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Extracellular Vesicles in Autoimmune Vasculitis - Little dirt lights the fire in blood vessels

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Abstract

Systemic vasculitis is diverse group of autoimmune disorders which are characterized by inflammation of blood vessel walls with deep aching and burning pain. Their underlying etiology and pathophysiology still remain poorly understood. Extracellular vesicles (EVs), including exosomes, microvesicles (MVs), and apoptotic bodies, are membrane vesicular structures that are released either during cell activation, or when cells undergo programmed cell death, including apoptosis, necroptosis, and pyroptosis. Although EVs were thought as cell dusts, but now they have been found to be potently active since they harbor bioactive molecules, such as proteins, lipids, nucleic acids, or multi-molecular complexes. EVs can serve as novel mediators for cell-to-cell communications by delivery bioactive molecules from their parental cells to the recipient cells. Earlier studies mainly focused on MVs budding from membrane surface. Recent studies demonstrated that EVs may also carry molecules from cytoplasm or even from nucleus of their parental cells, and these EVs may carry autoantigens and are important in vasculitis. EVs may play important roles in vasculitis through their potential pathogenic involvements in inflammation, autoimmune responses, procoagulation, endothelial dysfunction/damage, angiogenesis, and intimal hyperplasia. EVs have also been used as specific biomarkers for diagnostic use or disease severity monitoring. In this review, we have focused on the aspects of EV biology most relevant to the pathogenesis of vasculitis, discussed their perspective insights, and summarized the exist literature on EV relevant studies in vasculitis, therefore provides an integration of current knowledge regarding the novel role of EVs in systemic vasculitis.

Keywords

extracellular vesicles; systemic vasculitis; inflammation; autoimmunity

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1. Introduction

Systemic vasculitis is a multisystem autoimmune disorder characterized by inflammation of blood vessel walls, including weakening, thickening, narrowing or scarring, in any type, size and location of blood vessels, leading to aneurysm, stenosis, occlusion, thrombosis and superficial phlebitis. Systemic vasculitis is a heterogeneous group of diseases, including takayasu arteritis (TA), giant cell arteritis (GCA), polyarteritis nodosa (PAN), Kawasaki disease (KD), antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV), Behçet's disease (BD), etc [1, 2]. Though the progress has been continuously made in the past few decades, many aspects of the systemic vasculitis, including the etiology and pathogenic mechanisms, potential diagnostic biomarkers and therapeutic targets still remain poorly understood.

Genetic susceptibility, environmental factors, as well as abnormal innate and acquired immunity play important roles in pathogenesis of systemic vasculitis [3, 4]. A meta-analysis study reported that 33 genetic variants were identified to be associated with AAV [5]. Different AAV serotypes have different genetic variants [6]. Human leukocyte antigen (HLA) *52:01 and non-HLA genes, the IL-12B region, were associated with TA susceptibility [7]. However, GCA had a significant association with HLA-DR4, which is different from TA [7]. In addition, environmental toxins, pharmacological therapies, and infections can act as triggers of vasculitis and contribute to the disease onset [8]. Furthermore, abnormal activation of innate immune cells, such as neutrophils, monocytes and dendritic cells (DC), can release pro-inflammatory cytokines and activate adaptive immunity excessively [9]. A predominance of the T-help (Th)1 and Th17 cells and reduced number or functional impairment of T regulatory (Treg) cells promote the development of systemic vasculitis [10–12].

When cells die, they can trigger inflammatory responses in the body. The dead cells can always be found in the site of inflammation [13, 14]. Extracellular vesicles (EVs) are membrane vesicles which can be released by a variety of cell types during cell activation or programmed cell death [15–17]. EVs have been shown to mediate intercellular communications, and are involved in various physiological and pathological processes [17–19], including inflammation, autoimmune responses, endothelial dysfunction/damage, procoagulation, angiogenesis and intimal hyperplasia, the pathological conditions are known to be involved in vasculitis. In this review, we will summarize the latest literature on recent advances in our understanding of the biological characteristics and pathogenic functions of EVs, and their roles of EVs in pathogenesis of systemic vasculitis.

2. Classification and characteristics of extracellular vesicles

EVs are composed of a phospholipid bilayer and cytoplasmic components, including proteins, lipids, DNA, mRNA, microRNAs [17], or multi-molecular complexes [20] derived from their parental cells. EVs can be released from different cell types, including normal cells (platelets, erythrocytes, endothelial cells, monocytes, lymphocytes, etc) and malignant cells during cell activation, or undergo programmed cell death [15–17]. EVs can be found in blood, urine, synovial fluid, and other body fluids, many diseased organ/tissues, and even

feces [21, 22]. EVs are in low concentrations under normal physiological conditions, while the levels of EVs may be increased in various pathologic conditions or diseases, such as cancer, inflammatory, autoimmune, cardio-metabolic diseases [18–23]. EVs are always heterogeneous and can be classified into three types, namely exosomes, microvesicles (MVs), and apoptotic bodies, according to their size and different biogenesis [19] (Figure 1).

Exosomes are the smallest membrane vesicles, ranging 30 nm to 100 nm in diameter. Exosomes were first described by the Johnstone group in the 1980s, and they found that transferrin receptor was shed from multivesicular elements during reticulocyte maturation [24, 25]. Exosomes are derived from the inward budding of endosomal compartments, then resulting in the formation of multivesicular bodies and released to the extracellular milieu by exocytosis [26]. The formation of exosome can be regulated by Endosomal Sorting Complex Required for Transport (ESCRT) machinery [27, 28] or ESCRT-independent machinery, such as sphingolipid ceramide [29] and the tetraspanin CD63 [30]. Composition of exosomes is variable owing to their cellular origin. Exosomes are rich in lipids, such as cholesterol and sphingolipids, but phosphatidylserine (PS) is usually not present on exosomes as they are not budding from plasma membrane surface [31]. However, some studies report different results which may be due to their different cell origin [32, 33]. In addition, MHC molecules, adhesion molecules, tetraspanins (CD63, CD9, CD81, CD82), transferrin receptors have been identified on the surface of exosomes [26]. Exosomes may also contain nucleic acids, including messenger RNA, microRNA and DNA, originated from their parental cells [33].

MVs, also called microparticles, are small heterogeneous membranous vesicles which are approximately 100–1,000 nm in diameter. MVs were first described by Peter Wolf in 1967 as “platelet dusts” which were originated from platelets [34]. MVs are generated by budding directly from cell plasma membrane [17]. Numerous signal transduction pathways participate in the production of MVs from activated cells, including activation-induced protein tyrosine dephosphorylation, protein phosphorylation, and calmodulin activation [35]. Procoagulation is one of the most studied functions of MVs due to their transport of tissue factor (TF), a transmembrane molecule that is responsible for the initiation of the extrinsic coagulation cascade and thrombus formation [36]. Our previous publications have reported the release of prothrombotic TF-positive MVs from human monocytes when they are exposed to high levels of cholesterol [37] or tobacco smoke extract [38]. The majority of MVs expose PS on their surface, which is also involved in blood coagulation by providing phospholipid surface [39]. Since MVs have specific cell surface glycoproteins from their parental cells, these cell surface markers can be used for identification of their cellular origin. For example, endothelial cells-derived MVs carry CD105 (Endoglin), CD31, CD34, CD51, CD146 or CD62 (E-Selectin), lymphocyte carry CD45, erythrocyte carry glycophorin, while platelet-derived MVs are characterized by glycoprotein Iba polypeptide (GPIIb/CD42b), P-selectin and CD61 (GPIIb, integrin β 3) [17, 40, 41].

In addition to the classically discussed apoptotic MVs [17], several recent studies have also shown MVs can also be released from necroptotic [16, 42], and pyroptotic [15, 43, 44] cells, two novel types of programmed cell death, which are known to be related to inflammation and autoimmune diseases, including vasculitis [45]. It has been shown that pyroptotic cells

can release cytokine-containing MVs [15], i.e. IL-1 β containing pyroptotic MVs [43, 44]. Studies have demonstrated that both ANCA vasculitis [46] and vasculitis syndrome – KD [47] are related to IL-1 β . However, there is no direct investigation yet to determine if pyroptotic or necroptotic MVs are involved in the pathogenesis of vasculitis. The majority of necroptotic MVs are approximately 0.2–0.8 μ m in size [16, 42], similar to apoptotic MVs [17]. Necroptotic cells also expose PS on their plasma membrane surface in which externalization of PS is regulated by phosphorylated mixed lineage kinase-like (MLKL) when it translocates to cell membrane [42]. MLKL can also regulate endosomal trafficking and promote the release of EVs [16]. In addition, necroptotic cells lead to the release of danger-associated molecular patterns (DAMPs) from different intracellular compartments [13].

In contrast to the above mentioned several types of cell death, NETosis is a unique form of neutrophil cell death in which nuclear DNA is decondensed and released to form neutrophil extracellular traps (NETs) [48, 49] from ruptured nuclear envelope [50] and broken plasma membranes. As we discussed in the above, EVs can serve as an extracellular platform for intercellular transferring of bioactive molecules. Similarly, neutrophil NETs can also serve as a novel type of extracellular platform for transferring of nuclear DNA and histone from nucleus, as well as the NET-associated MPO or PR3 from cytoplasm of neutrophils, and therefore play important roles in pathogenesis of vasculitis [51].

DAMPs compose of a group of heterogenous molecules, such as DNA/RNA, high mobility group protein B1 (HMGB1), histones, heat shock proteins, biglycan, decorin and fibronectin [52, 53]. These DAMP molecules, when released to the extracellular space, can be carried by both EVs and NETs. When neutrophils undergo NETosis, nuclear DNA and histones in the decondensed chromatin are released from ruptured nuclear and plasma membranes forming ‘net-like’ extracellular trap structure. Therefore, as the major components of the NETs, DNA and histones are associated with NETs and play an important role in autoimmune diseases [51]. Our recent publication indicated that HMGB1 can be externalized and released with MVs [54]. On the other hand, HMGB1 could exaggerate NET formation by interaction with TLR2, TLR4 and the receptor for advanced glycation end products (RAGE) reported by Ma and colleagues [55]. DAMPs can trigger autoimmune response and promote inflammatory response by acting as auto-adjuvant and auto-antigen and activating pattern recognition receptors (PRRs), including toll-like receptors (TLRs), purinergic receptors, and inflammasomes [56, 57]. DAMPs can also drive tissue regeneration and fibrosis by promoting TGF- β receptor signaling [58].

Apoptotic bodies are dead corpse with approximately 1000–5000 nm in diameter, and they are released in the late stage apoptotic cells [59]. Apoptosis is a programmed cell death, and occurs in various physiological and pathological processes, such as cell turnover in physiological condition, embryonic, and immune system development [60]. Rho kinase plays a crucial role in regulation of the formation of membrane blebs and re-localization of fragmented DNA into blebs and apoptotic bodies [61]. Apoptotic bodies are recognized and cleared by phagocytes under physiological condition [62]. PS is located almost exclusively in the inner leaflet of the double layer cell membrane in resting conditions, while flip to the outer leaflet membrane, therefore exposed on the membrane surface during the formation of

apoptotic cells [63, 64]. PS serves as a “eat me” signal and is critical to drive recognition and phagocytosis. However, the phagocytic capability can be impaired in pathologic conditions, i.e. in patients with autoimmune diseases [65].

3. Detection of extracellular vesicles

Source identification of EVs is important for clinical studies. However, there are no standardized methods for the purification and detection of EVs until now [17]. Flow cytometry is the most widely used to detect cell-derived EVs according to their phenotype and size [17]. However, it is well known that conventional flow cytometry detect particles based on light scattering signal, and it is unable to detect EVs smaller than 200nm because these particles cannot be distinguished from background noise [22, 66], however fluorescent-labeled antibody against specific cell surface marker would be helpful. Along with the advance of biotechnology, a high-resolution flow cytometer which detects vesicles based on fluorescence intensity is introduced. High-resolution flow cytometer bright fluorescent labeling of cell-derived vesicles and can detect EVs with a diameter of 100nm [67, 68]. Other methods are also available for detecting EVs, including nanoparticle tracking analysis, electron microscopy, confocal fluorescent microscopy, fluorescence-based antibody array system, enzyme-linked immunosorbent assay (ELISA), western blotting, immune electrophoresis, have been used by our [17, 37, 38, 54] and other groups [69–71].

In addition, the process of EVs analysis can be greatly affected by many factors, including sampling process, the type of collection tube, transport conditions, phlebotomy conditions, centrifugation steps, and freezing conditions, which need to be carefully conducted [72]. Lacroix, et al. reported that three major pre-analytical parameters were the delay before the first centrifugation, agitation of the tubes during transportation and the centrifugation protocol. A double centrifugation of whole blood at 2500 – g for 15 min at room temperature can generate less artificial MVs [73]. Exosomes are generally isolated by sequential centrifugation [74].

4. Cell-Derived extracellular vesicles that are related to vasculitis

EVs derived from different cell types carry various different molecules and display different characteristics and functions. Here, we discuss several subsets of EVs with different cellular origin, i.e. platelets, endothelial cells, monocyte/ macrophages, neutrophils, and lymphocytes, which might be involved in vasculitis.

4.1. Platelet-derived extracellular vesicles

Platelets are the first responders in endothelial disruption and accumulates at the sites of vascular injury [75]. Platelet counts closely correlate with disease activity and may serve as a marker of vasculitis [76]. Platelet-derived EVs are released from activated platelets and involve in various pathophysiological processes, including thrombosis formation, inflammatory responses and intercellular signal transduction [77, 78]. Platelet-derived MVs may play more important roles in thrombogenesis than platelet-derived exosomes as MVs have more PS exposure on their surface [79]. Increased levels of platelet-derived MVs have been reported in patients with acute-phase KD, while anti-platelet therapy can decrease the

MVs levels [80, 81]. Furthermore, platelet-derived MVs can be used as biomarkers for evaluating platelet activation dynamics [80] and the effect of anti-platelet therapy in KD [81]. Daniel et al. have reported the elevated levels of platelet-derived MVs, neutrophil-derived MVs, and aggregates of neutrophil/platelet MVs in acute vasculitis, therefore these MVs can be considered as non-specific markers of neutrophil activation in acute vasculitis [82]. Moreover, platelet-derived MVs can enhance leukocyte-leukocyte interactions through P-selectin, leading to the activation and aggregation of inflammatory cells [83, 84]. Platelet-derived MVs also employ effects on vascular inflammation by transferring pro-inflammatory mediators to endothelial cells, and promoting secretion of pro-inflammatory cytokines and chemokines [85]. Interestingly, aspirin has been reported to decrease the expression of HMGB1 and α -granule chemokines CXCL4 and CXCL7 on platelet-derived exosomes [86]. So platelet-derived EVs can serve as biomarkers of platelet and neutrophil activation, and play key roles in the pathogenesis of vasculitis by promoting thrombogenesis and inflammatory responses.

4.2. Endothelial cell-derived extracellular vesicles

Many studies have reported to use endothelial cell-derived EVs as markers of endothelial activation/dysfunction or vascular injury [40, 87, 88]. Increased levels of endothelial cell-derived EVs has been found in patients with systemic vasculitis, and these EVs can serve as biological markers for monitoring disease activity in systemic vasculitis [40, 87–90]. Circulating endothelial cell-derived MVs can stimulate vascular endothelium by impairing acetylcholine-induced vaso-relaxation and nitric oxide (NO) production in a concentration-dependent fashion [91]. In addition, Taguchi K et al. reported that endothelial cell-derived MVs could decrease the expression of endothelial NO synthase protein through activation of ERK1/2 [92]. Furthermore, endothelial cell-derived EVs also have prothrombotic and proinflammatory effects [93, 94], and endothelial cell-derived EVs have significant procoagulant activity that can be partly blocked by an anti-TF antibody [95]. Therefore, endothelial cell-derived EVs can lead to endothelial dysfunction through various mechanisms, which are important in pathology of vasculitis.

4.3. Monocyte/macrophage-derived extracellular vesicles

Monocyte/macrophage-derived EVs are critical players in procoagulation, endothelial activation/damage, inflammation and, vascular complications [96, 97]. Our published works reported that monocyte-derived EVs carry both TF and PS on their surface, therefore demonstrate procoagulant activities [98, 99]. Aharon and colleagues confirmed our findings that monocyte-derived EVs harbor TF [96], and also enhance endothelial expression of TF, and decrease the levels of anticoagulant tissue factor pathway inhibitor and thrombomodulin [96]. Our study also found that cholesterol-induced monocyte MVs containing biologically active danger signals could stimulate endothelial activation and leukocyte recruitment to vasculature [56]. Nair et al reported that EVs released from LPS-stimulated macrophages can be coated with histones [100], which are recently shown to induce endothelial dysfunction or damage [101, 102]. On the other hand, our published work demonstrated that MVs from monocyte/macrophages can carry proteolytic MMP14 which can degrade collagen, therefore contribute to the weakness/rupture of vascular wall [37]. Furthermore, monocyte-derived MVs may carry IL-1 β and inflammasome that can amplify inflammatory

responses through activation of ERK1/2 and NF- κ B pathways [103]. Since pyroptotic monocytes can release IL-1 β and MVs [15], one can propose that there might be IL-1 β containing pyroptotic MVs. However, there is very little published work about pyroptotic MVs. In addition, exosomes released from monocyte-derived DCs can promote adaptive immune responses by enhancing the survival and activation of naive CD4⁺ T cells [104, 105]. Our study indicates that HMGB1 can be released with EVs into extracellular milieu from activated monocytes [54], and HMGB1-positive EVs may be important in the development of inflammatory and autoimmune diseases [54]. So monocyte-derived EVs may participate pathogenesis and development of vasculitis due to their roles in coagulation, inflammation and immune responses.

4.4. Neutrophil-derived extracellular vesicles

Neutrophils and their cytoplasmic proteinase 3 (PR3) or myeloperoxidase (MPO) are important in AAV [106–108]. Both PR3 [106] and MPO [107, 108] can be carried by EVs, and these EV-associated PR3 and MPO may serve as autoantigens to trigger production of the corresponding anti-PR3 and anti-MPO autoantibodies, therefore contributing to the development of AAV. Furthermore, PR3-ANCA and MPO-ANCA can induce neutrophil activation and stimulate the release of NETs, which also contain auto-antigens, including PR3 and MPO [51, 109, 110]. These process propagates the release of autoantigens, therefore, further enhance ANCA-associated vasculitis. On the other hand, Pitanga and colleagues reported that neutrophil-derived MVs can lead to endothelial damage in MPO-dependent manner [107]. Similar to neutrophil NETosis, neutrophil EVs may also carry nuclear molecules, including DNA/histone, from their parental cells, or even other cells [111]. EV-associated histones may also cause endothelial damage [101, 102]. Circulating levels of TF-positive neutrophil EVs are increased in patients with active AAV, suggesting the potential association between these pro-thrombotic and pro-inflammatory EVs with active AAV [112]. Furthermore, neutrophil-derived MVs express active and functional integrin $\alpha_M\beta_2$ that can also activate resting platelets and promote pro-thrombotic effects through AKT dependent pathway [113]. Neutrophil-derived MVs can promote the release of IL-6 and IL-8 and expression of intercellular adhesion molecule-1 adhesion molecule in endothelial cells, contributing to inflammatory response in AAV [114]. However, neutrophil-derived EVs may also enhance the release of anti-inflammatory factors, suggesting their role in regulation of the balance of pro-inflammatory and anti-inflammatory responses [115, 116]. In addition, Our study found that neutrophil MVs carry enzymatically active a disintegrin and metalloprotease-10 (ADAM10) and ADAM17 that could contribute to the damage/weakness of aortic wall development of human abdominal aortic aneurysm [38]. Therefore, neutrophil-derived EVs may contribute to the pathogenesis of systemic vasculitis through various mechanisms.

4.5. T-cell and B-cell derived extracellular vesicles

Lymphocytes are also important in vasculitis. The levels of T-cell, but not B-cell, derived EVs are increased in KD patients compared to healthy controls, while intravenous immunoglobulin administration results in the reduced levels of both T-cell and B-cell derived MVs [117]. Shefler et al reported that EVs derived from activated T cells contain functional miR-4443 and these EVs can activate mast cells to release proinflammatory

cytokine IL-8 through down-regulation of the tyrosine phosphatase protein tyrosine phosphatase receptor type J [118]. In contrast, CD4⁺ T cell derived exosomes may have immunosuppressive effects for inhibition of CD8⁺ cytotoxic T-lymphocyte responses [119]. Exosomes derived from Treg cells can suppress Th1 cell proliferation and cytokine production by transferring microRNA Let-7d to Th1 cells [120]. Furthermore, B-cell derived EVs can carry multiple short noncoding RNA molecules that may be involved in a variety of processes in immunity and inflammation [121]. In addition, B cell-derived exosomes express functional integrins that can mediate cell-cell adherence during inflammation [122]. So T-cell and B-cell derived EVs may be involved in pathophysiology of systemic vasculitis through promoting inflammatory and autoimmune responses.

5. Potential pathologic effects of Extracellular Vesicles on systemic vasculitis

EVs can mediate cell-cell communication by transferring bioactive molecules from their parental cells to target cells through different mechanisms. Ligands or other cell surface molecules released with EVs can bind to receptors of target cells, and then trigger intracellular signaling, and result in proinflammatory responses or cytokine release. Other cellular components, namely proteins, lipids, DNA, mRNA and microRNA, multimolecular complexes, can also be transferred to target cells and promote inflammatory responses [123, 124]. EVs may be involved in pathogenesis of systemic vasculitis through their role in inflammation, autoimmunity, pro-coagulation, endothelial damage/dysfunction, angiogenesis, and intimal hyperplasia [17, 18, 125–127].

5.1. Inflammation

EVs may exert either pro-inflammatory or anti-inflammatory properties. As summarized in figure 2, EVs can transport cytokines, chemokines or other pro-inflammatory molecules, therefore emerged as a novel mechanism for spread and maintenance of inflammation. It has been reported that EVs derived from activated monocytes/macrophages contain IL-1 β and inflammasome, therefore promote inflammation responses [20, 103, 128, 129]. On the other hand, EVs can stimulate the synthesis and secretion of proinflammatory mediators from immune cells through cell-cell interactions [130]. EVs have been shown to enhance the synthesis of IL-1 β , TNF α , IL-6, IL-8, monocyte chemoattractant protein 1 (MCP-1), and MCP-2 [131–133]. Our recent studies also showed that MVs could induce the release of TNF- α from cultured peripheral blood mononuclear cells [134, 135]. In addition, Esser J et al. have reported that macrophages and DCs derived exosomes containing functional enzymes can induce the biosynthesis of leukotrienes which are potent proinflammatory lipid mediators [136]. A recent work reported that PS-exposed necroptotic cells can be phagocytosed and led to the secretion of pro-inflammatory cytokines IL-6 and TNF α [42]. Necroptotic bodies released from necroptotic cells contain PS that may serve as specific “find me” and “eat me” signals and modulate the inflammatory response [42], while these results remain to be confirmed in future [42]. In contrast, neutrophil-derived EVs can enhance the release of potent anti-inflammatory cytokine transforming growth factor β 1 (TGF β 1) in activated or resting macrophages, and inhibit the secretion of pro-inflammatory cytokine, IL-8 and TNF α , in activated macrophages, thus suppress the inflammatory process

in the early stage of inflammation [115, 116]. Furthermore, neutrophil NETs can trigger plasmacytoid DCs and other immune cells for production of proinflammatory cytokines, thus involve in pathogenesis of systemic vasculitis [51, 137–140].

5.2. Autoimmunity

In the field of autoimmune diseases, increased levels of circulating EVs have been described in systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome, systemic sclerosis, and systemic vasculitis [17, 125] [141, 142]. Our recent publication systematically reviewed the role of MVs in autoimmune diseases [17]. EVs have been shown to affect immune responses through various mechanisms. EVs can activate both innate and adaptive immune cells as EVs have been found to express various immune response molecules, including auto-antigens and peptide–MHC complexes [143]. It has been reported that both PR3 and MPO can be associated with EVs, and these PR3-positive EVs [106] and MPO-positive EVs [107, 108] may serve as auto-antigens and contribute to autoimmune responses in AAV. Similarly, neutrophil NET-associated extracellular PR3 and MPO can also serve as autoantigens and play an important role in autoimmunity in vasculitis [51]. In addition, EVs may also participate in the formation of immune complexes (ICs) which can stimulate inflammatory responses and development of autoimmune diseases. Cloutier et al. found that ICs containing EVs existed in synovial fluid of RA patients [144]. The EVs could serve as antigenic surface for formation of functional ICs by recognition of citrullinated autoantigens [144]. Similarly, EVs exhibit nucleosomal molecules in an antigenic form and serve as a source of ICs in SLE [145]. The levels of IgG, IgM, and C1q are increased in circulating EVs in SLE patients, and IgG-EVs are associated with autoantibodies, such as anti-dsDNA, anti-ENA and anti-histone antibodies [145, 146]. Furthermore, EV-associated ICs may interrupt the recognition and clearance of these EVs by phagocytes [147]. Our unpublished works suggested EVs may carry CD47 and affect cell phagocytosis through CD47-signal regulatory protein α (SIRP α)–mediated inhibition [148]. Attenuated phagocytosis may result in the accumulation of proinflammatory EVs, debris and dead cells, and contribute to the propagation of autoimmune responses. EVs derived from infected cells and stressed or injured tissues carry pathogen-associated molecular patterns (PAMPs) and DAMPs, and these EVs can activate PRRs in recipient cells, and then induce inflammatory and autoimmune responses [149–151]. In addition, EVs can increase the expression of T-bet mRNA and protein, and then significantly enhance the differentiation of Th1 cells [152]. Circulating EVs can also inhibit the differentiation of Foxp3⁺ Tregs and induce the expression of interferon- γ by CD4⁺ lymphocytes as well as the differentiation of Th17 pathogenic cells [153]. Similar to EVs, NETs can also promote CD4⁺ T cells and CD19⁺ B cells proliferation, B cells maturation, and autoreactive B cell activation, thus contribute to autoimmune responses in vasculitis [51, 154].

5.3. Procoagulation

Elevated levels of circulating EVs have been reported in thrombotic disorders, particularly in cardiovascular and autoimmune diseases [17, 155]. EVs are associated with thrombotic diseases due to their carriage of procoagulant PS and TF. Platelets are the major source of PS⁺-EVs in blood, and EVs derived from monocytes, lymphocytes, or endothelial cells also present PS on their surface [155, 156]. The plasma membrane of normal cells has an

asymmetrical distribution of lipids between the inner and outer plasma membranes in which PS is localized in the inner plasma membrane. PS externalization can lead to the procoagulant activity of EVs due to an electrostatic interaction between positively charged carboxyglutamic acid in the clotting proteins and negatively charged PS on the membrane [157]. TF, released with monocytic EVs [98, 99], triggers extrinsic blood coagulation cascade, so the presence of TF on EVs dramatically increases their procoagulant activity in the studies from our [98, 99] and others [158]. Furthermore, the structural shape of the NET network, NET-associated DNA, histones, and TF, also provide procoagulant activities of neutrophil NETs, therefore contribute to vasculitis [51, 159–163]. It is reported that platelet-derived EVs have higher procoagulant activity than activated platelets [164]. However, Berckmans et al. found that EVs originated from platelets, erythrocytes, granulocytes and endothelial cells in healthy individuals had negative correlation with the concentrations of thrombin-antithrombin complex in plasma, indicating EVs also had an anticoagulant function [165].

5.4. Endothelial dysfunction/damage

Endothelial dysfunction characterized by reduced vasodilation and increased pro-inflammatory and pro-thrombic properties is an important manifestation in systemic vasculitis [166, 167]. Plasma levels of EVs are elevated when vascular endothelium are activated or damaged, thus EVs can serve as potential markers of endothelial dysfunction [92, 168]. EVs may be directly implicated in endothelial dysfunction by impairing endothelium-dependent vasorelaxation and reducing the production and bioavailability of NO [91]. In addition, EVs can bind to and activate endothelium through increasing the production of reactive oxygen species (ROS) [114]. MVs derived from active neutrophils expressing active MPO can activate myeloperoxidase-hydrogen peroxide-chloride pathway, leading to endothelial cell injury in vasculitis [107]. Furthermore, macrophage-derived EVs may carry nuclear histones from their parental cells [100]. These EV-associated histones have been recently shown to induce endothelial dysfunction or damages [101, 102], thus result in the onset of vasculitis. Since neutrophil NETs also contain histones, that may result in the induction of endothelial damage [159, 169]. In addition, NETs may also induce endothelial dysfunction through activation of MMP2 [169], thus contribute to vasculitis. However, further studies are still needed regarding the roles of EVs or NETs in the pathogenesis of vasculitis.

5.5. Angiogenesis

Angiogenesis, defined as the formation of new blood vessels from pre-existing vessels, plays a vital role in the pathogenesis of systemic vasculitis [170–172]. Increased circulating markers of angiogenesis, such as matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF), have been demonstrated in vasculitis, and MMP-2/9 are strongly associated with aneurysm formation [170–173]. It has been reported that EVs can exert pro-angiogenic and anti-angiogenic effects via a variety of mechanisms [174]. Endothelial- or macrophage-derived EVs contain pro-angiogenic mediators MMP-2, MMP-9 [175], and MT1-MMP [37], which can cause extracellular matrix remodeling that leads to endothelium invasion and revascularization [175]. Platelet-derived EVs can trigger an angiogenic response due to the expression of VEGF, basic fibroblast growth factor

(bFGF) and platelet derived growth factor (PDGF), which can activate PI3-kinase, Src kinase and ERK [176]. MiR-125a and miRNA-31, while suppress antiangiogenic genes-factor inhibiting HIF-1 (FIH1) and delta-like 4 (Dll4), also can be transferred to vascular endothelial cells via EVs [177, 178]. In contrast, lymphocyte-derived EVs can suppress angiogenesis by augmenting ROS generation and down-regulating the VEGF signaling pathway [179]. Furthermore, Aldabbous et al have reported the role of neutrophil NETs in angiogenesis and provide a functional link between NETs and inflammatory angiogenesis [180]. Lavoie also reported that NETs may contribute to angiopoietin-mediated pro-angiogenic activities [181]. Therefore, NETs may involve in vasculitis through promotion of angiogenesis.

5.6. Intimal hyperplasia

Intimal hyperplasia is the thickening of the tunica intima of a blood vessel as a complication of a reconstruction procedure, which eventually results in narrowing and obstruction of the vasculature in vasculitis [182]. In lesions of vasculitis, activated DCs recruit T cells and macrophages, and dysregulated vascular smooth muscle cells (VSMCs) migrate towards the lumen, eventually leading to luminal stenosis or occlusion [182, 183]. HMGB1 has been reported to drive inflammation and intimal hyperplasia after arterial injury [184]. Our recent publication indicated that HMGB1 can be externalized and release with MVs from macrophages [54]. In contrast, Liu R et al. has reported that EVs derived from adipose mesenchymal stem cells (ADMESCs-EVs) can inhibit intimal hyperplasia by interfering VSMCs proliferation and migration, as well as macrophage migration and inflammatory cytokine expression [127]. Therefore, EVs may be involved in vasculitis through their effects on intimal hyperplasia in different aspect.

6. Extracellular vesicles in systemic vasculitis

Over the past decade, increasing numbers of publications have demonstrated that EVs may play important roles in the pathogenesis of systemic vasculitis as we summarized in Table 1 [40, 80–82, 87–90, 112, 114, 117, 162, 185–201]. Brogan, et al. first reported that the plasma levels of MVs in children with active vasculitis were significantly higher than those in children with inactive vasculitis group and healthy controls, including platelet MVs expressing CD42a and endothelial MVs expressing CD105 or E-selectin. Endothelial MVs levels were closely associated with Birmingham Vasculitis Activity Score (BVAS) and the acute-phase reactant levels, suggesting that these MVs may be useful biomarkers for diagnosing and monitoring disease activity in children with systemic vasculitis [185]. It is generally known that endothelial activation or injury contribute to the development of vasculitis [166, 167]. MVs isolated from GPA patients have been shown to activate vascular endothelial cells and platelets in vitro [186]. The numbers of CD144⁺ endothelial MVs, circulating endothelial cells, VEGF, and endothelial progenitor cells are increased in children with active vasculitis as compared to their healthy controls, and declined after effective treatments [90]. The levels of endothelial cell-derived MVs are increased in KD patients [87, 88, 187–189], particularly in patients with coronary artery aneurysms [189], and these MVs are closely related to decreased values of flow-mediated dilation [88]. Dursun et al. reported that circulating endothelial MV levels are significantly higher in

children with Henoch-Schonlein Purpura (HSP) in both active and remission periods as compared to healthy controls, suggesting that circulating endothelial MVs may serve as biomarkers for subclinical inflammation in HSP [190]. Similarly, endothelial cell-derived MVs are also increased in adult patients with active AAV as compared to their healthy controls and the inactive AAV patients, and the levels of these MVs are correlated with the disease activity [40]. Therefore circulating endothelial cell-derived MVs may be a sensitive biomarker for identification of endothelial dysfunction and inflammation in patients with vasculitis.

The current investigations showed a potential role of neutrophil and platelet-derived MVs in the pathogenesis of vasculitis [80–82, 114]. Danie et al. reported that the levels of plasma neutrophil MVs, platelet MVs and neutrophil/platelet mixed MVs were strikingly higher in patients with acute vasculitis as compared to those in healthy controls [82]. It demonstrates that neutrophil and platelet-derived MVs are non-specific markers of neutrophil activation in acute phase of vasculitis. Polyclonal PR3-ANCA and MPO-ANCA from AAV patients and chimeric PR3-ANCA can induce the release of neutrophil MVs from cytokine-primed neutrophils in vitro [114]. The plasma levels of neutrophil MVs in children with active AAV are higher than those in healthy controls [114]. These neutrophil MVs can bind to endothelial cells in a CD18-dependent manner, resulting in the release of pro-inflammatory cytokines IL-6 and IL-8, increased expression of intercellular adhesion molecule-1 and production of reactive oxygen species [114]. PR3 and MPO can be carried by EVs, and these EVs can promote endothelial cell damage and inflammation reactions in AAV [106–108]. The levels of platelet-derived MVs are significantly higher in acute-phase KD patients as compared to those in healthy controls [80]. Platelet-derived MVs could be used as a biomarker for evaluation of platelet activation dynamics [80], and the therapeutic responses to anti-platelet therapy in KD [81]. Furthermore, ANCA-positive IgG can promote C5a-primed neutrophils to release TF-expressing MVs or neutrophil extracellular traps which may promote hypercoagulability in AAV [162]. Compared to children with inactive vasculitis and healthy controls, higher peak thrombin has been shown in children with active vasculitis, especially in the active vasculitis with thrombosis [191], and peak thrombin is associated with the levels of MVs. Therefore, MVs-mediated thrombin generation can be used as a biomarker to assess clinical thrombotic events in children with vasculitis [191].

The kinin system, also called the contact system, contributes to inflammatory responses and the development of vasculitis [202, 203]. Activation of the kinin system has been reported in children and adults with vasculitis [202, 203]. Kahn and colleagues recently reported that the leukocyte-derived MVs were associated with kinin B1-receptors [192], which played an important role in neutrophil chemotaxis and chronic inflammation [204]. Functional B1-receptors can be transferred to target organ cells via MVs and then promote kinin-associated inflammation [192]. Interestingly, plasma levels of the kinin B1-receptor-positive MVs are increased in patients with vasculitis as compared to the healthy controls [192]. C1-inhibitor is the major inhibitor of the kinin system that can inhibit the release of B1-receptor-positive MVs during vascular inflammation [193]. Thus, blockage of the B1-receptor may be potential new therapeutic strategy for vasculitis.

The numbers of total MVs are significantly increased in the acute phase of KD [117]. These MVs are derived from endothelial cells, platelet and T cells, and these MVs are decreased after intravenous immunoglobulin treatment [117]. Four serum exosomal microRNAs, namely miR-1246, miR-4436b-5p, miR-197-3p and miR-671-5p, have been identified as diagnostic biomarkers for KD [194]. Zhang et al. also explored serum exosomal microRNAs associated with KD [195]. It demonstrated that serum exosomal miR-328, miR-575, miR-134 and miR-671-5p could affect the expression of inflammatory genes, and these microRNAs might be used as potential biomarkers for the diagnosis of KD and the prediction of therapeutic outcomes of intravenous immunoglobulin therapy [195]. In addition, sixty nine differential proteins have been found in serum exosomes from KD patients compared to healthy controls, and some protein levels changed after intravenous immunoglobulin therapy, i.e. complement C3, apolipoprotein A-IV, and insulin-like growth factor-binding protein complex acid labile subunit. So these proteins may be used as potential biomarkers for monitoring intravenous immunoglobulin therapy in KD [196]. Zhang et al. reported that the proteome profile of serum exosomes was associated with coronary artery dilatation which was caused by KD [197]. They found 13 up-regulated proteins and 25 down-regulated proteins in patients with coronary artery dilatation caused by KD as compared to healthy controls, and most of the differentially expressed proteins are involved in inflammation response and coagulation cascades [197]. Kumpers et al. reported that endothelial, leukocytic and platelet-derived MVs markedly increased on initial stage in a patient with Churg-Strauss vasculitis-induced cardiomyopathy, while these MVs decreased during disease remission [89]. Therefore, the levels of these MVs subtypes are closely associated with the disease activity [89]. The endothelial MVs might be an important biomarker for diagnose and monitoring of the suspected vasculitic cardiac involvement in Churg-Strauss syndrome (CSS) [89]. The above studies suggested that EVs may serve as a novel biomarker for clinical application in diagnosis and monitoring of the disease activity and therapy.

In contrast, plasma levels of procoagulant MVs were increased in BD patients, but they were not related to gender or any clinical manifestations, including thrombotic events [198]. There was no significant difference between the levels of MVs after the previous or current treatments [198], indicating that MVs levels cannot be used for therapeutic monitoring. Khan et al. reported that BD patients had significantly higher plasma levels of total MVs and TF-positive MVs as compared to healthy controls, especially in BD patients with a history of thrombosis. A low balance between tissue factor pathway inhibitor (TFPI)-positive MVs and TF-positive MVs may predispose to thrombosis. So the balance between TF-positive and TFPI-positive MVs may be used to assess the risk of thrombosis in BD [199]. But there was no difference in the numbers of platelet-derived MVs between BD patients and healthy controls [200]. Another study showed that the levels of CD62P⁺ platelet MVs were significantly increased in patients in 20–50-year of age, but not in the older BD patients >50 years old. These results might suggest that activity of this disease was decreasing with age [201].

7. Conclusion

EVs release from various cell types, and composition of MVs is different owing to their different cell origin and biological status. EVs can act as important mediators of intercellular communication through a variety of approaches, such as cell-to-cell direct contact and secretion of soluble mediators and effectors. Increasing evidences suggest that EVs play important roles in inflammation, autoimmune responses, pro-coagulation, endothelial dysfunction/damage, angiogenesis and intimal hyperplasia, the crucial pathological processes in vasculitis. As another extracellular platform, NETs may also involve in vasculitis by affecting the above mentioned pathologic processes. EVs may serve as biomarkers for diagnosing and monitoring disease activity and therapeutic efficacy in patients with systemic vasculitis. However, whether EVs can be the therapeutic targets in systemic vasculitis are not fully understood.

Further studies are necessary to standardize the methodology for measurement of clinical samples, explore the potential novel therapeutic strategy to target EVs, and search the signalling pathways activated by EVs in vasculitis. The long-time follow-up studies are also needed to evaluate the possible roles of EVs as biomarkers to define any clinical manifestations and predict prognostic outcomes in vasculitis.

Abbreviations:

ANCA	antineutrophil cytoplasmic autoantibody
AAV	antineutrophil cytoplasmic autoantibody-associated vasculitis
ADMSC	adipose mesenchymal stem cells
ADAM	a disintegrin and metalloprotease-10
BD	Behçet's disease
BVAS	Birmingham Vasculitis Activity Score
bFGF	basic fibroblast growth factor
CRP	C reactive protein
CAD	coronary artery dilatation
CSS	Churg-Strauss syndrome
DC	dendritic cells
DAMPs	danger-associated molecular patterns
DII4	delta-like lagand 4
EVs	extracellular vesicles
ESR	erythrocyte sedimentation rate

ELISA	enzyme-linked immunosorbent assay
ESCRT	Endosomal Sorting Complex Required for Transport
FIH1	factor inhibiting HIF-1
GCA	giant cell arteritis
GPA	granulomatosis with polyangiitis
HLA	human leukocyte antigen
HMGB1	high mobility group protein B 1
HSP	Henoch–Schönlein purpura
ICs	immune complexe
ICAM-1	intercellular adhesion molecule 1
KD	Kawasaki disease
MVs	microvesicles
MPO	myeloperoxidase
MMPs	matrix metalloproteinases
MLKL	mixed lineage kinase-like
MCP	chemoattractant protein
MPA	microscopic polyangiitis
MALDI-TOF	matrix-assisted laser desorption/ionization-time-of-flight
ND	not detectable
NO	nitric oxide
ND	not detectable
NETs	neutrophil extracellular traps
PAN	polyarteritis nodosa
PRRs	pattern recognition receptors
PAMPs	pathogen-associated molecular patterns
PS	phosphatidylserine
PR3	proteinase 3
PDGF	platelet derived growth factor
PAN	polyarteritis nodosa

RA	rheumatoid arthritis
ROS	reactive oxygen species
RAGE	receptor for advanced glycation end products
SLE	systemic lupus erythematosus
TA	takayasu arteritis
TF	tissue factor
TFPI	tissue factor pathway inhibitor
TLRs	toll-like receptors
TGF-β	transforming growth factor β
Treg	T regulatory
Th	T-help
UCV	unclassified vasculitis
VEGF	vascular endothelial growth factor
VSMCs	vascular smooth muscle cells
2D	two dimensional

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Take-home messages:

- Extracellular vesicles, including exosomes, microvesicles and apoptotic bodies, are membrane vesicles released from various cell types during cell activation or programmed cell death.
- Extracellular vesicles can act as mediators for cell-to-cell communications by transferring membrane surface molecules, cytoplasmic or nuclear components from their parental cells to target cells via different mechanisms.
- Extracellular vesicles are involved in pathophysiology of systemic vasculitis through their effects on inflammation, autoimmunity, pro-coagulation, endothelial dysfunction/damage, angiogenesis, and intimal hyperplasia.

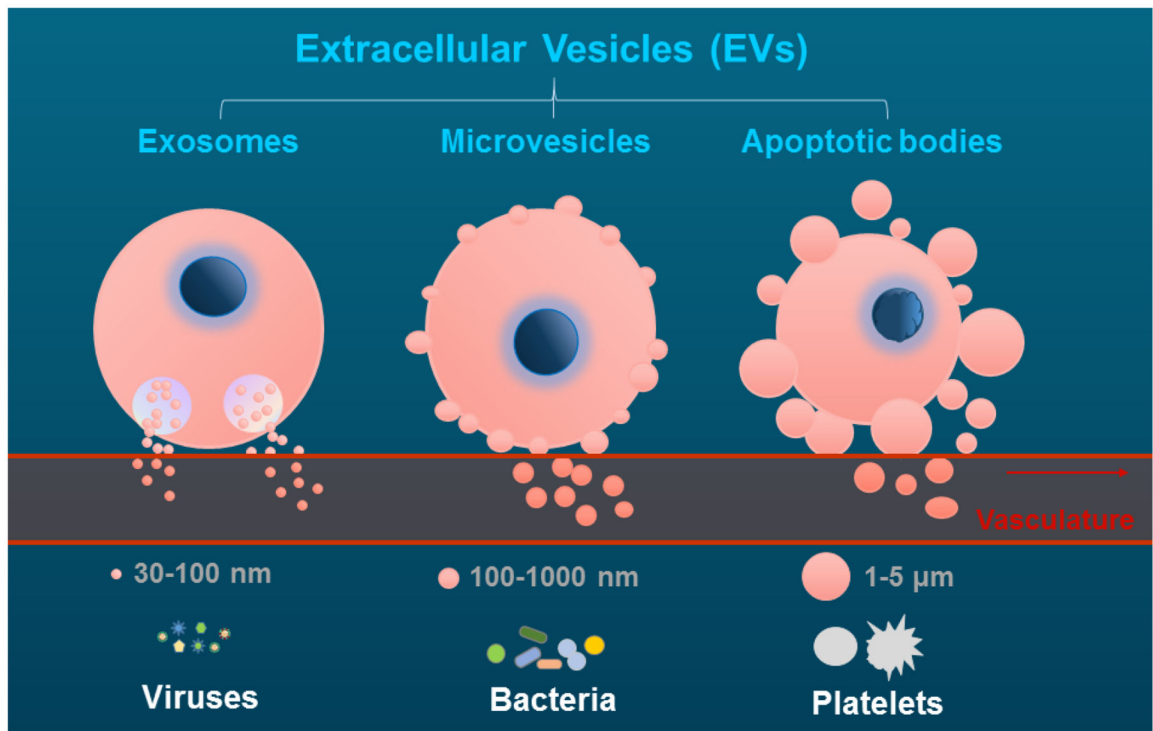


Figure 1. The formation and release of extracellular vesicles
 The schematic figure indicates the release of exosomes, microvesicles and apoptotic bodies, from their parental cells, as well as their corresponding comparisons in size to viruses, bacteria, and platelets.

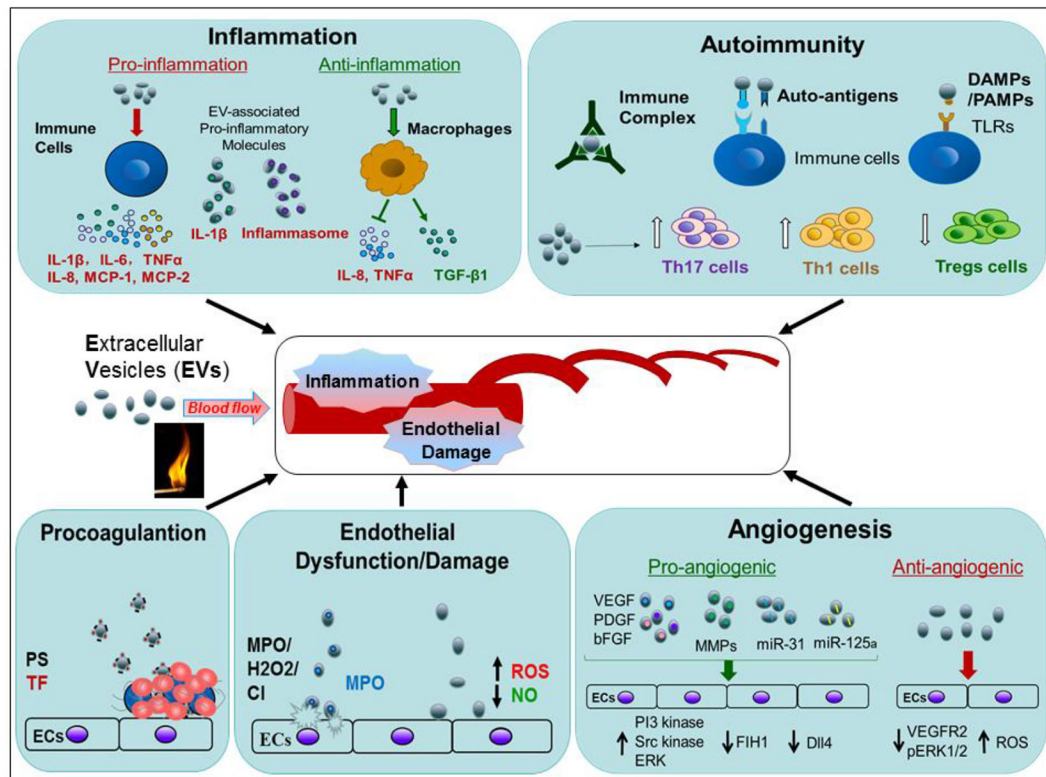


Figure 2.

Roles of extracellular vesicles in systemic vasculitis The schematic figures indicate the potential pathogenic effects of EVs on systemic vasculitis through their roles in inflammation, autoimmunity, procoagulation, endothelial dysfunction/damage, and angiogenesis. EVs can enhance inflammation through their associated proinflammatory cytokines/molecules, i.e. IL-1 β and inflammasome, and promotion the release of proinflammatory cytokines, i.e. IL-1 β , IL-6, TNF α , IL-8, MCP-1 and MCP-2. In contrast, EVs can also exert anti-inflammatory effects by stimulation of release of anti-inflammatory molecules, i.e. TGF β 1 or inhibition of the secretion of pro-inflammatory IL-8 and TNF α from macrophages. For the roles in autoimmunity, EVs can activate immune cells by EV-associated immune complexes, auto-antigens, and DAMPs/PAMPs. The EVs can serve as antigenic surface for formation of functional ICs. EVs can also enhance differentiation of Th1/17 cells, and inhibit the differentiation of Tregs. Increased procoagulant activity is important in pathogenesis of vasculitis, and EVs are highly procoagulant due to their associated PS and TF. As the important pathologic changes in vasculitis, endothelial dysfunction/damage can also be induced by EVs through increasing ROS production, reducing NO production and bioavailability, as well as the EVs-associated bioactive MPO or nuclear histone. In addition, EVs can not only exert pro-angiogenic function owing to their expression of VEGF, bFGF, PDGF, MMPs, MiR-125a and miRNA-31, but also suppress angiogenesis through augmentation of ROS generation and down-regulation of the protein levels of VEGFR2 and pERK1/2.

Table 1.

Summary of the Published Studies about EVs in Vasculitis

EV origin	Cell origin and markers	Associated Specific component	Samples	Techniques	Diseased/affected vessels	Presumed Pathogenic involvements	References
MVs	Endothelial cells CD144	miR-145-5p, miR-320a	Platelet-poor plasma	Flow cytometry	KD/ medium vessels	Upregulation of proinflammatory cytokine	187
MVs	Leukocytes CD45, neutrophils CD66	B1-receptors	Plasma, renal biopsies	Flow cytometry, electron microscopy	AAV, HSP/ small vessels	Kinin-associated inflammation	192
MVs	Endothelial cells CD105/CD144	B1-receptors	Platelet-free plasma	Flow cytometry	MPA, GPA, HSP/ small vessels	Kinin-associated inflammation	193
MVs	Platelets CD42a/CD62P	ND	Plasma	Flow cytometry	BD/ vessels of any sizes	Inflammation	201
MVs	Endothelial cells CD144/CD146	ND	Platelet-free plasma	Flow cytometry	HSP/ small vessels	Inflammation	190
Exosomes	ND	miR-328, miR-575, miR-134, miR-671-5p	Serum	Microarray	KD/ medium vessels	Inflammation	195
MVs	Neutrophils CD66b platelets CD41a	ND	Platelet-poor plasma	Flow cytometry	AAV/ small vessels	Inflammation and procoagulation	87
MVs	Neutrophils annexin V/CD66b	Tissue factor	Serum	Flow cytometry	AAV/ small vessels	Thrombosis and inflammation	112
Exosomes	CD9/CD81/flotillin	38 differentially expressed proteins	Platelet-poor plasma	Electron microscopy, Western blot, 2D-electrophoresis/ MALDI-TOF mass spectrometry	KD/ medium vessels	Inflammation and procoagulation	197
Exosomes	CD9/flotillin	69 differential proteins	Serum	Electron microscopy, Western blot, 2D-electrophoresis/ MALDI-TOF mass spectrometry	KD/ medium vessels	Inflammation, procoagulation and autoimmunity	196
MVs	Annexin V neutrophils CD66b/CD11b/MPO/PR3/CD18	CD18/CD11b/PR3/MPO/PS	Supernatant of cultured neutrophils, platelet-poor plasma	Flow cytometry	AAV/ small vessels	Inflammation, procoagulation and endothelium activation	114
MVs	Annexin V	Tissue factor	Supernatant of cultured neutrophils	Flow cytometry	AAV/ small vessels	Procoagulation	162
MVs	Annexin V	ND	Platelet-free plasma	ELISA	BD/ vessels of any sizes	Procoagulation	198

EV origin	Cell origin and markers	Associated Specific component	Samples	Techniques	Diseased/affected vessels	Presumed Pathogenic involvements	References
MVs	Annexin V	Tissue factor	Platelet-poor plasma	Flow cytometry	BD/ vessels of any sizes	Procoagulation	199
MVs	Annexin V, Platelets CD41, endothelial cells CD62E, neutrophils CD11b	Tissue factor/PS	Platelet-poor plasma	Flow cytometry	MPA, GPA, PAN, KD, BD,UCV / vessels of any sizes	Procoagulation	191
MVs	Platelets CD62	ND	Whole blood	Flow cytometry	BD/ vessels of any sizes	Procoagulation	200
MVs	Platelets CD42b/CD42a	ND	Platelet-poor plasma	ELISA	KD/ medium vessels	Evaluation of platelet activation	80
MVs	Annexin V	ND	Platelet-poor plasma	ELISA	KD/medium vessels	Evaluation of platelet activation	81
MVs	Annexin V, endothelial cells CD 105/ CD 144, platelets CD41, leukocytes CD 18, neutrophils CD 16b, monocytes CD 14	ND	Platelet-poor plasma	Flow cytometry	GPA/ small vessels	Platelets activation and endothelial activation	186
MVs	Annexin V/CD62E/CD31	ND	The supernatant of cultured HUVECs	Flow cytometry, ELISA, electron microscopy	KD/ medium vessels	Endothelial damage	87
MVs	Annexin V, endothelial cells CD105/CD62E, platelets CD42a/ CD62P, leukocytes CD45/ CD11b	ND	Platelet-poor plasma	Flow cytometry	AAV/ small vessels	Endothelial damage	41
MVs	Endothelial cells CD144 ⁺ /CD42b ⁻ , CD62E, CD105	ND	Platelet-poor plasma	Flow cytometry	KD/ medium vessels	Endothelial dysfunction	88
MVs	Annexin V, endothelial cells vWF/CD105/CD62e, leukocytes CD45/ CD11b, platelets CD42a/CD62P	ND	Platelet-poor plasma	Flow cytometry	CSS/ small vessels	Endothelial damage	89
MVs	Annexin V, endothelial cells CD 144/CD62E	ND	Platelet-poor plasma	Flow cytometry	MPA, GPA, PAN, KD, TA,BD, UCV/ vessels of any sizes	Endothelial damage	90
MVs	Platelets CD42, erythrocytes CD235, neutrophils CD66b, monocytes CD 14, T cells CD3, B cells CD 19, endothelial cells CD 144	ND	Platelet-poor plasma	Flow cytometry	KD/ medium vessels	Endothelial damage	117

EV origin	Cell origin and markers	Associated Specific component	Samples	Techniques	Diseased/affected vessels	Presumed Pathogenic involvements	References
MVs	Annexin V, platelets CD42a/CD62P endothelial cells CD62E CD105/ICAM-1/VCAM-1	ND	Platelet-poor plasma	Flow cytometry	MPA, GPA, PAN, KD/ small and medium vessels	Endothelial activation	185
MVs	Endothelial cells CD31/CD146	ND	Platelet-poor plasma	Flow cytometry	KD/ medium vessels	Endothelial damage	188
MVs	Endothelial cells CD 105/CD62E/ICAM-1/VCAM-1/CD144/CD31	ND	Platelet-poor plasma	Flow cytometry	KD/ medium vessels	Endothelial damage	189
Exosomes	CD9/CD81/TSG101	miR-1246, miR-4436b-5p, miR-197-3p, miR-671-5p	Serum	Microarray, real-time quantitative PCR, western blot	KD/ medium vessels	Diagnostic biomarkers	194