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Microparticles, Vascular Function and Atherothrombosis

Formation, Fate and Function of Platelet Microparticles
Leukocyte-derived Microparticles in Vascular Homeostasis

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Microparticles, Vascular Function, and Atherothrombosis

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Abstract: **Membrane-shed submicron microparticles (MPs) are released after cell activation or apoptosis. High levels of MPs circulate in the blood of patients with atherothrombotic diseases, where they could serve as a useful biomarker of vascular injury and a potential predictor of cardiovascular mortality and major adverse cardiovascular events. Atherosclerotic lesions also accumulate large numbers of MPs of leukocyte, smooth muscle cell, endothelial, and erythrocyte origin. A large body of evidence supports the role of MPs at different steps of atherosclerosis development, progression, and complications. Circulating MPs impair the atheroprotective function of the vascular endothelium, at least partly, by decreased nitric oxide synthesis. Plaque MPs favor local inflammation by augmenting the expression of adhesion molecule, such as intercellular adhesion molecule-1 at the surface of endothelial cell, and monocyte recruitment within the lesion. In addition, plaque MPs stimulate angiogenesis, a key event in the transition from stable to unstable lesions. MPs also may promote local cell apoptosis, leading to the release and accumulation of new MPs, and thus creating a vicious circle. Furthermore, highly thrombogenic plaque MPs could increase thrombus formation at the time of rupture, together with circulating MPs released in this context by activated platelets and leukocytes. Finally, MPs also could participate in repairing the consequences of arterial occlusion and tissue ischemia by promoting postischemic neovascularization. (*Circ Res*. 2011;109:593-606.)**

Key Words: angiogenesis ■ atherosclerosis ■ endothelial microparticles ■ microparticle ■ progenitor cells ■ thrombosis

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Atherosclerosis is a pathological condition that underlies several important adverse vascular events, including coronary artery disease, stroke, and peripheral arterial disease, responsible for most of the cardiovascular morbidity and mortality in the Western world today. Epidemiological studies indicate that the prevalence of atherosclerosis is increasing all over the world because of the adoption of Western lifestyle and is likely to reach epidemic proportions in the coming decades.[1,2]

Atherosclerosis was described as a simple proliferative process, with passive deposition of lipid debris on the arterial

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wall.[3] Within the past three decades, atherosclerosis emerged as a chronic inflammatory disease, involving increased endothelial cell permeability, accumulation of low-density lipoproteins (LDL) in the subendothelial space, followed by the diapedesis of leukocytes and formation of foam cells, migration and proliferation of smooth muscle cells, production of connective tissue, and neovascularization. The culminating point of this slowly developing disease is plaque rupture or erosion, resulting in thrombosis and arterial occlusion.[4]

Microparticles (MPs) are 0.1- to 1 μm membrane vesicles released in extracellular space after cell activation or apoptosis.[5] They harbor at their surface most of the membrane-associated proteins of the cells they stem from and are characterized by the loss of plasma membrane asymmetry, resulting in the exposure of phosphatidylserine on their outer leaflet.[6] A large number of studies have proposed that MPs may contribute to atherosclerotic plaque development, progression, and complications. This review summarizes the possible implication of MPs at different steps of atherosclerosis development. Noteworthy, investigation of MP role relies on either MPs generated in vitro from cultured cells or MPs isolated from the blood or tissues of patients and animal models. Although the information provided by in vitro generated MPs brings highly valued mechanical insights, the transfer of such results in vivo might be limited because the biological effects and composition of MPs greatly vary depending on the stimulus initiating MP release.[7–11]

Non-standard Abbreviations and Acronyms

HUVEC	human umbilical vein endothelial cell
ICAM	intercellular adhesion molecule
IL	interleukin
LDL	low-density lipoproteins
MMP	matrix metalloproteinases
MP	microparticle
PSGL-1	P-selectin glycoprotein ligand-1
TNF-α	tumor necrosis factor-α

Microparticle Generation and Cardiovascular Risk Factors

The general consensus is that most cell types, including circulating cells and cells present in the vessel wall, are capable to vesiculate and release membrane-shed MPs in the extracellular medium in response to cell activation or apoptosis. Several factors involved in the development of atherosclerotic lesions, such as lipoproteins, cytokines, oxidative stress, or shear stress level, increase in vitro the release of MPs from vascular and/or circulating cells (Table).

Based on the knowledge gathered from experiments on platelet, MP formation at the plasma membrane of the cell appears to require some specific modifications. First, intracellular calcium and caspase-dependent mechanisms are major determinants of the loss of membrane asymmetry.[6] Disruption of phospholipid membrane asymmetry leads to exposure of phosphatidylserine on the outer leaflet. This is a consequence of the calcium-dependent upregulation of scramblase and inhibition floppase/ABC1 and translocase/flippase activities.[6] Second, blebbing requires cytoskeletal reorganization. During apoptosis, bleb formation depends on actin cytoskeleton and actin-myosin contraction, which is regulated by caspase 3-induced Rho kinase I and II activation.[12,13] Caspase activities have been identified in different MPs, and thus could represent an attempt for cells to escape cellular apoptosis.[14–16]

Table. Relevant Stimuli for Atherosclerosis Leading to Microparticle Release From Circulating or Vascular Cells

	Cell Type			
Stimuli	Endothelial Cell	Platelet	Smooth Muscle Cell	Monocyte/Macrophage
Cigarette extract	…	…	…	Li et al[24]
Modified LDL	Nomura et al[173]	…	Llorente-Cortes et al[174]	…
HDL cholesterol	Liu et al[175]	…	…	Liu et al[175]
Uremic toxin	Faure et al[176]	…	…	…
Flow conditions	Ramkhelawon et al[177]	Nomura et al[178]	Stampfuss et al[179]	…
Thrombin	Sapet et al,[18] Simoncini et al[20]	Barry et al,[60] Dale et al,[180] Chang et al,[181]	…	…
Collagen	…	Barry et al,[60] Boilard et al,[182] Chang et al,[181]	…	…
Homocysteine	Sekula et al[183]	Olas et al[184]	…	…
Activated Protein C	Pérez-Casal et al[185]	…	…	Pérez-Casal et al[185]
PAI-1	Brodsky et al[186]			
Proinflammatory cytokines (TNFα, IL1β) and CRP	Combes et al,[187] Curtis et al,[19] Abid Hussein et al,[15] Wang et al,[22]	Nomura et al,[178] Piguet et al[188]	Schecter et al[189]	Jungel et al[190]
Oxidative stress	Vince et al,[191] Szotowski et al[192]	…	…	…
Fas ligand	…	…	Essayagh et al 2005[193]	…
PDGF	…	…	Schecter et al[194]	…

CRP indicates C-reactive protein; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; PAI, plasminogen activator inhibitor; PDGF, platelet-derived growth factor; TNF, tumor necrosis factor.

Only studies showing a significant increase in MPs release as compared to basal condition have been included.

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Endothelial MP formation and release have received significant attention over the past recent years, and different signaling pathways have been identified depending on the stimuli (Table).[17] Clearly, endothelial MP shedding can occur independently of endothelial apoptosis.[18] Curtis et al[19] identified p38 mitogen-activated protein kinase as a key factor for the shedding of endothelial cells under tumor necrosis factor-α (TNF-α) stimulation. In opposition, thrombin stimulation of endothelial cells induces a complex biphasic release of endothelial MPs.[18] Several different mechanisms concur to vesiculation. First, thrombin binds to its protease-activated receptor-1, followed by Rho kinase II activation. Second, a later pathway involves TRAIL/Apo2L, a cytokine that belongs to the TNF-α superfamily,[20] followed by interleukin (IL)-1 release and IL-1 receptor activation.[21] The second phase is characterized by an amplification loop based on the release by endothelial cells stimulated by thrombin of soluble forms of TRAIL and of IL-1 that act in an autocrine or paracrine manner on endothelial cells and stimulate MP shedding. Interestingly, these findings demonstrate that thrombin-induced activation of endothelial cells leads to the release of MPs of different composition. Endogenous nitric oxide appears to play a protective role against endothelial MP formation by a mechanism involving tetrahydro-biopterin, as observed after C-reactive protein endothelial activation.[22] No other study has addressed the potential effects of nitric oxide on endothelial MP formation and release.

Monocyte-macrophages also release MPs on activation (Table). In addition, endotoxin stimulates macrophage MPs formation via a pathway requiring inducible nitric oxide synthase activation.[23] Furthermore, tobacco smoke provokes the generation of highly procoagulant monocytic MPs in a process requiring ERK1/2 activation and caspase 3-dependent apoptosis.[24]

Although enucleated cells, such as erythrocytes and platelets, cannot undergo classical apoptosis associated with nuclear fragmentation, MPs expressing specific markers of platelets or red blood cells have been detected in human and animal plasma.[25] Contrary to platelet MP release, little information is available on the molecular mechanisms leading to the phosphatidylserine exposure and MP formation in erythrocytes. Increases in intracellular calcium and oxidative stress promote erythrocyte MP release.[26,27] In addition, senescence of erythrocyte, as well as of platelets, leads to phosphatidylserine exposure on the cell membrane outer leaflet and release of MPs.[28] In senescent platelets, the release of MPs depends on cytochrome c release and subsequent activation of caspase 3 and Rho kinase I.[28]

Lesion Initiation

A primary event in the development of atherosclerotic lesions is the accumulation of low-density lipoprotein in the subendothelial matrix.[4] This occurs in precise sites within the arteries such as arterial branching or curvature where hemodynamic forces and endothelial shear stress is disturbed.[4] In these areas, endothelial cells are not aligned in the direction of flow but rather have polygonal shapes and no particular orientation.[4] Endothelial permeability is also increased, allowing for the diffusion of macromolecules such as LDL through endothelial cell junctions.[29,30] Then, LDL undergoes several modifications, including oxidation, lipolysis, proteolysis, or aggregation in the subendothelial space, where they are removed by macrophages and foam cells.[4] The implication, if any, of MPs in these initial stages of atherosclerosis has never been directly assessed, but several findings indicate it may be likely.

First, MPs, and particularly endothelial MPs, are released and their circulating levels increase early in atherosclerotic process. Cardiovascular risk factors may trigger endothelial MP release, in addition to the well-known effects of cytokines and proapoptotic and procoagulant stimuli (Table). For instance, smoking, as well as second-hand smoking or enforced physical inactivity, are associated with increases in circulating endothelial MP in healthy subjects.[31–33] Increases in plasma endothelial MPs are also observed after high-fat meals with augmented circulating levels of modified LDL and triglycerides, whereas a Mediterranean regimen lowers levels of circulating endothelial MPs.[34–36]

Second, a paracrine effect of endothelium-derived MPs in atherosclerotic-prone areas is possible. Mechanical factors may be involved in the regulation of endothelial MPs release. In vitro and in vivo studies demonstrate that turbulent shear stress is accompanied by endothelial apoptosis, whereas laminar shear stress protects endothelial cells from apoptosis.[37,38] Apoptosis being a well-known stimulus of MP release, endothelial MPs are likely to be released in turbulent or low shear stress areas. This hypothesis is reinforced by the inverse correlation observed in patients with end-stage renal disease between endothelial MPs and basal arterial laminar shear stress.[39] Because endothelial MPs released from apoptotic cultured endothelial cells harbor caspase-3 activity, endothelial cells from low or disturbed shear stress areas may use MP release to expel proapoptotic proteins from their cell body in a last attempt to escape programmed cell death.[16] This interpretation therefore would support a beneficial role of endothelial MP by maintaining a protective endothelial lining of the blood vessel wall. However, because shear stress is low in atherosclerotic-prone areas, local endothelial MP concentrations are likely to be elevated and could affect neighboring endothelial cells.

Third, several recent findings indicate that endothelial MPs hamper the atheroprotective function of the endothelial lining of blood vessels. Endothelial MPs impair endothelial nitric oxide bioavailability by either stimulating free radical generation or decreasing Ser1179- endothelial nitric oxide synthase phosphorylation[40–43] (Figure A). Furthermore, endothelial MPs may directly increase endothelial permeability.[41] This effect was initially reported in pulmonary capillaries of C57BL/6 mice injected with endothelial MPs but was not observed for MPs carrying endothelial protein C receptor-generated after exposure of endothelial cells to exogenous activated protein C.[44] The increased permeability could also result from increased CD11b expression at the surface of leukocytes exposed to endothelial MPs.[45] Interestingly, several findings suggest that the release of endothelial MP may be concomitant with increased endothelial permeability. Stimuli such as thrombin or TNF-α induce both MP generation and endothelial permeability.[17,46,47] Stimuli inhibiting

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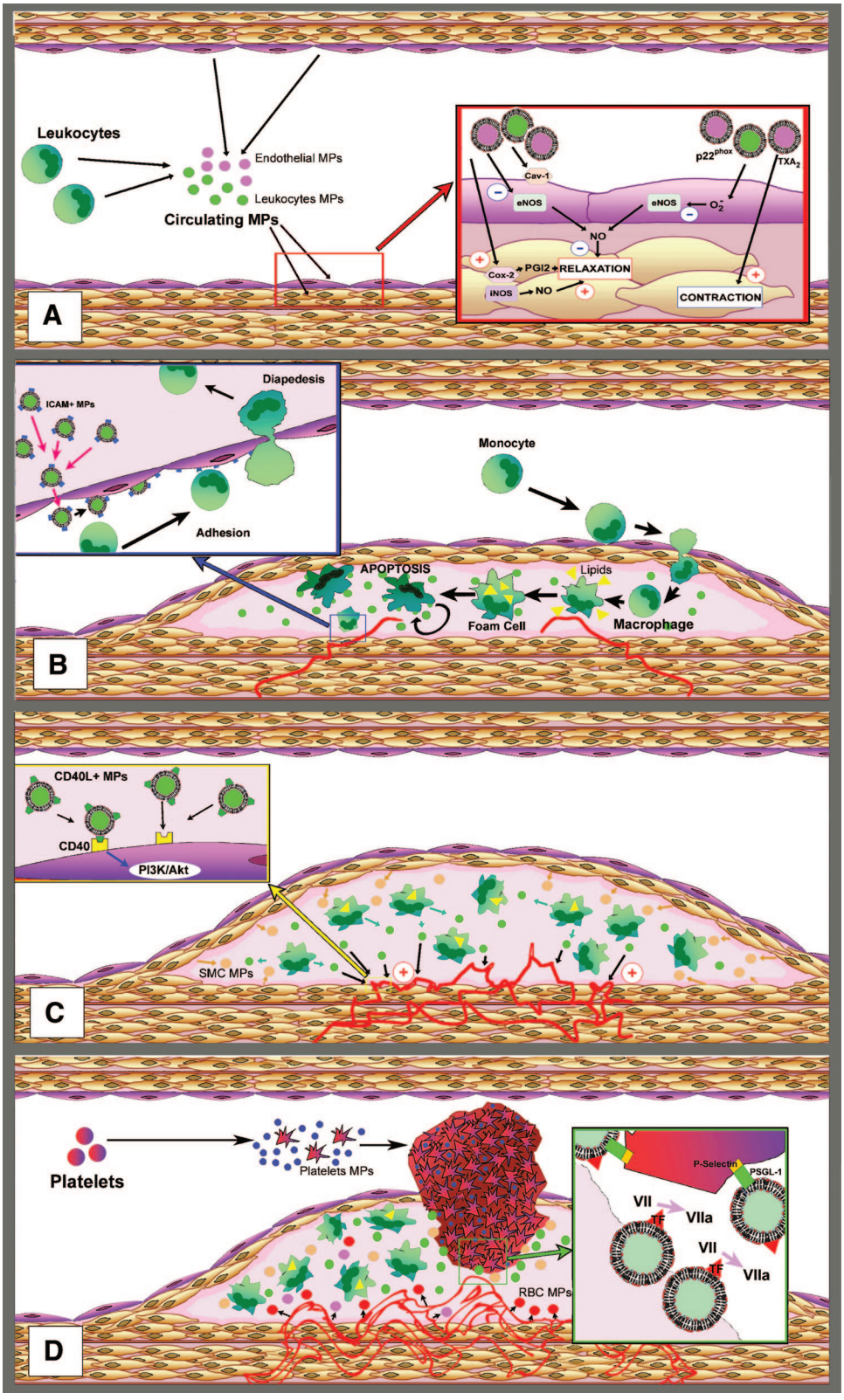

Figure. Implication of MPs in the development, progression, and complications of atherosclerotic plaques. A, Lesion initiation. Circulating endothelial and leukocyte microparticles (MPs) are increased in patients with high atherothrombotic risk. These MPs induce endothelial dysfunction by decreasing nitric oxide (NO) synthesis as the result of an inhibition of the endothelial nitric oxide synthase (eNOS) function and/or an increase in caveolin-1, increasing oxidative stress and production of endothelial superoxide anion (O_2^-). The highly unstable O_2^- anion can uncouple the eNOS, thus decreasing NO production. Some MP subpopulations also can enhance thromboxane A2 (TXA2) production and thus vascular contraction. Opposite effects also have been reported with MPs derived from lymphocytes as they increase the inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) enzymes expression in the smooth muscle cells, thus increasing vasodilatory mediators concentration within the media and decreasing contractile force. **B**, Lesion progression. Human atherosclerotic plaques contain high levels of MPs. These MPs are in the vicinity of the immature and leaky neovessels contained in plaques. MPs harbor intercellular adhesion molecule (ICAM)-1 and transfer this adhesion molecule to endothelial cell membrane, thus enhancing the recruitment of monocytes into plaques. Once transmigrated, monocytes differentiate into macrophages and foam cells, leading to phagocytosis of modified low-density lipoprotein (LDL, yellow triangles). MPs could contribute to macrophages and foam cells apoptosis, leading to the release of MPs and creating a vicious circle. **C**, Advanced lesions. Vulnerable atherosclerotic plaques prone to rupture are characterized by an increased number of vasa vasorum and frequent intraplaque hemorrhage. Plaque MPs express CD40 ligand (CD40L), stimulate endothelial cell proliferation after CD40 ligation, and promote angiogenesis. Therefore, plaque MPs likely contribute to the transition from stable to unstable plaques. **D**, Plaque rupture. Plaque MPs expose at their surface phosphatidylserine (PS) and, frequently, tissue factor (TF), conferring MPs a high procoagulant activity. At the time of plaque rupture, locally released plaque MPs stimulate thrombus formation. Platelet MPs are shed from activated platelets and participate in thrombus. The interactions between P-selectin glycoprotein ligand-1 (PSGL-1) carried by leukocyte MPs and platelet P-selectin are necessary to concentrate TF activity at the thrombus edge. Endothelial, leukocyte, smooth muscle cell, erythrocyte, and platelet MPs are represented by pink, green, brown, red, and blue circles, respectively.

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MP formation also decrease permeability.[48,49] In addition, Rho-associated protein kinase and p38 mitogen-activated protein kinase pathways have been implicated in both endothelial MP release and endothelial hyperpermeability.[17,19,47,50] Finally, MP formation and changes in endothelial permeability require cytoskeletal protein reorganization.[17,47]

Fourth, MPs of non-endothelial origin also may contribute to the loss of the vasculoprotective effect of endothelial cells. For instance, MPs circulating in patients with coronary artery disease impair endothelial nitric oxide-dependent relaxations in healthy arteries, suggesting that plasma MPs may contribute to the generalization of endothelial dysfunction in this disease.[51] Similar findings were observed using MPs generated in vitro from lymphocytes[52] (Figure A). Furthermore, endothelial permeability might be affected by circulating MPs other than endothelial MPs. Dean et al[53] have recently shown that platelet MPs of large diameter impair endothelial cell barrier integrity, whereas small platelet MPs have an opposite effect.

Taken together, these findings suggest that circulating endothelial MPs, as well as MPs of nonendothelial origin, may contribute to the overall proatherogenic phenotype of endothelial cells in atheroprone areas of the vasculature.

Plaque Progression

Endothelium and Recruitment of Inflammatory Cells

Considerable evidence supports the early involvement of the monocyte/macrophage, the most prominent cellular component of the innate immune response, during atherogenesis. Observations in human arterial specimens and many experimental models of atherosclerosis have identified monocyte recruitment as an early event in atherogenesis.[54] This recruitment involves attachment to activated endothelial cells by leukocyte adhesion molecules such as intercellular adhesion molecule (ICAM)-1, vascular adhesion molecule-1, E-selectin, and P-selectin. Several cytokines, including monocyte chemotactic protein-1, IL-6, and IL-8, direct cell migration of monocytes into the intima.[55] Interestingly, monocyte entry to atherosclerotic plaques not only occurs during the initial stages of lesion formation but also continues even in established lesions.[54,56]

Several studies using in vitro-generated MPs of different cellular origins support the concept that MPs increase the synthesis and the release of proinflammatory cytokines by endothelial cells and leukocytes.[57–59] The release of IL-6 and IL-8 could subsequently favor the recruitment of leukocytes at the site of injury, and even monocyte chemotaxis.[60] Interestingly, high-density lipoprotein impairs MP binding to endothelial cells, therefore limiting their proinflammatory effect.[59,61] This observation could contribute to the beneficial effect of high-density lipoprotein in atherosclerosis.

In vitro-generated MPs also stimulate the expression of adhesion molecules, particularly ICAM-1, at the surface of endothelial cells.[19,57,62] MPs also augment the expression of adhesion molecules counter-receptors, such as CD11a, on monocytes.[57] The mechanisms involve arachidonic acid or oxidized phospholipids carried by MPs.[10,57] The in vitro transfer of proatherogenic chemokine RANTES (CCL5) from platelet-derived MPs to endothelial cells could lead to monocyte attraction and activation, possibly promoting their recruitment into the lesions.[63] Platelet MPs expressing P-selectin also may contribute to monocyte infiltration by favoring leukocyte–leukocyte interaction under unfavorable flow conditions.[64] Whether these observations occur during the early stages of atherosclerotic plaque development remains unknown.

A recent study using human MPs isolated from advanced atherosclerotic plaques confirmed some of these results obtained with in vitro-generated MPs, supporting the hypothesis that MP proinflammatory effects may occur throughout the development of atherosclerotic lesions.[9] Atherosclerotic plaques contain large amounts of MPs, mostly originating from leukocytes.[65,66] Although the mechanisms leading to MPs formation in atherosclerotic lesions are unknown, oxidized or modified lipids as well as oxidative stress and proinflammatory cytokines may locally contribute to MP release from vascular cells or from monocytes (Table). MPs isolated from human plaques augment endothelial expression of ICAM-1 and subsequently enhance monocytes adhesion and transmigration under flow conditions, whereas MPs isolated from plasma has no such effects.[9] The increased endothelial ICAM-1 induced by plaque MPs results from the transfer of the adhesion molecule from MPs to endothelial cells in a phosphatidylserine-dependent manner.[9] Contrary to in vitro-generated MPs, MPs isolated from advanced human plaques does not affect endothelial release of IL-6, IL-8, or monocyte chemotactic protein-1, or endothelial expression of vascular adhesion molecule-1 or E-selectin.[9] The effect of plaque MPs on ICAM-1–dependent recruitment of inflammatory cells might be particularly relevant at the level of vasa vasorum invading the advanced atherosclerotic lesions, because intimal neovessels express much greater levels of vascular cell adhesion proteins than the luminal arterial endothelium.[67] These abnormal microvessels are characterized by disorganized branching and immature endothelial tubes with leaky imperfect linings.[68,69] Therefore, MPs bearing ICAM-1 present in plaques may diffuse within blood stream and thus transfer ICAM-1 to the endothelial cell surface in a "paracrine" manner[9] (Figure B). Whether plaque MPs promote monocyte recruitment in fatty streaks at early stages of atherosclerosis plaque development is unknown.

The lack of effect of circulating MPs on monocyte recruitment may result from the concomitant proinflammatory and antiinflammatory effects of MPs. For instance, neutrophil MPs augment the release of the antiinflammatory cytokine transforming growth factor-β1 from macrophages, suggesting that MPs down-modulate cellular activation in macrophages.[70] In addition, exposure of leukocyte-derived MPs to recipient leukocytes before flowing over endothelial cell monolayers significantly inhibits their adhesion in an annexin-1–dependent manner.[71] It should be noted that plaque MPs isolated from human plaques harbor annexin-1, but no information is available indicating whether this protein is bioactive or in a sufficient amount to confer plaque MP antiinflammatory properties or phospholipase A2 inhibitory activity.[9,72]

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Monocytes/Macrophages

The transformation of recruited monocytes into lipid-laden macrophages or "foam cells" by modified or oxidized LDL trapped in the subendothelium is central to the development of atherosclerotic lesions.[73] Dysregulated uptake of modified LDL via scavenger receptors such as CD36 determines foam cells formation in vivo.[74,75] Scavenger receptors also may be involved in MP uptake and phagocytosis, because MP binding to platelet CD36 has been reported, leading to their activation.[76] However, there is no information regarding MP interaction with monocytes–macrophages CD36 in atherosclerotic lesions and its potential functional consequences.

Recent findings demonstrate that the monocyte population is not homogeneous with respect to its proinflammatory properties. Besides the classical M1 activation of monocytes responsible for a harsh proinflammatory activity, M2 alternative activation, associated with an antiinflammatory response, is also implicated in atherosclerosis.[77] No study has yet evaluated the role of MPs in the regulation of monocyte/macrophages subsets. Another area requiring further investigations is the potential effect of MPs in monocyte proliferation, which would contribute to the increase in plaque cellularity and therefore to plaque progression.[56,78–80]

Macrophages and foam cells then undergo cell death leading to the deposition of a growing mass of extracellular lipids forming the lipid core.[4] Several reports have suggested that MPs could contribute to monocyte and macrophage apoptosis[14,81,82] (Figure B). If this was demonstrated in advanced atherosclerotic lesions, then MPs could contribute to a characteristic of lesions prone to rupture.[83] Two mechanisms have been proposed for the proapoptotic effect of MPs. The first one implicates the phagocytosis of monocyte-derived or T-lymphocyte–derived MPs by monocytes and macrophages, leading to an increased cellular content of membrane phospholipids, which are likely cleaved by phospholipases A2 into arachidonic acid. Arachidonic acid is a strong activator of acid sphingomyelinase that metabolizes sphingomyelin in proapoptotic ceramides, resultingin both caspase-dependent and caspase-independent cell death.[81,82] The second mechanism relies on the presence in endothelial-derived, erythrocyte-derived, platelet-derived, monocyte-derived, and dendritic cell-derived MPs of caspase-3 or caspase-1, which may induce target cells apoptosis.[14,15,84,85] MP encapsulation appears required for inducing apoptosis because disruption of MP integrity suppresses the apoptotic activity.[84]

Increased monocyte and macrophage apoptosis is likely to be associated with the release of MPs, further increasing plaque MP accumulation (Figure B). This leads to a vicious circle responsible for more monocyte recruitment and more macrophage apoptosis. The large MP concentration in human atherosclerotic plaque also may result from the decrease in macrophages phagocytic activity in plaques.[66,86] At least in normal conditions, macrophages seem to play a key role in MP clearance through lactadherin and phosphatidylserine. These data, obtained with platelet-derived and erythrocyte-derived MPs, need to be confirmed with other cell-derived MPs, particularly endothelial cell and monocytes.[87–89] Interestingly, lactadherin-deficient apolipoprotein $E^{-/-}$ mice have more extensive atherosclerotic lesions and have greater levels of circulating MPs.[90] The mechanism for this decreased macrophages phagocytic activity in plaques could be a competition of MPs with apoptotic bodies and oxidized LDL.[91]

Dendritic Cells, Lymphocytes, and Mast Cells

In atherosclerosis, the innate response is rapidly followed by an adaptive immune response to an array of potential antigens presented to effector T-lymphocytes by antigen-presenting cells, such as dendritic cells. Dendritic cells populate atherosclerotic plaques and regional draining lymph nodes, where they can present antigens to T cells with costimulatory molecules.[54] Their maturation requires the coordinated action of a number of cytokines and growth factors. Several molecules, including CD40, TNF receptor, and IL-1 receptor, have been shown to activate dendritic cells and to trigger their transition from immature antigen-capturing cells to mature antigen presenting cells.[55] Interestingly, in vitro-generated endothelial MPs, but not platelet-derived or lymphocyte-derived MPs, induce plasmocytoid dendritic cell maturation with production of inflammatory cytokines (IL-6 and IL-8).[92] However, the opposite effect has been reported with polymorphonuclear neutrophil-derived MPs.[93] The possible role of MPs in the migratory capacity of dendritic cells is unknown.

Several studies concur in demonstrating a role of MPs in lymphocyte proliferation. Both in vitro-generated and human atherosclerotic plaques MPs are able to stimulate T-lymphocyte proliferation.[72,94] One likely mechanism could implicate the major histocompatibility complex class II expressed, together with potent costimulatory molecules such as CD40 ligand, at the surface of MPs isolated from human plaques.[11,72] Macrophages or dendritic cells must be the source for MPs harboring major histocompatibility complex class II, which are responsible for lymphocyte proliferation.[94,95] MPs could also promote the differentiation of lymphocytes toward a proatherogenic T helper lineage. Naive $CD4^+$ T cells primed in the presence of plasmocytoid dendritic cell maturated with endothelial MPs produce mainly T helper-1 cytokines (interferon-γ and TNF-α).[92] However, these data need to be confirmed using human plaque MPs. This proatherogenic response is controlled by various T-regulatory cells and by T helper-2–related cytokines.[96] Except for studies using tumor-derived MPs, response of T-regulatory cells to MPs has not been evaluated.[97,98]

Mast cells are inflammatory cells present in the arterial wall, where they form part of the inflammatory cell infiltrate and may contribute to atherosclerosis.[55] Mast cells might be an additional source of inflammatory cytokines within the plaque. MPs released from activated T cells induce mast cell activation, degranulation, and cytokine release in a mitogen-activated protein kinase-dependent mechanism.[99] Although platelet MPs cannot be identified in atherosclerotic lesions, platelet MPs may be taken-up by mast cells, leading to the regulation of inflammatory cytokines.[66,100] Regulation of mast cells by plaque MPs is an additional area requiring further investigations.

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Smooth Muscle Cell Migration, Proliferation, and Phenotype

Smooth muscle cells play a key role in atherosclerosis both in early and in late stages.[101] In early stages, smooth muscle cells migrate from the media to the intima, where they are trapped and proliferate to contribute to the development of plaque. The stimuli initiating smooth muscle cell migration and proliferation are not well-elucidated.[101] The effects of MPs on smooth muscle cell proliferation depend on their cell origin. In vitro-generated platelet MPs increase smooth muscle cell proliferation in a platelet-derived growth factor-independent mechanism, but they had only minor migratory activity.[102,103] Conversely, endotoxin-stimulated monocyte MPs contain functional caspase-1 and induce smooth muscle cell death.[84] Studies using MPs obtained from atherosclerotic plaques would be useful to clarify this point. Given the high proportion of tissue factor-positive MPs in human plaques, a promigratory effect of plaque MPs would be expected.[66] Indeed, vascular smooth muscle cells express protease-activated receptor-2 that can be activated by tissue factor-coagulation factor VIIa, leading to vascular smooth muscle cell migration.[104–106]

In late stages, intimal smooth muscle cells differ significantly from their medial counterparts and as such have unique atherogenic properties that make them fertile ground for the initiation of plaques.[107] Whereas human medial smooth muscle cells predominantly express proteins involved in contractile function, such as smooth muscle myosin heavy chain or smooth muscle α-actin, smooth muscle cells found in the intima express lower levels of these proteins, have a higher proliferative index, and have a greater synthetic capacity for extracellular matrix, particularly collagen, proteases, and cytokines.[108,109] The potential role of MPs in this transition to the "synthetic" state that facilitates many of the pathogenic roles of vascular smooth muscle cells has not been investigated.

Neovessel Formation

The intima of normal human arteries lack vasa vasorum, whereas the adventitia and outer media possess a vascular network.[68] As atherosclerosis progresses, the intima thickens and intimal neoangiogenesis increases, likely arising from the adventitia.[69,110–112] This network of leaky neovessels allows extravasation of erythrocytes into the atherosclerotic lesion, providing erythrocyte-derived free cholesterol within the lipid core, favoring excessive macrophage infiltration and therefore promoting the transition from stable to unstable plaques.[68,113] The density of intraplaque neovessels increases the risk of plaque rupture and is an independent predictor of systemic cardiovascular outcome.[114,115]

The molecular mechanisms responsible for neovessel formation relate predominantly to hypoxia because of impaired oxygen diffusion in the thickened plaque but could also be driven by inflammation and Toll-like receptor activation.[69] MPs may contribute to intraplaque neovascularization at different steps of the process: disruption of cell–cell contacts, degradation of extracellular matrix, proliferation and migration and capillary tube formation of endothelial cells. First, as discussed, MPs might increase endothelial permeability, although data are scarce and controversial.[41,53] Second, proteolysis of basement membrane matrix cellular components is necessary to promote endothelial invasion into the surrounding interstitial matrix. As detailed below (see paragraph on fibrous cap weakening), human plaque MPs harbor active proteases[116] and MPs of different cellular origin are able to induce the release of metalloproteases by different cell types.[117–120] Third, MPs regulate endothelial proliferation and capillary tube formation. Human plaque MPs isolated from endarterectomy specimens surgically obtained from patients increase endothelial cell proliferation in vitro as well as in vivo in matrigel plugs.[11,121] This effect relies on the presence of CD40 ligand at the surface of plaque MPs, interacting with endothelial CD40 to mediate proliferation by a vascular endothelial growth factor receptor and PI3-kinase/Akt–dependent pathway[122–124] (Figure C). Most plaque CD40 ligand-positive MPs appear to be of macrophage origin. The proliferative effect of MPs isolated from human lesions is more potent for those obtained from symptomatic (ie, patients who experienced stroke or transient ischemic attack) than asymptomatic patients.[11] Interestingly, lesions from symptomatic patients have significantly more CD40 ligand-positive MPs than those from asymptomatic patients.[11] The effect of in vitro-generated MPs on endothelial cells proliferation depends on the type of endothelial cells used,[125] as well as the concentration[120,126] and the cellular origin of MPs. Platelet MPs stimulate angiogenesis both in vitro and in vivo through growth factors such as vascular endothelial growth factor.[127,128] On the contrary, lymphocyte MPs inhibit endothelial cell proliferation by augmenting reactive oxygen species generation and by interfering with the vascular endothelial growth factor signaling pathway.[62,129,130] Endothelial MPs also decrease endothelial cell proliferation by lowering endothelial nitric oxide synthase activity[131] or by increasing oxidative stress.[42] Opposite conclusions were drawn from experiments using MPs derived from endothelial cells overexpressing high levels of tissue factor[132] or T-cadherin.[133] The MP composition of human atherosclerotic plaque reconciles the different results obtained from studies using human plaque and in vitro-generated MPs. No platelet MPs could be identified in human plaque, and endothelial and lymphocyte MPs represent only 8% and 15% of all plaque MPs.[66] Conversely, the effect on endothelial cell proliferation of MPs generated from erythrocytes, macrophages/granulocytes, and smooth muscle cell MPs (representing, respectively, 27%, 37%, and 13% of all plaque MPs) has never been tested. Moreover, circulating MPs from patients with advanced atherosclerosis have no effect on endothelial cell proliferation.[11] In these patients, the proportion of platelet, lymphocyte, and endothelial MPs are in the same range (29%, 13%, and 9%, respectively), suggesting a balance between proangiogenic and antiangiogenic activities of MPs of different cellular origin present in human plasma.[66]

Complications and Repair Mechanisms

Fibrous Cap Weakening

Atherosclerotic plaques exist under two major phenotypes: (1) stable plaques, characterized for the most part by a thick

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fibrous cap isolating a relatively small lipid core from the lumen, which are associated with a very low risk of thromboembolic complications; and (2) unstable plaques, most of which are characterized by a large lipid core covered by a thin fibrous cap prone to rupture and thrombus formation and are thought to be associated with a higher risk for thromboembolic complications.[134,135] Weakening of the fibrous cap presumably results from extracellular matrix proteins degradation and from smooth muscle cell disappearance likely after cell apoptosis.[136]

Plaque MPs likely contribute to matrix degradation. Human atherosclerotic plaques carry active proteases.[116] TNF-α–converting enzyme (ADAM-17) has been identified, but other proteases are also present on plaque MPs because these MPs are able to cleave one peptide targeted by a large panel of matrix metalloproteinases (MMPs), such as MMP-1, MMP-2, MMP-7, MMP-8, MMP-9, MMP-12, MMP-13, MMP-14, MMP-15, and MMP-16, and another one preferentially cleaved by MMP-3 and MMP-10.[116] Proteases also have been detected on MPs generated in vitro from various cell types. For instance, in vitro-generated endothelial cell MPs carry MMP-2, MMP-9, and MT1-MMP proenzyme and also harbor proteases inhibitors (tissue inhibitor of MMPs-1 and tissue inhibitor of MMPS-2).[126] Furthermore, adipocyte-derived MPs carry MMP-2 and MMP-9,[137] and neutrophil MPs expose active MMP-9.[138] In addition, plaque MPs might stimulate the release of MMPs by cells present in atherosclerotic plaques as shown for other MPs. Platelet-derived MPs stimulate the secretion of active MMP-2 by prostate cancer cells.[117] Furthermore, T cells and monocytes MPs induce the synthesis of MMP-1, MMP-3, MMP-9, and MMP-13 in fibroblasts.[118] Finally, T-cell–derived MPs upregulate MMP-1, MMP-3, MMP-9, and MMP-13 genes in hepatic stellate cells.[119]

Whether plaque MPs also could contribute to fibrous cap weakening through induction of smooth muscle cell apoptosis remains to be determined. As mentioned, conflicting data have been reported regarding the effect of MPs on smooth muscle cell survival.

Plaque Rupture and Thrombosis

Studies in apolipoprotein E-deficient mice have shown that activated endothelial cells, either covering the lesion or present in neovessels, could promote thrombus formation and fibrin deposition by shedding soluble P-selectin, which then increases circulating levels of procoagulant tissue-factor positive MPs.[139] Physical disruption of plaques may also trigger thrombosis and promote downstream ischemic event. Three types of physical disruption may occur.[140] First, superficial erosion or microscopic areas of desquamation of endothelial cells account for approximately one-quarter of fatal coronary thromboses. There is currently no evidence suggesting an implication of MPs in this process except for one study showing that MPs generated in vitro from the monocytic cells line THP-1 induce endothelial cell apoptosis.[141] Second, disruption of the microvessels that form in atherosclerotic plaques furnishes another scenario for sudden plaque progression. The new blood vessels in the plaque may be particularly fragile and prone to microhemorrhage.[68] The role of plaque MPs in intraplaque neoangiogenesis seems crucial and has been discussed. The third and most common mechanism of plaque disruption is a fracture of the plaque's fibrous cap. This allows the circulating blood contact with plaque MPs. Human plaque MPs are particularly prothrombogenic because they generate twice as much thrombin as plasma MPs from the same patients.[66] This procoagulant activity is related to the exposure at their surface of phosphatidylserine because components of the clotting cascade assemble on membrane surfaces containing phosphatidylserine. Additionally, a large number of human plaque MPs harbor tissue factor. This transmembrane receptor for plasma coagulation factor VII/VIIa dramatically increases their procoagulant activity. The high concentration of highly procoagulant MPs in plaques supports their crucial role in thrombus formation at the time of rupture (Figure D). Circulating MPs, and particularly tissue factor-positive MPs, also contribute to arterial thrombosis.[142] MP accumulation and subsequent formation of fibrin are dependent on interaction of MPs P-selectin glycoprotein ligand-1 with platelet P-selectin.[143] MPs released by activated platelets may also contribute to thrombus formation.[144] More details on the procoagulant activity of MPs appear in the review by Owens and Mackman.[145]

Repair Mechanisms: Postischemic Neovascularization

Neovascularization after vascular occlusion involves vascular progenitor cells of bone marrow and nonbone marrow origin.[146,147] After acute ischemia, such as acute coronary syndrome[51,148–150] or acute stroke,[151] circulating levels of platelet and endothelial MPs are increased in patients. Moreover, high amounts of MPs, mainly from endothelial cells (70% of MPs are $CD144^+$), are detected in mouse hind-limb muscle 48 hours after unilateral femoral artery ligation.[152] These observations prompted the investigation of the role of MPs in postnatal neovascularization. Such neovascularization is augmented by MPs originating from platelets[153–155] or endothelial progenitor cells,[156] as well as by MPs isolated from mouse plasma[157] or ischemic hind-limb muscle.[151] However, conflicting results were obtained using MPs from lymphocytes.[129,158]

MPs act at different steps of the neovascularization process. First, after ischemia, local tissue injury alters the vascular endothelium to arrest progenitor cells in regions where endothelium regeneration is needed.[159] Besides hypoxic gradients via hypoxia-induced factor-1α–induced expression of CXCL12,[160] platelet MPs also contribute to chemoattract progenitor cells.[154] A recent study has demonstrated that endothelial cell-derived apoptotic bodies can be transferred to recipient cells to induce the expression of CXCL12. This effect is mediated through miRNA-126, which is enriched in the apoptotic bodies and acts by knocking down the negative regulator RGS16 and enabling CXCR4 to stimulate an autoregulatory feedback loop that enhances ERK1/2 phosphorylation and further increases CXCL12 production. Repetitive in vivo injections of endothelial apoptotic bodies have an atheroprotective effect by promoting the mobilization and incorporation of progenitor cells to the plaque.[161] MPs also carry miRNA.[5] Whether

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endothelial MPs have the same effect as apoptotic bodies in this setting remains to be determined.

Another step of the neovascularization process in which MPs could interfere might be the adhesion of progenitor cells to activated endothelium or subendothelial components of the extracellular matrix exposed at sites of vascular injury. Platelet MPs are also implicated at this level because they enhance adhesion and migration of progenitor cells.[154,155] This effect seems to be attributable to phenotypic alterations of progenitor cells exposed to platelet MPs with increased expression of endothelial cell markers and transfer of the chemokine receptor CXCR4 to progenitor cells, which enhances responsiveness to its ligand CXCL12.[63] Several cellular mechanisms could mediate progenitor cell vasoregenerative capacity after their adhesion. Progenitor cells may deliver angiogenic factors to pathological tissues and contribute to neovascularization and tissue/vessel remodeling through paracrine effects.[162,163] Progenitor cell MPs could be one of these factors. These MPs can be incorporated in endothelial cells by interaction with α-4 and β-1 integrins expressed on the MP surface. These MPs promote endothelial cell survival, proliferation, and organization in capillary-like structures through mRNA transfer, with a critical role of PI3K and endothelial nitric oxide synthase.[156]

Finally, progenitor cells may incorporate into blood vessels and regenerate the vascular endothelial barrier. MPs contribute to this step by promoting the differentiation of progenitor cells into cells with endothelial phenotype.[152] The mechanism implicated depends on the origin of MPs: a binding of MP to progenitor cells that changes their phenotype for platelet MPs;[155] reactive oxygen species for MPs isolated from ischemic muscles;[152] or peroxisome proliferator-activated receptor-α for plasma MPs.[157] It should be highlighted that these positive effects of MPs on postischemic neovascularization have been documented not only in vitro but also in vivo in unilateral femoral artery ligation[152,158] and arterial wire-induced injury murine models.[155]

Microparticles: Biomarkers of Cardiovascular Disease Progression

MPs originating from different cell types can be detected in the plasma of healthy subjects, where they result from the active balance between MP generation and clearance. Over the past decade, numerous studies have shown that circulating MPs levels increase in a wide range of cardiovascular diseases, including uncontrolled cardiovascular risk factors, atherosclerotic lesion progression, heart failure, thrombosis, arrhythmias, and inflammatory vascular diseases.[25] Changes in circulating levels of MPs might provide important clinical information in healthy subjects and in patients with cardiovascular disorders. Circulating levels of leukocyte-derived MPs, possibly reflecting the increased vascular inflammation, are independently associated with subclinical atherosclerosis[164] and inward carotid artery remodeling in asymptomatic subjects.[165] Several studies identify plasma levels of endothelial MPs as a surrogate marker of vascular function. In patients with established endothelial dysfunction, levels of circulating endothelial MPs are inversely correlated with the amplitude of flow-mediated dilatation, independently of age and pressure.[40,166,167] Recent findings also support the prognostic value of circulating MP levels. Circulating levels of endothelial MPs, but not MPs of other cellular origin, appear as a robust predictor of cardiovascular mortality and major adverse cardiovascular events in patients with coronary artery disease, pulmonary hypertension, or end-stage renal failure.[168–171] Whether measuring MP plasma levels would be useful to better-assess cardiovascular risk in primary prevention is not known at the moment because of the lack of evidence of its predictive value, discrimination, and reclassification power that are required to confer clinical utility to a biomarker.[172]

Conclusion

Increasing numbers of studies point out the possible contribution of MPs in different stages of atherothrombotic disease, from initiation of endothelial dysfunction to atherosclerotic plaque rupture and thrombosis. However, the extent of their contribution remains uncertain for the time being because animal models of atherosclerosis with selective defect in MP generation or uptake are unfortunately lacking. Clearly, this will be possible once the molecular mechanisms governing MP formation and release are dissected. Then, one could envisage therapeutic avenues designed to either prevent their deleterious effects or promote their repair capacity.

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Disclosures

None.

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