

# Cellular microparticles: what are they bad or good for?

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**Summary.** Microparticles are fragments released from the plasma membrane of most stimulated or apoptotic cells. After having long been considered inert cell debris, of possible value for the diagnosis of cell activation or death, there is increasing documented evidence that they can interact with neighboring or remote cells, in which case they acquire a pathophysiologic potential. On the one hand, deleterious microparticles stemming from activated cells can elicit an adverse response from other cells, themselves undergoing membrane vesiculation, leading to pathogenic amplification. On the other hand, since they are thought to reflect a balance between cell stimulation, proliferation, and death, it is conceivable that they are discerned as sensors for the maintenance of homeostasis in multicellular organisms. Because vesiculation is an integral part of the plasma-membrane remodeling process, with the transverse migration of procoagulant phosphatidylserine from the cytoplasmic to the exoplasmic leaflet as the central event, the majority of released microparticles are thought to fulfill a homeostatic function under physiologic conditions. This is particularly true when they originate from platelets, with possible deviation towards thrombosis when produced in excess. Owing to these procoagulant properties, the hemostasis laboratory offers the most appropriate tools for the assessment of the *in vivo* significance of microparticles.

**Keywords:** apoptosis, cell activation, membrane microvesicles, thrombosis.

## Introduction

Cellular microparticles (MPs), also referred to as microvesicles, are fragments shed almost spontaneously from the plasma membrane blebs of virtually all cell types when submitted to a number of stress conditions, including apoptosis. MP release is an integral part of the membrane-remodeling process in which the asymmetric distribution of constitutive phospholipids (PL) between the two leaflets is lost. After having long been considered 'cell dust', MPs have more recently been shown to reflect *in vitro* cell stimulation, and testify to cellular activation and/or tissue degeneration occurring *in vivo* under a variety of

pathophysiologic circumstances. Besides their marker characteristics, MPs have been identified as true vectors in the transcellular exchange of biologic information. Owing to the deleterious potential associated with MPs detected in pathology, one would be tempted to treat them as 'bad'. There are, however, biologic situations where MPs should play a beneficial role, in which case they can be viewed 'good'.

The aim of the present review is to examine the significance of MPs as a function of the diverse modes of cellular stimulation at their origin, and not to catalog the pathologic situations in which they have been detected (electronic bibliographic searches are more efficient for this purpose). The review will serve to briefly introduce key features of the membrane plasticity in basic physiologic responses, e.g. blood coagulation and phagocytosis, which involve shared participants.

## Membrane remodeling and vesiculation

In resting mammalian cells, the constitutive aminophospholipids, phosphatidylserine (PS) and phosphatidylethanolamine (PE), are mainly sequestered in the inner (cytoplasmic) leaflet of the plasma membrane, whereas sphingomyelin (SM) and phosphatidylcholine (PC) constitute the majority of the outer (exoplasmic) leaflet PLs [1,2]. This asymmetric distribution is under the control of an inward aminophospholipid translocase (flippase), of as yet elusive nature [3].

## Loss of phospholipid asymmetry and consequences

When subjected to procoagulant, pro-inflammatory or apoptogenic stimulation, cells of the vascular compartment, the territory of prime interest here, show specific responses according to lineage and stimulus, but in most of them a spontaneous collapse of their membrane asymmetry happens [2,3]. One of the resulting universal hallmarks is the occurrence of PS in the exoplasmic leaflet where it expresses two of its properties as a multifunctional membrane effector. Once accessible to circulating blood clotting factors, this anionic PL can promote the assembly of the characteristic enzyme complexes of the cascade by interacting with enzymes, cofactors and substrates in order that their local concentrations fulfill the kinetic requisites for optimal activity, culminating in the generation of sufficient thrombin for efficient hemostasis [4]. The prothrombotic consequence of excess thrombin formation necessitates a tight control exerted by the natural anticoagulant systems [4] and by phagocytes in charge of the elimination of potentially

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thrombogenic activated cells. In the latter process, PS acts as a recognition determinant [5,6], involving an apparently specific receptor [7], and/or milk fat globule-epidermal growth factor 8 (MFG-E8; lactadherin), a peripheral membrane protein that links activated/apoptotic cells to phagocytes [8]. Interestingly, MFG-E8 contains epidermal growth factor-like and factor V/VIII-like sequences, with RGD (Arg-Gly-Asp) and PS-binding motifs, respectively [9]. In addition to regulating blood coagulation, protein S, the vitamin K-dependent cofactor of anticoagulant activated protein C, may also facilitate clearance of early apoptotic cells through interaction with PS [10].

PS exposure is not restricted to multicellular organisms, as it occurs in apoptotic yeast, suggesting that PS is not primarily expressed for attracting macrophages, but rather, that evolution has led phagocytic cells to interpret this determinant as a signal for engulfment [11]. In the nematode *Caenorhabditis elegans*, there is no elaborated coagulation system, suggesting that the engulfment signal function of PS occurred well before its procoagulant function during evolution.

### Membrane vesiculation

The transverse migration of PS is generally coincident with membrane blebbing. Blebs are thought to result from a transient overload of the outer leaflet at the expense of the inner one. When the cytoskeleton is no longer able to counteract the surface tension, shedding of MPs takes place [12]. MPs not only carry accessible PS, but also membrane antigens including adhesion proteins or complexes, which can be active, and other procoagulant entities such as tissue factor (TF), when they stem from cells expressing these substances [13]. MPs are rather heterogeneous in size (0.05–1 µm), and in protein and lipid composition. In that, they differ from exosomes (0.03–0.1 µm), which originate through exocytosis of endocytic multivesicular bodies and play a role in antigen presentation. Exosomes are particularly enriched in MFG-E8, tetraspanins (CD9, CD63, CD81, CD82), and MHC class II molecules [14].

Membrane vesiculation in platelets may be viewed as a method to increase the procoagulant surface for optimal spatially limited hemostasis, provided MPs are retained at the site of platelet adhesion and activation. Functional adhesion complexes, such as GPIIb-IIIa present on MPs derived from platelets activated by the physiologic thrombin + collagen combination, are believed to mediate MP attachment together with fibrin(ogen) and/or von Willebrand factor, depending on shear-stress conditions [15–17]. In contrast, in pathologic situations where platelets are subjected to more drastic activation, e.g. by the C5b9 membrane attack complex of complement MP, GPIIb-IIIa is not activated [18], in which case it is reasonable to assume that MPs can be more easily swept along by the blood flow and disseminate their associated procoagulant potential. This is illustrated by two reports. In the first, concerning autoimmune thrombocytopaenias, higher MP levels were detected in patients apparently free of bleeding complications, suggesting a beneficial hemostatic role for MPs, but with possible thrombogenic implication in certain cases [19]. The

second one was devoted to paroxysmal nocturnal hemoglobinuria (PNH), a clonal disorder associated with an increased incidence of thrombotic events, and where complement attack is highly probable owing to the disappearance of protective membrane antigens. In PNH patients, MP levels were indeed different according to the state of disease evolution and corresponding cell counts, and could help to draw a type of 'decisional chart'. For those with pancytopenia and high MP levels, MPs appeared protective against bleeding, whereas low levels of MPs represented a risk for hemorrhage. With normal cell count, high MP levels seem to reflect a thrombotic risk, while normal or low levels testify to quiescence [20]. Activated platelets also release exosomes, but these smaller microvesicles have a much lower procoagulant potential than MPs [21].

In cultured nucleated cells, MP levels released into the supernatant have been shown to be correlated with the degree of apoptosis [22,23], and are therefore believed to constitute an *in vivo* marker of cell death since counterpart cells are hardly detectable owing to the extreme efficiency of phagocytic elimination (see above). MPs are smaller than cells and are certainly more diffusible, explaining their greater ability to escape phagocytosis. The number of patho(physio)logic circumstances under which MPs are produced, and their potentially deleterious or beneficial roles, justify a closer examination of the mechanisms governing the remodeling of the cell plasma membrane.

### Mechanisms governing the remodeling of the plasma membrane

Genetic variants are of prime interest for the elucidation of basic physiologic functions. Among the hereditary hemostasis defects, Scott syndrome is probably by far the rarest one, with only three cases as yet clearly identified [24–26]. The primary phenotype is a lack of ability of platelets, and also other blood cells, to externalize PS and shed MPs, associated with severe to moderate hemorrhagic episodes. Hence, Scott syndrome demonstrates the essential character of PS for normal hemostasis, and the intimate link between PS exposure and membrane vesiculation.

It is generally agreed that a sustained increase in bulk cytosolic  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) is necessary to allow PS transmembrane redistribution in a variety of mammalian cells [2]. A defect of store-operated  $Ca^{2+}$  entry (SOCE), also referred to as capacitative  $Ca^{2+}$  entry [27], has been reported in Epstein-Barr virus-infected Scott B lymphocytes [28]. In addition, the partial blockage of channel(s) mediating this particular mode of  $Ca^{2+}$  entry in megakaryocytic HEL cells resulted in significantly decreased ability to externalize PS. The integrity of the cytoskeleton was also found to be necessary for the completion of the process, at least until vesiculation is triggered [29]. In the light of these observations, defective SOCE could be part of the Scott phenotype.

Intracellular signaling has been shown to involve the plasma membrane, and more particularly lipid microdomains, termed rafts, able to recruit signaling molecules [30]. Because rafts are defined by the methods used to demonstrate them, such structures may be considered 'evanescent associations' [31], the

most common feature being enrichment in sphingolipids and cholesterol, explaining why rafts are less fluid than the rest of the membrane environment [30]. Raft integrity is essential for normal SOCE and consecutive PS transverse redistribution in stimulated megakaryocytic HBL cells, which also depend on the ERK pathway, at least in part itself, associated with rafts. In these experiments, rafts were disrupted by using the non-invasive cholesterol acceptor methyl- $\beta$ -cyclodextrin [32].

Although the above investigations provide a rational link between basic signaling pathways and PS transmembrane migration, no PS transporter or store-operated channel has been as yet definitely identified. Figure 1 summarizes our actual view of the mechanisms involved in the plasma membrane response to cell stress or stimulation. It has however, to be mentioned that, according to several groups, cytosolic  $\text{Ca}^{2+}$  may well not be the sole inducer of the collapse of membrane asymmetry. Phosphatidylinositol 4,5-bisphosphate has been reported to promote egress of PS in red blood cells [33] and platelets [34]. Under certain conditions, ceramide, stemming from SM conversion, could play a physical role rather than behaving as a second messenger, with consequences for membrane transverse organization [35]. Finally, it has to be kept in mind that all cell types do not elicit identical responses to the same stimulus [36].

#### Candidate genes for phosphatidylserine transport and capacitative calcium entry

The nature of the transporter(s) or molecule(s) mediating PS redistribution between the two leaflets of the plasma membrane is still elusive, perhaps owing to the fact that two processes remain possible. A non-specific bidirectional scrambling, under the dependence of the so-called PL scramblases (PLSCR), has

been proposed to account for  $\text{Ca}^{2+}$  dependence and independence of adenosine triphosphate (ATP) [3]. PLSCR1 was first characterized [37], and three new members were subsequently identified in this family of endofacial plasma membrane proteins [38]. PLSCR1 knockout mice do not show any impairment of hemostatic function, but present defective hematopoietic response to growth factors [39]. A transient outward vectorial transport has been reported, explaining the rapid expression of PS-dependent procoagulant activity in stimulated platelets, and suggesting the existence of a floppase specific for aminophospholipids [40]. Multidrug-resistance proteins (MDR) 1 and 3, members of the ATP-binding cassette (ABC) family of transporters, have been shown to transport different PL across the plasma membrane, but not endogenous PS [41]. More recently, knockout of the *ABCA1* gene, another member of the ABC family (mutated in Tangier disease, a disorder of free cholesterol efflux to high density lipoprotein [42]), led to decreased ( $\sim 70\%$ ) PS externalization ability and consecutive membrane vesiculation in ionophore-stimulated red blood cells of transgenic mice. Furthermore, PS redistribution was abnormal in such cells, whereas that of PC was normal, and ionophore-stimulated embryonic fibroblasts from *ABCA1*<sup>-/-</sup> mice exposed significantly less PS than counterpart cells from wild-type animals [43]. Despite the fact that ABCA1 can function only as a regulator/adaptor protein rather than as a true transporter [44], the results of its knockout clearly point at the ABCA subfamily for the search for candidate gene(s). Furthermore, ABCA1 provides an objective link between intimately related cardiovascular disorders such as atherogenesis resulting from defects of cholesterol metabolism and thrombosis, implicating several activated cell types bearing exposed PS and releasing procoagulant MPs.

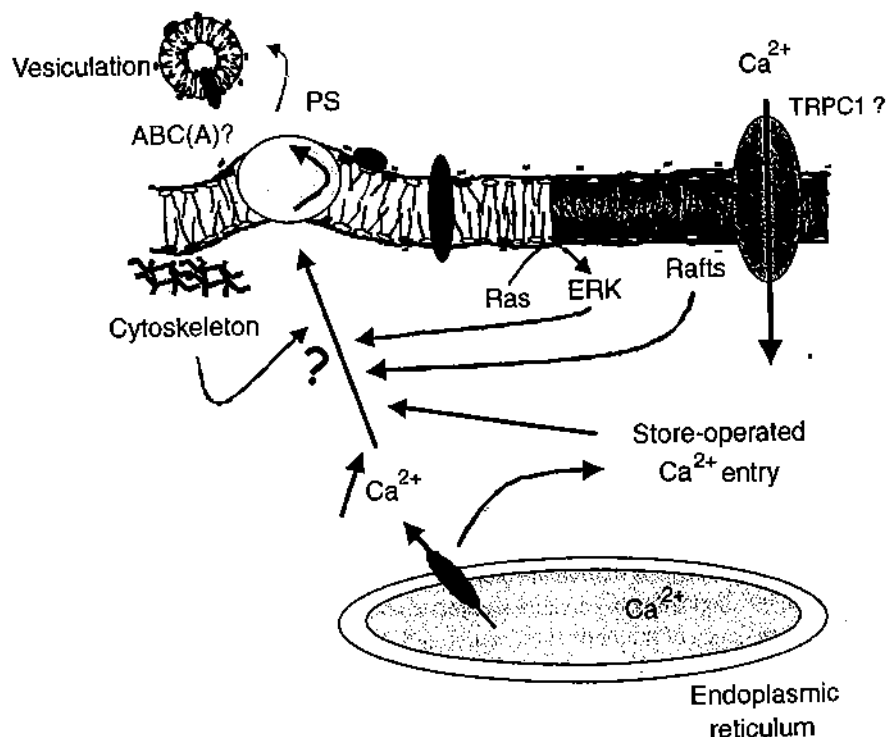


Fig. 1. Mechanisms participating in the regulation of the transmembrane migration of phosphatidylserine (PS) in activated cells, followed by microparticle shedding. After stimulation, calcium is released from intracellular stores. Calcium depletion induces the activation of store-operated calcium entry (SOCE) through channels in the plasma membrane. Transient receptor potential channel (TRPC) proteins have been proposed as the candidates mediating SOCE. The transverse redistribution of PS is under the control of SOCE. Raft integrity, cytoskeleton organization and MAP kinase pathway (Ras-ERK) are also involved in membrane remodeling. ABCA1, a member of the ATP-binding cassette family of transporters, is a potential candidate for the transport of PS. (PS is distinguished by its net negative charge.)

Concerning capacitative  $\text{Ca}^{2+}$  entry, a candidate gene family to be considered is that of transient receptor potential channels (TRPC), thought to mediate SOCE [27]. In our experience, a polyclonal antibody directed to TRPC1 reduced SOCE and the degree of PS exposure in HEL cells [32]. The expression of TRP proteins has also been assessed in detail in differentiating human stem cells and platelets [45]. Again, it has to be emphasized that the expression and function of the different members of a gene family can be expected to vary according to the cell type.

Once a candidate gene or genes have been confirmed, the next obvious step will be to search for the mechanism linking cytosolic  $\text{Ca}^{2+}$  and PS transport. Such a link is suggested by our observations of the colocalization of PS and TRPC1 with rafts in stimulated megakaryocytic HEL cells (C. Kunzelmann, J.-M. Freyssinet, M.C. Martinez, unpublished data).

### The pathophysiologic significance of cellular MPs

Because stimulated or damaged cells are hardly accessible *in vivo* owing to the efficiency of the clearance systems, MPs remain the objective parameter testifying to deleterious cellular alterations. Despite the fact that PS is probably not translocated across the plasma membrane of apoptotic cells or activated cells other than platelets to promote coagulation (see above), derived MPs are most probably procoagulant *de facto*, as are those circulating in normal individuals [46]. As for other thrombotic risk factors, it has however, to be emphasized that elevated MPs do not necessarily lead to thrombosis, as they can also promote the expression of the anticoagulant activity of activated protein C [47] and the PS content may vary according to the cell type, subjects and conditions of induction [48].

Several groups have designed methods aimed at measuring MPs, mainly based on size and phenotypic analyses by flow cytometry [49], while we have set up an MP capture assay enabling assessment of the associated procoagulant potential(s) and the presence of specific antigens. The capture of MPs can be achieved through either interaction of PS with immobilized annexin V or antigens with immobilized specific antibodies [22,50]. Each method has its inherent drawbacks, and often depends on the quality of reagents, usually antibodies. In addition, the possible interference of soluble antigens released from the membrane of stimulated cells may lead to underestimation of MP levels by flow cytometry or by antigenic capture, while capture by annexin V usually requires the presence of accessible PS, as PS exposure and consecutive vesiculation are generally accepted hallmarks of activated cells (see above).

Numerous reports have now been published on the presence of high levels of MPs in various disorders, clearly demonstrating their marker value. Most of these studies, if not all, concern pathologic situations where functional cells disappear, with the corollary that a beneficial treatment should lower MPs. On the other hand, it is logically conceivable that increased MPs could reflect the efficiency of tumor or autoreactive cell destruction in cancer or autoimmune disease therapy.

Whatever the situations leading to MP release, there is increasing evidence that they are detected by cells of various

types, often in remote tissues, different from those from which they stem, and with which they can interact through surface-borne antigens and/or to which they can transfer new phenotype(s) after membrane fusion [51]. The relevant literature suggests that the mechanisms governing MP-cell interactions are certainly multiple. In the first instance, it could be assumed that concentration is the determining physical factor, implying that MP composition in biologic effectors becomes important beyond a basal threshold. This seems reasonable in the light of homeostasis, where basal levels of MPs are thought to reflect a balance between cell proliferation, stimulation and death. Any excess in one of these directions usually leads to a pathologic disorder with significant variations in MP composition depending on the nature of the stimulus. At this stage, newly generated MPs are generally 'bad', in the sense they can elicit harmful responses in 'target' or recipient cells, whereas MPs detected in normal individuals are generally positive. The presence of the latter during homeostasis suggests that most are 'good' as long as they are kept around the basal threshold. The maintenance of homeostasis implies sensitivity to MP decrease, with an appropriate response being to restore normal basal level by destroying deviant cells that run the risk of acquiring a dangerous character due to abnormal survival, leading to, for example, tumorigenesis. In this general context, MPs can be considered relevant biologic sensors.

### 'Bad' cellular MPs

The following overview is not intended to be exhaustive, but rather aims to highlight the most surprising effects of MPs.

The vascular compartment has been the favorite territory of investigation, probably owing to the facility of MP extraction and analysis from peripheral blood. Hence, platelet MPs have been observed to activate platelets and endothelial cells [52], modulate monocyte-endothelium interactions [53], and mediate leukocyte-leukocyte interactions under flow [54]. These MPs contain arachidonic acid, which can trigger a variety of responses [55]. Lipopolysaccharide-stimulated monocytes release MPs bearing TF and active adhesion complexes, making them able to disseminate a double procoagulant potential [13], whereas in those stemming from non-stimulated monocytes, thrombomodulin is thought to counteract PS-dependent procoagulant activity, at least in part [56]. Leukocytic MPs activate endothelial cells and stimulate TF induction [57,58], and conversely, endothelial MPs, induce monocyte activation and TF expression [59]. MPs of endothelial origin have been shown to contain matrix metalloproteinases with an impact on angiogenesis [60], and plasminogen activator inhibitor-1 has been found to promote procoagulant MP generation in endothelial cells [61]. Circulating MPs from patients with chronic heart failure [62] or myocardial infarction [63] cause endothelial dysfunction, this harmful potential being significantly attenuated after appropriate treatment of the patients resulting in MP decrease [64]. Two challenging issues in the vascular context are the relationship between high levels of soluble P-selectin and MPs [65], and the role of MP-MP and MP-cell interactions in

decrypting blood-borne or membrane-encrypted TF [66–68], both being tightly associated with vascular and thrombotic diseases. It has been proposed that the transfer of TF from platelets to monocytes occurs via platelet-derived MPs and depends on P-selectin (CD62P) [69]. Platelet MPs have been observed to be the major location of plasma TF, which could acquire functional competence upon interaction of such MPs and platelets with neutrophils, mediated by P-selectin and CD18 integrins [70]. Furthermore, *in vivo* experimental evidence exists that blood-borne TF is a major determinant of perfusion in the coronary circulation [71]. In general, because of their procoagulant [46,72] and/or pro-adhesive [13,70] characters, most MPs can be considered 'bad' when in excess under pathologic circumstances complicated by thrombotic events in which they can participate.

In blood vessels or intercellular space in solid tissues, MPs are deleterious when they carry Fas ligand (FasL) and induce apoptosis of beneficial cells, e.g. killer T cells normally in charge of triggering death of dangerous cells. This has been reported for MPs derived from tumor cells [73,74], providing a realistic explanation for tumor escape. In addition, tumor MPs can promote angiogenesis [75], and apoptotic bodies from tumor cells (among which there is probably a proportion of MPs) have been shown to induce *in vivo* tumor formation through oncogene transfer [76]. Interestingly, circulating platelet MPs have been shown to be elevated in patients with gastric cancer, together with VEGF, interleukin (IL)-6 and RANTES, and could constitute a metastasis predictor [77]. As already stated, one of the most intriguing features of MPs is their capacity to deliver phenotypes to cells that do not normally express them, and hence their ability to elicit part of the responses mediated by the latter [51]. Under such circumstances, the chemokine receptor CCR5, which acts as a coreceptor for human immunodeficiency virus, could confer susceptibility to infection to endothelial cells when acquired through MPs stemming from peripheral blood mononuclear cells [78]. The same might be true for hepatitis C virus and CD81, provided this membrane antigen is confirmed as receptor for the virus [79]. The presence of the normal cellular form of the prion protein, PrP<sup>c</sup>, on MPs of lymphocytic [80] or endothelial [81] origin raises questions regarding prion transmission.

Secretion of protein 'messengers' that lack a secretion peptide may well occur via membrane vesiculation, as demonstrated for IL-1 $\beta$  originating from monocytic cells stimulated by extracellular ATP acting on P2X<sub>7</sub> receptors [82].

Another possible consequence of the longer half-life of MPs is the development of an immune response directed to these cellular fragments, providing a template for PS-protein interactions to form protein-PL antigen complexes. The resulting antibodies are thought to be part of the PL-binding antibodies evidenced in the antiphospholipid syndrome [83,84]. Increased MPs of endothelial origin have indeed been detected in patients with lupus anticoagulant, a subgroup of anti-PL antibodies [85].

Finally, it should be mentioned that MPs represent a source of aminophospholipid substrates for secretory phospholipase A<sub>2</sub> to generate lysophosphatidic acid, a potent pro-inflammatory

mediator and platelet agonist [86]. This may explain abnormally low MP levels [72], even their absence (B. Hugel & J-M. Freyssinet, unpublished data), in multiple organ failure or septic shock complicated by disseminated intravascular coagulation, in which extremely high concentrations of MPs would normally be expected; they may be destroyed by phospholipase A<sub>2</sub>.

### 'Good' cellular microparticles

'Good' MPs are those fulfilling a function in hemostasis, in which platelet MPs are primarily believed to act (see above). This is illustrated in hemophilia where MP levels are higher than normal, and can be further elevated in case of acute bleeding [87], reflecting a permanent mobilization of the hemostatic system, although without the threat of thrombotic complications since there is insufficient thrombin formed. MPs of non-platelet origins may also behave as occasional hemostatic agents although they have not been produced for this purpose, and they can become 'bad' in other situations previously described.

Examples of MPs with a positive role are much more restricted than for 'bad' ones, probably due to the lack of studies in this direction. Although FasL-bearing MPs can be 'bad' (see above section), FasL fulfills a number of fundamental biologic functions, hence those of FasL<sup>+</sup> MPs contributing to the turnover of cell-associated FasL can however, be quoted in the 'good' category [88]. Secretion of anti-inflammatory cytokines through MP release is another case, likewise IL-1 $\beta$  release [82] but with the opposite function, illustrating the possible dual role of MPs.

In the light of the discussion introducing this paragraph, it is conceivable that 'good' MPs are those with beneficial associated information, e.g. those with a rescue or reorientation message, which can be of two different natures. In the case of excessive degeneration of certain cells or tissues, homeostasis could be restored after interpretation of corresponding MPs by relevant stem cells, which could be stimulated to differentiate into appropriate lineage to counterbalance cell death. On the other hand, abnormal survival of cells in the process of acquisition of a dangerous character could be discerned by bystander cells through a decrease of MPs stemming from the former (proliferating) ones. Hence, 'good' MPs should play a part in development, angiogenesis, wound healing, and more generally in tissue remodeling, under the form of positive or negative gradients of information delivered to neighboring cells. The possibility of induction of distinct patterns of genes by plasma membrane fragments has indeed been considered in development [89].

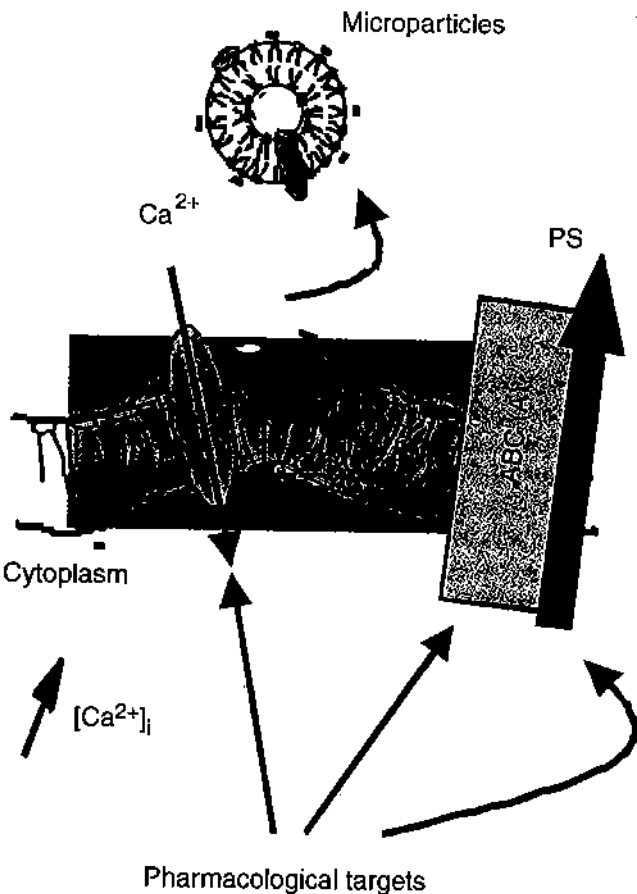
### Impact in pharmacology

The marker character of MPs makes them of particular interest for the follow-up of the efficiency of therapeutic treatments, as already discussed. In addition, it can be mentioned that, owing to the lack of species specificity of PS, MPs can be assessed in animal models.

Improvements in understanding of the mechanisms governing PS exposure process offer an opportunity for a new pharmacologic approach to thrombotic risk based on the control of PS

available at the activated cell surface and on the degree of consecutive membrane vesiculation [90]. In this context, the triangular relationship involving  $\text{Ca}^{2+}$  entry, PS externalization, and perhaps cholesterol efflux is rather novel in that it provides a rational link between disorders of cholesterol metabolism leading to atherogenesis and those of excessive PS exposure at the origin of thrombosis. Figure 2 summarizes possible targets that are valid for the modulation of the release of MPs themselves.

A general feature of pharmacologic mediation is that it must offer an unquestionable benefit/risk ratio. With high levels of 'bad' MPs associated with an increased thrombotic propensity, the benefit is obvious, provided that 'good' MPs are not dramatically decreased. The ideal situation would be the possibility of a selective control of the degree of PS exposure and membrane vesiculation in a particular lineage, without interference in other cell types. In thrombosis, platelets are of course of prime interest, and the absence of clinical manifestations other than bleeding in homozygous-type *Scott* patients and the lack of symptoms in heterozygous-type offspring [24,25] are reassuring in this sense.



**Fig. 2.** The identification of the regulatory mechanisms of phosphatidylserine (PS) transmembrane migration is a key step in developing a new pharmacologic approach to the thrombotic risk based on the modulation of procoagulant potential associated with an excess of externalization of PS and consecutive release of microparticles. Besides their procoagulant character, microparticles are of course of value for diagnosis.

## Conclusions

MPs are not only procoagulant plasma membrane fragments with prothrombotic potential, but also behave as true diffusible vectors in the transcellular exchange of biologic information. One of the most exciting issues is to establish whether MPs may constitute genuine biologic entities, productively interpreted throughout evolution of multicellular organisms, rather than simple cell debris, and able to transfer information and phenotypes, therefore modifying the intrinsic properties of recipient cells. This implies that more investigations are carried out in order to prove this is a general characteristic with *in vivo* relevance. In this respect, a challenging question is that of the transfer of functional rafts, or at least of elements capable of inducing the structuring of such membrane domains, with consequences for signaling in acceptor cells.

Whatever the nature of the problem involving MPs as markers or participants, they have to be measured, and, for this primary purpose, the hemostasis laboratory offers the most appropriate tools.

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