Extracellular vesicles and systemic lupus erythromatosus

Rasha N. Omran[a], Emad M. El Shebini[b], Enas S. Zahran[b], Sabry A. Shoeib[b]

[a]Department of Internal Medicine, [b]Rheumatology and Immunology Unit, Internal Medicine Department, Faculty of Medicine, Menoufia University, Shebeen El-Kom, Egypt

Correspondence to Rasha N. Omran, BSc, Berkat Al Sabaar Hospital, Menoufia, Egypt. Tel: +20 102 250 4370; e-mail: dr_rno36@yahoo.com

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Introduction
Extracellular vesicles (EV) have emerged as important 'nanoshuttles' of information between cells, carrying proteins, genetic information, and bioactive lipids to modify the phenotype and function of recipient cells. EVs are potential regulators in autoimmune disorders, playing a determinant role in the appearance and maintenance of inflammation.
Objective
This study aimed to carry out an up-to-date review of the EVs and their relationship with systemic lupus erythromatosus.
Data sources
Medline databases (PubMed, Medscape, ScienceDirect, EMF-Portal) and all materials available in the Internet till 2018.
Study selection
This search yielded 275 articles. The articles were studied to perform an up-to-date review of the extracellular vesicles and their relationship with systemic lupus erythromatosus.
Data extraction
If the studies did not fulfill the inclusion criteria, they were excluded. Study quality assessment included whether ethical approval was obtained, the eligibility criteria specified, appropriate controls, and adequate information and defined assessment measures.
Data synthesis
Comparisons were made by a structured review, with the results tabulated.
Conclusion
We can safely conclude that EVs play an important role in the complex pathogenesis and management of systemic lupus erythematosus.

Keywords:
avascular necrosis, extracellular vesicles, systemic lupus erythromatosus

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Introduction

Extracellular vesicles (EV) are a heterogeneous group of membrane-limited vesicles loaded with various proteins, lipids, and nucleic acids. The release of EVs from its cell of origin occurs either through the outward budding of the plasma membrane or through the inward budding of the endosomal membrane, resulting in the formation of multivesicular bodies, which release vesicles upon fusion with the plasma membrane. The release of vesicles can facilitate intercellular communication by contact with or by internalization of contents, either by fusion with the plasma membrane or by endocytosis into 'recipient' cells. Although the interest in EV research is increasing, there are still no real standards in place to separate or classify the different types of vesicles [1].

EVs such as exosomes and microvesicles are phospholipid bilayer-enclosed vesicles that are recognized as novel tools for intercellular communications and as biomarkers for several diseases. They contain various DNAs, proteins, mRNAs, and microRNAs (miRNAs) that have potential diagnostic and therapeutic purposes [2].

Systemic lupus erythematosus (SLE) is a complex autoimmune disease associated with hormonal, environmental, and genetic factors and is linked to the tolerance breakdown of B and T cells to self-antigens. SLE is characterized by the presence in patient serum of autoantibodies raised against nuclear components. The association of these antibodies with self-antigens, complement factors, DNA, and particular proteins will lead to the formation of circulating immune complexes, which can deposit in several organs, causing tissue damage and clinical manifestations. Historically, SLE is considered an adaptive immune system disorder. Over the past decade, advances in the understanding

of SLE pathogenesis placed the innate immune system as a key player in perpetuating and amplifying this disease [3].

In rheumatic diseases, EVs have been isolated from synovial fluid and have been shown to play pathogenic roles contributing to the progression of rheumatic diseases. By contrast, EVs may have therapeutic effects through the delivery of molecules that may stop disease evolution. In particular, EV-derived from mesenchymal stem cells (MSCs) reproduce the main functions of the parental cells and therefore represent the ideal type of EV for modulating the course of either disease [4].

In SLE, EVs can work as autoadjuvants, enhance immune complex formation, and maintain an inflammation state. Over the last few years, EVs derived from MSCs and antigen-presenting cells (APCs) have emerged as cell-free therapeutic agents for the treatment of autoimmune and inflammatory diseases. In this review, we summarize the current therapeutic applications of EVs to regulate immune responses and to ameliorate disease activity in SLE and other autoimmune disorders [5].

The aim of this review was to discuss the function of EVs and their miRNAs in SLE, with a focus on the biological properties, biogenesis, pathophysiologic roles, and their potential use as biomarkers and therapies for SLE.

Aim

The aim of this study was to carry out an up-to-date review of the EV and their relationship with SLE.

Review of literature

Extracellular vesicles in systemic lupus erythromatosus

In all of medicine, there is no disorder with clinical manifestations as protean as those of lupus which can affect essentially any organ. In the pathogenesis of this condition, genetic and epigenetic factors interact with factors from the environment to induce immune cell dysfunction, including the production of type 1 interferons, and aberrant antibody responses that manifest mostly by the production of anti-nuclear antibodies, of which antibodies to DNA are most characteristic [6].

As anti-nuclear antibodies have features of antigen selection, a key issue in understanding lupus is related to the origin and properties of the nuclear molecules that drive autoantibody production; a related issue is the nuclear molecules that form immune complexes that deposit in the tissue or drive cytokine production. Recent studies decisively point to the role of EVs as both autoantigens and autoadjuvants that can play these critical roles [7].

Like many autoimmune disorders, SLE is a multifactorial disease in which genetic and environmental factors interact to modulate the final phenotype. Some loci have been associated with an increase in the risk of SLE (complement components C1q and C4), whereas others are generally related to several autoimmune diseases, such as diabetes or rheumatoid arthritis (e.g. PTPN22 and STAT4) [8].

Moreover, an epigenetic dysregulation, found in many SLE patients, has been proposed to be crucial in the initiation and progression of the disease. Thus, several studies on DNA methylation, histone acetylation, and miRNAs have reported epigenetic cross talk. Furthermore, environmental factors (Epstein–Bar virus and pesticides) and hormones may trigger autoimmune responses and modulate the alternating periods of SLE flares [9].

One of the most affected organs in SLE is the kidney. The deposition of immune complexes, activation of complements and macrophages, and production of proinflammatory cytokines and chemokines lead to lupus nephritis (LN). LN is estimated to affect almost two-thirds of SLE patients during their lifetime, of whom up to 30% progress to end-stage renal disease [10]. In particular, the clinical manifestations of active LN include proteinuria, active urinary sediments, and progressive renal dysfunction. Currently, the invasive procedure of renal biopsy provides a direct visualization of renal damage. A recent work, however, showed no correlation between clinical and histological remission, which discards this procedure as a prognostic tool [11].

Despite being well established and easy to measure, complements C3 and C4, proteinuria, anti-double stranded DNA, or creatinine clearance are not as specific or as sensitive as desired. Currently, the SLE Disease Activity Index is the most commonly used indicator. It consists of a list of 24 items, of which 16 are clinical variables and eight are laboratory tests such as urinalysis, blood complement levels, increased antibodies to DNA antibody levels, and low platelet and white blood cell counts. A final score of 6 or higher seems to be consistent with an active disease state [9].

Also, despite the continuous improvements in the diagnosis and prevention of SLE flares, laboratory markers are still unsatisfactory. Over the last few years, the EV, which carry nucleic acids, proteins, and lipids, have been described as essential players in several cellular processes [12].

The study of EVs in the plasma of SLE patients has outlined novel subpopulations of platelet, endothelial, and leukocyte-derived vesicles, some of which have clinical and serological correlations. Cytometry studies carried out by Nielsen *et al.* [13] showed correlations between a population of EVs of endothelial origin (AnxV-CDMPs) and disease activity measures, glomerulonephritis, and vascular dysfunction. Thus, the phenotype of endothelial molecular protein (MP) may have strong potential as a specific biomarker of vascular pathology associated with SLE. Confirming this hypothesis, Parker *et al.* [14] have shown an increase in endothelial EV with active SLE compared with the controls. Immunosuppressive therapy reduced the cardiovascular risk by reducing the number of circulating endothelial EVs [9].

Moreover, the protein signature of these EV reveals specific patterns that could be used as biomarkers of the activity and progression of SLE. Østergaard *et al.* [15] have shown a special spectrum of EV in SLE patients, with a particularly unbalanced and decreased microtubule and cytoskeletal composition, which differs from healthy individuals or even rheumatoid arthritis patients. Therefore, the amounts and characteristics of circulating EVs provide new targets for assessing SLE pathogenicity and treatments.

Nevertheless, LN is still a major cause of the morbidity and mortality of SLE, with 10–30% of all cases progressing to end-stage renal disease. The investigation of new biomarkers to assess glomerular damage without invasive biopsy has become essential to monitor disease progression. In that sense, urine is the ideal biological fluid for new biomarkers because of its uncomplicated and noninvasive way of collection. Since the identification and characterization of urinary exosomes by Zhou *et al.* [16], many studies focusing on urinary exosomes as a source of biomarkers in renal, systemic, and urogenital diseases have been carried out [17,18].

Recently, the use of novel immunotherapies in SLE has been based on targeting the biological pathways involved in oncology, transplantation, and other autoimmune diseases such as RA. Therefore, targeted immunotherapy includes different approaches such as B-cell depletion/survival (rituximab, bortezomib), anti-cytokine therapies (tocilizumab, secukinumab), JAK kinase inhibitors (tofacitinib), and immune-modulating peptides (forigerimod) [19].

Although survival rates and longevity have increased, current therapeutic molecules lead to adverse side effects and are partially effective only with some patient subgroups, such as low interferon signatures or active SLE without nephritis [20]. In addition, these new drugs will have a high impact on the long-term medical costs associated with the disease [21].

Despite the improvement in the understanding of SLE pathogenesis and the presence of more specific therapies, these are still unsatisfactory. Over the last few years, the EV have been described as biological essential players in several cellular processes and carriers of nucleic acids, proteins, and lipids [22].

Extracellular vesicles as an immune-modulator

Most autoimmune disorders are characterized by a chronic inflammatory state; thus, the reduction of inflammation becomes essential to treat the patient's condition. EVs may be involved in the development and maintenance of exacerbated immune responses by working as autoadjuvants, initiating and promoting autoantibody production [5]. EVs can also transport and transfer a broad range of cytokines and chemokines, inducing and maintaining inflammation. Thus, Lee *et al.* [23] have reported that serum exosomes isolated from SLE patients could induce high cytokine production in healthy peripheral blood mononuclear cells. Interestingly, this proinflammatory response disappeared when exosome preparations were disrupted mechanically.

Recently, some studies have shown the relationship between pathogen and damage-associated molecular patterns and EV transport. These molecules are normally found inside cells, but may trigger abnormal immune responses after cellular stress, infection, or injury conditions. Therefore, EVs are likely to participate actively in the persistence of inflammation by promoting the activation of lymphocytes and the release of proinflammatory cytokines. Thus, EVs are released after injury or stress and can carry nuclear proteins such as HMGB1 or S100 proteins (group of ligands of toll-like receptors), leading to proinflammatory cytokine release. At the same time, EVs can also act as pathogen associated molecular patterns themselves after pathogen infection, being recognized by APCs [5].

However, under natural circumstances, there is also a release of immunosuppressive EVs, which are also involved in the maintenance of immunological tolerance. For instance, trophoblast-derived EVs have been shown to ameliorate severity in multiple sclerosis and RA patients. In addition, exposure to specific antigens has stimulated EV production with anti-allergic properties in bronchoalveolar lavage fluid. Interestingly, Ostman and Heldin [24] showed how intestinal epithelial cells could secrete exosome-like structures named 'tolerosomes', and expressed major histocompatibility complex proteins, able to induce food antigen tolerance in the gastrointestinal tract [25].

Extracellular vesicles as biomarkers of systemic lupus erythromatosus

EVs have been reported as reliable biomarkers of activity disease besides their role in regulating immune responses, offering a valuable complement to classical laboratory markers. In this respect, circulating MP of SLE patients have been associated with clinical features, suggesting an important role in driving the activation of dendritic cells (DCs) and pathological responses. Thus, novel subpopulations of platelet, endothelial, and leukocyte-derived MP have shown a specific MP signature that could become a diagnostic and prognostic tool. Studies done by, Nielsen *et al.* [13] showed a correlation between a subset of MP of endothelial origin with disease activity, glomerulonephritis, and vascular dysfunction.

Similarly, another study has shown an increase endothelial MP with active SLE compared with controls, and immunosuppressive therapy reduced the cardiovascular risk by decreasing the number of circulating endothelial MP [14]. Also, another study has shown an increase in plasma levels of monocytic CD14+ MP in active patients and a strong correlation with SLE disease activity [26]. Interestingly, circulating apoptotic MP in plasma exerted high proinflammatory effects by stimulating cytokine release [lnterleukin 6 (IL-6), tumor necrosis factor (TNF), and INF-α] in some subsets of DCs. Alternatively, MP from healthy individuals or patients with other autoimmune diseases did not show the same response [27].

However, EVs transport miRNAs that can be used as cell-free biomarkers. In this sense, exosomes in urine constitute an interesting approach to study renal and urogenital diseases because of their easy access and easy collection [17,18].

Furthermore, characterization of exosomal miRNAs compared with intracellular miRNAs by next-generation sequencing has confirmed urinary exosomes as a stable source of miRNA biomarkers [28]. In sense, some studies have reported changes in the amount of exosomal miRNAs in urine from LN patients. Concretely, miR-26a was found to be augmented and miR-29c reduced compared with healthy controls. Moreover, those levels showed a correlation with urinary protein levels and renal fibrosis, suggesting a predictive role of podocyte injury and renal function. Perez-Hernandez *et al.* [9] found much higher levels of miR-146a within urinary exosomes of LN patients compared with controls or SLE without renal affection.

Finally, protein levels of the adhesion molecule ADAM10 were found to be higher in the exosomes of patients with glomerular disease, including LN [29]. An important substrate of this protein is the Notch receptor, which is not only involved in podocyte development but also plays a role in glomerular disease [30]. Moreover, transcription factors related to early podocyte injury were found in urinary EVs, but not in the whole urine of acute and chronic renal patients [16].

Thus, the analysis of urinary exosomes could be considered a reliable, noninvasive approach to the physiological state, offering complementary information to the invasive kidney biopsies. Exosomes are likely to replace biopsies in the future [9]. In summary, the amount and phenotype of circulating EVs may be used as new biomarkers of the activity and progression of SLE and could provide a new therapeutic approach [5].

Extracellular vesicles as a therapeutic approach

Leaving aside their promising future as biomarkers, EVs and their cargo could be used for therapeutic purposes in a broad range of procedures [9].

Because of the fundamental role of EVs in regulating biological processes and promoting inflammation and tumor growth under pathophysiological conditions, therapeutic actions are being developed to reduce the load of circulating EVs using different strategies: by inhibiting EV formation and release, by blocking EV-specific components with small interfering RNA, and by inhibiting EV uptake [31]. Although reducing the amount of apoptotic bodies or MPs is especially attractive for autoimmune disorders with a high inflammatory component such as SLE, interfering with the general aspects of biogenesis could lead to

undesirable off-target effects. Therefore, such actions would require a targeting system capable of selecting EV cell-specific populations [32].

There has also been a increasing number of studies investigating the role of EVs in the modulation of the immune system. Thus, EVs containing anti-inflammatory substances could be used as therapeutic agents to promote immunosuppressive responses. Some studies have shown these types of compounds to have a longer half-life when encapsulated in EVs, increasing the survival of mice after LPS-induced septic shock. Therefore, these vesicles could work as immunomodulatory agents for autoimmune, inflammatory, and hypersensitivity disorders [33].

Several studies have been carried out to examine the immunomodulatory action of DC-derived exosomes. For instance, bone marrow (BM)-derived DCs were treated with IL-10, and the reduction of autoimmunity was evaluated in some murine models of disease, such as collagen-induced arthritis and delayed hypersensitivity. Further work, however, will be required before the production of well-defined EV therapeutic agents that are safe in the long run [34,35].

In terms of drug delivery, patient-derived EVs could be used to package molecules capable of avoiding immune responses. Exosomes are ideal for transfer purposes and may become a useful delivery tool for pharmacological agents. Because of their bilipidic structure, they are flexible vectors with the ability to carry select nucleic acids (miRNA, siRNA, and mRNA), proteins, and active chemical drugs across biological barriers [33]. Altogether, this information underscores the broad potential of EV for the treatment and prevention of flares in autoimmune disorders such as SLE. Nonetheless, further investigation is needed to determine the exact effect of EV treatment with immunomodulatory purposes [5].

New insights and future directions

Novel therapeutic options

Mesenchymal stem cell-derived extracellular vesicles

MSCs are a population of adult multipotent cells that have the ability to differentiate into mesodermal tissues. MSCs were originally described in the BM-MSCs, but can be isolated from many sources such as adipose tissue, dental pulp, umbilical cord blood, and placenta [36]. Because of their high proliferative potential, easy access, and immunosuppressive properties, MSCs have been proposed for the treatment of diverse pathological conditions [37].

In the past few years, MSC potency in tissue reparation has been related to the secretion of bioactive components rather than cell differentiation and engraftment [38]. Besides the role of classical soluble factors in the paracrine action such as cytokines and growing factors, EVs derived from MSCs (MSC-EVs) have emerged as major components of the MSC secretome.

Several preclinical studies have explored the MSC-EVs potential in regeneration using both in-vivo and in-vitro models. These include a broad range of diseases including myocardial infarction or ischemia, acute kidney injury, fibrotic liver, and neurodegenerative diseases. In the context of chronic inflammation and autoimmune disorders, MSC-EVs are immunosuppressive probably because of RNA and protein transfer. Therefore, they may act as immunological active agents by inducing anti-inflammatory cytokine release and also modulating Toll-like receptor signaling [5].

In terms of autoimmunity and SLE, many studies have assessed MSCs transplantation in murine models, but very few attempted to use direct 'conditioned medium' (based on cell-free MSCs secretome) or directly purified MSC-EVs [39]. In a model of multiple sclerosis, MSC-microvesicles were responsible for inhibiting auto-reactive lymphocyte proliferation and also promoting the secretion of anti-inflammatory cytokines, IL-10, and tumor growth factor (TGF-β) [40]. Similarly, Liu *et al.* [41] team found that MSC-exosomes after cell transplantation were key to rescue BM-MSC function in a lupus murine model. Thus, the Fas receptor was transferred by exosomes, which helped recipient cells to reduce intracellular miR-29b levels and ameliorated osteopenia. Another treatment attempt showed the clinical efficacy of MSC-exosomes in a patient with therapy-refractory graft-versus-host disease. After 4 months of MSC-exosome therapy, cutaneous and mucosal manifestations still ameliorated and steroid administration was reduced [42].

Extracellular vesicles derived from professional antigen parent cells

In the last few years, the ability of EV-derived from professional APCs to regulate immune responses has been studied. To date, the best examples have focused on dendritic cells-derived EVs (DC-EVs), in which DCs can be cultured under specific conditions to alter the EV population released. Therefore, DCs treated

in-vitro with immunosuppressive drugs or cytokines enable them able to suppress immune reactions [5].

For instance, BM-derived DCs that were treated with IL-10 and IL-4 could reduce inflammation in collagen-induced arthritis by exosome signaling. Thus, exosomes were shown to be as therapeutic as the parental DCs, which may show that DC-EVs could be a good approach to treat arthritis and other autoimmune disorders [35].

Furthermore, modification of APC can also be engineered genetically to improve immune-regulatory EV production for therapeutics. In this sense, gene transfer of cytokines such as TGF-β, IL-4, or IL-10 leads to more powerful anti-inflammatory DC-EVs. Similarly, genetic modification to express specific antigens, which are not naturally present on EVs, can enhance immunogenicity and may constitute a new type of cell-free system for vaccination [43].

Do extracellular vesicles function as an organelle (a communicasome)?

Recent progress in the field of EVs supports the concept that EV-mediated communications are evolutionarily conserved phenomena. For intercellular communication, almost all mammalian cells in the body, as well as bacteria living in or on it, shed EVs containing cell-type specific proteins, genetic materials, lipids, and metabolites. Notably, these vesicular cargos are linked to their pathophysiological functions and have been proven to be a rich source of biomarkers in various human diseases such as cancer [44].

For example, mammalian EVs are mainly derived from plasma membranes: their lipid bilayers are composed of phospholipids, especially enriched with sphingolipids and cholesterol, and also harbor plasma membrane proteins such as tetraspanins, receptors, and integrins, and cell adhesion molecules that may be involved in their tropisms by targeting specific cells or tissues [45]. Lumina of mammalian EVs also harbor various forms of bioactive materials such as cytosolic proteins including metabolic enzymes, mRNAs, miRNAs, other RNAs, and numerous metabolites [46].

Accumulating evidence suggests that EVs are critically involved in the body's homeostatic regulation under normal physiological conditions, and also have diverse pathological functions by facilitating angiogenesis, immune suppression, or inflammation. Thus, together with the complexity of vesicular cargos, EVs are intercellular communicasomes, that is, complex extracellular organelles that play diverse pathophysiological roles in intercellular and interkingdom communication [44].

Future research

EVs are key modulators of immune responses and have a vast potential as therapeutic agents for treating a variety of human disorders, including autoimmune diseases. Given their ability to modulate immune responses, EVs from MSC and APC represent a promising therapeutic approach in immune therapy. EVs can be generated safely and used as a tool to package small RNAs and other pharmacological and bioactive molecules to avoid immune response and enhance the therapeutic activity. In this respect, Alvarez-Erviti *et al.* [47] used self-derived DCs to produce targeted exosomes (neuron-specific peptide), which were loaded with siRNA and injected into mice, where they delivered their content into the brain. Similarly, patient-derived APCs could be modified in-vitro to improve the immune regulation by releasing EVs with high levels of co-stimulatory ligands [5]. However, there are some issues that have to be more tests before an extensive EV implementation in the clinics. First of all, targeting EVs for a specific uptake has not been evaluated systematically. For instance, mature DC-EVs are efficiently internalized by activated T cells, whereas infected B cells release EVs that bind more specifically to other B cells. Similarly, EV uptake might not be substitute of functional delivery into the recipient cells (e.g. endosomal membrane breakdown); thus, quantitative analysis of EV delivery will be required for different cell types [48].

In addition, an EV-based treatment or drug is considered to be a 'biological medicinal product'; thus, we have to differentiate between the 'active substances' and 'excipients' to establish a 'mode or mechanism of action'. In this sense, evaluation of EV-loaded products and potency is crucial and may be challenging. Determination of total protein, lipids, or RNA content as well as vesicle dosage will require the development of new methods and quality control of vesicle storage and stability [49].

Conclusion

We can safely conclude that EVs play an important role in the complex pathogenesis and management of SLE.

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Conflicts of interest

There are no conflicts of interest.

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