REVIEW

Extracellular vesicle– and particle-mediated communication shapes innate and adaptive immune responses

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Intercellular communication among immune cells is vital for the coordination of proper immune responses. Extracellular vesicles and particles (EVPs) act as messengers in intercellular communication, with important consequences for target cell and organ physiology in both health and disease. Under normal physiological conditions, immune cell–derived EVPs participate in immune responses by regulating innate and adaptive immune responses. EVPs play a major role in antigen presentation and immune activation. On the other hand, immune cell–derived EVPs exert immunosuppressive and regulatory effects. Consequently, EVPs may contribute to pathological conditions, such as autoimmune and inflammatory diseases, graft rejection, and cancer progression and metastasis. Here, we provide an overview of the role of EVPs in immune homeostasis and pathophysiology, with a particular focus on their contribution to innate and adaptive immunity and their potential use for immunotherapies.

Introduction

Intercellular communication among immune cells is vital for the coordination of proper immune responses. The ability of immune cells to respond to and integrate signals from other immune and nonimmune cells allows them to perform diverse tasks that orchestrate complex processes from embryogenesis to defense against pathogens and cancers in adults. Classically, soluble factors, such as cytokines and chemokines, have been considered the main mediators of cell–cell communication in the immune system (Janeway et al., 1985; Xie et al., 2013). However, target cell behavior is also modulated through paracrine receptor–ligand interactions (van Niel et al., 2018) and extracellular vesicle (EV)–mediated transfer of molecules to recipient cells (reviewed in Cocucci et al., 2009; Ratajczak et al., 2006b). EVs are classified based on their biogenesis, heterogeneous size, and function. Microvesicles (MVs; 150–1,000 nm) are generated by direct budding from the cell membrane and function mainly in local intercellular communication (Cocucci et al., 2009; Zijlstra and Di Vizio, 2018). In contrast, exosomes (30–150 nm) are derived from the perinuclear luminal membrane of late endosomes/multivesicular bodies and released via multivesicular

body fusion with the cell membrane. Due to their small size, protective lipid bilayer, and surface receptors, exosomes can mediate long-range, interorgan systemic crosstalk (Hoshino et al., 2015; van Niel et al., 2018; Mathieu et al., 2019). Recent technological advances enabled further dissection of exosome heterogeneity, leading to the identification of three distinct subclasses of vesicles and particles: exo-large vesicles (90–120 nm), exo-small vesicles (60–80 nm), and membrane-less exomere particles (<50 nm; Zhang et al., 2018; Zhang and Lyden, 2019; Jeppesen et al., 2019). In this review, we collectively refer to MVs, exosomes, exo-large, exo-small vesicles, and exomere particles as EVs and particles (EVPs; Hoshino et al., 2020).

A decade after the discovery of reticulocyte exosomes (Harding et al., 1983; Pan et al., 1985), it was shown that adaptive immune cells, such as B lymphocytes, also secreted biologically active EVPs (Raposo et al., 1996) that perform a variety of extracellular functions (Fig. 1). Although early studies focused on immune cell–derived EVs and exosomes, critical evidence regarding the role of EVPs in systemic communication came from observations in systemic diseases, including cancer. The

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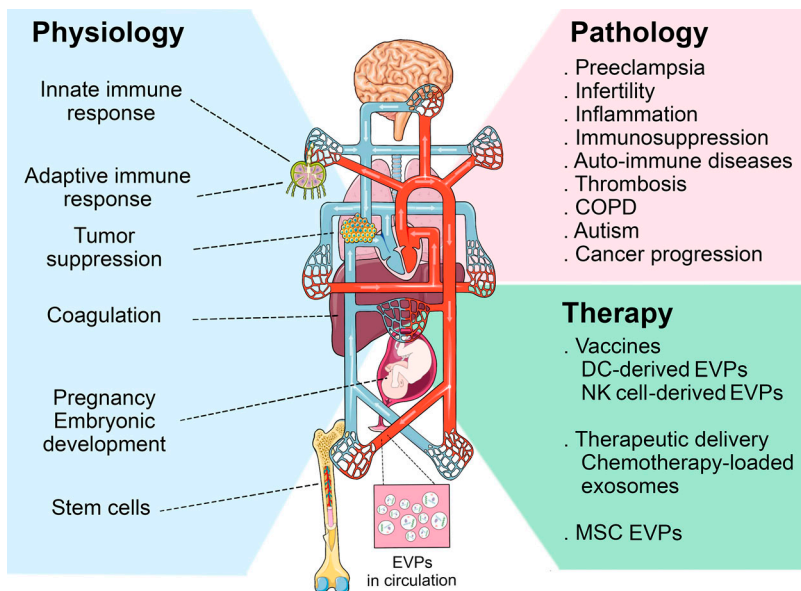
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Figure 1. **Pleiotropic roles of immune cell–derived EVPs in physiology and pathology.** EVPs are secreted by all cells, including immune cells, circulate in the blood, and are critical in short- and long-range intercellular communication. EVPs exert a variety of functions depending on the physiological context and the state of the secreting cells. Under normal physiological conditions, immune cell–derived EVPs participate in immune homeostasis by regulating innate and adaptive immune responses, maintaining stem cell homeostasis, promoting tumor suppression, and controlling coagulation. It is likely that immune cell–derived EVPs maintain the precise immune equilibrium to maintain healthy pregnancy and embryonic development. On the other hand, immune cell–derived EVPs play important roles in pathological conditions such as thrombosis, autoimmune diseases, and cancer progression and metastasis. Thus, understanding the functions of EVPs in physiological and pathological states could lead to the development of EVP-based biomarkers and therapies for a variety of diseases. COPD, chronic obstructive pulmonary disease; MSC, mesenchymal stem cell.

contribution of EVP cargo to cancer progression, including pre-metastatic niche establishment and organotropic metastasis, is described elsewhere (Wortzel et al., 2019; Sheehan and D'Souza-Schorey, 2019; Peinado et al., 2017). The critical breakthrough in understanding the role of EVPs in disease progression was the finding that they contain functional proteins, lipids, and metabolites (Kalluri and LeBleu, 2020; Zhang et al., 2019), as well as DNA (Thakur et al., 2014), mRNA, and various noncoding RNAs (e.g., microRNA [miRNA]; Jeppesen et al., 2019; Ratajczak et al., 2006a), which reflect their potential as vehicles for horizontal transfer of information (Fig. 2) and, as a consequence, to alter the function of target cells (Sullivan et al., 2017). Recent years have marked renewed interest in the role of immune cell–derived EVPs in health and disease (reviewed in Veerman et al., 2019; Robbins and Morelli, 2014). Understanding how immune cell–derived EVPs promote or inhibit immune responses is crucial to unlock their therapeutic potential. This review focuses on EVP-mediated immune homeostasis in normal physiology and its dysregulation in disease.

EVP-mediated immune regulation in normal physiology
Adult HSC–derived EVPs
Despite the lack of understanding regarding EVP contributions to hematopoiesis and immune crosstalk during embryonic development, accumulating evidence supports important roles for EVPs throughout hematopoiesis in the adult bone marrow (BM; Gu et al., 2016; Kumar et al., 2018). The complex BM microenvironment dynamically regulates hematopoiesis to ensure adequate formation of mature blood cells from hematopoietic stem cells (HSCs), controlling their egress in response to various stressors and stimuli (Morrison and Scadden, 2014). Interestingly, CD34+ HSC-derived EVPs promote ischemic tissue repair and angiogenesis (Mathiyalagan et al., 2017). Conversely, EVPs released by peripheral blood mononuclear cells may be involved in HSC mobilization through modulation of vascular cell adhesion molecule 1 expression, which is critical for the retention of HSCs in the BM (Lévesque et al., 2001). Specifically, G-CSF,

commonly used to mobilize BM HSCs into the peripheral blood, promoted the accumulation of miR126-containing EVPs in the BM extracellular compartment, which, upon uptake by HSCs and stromal and endothelial cells, reduced surface vascular cell adhesion molecule 1 expression, leading to HSC egress (Salvucci et al., 2012). Due to challenges posed by obtaining large enough HSC numbers and maintaining their stemness ex vivo, most of our knowledge regarding HSC EVPs is derived from studies of leukemia stem/initiating cells (reviewed in Butler et al., 2018).

EVPs in the circulation: Complement activation and coagulation
The complement cascade is a critical innate immune component that enhances the ability of antibodies and phagocytic cells to clear microbes and damaged cells. Not only does the cargo of EVPs isolated from plasma include complement components (Hoshino et al., 2020), but EVPs also crosstalk with the complement system (Karasu et al., 2018). Complement may be directly activated by EVP cargo, such as C3 and C4a, in human primary dendritic cells (DCs; Kowal et al., 2016), or by binding immunoglobulins (Huang et al., 2018; Huang et al., 2020), while expression of complement regulators CD55 and CD59 on APC-derived EVPs allows them to escape from complement-mediated lysis (Clayton et al., 2003). However, the precise mechanistic interactions between complement and EVPs and the specific functions of EVP complement cargo remain to be determined.

In the circulation, over 50% of EVPs originate from platelets or their BM precursors, megakaryocytes (MKs; Berckmans et al., 2019). In response to physiological signals, such as chemokines, apoptosis, or increased sheer stress (Shai et al., 2012), activated platelets release EVPs from multivesicular bodies and α-granules. Platelet- and MK-derived EVPs are distinguished by surface expression of typical activation markers, such as P-selectin (CD62P; Heijnen et al., 1999; Guo et al., 2017). Both MK- and platelet-derived EVPs contain prothrombotic molecules, including tissue factor and phosphatidylserine, that trigger coagulation and thrombosis, supplementing tissue factor release
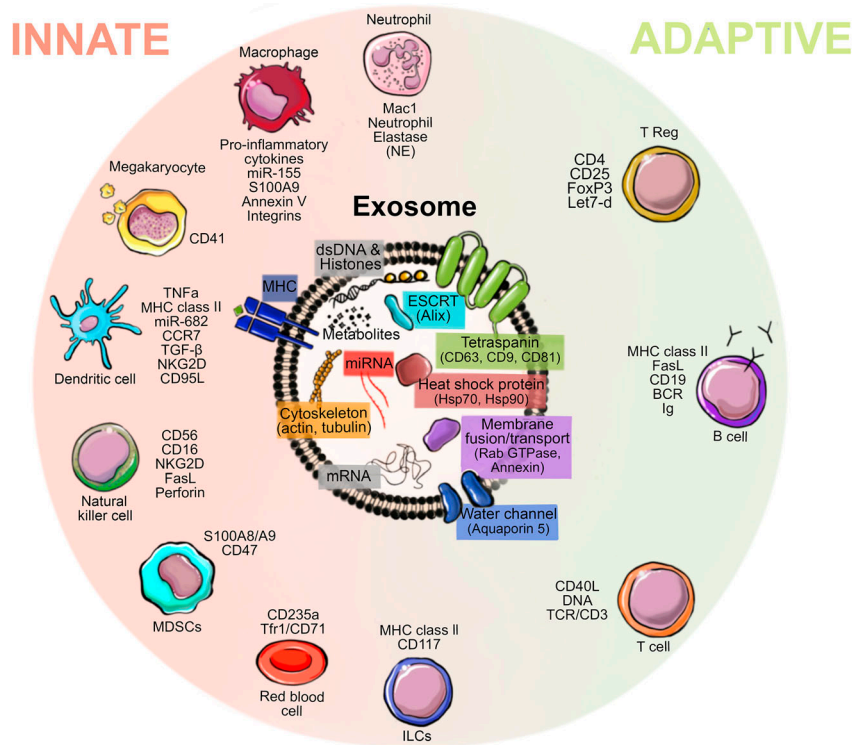
from perivascular tissue following endothelial disruption (Flaumenhaft et al., 2009; Müller et al., 2003). In addition, platelet-derived EVPs express several membrane glycoproteins that interact with target cells, such as monocytes, neutrophils, endothelial cells, and cancer cells, during various pathological processes (Perez-Pujol et al., 2007; Wu et al., 2020). Indeed, we found that proteins involved in thrombosis, such as factors II, III, and IX and thrombospondin 2, are highly packaged in EVPs isolated from cancer patient tumors (Hoshino et al., 2020).

Thrombus formation and the resolution of thrombosis by platelets are also modulated by interactions with other components of the immune system (Branchford and Carpenter, 2018) and potentially by EVPs. Platelets can also modulate inflammation and adaptive immunity through membrane-derived vesicles. CD154 (CD40 ligand), a costimulatory ligand present on activated T cells, is also present on platelet-derived EVPs and is sufficient to stimulate antigen-specific IgG production and modulate germinal center formation (Sprague et al., 2008). These studies reveal the critical roles of EVP-mediated platelet interactions with other immune system components, thereby supporting the development of novel therapeutic strategies to treat coagulopathies by modulating this crosstalk.

EVPs as natural tumor suppressors

In addition to systemic immune responses, the local microenvironment also constrains malignant progression (Bissell and Hines, 2011). While it is well established that tumor-derived EVPs suppress anticancer immunity and stimulate protumorigenic processes, there is growing interest in understanding the mechanisms through which normal EVPs, be they stromal or immune system derived, may impart protection against tumor

progression. Normal EVPs may exert a direct tumor-suppressive role by altering the behavior of tumor cells. Conversely, both tumor-derived and immune cell–derived EVPs can exert a systemic tumor-suppressive role via activation of the immune system. For example, Hsp70 in EVPs isolated from Hsp70-overexpressing melanoma cells activated NK cells, decreasing primary tumor size and metastatic outcome in a murine melanoma model (Elsner et al., 2007). Moreover, EVPs isolated from a low metastatic strain of mouse melanoma stimulated $Ly6C^{low}$ patrolling monocytes, inhibiting lung metastasis (Plebanek et al., 2017). Conversely, in vivo studies showed that upon exposure to tumor antigens, activated DC-derived EVPs induce T cell–mediated antitumor immune responses (Zitvogel et al., 1998), while ADAM15-containing macrophage-derived EVPs successfully suppressed ovarian cancer cell growth and migration (Lee et al., 2012). Recent studies also showed an exercise-induced increase in EVP secretion in healthy subjects, which may in turn promote beneficial systemic effects through inter-organ communication and immune system activation (Whitham et al., 2018; Frühbeis et al., 2015), potentiating antitumor responses. Therefore, immune cell–derived EVPs represent an attractive tool for maintaining tissue homeostasis and suppressing cancer progression.

Immune cell–derived EVPs and immune responses

Given the numerous types of immune cells releasing EVPs and the various recipient cell types, the physiological role of the EVPs within the immune system is complex. EVPs contain molecules critical for the initiation of immune responses and antigen presentation, such as HLA molecules, and can mediate the exchange of both membrane and cytosolic components without cells actually being in proximity (Lamparski et al., 2002;

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Lynch et al., 2009; Kowal and Tkach, 2019; Buschow et al., 2009). Besides MHC-encoded proteins, other proteins, both cytosolic and membranar, may be selectively packaged in EVPs, resulting in targeting to specific recipient cells and immune activation. The Amigorena group was the first to show, in the late 1990s, that DC-derived EVPs elicited antitumor effects in vivo, delaying tumor growth in a T cell–dependent manner, most likely through EVP heat shock proteins and integrins (Théry et al., 1999; Zitvogel et al., 1998). Additional studies published over the last two decades have revealed a plethora of other physiological and pathological functions of EVPs in both adaptive and innate immunity (Fig. 1; Denzer et al., 2000a; Pitt et al., 2016; Kowal and Tkach, 2019; Li et al., 2019; Robbins and Morelli, 2014).

The immunomodulatory properties of APC-derived EVPs

Professional APCs initiate the multistep adaptive immune response. DCs are the most potent APC, efficiently triggering the proliferation, activation, and differentiation of naive T cells (Steinman and Witmer, 1978; Banchereau et al., 2000). The immune synapse, a specialized contact between T cells and APCs, concentrates the structures and mechanisms for communication between immune cells through contact-dependent antigen presentation (Dustin et al., 1998; Monks et al., 1998). Importantly, EVPs carry a combination of ligands and receptors that simultaneously interact with cell-surface molecules on recipient cells, allowing for typical contact-dependent cellular activation (Robbins and Morelli, 2014). DC-derived EVPs were among the first EVs described by Zitvogel et al. and were subsequently most extensively studied in the context of immune responses (Zitvogel et al., 1998; Théry et al., 1999; André et al., 2004). The observation that a DC–EVP exchange enhanced T cell stimulation efficiency by DCs (Bedford et al., 1999; Knight et al., 1998; Vallhov et al., 2015) illustrated the critical role of EVPs in coordinating immune function. Importantly, the mechanisms of TCR sorting and release into EVPs present at the immune synapse depend upon the process of endosomal sorting and the complexes required for transport (Choudhuri et al., 2014). Consequently, the accumulation of TCRs on the surface of EVPs, along with associated downstream signaling molecules (e.g., RAS), mediates antigen-dependent interactions with cognate MHC complexes on APCs (Choudhuri et al., 2014). Alternative sorting of MHC, along with CD9, into multivesicular body luminal vesicles that are secreted as EVPs by APCs results in transfer of these molecules to interacting T cells (Buschow et al., 2009). DC-derived EVPs can activate T cells through stable interactions with TCR complexes even in the absence of intact APCs (Théry et al., 2002; Sprent, 2005). APC-derived EVPs maintain the original topology of the APCs and therefore expose the extracellular domain of MHC molecules on the surface of the vesicle. Théry et al. proposed that EVPs derived from peripheral DCs sensitize immature lymph node–resident DCs with specific peptide–MHC complexes before their arrival at lymph nodes (reviewed in Théry et al., 2002). The EVP-mediated spreading of antigen-specific MHC complexes between DCs ultimately amplified T cell activation and induced potent immune responses. EVPs derived from other APCs, such as macrophages, which also contain MHC class II heterodimers bound to antigenic peptides, perform similar functions. This in turn elicits the activation of primed CD4^{+} and CD8^{+} T cells through specific TCR–peptide interactions (Patel et al., 1999; Arnold and Mannie, 1999; Lindenbergh and Stoorvogel, 2018). Furthermore, EVP-mediated antigen presentation includes the transfer of specific peptide–MHC complexes to follicular DCs and stromal cells in lymph nodes, priming CD4 T cells and activating B cells (Mallegol et al., 2007; Denzer et al., 2000b).

On the other hand, several studies have focused on the ability of DC-derived EVPs and MVs to elicit both cognate T cell activation and humoral responses through MHC class I and II molecules (Pitt et al., 2016; Kowal and Tkach, 2019). Importantly, the extent of EVP-mediated T cell activation depends on DC developmental stage, with mature DCs activating T cells more efficiently than immature DCs, both in vitro and in vivo (Admyre et al., 2006; Segura et al., 2007; Montecalvo et al., 2008).

Collectively, these studies emphasize that whether their effects are direct or indirect, APC-derived EVPs play a major role in antigen presentation and immune activation.

Adaptive immune cell–derived EVPs orchestrate immune responses

T cell–derived EVPs. Crosstalk mediated by T cell–derived EVPs can also activate various innate immune cells. T cell–derived EVPs containing LFA-1 molecules stimulated human mast cells, inducing degranulation and release of IL-8 and oncostatin M in a MAPK signaling–dependent manner, suggesting that these EVPs carried mast cell–activating factors similar to the cells from which they originated (Shefler et al., 2010). In contrast, uptake of T cell–derived EVPs by DCs modulated their function, leading to down-regulation of peptide–MHC I complex expression and induction of DCs apoptosis via Fas/Fas ligand (FasL) pathway (Xie et al., 2010).

Despite several studies investigating the effect of T cell–derived EVPs on immune responses, little is known about the underlying molecular mechanisms. Human resting T cell–derived EVP cargo included proteins related to cytoskeletal organization, while activated T cell–derived EVPs packaged proteins involved in immune receptor signaling and metabolism, including Ras-associated proteins, such as ZAP70, RASGRP1, and AKT. Activated T cell–derived EVPs enriched in RAS signaling pathway molecules activated MAPK/ERK in recipient nonactivated T cells and mast cells (Azoulay-Alfaguter and Mor, 2018). The paucity of studies on how T and B cell–derived EVPs exert their function highlights the importance of elucidating these mechanisms, allowing their modulation, which can in turn promote or dampen immune responses for therapeutic benefit.

B cell–derived EVPs. B cell–derived EVPs have been greatly understudied. EVPs from activated B cells efficiently stimulated primed antigen-specific T cells, suggesting a role for B cell–derived EVPs as modulators of an ongoing immune response (Muntasell et al., 2007; Raposo et al., 1996). Recently, B cell–derived EVPs were shown to mediate cytotoxic T cell immunity in vivo, as MHC class I molecules expressed on B cell–derived EVPs cooperated with host MHC class I expressed on splenic

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langerin+ CD8α+ DCs in antigen presentation to CD8+ T cells (Saunderson and McLellan, 2017). B cell receptor stimulation of follicular B cell lymphomas polarized the CD63+ MHC class II compartment and EVP release and induced the routing of antigen-bound IgG to EVPs (Rialland et al., 2006). Interestingly, the presence of B cell–derived EVPs in plasma of patients with solid tumors correlated with B cell responses and responsiveness to immunotherapies (Helmink et al., 2020). We have recently demonstrated that immunoglobulins are the most abundant family of proteins packaged in EVPs isolated from the plasma of cancer patients as well as healthy subjects and that specific immunoglobulins can distinguish between healthy controls and cancer patients (Hoshino et al., 2020). Therefore, EVPs derived from adaptive immune cells can mediate the transport of immunomodulatory components among various cell types (Fig. 2), supporting novel mechanisms to enhance immune responses and may be used as disease-specific biomarkers.

The diverse cargo of immune cell–derived EVPs

The pleiotropic functions of immune cell–derived EVPs are exerted through their diverse cargo, which includes proteins, cytokines, nucleic acids, and lipids. Cytokines packaged in EVPs, either surface bound or encapsulated (Fitzgerald et al., 2018), depending on the context, can represent another mechanism of EVP-mediated crosstalk and activation of immune cells. EVPs may protect certain cytokines from degradation, thus increasing their half-lives, and target specific cells for delivery. For example, both the pro-form and mature pro-inflammatory IL-1β and IL-18 are packaged in EVPs released from DCs and macrophages through distinct pathways (Pizzirani et al., 2007; Gulinelli et al., 2012; Yoon et al., 2017). Interestingly, the inflammasome, a multiprotein intracellular complex that detects pathogenic microorganisms and sterile stressors, is required for IL-1β and IL-18 activation. Sequestration of the activated inflammasome complex together with IL-1β and IL-18 precursors and their secretion in EVPs may precede inflammasome activation, thereby proposing an attractive explanation for the detection of both immature and mature cytokine forms in EVPs (Prada et al., 2013).

EVP cargo is enriched in noncoding RNAs, of which miRNAs received the most scrutiny due to their well-described regulatory functions. Nevertheless, the mechanisms regulating selective miRNA packaging into EVPs have only recently begun to be explored (Clancy et al., 2019). Among immune cells, mast cells were the first described source of secreted EVP mRNA and miRNA that could be shuttled between cells, resulting in activation and degranulation of recipient cells (Valadi et al., 2007; Vukman et al., 2017). It is now clear that each immune cell type EVPs package miRNAs with specific targets and functions and that miRNA content is cell-state dependent and can be modulated by extrinsic cues. For instance, in response to endotoxin stimulation, macrophages secrete EVPs containing miR-155, which in turn promotes inflammatory responses to LPS in vivo (Alexander et al., 2015; Vigorito et al., 2013). Furthermore, EBV-infected B cell EVPs cause miRNA-mediated repression of the immunoregulatory *CXCL11/ITAC* genes once captured by circulating host DCs (Pegtel et al., 2010). These studies provide
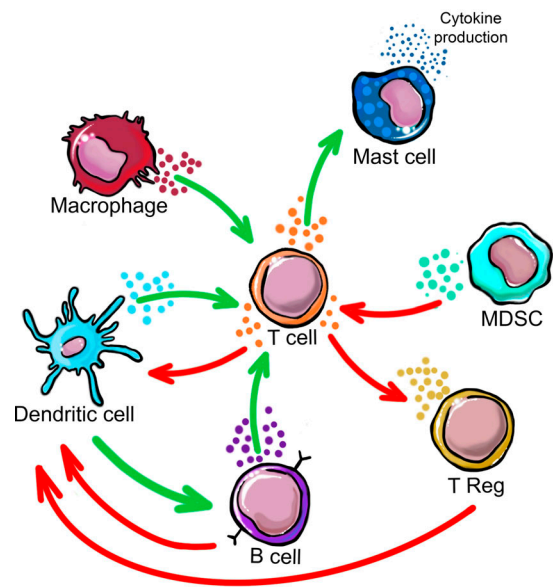

Figure 3. **Immune cell–derived EVPs orchestrate immune responses.** Immune cell–derived EVPs (depicted as small vesicles) maintain the balance between immune activation (green arrows) and immune suppression (red arrows). EVPs participate in the crosstalk between innate and adaptive immune responses and promote cytokine production as highlighted by mast cells.

evidence that EVPs offer a means of miRNA transfer between immune cells, facilitating the regulation of gene expression and the generation of proper inflammatory responses.

Lipids, such as ceramides, sphingolipids, and the lipid raft component cholesterol, are not only essential components of EVP membranes but also enriched within EVPs (Wubbolts et al., 2003; Trajkovic et al., 2008). For example, EVPs from mast cells and DCs are enriched in sphingomyelin and phosphatidylethanolamines, but not in cholesterol, phosphatidylcholine, or lyso(bis)phosphatidic acid, a composition that could be altered by pH changes (Laulagnier et al., 2004). EVP lipid composition may affect their function, as their alteration was observed in asthmatic patients during chronic airway inflammation (Hough et al., 2018). Recently, in addition to lipidomics, metabolomic analysis of EVPs revealed a complex set of molecules, including amides, amino acids, carboxylic acids, sugars, and others (Altadill et al., 2016; Luo et al., 2018). While knowledge of the physiological role of various metabolites in EVPs, especially those derived from immune cells, remains rather limited, it will be important to determine if they can act as secondary messengers and influence immune and target cell metabolism.

Immunosuppressive functions of immune and tumor cell–derived EVPs

Immune cell–derived EVPs can also exert immunosuppressive effects in response to various stimuli, including pathogens or the presence of a tumor, to prevent or mitigate autoimmunity (Fig. 3). Immunosuppressive regulatory T (T reg) cells are critical for maintaining self-tolerance and dampening immune responses; therefore, it is not surprising that T reg cell–derived EVPs also possess the capacity to suppress T helper 1 (Th1) cell

proliferation and IFN-γ cytokine secretion (Okoye et al., 2014) and induce tolerogenic DCs (Tung et al., 2018). Myeloid-derived suppressor cells (MDSCs), on the other hand, regulate immune suppression by preventing T cell activation and polarizing macrophages toward a tumor-promoting phenotype (Veglia et al., 2018). MDSCs mediate their suppressive effects not only directly but also indirectly via EVPs (reviewed in Ostrand-Rosenberg and Fenselau, 2018). Specifically, expression of CD47, which protects cells from phagocytosis, on MDSC-derived EVPs chemoattracts MDSCs (Chauhan et al., 2017).

In response to pathogen-associated molecular patterns, such as bacterial endotoxins, the exchange of EVP miR-146a between BM-derived DCs inhibited the immune response to endotoxin in vivo (Alexander et al., 2015). In response to tumors, immature DC-derived EVPs expressed immunosuppressive molecules, such as TGF-β, NKG2D, and death ligand FasL, which can inhibit natural killer (NK) cells, macrophages, and neutrophils (Batlle and Massagué, 2019; Kim et al., 2006). A crucial immune evasion mechanism is the up-regulation of programmed death ligand 1 (PD-L1) and interaction with the corresponding PD-1 receptor on T cells. Tumors have hijacked this regulatory mechanism, and anti–PD-1 antibodies are promising immunotherapies (Sharma and Allison, 2020). Interestingly, the presence of PD-L1 on melanoma, lung, and prostate cancer EVPs suppresses T cell activation, reducing the release of anti-tumor IL-2 and IFN-γ, thus enabling immune escape (Xie et al., 2019; Kim et al., 2019; Chen et al., 2018a). Consequently, in prostate cancer, which is resistant to anti–PD-L1 therapy, loss of PD-L1 in tumor EVPs inhibits tumor growth and promotes T cell activation in draining lymph nodes (Poggio et al., 2019). Understanding and altering the mechanism of selective PD-L1 packaging into EVPs could overcome resistance to anti–PD-1/PD-L1 antibody therapy and greatly impact the success of immunotherapies. To achieve this, it is critical to first determine the role of EVP PD-L1 in normal immune responses.

The role of immune cell–derived EVPs in pathology
EVP contributions to inflammatory responses
EVP-mediated communication between immune cells also participates in immune dysfunction associated with the development and progression of disease, such as neurodegenerative, infectious, and autoimmune disorders (Fig. 1) in which a common denominator is inflammation, driven or sustained by EVPs. For example, autism spectrum disorder is also associated with microglial activation and brain inflammation. The protein and mitochondrial DNA content of EVPs isolated from the serum of children with autism spectrum disorder is increased and induces the production of pro-inflammatory IL-1β in human microglia in vitro (Tsilioni and Theoharides, 2018; Abal, 2017). During infection, EVPs derived from pathogen-infected cells, such as macrophages infected with *Mycobacterium avium*, a known pathogen in HIV-positive individuals, can elicit proinflammatory responses measured by increased levels of TNF-α and RANTES upon fusion with uninfected macrophages, which in turn contributed to HIV and CMV pathogenesis (Bhatnagar and Schorey, 2007; Walker et al., 2009). EVP-mediated inflammasome activation can also

occur in the context of central nervous system (CNS) trauma, when the secretion of CNS-derived inflammasome-containing EVPs into the cerebral spinal fluid may activate innate immune responses upon fusion with target cells in peripheral tissues (de Rivero Vaccari et al., 2016).

EVPs derived from activated neutrophils also contribute to lung inflammation by expressing αM integrin and surface neutrophil elastase in a favorable orientation that renders them resistant to a naturally occurring protease, α1-antitrypsin. When released, these vesicles degrade collagen within the extracellular matrix, inducing proteolytic damage in patients with neutrophil-driven diseases, including chronic obstructive pulmonary disease and bronchopulmonary dysplasia (Genschmer et al., 2019). Thus, innate immune EVP-mediated tissue remodeling could initiate inflammation and drive pathophysiology.

The role of EVPs in transplantation tolerance and rejection
Allograft rejection remains a serious complication after organ transplantation. EVPs can both directly and indirectly activate T cells and therefore can participate in graft rejection/tolerance. This occurs through crosstalk between recipient APCs and donor MHC antigens, followed by T cell activation. In murine models of renal allografts, miR-682, highly enriched in immature DC–derived EVPs, suppressed IL17+CD4+ T cells and promoted Foxp3+CD4+ regulatory T cell development, in contrast to mature DC–derived EVPs, which induced T cell immunity (Pang et al., 2019). Moreover, follicular helper T (Tfh) cell–derived EVPs regulated B cell proliferation and differentiation, revealing a novel crosstalk mechanism in renal transplantation patients with antibody-mediated rejection (Yang et al., 2019). Given the ability of professional APC EVPs to augment specific immune responses and facilitate tolerance, they represent an attractive therapeutic strategy as vaccine adjuvants or anti–allograft rejection treatments. For instance, injection of immature DC–derived EVPs prolonged survival following cardiac, intestinal (Pêche et al., 2006; Yang et al., 2011), and renal transplantation (Pang et al., 2019). Although preclinical studies have shown promising results, no clinical trials testing the therapeutic efficacy of immature DC–derived EVPs have been conducted in autoimmune diseases or transplant settings to date.

EVP contributions to autoimmune diseases
In addition to the pleiotropic functions they play in the immune system, EVPs have been implicated in immune modulation associated with autoimmune diseases (Buzas et al., 2014), such as rheumatoid arthritis (RA), multiple sclerosis (MS), and systemic lupus erythematosus (SLE). EVPs and their cargo, circulating systemically, could be targets of autoreactive recognition that can trigger autoimmunity. In RA, which is characterized by joint and systemic inflammation, synovial EVPs contain autoantigens that contribute to the pathogenesis of RA, such as citrullinated fibrin α-and β-chain fragments, fibrinogen β-chain precursor, fibrinogen D fragment, and the Spα (CD5 antigen-like protein) receptor, which may result in increased inflammation and cartilage degradation (Skriner et al., 2006). Moreover, both EVPs derived from immature DCs (Kim et al., 2006) and DCs engineered to produce FasL have anti-inflammatory activity in RA

(Min et al., 2000). Vaccination with EVPs isolated from IL-4– or IL-10–stimulated DC also reduced clinical manifestations of RA in mice (Kim et al., 2007; Kim et al., 2005). Therefore, the cell of origin producing the EVPs, its activation status, and microenvironmental interactions may determine whether they promote or dampen synovial inflammation.

In MS pathogenesis, a chronic inflammatory demyelinating disease of the CNS, EVPs can have opposite functions depending on their cargo and the recipient cells. Specifically, microglia, the resident immune cells of the CNS, release EVPs involved in physiological and pathological conditions (reviewed in Dolcetti et al., 2020). In particular, EVPs from MS patients are able to inhibit T reg cell differentiation from naive CD4+ T cells (Kimura et al., 2018). However, DCs can release EVPs that increase remyelination, which is lost in MS and significantly contributes to disease progression (Pusic et al., 2014). These observations reinforce the importance of providing a deeper characterization of EVPs in both physiological and pathological conditions to potentially provide targeted therapeutic interventions.

SLE is a prototypic systemic autoimmune disease characterized by the production of an array of pathogenic autoantibodies that recognize, among others, DNA and chromatin, forming immune complexes. Immune complexes deposit in the kidneys and lead to lupus nephritis (LN), a major cause of morbidity and mortality (Zan et al., 2014). EVP levels were significantly higher in SLE patients and induced the production of IFN-α, TNF-α, IL-1β, and IL-6 in peripheral blood mononuclear cells (Lee et al., 2016). Several EVP miRNAs have been implicated in SLE pathology (Perez-Hernandez et al., 2015). EVP miR-29c, which exerts antifibrotic effects by regulating collagens, fibronectin, laminin, and matrix metalloproteinase-2, was down-regulated in patients with LN compared with healthy controls and correlated with renal fibrosis and kidney damage (Solé et al., 2015). In contrast, elevated EVP miR-26a in patients with LN correlated with proteinuria and was predictive of podocyte injury. Given the importance of miR-26a in regulating podocyte differentiation and cytoskeleton genes, these findings are consistent with EVP miRNAs contributing to the progression of podocyte injury in LN (Ichii et al., 2014). A landmark study from the Reizis laboratory showed that an excess of DNA within EVPs is sufficient to break tolerance and induce the production of anti-DNA autoantibodies in both mice and humans with inactivating mutations in a circulating DNase that normally degrades EVP-associated and extracellular DNA (Sisirak et al., 2016). In addition to nucleic acids, SLE EVPs also contain autoantigens, such as the cytosolic E3 ubiquitin–protein ligase Ro(SS-A)/TRIM-21 and La (SS-B) and the nuclear Smith antigens, targets of the autoimmune process in this disease (Kapsogeorgou et al., 2005). Thus, one could speculate that EVP cargo might participate in the breakdown of tolerance to cytosolic and nuclear antigens in SLE through mechanisms that remain to be elucidated. Moreover, circulating EVPs might serve as valuable diagnostic markers and tools for predicting disease severity and response to treatment, all of which are direly needed in SLE.

EVPs may also play a role in the pathogenesis of inflammatory bowel disease, including Crohn's disease and ulcerative colitis (Yang and Merlin, 2019). Clinical and pathological outcomes differ between these two diseases (Neurath, 2014), and EVPs may play distinct roles in their respective pathogenesis. Indeed, in a colitis mouse model, serum-derived EVPs hyperactivated macrophages, exacerbating disease (Wong et al., 2016). In contrast, EVPs derived from IL-10–treated DCs suppressed acute trinitrobenzene sulfonic acid–induced colitis, potentially representing a promising therapeutic strategy for inflammatory bowel disease (Yang et al., 2010). Collectively, these findings indicated that EVPs, including immune cell–derived EVPs, not only directly contribute to the pathogenesis of chronic autoimmune and inflammatory diseases but also have great potential as new biomarkers or therapeutic targets of these diseases.

The future of EVP-based immunotherapies
Immune EVPs in vaccine development
EVPs are crucial mediators in cell–cell communication and orchestrate a variety of immune responses, making them attractive candidates for immunotherapies (Fig. 1). Research on immunotherapeutic applications of EVPs has focused largely on DC-derived EVPs due to their immunogenicity and ability to carry MHC class I and II molecules together with costimulatory molecules (CD40, CD80, and CD86) that induce a potent antigen-specific T cell response (Théry et al., 1999; Sheng et al., 2013) and tumor suppression in vivo (Zitvogel et al., 1998). Two phase 1 clinical trials exploring the efficacy of immature DC-derived EVPs loaded with tumor-associated antigen peptides in melanoma and non-small cell lung cancer (NSCLC) patients (Table 1; Escudier et al., 2005; Morse et al., 2005) revealed that while nontoxic, DC-derived EVPs provided little therapeutic benefit due to poor immunostimulation. In phase 2 clinical trials, patients with NSCLC tolerated IFN-γ–matured DC-derived EVPs well, but activation of NK cells and T cell stimulation were limited (Besse et al., 2016). Thus, the efficiency of DC-derived EVPs can be enhanced by cytokine cocktails that up-regulate costimulatory signals and reduce immunoregulatory molecules, such as PD-L1. Maturation of DCs with TLR-4 or -9 ligands, LPS and CpG, respectively, or the TLR-3 inducer poly(I:C) produced EVPs with higher concentrations of MHCs that potentiated CD8+ T cell responses in a murine melanoma model (Damo et al., 2015). Moreover, DC-derived EVPs engineered to activate invariant NK T cells through α-galactosylceramide induced potent antigen-specific antitumor immune responses in murine melanoma (Gehrmann et al., 2013). More recently, EVPs derived from α-fetoprotein–expressing DCs showed robust antigen-specific antitumor immune responses in various hepatocellular carcinoma mouse models (Lu et al., 2017). In human papillomavirus (HPV)–driven cervical cancer, HPV early antigen 7 peptide (E749–57) loaded into murine DC-derived EVPs induced an anti-tumor cytotoxic T lymphocyte (CTL) response and activated potent protective and therapeutic immune responses against cervical cancer in vivo (Chen et al., 2018b). These approaches could be used to generate more potent immunostimulatory EVPs for clinical use.

In addition to DC-derived EVPs, NK cell–derived EVPs also represent a potential suitable tool for anti-tumor therapy. NK cell–derived EVPs package FasL and perforin, which mediated

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Table 1. Ongoing and completed National Institutes of Health–registered clinical trials investigating EVP-based therapeutics

Disease	Phase	Cellular source	Route of administration	Isolation method	Modification	Status	Reference
Melanoma	1	Monocyte-derived DCs	SC	UF/UC sucrose cushion	Melanoma antigen loaded	Completed	Escudier et al., 2005
NSCLC	1	Monocyte-derived DCs	SC and intradermal	UF/UC sucrose cushion	Peptide loaded	Completed	Morse et al., 2005
NSCLC	2	Monocyte-derived DCs	Intradermal	UF/UC sucrose cushion	Peptide loaded	Completed	Besse et al., 2016
Cutaneous wound healing (ulcer)	1	Autologous plasma-derived exosomes	Intradermal	0.02-µm filter	None	Open	NCT02565264
SARS-CoV-2	1/2	T cells	Aerosol inhalation	Not specified	COVID-19–specific T cells	Open	NCT04389385

COVID-19; coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SC, subcutaneous; UC, ultracentrifugation; UF, ultrafiltration.

anti-tumor effects in glioblastoma and melanoma xenograft models (Zhu et al., 2017). Of note, NK cell–derived EVPs were taken up by tumor cells, but not immune cells, resulting in selective antitumor cytotoxicity (Lugini et al., 2012), suggesting NK cell–derived EVPs could be vehicles for targeted therapy and drug delivery.

In the context of the current ongoing pandemic, immune cell–derived EVPs are being tested in a phase 1/2 clinical trial for the treatment of early-stage pneumonia induced by severe acute respiratory syndrome coronavirus 2. The premise of the study is that donor T cells activated and expanded in vitro in response to viral peptide fragments and cytokines secrete EVPs containing potent immune mediators, including IFN-γ, that could control disease progression if administered early in the course of the disease (NCT04389385).

Immune EVPs as delivery vectors for therapeutic agents

The use of immune EVPs for therapeutic purposes offers numerous advantages over cell-based therapies (Yang et al., 2020; Li et al., 2020). Unlike cells, EVPs do not replicate after injection reducing the risk of tumor formation. Moreover, the lipid bilayer-membrane increases their stability and protects their content from degradation in the extracellular space, a characteristic required for an efficient delivery system. EVPs can be engineered to carry exogenous proteins, miRNA, mRNA, or chemotherapies (Delcayre et al., 2005). Initial studies on DC-derived EVPs as therapeutic vehicles revealed efficient gene delivery in vivo and their capacity to cross the blood–brain barrier (Alvarez-Erviti et al., 2011; Ha et al., 2016). In a breast cancer xenograft model, DC-derived EVPs loaded with doxorubicin were targeted to tumors through expression of a Lamp2b-αv integrin-specific iRGD peptide fusion protein (Tian et al., 2014). More recently, macrophage cell line–derived EVPs loaded with paclitaxel were taken up by cancer cells and inhibited tumor growth in a lung cancer mouse model (Kim et al., 2016). In addition, modification of these EVPs with aminoethylanisamide-polyethylene glycol reduced their immunogenicity, thus increasing their circulation time in mice (Kim et al., 2018).

Due to their capacity for selective cell targeting and immune stimulation, the potential of EVPs in drug delivery and immune therapy is immense, but limitations, such as large-scale production of good manufacturing practice (GMP)–grade EVPs and possible tumorigenic effects, need to be overcome (Yeo et al., 2013). Platelet-derived EVPs represent an alternative, advantageous delivery system, since these cells are anucleated, thus reducing safety concerns, and can be directly produced from collected platelet concentrates, bypassing the need for a GMP cell culture facility. Furthermore, the availability of autologous platelet-derived EVPs could reduce immunogenicity concerns (Johnson et al., 2021).

In conclusion, methods for EVP generation and isolation for therapeutic purposes should be optimized, since ultracentrifugation and precipitation, the most common techniques used to isolate EVPs, are nonscalable. Rigorous investigations are needed to identify a cost-effective alternative to engineer EVPs for large-scale clinical trials and therapeutic applications.

Conclusions

Immune cell–derived EVPs may regulate a myriad of physiological processes, including tolerance induction during pregnancy, orchestrating fetal hematopoiesis, and coordinating adult immune system development and function. Alterations in EVP-mediated crosstalk among immune cells are associated with local and systemic inflammation, autoimmunity, cancer, and neurodegeneration. While the vast majority of research to date has focused on cancer cell–derived EVPs, it is becoming clear that understanding the function of immune cell–derived EVPs in health and disease will pave the way to understanding the systemic nature of these diseases.

How is innate and adaptive immune cell–derived EVP-mediated communication orchestrated in normal physiology? It is unclear whether the cargo of circulating immune cell–derived EVPs is influenced by diet, circadian rhythm, or exercise. In particular, exercise confers multisystemic benefits for human health, mitigating the effects of metabolic disease and cancer (Darkwah et al., 2021), and recent studies have indeed demonstrated that plasma circulating EVP cargo increases in an intensity-dependent manner in response to endurance exercise (Frühbeis et al., 2015; Brahmer et al., 2019). Thus, immune cell

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EVPs could mediate the systemic benefits of exercise and could have therapeutic utility in the treatment of cancer and metabolic diseases. Another outstanding question is what are the consequences of EVP binding or uptake for target immune cells? Do these interactions lead to only short-lived functional changes, or do they result in long-term reprogramming, perhaps via epigenetic changes? What determines one outcome versus the other?

Understanding the mechanisms involved in EVP-mediated reprogramming of immune responses would enable the engineering of EVPs that could boost or inhibit particular target immune cell populations in a disease-specific context, for example stimulating/priming antitumor immune responses in cancer patients or dampening autoimmune responses in systemic autoimmune diseases. In this context, sustained signals could enhance normal immune function through signal amplification and faster responses during infection or anticancer treatments. Moreover, uncovering how immune cells differentiate between canonical and noncanonical EVP antigen presentation could lead to the development of specific inhibitors as innovative therapeutic strategies in autoimmune diseases.

Our knowledge of immune cell–derived EVP cargo is still limited, hindering our understanding of their function. Moreover, most of the research to date has focused on EVP protein and miRNA cargo, but very little is known about the role of EVP DNA in selecting an immune repertoire, establishing tolerance during normal immune development, and driving immune-related pathologies. EVP DNA may represent an important signal that can modulate immune cell activation and a target for novel therapies in cancer and autoimmune diseases. Therefore, the complexity of EVP-mediated communication in the immune system awaits deconvolution but also holds many opportunities to be exploited for therapeutic benefits in patients.

Acknowledgments

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