Stimulation of a variety of cell types leads to the release of submicron vesicles that bud off from the plasma membrane. These so-called microparticles (MPs) or microvesicles are defined by their small size (0.1 to 1 μm) and the presence of surface antigens from the parental cells.1,2 MPs contribute to a variety of physiological and pathological processes. For instance, they appear to play a role in both hemostasis and thrombosis. MPs released into the bloodstream can act as messengers delivering a variety of cargos, such as cell surface receptors, proinflammatory cytokines, signaling molecules, and even mRNA, to distal cells.1,2 They may also contribute to disease by transporting viruses and prions.1,2 In addition, in vitro studies have shown that binding of MPs to endothelial cells and monocytes induces the expression of proinflammatory and procoagulant molecules (Figure 1). In this issue of Circulation Research, Simoncini et al explore the inflammatory pathways that trigger the release of MPs from endothelial cells.3

MPs are generated from a variety of different vascular cell types, including endothelial cells, monocytes, and platelets. Early studies on MPs focused on platelets because these cells readily generated MPs on activation. Indeed, the original term for MPs was “platelet dust” and platelets are the major source of MPs in the blood of healthy individuals.4 Importantly, the procoagulant activity of MPs can be significantly increased by altering the phospholipid composition of the membrane surface. Under normal conditions the distribution of phospholipids moieties in the plasma membrane of cells is asymmetrical due to the activity of several enzymes whose net effect is to maintain phosphatidylcholine and sphingomyelin in the outer layer and phosphatidylserine (PS) and phosphatidylethanolamine in the inner layer. During formation of MPs, membrane asymmetry may be lost, resulting in the exposure of PS on the surface of the MPs. PS is an anionic phospholipid that binds the positively charged “Gla” domain of coagulation proteins. This means that PS-positive MPs are more procoagulant than PS-negative MPs. Interestingly, patients with Scott’s syndrome have a defect in platelet prothrombinase activity and a bleeding phenotype that appears to be attributable to a defect in PS translocation to the surface of platelets and presumably platelet MPs.2 These data suggest that platelet MPs may play a role in hemostasis in healthy individuals. Another study showed that stimulation of monocytes with a P-selectin and immunoglobulin chimera increased the number of circulating monocyte MPs and restored hemostasis in hemophilia A mice, leading the authors to propose that MPs could be used to treat patients with hemophilia.

Elevated numbers of MPs have been reported in a variety of diseases, including patients with acute coronary syndromes, cancer, anti-phospholipid antibody syndrome, sickle cell disease, sepsis, and diabetes.1 Although all MPs are procoagulant, those with the highest procoagulant activity are MPs derived from activated monocytes because of the presence of tissue factor. This transmembrane receptor is a potent activator of the coagulation cascade.6 Endothelial cells may also contribute to the pool of tissue factor–positive MPs in different diseases, such as sickle cell disease.7 Lipopolysaccharide stimulation of human monocytic cells induced the release of MPs from cholesterol-rich lipid rafts in the membrane.8 These MPs were selectively enriched for tissue factor and P-selectin glycoprotein ligand (PSGL)-1 and were deficient in CD45. These data indicate that MPs are not simply generated randomly from the plasma membrane but instead are formed in a selective and regulated manner. In a mouse thrombosis model, leukocyte-derived, tissue factor–positive MPs contributed to the growth of the thrombus.9 Accumulation of these monocyte MPs was mediated by the binding of PSGL-1 on the MPs to P-selectin exposed on the surface of activated platelets.10 These results suggest that elevated numbers of MPs in the blood, particularly monocyte MPs, may trigger thrombosis. Tumor-derived MPs also express tissue factor and may increase the risk of venous thrombosis in cancer patients.11,12 Tissue factor–positive MPs may ultimately prove to be a useful biomarker to identify patients at risk for thrombosis.

Inflammation and coagulation are linked processes in many diseases and MPs may amplify the responses by activating the endothelium (Figure 1). In addition, proinflammatory mediators directly induce tissue factor expression in endothelial cells, and the coagulation protease thrombin directly induces the expression of proinflammatory mediators in endothelial cells. This results in elevated levels of endothelial cell–derived MPs, so-called EMPs, in many disease states. The presence of these EMPs in blood can be used as biomarkers of endothelial cell injury. In vitro studies have shown that a variety of proinflammatory agents, such as tumor necrosis factor (TNF)α, will activate endothelial cells and induce the release of MPs. Interestingly, MPs generated from apoptotic endothelial cells have higher levels of PS on their surface compared with MPs generated from activated endothelial cells, suggesting that there are distinct mechanisms for the formation of MPs in apoptotic and activated cells.13 A recent study showed that treatment of endothelial

On the Trail of Microparticles

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cells with the cancer chemotherapy drug cisplatin induced the release of tissue factor-negative MPs. Further studies are required to compare the protein and lipid composition of MPs formed by exposure of endothelial cells to different agents and those formed in vivo in different diseases.

There are very few studies that have analyzed the mechanisms leading to the formation and release of MPs from endothelial cells. An early study showed that fluvastatin decreased the generation of MPs from TNFα-stimulated human coronary artery endothelial cells. Importantly, a Rho-kinase inhibitor produced a similar suppression of MPs, indicating a critical role for this kinase in the formation of MPs. Another study found that the Rho-kinase ROCK-II was required for the generation of MPs from thrombin stimulated human microvascular endothelial cell line 1. Inhibition of caspase 2 also inhibited the release of MPs, and it was proposed that the proteolytic activity of caspase 2 was involved in the release of MPs. In platelets, proteolytic cleavage of proteins that anchor the plasma membrane to the cytoskeleton is required for the release of MPs. It is possible that caspase 2 plays a similar role in the release of MPs from endothelial cells.

In this present study, Simoncini et al. analyzed the mechanism of MP formation in thrombin stimulated human endothelial cells. The authors found that thrombin induced the expression of both cell associated and soluble forms of TRAIL/Apo2L, a member of the TNF family, in endothelial cells. Monocyte-derived MPs also bind to activated platelets. MPs derived from endothelial precursor cells (EPC) bind to endothelial cells and enhance angiogenesis. TF indicates tissue factor.

Figure 1. MPs derived from different cell types induce the expression of proinflammatory and procoagulant molecules in endothelial cells, monocytes, and epithelial cells. Monocyte-derived MPs also bind to activated platelets. MPs derived from endothelial precursor cells (EPC) bind to endothelial cells and enhance angiogenesis. TF indicates tissue factor.

Figure 2. Thrombin stimulation of endothelial cells induces the expression of proinflammatory mediators and tissue factor (TF) and the release of procoagulant EMPs via direct and indirect activation of NF-κB.

activation of NF-κB and, as expected, inhibition of nuclear factor (NF)-κB also reduced the generation of MPs. The transcription factor NF-κB was required for both the early and late production of MPs in thrombin-stimulated cells (Figure 2). The early pathway involves direct activation of NF-κB and may involve the Rho kinase ROCK-II, whereas the late pathway indirectly activates NF-κB by binding of newly synthesized sTRAIL to TRAIL-R2. Importantly, both pathways were involved in the expression of proinflammatory mediators (interleukin [IL]-8 and intercellular adhesion molecule [ICAM]-1) and tissue factor. Thus, thrombin production of MP and the expression of inflammatory mediators and tissue factor appear to be coordinately regulated in endothelial cells.

Further studies are needed to determine whether intracellular pathways regulating the release of MP from endothelial cells can be separated from the general inflammatory response that requires activation of NF-κB. Interestingly, a recent study demonstrated that inhibition of the p38 mitogen-activated protein kinase (MAPK) reduced MP production in TNFα-stimulated human aortic endothelial cells. In contrast, inhibition of the extracellular signal-regulated kinase and c-Jun N-terminal kinase MAPKs did not affect the generation of MPs. At present, the pathways downstream of p38 MAPK that regulate MP formation have not been defined. Nevertheless, targeted inhibition of the generation and release of MPs may represent a novel therapeutic strategy for the treatment of inflammatory and thrombotic diseases.

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