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Endothelial microparticles in transplant patients – great potential but a long way to go

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1. ABSTRACT

This overview provides information on the current state of endothelial microparticle research. Microparticles are small membrane vesicles shed by different cell types, which contain cell surface proteins and cytoplasmic components of the original cell. The microparticle production is a part of normal cell function, but it increases by apoptotic cells and cells under stress. Numerous evidences suggest that circulating endothelial microparticles may be a marker of endothelial cell function. Endothelial cells play an important role in the pathogenesis of allograft dysfunction and rejection. The endothelium is one of the main targets in allograft rejection. Recent studies have indicated that levels of circulating endothelial microparticles change in patients with solid organ transplants, including kidneys. We believe that circulating endothelial microparticles may be a useful tool of allograft function monitoring and/or a diagnostic marker of allograft rejection.

2. INTRODUCTION

Endothelial cells (EC) play an important role in the pathogenesis of allograft dysfunction and rejection. With improved immunosuppression and surgical techniques, graft survival has significantly increased. Nevertheless, allograft rejection remains one of the main causes for allograft failure. Typically, renal allograft function is evaluated by serum creatinine (SC) levels. Unfortunately, SC level elevation is a non-sensitive and non-specific marker of renal allograft dysfunction. By the time the serum creatinine levels become elevated, damage to the renal allograft may be significant.

Microparticles are small membrane vesicles shed by different cell types, which contain cell surface proteins and cytoplasmic components of the original cell. Microparticle production is a part of normal cell function, but it increases by apoptotic cells and cells under stress. Others and we had previously demonstrated that levels of

circulating endothelial microparticles may be used as a surrogate marker of EC dysfunction. Recent studies have indicated changes in circulating endothelial microparticles levels in patients with solid organ transplants, suggesting that the levels of circulating endothelial microparticles may be useful as a biomarker of EC function in patients with solid organ transplants.

3. THE ENDOTHELIUM AS AN ORGAN

Since the first description of blood circulation by Harvey in the 17th century and the portrayal of the connection between arteries and veins by capillaries in Marcello Malphigi' and Antoni van Leeuwenhoek's classic studies, more than two centuries have passed before Friedrich von Recklinghausen described in 1860 that capillary walls are lined by cells, which was named by Wilhelm His as endothelium in 1865. A few years later, Charles Rouget first described contractile capillary cells, which were named pericytes by Zimmerman in 1886. Von Recklinghausen and later Heidenhahn suggested that the endothelium is not only a physical barrier between the blood and interstitium, but also had properties consistent with an active cell system (1). However, it took another 60 years before electron microscopy studies of the vascular wall by George Palade and lymphocyte-endothelium interaction studies by James Gowans led to the modern understanding of the endothelium as an organ, which is responsible for regulation of the hemodynamics, angiogenic vascular remodeling, metabolic, synthetic, inflammatory, anti- and pro-thrombogenic processes (2).

The endothelium is one of the largest organs in the human body, weighing about 1 kg and covering up to 7 m^2 of the surface area. The endothelium lines the entire circulatory system. However, despite the common anatomical location inside of the vessel wall, physiologically endothelium is quite diverse. Endothelial cells have different function and structure in different vascular beds (3).

Endothelial cells play an important role in regulation of many vascular functions, including regulation of vascular tone and blood pressure by production of vasoconstricting [endothelin-1, platelet-derived growth factor (PDGF), platelet-activating factor (PAF), leukotriene C4] and vasodilating [nitric oxide, prostacyclin (PGI_2), prostaglandin E_2)] biologically active substances; regulation of coagulation (thrombosis & fibrinolysis); formation of new blood vessels (angiogenesis); regulation of inflammatory processes, including atherogenesis. Endothelial cells form a selective barrier between the blood and interstitium and they control the passage of different molecules and the transit of white blood cells through the capillary wall (5).

4. ENDOTHELIAL MICROPARTICLES

4.1. Microparticle formation as a part of normal cellular function

Since the first description of "platelet dust" in 1967 (5), the ability of eukaryotic cells to shed small fragments of the cytoplasm encapsulated by the plasma membrane into the extracellular space has been established for many cell types (6-9). Such fragments, also known as microparticles, typically range in size from 0.1 to 1 mcm (some authors recognize micropartilce size up to 2 mcm) and they contain cell surface proteins and cytoplasmic components of the original cell. This property makes possible a separation and analysis of microparticles of different origin using different antigen-antibody-based methods, including **f**luorescence-activated cell sorting (FACS), enzyme-linked immunosorbent assay (ELISA) and other methods (9-17). For example, microparticles shed by polymorphonuclear leukocytes express selectins and integrins, complement regulators, proteins of the human leukocyte antigen system (HLA) and other markers of neutrophils (18), whereas microparticles derived from endothelial cells express CD31, CD54, CD62E, alphaVbeta3 integrins, etc (19, 20). Shedding of membrane-associated endothelial cell surface elements containing beta1 integrins and different matrix metalloproteinases, including MMP-2, MMP-9 and MT1-MM has been observed *in vitro* (21).

Formation of microparticles is a part of normal cellular function (10), although it increases in cell stress and apoptosis (22). The pool of circulating microparticles contains microparticles shed by different cell types, including platelets, leukocytes and endothelial cells. The endothelium-derived microparticles represent about 10-15% of circulating microparticles (9, 23). In healthy people, circulating microparticles of endothelial origin are present within the concentration range of 1-70x10^3/ml (8, 24, 25). These numbers significantly change in many different conditions. Increased numbers of circulating endothelial microparticles have been documented in patients with lupus anticoagulant (19), acute coronary syndromes (26), during cardiopulmonary bypass (27), unstable angina and lacunar infarcts (28, 29), or diabetes mellitus (30). The levels of circulating microparticles may be gender-specific and even change within the menstrual cycle. Thus, women generally have an increased number of circulating platelet- and endothelium-derived microparticles, as compared to men. Moreover, the number of these microparticles is elevated during the luteal phase of the menstrual cycle (31).

The mechanisms of microparticle formation are not completely clarified. During cell activation, a remodeling of the plasma membrane may lead to externalization of aminophospholipids, such as phosphatidylserine. These aminophospholipids are almost exclusively present in inner leaflets of the plasma membrane in quiescent cells. A group of enzymes is involved in the regulation of the cell membrane asymmetry, including gelsolin (identified in platelets only), aminophospholipid translocase, floppase, scramblase and calpain. These enzymes maintain the dynamic asymmetry of the cell membrane, controlling traffic of phospholipids and aminophospholipids between the outer and inner sides of the cell membrane. A Ca^{2+} influx changes this stable state and results in a relocation of aminophospholipids to the outer side of the cell membrane and possibly initiates

the formation of microparticles (32-35). An increased formation of endothelial microparticles *in vitro* was observed after a treatment with Ca^{2+} ionophore A23187 (36). Scanning electron microscopy provided remarkable images of microparticle shedding from cultured cells (21).

Formation of microparticles *in vivo* is a complex process, regulated not only by numerous cytokines, but physical factors as well. It has been reported that shear stress plays an important role in the formation of circulating microparticles by circulating blood components and endothelial cells. Thus, the microparticle formation by platelets *in vitro* was increased by high shear stress, while an activation of protein kinase C (PKC) promoted the shear-dependent formation of microparticles (37). Microparticle formation was mediated via platelet glycoprotein Ib (GPIb-alpha) receptor - von Willebrand factor interaction (38) and it was accompanied by the cleavage of platelet endothelial cell adhesion molecule-1 (PECAM-1) from the platelets (39). The levels of circulating endothelial microparticles were inversely correlated with brachial artery and aortic shear stress in patients with end-stage renal disease (40).

Abid Hussein *et al* (41) demonstrated that microparticle formation plays an important role in normal function of endothelial cells. An inhibition of microparticle formation by calpeptin (a calpain inhibitor) in quiescent cultured human umbilical vein endothelial cells (HUVEC) resulted in an increased detachment of HUVEC from the culture dish, accompanied by caspase 3 accumulation in HUVEC. Co-incubation of these cells with calpeptin and the apoptosis-inducing material staurosporin (ATP-competitive kinase inhibitor) resulted in a dramatic increase in the number of detached HUVEC. Caspase 3 accumulated in adherent cells and >90% of the cells detached within 48 hours. The authors propose that the formation of microparticles allows cells to escape from accumulation of caspase-3, cell detachment and apoptosis; therefore, contributing to endothelial cell survival.

The formation of microparticles is facilitated by numerous different stimuli. We demonstrated that plasminogen activator inhibitor-1 (PAI-1) stimulates the formation of endothelial microparticles both *in vitro* and *in vivo* (9). PAI-1 treatment of cultured HUVEC resulted in a reduced expression of urokinase receptor **(**uPAR) and disrupted its co-localization with caveolin, accompanied by a uPAR increase in the culture medium. FACS analysis revealed that PAI-1 rapidly and dose-dependently increased the number of microparticles expressing uPAR and alphaVbeta3 integrin. This process was attenuated by pretreatment with neutralizing anti-uPAR antibodies (9). Interestingly, PAI-1 knockout mice showed a significantly decreased number of circulating endothelial microparticles as compared to wild-type mice. PAI-1 knockout mice responded to an intraperitoneal injection of PAI-1 with a more pronounced increase in the number of circulating endothelial microparticles. PAI-1 treatment increased the number of microparticles stained with Annexin V, an evidence for the expression of anionic phospholipids (9). Several studies demonstrated that TNF-alpha alone (19) or in combination with cycloheximide (42) also results in an increased production of endothelial microparticles *in vitro*.

Microparticles shed by activated or apoptotic cells are distinct both quantitatively and phenotypically. In apoptosis, the expression of constitutive markers (such as CD54, CD62E and CD106) was increased in endothelium-derived microparticles with a concomitant decrease of these markers in the cells of origin, whereas during an activation of endothelial cells, the expression of these markers was increased both in cells and microparticles (20).

Recent advances in proteomics research allowed detailed studies of the protein composition of microparticles. Thus, using a polyacrylamide gel electrophoresis followed by MALDI-TOF mass spectrometry, Banfi *et al* (43) demonstrated that TNF-α - stimulated endothelial microparticles had a complex protein structure, including metabolic enzymes, cytoskeleton associated proteins, adhesion receptors, members of protein folding elements, membrane fusion proteins and even nucleosome proteins (43). Another report described the protein composition of PAI-1 - stimulated endothelial microparticles. Using a similar experimental technique, the authors describe 58 proteins found in microparticles and confirmed that these proteins are derived from different cellular components (44). The protein composition of microparticles derived from endothelial cells by different stimuli was diverse, confirming previous observations (20). These data not only identified proteins involved in the known microparticle-cell interactions, but also demonstrate the complexity of endothelial microparticles and open a door for further investigations of the biological role of circulating microparticles in health and different pathological processes.

4.2. Endothelial microparticles as a marker of endothelial cell function

Endothelial cell dysfunction is a precursor and common denominator of many cardiovascular diseases, including atherosclerosis, diabetic vasculopathy, hypertension, and progressive cardiomyopathy. It has been well documented that altered function of endothelial nitric oxide synthase (eNOS) and/or decreased bio-availability of nitric oxide (NO) are fundamental abnormalities leading to the pathophysiologic manifestations of endothelial cell dysfunction (45-47). In fact, accumulated data suggest that many clinical manifestations of endothelial cell dysfunction are intimately linked to the expression and function of eNOS (3, 48, 49).

Many reports describe increased levels of circulating endothelial microparticles in the background of endothelial cell dysfunction. Increased numbers or proportions of circulating endothelial microparticles have been reported for patients with different cardiovascular diseases, including atherosclerosis (50-52), coronary artery diseases (26, 53-55), congestive heart failure (56), nonvalvular atrial fibrillation (57), systemic hypertension (58, 59) and pulmonary hypertension (60). The levels of endothelium-derived microparticles were altered in patients after a cardiovascular surgery, including cardiopulmonary

bypass (27) and heart transplantation (61). In addition, the levels of circulating endothelial microparticles was elevated in diseases which are well known to be accompanied by endothelial cell dysfunction, such as metabolic syndrome and obesity (62-64), diabetes mellitus type 1 (65) and type 2 (51, 64, 66), stroke (67), pre-eclampsia (23, 24, 68), end stage renal disease (69, 70) and sepsis (71-73). Interestingly, hematological disorders also are accompanied by an altered number of circulating endothelial microparticles, which includes lupus anticoagulant (19), sickle cell disease (74), thrombotic thrombocytopenic purpura (25), antiphospholipid syndrome (75) and venous thromboembolism (76). Data from experimental animals also reported an increased number of circulating endothelial microparticles in rats with type 2 diabetes (77) and in mice after treatment with PAI-1 (9). In addition, endothelial microparticles were reported to induce endothelial cell dysfunction and acute lung injury after injections in rats and mice (78, 79). It has been reported that a large amounts of phosphatidylserine-bearing microparticles are produced in human atherosclerotic plaques. These microparticles were produced mostly by monocytes and T-lymphocytes (52). The presence of endothelial microparticles in atherosclerotic plaques was identified recently by Leroyer *et al.* (80). In addition to already described populations of plaque microparticles, the authors reported a presence of microparticles derived from macrophages, granulocytes, erythrocytes and smooth muscle cells. Plaques from symptomatic and asymptomatic patients showed no differences in microparticles origin (80). Interestingly, microparticles originated from atherosclerotic plaques enhance the shedding of the TNF receptor-1 and endothelial protein C receptor from cultured endothelial cells (81).

Endothelial microparticles may induce endothelial cell dysfunction both in *in vitro* and *in vivo* setting. Circulating microparticles isolated from the blood of preeclamptic women directly affect the endothelium, impairing endothelium-dependent relaxation *in vitro* in isolated myometrial arteries. In contrast, the blood from healthy pregnant women had no effect on the endothelium-dependent vasorelaxation in the same experimental setup (23). Moreover, rat aortic rings exposed for 24 hours to circulating microparticles obtained from patients with acute myocardial infarction showed significant impairment in the endothelium-dependent relaxation, whereas microparticles obtained from patients with non-ischemic cardiovascular diseases had no effect on the endothelium-dependent relaxation (82). Evidences that microparticles originated from different cell types may induce endothelial cell dysfunction have been reported. Thus, microparticles from apoptotic smooth muscle cells dose-dependently reduced the vasodilatory response to acetylcholine by murine aortic rings and this phenomenon was mediated via beta3-integrins (83).

We have demonstrated that endothelial microparticles obtained from cultured cells may impair endothelium-dependent relaxation induced by acetylcholine at concentrations, which are similar to those observed in patients with various cardiovascular diseases (84). Thus, 3-hour incubation of aortic rings with high concentrations of endothelial microparticles lead to a significant impairment of acetylcholine-induced relaxation of aortic rings. However, incubation of aortic rings with endothelial microparticles in concentrations, which are seen in healthy people, did not affect acetylcholine-dependent relaxation of aortic rings. In addition, endothelial microparticles increased superoxide production in cultured endothelial cells, as well as by the endothelium of aortic rings *in vitro*. Possible pathophysiologic mechanisms of this phenomenon may include an uncoupling of eNOS, which lead to switch from the nitric oxide to superoxide production by this enzyme (3, 49, 85). In fact, inhibition of eNOS by L-NAME partially inhibited the microparticle-induced release of superoxide, suggesting an important role of eNOS in the increase superoxide production. The increase in superoxide production induced by endothelial microparticles may play an important role in the pathogenesis of impaired endothelium-dependent relaxation. Indeed, treatment with the superoxide dismutase (SOD)-mimetic MnTBAP restored not only acetylcholine-induced relaxation of aortic rings impaired by endothelial microparticles, but NO production as well. Additionally, endothelial microparticles *per se* can produce detectable amounts of superoxide and they contain subunits of NADPH-oxidase (84).

Furthermore, we found that isolated endothelial microparticles in pathophysiological concentrations impair angiogenesis *in vitro*, affecting the capillary-like network formation by cultured endothelial cells (86). Thus, using the Matrigel assay of angiogenesis *in vitro* and a topological analysis of the capillary-like network by HUVEC, we demonstrated that endothelial microparticles affect all parameters of angiogenesis, including decreased total capillary length, number of meshes and branching points, and increased mesh area. The positional and topological order indicated that endothelial microparticles affect angiogenic parameters uniformly over the capillary network. Treatment with the cell-permeable SOD-mimetic MnTBAP partially or completely restored all parameters of angiogenesis affected by endothelial microparticles. Endothelial microparticles also reduced the cell proliferation rate *in vitro*. The apoptosis rate in cultured HUVEC incubated with endothelial microparticles was increased in a time- and dose dependent manner, a phenomenon which was also prevented by MnTBAP (86). Endothelium-derived microparticles dose-dependently inhibited bFGF- and VEGF-induced proliferation and migration of cultured human cardiac valve endothelial cells (87). Another study demonstrated that in "physiological" concentrations endothelial microparticles may promote capillary tube formation by cultured HUVEC. This proangiogenic activity of endothelial microparticles was attributed to matrix metallolproteases MMP-2, MMP-9 and MT1-MMP, which were found in shed microparticles. The metalloproteases delivered by microparticles to cultured endothelial cells may facilitate the formation of capillary tubes by proteolysis of the basement membrane (21). Another possible mechanism of this augmentation of angiogenesis by endothelial microparticles may be an activation of plasminogen into plasmin at the surface of

endothelial microparticles, as it has recently been described (88, 89).

Circulating endothelial microparticles may be used as a potential hallmark of endothelial cell dysfunction *in vivo*. One of the early markers of endothelial injury in patients with coronary artery disease, even before the onset of myocardial infarction, may be endothelial microparticles. High levels of procoagulant endothelial microparticles were described in the circulating blood of patients with acute coronary syndromes, which may contribute to the generation and perpetuation of intracoronary thrombi (26). Later it was adjoined with angiographic data, when the levels of endothelial microparticles were correlated with high-risk angiographic lesions, including eccentric type 2 lesions, multiple irregular lesions and lesions with thrombi in patients with acute coronary syndromes (53, 54). These microparticles may be produced by apoptotic endothelial cells, since the number of CD31+/Annexin V+ microparticles was correlated with the impairment of coronary endothelial function, as it was assessed by the endothelial-dependent vasodilatation in patients with coronary artery disease (55). Moreover, an increased ratio of endothelial microparticles to endothelial progenitor cells was found in patients with hypercholesterolemia, which may contribute to aortic stiffness in these patients (90). Also, the numbers of circulating CD31+/CD42- endothelial microparticles were correlated with the deterioration of artery elasticity profiles in healthy subjects, as it was evaluated by pulse-wave analysis (91). This correlation was independent of other important covariates of arterial stiffening, like age and blood pressure levels, suggesting that an increased production of endothelial microparticles and arterial wall stiffness may coexist in early stages of endothelial cell dysfunction (92). Also, we demonstrated that levels of circulating endothelial microparticles in the blood obtained from 8- and 22-week old Zucker Diabetic Fat (ZDF) and their counter pairs ZL rats are associated with endothelial cell function in these animals. Levels of circulating endothelial microparticles were significantly increased in ZDF rats at 22 weeks, as compared to ZL rats of the same age. Treatment with the peroxynitrite scavenger/antioxidant Ebselen significantly attenuated the increase in circulating endothelial microparticles in ZDF rats. The number of circulating endothelial microparticles was associated with the severity of endothelial cell dysfunction in ZDF rats, evaluated by the acetylcholine-induced vasorelaxation and nitric oxide production (77).

Recently a new potential area of microparticles research has been discovered. Patients with increased cardiovascular risk factors have an increased release of pro-atherogenic microparticles from endothelial cells and reduced number of the atheroprotective endothelial progenitor cells. Pirro *et al* (93) reported that the levels of circulating microparticles derived from endothelial progenitor cells are correlated with Framingham risk score in patients with various degrees of cardiovascular risk. The authors propose that an increased microparticle shedding from endothelial progenitor cells may reduce the number of these cells in the circulation and may contribute to increase aortic stiffness beside traditional risk factors (92, 93).

Growing evidences support an important role of endothelial microparticles as a marker of endothelial cell dysfunction. On other hand, endothelial microparticles themselves in high concentrations may induce dysfunction in endothelial cells, at least *in vitro*. These lead us to hypothesis that circulating endothelial microparticles are not only markers of endothelial cell dysfunction, but also play an important role in the pathogenesis of endothelial cell dysfunction (Figure 1).

4.3. Endothelial microparticles in patients with solid organ transplantation

More than 27000 solid organ transplants are performed annually in the USA. This includes more than 18000 kidney transplants, more than 6000 liver transplants and more than 2000 heart transplants (94). With improved immunosuppression and surgical techniques, graft survival has significantly increased and the population of patients with a functioning solid allograft is increasing. Nevertheless, allograft rejection remains one of the main causes for allograft failure. The endothelium is one of the main targets in allograft rejection, especially antibody-mediated rejection (AMR). Altered expression of endothelial genes due to alloantibodies acting on the microcirculation was noted in patients with antibody mediated rejection. Sis *et al* (95) performed analysis of microarray expression of 119 endothelial-associated transcripts (ENDATs) in renal allograft biopsies. They found that mean ENDATs expression was increased in both cellular and antibody mediated rejections, but it was higher in antibody mediated rejection than in T-cell-mediated rejection. Increase in ENDATs expression was correlated with histopathologic findings. Many individual ENDATs were increased in antibody mediated rejection and it was correlated with allograft loss. Only 40% of kidneys with high ENDATs expression and chronic antibody mediated rejection had positive PTC C4d staining. The authors conclude that high renal endothelial transcript expression in patients with alloantibodies is indicator of active antibody-mediated allograft damage and poor graft outcome (95).

Interestingly, endothelial damage may pre-exist in renal allografts. Thus, biopsies from cadaveric renal allografts prior to exposure to cyclosporine or other known endothelial toxins revealed loss of glomerular endothelial cells on light microscopy and shrinkage of the endothelial cells away from the glomerular basement membrane by ultrastructural examination. Extensive microvascular thrombi were seen as well (96).

Usually renal allograft function is evaluated by SC levels. Unfortunately, SC level elevation is a non-sensitive and non-specific marker of renal allograft dysfunction, and it may occur in many different conditions, including acute kidney injury secondary to disturbances in systemic circulation and renal blood flow, urinary outlet obstruction or infection (97). The “gold standard” test for the assessment of allograft rejection is the renal allograft

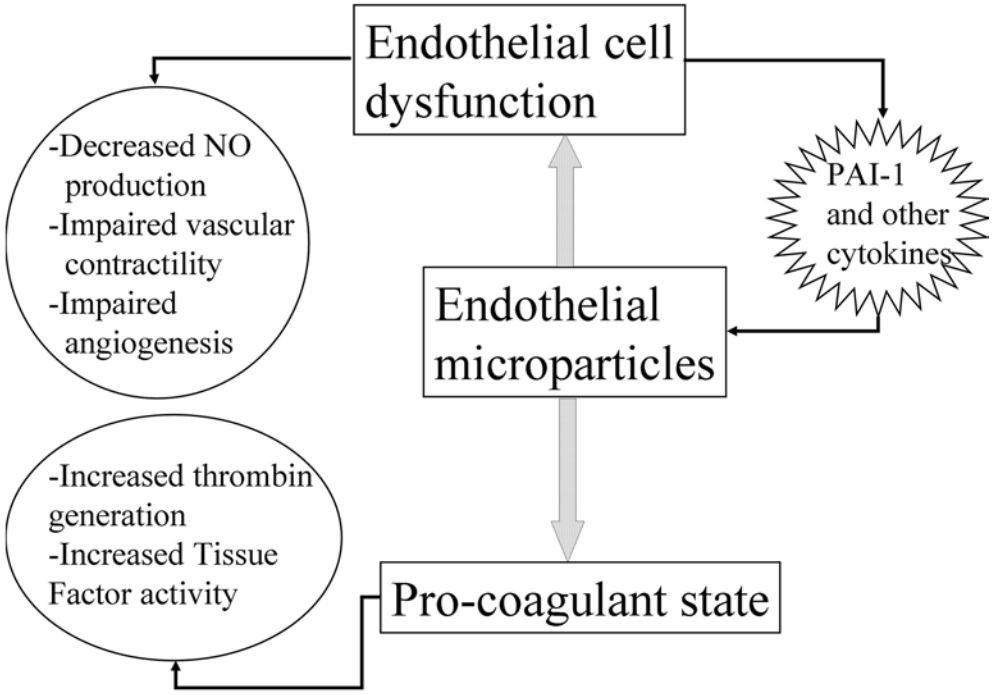

Figure 1. Proposed role of endothelial microparticles in the pathogenesis of endothelial cell dysfunction. Endothelial microparticles play an important role in the pathogenesis of endothelial cell dysfunction. Increased production of endothelial microparticles by endothelial cells is mediated by plasminogen activator inhibitor 1 (PAI-1) and other cytokines. Elevated numbers of circulating endothelial microparticles by themselves may induce endothelial cell dysfunction, resulting in decreased nitric oxide (NO) production and impaired angiogenesis. Also, increased numbers of endothelial microparticles result in procoagulant stage.

biopsy, which is an invasive, expensive and relatively risky procedure. The patterns of acute cellular rejection (ACR) are well-documented and broadly recognized (98, 99). However, diagnosis of AMR is one of the most challenging in renal pathology (99). Peritubular capillary (PTC) C4d staining is one of the markers of AMR. Whereas PTC C4d itself is not diagnostic of AMR, it is often accompanied by histologic features of acute or chronic AMR. However, some biopsies, mainly from ABO-incompatible renal allografts, show C4d staining without histologic findings of AMR or ACR (100). Recently, evidence that some forms of AMR may be negative for C4d staining has been described (101). Therefore, the need for a reliable and clinically significant marker of renal allograft rejection is emerging.

Solid organ transplantation creates the unique situation, where a new organ with clinically normal endothelium replaces the organ with malfunctioning endothelium. Therefore, the levels of circulating endothelial microparticles are dynamically changed after the surgery and they may reflect the functional status of the endothelium. This area of research is in a beginning stage of exploration, and only few studies about the levels of circulating microparticles in patients with solid organ transplantation are available. Thus, we had previously demonstrated that levels of circulating endothelial microparticles are increased in patients with Hepatitis C cirrhosis with and without hepatocellular carcinoma (HCC). Dynamics of circulating endothelial microparticles correlated well with the pathophysiological changes occurring after liver transplantation. The levels of circulating endothelial microparticles, initially elevated in patients with Hepatitis C cirrhosis, were rapidly decreased after liver transplantation, reaching the levels observed in healthy people within two weeks after the surgery (102).

Garcia *et al* described changes in circulating endothelial microparticles in patients with heart transplants. The levels of circulating endothelial microparticles were significantly elevated in patients with heart failure, as compared to control healthy people. These levels remained elevated after heart transplantation, but the levels of endothelial microparticles expressing CD62E (E-selectin, a marker of endothelial cell activation) were significantly decreased after heart transplantation. The authors conclude that heart transplantation is associated with a different pattern of endothelial injury than that seen in patients with heart failure and endothelial cell apoptotic activity is increased in post-transplant patients (61).

Levels of endothelial microparticles are changed after hematopoietic stem cell transplantation as well; the levels of endothelial microparticles were elevated in patients with allogeneic stem cell transplantation, as compared to patients with autologous stem cell transplantation. This was accompanied by increase in soluble Fas ligand, interleukin 6 (IL-6), tumor necrosis factor (TNF)-alpha (markers of graft versus host disease)

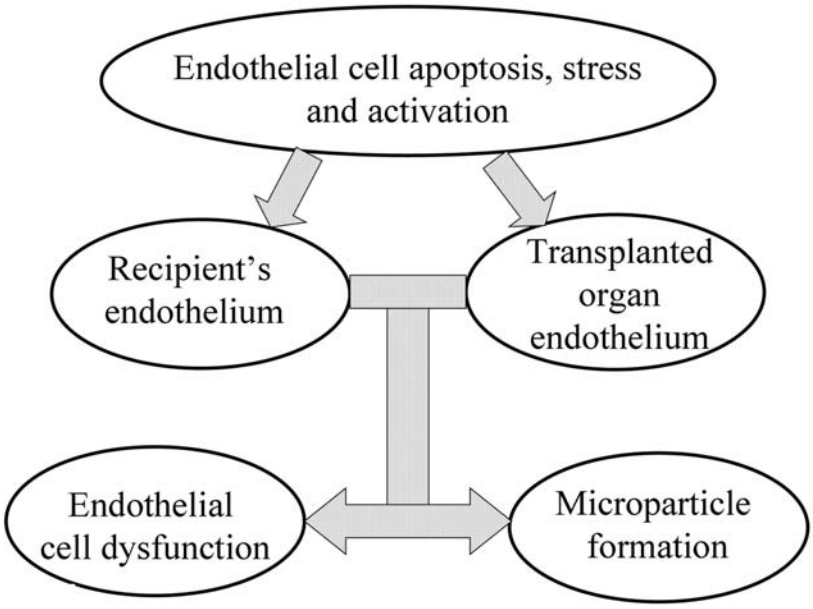

Figure 2. Proposed pathogenesis of endothelial microparticles formation in solid organ transplant patients. Increased formation of endothelial microparticles occurs during endothelial cell activation, stress or apoptosis, which is often seen during rejection episodes. Endothelial microparticles can be formed in different endothelial compartments, such as a transplant recipient or the allograft endothelium. Elevated numbers of endothelial microparticles may not only indicate functional status of endothelial cells in different compartments, but by themselves induce endothelial cell dysfunction.

and angiopoietin 2 (103). These data also suggest that levels of endothelial microparticles may be useful to assess different posttransplant complications.

In kidney transplant recipients, levels of endothelial microparticles were significantly decreased one year after transplantation, as compared to pretransplant values in the same patients. Interestingly, patients on cyclosporine and azathioprine immunosuppressive regimen had lower levels of endothelial microparticles posttransplant, as compared to patients who were on tacrolimus and mycophenolate immunosuppressive treatment. These data suggest that levels of circulating endothelial microparticles in renal transplant patients depend not only on the functional status of the endothelium, but on endothelial toxicity of immunosuppressive medications as well (104). The same group reported that the levels and procoagulant activity of circulating microparticles of non-endothelial origin after renal transplantation are also decreased. The decrease in circulating microparticle levels was more pronounced at 12 month posttransplant in patients without history of cardiovascular diseases, suggesting that improvement in vascular function after the transplantation plays an important role in the normalization of circulating microparticle levels (105).

Microparticles, shed by different cell types, express a subset of cell surface proteins similar to the plasma membrane of the original cell, including major histocompatibility complex (MHC) antigens (18). It is possible that microparticles originated from recipient and allograft endothelial cells have different protein composition. The pool of circulating microparticles is heterogeneous by its nature. First, microparticles are produced by different cell types. Second, endothelial microparticles, released from activated or apoptotic EC are distinct, both quantitatively and phenotypically. In apoptosis, the expression of constitutive markers (such as CD54, CD62E and CD106) increases in endothelium-derived microparticles with a concomitant decrease of these markers in the cells of origin, whereas during EC activation, the expression of these markers increases both in cells and microparticles (20). Third, microparticle formation *in vivo* is a complex process, regulated not only by numerous cytokines, but physical factors, such as shear stress, as well. Circulating endothelial microparticles in patients with solid organ transplantation are produced by the recipient endothelium and the endothelium of the allograft. The recent advantages in new and more powerful immunosuppressive regimens have increased the survival for MHC mismatched recipients and the number of these patients is significantly increased. It is possible that using different mismatched MHC antigens as a target for immunostaining, one can separate microparticles produced by the recipient and allograft endothelium and study its dynamic posttransplant. We hypothesize that the levels of circulating endothelial microparticles may be useful as a biomarker of EC function in patients with solid organ transplants (Figure 2). A hypothesis that endothelial microparticle composition is different in cyclosporin toxicity and acute rejection has recently been proposed. The authors propose that using an animal model it would be possible to distinguish endothelial microparticles populations in acute renal allograft rejection and calcineurin inhibitor nephrotoxicity (106).

In conclusion, the microparticle research is currently emerging to a new level. The number of manuscripts related to a microparticle research almost doubles every year. However, these reports mainly describe quantitative changes in the levels of circulating microparticles in patients with different disorders, not revealing the pathophysiological mechanisms leading to alterations in microparticle levels *in vivo*. One of the major questions, if circulating endothelial microparticles are one of the links in the chain of events in the development of endothelial cell dysfunction or they are just markers of dysfunctional endothelial cells, still remains unanswered. Also, it is possible that the levels of circulating endothelial microparticles from a transplant recipient and from the solid organ allograft are changing differently, especially during episodes of rejections, which would allow us using levels and quality of circulating endothelial microparticles as a useful tool of allograft function monitoring and/or as a diagnostic marker. This could be a next step in the area of microparticle research.

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