1. ABSTRACT

Microparticles (MPs) are submicron vesicles released from stimulated or apoptotic cells after plasma membrane remodeling. In body fluids, they constitute relevant hallmarks of cell damage. Having long been considered inert debris reflecting cellular activation or damage, MPs are now considered as cellular effectors involved in cell–cell crosstalk. This review focuses on the pathophysiologic significance of MPs in the particular setting of solid graft and cellular transplantation.

2. INTRODUCTION

In the vessel microparticles (MPs), also referred to as microvesicles, are submicron fragments shed from the plasma membrane of stimulated or apoptotic cells (1-3). MPs constitute relevant hallmarks of cellular activation or damage (4). They also act as transcellular effectors in hemostasis, thrombosis, atherosclerosis, arterial wall vasomotoricity, vascular remodeling, inflammation, cellular adhesion and angiogenesis (2, 5-10). MPs of various cellular origin (platelets, leukocytes, endothelial cells) are detectable in small amounts in the plasma of healthy subjects that may increase in various pathophysiologic circumstances such as the evidence of cardiovascular risk factors (diabetes, hypertension, hyperglycemia), cardiovascular diseases (acute coronary syndrome, pulmonary embolism, pulmonary arterial hypertension, heart failure), stroke, kidney failure, hemodialysis, cirrhosis, and cancer among the most investigated (Table 1).

During the last sixty years, developments of allogeneic transplantation techniques and immunosuppressive therapies have improved survival of patients with severe organ failure generally associated with apoptosis and cell activation. Nowadays kidney, heart, liver, pulmonary, pancreatic islet, bone marrow and stem cells transplants are grafted and the vessel remains a central element connecting donor and host tissues. The monitoring of transplanted patients relies on the clinical and biological follow-up, and on biopsies to confirm complication and graft rejection. An earlier detection of graft rejection is one of the crucial goal for efficient patients’ caring and immunosuppressive therapy management.

The present review focuses on the pathophysiologic significance of MPs in solid graft and cellular transplantation. The incidence of immunosuppressive therapy on MP level is discussed.
3. BIOGENESIS AND BIOLOGY OF MICROPARTICLES

3.1. Microparticle generation

Knowledge of the general molecular mechanisms leading to the MP release from the budding plasma membrane mainly comes from in vitro studies on platelets (3, 4, 11, 12) (Figure 1). MP shedding is the ultimate consequence of the membrane remodeling initiated by cell stimulation. Membrane response to stress is characterized by phosphatidylserine (PtdSer) translocation from the inner to the outer leaflet under the control of specific transporters termed floppase and flippase that are believed to govern the aminophospholipid distribution across the bilayer (13-15). Calcium influx is another feature of cell activation or apoptosis enabling the cleavage of the cytoskeleton by calcium-dependent proteolysis. Cytoskeleton cleavage and reorganization, the latter being under the dependence of ROCK-I, a Rho-kinase acting by myosine light chain phosphorylation that induces cell membrane contraction, promote membrane blebbing and MP release into the extracellular fluid (Figure 1) (16, 17).

3.2. Composition of microparticles and functional characteristics

MPs bear or contain functional membrane glycoproteins (selectins, adhesion molecules, CDs…), bioactive phospholipids, cytoplasmic components (caspase-3, DNA, RNA, …), and various antigens characteristic of the cells they are derived from (Figure 1). MP protein composition may also vary with the cell type and applied stimulus (apoptosis or stimulation) (3, 4, 18-20). When harboring appropriate membrane ligands, MPs can behave as vectors of transcellular exchanges of biological information or of procoagulant potential, addressing their “message” to target cells expressing the corresponding counter-receptors (12).

MPs are considered procoagulant, because they constitute an additional procoagulant phospholipid surface for the assembly of the clotting enzyme complexes promoting thrombin generation. Indeed membrane remodeling and MP shedding in plasma provide accessible PtdSer, a procoagulant anionic aminophospholipid (21). This ubiquitous MP property is reinforced when cells express tissue factor (TF), the major cellular initiator of coagulation, up-regulated in stimulated monocytes and endothelial cell (22). The detection of proteins inhibiting coagulation that are either harbored or present in MP membrane such as tissue factor pathway inhibitor, protein C or thrombomodulin has raised the question of an eventual MP contribution to anticoagulant pathways, (23-26). Furthermore, anionic phospholipids promote the assembly of the protein C anticoagulant complexe, at concentrations 10-fold higher than those required for procoagulant activity. At the MP surface, it is reasonable to assume that

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**Table 1. Diseases presenting enhanced microparticle levels**

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Microparticles in transplantation

Figure 1. Membrane remodeling and MP generation. In resting cells, constitutive aminophospholipids, phosphatidylserine (PhtdSer) with its net negative charge, and globally neutral phosphatidylethanolamine, are mostly sequestered in the inner (cytoplasmic) leaflet of the plasma membrane, whereas sphingomyelin and phosphatidylcholine constitute the majority of the outer (exoplasmic) leaflet phospholipids. This asymmetric distribution is under the control of an inward aminophospholipid translocase (flippase), floppase and scramblase. When subjected to procoagulant, pro-inflammatory or apoptogenic stimulation, a sudden increase in cytosolic calcium, calpain activation and cytoskeleton proteolysis are observed leading to the loss of membrane asymmetry. ROCK-I activation leads to myosin light chain phosphorylation and cytoskeleton contraction. The swift egress of aminophospholipids to the exoplasmic leaflet is not compensated rapidly enough by other phospholipid inward redistribution, resulting in a transient overload of the outer leaflet at the expense of the inner one, explaining bleb formation and MP shedding.

3.3. Cellular origin of membrane microparticles and pathophysiologic background

Most MPs have been characterized from body fluids (27). Because they survive longer the activated cells they are stemming from, probably owing to their smaller size and greater ability to diffuse and transiently escape phagocytosis, MPs constitute relevant hallmarks of cell damage (4). With the exception of the atherosclerotic plaque, data from the literature mostly concern circulating MPs that can be considered a specific storage pool (6, 22). In the blood flow, MPs are mainly of platelet origin. Depending on the pathophysiologic context, endothelial cells, leukocytes, monocytes, erythrocytes, smooth muscle cells and macrophages represent other possible contributors to this circulating pool (3). Antigens specifically expressed during cell activation could prove useful in discriminating underlying pathologies and associated damages.

Recent advances suggest that MPs could reflect the pathways tuning their biogenesis and release. Of particular relevance in cardiovascular disorders, endothelial antigens CD31 and CD62E borne by circulating MPs were reported to discriminate apoptosis or cytokine stimulation (18, 20, 28). Additional complexity was recently suggested as circulating MPs may bear antigens from different cellular origins, pointing to multiple transcellular MP-mediated exchanges (29, 30).

3.4. Clearance of microparticles

Little is known about MP clearance, of major importance in the deciphering of MP effects. MP levels reflect the balance between production and clearance, a crucial element for their assessment in body fluids. The
Microparticles in transplantation

Major determinant of MP clearance is related to Phldser membrane exposure (31-34). The specific receptor of Phldser, expressed by macrophages, fibroblasts and epithelial cells, contributes to apoptotic cells and bodies recognition and phagocytosis. Other cellular actors in MP clearance have been described, including annexin-I, CD14, CD36, LOX-1, β₂-glycoprotein 1, lactadherin, vitronectin receptor, Mer tyrosine kinase, and apolipoprotein E (35-38). MP clearance may also involve plasma phospholipase A2 (39). In the assessment of circulating MPs, misleading quantification or phenotypes could result from accelerated degradation by secretory phospholipase A2 (39, 40), interactions with the vascular wall (41), or trapping in cell–cell aggregates or within the thrombus (42).

3.5. Detection and measurement of microparticles

MPs are highly heterogeneous in size (0.1 - 1 μm) and composition, depending on the stimulus and cells at their origin. In these respects, MPs have to be discriminated from exosomes, another form of cellular vesicles released from large multivesicular bodies. Of smaller size and more homogeneous composition, they act as conveyors of immune responses and expose little if any Phldser (43).

Because the conditions of isolation, mainly depending on centrifugation steps, are crucial for MP characterization, there is no consensus on their size distribution. Therefore, values yielded by the different methods of determination proposed so far may vary on a wide range (44), according to physical and/or biological parameters they are relying on (45). Nine methods have been described including flow cytometry (44, 46), solid phase capture with functional read out (4, 47), thrombin generation tests (48), ELISA (49), impedance-based flow cytometry, atomic force microscopy (50), dynamic light scattering (51), fluorescent single particle tracking, and proteomics, lipidomics analysis (52, 53). Flow cytometry is widely used for phenotyping MP. The discrepancy between its theoretical size limitation determined by laser wavelength (460 to 700 nm) and microparticle size raises the question of MP phenotype distribution as a function of size range. Nevertheless, variations in the tiny visible part of the MP « ice berg » detected by flow cytometry might remain statistically significant in clinical and biological studies (45).

Global assessment of MPs is performed through annexin A5, a highly specific probe for Phldser. Specific antibodies are used to determine MP cellular origin. For instance, CD41 (GPIIb) or CD42 (GPIb) reveal a platelet origin, whilst CD62E (E-selectin), CD144 (VE-cadherin), CD146 (s-endo), CD105 (endoglin), CD54 (ICAM-1), CD51 (α₅ integrin) are common markers of endothelial MPs. CD31 (PECAM) is present on platelets and endothelial cells. However, considering its low representation on platelets, it remains a reliable endothelial marker, as proven by the discrepancy between platelet and endothelial MP levels in plasma, the latter being far more scarce.

3.6. Paradoxical cytoprotective effects of microparticles

Due to their association with thrombotic, inflammatory and degenerative diseases, the deleterious effects of MPs have been extensively investigated, primarily as procoagulant entities (2, 3, 5, 54). Nevertheless, their participation in anticoagulation has been shown (25), and their cytoprotective properties recently suggested (1, 55, 56).

Scarce data on the absence of MP-mediated apoptosis or on their direct cytoprotective abilities can be noticed in the literature. HUVEC, dermal or synovial fibroblasts treated by leukocyte-derived MPs show no increased apoptosis (57, 58). Suspensions containing a proportion of mesenchymal stem cell-derived MPs were found cytoprotective on kidney tubular cells through CD44 and β₁ integrin-dependent binding, as they promoted cell proliferation and accelerated functional recovery after acute kidney tubular injury (59). Cytoprotection by modification of gene profiling in target cells is illustrated by the action of activated protein C (APC) recruited by EPCR on MPs, that appeared to tune endothelial cell apoptosis through Bax down-regulation and the up-regulation of Bel-X, inflammatory genes being controlled as well (23, 56, 60). Indeed, besides its anti-coagulant function, APC shows anti-inflammatory and anti-apoptotic properties, modulates TNF-α-induced NF-κB pathway and downstream expression of ICAM, VCAM, E-selectin and fractalkine (61). The anti-apoptotic effects of APC were suggested to be dependent on protease-activated receptor-1 (PAR-1) and to alter the expression of Bel-2 homologue and inhibitors of apoptosis (IAPs) proteins. In addition, proteolysis of cytotoxic extracellular histones by APC could also be beneficial (62).

Cytoprotection may also result from two other complementary mechanisms: the MP-mediated sorting out of deleterious molecules such as caspase-3 from challenged parental cells (63) or the delivery of apoptosis modulators embedded within MPs with eventual cytoprotective effects on proximal target cells. The latter hypothesis relies on proteomic analysis showing that MP proteins vary with the stimulus and that a proportion of anti-apoptotic proteins is detectable in apoptotic lymphocyte-derived MPs (52, 53, 64).

Taken together, these data are in favor of a role of MPs in the cellular tuning between survival or death.

3.7. Other beneficial effects of microparticles in the maintenance of vascular integrity and function

Although MPs were mainly considered deleterious effectors leading to vascular dysfunction, several MP types were shown able to limit vascular damage, favor vascular repair and promote angiogenesis, raising the question of their contribution to vascular homeostasis and potential role in graft survival. Indeed, platelet-derived MPs also favor the endothelial homing through the delivery of platelet adhesion receptors to hematopoietic stem cells promoting chemotaxis, cell adhesion, proliferation and survival. In rats, locally injected platelet-derived MPs improved revascularization of the ischemic myocardium in a growth factor-dependent mechanism (65, 66).

Furthermore, MPs contribute to various storage pools. In atherosclerosis, sequestered MP-driven
neovascularization might thus be counterbeneficial by promoting intraplaque hemorrhage and consecutive vulnerability. Indeed, MPs from atheromatous plaques were demonstrated to up-regulate VEGF in endothelial cells, and promote endothelial proliferation and neovessel formation in a CD40L dependent process (67). Conversely, in a mouse model of post-natal vasculogenesis, endothelial MPs from the ischemic muscle appeared determinant for progenitor cell differentiation (68).

In septic shock, hyporeactivity together with tissue hypoperfusion and hypoxia account for severe hypotension. Beneficial effects of MPs in patients with septic shock were recently suggested by Soriano et al. (69). In their study, lower levels of endothelial-, platelet- and leukocyte-derived microparticles were associated with higher mortality rates and organ dysfunction. The cytoprotective effect of MPs was suggested to rely on their ability to maintain a tonic vasopressor response. This challenging hypothesis was supported by the demonstration that MPs from patients with septic shock prevent vascular hyporeactivity in LPS-treated mice through thromboxane A2 delivery, accounting for enhanced aortic contraction (70).

4. MICROPARTICLES IN TRANSPLANTATION

4.1. Kidney transplantation

Apart from the excretion of waste products, kidney functions include the maintenance of fluid, electrolyte and salt balances, hormone secretion, and blood pressure control. The worsened evolution of severe chronic renal failure (CRF) toward end-stage renal disease is of poor prognosis in the absence of dialysis, and often associated with cardiovascular events, accounting for more than 50% of all deaths (71). Indicative of major vascular damage, CRF patients display endothelial dysfunction and accelerated atherosclerosis. Diabetes mellitus, one of the main causes of CRF, is another disease commonly associated with endothelial dysfunction, dialysis and transplantation remaining ultimate treatments.

Elevated levels of circulating MPs characterize the chronic vascular damage observed in severe renal diseases. Elevated endothelial-, platelet-, erythrocyte-, leukocyte- and neutrophil-derived MPs have been described in CRF and hemodialysed patients (72-76). In CRF patients, endothelial MP levels were tightly associated with arterial dysfunction characterized by the loss of flow-mediated dilation, increased aortic pulse wave velocity and common carotid artery index. Underlying mechanisms involved impairment of endothelium-dependent relaxation and cyclic guanosine monophosphate generation (73).

In CRF patients, circulating endothelial MPs may also reflect the deleterious effects of uremic toxins like p-cresol and indoxyl sulfate reported to increase MP generation from treated endothelial cells (76).

CRF is the consequence of intricated processes leading to the progressive decline of renal function associated with increased inflammation and accelerated atherosclerosis (77). Aside from endothelial MPs typical of vascular damage in CRF patients, elevated MPs of leukocytic, neutrophil and platelet origin suggest additional contribution of inflammation, atherosclerosis and thrombosis to the progression of the disease (78, 79). Because of the frequent association between renal failure and cardiovascular diseases with similar confounding risk factors predisposing to both diseases, like smoking, hypertension, diabetes mellitus, caution should be taken when interpreting MP levels. Furthermore, the question of possible underscored MP levels by flow cytometry due to the presence of neutrophil- and platelet-derived MPs aggregates has been evoked in CRF and hemodialysed patients since each multi-MPs aggregate could be recorded as a single event (78). In cardiovascular issues circulating multi-MP aggregates combining platelets, MPs of platelet, endothelial or monocyte origin have been reported. (42, 80). In addition, a proportion of hybrid MPs cumulating markers of different cell lineages could also contribute to the underestimation of a specific MP phenotype (30).

In CRF and relative to hemodialysis, kidney transplantation greatly improves survival by decreasing the worsening of cardiovascular diseases and mortality (81-83). Kidney transplantation was associated with decreased MP levels that remained stable at least one year, regardless of the cellular origin (leukocytes, platelets, erythrocytes, granulocytes). Nevertheless, procoagulant activity borne by MPs was still higher than in healthy controls, possibly reflecting hypercoagulability and cardiovascular background in CRF patients (Table 2) (72, 84). Indeed, the decrease of MP levels was more pronounced in patients without clinical history of cardiovascular diseases. Such observations, illustrate the importance of the recording of clinical background on the design of a clinical study. Indeed, variations in circulating MP levels may be consecutive to multiple amplification loops. Aside from cardiovascular background, dialysis arrest could per se contribute to the reduced MP levels reported in kidney-transplanted patients. Indeed, hemodialysis sessions favor the generation of platelet- and neutrophil-derived MPs owing to dialysis membrane-induced complement activation and to extracorporeal dialysers with consequences on the inflammatory status varying with membrane type (celluloid vs. synthetic) (78, 85, 86). Taken together, decreased MP levels could be indicators in the follow-up of restored renal function after transplantation. Indeed, circulating MPs may be associated with acute rejection episodes leading to graft function loss, as suggested by immunohistochemical studies. Platelet-derived MPs, assessed by CD61, were detected in glomerular and peritubular capillaries of allograft nephrectomy with hyperacute humoral rejection and severe endothelial injury (87, 88). To decipher the role of MPs as pathogenic markers in acute humoral or cellular rejections, further studies aimed at the characterization of specific circulating MP profiles are needed. In this respect, advantage could be taken from the recent immunohistological BANF-H classification (89).

4.2. Heart transplantation

Heart transplantation is the ultimate treatment of severe congestive heart failure (CHF), mainly related to coronary artery diseases. The raise in endothelial and
platelet MPs is a characteristic feature of heart failure (28, 90-92). CHF is also associated with elevated plasma C-C chemokines and pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α, that cause endothelial cell dysfunction and apoptosis. On the basis of the direct or reactive oxygen species-mediated deleterious effects of such cytokines, a pathophysiologic link between the loss of normal endothelial function and congestive heart failure has been proposed (90). Interestingly, in CHF patients, anti-oxidant treatments by vitamin C and carvedilol promoted endothelial cytoprotection and decreased MP levels, suggesting that apoptosis contributes to the generation of the endothelial MP pool (90-92). The combined assessment of endothelial MP phenotype and plasma biomarkers such as soluble selectins might bring new hints on the molecular mechanisms underlying endothelial damage in CHF. Indeed, In vitro studies have shown that the phenotypic profile of endothelial-derived MPs might characterize the parental cell stress. MPs with constitutive markers, such as CD31, are markedly increased in apoptosis, whereas those expressing inducible markers, such as CD54 and E-selectin (CD62E), are prominent after cell activation (20). In patients, the phenotypic assessment of endothelial cell surface components would thus provide information on the functional status of the endothelium. After heart transplantation a different pattern of endothelial MPs is observed, MPs bearing CD62-E are decreased, suggesting a diminished endothelial injury by comparison with CHF, and a lower ratio between E-selectin- and CD31-bearing MPs is detected, consistent with a higher contribution of apoptosis to the endothelial MP pool (Table 2) (28, 90-92).

In heart transplant recipients, acute rejection remains the leading cause of death within the following year (93). Although immunologic or non-immunologic markers have been widely explored (94-97), diagnosis is still based on immunohistological analysis of endomyocardial biopsies (98). Endothelial cells contribute to the procoagulant vascular response during acute allograft rejection and are possible targets for the alloimmune reaction leading to apoptosis (99). In a prospective study, we observed a specific MP pattern in patients undergoing a first episode of acute cardiac rejection (100). A significant elevation of MPs bearing tissue factor (TF), Fas or E-selectin could be associated with rejection and suggested endothelial cell activation and Fas-mediated apoptosis. Furthermore, E-selectin-bearing MPs appeared an independent marker of acute allograft rejection that was still informative after adjustment for graft characteristics (time elapsed from heart transplantation, cold ischemia time, TF- and Fas-bearing MP levels). All together, MP assessment could provide an early, accurate, and easy-to-assess marker for the detection of acute rejection. Additional data from larger cohorts with different rejection grades will be helpful to decipher the mechanisms at the origin of endothelial MP release. Animal models will be needed to evaluate the incidence of endothelial apoptosis and activation in graft survival.

4.3. Liver transplantation

Liver is the main provider of blood coagulation factors and plays a major role in metabolism and plasma detoxification. Serious liver diseases lead to cirrhosis, a disorganization of liver structure characterized by cell death and organ fibrosis, generally occurring as a consequence of chronic viral hepatitis, alcoholism, or biliary ducts diseases. Liver transplantation then appears the ultimate treatment.

In alcoholic fatty liver, alcoholic liver cirrhosis or hepatitis-C liver cirrhosis with thrombocytopenia, the inverse correlation between platelet counts and elevated platelet-derived MPs plasma levels, reflects platelet activation (101, 102). In hepatitis-C liver cirrhosis patients, platelet activation assessed through platelet-derived MPs is correlated to liver fibrosis biological markers and associated with the fibrosis histological scoring, suggesting a relationship between platelet activation and liver fibrosis (101). Interestingly, in alcoholic liver cirrhosis, a ten days discontinuation of alcohol intake reduced platelet-derived MPs, suggesting its specific role in platelet activation (102). Compared to healthy control, hepatitis C patients with or without hepatocellular carcinoma, show elevated MPs, including endothelial- and hepatic- derived MPs (103). Within the first week after liver transplantation, a raise in circulating MPs was reported, values of total and endothelial MPs returning to the baseline observed in healthy controls after day 14 (Table 2) (103). Interestingly, this prospective study of a small cohort, showed a decrease in circulating MPs bearing hepatic markers that was less pronounced at D14. With respect to the other cell origins of MPs, it could be hypothetized that specific dynamics in the release of hepatic MPs occurs after transplantation that surgery could not account for. Indeed, hepatic MP levels in patients with partial hepatectomy remain at baseline values after surgery. In liver transplantation, dynamics of MP generation thus follows a general profile mainly characterized by a 2 weeks transient elevation. Despite a low rate of complications, it was observed that patients with poor clinical outcome after transplantation may escape this pattern, MP levels remaining elevated in sepsis and acute rejection (103).

The interest of MPs as possible indicators of the liver function remains to be confirmed, especially with respect to known variations of other biological markers such as ASAT, ALAT or coagulation factor V. For instance, one could expect that values of ASAT and ALAT reflecting cytolyisis are correlated with circulating MPs released in blood flow owing to massive cellular loss. Similar comparison between MP values and biological markers of the hepatic function are needed to confirm the usefulness of MPs in the follow-up of the transplanted liver status and in the assessment of graft rejection.

4.4. Pancreatic islet transplantation

Islets of Langherans are highly vascularized and specialized units of the endocrine pancreas. Constitutive cell lineages of the islets are dedicated to the production of hormones such as insulin and glucagon. In type 1 diabetes, pancreatic islets are the target of autoimmune reactions leading to endocrine cell death and dysregulation of insulin secretion. Despite progress in clinical management (insulin therapy, diet), diabetes remains a chronic disease associated
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Table 2. Microparticle variations in corresponding transplantation issues

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with severe vascular complications and endothelial damage including micro and macroangiopathies in kidney (diabetic nephropathy), lower extremities (diabetic foot ulcers), eyes (diabetic retinopathy), coronary arteries, and brain. In patients with end-stage type I diabetes, the restoration of a physiological endocrine secretion by pancreatic islet transplantation is of prime interest. Recently, the success of the Edmonton approach to islet transplantation, through a steroid-free immunosuppression, gave rise to particular interest (104-110). Pancreatic islets, isolated from the donor organ after collagenase digestion and sequential purification steps, are transplanted in the liver by injection into the portal vein. Only a small fraction of the transplanted islets successfully engraft (111) explaining that the functional capacity of the transplanted islets only reaches ~20% of that observed in non diabetic subjects (112). Among the reasons for the incomplete success, difficulties in islet isolation, efficacy of immunosuppression to prevent local inflammation and immunoreactivity, activation of the coagulation cascade, absence of islet regeneration, immunosuppression side effects, poor engraftment, and the absence of beta cell mass markers should be mentioned (105-110). Low engraftment efficacy may be related to the occurrence of local coagulation and to the rapid local inflammatory response termed IBMIR (instant blood-mediated inflammatory reaction) resulting from the contact between injected islets and blood. Beside allograft rejection, this local inflammatory reaction at the site of islet transplantation is often associated with negative clinical outcome and seems to be a major determinant of the partial loss of beta cell mass in a short time. IBMIR is characterized by the recruitment of platelets to the islet and by leukocyte infiltration leading to islet integrity disruption and loss. In isolated and in situ pancreatic islets, active TF borne by MPs has been reported in α and β endocrine cells whereas endothelial cells and exocrine cells from pancreatic sections do not express TF, indicating the absence of inflammatory signal-mediated TF up-regulation (Table 2) (111, 113, 114). IBMIR is prevented after TF blockade by active site-blocked factor. Hyperglycemia and plasma C peptide levels do not allow the early evaluation of the loss of beta cell mass and function. Easy-to access biological markers of early islet disruption are still lacking. Molecular detection of circulating beta-cells by RT-PCR of insulin mRNA has been proposed for the follow-up of islet transplantation (108). Recently we could evidence elevated levels of circulating MPs in patients that underwent islet loss. Interestingly, a peak in MPs appeared one month before the c-peptide drop and the raise in insulin needs. Furthermore, MP levels returned to baseline after a second transplantation that restored islet function and normal c-peptide levels. Further investigations are indeed needed to confirm the prognosis value of MPs in islet transplantation and to characterize the damaged pancreatic cell lineages that might contribute to MP shedding (115).

4.5. Hematopoietic stem cell transplantation

The bone marrow is a tissue of great immune potency. Allogeneic hematopoietic stem cells transplantation (HSCT) into the bone marrow involves the transfer of a potent immune system. This kind of transplantation is mainly performed in severe genetic diseases with deep immunodeficiency or enzyme defects, in aplastic anemia, in blood cancer like leukemia, or in solid malignant tumors. Rejection or the development of a graft-versus-host disease (GVHD) are threatening issues in stem cell transplantation. Prevention is usually achieved by a conditioning regimen preceding HSCT that includes different steps (chemotherapy, total body irradiation, treatment by anti-thymocyte globulin). HSCT is frequently associated with hypercoagulability, with an eventual role of the conditioning regimens that is still debated (116-119). In line with the increased thrombosis risk, elevated levels of circulating platelet-derived MPs were observed after the chemotherapy step (117). Other authors reported an
absence of significant difference in total or endothelial-derived MPs during the conditioning period (116). Indeed, in patients undergoing HSCT and GVHD, a continuous increase in platelet-, monocyte- and endothelial-derived MPs, as well as soluble VCAM-1, E-selectin, and IL-2R, a GVHD marker, is observed during the first month (Table 2) (120). The raise in plasma endothelial markers and MPs may reflect endothelial injury which is a common feature of GVHD (118, 121). Endothelial MP levels could even be viewed as indicators of the severity of GVHD since they appear to discriminate higher grades (122). Interestingly, in the particular settings of HSCT, platelet-derived MPs that are able to bind and transfer adhesion receptors to hematopoietic cells, may prove beneficial in situ by promoting cell engraftment and accelerating bone marrow regeneration (66, 123-125).

4.6. Pharmacological and immunosuppressive treatments

Several therapies known to be beneficial in cardiovascular disorders were reported to reduce the concentration of circulating procoagulant MPs (7). Indeed treatments with statins (simvastatin, pravastatin, fluvastatin) (126-128), anti-platelet therapies (ticlopidine, clopidogrel, aspirin, cilostazol, abximacib) (92, 129-133), beta-blockers (carvedilol) (90), anti-oxidants (vitamine C) (91, 134), anti-hypertensive therapies (AT II receptor antagonists, calcium channel antagonists) (126, 135-139), or prostaglandins (140) were associated with a decrease in MP levels. These observations support the hypothesis that part of the beneficial effect of treatments is linked to decreased MP pathogenicity, at quantitative or qualitative levels. Conversely, atrial fibrillation treatment by digitalis glycosides was associated with increased MP levels. Their molecular mode of action would involve the inhibition of membrane Na+/K+ channels promoting the raise in sodium and calcium intracellular concentrations and consecutive MP shedding (141).

Although immunosuppressive therapy is a key treatment in graft transplantation management, little is known about its eventual incidence on MP levels. One could expect that control of rejection would protect the graft from cell damage and therefore avoid the release of MPs associated with hypercoagulable states impairing the integrity of graft vessels. On the other hand, platelet MPs were proven to behave in situ as proangiogenic factors promoting neovascularization (65). In fact, variations in circulating MP levels after transplantation probably combine indications on the vascular host and graft status, and on the tissue remodelling or cell loss. Thus, caution should be taken in the assessment of the mechanisms at the origin of MP release with respect to each clinical background and immunosuppressive therapy. For instance, calcineurin inhibitors (cyclosporin, tacrolimus) may be cytotoxic. The immunosuppressive regimens, also including proliferation inhibitors (azathioprine, mycophenolate mofetil) and steroids, can cause, favor or worsen hyperlipidemia, hypertension, anemia, diabetes, infections, nephrotoxicity or cancer (81). Indeed, during the acute phase of GVHD in hematopoietic cells transplantation, a trend for higher endothelial MP levels has been reported after corticosteroid treatment (prednisolone) whereas no significant variation could be shown using mycophenolate mofetil or cyclosporin A (122). In the setting of kidney transplantation, Al-Massarani et al. compared the impact of two immunosuppressive regimens, cyclosporin/azathioprine or tacrolimus/mycophenolate mofetil, on vascular toxicity markers, among which endothelial MPs, circulating endothelial cells and sVCAM-1 (84). After 1 year follow-up, MP levels showed a continuous decrease, but no significant variation could be observed with respect to the treatment regimen. The absence of endothelial MP or sVCAM-1 variations appears intriguing because the authors detected a significant difference in circulating endothelial cells that were elevated after tacrolimus/mycophenolate mofetil treatment, suggesting an underlying endothelial damage. Such data point at a possible limitation in the use of endothelial MPs for the monitoring of transplanted patients, partly because of the small proportion of circulating endothelial MPs and of the technical difficulty in the assessment of smaller MPs by conventional flow cytometry. In this interesting prospective study, the constant reduction in circulating endothelial cells observed in the cyclosporin/azathioprine subset was in favor of a diminished endothelial damage, even after adjustment for diabetes. These data point at circulating endothelial cells as reliable indicators of endothelial injury while MPs or sVCAM-1 appear more delicate to assess.

5. CONCLUSION

MPs are circulating indicators of cellular stress when parental cells are hardly accessible. In the particular settings of transplantation, their variations appear a challenging approach for the non invasive investigation of the graft status. Further studies are however needed to determine whether MP profiles are characteristic of cell activation or of cell loss at the onset of rejection and if they could be useful in the monitoring of patients. Transplantation is the ultimate treatment of severe chronical diseases often associated with intricated cardiovascular damages and heterogeneous treatments that may be confounding factors in the interpretation of MP levels. Interesting data point at the eventual role of MPs as cellular and tissular actors in the tuning of cell response. Distal or local MP-mediated effects remain to be ascertain in cellular crosstalk models and in small animal models of transplantation. Histological assessment and scoring remain to be correlated to MP levels, possibly indicative of the cellular orchestration of graft survival or death.

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