Circulating microparticles in renal diseases

Laurent Daniel1, Laetitia Dou3, Yvon Berland4, Philippe Lesavre5, Lise Mecarelli-Halbwachs5 and Francoise Dignat-George3

1Department of Pathology, CHU Timone-Adultes, 2UMR 911 INSERM, Université de la Méditerranée, 3UMR-S 608 INSERM, Université de la Méditerranée, 4Department of Nephrology, CHU Conception, Marseille, 5Department of Nephrology, CHU Necker and INSERM 507, Paris and 6Department of Haematology, CHU Conception, Marseille, France

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Introduction

Cellular microparticles (MP) are submicrometric fragments resulting from the remodelling of the plasma membrane in response to numerous conditions, including activation and apoptosis [1]. The general consensus is that MP are small (<1 µm), expose the anionic phospholipid phosphatidylserine (Phosphatidylserine) and express membrane antigens that reflect their cellular origin. These characteristics discriminate MP from exosomes, which are smaller (<0.1 µm), originate from intracellular multivesicular bodies and differ in antigenic composition [2].

MP formation, composition and functions

All cell types shed MP according to an active process controlling plasma membrane remodelling and antigenic turnover. Among the mechanisms proposed, disruption of the natural asymmetric distribution of membrane phospholipids is thought to be an important step explaining the translocation of Phosphatidylserine to the exoplasmic leaflet before membrane blebbing and MP shedding. Although calcium entry, activation of calpains and scramblase activity have been reported to be important processes [3], the exact mechanisms governing MP formation are not completely understood.

MP phospholipid composition, phenotypes and content vary according to the cellular origin and the inducer triggering their formation (Table 1). Determination of the cellular origin of MP relies on the expression of specific antigens. According to the cell type, their formation occurs following exposure to various agents, including calcium ionophore, ATP depletion, lipopolysaccharides, thrombin, cytokines and autoantibodies (Table 1). Therefore, vesiculation is a well-regulated process generating MP that present specific features, with significant implications on their functional activity.

Indeed, MP behave as vectors of bioactive molecules able to disseminate biological information in the vascular compartment. Due to the expression of both membrane Phosphatidylserine and functional tissue factor (TF), MP are catalytic procoagulant surfaces involved in thrombogenesis. They also harbour inflammatory components (LpA, IL-1 and chemokine receptor CCR5) [4,5], growth factors (TGF-beta and VEGF) and proteases (uPA and MMP) that are related to their involvement in inflammation, immune response, angiogenesis and cancer [6–8].

MP measurement and variations in atherosclerotic vascular diseases

Different methodologies, which were reported in a forum [9], are available for MP determination. Antibody-capture ELISA and flow cytometry rely on the antigenic composition of MP and allow them to be enumerated according to their cellular origin. Besides, functional assays measuring MP-associated procoagulant activity are based on Phosphatidylserine and TF expression. Finally, other assays combine ELISA capture on annexin or antibody and determination of procoagulant activity of MP.

MP are detectable in the peripheral blood of healthy volunteers. Elevated levels of circulating platelet-, monocyte- or endothelial-derived MP have been reported in cardiovascular disorders such as diabetes [10], acute coronary syndromes, hypertension [11], metabolic syndrome, heart transplantation and pulmonary or venous embolism [12]. It is now obvious that MP levels are associated with cardiovascular risk factors and most often correlate with disease severity and clinical outcome [1].

MP in renal diseases

Elevated levels of MP have been detected throughout the entire process of vascular damage associated with renal diseases.

During renal diseases with acute vascular lesions, such as Wegener granulomatosis or micro-polyangiitis, MP originating from platelets and neutrophils increase
dramatically. These levels, which were significantly higher than those reported in chronic vasculitis, may be considered as markers of disease activity [13]. In children with acute vasculitis, increased levels of endothelial MP (EMP) correlated with the Birmingham Vasculitis Activity Score and the acute-phase reactant levels, suggesting that they could be used as diagnostic tool in the context of febrile diseases [14].

A vesiculation process, reflecting endothelial injury and platelet activation, is also a feature of vascular disorders associated with immune-mediated renal lesions. In agreement, high platelet MP (PMP) levels are observed in microangiopathies, whereas EMP vary as lactate dehydrogenase levels over the course of acute phase. Elevation of EMP has also been reported in antiphospholipid syndrome, where they strongly correlate with procoagulant activity of lupus anticoagulant [15].

MP levels are increased during chronic renal failure (CRF), suggesting that vesiculation may participate in thrombogenesis and compensation for the bleeding tendency in CRF. Elevated PMP counts have been reported in CRF patients (pre-dialyzed, under haemodialysis and under ambulatory peritoneal dialysis), with significantly higher levels in patients with thrombotic events. The existence of an arteriovenous fistula did not affect PMP count [16]. It is well established that CRF patients display accelerated atherosclerosis related to endothelial dysfunction. Hence, high EMP levels, detected in uremic patients in the absence of a clinical history of vascular disease, may constitute an early indicator of vascular injury [17].

Diabetes is a confounding factor during CRF, but PMP levels are equivalent during end-stage CRF whatever be the initial diseases, i.e. diabetic nephrophathies or others. Additionally, the increase in EMP levels in series remains significant after exclusion of diabetic patients. Finally, among diabetic patients, PMP and EMP levels are higher in those presenting nephropathies [18].

High PMP levels are clearly a risk factor for thrombotic events in non-CRF patients. Although it is well known that the main drugs used for cardiovascular diseases and CRF are able to decrease MP number and procoagulant activity, one should also keep in mind that they have not been evaluated in the main series. This is particularly true for statins [19] and nifedipine [20], which decrease, respectively, leukocyte-platelet MP and PMP.

In patients with end-stage CRF, platelet, erythrocyte, and endothelial MP are increased [21]. However, only EMP levels correlate with a loss of flow-mediated dilatation and increased aortic pulse wave velocity, suggesting that they are highly associated with arterial and endothelial dysfunction in end-stage CRF [22].

**MP are both causes and consequences of vascular dysfunction (Figure 1)**

In acute vascular lesions, numerous *in vitro* studies have accounted for a direct role of MP in disease progression. Renal microvessel cells exposed to plasma of patients with thrombotic thrombopenic purpura shed prothrombotic MP expressing von Willebrand Factor (VWF) that can spread into the circulation of the ultralarge VWF multimers resulting from ADAMTS-13 deficiency. In immune-mediated acquired renal disease, MP bear procoagulant activity detected in patients with antiphospholipid syndrome. MP may be pathogenic when they bear proteinase 3, as in antineutrophil cytoplasmic antibody (ANCA)-vasculitis [15].

In patients with CRF, EMP levels may reflect subclinical lesions because their levels correlate with arterial stiffness and vasodilatation changes [21]. In this context, MP could also participate in the progression of vascular lesions because a decrease in endothelial nitric oxide is detected...
in vitro when EMP from uraemic patients are added on rodent aorta rings in culture.

Besides endothelial-dependent vasodilatation changes, other vascular abnormalities reflected by the alteration of VWF and thrombomodulin concentrations have been correlated with MP levels in CRF. Increased PMP levels may be a crucial step for arteriosclerosis, because PMP promote chemokine Rantes deposits on endothelial cells and favour leukocyte recruitment, particularly, in the context of pre-existing atherosclerosis as in CRF [23].

In patients with CRF, one could speculate that the initial stimuli that lead to MP generation could result from a release of LPS, AGEs, proinflammatory cytokines (TNFα and IL-1) or oxidized LDL, reflecting the oxidant stress. Uraemic toxins might also be involved because para-cresol and indoxyl sulphate induce vesiculation by HUVEC (human umbilical vein endothelial cell) [17].

Haemodialysis treatment is among the stimuli inducing MP generation. A dramatic increase in PMP is observed at the end of a dialysis session. This is probably explained by the membrane-induced complement activation and biological effects of extracorporeal dialysers. Results are not similar for EMP. As dialysis restores v viscosity, which is reflected by haematocrit, it also restores laminar shear stress and thus may decrease EMP levels, because it is well known that a lower local shear stress is associated with endothelial apoptosis and MP release [21]. The complement is another factor of vesiculation, since a membrane attack complex is formed during the first minutes of dialysis. Nevertheless, the membrane type (synthetic or celluloid) has no significant effect on circulating MP levels [13]. Another cause for MP release is the endotoxins present in the dialysate fluid.

Conclusion

MP behave as effectors that play a deleterious role in renal diseases. They also represent novel biomarkers that are useful to appreciate cardiovascular risk associated with CRF and sometimes disease activity. The pharmacological control of MP is the next challenge in the control of CRF-vascular complications.

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References


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