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MINI REVIEW

Role of extracellular membrane vesicles in the pathogenesis of various diseases, including cancer, renal diseases, atherosclerosis, and arthritis

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Extracellular membrane vesicles (MVs) 30–1000 nm in diameter and of varying cellular origins are increasingly recognized for their participation in a range of processes, including the pathogenesis of various diseases, such as: (1) atherosclerosis, (2) thromboembolism, (3) osteoarthritis (OA), (4) chronic renal disease and pulmonary hypertension, (5) tissue invasion and metastasis by cancer cells, (6) gastric ulcers and bacterial infections, and (7) periodontitis. MVs are derived from many different cell types and intracellular mechanisms, and perform different metabolic functions or roles, depending on the cell of origin.The presence of a metabolically active, outer membrane is a distinguishing feature of all MVs, regardless of their cell type of origin and irrespective of terminologies applied to them such as exosomes, microparticles, or matrix vesicles. The MV membrane provides one of the few protected and controlled internal microenvironments outside cells in which specific metabolic objectives of the host cell may be pursued vigorously at a distance from the host cell. MVs are also involved in various forms of normal and abnormal intercellular communication. Evidence is emerging that circulating MVs are good predictors of the severity of several diseases. In addition, recently, the role of MVs in inducing immunity against cancer cells and bacterial infections has become a topic of interest to researchers in the area of therapeutics. The main objective of this review is to list and briefly describe the increasingly well-defined roles of MVs in selected diseases in which they seem to have a significant role in pathogenesis.

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INTRODUCTION TO THE MV ENVIRONMENT

Extracellular membrane vesicles (MVs) are diverse in the human body.[1] The MV outer membrane provides one of the few protected and controlled internal microenvironments that exist outside cellular environments, in which complex interactions between, eg, hundreds of proteins, minerals, and proinflammatory molecules can occur, protected from, but still able to interact with, the extracellular environment. Outer membrane composition can not only be derived directly from the outer membrane of the host cell but can also contain intracellular membrane components. Shedding of MVs was once considered to be limited to disposing of cellular debris, but it is becoming increasingly evident that MVs are also programmed, whereby metabolic objectives of the host cell may be pursued vigorously at a distance from the host cell and, eg, can be transferred by MVs into other cells. This characteristic gives MVs a potentially high relevance to human clinical disease and the mechanisms of disease processes.

THE EXAMPLE OF PATHOLOGICAL CALCIFICATION

In healthy skeletal mineralization and in various 'calcific diseases' characterized by abnormal mineralization, extracellular matrix vesicles initiate and participate in calcification,[2] (Table 1). Calcific diseases are those in which: (1) Ca^{2+} uptake occurs early in the course of the disease, (2) calcification is importantly related to dysfunction, and (3)

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Table 1 Calcific diseases associated with extracellular membrane vesicles

Cardiovascular	Miscellaneous
Atherosclerosis[3,4]	Renal, metastatic calcification[5]
Calcific valvular stenosis[6]	Nonskeletal neoplasms[2]
Bones and joints	Tympanosclerosis (deafness)[7]
Osteoarthritis[8]	Calcinosis cutis[9]
Calcifying tendonitis[118]	Artificial heart valves[10]
Ectopic bone formation[11]	Dental plaque, Calculus[2]
Osteosarcoma and chondrosarcoma[12,13]	

therapeutic control of calcification may lead to decreased morbidity and/or enhanced diagnostic capability.[2]

In all of the calcific diseases cited in Table 1, extracellular MVs initiate and/or promote pathological calcification. Interestingly, in several of the diseases listed in Table 1, calcification may also commence within the cytoplasm of injured cells, in association with the mitochondria.[2] Mitochondrial initiation of pathological, intracellular calcification is especially important in renal calcification[5] in which injured cells absorb Ca^{2+} in excess of the normal, very low levels in the cell cytoplasm. This allows the mitochondria, residing in the renal cell cytoplasm, to actively accumulate Ca^{2+}, thus forming precipitable amounts of pathological intracellular $CaPO_4$.[2,5,14] Extracellular renal calcification appears to be initiated by protrusion of cytoplasmic buds from renal tubular epithelial cells, followed by their release as extracellular MVs known as 'ovoid bodies.' The latter become embedded in the renal tubular basement membrane and serve as a nidus for further extensive renal calcification.[5]

DISEASES IN WHICH MVS HAVE A ROLE IN PATHOGENESIS

Atherosclerosis

Identification of calcifying MVs in the vasculature occurred as early as 1976.[6] In atherosclerosis, MVs are released from intimal smooth muscle cells and/or macrophages (Table 2). The initial electron dense, calcific deposits occur within these 100–700 nm vesicles (Figure 1).[3,4,55] In 1996, the American Heart Association published a statement for health professionals drawing attention to the possible role of MVs in atherosclerosis, citing evidence that hydroxyapatite is formed in vesicles that pinch off by budding from the plasma membrane of arterial wall cells,[56] similar to the manner by which matrix vesicles pinch off from osteoblasts in the developing bone.[57] Recently, this has been followed up with evidence of a link between MVs, inflammation, and calcification.[58] Studies by Demer and Tintut[22] and Aikawa *et al*[59] demonstrated that inflammation triggers osteoblastic activity, leading to calcification during the early stages of atherosclerosis. Studies from Shanahan[16] demonstrate the complexity of the cycle of inflammation and calcification events associated with atherosclerosis. Studies with matrix vesicles isolated from vascular smooth muscle cells suggest that MV-mediated calcification events are regulated by a balance of mineralization promoters and inhibitors within and outside MVs,[15] in turn suggesting that calcification is an adaptive or protective response toward inflammation. It is not clear as yet, whether the mineral present inside or released from MVs can directly stimulate inflammation. More research is required to address whether calcification is an adaptive/protective response to inflammation and/or directly induces inflammation. The same pattern of pathological mineral initiation by extracellular plasma membrane-derived MVs is seen in calcific valvular stenosis.[6] However, in both calcific valvular stenosis and calcification of artificial heart valves, calcifying extracellular vesicles are derived not only from exfoliated surface vesicles but also from a mix of intracellular organelles, which are released from devitalized connective tissue cells.[6,10]

Thromboembolic Diseases

Extracellular microvesicles released from platelets[60] and/or from endothelial cells[28] have important roles in promoting normal blood coagulation in hemostasis, as well as in the pathogenesis of thromboembolic diseases.[28,61] Thrombogenic microvesicles are also known as microparticles (MPs) or as 'shedding vesicles.' They usually measure from 100 to 500 nm in diameter, and are released by budding from the surface membranes of activated platelets, after platelet attachment to endothelial-lined inner surfaces of the vessel wall, during normal hemostasis[62] (Table 2). First referred to as 'platelet dust,' circulating MVs are CD 42 positive (indicating their origin from platelets) and contain platelet-derived tissue factor (TF) and von Willebrand's factor (VWF),[30,63] which are the major initiators of intravascular coagulation in normal processes and in thromboembolic diseases.[30] Platelet release of extracellular MPs also increases during the tightly regulated process of platelet apoptosis, and is dependent on the formation of mitochondrial permeability transition pore.[64]

Disseminated intravascular coagulation (DIC) is a very prevalent, often fatal disorder, that occurs as a complication of various medical conditions, including septicemia, metastatic cancer, and ischemic cardiovascular disease. DIC occurs primarily in the peripheral circulation (in arterioles, venules, and capillaries). The extensive intravascular coagulation that occurs with DIC, leads to consumption of clotting factors, including platelets and fibrinogen. The final event is a hemorrhagic state, associated with widespread intravascular hemorrhage. Most forms of DIC appear to be related to dysfunction triggered by circulating, platelet- and endothelial-derived MVs. In cancer-associated DIC isolation of MVs from patients' blood plasma, differential centrifugation showed a marked increase in the number and procoagulant activity of the circulating MVs that were released from the surface membranes of cancer cells.[65]

Table 2 Characteristics of MVs associated with different diseases

Disease	Membrane vesicle-associated molecules	Source of MVs	Citations (including MV ultra-structure and cell of origin)
Atherosclerosis and vascular calcification (chronic kidney disease)	ALP,[4,15] annexins,[15] fetuin-A,[16,17] HAP,[4] matrix Gla protein,[17,18] phosphatidyl serine,[19] sodium-dependent phosphate cotransporters[20,21]	Vascular smooth muscle cells; calcifying vascular cells (pericytes, aortic-mesoangioblasts, and myofibroblasts)	Bobryshev *et al*,[18] Chen *et al*,[15] Demer and Tintut,[22] Giachelli,[21] Hsu and Camacho,[4] Kim,[19] Li *et al*,[20] Reynolds *et al*,[17] Shao *et al*,[23] Tanimura *et al*[3]
Osteoarthritis	ALP,[8,24] annexins,[24,25] glycosidases,[26] HAP,[8,24] type II and X collagen[24]	Articular chondrocytes, synovial fibroblasts	Ali and Griffiths,[8] Kirsch *et al*,[24,25] Pasztoi *et al*[26]
Pulmonary hypertension and thrombosis	Annexin-V,[27] cell surface molecular markers,[27–29] Phosphatidyl serine,[28,30,27,31] thrombomodulin,[32] TF[27,30]	Endothelial cells, platelets, leukocytes, monocytes	Amabile *et al*,[29] Bakouboula *et al*,[27] Chironi *et al*,[28] Dvorak,[30] Enjeti *et al*,[33] Satta *et al*[32]
Cancers	Angiogenic molecules such as TF,[30,34] VEGF,[30,35] cell surface adhesion molecular markers,[36–38] immune suppressing cytokines,[39] miRNA,[35] sphingomyelin,[40] tumor surface antigens[41–43]	DCs, endothelial cells, epithelial cells, hematopoietic cells, platelets, tumor cells	Andre *et al*,[44] Choi *et al*,[36] Dolo *et al*,[45] Dolo *et al*,[38] Dvorak,[30] Giusti *et al*,[37] Kim *et al*,[40] Koga *et al*,[43] Mitchell *et al*,[41] Osterud,[34] Skog *et al*,[35] Taraboletti *et al*,[46] Valenti *et al*[39]
Bacterial infections	Cytokines,[47–49] glycoproteolipids,[50] lipopolysaccharides,[50] quorum-sensing agents,[51] virulence factors,[52,53] TLR ligands[48,50]	Pathogenic bacteria, host macrophages	Alaniz *et al*,[49] Bhatnagar *et al*,[50] Ismail *et al*,[47] Kuehn and Kesty,[48] Li *et al*,[54] Nowotny *et al*,[52] Mashburn and Whiteley[51]

Thrombotic thrombocytopenic purpura (TTP) is a rare disease characterized by the presence of microangiopathic hemolytic anemia, decreased platelet counts in the peripheral blood (thrombocytopenia), and small foci of bleeding under the skin (purpura). These characteristics of TTP are due to the deposition of small, platelet-rich thrombi, deposited in the peripheral microcirculation (ie, in arterioles, capillaries, and venules). Such small thrombi obstruct the microcirculation at multiple sites, causing local ischemia, associated with the necrotic breakdown of microvessels and focal hemorrhages (petechiae). The final common path in causation of TTP involves the activation of platelets to form platelet-rich microthrombi in the peripheral circulation. This process is accompanied by shedding of procoagulant microvesicles from the outer membrane of endothelial cells[28,66] and platelets,[67] into the peripheral circulation where many microthrombi are formed. The number of circulating microvesicles (also referred to as MPs),[67] are greatly increased in patients with TTP *vs* in normal control subjects, and increase in number before the onset of symptoms of TTP.[28]

Trousseau's syndrome is a prothrombotic condition existing in the blood of cancer patients. It is more common in association with adenocarcinomas of the pancreas, stomach, ovary, or lung. Often, a hypercoagulable state is present in patients even before the causative malignant tumor becomes clinically evident. In Trousseau's syndrome, there is a marked increase in circulating, procoagulant microvesicles, and these MVs are mostly derived by shedding from the outer plasma membranes of malignant tumor cells themselves.[30,68] The main procoagulant factor in tumor-generated microvesicles appears to be TF which, in Trousseau's syndrome, activates platelets, causing them to aggregate and initiate thrombosis of major (large-sized) veins and arteries. In Trousseau's syndrome, widespread thromboembolism becomes the dominant feature of the patient's cancer-related illness, and often serves as the cause of death.[68]

Osteoarthritis

Osteoarthritis (OA) is a progressive disease of unknown etiology characterized by degeneration of articular cartilage, particularly in large, weight-bearing joints (namely the knee

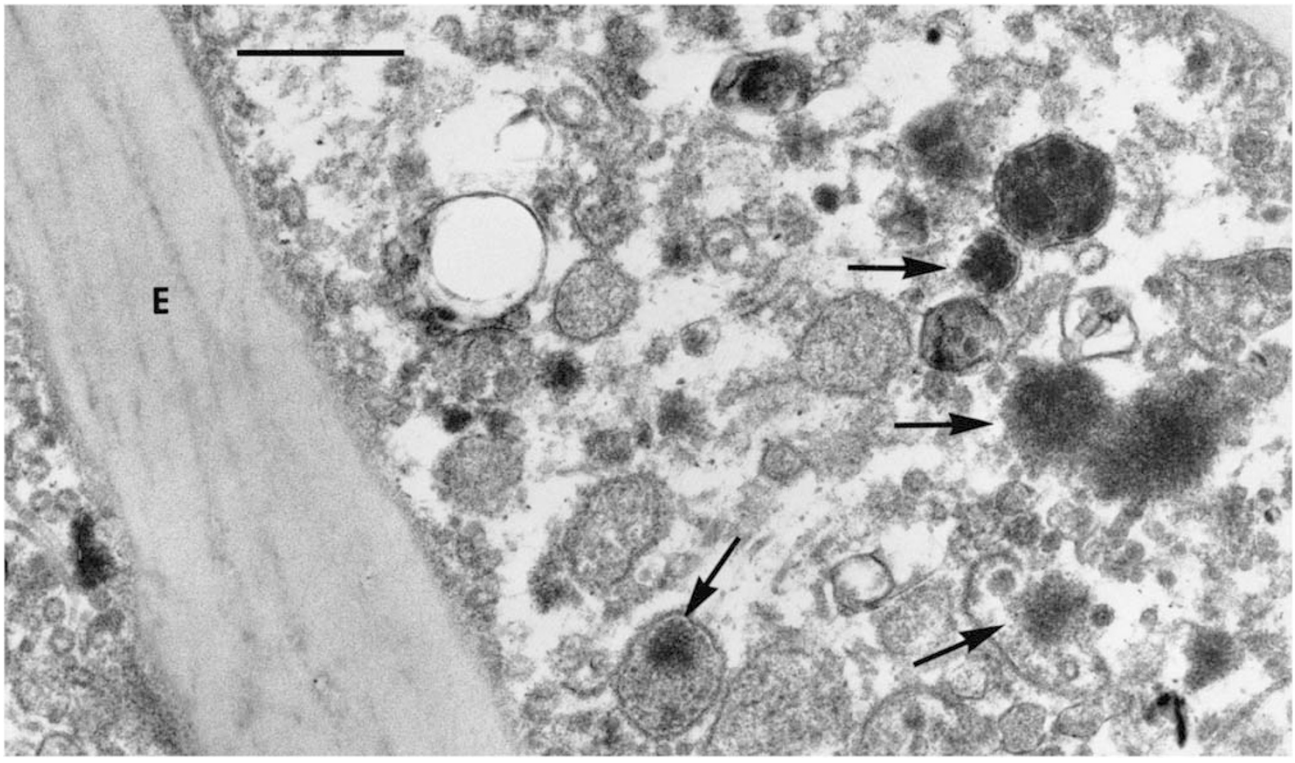

Figure 1 Human aortic intima by transmission electron microscopy. Adjacent to an elastic fiber (E), are many extracellular vesicles, ranging from 100 to 1000 nm in size. The vesicles are encased by a single, trilaminar membrane, and contain finely granular electron-dense calcific deposits (arrows) (scale bar = 500 nm, × 76 000). (Modified from Tanimura *et al*[55] and used with permission.)

and hip). OA usually begins in the middle age and gradually worsens with age. A pathological feature of OA is irregular hypercalcification of the 'tidemark,' a calcified layer of cartilage matrix located at the junction of articular cartilage and subarticular bone.[69–71] During OA development, the tidemark and subchondral bone thicken, hypercalcify, and present an irregular surface to the overlying articular cartilage, resulting in abnormal transmission of mechanical stress, thus contributing to articular cartilage degeneration.[72]

Ali and Griffiths[8] showed that the articular cartilage of the knees and hips in OA patients, contains more extracellular 50–250 nm-diameter MVs than normal, especially in the deep cartilage near the tidemark (Table 2). OA MVs contain high alkaline phosphatase (ALP) enzymatic activity[8] and originate from the surface membranes of articular chondrocytes. Thus, it was proposed that in OA, MV-induced hypermineralization of the tidemark would tend to make the tidemark stiff and unyielding to downward articular pressures, thus increasing the mechanical stress upon overlying articular cartilage. Increased, MV-initiated calcifying activity may induce increases in Ca^{2+} concentration in the articular cartilage extracellular fluid. Kirsch[25] demonstrated the role of annexins in MV-mediated pathological mineralization of osteoarthritic chondrocytes. Annexins are calcium- and phospholipid-binding proteins that form calcium channels through MV membranes that promote both normal and pathological calcification. Kirsch *et al*[24,25] demonstrated that MVs derived from osteoarthritic chondrocytes contain annexins II, V, and VI, which have an important role in pathological mineral formation and destruction of articular chondrocytes in OA. Annexins II and VI are also important for pathological calcification of vascular smooth muscle cells.[15]

Nevertheless, it should be emphasized that OA is a multifactorial disease, in which pathogenic factors other than overactive MVs have important roles in pathogenesis. One characteristic abnormality in OA is increased proteolytic activity in articular cartilage, including increased matrix metalloproteinases (MMPs), MMP-3, MMP-8, MMP-9, and MMP-13.[73,74] Membranous microvesicles, 200–700 nm in diameter derived from plasma membranes of T cells and monocytes (also referred to as MPs) induce MMP-1, MMP-3, and MMP-9 production in synovial fibroblasts.[75,76] Increased proteases released by synovial fibroblasts in OA[75] lyse collagen and proteoglycans of the articular cartilage, thus decreasing resistance to mechanical forces generated by weight-bearing activities. Genetic factors also increase the risk of OA.[77]

Chronic Renal Disease and Pulmonary Hypertension

Circulating, plasma membrane-derived MPs, 100–1000 nm in diameter, and carrying bioactive molecules known to cause thrombogenesis, inflammation, and atherogenesis, are present in increased numbers in chronic renal disease (CRD) (Table 2). CRD may result from diabetes, autoimmune renal diseases (eg, in Wegener's granulomatosis), or hypertensive

or ischemic heart disease.[78] In many such diseases, the number of circulating MPs is directly correlated with the severity of the associated chronic renal failure.[79] Pathogenetic effects of circulating membrane particles in CRD have yet to be completely defined. It is possible that they are involved in promoting accelerated arterial calcification and sclerosis, as seen, not only in the affected kidneys but also at multiple sites in arteries throughout the body.[80,81] In CRD, cell-damaging agents such as uremic toxins, low shear stress, and increased arterial stiffness contribute to endothelial apoptosis with a substantial release of endothelial MPs (EMPs) ie, MVs.[81] Under normal physiological conditions, EMP release is local and quite low in the peripheral circulation[82] in contrast to pathological conditions such as endothelial dysfunction in end-stage renal disease[29] and pulmonary arterial hypertension (PAH)[27] in which EMPs are found in high numbers.[27,29]

Recent studies have shown that an increased number of plasma membrane-derived microvesicles (200–1000 nm in diameter) in the pulmonary circulation (referred to as 'MPs') represent an excellent biomarker for determining the severity of PAH.[27,28] The largest number of such microvesicles or MPs is usually in the pulmonary circulation.[27] In PAH, such circulating MPs are mostly derived from endothelial cells, and are procoagulant, mainly because they carry concentrated phosphatidyl serine and TF. It seems that MPs in the pulmonary circulation promote a cascade of prothrombotic events leading to local thrombosis and ischemia, endothelial dysfunction, and vascular remodeling, which ultimately results in pulmonary hypertension.[27,28]

Nephrolithiasis (kidney stone formation) is a chronic renal condition that is associated with the formation of calcium oxalate (CaOx), calcium phosphate (CaP), or urate crystals in the kidneys.[83] Crystal deposition in the kidneys can induce tubulointerstitial damage and inflammation, leading to fibrosis, loss of nephrons, and chronic renal failure.[84] *In vitro* studies have demonstrated that renal brush border MVs $\sim$100 nm in diameter,[85] can induce and promote CaOx crystallization.[86] It is not known whether such MVs also initiate CaP or urate crystal stones.

Cancers

Tumor cell-generated microvesicles (TCMVs) are protective for the cancer cell, and support tumor cell survival, growth, host tissue invasion, and metastasis. An important function of tumor cell-generated MVs is to evade host immunity.[39] TCMVs have diameters in the size range of 30–100 nm,[39] and are generated by fusion of endosomal membranes (eg, from multivesicular bodies[87]) with the plasma membrane, which then exfoliates PM-derived MVs with various functions, including suppression of antitumor cell immunity[39] (Table 2). TCMVs also promote tissue invasion by transport and release of proteases, which digest host tissues at the site of invasion.[88] Tumor cell invasion from the blood stream is augmented by MV activation of local blood coagulation, which causes tumor cells to adhere to the endothelial-lined surfaces of vessels, and promotes tissue invasion at the site of adherence.[30] Once into the perivascular tissue, the release of MV proteases promotes local tissue invasion.[88] Survival of tumor cells at an ectopic site, such as the lungs, liver, or bone marrow requires the development of a new tumor blood supply (neoangiogenesis), to provide metastatic tumor cells with oxygen and nutrients on a permanent basis. To activate neoangiogenesis, tumor cells release MVs enriched in epithelial growth factor receptor (EGFR),[89] TF,[34] or developmental endothelial locus-1 protein.[90] Released MVs then fuse with local endothelial cells and stimulate the expression and release of vascular endothelial growth factor (VEGF), a potent, neoangiogenic factor.[89] Invading tumor cells also promote neoangiogenesis indirectly by stimulating the release of platelet-derived microvesicles, which then stimulate tumor cell expression and release of angiogenic factors, eg, VEGF.[91] Molecular analysis of glioblastoma microvesicle total protein, using a human angiogenesis array showed an enrichment of angiogenic regulators such as angiogenin, interleukin (IL)-6, IL-8, TIMP-1, TIMP-2, VEGF, and an abundance of miRNA 21.[35] In a recent study, Hood *et al*[92] demonstrated that melanoma cells release 30–100 nm microvesicles (known as 'exosomes'), which stimulate neovasculogenesis by endothelial cells *in vitro*.

Detection of tumor-specific markers such as EGFR VIII in glioblastoma microvesicles,[35] 5T4 (oncofetal protein), prostate-specific antigen, and prostate-specific membrane antigen[41] and prostate cancer (PC) mRNA markers[93] in urinary exosomes of PC, and tetraspanin-8 in MVs of pancreatic adenocarcinoma[94] suggest that the presence of MV-associated proteins may have a promising role as markers of malignancy in diagnostic blood or urine tests.

Recent studies with TCMVs have suggested an immunogenic role for such vesicles, owing to the presence of specific antigenic markers on tumor cells, which then trigger the immune activation of T cells, thereby resulting in tumor rejection.[95–97] The protumorigenic *vs* antitumorigenic roles of TCMVs are governed by the immune status of the patient and the stage of cancer progression.[96] Microvesicles generated from other cells such as platelets, dendritic cells, lymphocytes, monocytes, and stromal fibroblasts in the tumor microenvironment have been observed to modulate progression of the tumor[91,98] (Table 2).

Gastric Ulcers and Bacterial Infections

Since the 1980s, researchers have known that toxic MVs are released from oral, gastric, and other pathogenic bacteria.[52] Ismail *et al*[47] demonstrated that outer membrane-derived MVs from *Helicobacter pylori* increased proliferation of gastric epithelial cells *in vitro*, and increased *H. pylori* toxicity and IL-8 production.[48] MVs released by *H. pylori* are also endowed with proinflammatory molecules such as lipopolysaccharides (LPSs), lipoproteins, glycoproteolipids, ligands for membrane receptors (eg, TLR ligands), and tumor necrosis factor-α, etc,, all of which are capable of inducing

a damaging inflammatory response in the host.[47,48] A possible proinflammatory function of MVs released from macrophages, infected by certain bacteria, was also reported by Bhatnagar *et al*,[50] who demonstrated that MVs, released by macrophages infected with *Mycobacterium avium*, contain glycopeptidolipids and other TLR ligands, which could stimulate a proinflammatory response in resting macrophages.

BACTERIAL MICROVESICLES IN PERIODONTITIS AND ASSOCIATED ATHEROSCLEROSIS

Gram-negative oral bacteria such as *Actinobacillus actinomycetemcomitans*, *Bacteriodes gingivalis*, and other *Bacteriodes* species produce extracellular vesicles in the range of 30–200 nm in size.[99] The release of vesicles from the bacterial outer cell membrane seems to be dependent on the bacterial strain and nutrient availability.[100] The shed vesicles have an important role in periodontitis by serving as reservoirs for bacterial virulence factors, such as proteolytic enzymes, toxins,[53] and LPS (Table 2). These vesicles also contain factors that promote bacterial adherence to the infection site.[53] *Pseudomonas aeruginosa*, an opportunistic pathogen commonly associated with oral infections, sheds MVs that utilize 'quorum sensing' to regulate bacterial growth cell density and virulence.[51] Recently, it was shown that MVs of *P. aeruginosa* upon contact with the host plasma membrane lipid rafts, release virulence factors such as β-lactamase, ALP, hemolytic phospholipase C, and cystic fibrosis transmembrane factors (CiF) into the cytoplasm of the host cell.[101] Furuta *et al*[102] consider MVs of *Porphyromonas gingivalis* as 'targeted transport vehicles' that facilitate entry of virulence factors such as gingipains, which degrade host membrane proteins such as transferrin receptor, paxillin, and focal adhesion kinase, ultimately leading to cellular disruption. The shedding and release of such microvesicles in dental plaque accelerates inflammation in the surrounding host tissue through the release of proinflammatory mediators, thus leading to periodontitis.

Interesting recent studies have shown that microvesicles, released by bacteria in periodontitis, can also contribute to the progression of atherosclerosis in adjacent and distant arteries.[103] In this case, bacterial microvesicles from the gums carrying LPS and other proinflammatory agents are released into the circulation. They enter the walls of arteries where they initiate local inflammation associated with early stages of atherosclerosis.[104] Qi *et al*[105] reported that vesicles and LPS, released by *P. gingivalis*, activate macrophages to form foam cells, which are important mediators of the cascade of pathological events occurring in atherosclerosis.

MVs as Therapeutic Agents

MVs are currently being tested as immunotherapeutic agents, especially in the treatment of cancer, in which 60–90 nm-diameter MVs, also known as 'exosomes', have been shown (in the presence of GM-CSF) to stimulate beneficial tumor-specific immunity against colorectal cancer in human patients.[106] The rationale for this approach to cancer therapy relies on the fact that exosomes, derived either from immunocompetent T cells or tumor cells, can serve as a cell-free vaccine and can induce potent, specific antitumor immunity in animal models.[107] MV-based vaccines also have been used, with significant success, in clinical trials against bacterial infections.[108]

CURRENT DIAGNOSTIC TESTS FOR DISEASE-ASSOCIATED MPS AND MVS

MVs referred in various studies as MPs or microvesicles (MVs) in recent years have gained attention for their role as diagnostic markers for cardiovascular and renal diseases. Simak and Gelderman[109] and Simak *et al*[109] summarize various types of laboratory techniques used to analyze MPs found in blood. These detection techniques range from microplate affinity assays to immunolabeling and flow cytometry. One of the questions often raised, is where MVs are likely to settle during centrifugation of blood samples. Hornsey *et al*[111] demonstrated that during centrifugation of blood, MVs precipitate in the buffy coat. Krailadsiri *et al* performed quantitation of platelet- and RBC-derived microvesicles during WBC reduction using different filter/storage combinations. They observed that platelet-derived MVs increased during storage irrespective of whether they were filtered or unfiltered (control). Interestingly, the levels of RBC-derived microvesicles remained constant in the filtered products, whereas in the unfiltered control, their number increased significantly.[112] Thus, on the basis of the above studies, MVs are detected in association with both platelets and RBCs.

An important challenge associated with the diagnostic tests for MVs is the ability to distinguish different types of MPs. Mayr *et al*[113] used contemporary techniques such as tandem mass spectroscopy, high-resolution nuclear magnetic resonance spectroscopy, and combinatorial antigen libraries to determine the role of MPs in atherogenesis. These MPs are derived from plaque macrophages, but enter the circulation as the atherosclerotic lesion progresses. As their antibody composition is different from that of other circulating antibodies, they could serve as possible diagnostic markers of atherosclerosis.[113] An important refinement in the current laboratory tests is the ability to identify circulating endothelial-derived MPs, and measure their occurrence in diseased patients. Amabile *et al*[29,81,114] demonstrated the potential of circulating MVs as diagnostic markers to assess cardiovascular events in hypertension and in renal disease by evaluating the number of circulating endothelial MPs. Beyer and Pisetsky[115] summarized the state of the art diagnostic techniques in defining the role of MPs as biomarkers for vasculitides, PAH, and systemic sclerosis.

The question of whether MV proteins are hidden from test antibodies is addressed in studies investigating how MVs might participate in infection by evading immune system detection. Gould *et al*[116] proposed the 'Trojan exosome hypothesis' and provided evidence to demonstrate that retroviruses use pathways for nonviral or host exosome biogenesis

for the formation of infectious particles and mode of infection, thereby evading adaptive immune response.

Another intriguing field of investigation is to ascertain the role of MPs as possible contaminating agents in clinical laboratory samples, thus generating inaccurate test results. Prokopi *et al*, using tandem liquid chromatography and mass spectroscopy, demonstrated that platelet MPs, when taken up by mononuclear cells, result in endothelial progenitor cell phenotype. They reported the presence of endothelial biomarkers such as CD31 and VWF in mononuclear cells containing platelet MPs.[117]

Finally, as discussed above, the levels of circulating MPs in the peripheral blood measured by flow cytometry have been used to assess the clinical status of patients with thromboembolic diseases, such as TTP and Trousseau's syndrome.[27,29,66,67] To date, the use of clinical laboratory tests to measure circulating, disease-associated MVs has just begun, but will undoubtedly be more frequently used in future to assess diagnosis and prognosis in various diseases.

SUMMARY

A growing body of observational, biochemical, and proteomic evidence suggests that MVs are participants in pathological processes and can be utilized for diagnosis and therapy. However, despite four decades of well-documented investigations into MVs and their presence in disease, these are still early days in the understanding of their contribution to and defense against disease processes. The multiple origins of MVs from different parts of diverse cells, coupled with incomplete investigations into MV proteomic characteristics, leave much to be investigated regarding MV functions. In particular, the seemingly contradictory role of MVs as both defenders against and participants in disease remains underexplored. Evidence suggests that MVs provoke chronic inflammation as a defensive response, but this may also lead to a pathological outcome, eg, in atherosclerosis. The number of MVs circulating in the peripheral blood has been recognized as a good index to measure the severity of clinical PAH[27] and thromboembolic diseases, eg, in TTP.[66] Ratajczak *et al*[98] who studied the role of MVs in cancer, observed that MVs have a range of functions and could serve as potential diagnostic markers in laboratory medicine.[98] Recently, MV-based vaccines have been used with some success in clinical trials against colorectal cancers[106] and bacterial infections.[108]

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DISCLOSURE/CONFLICT OF INTEREST

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