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Extracellular Vesicles: Evolving Contributors in Autoimmunity

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Abstract

Extracellular vesicles, including microvesicles, exosomes and apoptotic bodies are recognized as carriers of pathogen-associated molecules with direct involvement in immune signaling and inflammation. Those observations have enforced the way these membranous vesicles are being considered as promising immunotherapeutic targets. In this review, we discuss the emerging roles of extracellular vesicles in autoimmunity and highlights their potential use as disease biomarkers as well as targets for the treatment and prevention of autoimmune diseases.

Keywords

apoptotic bodies; autoimmunity; exosomes; extracellular vesicles; immunity; microvesicles

I. INTRODUCTION

The immune system embodies several biological processes, making it capable of recognizing and responding to the extracellular environment. In the context of autoimmunity, immune tolerance to harmless self-antigens is abolished, resulting in a dysregulated immune homeostasis. Multiple immune cell types mediate autoimmune responses, including autoreactive and regulatory T lymphocytes, B lymphocytes, and DCs.^{1,2} Autoimmune diseases affect approximately 5% of the general population, mostly women, and these diseases comprise a heterogeneous group of poorly understood disorders.^{3,4}

Recently, the role of extracellular vesicles (EVs), particularly exosomes and microvesicles, which are secreted by several immune cells in modulating the auto-inflammatory response, has been the subject of much research. Acting as both local and distal mediators of intercellular communication, those vesicles represent a relatively newly discovered biological player which shows promise in both diagnostics and therapeutics of autoimmune diseases.⁵

Almost all cell types examined to date secrete some form of EVs, which have a wide range of important physiological and pathophysiological effects in the microenvironment as well as the distal sites of the body.⁶ In the past decade, our understanding of the significant role that EVs play in many aspects of biologic function and communication has advanced significantly. In this rapidly progressing field, most attention has focused on tumor-derived EVs, and little is known about their role in autoimmune inflammation. This review outlines the current knowledge of EVs in autoimmune inflammatory diseases and highlights their potential directions.

II. BIOGENESIS, TRAFFICKING, AND CLASSIFICATION

Extracellular vesicles are a heterogeneous population of small, membranous, spherical structures composed of a bilayer membrane that are released by several different kinds of cells.⁶ EVs were found in almost all biofluids such as urine, plasma, saliva, cerebrospinal fluid, synovial fluid, semen, and breast milk.⁷

Among EVs, exosomes are the smallest vesicles, ranging from 30 nm to 120 nm; they are derived from endosomal budding and are released into the lumen through exocytosis.^{8,9} Microvesicles (MVs) are small, intact, extracellular vesicles larger in size than exosomes, up to 1,000 nm, that are also released by most cell types.^{6,9} These vesicles have been shown to bleb off the surface of several cell types and are distinct in composition and size from other subcellular structures, such as apoptotic bodies.¹⁰ Finally, apoptotic bodies are the larger EVs, with a diameter ranging from 1000 nm to 5000 nm, and they are released during cell apoptosis.^{10,11} Collectively, the general term 'EV' refers to all of the different types of vesicles mentioned above; they often overlap in size, and they are classified using different parameters such as size, biogenesis, trafficking, release, morphology, and cargo.

Currently, there is no consensus on a gold standard method for the isolation and characterization of EVs. EVs are generally isolated by ultracentrifugation after multiple low-speed centrifugations and/or filtration steps to increase purity.^{12,13} Alternatively, EVs can be isolated using immunoaffinity magnetic beads against membrane proteins such as CD63.¹⁴ The International Society for Extracellular Vesicles has recently published a position statement regarding minimum experimental requirements for EV isolation and characterization.¹⁵

III. CARGO

The content of the EVs mostly reflects that of the parent cells, ranging from cytoplasmic and nuclear components to surface proteins.^{7,16} The cargo of different types of EVs is enriched in certain molecules, including adhesion molecules, membrane trafficking molecules, cytoskeleton molecules, heat shock proteins, cytoplasmic enzymes, signal transduction proteins, cytokines, chemokines, proteinases and cell-specific antigens.^{17,18} Moreover, EVs contain mRNAs, non-coding RNAs (ncRNAs), including microRNAs (miRNAs), and extra-chromosomal DNA.^{19–22} Extracellular vesicles participate in important biological functions, acting as a mode of communication between cells. This intercellular communication can be conferred by mediators that are expressed either on the surface of the extracellular vesicles or that are contained within the extracellular vesicle lumen.¹⁹

IV. ACTIVE PLAYERS IN THE IMMUNE RESPONSE: EVS CONTAIN AUTOANTIGENS

Extracellular vesicles that are produced by both immune and non-immune cells have important roles in the regulation of immunity. Recent evidence suggests that EVs can mediate immune stimulation or suppression and they can drive inflammatory, autoimmune

and infectious disease pathology.²³ Thus, modulation of EVs has the potential to be used as therapeutic agents to.

One of the most challenging questions related to the role of EVs in autoimmunity is whether they are targets of autoreactive recognition and, therefore, capable of initiating and perpetuating pathological autoimmune responses or bystanders. EVs express both self-antigens and peptide–MHC complexes²⁴ and, therefore, they could represent a source of self-antigens and might activate autoreactive T cells in the context of MHC. A wide range of autoantigens are contained in EVs including Ro/SSA, La/SSB, histones, and a-enolase.^{25,26} In rheumatoid arthritis (RA), platelet EVs derived from the synovial fluid are autoantigen-expressing elements capable of perpetuating the formation of inflammatory immune complexes.²⁷ In another example, circulating EVs from SLE patients carry IgG, IgM, C1q, and IgG, and are associated with autoantibodies and complement activation.²⁸ Thus, EVs have attracted great interest as important contributors to the autoimmune response.

V. EVS IN AUTOIMMUNE DISEASES

A. Rheumatoid Arthritis

A number of studies have substantiated the role of EVs in RA: significantly higher levels of platelet-derived EVs were found in RA patients than in healthy controls, and the number of EVs correlated with disease activity.²⁹ In another study, platelet-derived as well as leucocyte-derived EVs were detected in the synovial fluid of patients with RA.³⁰ Platelet-derived EV counts were increased in both the circulation and the synovial fluid in a series of studies.^{31–33} Furthermore, joint inflammation can be the result of coagulation and fibrin deposition, which are induced by synovial EVs carrying proinflammatory cytokines.³⁰

Synovial fluid EVs exert profound biological functions, including the induction of BAFF³⁴ and increased production of IL-6, IL-8, RANTES, MCP1, ICAM-1, and VEGF by cultured synoviocytes.³⁵ In addition, monocyte-derived and T-lymphocyte-derived EVs up-regulate the production of PGE in synovial fibroblasts by inducing COX-2 and mPGES-1, providing evidence for a mechanism by which extracellular vesicles may contribute to inflammation in RA.³⁶

Several other studies have also suggested that exosomes from certain sources could contribute to disease progression. For example, synovial fibroblasts from RA patients produce exosomes that contain a membranous form of TNF- α , which may play a role in tissue destruction and autoimmune inflammation.³⁷ These TNF- α -positive exosomes can be effectively taken up by T cells, and in turn, they activate AKT and NF- κ B, rendering these T cells resistant to apoptosis.³⁷ In addition, citrullinated proteins, which are known to be autoantigens in RA, were found in exosomes from the synovial fluids of RA patients.³⁸ Therefore, exosomes carrying citrullinated peptides could play an important role in the induction and distribution of citrullinated proteins. Similarly, the autoantigen nuclear protein DEK, which contributes directly to joint inflammation in juvenile arthritis, were secreted in a vesicle-contained form in the synovial fluid of JA patients.³⁹ Finally, exosome-containing annexins, which are known to promote mineral formation and destruction of the articular

chondrocytes, were higher in the articular cartilage of osteoarthritis patients than in controls.⁴⁰

In summary, platelet-derived and leucocyte-derived MVs play a pivotal role in local inflammation and joint destruction through the activation of the synovial fibroblasts. The aforementioned studies demonstrate the presence of disease-contributing exosomes, which could be useful in arthritis therapy by using targeted selective depletion.

B. Systemic Lupus Erythematosus (SLE)

A key line of evidence suggesting a role for EVs as an antigenic stimuli comes from studies showing higher platelet-derived EV counts in patients with SLE than in healthy individuals.^{41,42} Sellam et al. reported increased levels of circulating EVs in SLE, primarily in patients with Sjogren's syndrome and RA.³² However, the level of EVs is low in the case of more severe autoimmune diseases, probably because of high secretory phospholipase A2³² activity, which leads to consumption of EVs. In patients with SLE, EVs contain DNA, which is antigenically active and can bind to lupus anti-DNA autoantibodies.^{43,44} These findings suggest that EVs are an important source of extracellular DNA that serves as an autoantigen and autoadjuvant in SLE.

Regarding the role of EV in epigenetic regulation, some EV-containing miRNAs, small noncoding RNAs that modulate gene expression, have been identified. Ichii et al. found that miR-26a levels in urinary exosomes were higher in patients with lupus nephritis than in healthy controls and that these levels correlated positively with urinary protein levels.⁴⁵ In a similar study, Solé et al. reported lower levels of miR-29c in lupus nephritis patients than in controls.⁴⁶ Moreover, those levels correlated with renal function and the degree of renal fibrosis.

C. Sjogren's Syndrome

In the context of Sjogren's syndrome, in one of the pioneering studies in the field, Kapsogeorgou et al. showed that EVs derived from cultured primary salivary gland epithelial cells contain the major autoantigens Ro/SSA, La/SSB, and Sm.²⁶ This finding might explain, at least in part, the mechanism by which intracellular autoantigens are presented to the immune system, leading to an immunogenic or tolerogenic outcome. More recently, Ro/SSA and La/SSB autoantigens were relocalized to apoptotic bodies during ER stress-induced apoptosis of salivary gland epithelial cells. These data further suggest that autoantigens present in EVs may be accessible to the immune system in patients with autoimmune diseases.⁴⁷

D. Systemic Sclerosis

The presence of EVs in systemic sclerosis (SSc) has been addressed by a few studies. Higher numbers of circulating EVs were found in patients with SSc, suggesting that the EV-mediated interaction between activated cell populations contributes to pathogenesis.^{48,49} In addition, high concentrations of circulating EVs can substantially modulate endothelial cell apoptosis, which has been suggested to be a primary pathogenic event in SSc.^{50,51} A more recent study showed that in systemic sclerosis, oxidized HMGB1 was associated with

platelet-derived EVs and increased the activation of neutrophils. These data implicate EVs in microvascular injury and inflammation. In fact, EVs from patients activated neutrophils *in vitro*, which could not be achieved by EVs from healthy individuals. Intriguingly, HMGB1 inhibitors reversed the effects of EVs, providing a potential therapeutic strategy for targeting this particular pathway.⁵²

E. Antiphospholipid Syndrome (APS)

The hallmark of this autoimmune disorder is the persistent presence of anti-phospholipid antibodies associated with thrombotic complications. EVs have been investigated in the context of APS due to their pro-coagulant function. Studies have shown elevated plasma levels of endothelial EVs in patients with APS,⁵³ and this finding is independent from previous history of thrombosis.⁵⁴ On the other hand, platelet EVs are higher in patients with a personal history of thrombosis.⁵⁴ Collectively, these data suggest that higher levels of endothelial EVs result from chronic activation of the endothelial cells by antiphospholipid antibodies.⁵⁵ Thus, platelet EVs could serve as a biomarker of the risk of thrombosis in patients with antiphospholipid antibodies.

VI. THERAPEUTIC POTENTIAL OF EVS

In addition to their promising use as biomarkers, the vast majority of the existing data indicates that EVs have a fundamental immunomodulatory potential for treating or inhibiting inflammatory diseases. EVs could also be used as drug delivery system; they are able to cross biological barriers, including the blood–brain barrier⁵⁶ and synovial membrane.⁵⁷ In the context of autoimmune diseases, therapeutic actions can be developed to reduce the load of circulating EVs using different strategies: (1) inhibition of EV generation and secretion, (2) blocking of EV-specific components using RNAi technology, and (3) inhibition of EV uptake by the target/recipient cells. Thus, EVs containing anti-inflammatory substances emerge as potential therapeutic agents.^{58,59} The first step in developing such agents is the genetic modification of APC, like DC, by the transfer of immune-related genes. The result of this modification is that the released EVs have the same suppressive properties as their parental cell. In a series of studies, it was shown that, in mice models of RA, the injection of EVs from IL-4, IL-10, FasL, and indoleamine 2,3-dioxygenase–modified DCs decreased the clinical manifestations of the disease.^{60–64} Similarly, mouse thymic EVs induce CD4+CD25+Foxp3+ regulatory T-cell differentiation in the periphery, which can be partly attributed to transforming growth factor (TGF)- β .⁶⁵

Moreover, exosomes from immature bone-marrow–derived DCs treated with IL-10 have robust immunosuppressive and anti-inflammatory properties, further highlighting their strong immunomodulatory activity.⁶⁶ Kim et al. showed that the systemic injection of IL-10–treated DC–derived exosomes suppressed the onset of collagen-induced arthritis and reduced the severity of established arthritis in mice. Thus, EVs are promising immunomodulatory constituents of future therapeutic strategies against autoimmune diseases.

VII. CONCLUSIONS AND FUTURE PERSPECTIVES

Extracellular vesicles have emerged as “nanoshuttles” that spread information among cells. Their cargo ranges from RNA and DNA to proteins and lipids, and this explains their wide range of pleiotropic effects, including initiation and perpetuation of the autoimmune response, and their participation in inflammatory and thrombotic phenomena and vascular dysfunction. In addition to their immunomodulatory functions, EVs can serve as reliable biomarkers for disease activity or even for disease onset. However, the variability of the data among similar studies represents the challenge of EV isolation and characterization through different biofluids and tissues and highlights the need for a consensus regarding those processes. Although the precise physiological and pathological functions of these EVs are not fully understood, a strong body of evidence indicates their pivotal roles in immune regulation, particularly in autoimmunity. Therefore, EVs have great potential as both therapeutic vehicles and as disease biomarkers.

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ABBREVIATIONS

APS	antiphospholipid syndrome
DC	dendritic cells
EV	extracellular vesicles
MV	microvesicles
RA	rheumatoid arthritis
SLE	systemic lupus erythematosus

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