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Microvesicles at the crossroads between infection and cardiovascular diseases

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Abstract

Observational and experimental studies continue to support the association of infection and infection-stimulated inflammation with development of cardiovascular disease (CVD) including atherosclerosis and thrombosis. Microvesicles (MV) are heterogeneous populations of sealed membrane-derived vesicles shed into circulation by activated mammalian cells and/or pathogenic microbes that may represent an interface between bacterial/microbial infection and increased risk of CVD. This review evaluates how MV act to modulate and intersect immunological and inflammatory responses to infection with particular attention to progression of CVD. While infection-related stimuli provoke release of MV from blood and vascular cells, MV express phosphatidylserine (PS) and other procoagulant factors on their surface which initiate and amplify blood coagulation. In addition, MV mediate cell-cell adhesion which may stimulate production of pro-inflammatory cytokines in vascular cells, which in turn aggravate progression of CVD and propagate atherothrombosis. MV transfer membrane receptors, RNA and proteins among cells, and present auto-antigens from their cells of origin to proximal or remote target cells. Because MV harbor cell surface proteins and contain cytoplasmic components of the parent cell, they mediate biological messages and play a pivotal role in the crossroad between infection-stimulated inflammation and cardiovascular diseases.

Keywords

atherosclerosis; infection; inflammation; leukocytes; platelets

Introduction

Both chronic and acute infection, especially when provoked by gram-negative bacteria, increase risk of cardiovascular events including instability of atherosclerotic plaques and formation of microvascular thrombi (1–3). Increased risk of thrombosis does not seem to be specific for one type of infection but rather seems to be a universal potential consequence of infection regardless of the organ system affected: dental infections including periodontitis (4), infection of the urinary tract (5) and upper airway (5,6) or systemic sepsis (7). In

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addition, exposure to infections and their subsequent inflammatory responses may contribute to increased incidence of venous thromboembolism with hospitalization (8).

In addition to risk for thrombosis, gram-negative bacteria, for example, *Chlamydia pneumoniae* (C. pneumoniae) that causes upper respiratory tract infection, may exacerbate coronary artery disease (CAD) as *C. pneumoniae* has been cultured from atherosclerotic plaque. Also found in monocytes, macrophages and foam cells, *C. pneumoniae* seems to be ubiquitous in all populations with CAD and contributes to the pathobiology of atherosclerotic plaque by causing inflammation and endothelial dysfunction (9). Other microbes may also participate in development of vascular disease processes as 26 different microbes including *Staphylococcus, Streptococcus, Escherichia coli, and Propionibacterium acnes*, have been cultured from calcific aortic aneurysms (10). However, mechanisms by which these microbes and their byproducts initiate atherosclerotic injury, facilitate its progression, or merely colonize preexisting atheromas to modify disease progression remain controversial (9). One mechanism that has emerged to link infection-stimulated inflammation with altered thrombotic potential of the blood and atherogenic processes is that infection increases production of activated cell membrane-derived pro-inflammatory and procoagulant microvesicles (MV) from invading microbes and host cells which then activate other host cellular and soluble components of the cardiovascular system (10–15). This review will focus first on the generation of MV during infection and then focus on direct and indirect impact of MV on the onset and progression of CVD. Understanding how infection contributes to production and propagation of MV may suggest possible new early diagnostic and prognostic tests and therapeutic targets for prevention and treatment to reduce progression of CVD.

Definition and properties

MV are heterogeneous populations of activated cell membrane-derived sealed vesicles, ranging from 20 nm to 1μm, shed from the cell surface in response to activation and apoptosis (16–20). The terms ‘microvesicles’ and ‘microparticles’ have been used interchangeably in the literature. But a distinction is warranted as microparticles and microvesicles may overlap in size, but a distinct double membranous border distinguishes MV from other similar size microparticles present in the blood including lipoproteins, protein aggregates, and protein-mineral aggregates (10,21–24). The literature is further confused by the term “ectosome” which refers to “membrane released vesicles with right-side-out oriented vesicles with cytoplasmic content” (25).

Because MV and ectosomes are sealed membrane vesicles, they bear antigens of their cellular origin and also may retain proteins, receptors and some functional properties of their parent cell (18,25,26). Thus, they facilitate transcellular communication throughout the circulation by delivering bioactive molecules and even genetic information when they come in contact with or bind to other cells in the circulation or the blood vessels (27). Elevated levels of circulating platelet-, monocyte- or endothelial-derived MV are associated with inflammation and CVD (11,28–32). Both mammalian cells and gram-negative bacteria and other microbial pathogens release MV (Table 1)(26). Thus, infectious agents could promote formation of MV from host cells; conversely, host-cell-derived MV could provide a feedback loop to either exacerbate or reduce infection, inflammation, vascular dysfunction and thrombotic processes at sites distant from the site of infection.

Mammalian cells

MV are released during physiological processes from certain types of cells and during pathological processes from every type of mammalian cells. For example, only platelet-derived MV are observed in blood of healthy humans and mice and are released following

activation of platelets by a variety of stimuli including adrenaline, ADP, thrombin, collagen, Ca^{2+} ionophore A23187, complement and shear stress (33,34). In general, formation of MV may begin within minutes after addition of an agonist, with the rise of intracellular calcium, which in platelets can be inhibited by calcium chelators (EDTA or EGTA) commonly used as anticoagulants (35,36). Increases in cytosolic calcium initiate kinase mediated reorganization of the cytoskeleton including myosin light-chain kinase (37), Rho-associated kinases (38) and proteases like calpain (39,40).

However, formation of MV is not a uniform process; release of MV differs quantitatively and phenotypically among stimuli. For example, endothelial cells release phenotypically different MV following exposure to the pro-inflammatory cytokine, tumor necrosis factor α (TNFα) which initiated receptor mediated intracellular signaling as compared to exposure to media stripped of growth factors which induced apoptosis (19). Endothelial cell-derived MV generated by plasminogen activator inhibitor -1 (PAI-1) and those produced by TNFα have overlapping but distinct protein compositions, with some proteins distinctively and exclusively related to one stimulus (41). Early studies which analyzed purified MV by SDS-PAGE revealed a pattern of limited complexity (42). However, proteomic analysis of the composition of MV from CEM T-lymphocytic cells following stimulation with phytohemagglutinin for 72 h or actinomycin for 18 h support the idea that the spectrum of proteins found in MV is influenced in part by the type of stimulus which stimulates their formation (43).

Confocal and electron microscopic images of MV formation provide evidence for selective arrangement and sorting of certain proteins. Thus, translocation of cellular molecules into MV might not be random (44). Additional work is needed to fully appreciate differential signaling systems induced by various stimuli and to determine if phenotypic assessment of MV might provide useful information reflecting the nature of cell activation and injury as a diagnostic or prognostic tool or treatment options (45).

Upon release, MV transport their biologically active contents including lipid, protein, RNA and cell surface receptors among cells (18). Specific surface receptors and cellular markers can be used to identify the origin and physiological status of the parent cell (Table 2). By identifying the cellular origin and establishing a reference range of blood borne MV in healthy individuals, it will be possible to understand which subpopulation may contribute to specific pathogenic processes.

Gram-negative Bacteria and MV

Bacterial derived MV are made up of an outer membrane which engulfs periplasmic components (46,47) and which serves as a delivery system of genetic material, protein, and lipopolysaccharide (LPS) (47–50), and promotes adherence of the bacterium to infect host cells (51) or other bacteria (47). MV formation may be a functional requirement for bacteria as DNA packaged within MV can be protected from degradation by soluble DNase (50,52). Resistance to antibiotics can be acquired by the recipient bacteria by integrating MV from the parental bacteria species (47).

MV released by bacteria may contribute to infection-induced cardiovascular diseases like coronary artery diseases since virulence genes, LPS and other toxins and enzymes carried by MV can be absorbed by host cells (53). MV from certain species of bacteria like *P. gingivalis* (a gram-negative bacteria related to chronic periodontitis) activate platelets (54,55), suggesting a possible mechanism by which bacteria-derived MV increase risk of thrombosis. However, even though bacterial-derived MV can be detected in infected human tissues (56), no studies have evaluated how bacteria-derived MV lead to onset and progression of CVD. In addition, most information regarding production of bacterial MV

was obtained during normal growth of bacteria *in vitro*. Little is known about mechanisms underlying production and release of bacterial derived MV, or about what and how stimuli regulate their production and composition by the host *in vivo* (49,54,57–59).

Host processes triggered by bacterial infection

Direct response of mammalian cells to LPS-receptor activation

Bacteria activate host cells mainly through the binding of LPS, the major pathogenic molecule of outer membrane component of gram-negative bacteria to toll-like receptor 4 (TLR4), a transmembrane glycoprotein present on platelets, leukocytes, lymphocytes, and endothelium (60–62). In pigs, infusion of LPS (10 mg/kg/ per hr) and in mice a single intra-peritoneal injection of endotoxin (7.5 mg/kg), increased platelet-derived MV within 3 and 6 hours, respectively (63–66). Experiments like these, while providing support for the concept that infection stimulates production of platelet-derived MV, should be interpreted with caution as measurement of a MV from a single cellular origin does not provide information about generation of MV from other cell types or interactions among MV of different cellular origin or interactions of MV with other blood cells. In addition, depending on the dose of LPS or type of bacterial infection, receptors other than TLR4 may be activated. Therefore, complement activation or cytokine-mediated mechanisms in vascular cells complicate unraveling mechanisms of LPS induced production of host MV (67–69).

In vitro studies can be used to identify effects of LPS on blood elements and cells of the vascular wall. In diluted blood which prevents cell-cell interactions, addition of LPS (500 ng or 1 μg/mL) increased the number of TF-bearing MV and MV from platelets after 4 hours of incubation (70). Incubation of human monocytes with LPS (5 μg/mL for 5 hours) increased the total number of MV, TF-bearing MV and those bearing phosphatidylserine (PS) and other adhesion molecules (42,71). MV formation visualized by fluorescence microscopy occurred in leukocytes and endothelial cells after incubation of LPS (5 μg/mL) for 90 min (72). Thus, LPS stimulates MV production from circulating blood cells and cells of the vascular wall.

In unpublished work by our group, incubation of whole blood with LPS for one hour at a dose which stimulated TLR-4 receptors did not increase the total number of MV. However, leukocyte-derived MV were associated with platelets which subsequently altered the reactivity status of the platelets (Figure 1). These data provide evidence as have other studies that MV generated by one cell type in response to LPS alter reactivity of other cell types within the circulation (69,73–76). Although existing data provide evidence that LPS induces MV release from isolated cell or cells *in vitro*, more studies are needed to understand how other bacterial proteins affect MV production from host cells *in vivo*. In particular, studies are needed to determine whether and how LPS, as a natural component of bacteria and a content of bacteria-derived MV, affects composition of infected mammalian cell-derived MV distinct from other stimuli.

Indirect response of mammalian cells to infection-associated processes

Mammalian cells release MV in response to a variety of stimuli associated with infection, many of which are considered conventional biomarkers for CVD including inflammatory cytokines, lipids and lipidperoxidation, oxidative stress and apoptosis. These stimuli are also important participants in the defense mechanisms and pathogenesis of infection.

Pro-inflammatory agents—Release of cytokines provoked in the host cells by invading micro-organisms may in turn stimulate release of MV from non-infected cells (9). For example, pro-inflammatory cytokines such as TNFα or interleukin-1 (IL-1) stimulate MV

production from endothelial cells and monocytes which contain tissue factor (TF) and have high procoagulant activity (75–77). Activated complement component (C5a) also increases the release of cellular MV from endothelia and platelets (78). Leukocyte-derived MV significantly increased after 1h incubation with 10μM fMLP(formylmethionyl-leucyl-phenylalanine, a formylated tripeptide originally isolated from bacterial filtrates) (79).

C-reactive protein (CRP), which promotes agglutination and bacterial phagocytosis, is an acute-phase response protein produced by the liver in response to infection as well as by cytokines IL-6, IL-1, and TNFα (80). CRP is elevated in individuals at risk for recurrent myocardial infarction, coronary heart disease or stroke and may predict the risk of future cardiovascular events (81). However, circulating CRP is used as a biomarker of inflammation but not as a specific biomarker linking the inflammation to an acute or chronic infection in CVD.

Oxidative stress and metabolic failure—Oxidative stress, characterized by disruption of oxidant and antioxidant systems within cells, increases with infection, inflammation and immunological disorders (82,83). Reactive oxygen species (ROS) damage cellular membrane structures leading to release of MV (39,84,85). Oxidative stress is a common mechanism underlying cardiovascular diseases (86,87), and diverse therapeutic interventions such as antioxidants can impede or delay the onset and progression of cardiovascular diseases (88,89).

Oxidative stress markers independently increase the total number of MV and MV derived from platelets (90). Energetic failure within platelets is also associated with increased shedding of thrombogenic MV, which may result from decreases in activity of enzymes of the mitochondrial electron transport chain (91). Oxidative stress also causes membrane phospholipid rearrangement and PS-bearing MV to be shed from red blood cells (92).

Oxidative stress in endothelial cells increases release of MV expressing vascular cell adhesion molecular-1 (VCAM-1) (93). Endothelium-derived MV released in response to oxidative stress stimulated neutrophil adhesion via platelet-activating factor (PAF) receptors (84). However, the exact mechanism of how oxidative stress and metabolic failure lead to MV release is not clear, but chronic treatment with antioxidants, such as vitamin C or carvedilol, decreases circulating endothelial MV in patients with heart failure and exhibits a protective effect (94,95).

Monocyte-derived MV released during apoptosis induce production of superoxide anion in endothelial cells (96), suggesting that production of reactive oxygen species are both the result of and stimulus for release of MV and thus, represent a mechanism of positive feedback to cellular injury. Reactive oxygen species promote a procoagulant phenotype through up-regulation of TF expression and activity in MV (97).

Apoptosis—Release of membrane blebs or MV are an early feature of apoptosis activated by various stressors including bacterial infection, cytokines and oxidative stress (83,98). Release of MV may be a protective mechanism against intracellular stress as MV containing caspase 3 prevent intracellular accumulation of the enzyme which could allow escape from apoptosis and thus, contribute to cell survival (99).

Mechanisms leading to release of MV upon apoptosis are only partly understood. Loss of tumor suppressor gene p53 leads to an increased release of TF-bearing MV (100). MV are not released from MCF-7 cells lacking caspase-3 but release is restored by transfection with functional caspase-3 (101). These results implicate both p53 and caspase-3 in formation of MV. As with oxidative stress, MV released as a result of apoptosis can stimulate apoptosis

in other cells as platelet-derived MV containing functional active caspase-3 induce apoptosis of human macrophages (102).

Contribution of MV to cardiovascular disease

CVD, in particular, atherosclerosis is described as an inflammatory disease (103). However, the source of inflammation is usually attributed to disruptions in metabolism related to altered lipid metabolism. The preceding discussion expands this view to include processes associated with infection and immunity. Indeed, pro-inflammatory cytokines are independent risk factors for CVD, and in persons with chronic infections, inflammatory responses increase risk of atherosclerosis (104). The number and type of MV generated during infection, inflammation, and thrombotic complications suggest that MV may provide an interface between infection, initiation and amplification of cardiovascular diseases (Figure 2).

MV in the initiation and amplification of coagulation and thrombosis

During formation of MV, the normal phospholipid asymmetry of the membrane is changed, exposing negatively charged phospholipids such as PS from the inner membrane leaflet to the outer membrane leaflet. Thrombin generation capacity of MV is proportional to the amount of PS bearing on the surface (105) or the number of MV bearing PS (106). Surface PS offers binding sites for coagulant factors II, Va, and Xa and provides a platform for the assembly of the prothrombinase complex, accelerating the conversion of prothrombin into thrombin and supporting a procoagulant phenotype (107,108).

MV also represent a reservoir of blood borne tissue factor (TF) activity (109,110). Monocytes and polymorphonuclear leukocytes are one source of TF-bearing MV, which when transferred or in association with platelets, trigger and propagate thrombosis (76). Whether platelets produce TF is controversial (109). There may be three distinct sources of platelet TF: 1) TF taken up from circulating monocyte-derived MV; 2) TF stored in the α-granules of platelets from precursor bone megakaryocytes or taken up from other sources; 3) TF that is synthesized and expressed on the plasma membrane of mature platelets (111,112).

The rate of accumulation of TF in platelet-rich thrombi cannot be explained by recruitment of leukocytes onto adherent platelets (113,114). One mechanism to explain this discrepancy is that TF-bearing MV adhere to platelets through binding of P-selectin glycoprotein ligand-1 (PSGL-1) on the MV and P-selectin on the platelets (113,115,116). Tissue factor exists in the blood in an inactive (latent) and active configuration (117) as TF-bearing MV circulate in healthy persons while TF activity was not detected (118). In addition to contributing to thrombin formation, fusion between TF-bearing MV and activated platelets facilitates transfer of both proteins and lipids to the platelet membrane (116). Therefore, MV modulate coagulation by directly initiating coagulation cascade or indirectly via activating platelets. It is proposed that TF bearing MV, mainly of monocytic and lymphocytic origin, contribute to thrombus formation following rupture of atherosclerotic plaque by binding to activated platelets at the site of plaque rupture (14,113,119).

MV and interactions with cells of the vascular wall

Using rat aortic rings as a bioassay, MV from patients with acute myocardial infarction initiated reduced endothelium-dependent relaxations and release of nitric oxide compared to MV from patients without ischemia (120). These reductions in endothelium-dependent relaxations are consistent with general vasomotor dysfunction observed after myocardial infarction, and potentially would contribute to increased formation of thrombus and decreased tissue perfusion.

Endothelium-dependent vasodilatation induced by acetylcholine was significantly reduced by circulating MV from patients with metabolic syndrome (121). MV derived from diabetic patients and HIV-infected individuals also reduce acetylcholine-induced and attenuate shear stress-induced vasodilatation through stimulating expression of caveolin-1 and down-regulation of endothelial nitric oxide synthase (eNOS) in cultured human endothelial cells (77). T-lymphocyte-derived MV may increase inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX2) through NF-κB - dependent transcription (122).

MV derived from monocytes and lymphocytes are found in atherosclerotic plaques in greater concentrations than those derived from platelets (14). This condition is opposite to the various numbers of MV found in blood and may reflect the activation of these cells as they migrate into vascular lesions or that the leukocyte-derive MV may be associated with platelets in the blood.

MV and intercellular communication

Regardless of their cellular origin (bacterial or host), MV can amplify progression of CVD through the transfer of biological information between cells. Transfer of integrins and selectins to target cells through MV facilitate leukocyte adhesion and interaction with vascular endothelium. In addition, platelet-derived MV enhance binding of neutrophils to other neutrophils under flow conditions via P-selectin on MV and PSGL-1 on neutrophils (123). Monocyte-endothelial interactions stimulated by platelet-derived MV up-regulated expression of cellular adhesion molecules (ICAM-1) on endothelium and CD11b/CD18 on monocytes, a process related to metabolism of arachidonic acid (124). Adhesion of monocytes and neutrophils to the endothelium is considered a crucial step in the early processes of atherosclerotic lesions (125).

It is likely that platelet-derived MV, rather than platelets deliver long-range signals to other cells throughout the circulation since the aggregation and binding properties of activated platelets make it difficult for them to travel through the circulatory system (126). Consequently, MV, instead of intact platelets, may be critical in enhancing thrombosis and modulating both innate and adaptive immune responses.

Processes of how MV attach to target cells is not completely understood. In addition to fusion of the cell membranes, receptor binding, as described in other sections, is important, and the receptors involved may differ depending upon the stimulus for MV generation. MV bearing ICAM-1 resulting from TNF-α stimulation of endothelial cells bind to β2 integrin on monocytes increasing TF gene expression in monocytes and induction of TF-dependent procoagulant activity (127). Alternatively, Fas/Fas ligand receptor interaction might be important in binding of apoptotic MV to target cells (122).

MV and amplification of infection-induced immune responses

As discussed above, LPS on the outer membrane of gram-negative bacteria or associated with bacterial derived MV activate host cells in part through TLR4 signaling mechanisms. Mutational deletion of TLR4 decreased risk of development of atherosclerosis but increased susceptibility for infection (128,129). Other genetic polymorphisms of monocyte chemo-attractant protein (MCP-1) and its receptor CCR2 (chemokine {C-C motif} receptor 2) may increase susceptibility for chronic stable angina pectoris and myocardial infarction (129,130). While pro-inflammatory cytokines stimulate MV production, MV in turn stimulate production of these same cytokines thus amplifying the signal through a type of positive feedback. For example, stimulation of leukocytes with endotoxin (a complex, not purified LPS) induced shedding of MV containing PAF, an agonist which stimulates innate immunity (72). Furthermore, after priming THP-1 monocytes with LPS, stimulation of those

cells with ATP generated MV which were both PS positive and contained bioactive IL-1β (131). This convergent response initiated by two different agonists suggests that generation and release of MV could be a general pathway for secretion of some cytoplasmic proteins which modulate the immune system. Stimulation of dendritic cells by IL-1β initiates acute inflammatory response (132).

In addition to initiating immune response, MV contribute to maturation of immune cells. MV generated from thrombin-activated platelets carry CD40L and initiate maturation of monocyte-derived dendritic cells which in turn activate naïve T-cells (133). CD40L in platelet MV can be transferred to the adaptive immune B-cell compartments that may be distant from the site of platelet activation. This “long-range” signaling results in production of antigen-specific IgG (134). MV generated from endothelial cells in response to bacterial infection induce maturation of plasmacytoid dendritic cells, secretion of inflammatory cytokine (IL-6 and IL-8, but not IFN-α) and proliferation of allogenic naïve CD4+ T-cell. These responses are not activated by either platelet or T-cell derived MV (75). Collectively these responses demonstrate a coordinated interaction among processes responding to infection, vascular cell activation and cytokines implicated in progression of cardiovascular disease.

Summary and future directions

MV derived from invading pathogens and host cells can be considered as vectors of trans-cellular exchange of signals common to infection, general inflammation, and procoagulant properties of the blood contributing to progression of CVD. The virulent potential of MV derived from pathogens may explain in part the consistent finding that the burden of infection increases development of CVD in the absence of consistent identification of specific pathogens within atherosclerotic lesions (9,135–137). While the literature abounds in studies of generation of cell-specific MV and their characterization in various diseases, general methodological challenges remain in the development of MV as a diagnostic or prognostic tool. These challenges include the need for standardized methods of blood collection and processing including issues related to anticoagulants and cell-free plasma preparation after blood collection which may themselves affect production, adhesion or secretion of MV in the collected sample (31,36). A second challenge is to better understand how different stimuli affect production and content of MV or to differentiate a non-specific “molecular switch” common to generation of MV from various cell types.

A third challenge is to define how sex and age modulate formation of MV in various cell types which might influence thrombosis, immunity and vascular reactivity. For example, estrogen receptor beta knockout female mice show higher PS-expressing platelet-derived MV in the plasma and thrombin-generating capacity compared with wild type mice (91). In addition, total numbers of MV, those derived from platelets, monocytes and vascular endothelium, and those positive for phosphatidylserine and tissue factor were significantly greater ($P<0.05$) in newly menopausal women (circulating estrogen < 20pg/ml) compared to age-matched women who had estrogen >40pg/mL (138). These results infer hormonal regulation on MV generation and thrombotic risk. Since incidence of immunologic disorders and cardiovascular risk diverge with age and sex (139,140), understanding the contribution of MV in association with these important variables will facilitate development of more effective preventive strategies for individuals.

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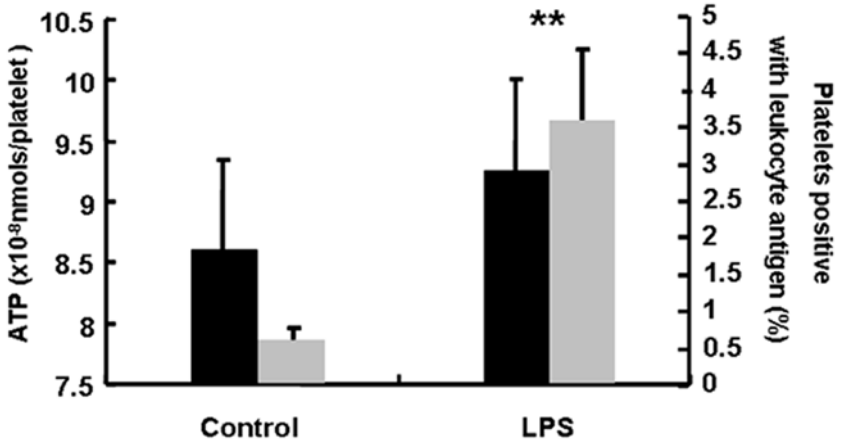

Figure 1.

ATP secretion in response to thrombin (0.1 U/mL) (black bars) and platelets associated with leukocyte-derived MV (gray bars) after whole blood from mice was incubated with LPS (5 μg/mL) or saline (control) for 1h. P<0.01 vs. Control.

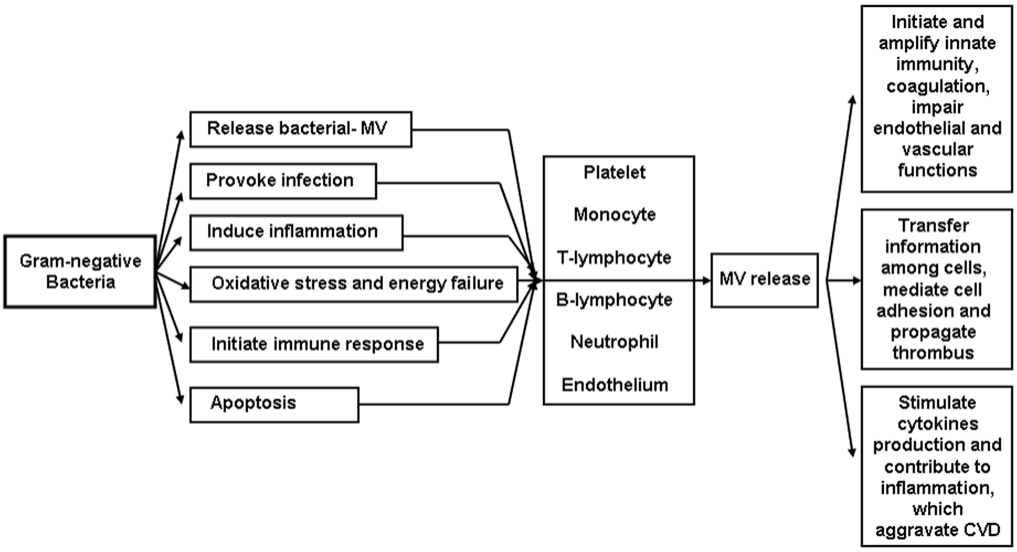

Figure 2.
Schematic of how MV of gram-negative bacteria may interface with host cells to increase progression of cardiovascular disease.

Table 1

Comparison between pathogen-derived and mammalian cell-derived MV

	Microbe-derived MV	**Mammalian cell-derived MV**
Origin	bacteria, fungus, protozoan pathogens	all mammalian cells
Size	20–250 nm	100 nm – 1 um
Formation	budding of outer membrane and vesiculation	cell membrane blebbing and vesiculation
Release	all stages of growth and activation	cell activation and apoptosis
Content	RNA, DNA, enzymes, LPS	RNA, protein and membrane receptors
Function	deliver of virulent factors and resistance to antibiotics; pro-inflammatory; immuno-stimulatory, or anti-tumor depending on target host cells	transfer of signaling molecules and functional genetic information; remove cellular waste; thrombogenic; pro-inflammatory and immuno-stimulatory depending upon stimuli to cell of origin

Table 2

Surface markers identifying cellular origin of blood borne microvesicles

Cell type	Surface marker
Polymorphonuclear cells and neutrophils	selectins and integrins complement regulators HLA-1
Lymphocytes	CD4, CD3, CD8 or CD 19
Platelets	GPIIb/IIIa (CD41/CD61), GPIX (CD42a)
Endothelial cells	CD31/-CD42a, CD62E or CD146