointing to cancer escape through MP-mediated induced apoptosis of cytotoxic T cells, recent papers describe the apoptogenic potential of a proportion of MP through Fas ligand-Fas interactions [74–77] (Tables 1, 4, 5).

Circulating microparticles: a new pharmacological target?

Although elevated levels of circulating MP testify to a prothrombotic status of poor prognosis in atherothrombosis [16,78], their procoagulant potential may be of therapeutic benefit in some bleeding disorders. Pioneering work in hemophilic mice, involving leukocyte-derived MP released after recombinant P-selectin infusion, highlights this issue. Harboring functional TF generated leukocyte-derived MP improved kinetics of fibrin formation therefore normalizing tail bleeding time. In blood from hemophilia A patients, a similar release was observed *ex vivo*, providing new insights in the management of bleeding disorders [9••]. Additionally, in some hemophilic patients, recombinant activated factor VII (rFVIIa) induced a transient release of platelet-derived MP, either directly or as a consequence of the thrombin burst. TF associated to a proportion of generated MP could thus support rFVIIa efficacy [79].

In cardiovascular diseases, the inhibition of the shedding of MP may account for the efficacy of various drugs [7•,8,80]. One benefit of abciximab, a platelet GPII-IIIa antagonist, may rely on the control of MP release by platelets at high shear stress, a common condition in the vicinity of stenotic plaques [7•] (Tables 1 and 2). This process could prove crucial in discriminating the efficiency of anti-GPIIb/IIIa treatments. For instance, during myocardial infarction, the initial decrease in platelet-derived MP observed in abciximab-treated patients could also have blunted an amplified inflammatory response [8]. Other antiplatelet agents like ticlopidine were shown to reduce platelet-derived MP, circulating chemokine levels or monocyte shedding [81,82]. Other amplification loops promoted by oxidative stress, catecholamine or angiotensin II activation, hyperglycemia or dyslipidemia, could possibly be targets in the pharmacological control of MP release [80,83–87].

Prognosis value of circulating microparticles

Because of their pleiotropic effects within the vascular compartment, the prognosis significance of circulating MP is of particular interest. Elevated MP levels were reported associated to worsened outcome in ACS [8,16,78] and microangiopathy [13,15]. Numerous studies associate MP with a prothrombotic context [1], emphasizing their significance as a relevant parameter in the identification of patients at higher risk of thrombotic recurrence [80], and for the follow-up of treatment efficiency. Further studies with large multicentric trials are necessary to define thresholds according to clinical backgrounds or treatments, and taking into account the specific incidence of the latter on MP clearance and kinetics.

Conclusion

Circulating MP behave as an actual storage pool, able to disseminate blood-borne TF activity and other bioactive effectors. Vascular responses thus integrate dynamic exchanges and transfer events between cells and MP. Behaving as vascular pathogenic markers, circulating MP can modulate or impair essential functions including inflammatory response, hemostasis and thrombosis, vasomotricity, vascular remodeling, cell survival, and apoptosis. The pharmacological control of MP-mediated vascular responses constitutes the next challenging issue.

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