Modulation of Immune Responses by Exosomes Derived from Antigen-Presenting Cells

Botros B. Shenoda and Seena K. Ajit

Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA, USA.

Supplementary Issue: Host Factors that Influence the Outcome of Pathological Diseases

ABSTRACT: Exosome-mediated signaling is important in mediating the inflammatory response. To exert their biological or pathophysiological functions in the recipient cells, exosomes deliver a diverse array of biomacromolecules including long and short coding and non-coding RNAs, proteins, and lipids. Exosomes secreted by antigen-presenting cells can confer therapeutic benefits by attenuating or stimulating the immune response. Exosomes play a crucial role in carrying and presenting functional major histocompatibility peptide complexes to modulate antigen-specific T cell responses. Exosomes from Dendritic Cells (DCs) can activate T and B cells and have been explored for their immunostimulatory properties in cancer therapy. The immunosuppressive properties of exosomes derived from macrophages and DCs can reduce inflammation in animal models for several inflammatory disorders. This review focuses on the protective role of exosomes in attenuating inflammation or augmenting immune response, emphasizing studies on exosomes derived from DCs and macrophages.

KEYWORDS: exosomes, inflammation, dendritic cells, macrophages

SUPPLEMENT: Host Factors that Influence the Outcome of Pathological Diseases

CITATION: Shenoda and Ajit. Modulation of Immune Responses by Exosomes Derived from Antigen-Presenting Cells. *Clinical Medicine Insights: Pathology* 2016:9(S1) 1–8 doi: 10.4137/CPath.S39925.

TYPE: Review

RECEIVED: July 01, 2016. **RESUBMITTED:** August 21, 2016. **ACCEPTED FOR PUBLICATION:** August 23, 2016.

ACADEMIC EDITOR: Dama Laxminarayana, Editor in Chief

PEER REVIEW: Five peer reviewers contributed to the peer review report. Reviewers' reports totaled 695 words, excluding any confidential comments to the academic editor.

FUNDING: Studies funded by grants from the NIH (NINDS 1R21NS082991-01) and Drexel Clinical & Translational Research Institute to Seena Ajit. Botros Shenoda is a recipient of the Fulbright Foreign Student Program fellowship funded by the US Department of State, Bureau of Educational and Cultural Affairs, and Dean's Fellowship for Excellence in Collaborative or Themed Research, Graduate School of Biomedical Sciences and Professional Studies, Drexel University College of Medicine. The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

CORRESPONDENCE: seena.ajit@drexelmed.edu

Introduction

Inflammatory response is a well-regulated process of integrated and complex network of cellular communication. Multiple modes of information exchange are employed through the secretion and subsequent receptor-mediated detection of biomolecules, including cytokines, chemokines, and even metabolites. Uni- and bidirectional communication exists between immune and non-immune cells during the process of initiating, maintaining, and resolving inflammation.[1] Most of the interactions between immune cells are mediated by cytokines released in response to different stimuli. Recent evidence has shed light on a novel mode of intercellular communication mediated by exosomes, a class of Extracellular Vesicles (EVs) in regulating inflammation.

Cells secrete different types of EVs that can be classified based on their size and subcellular origin. EVs formed by budding from the plasma membrane of cells are referred to as microvesicles or ectosomes. Exosomes differ from ectosomes in that they are derived from inward budding of the inner endosomal membrane to form multivesicular bodies, followed by the fusion of multivesicular bodies with the plasma membrane.[2] These vesicles range in size from 30 to 150 nm. Composed of a lipid bilayer, they contain both transmembrane and cytosolic proteins and enclose mRNAs and miRNAs.[3–8] In a recent editorial, the International Society for Extracellular Vesicles put forth a minimal set of biochemical, biophysical, and functional standards that should be used to attribute any specific biological cargo or functions to EVs.[9] After isolating exosomes, the purity of the exosomes can be tested using electron microscopy and western blotting. It has been reported that exosome processing including centrifugation, dehydration, and fixation for transmission electron microscopy may alter the size and morphology of vesicles. The cup-shaped morphology for exosomes shown in some studies has been attributed to differences in sample processing.[10,11] Proteins highly enriched in exosomes are used as markers to identify exosomes. Commonly used exosomal markers are Hsp70, tetraspannin family glycoproteins CD63, CD81, and CD914. Cells use these vesicles to communicate with both adjacent and distant cells. The molecules present on the surface of EVs

enable them to target recipient cells. These EVs can induce signaling by receptor-ligand interaction or can be internalized by endocytosis and/or phagocytosis, or even fuse with the membrane of the target cell, thereby delivering vesicular contents into the cytosol of the recipient/target/acceptor cell.[8]

The biological effects mediated by exosomal uptake in recipient cells depend on the donor cells secreting the exosomes.[12,13] The exosomal contents vary depending on the source and the physiological conditions of cells releasing them. Changes due to infection, inflammation, or transformation as in the case of tumor cells can influence and thereby alter the composition of exosomes. However, not everything that is present in the parent cell is incorporated into the exosomes, suggesting that this well-regulated process is dynamically altered by signaling cues. This variability in biomolecules within the exosomes is the basis for biological consequences upon their uptake, as well as their biomarker utility. Though the function of exosomes in normal cellular homeostasis is still being elucidated, their role in pathogenesis is well established; it is known that exosomes can be pathogenic or protective.[14] Here, we will focus primarily on the protective role of exosomes in attenuating inflammation or augmenting immune response, emphasizing studies on exosomes derived from Dendritic Cells (DCs) and macrophages.

Immunomodulatory Function of Exosomes

Innate and adaptive responses collectively contribute to immune response. The evolutionarily conserved innate immune system is found in all multicellular organisms, while the adaptive immune response is found only in vertebrates.[15] The activation of the innate immune system is mediated by a limited number of receptors that can recognize pathogen-associated molecular patterns or damage-associated molecular patterns.[16,17] Toll-Like Receptors (TLRs) are well characterized examples in mammals. Often referred to as pattern recognition receptors, these receptors are germline encoded. In adaptive immune systems, the antigen receptors are not germline encoded but generated through somatic hypermutations.[18] This results in a wide range of antigen receptors in the adaptive immune system, compared to the limited number of receptors for pathogen-associated molecular patterns or damage-associated molecular patterns in the innate immune system.[19] T and B cells are the two main cell types in the adaptive immune system. B cells can recognize antigens, while the T cells utilize the process of antigen presentation for antigen recognition. DCs and macrophages are Antigen-Presenting Cells (APCs). These APCs internalize foreign antigens and load the processed antigens on to major histocompatibility complexes I and II (MHCI and MHCII) molecules for presentation to naïve CD8+ and CD4+ T cells, respectively, imparting the memory of the antigen to the T cells.[20]

Exosomes play a crucial role in carrying and presenting functional MHC–peptide complexes to modulate antigen-specific CD8+ and CD4+ responses.[6,21] This presentation can be direct or occur as a cross-presentation. In direct presentation, MHC–peptide complexes on the exosomes are directly engaged by antigen-specific T cells, leading to T-cell activation. In cross-presentation, APCs acquire antigens carried by exosomes and after additional processing of these antigens, present their peptides to T cells. Cross-presentation can also occur when antigenic peptide–MHC complexes are together transferred onto DCs and then presented to T cells (termed cross-dressing).[6] These features thus form the basis of protective or therapeutic benefits conferred by exosomes.

Immunomodulatory Role of Exosomal RNA

RNA-sequencing analysis using exosomes from human plasma samples showed the presence of a wide variety of RNA species within these circulating vesicles.[22] These include mRNAs and noncoding regulatory RNAs including miRNAs and long noncoding RNAs.[3,23–27] The functionality of RNA in the recipient cell after uptake[4,28] has been demonstrated. RNA sequencing of naïve and activated macrophages has shown that inflammation alter the transcriptome. The timing of transcription factor activation, as well as the localization of nascent transcripts (chromatin-associated, nucleoplasmic, and cytoplasmic) play a role in spatial and temporal regulation of transcriptome changes resulting from inflammation.[29,30] Whether inflammation-induced alterations are reflected in exosomal transcriptome was determined in a separate study by sequencing exosomal RNA derived from naïve and lipopolysaccharide (LPS)-stimulated macrophages. Pathway analysis showed significant changes in both the adaptive and innate immune processes; specifically, pathways related to NF-κB activation and TLR cascades differed between exosomal mRNAs from naïve cells compared with those from LPS-stimulated cells.[31] Exosomal miRNAs miR-21 and miR-29a secreted by tumor cells can bind to TLR8 and TLR7 in immune cells, leading to NF-κB activation and secretion of inflammatory cytokines.[32]

RNA sequencing and profiling studies have shown that miRNAs are abundant in exosomes.[2,4] It is also known that the repertoire of exosomal miRNAs differ from that of the donor cell.[33] Recent studies have explored the mechanisms determining the incorporation of a subset of cellular miRNAs into exosomes suggesting that the sorting of specific miRNA species to exosomes may be actively regulated. One study showed that endogenous mRNAs modulate miRNA sorting to exosomes and transfer to acceptor cells.[34] This study implied that exosomal miRNA secretion is a mechanism by which cells rapidly dispose miRNAs in excess of their targets to adjust miRNA:mRNA homeostasis. Based on current research, four potential modes/mechanisms for miRNA sorting into exosomes are known. The sorting mechanism for exosomal miRNAs includes the following steps: (1) The neural sphingomyelinase 2 (nSMase2)-dependent pathway: overexpression of nSMase2 increased the number of exosomes secreted and exosomal miRNAs. Conversely, inhibition

of nSMase2 expression reduced the number of exosomal miRNAs.[35] (2) The miRNA motif and sumoylated heterogeneous nuclear ribonucleoproteins (hnRNPs)-dependent pathway: sumoylated hnRNPA2B1 recognized the GGAG motif (EXOmotif) in miRNA sequences and caused specific miRNAs to be packed into exosomes.[36] This short EXOmotif sequence is overrepresented in miRNAs that are commonly enriched in exosomes allowing exosomal miRNAs to be specifically recognized (and bound) by a transport protein, hnRNPA2B1. This motif controls the loading of these miRNAs into exosomes.[36] This specific 4 nucleotide sequence (GGAG) was overrepresented in miRNAs enriched in T-cell-derived exosomes. (3) The 3′ miRNA sequence-dependent pathway: adenylation and uridylation of miRNA at its 3′ end can contribute to direct miRNA sorting into exosomes suggesting that the 3′ end may harbor critical sorting signal.[37] (4) The miRNA-induced silencing complex (miRISC)-related pathway: mature miRNAs can interact with assembly proteins GW182, and AGO2 to form miRISC complex. miRISC components co-localized with multi vesicular bodies and there is a correlation between AGO2 and exosomal miRNA sorting. Knockout of AGO2 can decrease the types or abundance of the preferentially exported miRNAs.[38] In summary, specific sequences present in certain miRNAs may guide their incorporation into exosomes; certain enzymes or proteins may control the sorting of exosomal miRNAs[39] and mRNA targets of miRNA being sorted may also play a role.[34]

It is now well established that endogenous miRNAs are transferred between immune cells and functional in recipient cells, thereby contributing to the inflammatory response. Exosome-mediated transfer of miRNAs between T cells and APCs is enhanced by the formation of a functional immune synapse. Though first described in cells of the adaptive immune system including T and B cells, the description now encompass interactions involving innate immune cells such as Natural Killer (NK) cells.[40] The immune synapse promotes exchange of miRNA-loaded exosomes between a T cell and its cognate APC.[3]

Several of the LPS-responsive miRNAs detected in exosomes have validated mRNA targets that encode proteins involved in TLR signaling, chemokine signaling, and the transforming growth factor-beta (TGF-β) pathway.[41] Comparison of miRNA profile in exosomes purified from THP-1 cells, a human-derived monocytic cell line, and RAW 264.7 mouse macrophage, with and without LPS stimulation showed that the exosomal biomolecular signature will differ between cell types and between species. Thus, the species and the physiological state of the cells secreting the exosomes will influence the molecules in the vesicles.[31] The same study showed that there was a striking shift in the most abundant noncoding RNA populations after LPS stimulation of RAW 264.7 cells. The presence of pre-miRNAs and snoRNAs suggests that exosomes deliver molecules that could induce temporal epigenetic regulation in recipient cells regulating the course of inflammatory gene expression.[30] The influx of inflammatory-relevant pre-miRNAs in LPS-stimulated cells could indicate the need for a rapid response to inflammation rather than regulation at the nuclear level. Mature miRNAs may fine-tune the regulation of inflammation by altering the mRNA levels of inflammatory proteins immediately, while pre-miRNAs offer a second wave of regulation at a later time point. Exosomes have the advantage of delivering proteins and miRNAs that are primed to act directly and immediately along with mRNAs that can be readily translated.[31]

A recent study showed the presence of mRNA fragments enriched in the 3′ untranslated regions (UTR) in exosomes.[42] The 3′UTRs of mRNAs have multiple regulatory roles and harbor sequences for numerous RNA-binding proteins that modulate the stability and translational efficiency of mRNAs. They also have multiple miRNA target-binding sites that guide the RNA-induced silencing complex (RISC), resulting in miRNA binding by seed sequence complementarity, inducing degradation or translational repression. It was suggested that the mRNA fragments transported by exosomes may act as competing RNAs to regulate stability, localization, and translational activity of mRNAs in target cells.[42]

Immunomodulatory Role of Exosomal Proteins

Cells secrete a variety of vesicles that are heterogeneous in size and biogenesis. Protein markers are often used to differentiate EVs. A recent study systematically performed comparative analysis of the protein composition of all EVs recovered in the different steps of the differential ultracentrifugation protocol that is classically employed to isolate exosomes.[43] Human primary monocyte–derived DCs were used as source cells because of their ability to promote immune responses. Quantitative proteomic analysis demonstrated that some protein markers are unique to exosomes, whereas some others are generic. They identified three different tetraspanins –CD63, CD81, or CD9 – as specific protein markers for exosomes.[43]

Exosomes are enriched in proteins and they do not contain a random set of proteins, as would be the case for cell debris; the proteome of exosomes includes cytosolic, nuclear, endosomal, and membrane proteins.[44] Databases compiling protein composition of exosomes from various studies include Exocarta,[45] Vescilepedia,[46] and EVpedia.[47] A number of endosome-associated proteins (Rab GTPase, SNAREs, Annexins, and flotillin) and proteins involved in exosome biogenesis (ESCRT complex, ALIX, TSG101), tetraspanins mentioned above, heat shock proteins (HSP70, HSP90), and MHC are present in exosomes.[48] Exosomes are carriers of important soluble mediators such as cytokines, and for cytokines that lack an N-terminal signal peptide, exosomal release represents a form of leaderless secretion.[49] A list of cytokines secreted by EVs has been reported.[49] LPS stimulation of RAW 264.7 mouse macrophage cells can induce the secretion of cytokines into culture media after 24 hours. Exosomes derived from RAW 264.7 mouse macrophages after LPS stimulation

showed increased cytokines, predominantly chemokines. Of the 16 cytokines secreted by RAW 264.7 cells after LPS stimulation, 10 were detected in RAW 264.7 cell-derived exosomes.[31] Systematic studies have not been conducted to determine the complete spectrum of EV-associated cytokines. Additionally, the extent to which vesicular localization of cytokines affects conventional cytokine measurements has not been addressed.[49]

Therapeutic Benefits of Exosomes

Exosomes can confer therapeutic benefits by attenuating or stimulating the immune response.[5] DC-derived exosomes (Dex) can amplify immune responses *in vivo*, by transferring peptide–MHC complexes from DCs that have been exposed to an antigen to another DC that have not been in contact with the same antigen (Fig. 1).[5] The ability of the exosomes to stimulate immune responses is dependent on the maturation state of the DCs secreting them. Mature DCs carry more MHCII, intercellular adhesion molecule-1 (ICAM-1), and costimulatory molecules and are more potent T-cell stimulators.[50,51] Maturation state is also known to influence the miRNA profile of DCs-derived exosomes.[52]

The use of Dex has enabled immunotherapy and overcome some of the challenges associated with the use of DCs in clinical settings. In addition to being amenable to regulated manufacturing process and long-term storage, administering exosomes eliminate the risks associated with *in vivo* replication and lodging of cells in microvasculature.[15] Dex derived from tumor peptide–stimulated DCs can prime tumor-specific cytotoxic T lymphocyte responses *in vivo*, and a single intradermal injection resulted in delayed tumor growth or a complete eradication of established murine tumors.[53] Strategies harnessing DCs or their functions driving tumor-associated antigen (TAA)-specific T-cell responses have been successful in cancer immunotherapy.[7] Exosomes maintain the ability of DCs to present TAA and to activate TAA-specific responses.[54,55] The occurrence of antigen-presenting MHC I and II molecules along with the ICAM for adhesion and integrins for docking facilitates *in vivo* efficacy of exosomes. Two studies proved the feasibility of large-scale Dex production and confirmed the safety profile for Dex administration in patients.[56,57] Clinical trials were conducted on patients with advanced stage melanomas[56] or non-small cell lung carcinomas (NSCLC)[57] expressing melanoma-associated antigen. These studies using the first generation of Dex in end-stage cancer demonstrated that Dex exerted NK-cell effector functions in patients, but only minimal or no melanoma-associated antigen-specific T-cell responses were observed.[56,57]

The low immunogenic capacities of Dex led to the development of second-generation Dex, aimed at boosting NK and T-cell immune responses. Dex secreted by interferon-γ (IFN-γ)-treated DCs express higher levels of CD40, CD80, CD86, and ICAM-1 molecules compared with Dex from immature DCs, which in turn enhanced the immunogenicity.[58]

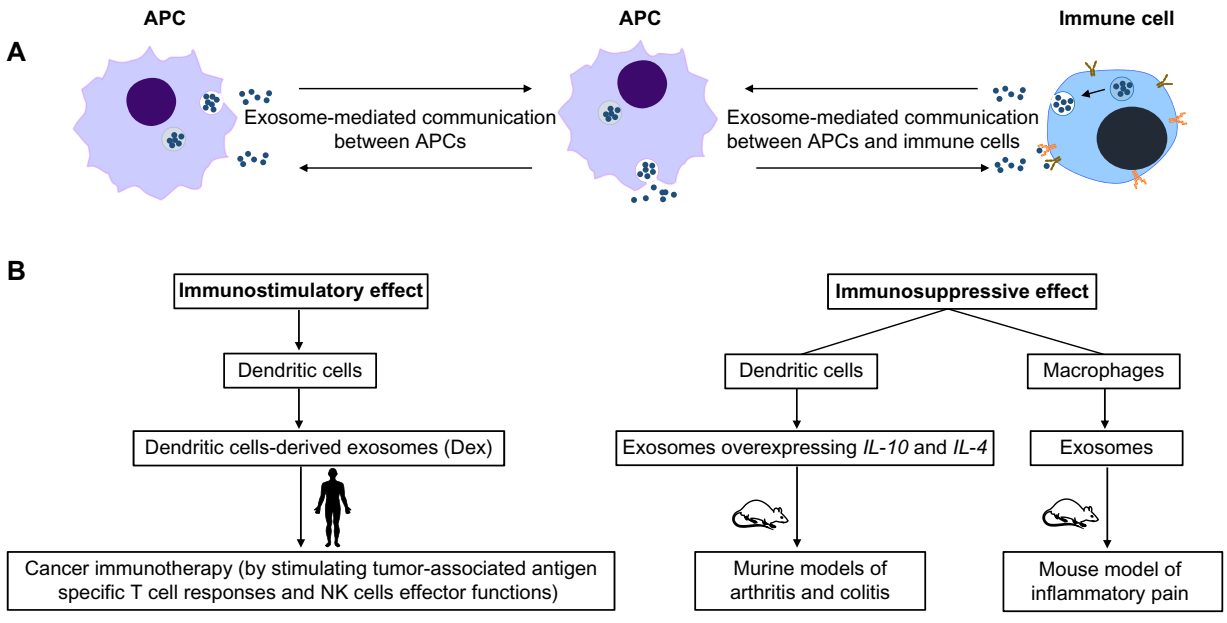

Figure 1. Immunomodulatory effects of exosomes derived from APCs.

Notes: (**A**) Exosomes released from APCs including Dendritic Cells (DCs) and macrophages can play a role in carrying and presenting functional MHC–peptide complexes. This presentation can be direct or occur as a cross-presentation. Exosomes can thus establish a bi-directional mode of communication between APCs and immune cells. (**B**) Exosomes secreted by APCs can have both immunostimulatory and immunosuppressive effects. Dex augment anticancer immune response by enhancing NK and T-cell effector functions. Immunosuppressive effects have been demonstrated for exosomes secreted by DCs and macrophages. Exosomes produced by DCs engineered to over express certain genes including *IL-10* and *IL-4* reduced inflammation in murine models of arthritis. Exosomes from LPS-stimulated macrophages can reduce thermal hyperalgesia and edema in a mouse model of inflammatory pain.

A phase-II clinical trial was conducted on patients with inoperable NSCLC without tumor progression. IFN-γ-Dex loaded with MHC class I- and class II-restricted cancer antigens were used as maintenance immunotherapy after induction chemotherapy. In this study that enrolled 22 patients, the primary endpoint, which was to observe at least 50% of patients with progression-free survival at four months after chemotherapy cessation was not met. However, this study confirmed that these Dex can boost the NK cell arm of antitumor immunity in patients with advanced NSCLC.[59]

Exosomes derived from APC have been shown to confer immunosuppressive effects in different disease models including Rheumatoid Arthritis (RA).[60] RA is a systemic autoimmune disorder characterized by synovial inflammation and hyperplasia. DCs transduced with adenovirus expressing the *IL-10* gene or treated with recombinant murine *IL-10* suppressed the onset of murine collagen-induced arthritis.[61,62] Several studies demonstrated positive outcomes using exosomes derived from DCs engineered to over express certain genes including *IL-10, IL-4*, FasL, and indoleamine 2,3 dioxygenase (IDO). A single dose of these exosomes systemically delivered after the onset of carrageenan-induced arthritis in mice effectively ameliorated disease progression, an effect that was absent with the direct injection of recombinant murine *IL–10*.[63] DCs genetically engineered to express the *IL-4* were effective in reducing inflammation in murine arthritis. Exosomes derived from these DCs were shown to be effective in reducing the severity and the incidence of established arthritis when delivered systemically and the effects were significantly higher than those observed after repeated injection of recombinant *IL-4*.[61] Exosomes derived from FasL-expressing DCs, similar to the effect of their parent cells, were able to suppress collagen-reactive T cells and inhibit the progression of murine carrageenan-induced arthritis after systemic injection.[62] FasL-expressing DCs also showed an anti-inflammatory effect in a murine delayed hypersensitivity model upon local administration.[64] Exosomes from DCs expressing IDO reduced inflammation, inhibited T cell activation, and suppressed T-cell responses to auto- and alloantigens by tryptophan starvation and/or production of toxic metabolites.[65] Exosomes derived from DCs treated with *IL-10* suppressed trinitrobenzene sulfonic acid (TNBS)-induced colitis.[66] Thus, these studies point to the immunosuppressive effects of exosomes derived from modified DCs, suggesting their potential therapeutic potential (Fig. 1).

Exosomes from LPS-stimulated macrophages significantly reduced paw swelling in mice caused by a single intraplantar injection of complete Freund's adjuvant (CFA). A single injection of exosomes attenuated thermal hyperalgesia in a mouse model of inflammatory pain, suggesting an immunoprotective role for macrophage-derived exosomes (Fig. 1). In CFA-treated animals, injection of exosomes purified from LPS-stimulated macrophages induced a transient increase in thermal hypersensitivity that was not observed in saline-treated animals. By 24 hours, CFA-treated animals that received injections of exosomes from LPS-stimulated RAW 264.7 cells had increased paw withdrawal latency and hence reduced thermal hyperalgesia compared with CFA-treated animals that received PBS. At 48 hours, CFA-treated animals displayed reduction in thermal hyperalgesia in response to exosome administration from both LPS-stimulated and naïve macrophages indicating that the reduction in thermal hypersensitivity observed after 48 hours was independent of the inflammatory status of the macrophages from which these

Table 1. Exosomes as potential biomarkers for inflammatory disorders.

DISEASE	MODEL	EXOSOME SOURCE	BIOMARKER	REFERENCE
Rheumatoid arthritis	Patients	Serum	Hotair, the HOX transcript antisense RNA	82
	Patients	Synovial	Citrullinated proteins	75
Systemic lupus	Patients	Urine	Lower levels of miR-26a, miR-29c, higher levels of miR-146a	69–71
Alcoholic hepatitis	Mouse model of alcoholic hepatitis and patients	Serum and plasma	miRNA-192 and miRNA-30a	83
Sjögren's syndrome	Patients	Saliva	miR-let7b, miR-let 7c, miR-128 hsa-miR-4524b-3p, hsa-miR-4524b-5p, hsa-miR-5571–3p, hsa-miR-5571–5p, hsa-miR-5100, and hsa-miR-5572	72,73
Inflammatory bowel disease	Dextran sulfate sodium (DSS) induced colitis mouse model	Serum	56 differentially expressed proteins identified by proteomics	84
	Patients	Serum	Annexin-A1	76
Chronic hepatitis C	Patients	Plasma	HCV RNA level in the exosomes was 3–20-fold higher than that in exosome-free fractions	85
Complex regional pain syndrome	Patients	Serum	miRNA profiling showed differential expression of 127 miRNAs compared to control	31
Systemic sepsis	Mouse cecal ligation and puncture	Serum	Increase in exosomal expression of miR-16, miR-17, miR-20a, miR-20b, miR-26a, and miR-26b	86

exosomes were derived. The absence of pain and swelling in saline-treated paws after exosome injection demonstrates that exosomal delivery does not produce a proinflammatory response. Attenuation of thermal hyperalgesia by macrophage-derived exosomes in CFA-treated animals could reflect the temporal regulation that exosomes can mediate by synergistically influencing multiple inflammatory pathways through the delivery of immediate-acting biomolecules such as cytokines and those that are translation dependent to induce changes in recipient cells by influencing gene transcription.[31]

There are also reports of anti-inflammatory drugs inducing alterations in exosome composition. Sulfasalazine and methotrexate are used in the management of RA due to their anti-inflammatory properties.[67] Treatments of human synovial sarcoma cell line SW982 with sulfasalazine and methotrexate altered the protein profiles of exosomes. Combination of the two drugs suppressed part of the protein profile changes induced by *IL-1β*. Most of the identified proteins were immunity- or anti-oxidation-related proteins[68] and the authors suggest that exosomes may have a role in mediating the actions of anti-inflammatory drugs.

Exosomal contents can also serve as biomarkers for inflammatory disorders. The presence of exosomes in bodily fluids, a biomolecular composition rich in RNA, proteins, and lipids, along with the plasticity of exosomal content in response to various physiological stimuli and pathological states render exosomes an ideal biomarker for disease state. Table 1 lists the inflammatory disorders for which exosomes were investigated as potential biomarkers. miRNAs transported by exosomes may serve as biomarkers for several inflammatory and autoimmune disorders including systemic lupus[69–71] and Sjögren's syndrome.[72,73] The number of miRNAs that are differentially expressed in exosomes from patients with complex regional pain syndrome were much higher than that observed in whole blood (18 in whole blood[74] vs 127 in exosomes[31]), suggesting that exosomes may be a better source for biomarker studies. Exosomes also transport important protein biomarkers such as citrullinated proteins in RA[75] and annexin-A1 in inflammatory bowel diseases.[76]

Conclusions

Immense interest in understanding the role of exosomes, and all EVs in general, in both normal physiology and disease states is continuing to grow. Immunomodulatory functions of exosomes from DCs have been explored in clinical trials for cancer and will provide guidance, as therapeutic approaches are being pursued for other inflammatory disorders. Exosomes hold immense promise for therapeutic drug delivery. They confer a number of advantages over other vector-or liposome-based delivery methods. They act as natural, non-toxic membranous nanocarriers of bio-macromolecules for effective delivery of the desired compound. Exosomes isolated from the recipient's own body fluids or cell culture (autologous exosomes) can be used for loading biological drugs.[77] Exosomal composition is also being explored as biomarkers and as a noninvasive approach for early diagnosis of various disorders.[78] The role of exosomes in normal physiology is less explored. There is concerted effort from the EVs community to standardize the purification methods and this is crucial in validating published reports.[79–81] Exosome biology will undoubtedly be important in the implementation of strategies to make precision medicine a reality for a broad spectrum of diseases.

Author Contributions

Wrote the first draft of the manuscript: BS, SA. Developed the structure and arguments for the paper: SA. Made critical revisions: SA. Both authors reviewed and approved of the final manuscript.

REFERENCES

1. Kreuger J, Phillipson M. Targeting vascular and leukocyte communication in angiogenesis, inflammation and fibrosis. *Nat Rev Drug Discov*. 2016; 15(2):125–42.
2. El Andaloussi S, Mager I, Breakefield XO, Wood MJA. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat Rev Drug Discov*. 2013;12(5):347–57.
3. Mittelbrunn M, Gutierrez-Vazquez C, Villarroya-Beltri C, et al. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. *Nat Commun*. 2011;2:282.
4. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. Jun. 2007;9(6):654–9.
5. Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol*. 2009;9(8):581–93.
6. Greening DW, Gopal SK, Xu R, Simpson RJ, Chen W. Exosomes and their roles in immune regulation and cancer. *Semin Cell Dev Biol*. 2015;40:72–81.
7. Pitt JM, Kroemer G, Zitvogel L. Extracellular vesicles: masters of intercellular communication and potential clinical interventions. *J Clin Invest*. 2016;126(4):1139–43.
8. Tkach M, Thery C. Communication by extracellular vesicles: where we are and where we need to go. *Cell*. 2016;164(6):1226–32.
9. Lötvall J, Hill AF, Hochberg F, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. *J Extracell Vesicles*. 2014;3:26913.
10. Thery C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol*. 2006;Chapter 3:Unit322.
11. Gyorgy B, Szabo TG, Pasztoi M, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell Mol Life Sci*. 2011;68(16): 2667–88.
12. Chalmin F, Ladoire S, Mignot G, et al. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. *J Clin Invest*. 2010;120(2):457–71.
13. Sun D, Zhuang X, Zhang S, et al. Exosomes are endogenous nanoparticles that can deliver biological information between cells. *Adv Drug Deliv Rev*. 2013;65(3):342–7.
14. Gangoda L, Boukouris S, Liem M, Kalra H, Mathivanan S. Extracellular vesicles including exosomes are mediators of signal transduction: are they protective or pathogenic? *Proteomics*. 2015;15(2–3):260–71.
15. Zhang B, Yin Y, Lai RC, Lim SK. Immunotherapeutic potential of extracellular vesicles. *Front Immunol*. 2014;5:518.
16. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol*. 2007;81(1):1–5.
17. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin Microbiol Rev*. 2009;22(2):240–73.
18. Vivier E, Malissen B. Innate and adaptive immunity: specificities and signaling hierarchies revisited. *Nat Immunol*. 2005;6(1):17–21.
19. Schenten D, Medzhitov R. Chapter 3 – the control of adaptive immune responses by the innate immune system. In: Frederick WA, ed. *Advances in Immunology*. Vol 109. Academic Press; 2011:87–124.
20. Théry C, Amigorena S. The cell biology of antigen presentation in dendritic cells. *Curr Opin Immunol*. 2001;13(1):45–51.

21. Chaput N, Théry C. Exosomes: immune properties and potential clinical implementations. *Semin Immunopathol*. 2011;33(5):419–40.
22. Huang X, Yuan T, Tschannen M, et al. Characterization of human plasma-derived exosomal RNAs by deep sequencing. *BMC Genomics*. 2013;14(1):1–14.
23. Koh W, Sheng CT, Tan B, et al. Analysis of deep sequencing microRNA expression profile from human embryonic stem cells derived mesenchymal stem cells reveals possible role of let-7 microRNA family in downstream targeting of hepatic nuclear factor 4 alpha. *BMC Genomics*. 2010;11(suppl 1):S6.
24. Nolte-'t Hoen EN, Buermans HP, Waasdorp M, Stoorvogel W, Wauben MH, t Hoen PA. Deep sequencing of RNA from immune cell-derived vesicles uncovers the selective incorporation of small non-coding RNA biotypes with potential regulatory functions. *Nucleic Acids Res*. 2012;40(18):9272–85.
25. Skog J, Würdinger T, Van Rijn S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol*. 2008;10(12):1470–6.
26. Hong BS, Cho JH, Kim H, et al. Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. *BMC Genomics*. 2009;10:556.
27. Xiao D, Ohlendorf J, Chen Y, et al. Identifying mRNA, microRNA and protein profiles of melanoma exosomes. *PLoS One*. 2012;7(10):e46874.
28. Skog J, Wurdinger T, van Rijn S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol*. 2008;10(12):1470–6.
29. Bhatt DM, Pandya-Jones A, Tong AJ, et al. Transcript dynamics of proinflammatory genes revealed by sequence analysis of subcellular RNA fractions. *Cell*. 2012;150(2):279–90.
30. McCall CE, El Gazzar M, Liu T, Vachharajani V, Yoza B. Epigenetics, bioenergetics, and microRNA coordinate gene-specific reprogramming during acute systemic inflammation. *J Leukoc Biol*. 2011;90(3):439–46.
31. McDonald MK, Tian Y, Qureshi RA, et al. Functional significance of macrophage-derived exosomes in inflammation and pain. *Pain*. 2014;155(8):1527–39.
32. Fabbri M, Paone A, Calore F, et al. MicroRNAs bind to toll-like receptors to induce prometastatic inflammatory response. *Proc Natl Acad Sci U S A*. 2012;109(31):E2110–6.
33. Stoorvogel W. Functional transfer of microRNA by exosomes. *Blood*. 2012; 119(3):646–8.
34. Squadrito Mario L, Baer C, Burdet F, et al. Endogenous RNAs modulate MicroRNA sorting to exosomes and transfer to acceptor cells. *Cell Rep*. 2014; 8(5):1432–46.
35. Kosaka N, Iguchi H, Hagiwara K, Yoshioka Y, Takeshita F, Ochiya T. Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic MicroRNAs regulate cancer cell metastasis. *J Biol Chem*. 2013;288(15):10849–59.
36. Villarroya-Beltri C, Baixauli F, Gutierrez-Vazquez C, Sanchez-Madrid F, Mittelbrunn M. Sorting it out: regulation of exosome loading. *Semin Cancer Biol*. 2014;28:3–13.
37. Koppers-Lalic D, Hackenberg M, Bijnsdorp Irene V, et al. Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes. *Cell Rep*. 2014;8(6):1649–58.
38. Gibbings DJ, Ciaudo C, Erhardt M, Voinnet O. Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nat Cell Biol*. 2009;11(9):1143–9.
39. Zhang J, Li S, Li L, et al. Exosome and exosomal MicroRNA: trafficking, sorting, and function. *Genomics Proteomics Bioinformatics*. 2015;13(1): 17–24.
40. Dustin ML. Signaling at neuro/immune synapses. *J Clin Invest*. 2012; 122(4):1149–55.
41. Hsu S-D, Lin F-M, Wu W-Y, et al. miRTarBase: a database curates experimentally validated microRNA–target interactions. *Nucleic Acids Res*. 2011;39(suppl 1): D163–9.
42. Batagov AO, Kurochkin IV. Exosomes secreted by human cells transport largely mRNA fragments that are enriched in the 3′-untranslated regions. *Biol Direct*. 2013;8(1):1–8.
43. Kowal J, Arras G, Colombo M, et al. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proc Natl Acad Sci U S A*. 2016;113(8):E968–77.
44. Kowal J, Tkach M, Théry C. Biogenesis and secretion of exosomes. *Curr Opin Cell Biol*. 2014;29:116–25.
45. Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. ExoCarta 2012: database of exosomal proteins, RNA and lipids. *Nucl Acids Res*. 2012;40(1):D1241–44.
46. Kalra H, Simpson RJ, Ji H, et al. Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. *PLoS Biol*. 2012;10(12): e1001450.
47. Kim D-K, Lee J, Simpson RJ, Lötvall J, Gho YS. EVpedia: a community web resource for prokaryotic and eukaryotic extracellular vesicles research. *Semin Cell Dev Biol*. 2015;40:4–7.
48. Colombo M, Raposo G, Thery C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol*. 2014;30:255–89.
49. Yáñez-Mó M, Siljander PR-M, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles*. 2015;4:27066.
50. Colino J, Snapper CM. Exosomes from bone marrow dendritic cells pulsed with diphtheria toxoid preferentially induce type 1 antigen-specific IgG responses in naive recipients in the absence of free antigen. *J Immunol*. 2006;177(6): 3757–62.
51. Segura E, Nicco C, Lombard B, et al. ICAM-1 on exosomes from mature dendritic cells is critical for efficient naive T-cell priming. *Blood*. 2005;106(1):216–23.
52. Montecalvo A, Larregina AT, Shufesky WJ, et al. Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. *Blood*. 2012;119(3):756–66.
53. Zitvogel L, Regnault A, Lozier A, et al. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat Med*. 1998;4(5):594–600.
54. Pitt JM, Charrier M, Viaud S, et al. Dendritic cell–derived exosomes as immunotherapies in the fight against cancer. *J Immunol*. 2014;193(3):1006–11.
55. Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol*. 2014;14(3):195–208.
56. Escudier B, Dorval T, Chaput N, et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of the first phase I clinical trial. *J Transl Med*. 2005;3(1):1–13.
57. Morse MA, Garst J, Osada T, et al. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. *J Transl Med*. 2005; 3(1):9.
58. Viaud S, Ploix S, Lapierre V, et al. Updated technology to produce highly immunogenic dendritic cell-derived exosomes of clinical grade: a critical role of interferon-gamma. *J Immunother*. 2011;34(1):65–75.
59. Besse B, Charrier M, Lapierre V, et al. Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC. *Oncoimmunology*. 2016;5(4):e1071008.
60. Yang C, Robbins PD. Immunosuppressive exosomes: a new approach for treating arthritis. *Int J Rheumatol*. 2012;2012:573528.
61. Kim SH, Kim S, Evans CH, Ghivizzani SC, Oligino T, Robbins PD. Effective treatment of established murine collagen-induced arthritis by systemic administration of dendritic cells genetically modified to express IL-4. *J Immunol*. 2001;166(5):3499–505.
62. Kim SH, Kim S, Oligino TJ, Robbins PD. Effective treatment of established mouse collagen-induced arthritis by systemic administration of dendritic cells genetically modified to express FasL. *Mol Ther*. 2002;6(5):584–90.
63. Kim SH, Lechman ER, Bianco N, et al. Exosomes derived from IL-10-treated dendritic cells can suppress inflammation and collagen-induced arthritis. *J Immunol*. 2005;174(10):6440–8.
64. Kim SH, Bianco N, Menon R, et al. Exosomes derived from genetically modified DC expressing FasL are anti-inflammatory and immunosuppressive. *Mol Ther*. 2006;13(2):289–300.
65. Bianco NR, Kim SH, Ruffner MA, Robbins PD. Therapeutic effect of exosomes from indoleamine 2,3-dioxygenase-positive dendritic cells in collagen-induced arthritis and delayed-type hypersensitivity disease models. *Arthritis Rheum*. 2009;60(2):380–9.
66. Yang X, Meng S, Jiang H, Chen T, Wu W. Exosomes derived from interleukin-10-treated dendritic cells can inhibit trinitrobenzene sulfonic acid-induced rat colitis. *Scand J Gastroenterol*. 2010;45(10):1168–77.
67. Dougados M, Combe B, Cantagrel A, et al. Combination therapy in early rheumatoid arthritis: a randomised, controlled, double blind 52 week clinical trial of sulphasalazine and methotrexate compared with the single components. *Ann Rheum Dis*. 1999;58(4):220–5.
68. Tsuno H, Suematsu N, Sato T, et al. Effects of methotrexate and salazosulfapyridine on protein profiles of exosomes derived from a human synovial sarcoma cell line of SW982. *Proteomics Clin Appl*. 2016;10(2):164–71.
69. Ichii O, Otsuka-Kanazawa S, Horino T, et al. Decreased miR-26a expression correlates with the progression of podocyte injury in autoimmune glomerulonephritis. *PLoS One*. 2014;9(10):e110383.
70. Sole C, Cortes-Hernandez J, Felip ML, Vidal M, Ordi-Ros J. miR-29c in urinary exosomes as predictor of early renal fibrosis in lupus nephritis. *Nephrol Dial Transplant*. 2015;30(9):1488–96.
71. Perez-Hernandez J, Forner MJ, Pinto C, Chaves FJ, Cortes R, Redon J. Increased urinary exosomal MicroRNAs in patients with systemic lupus erythematosus. *PLoS One*. 2015;10(9):e0138618.
72. Michael A, Bajracharya SD, Yuen PS, et al. Exosomes from human saliva as a source of microRNA biomarkers. *Oral Dis*. 2010;16(1):34–8.
73. Tandon M, Gallo A, Jang SI, Illei GG, Alevizos I. Deep sequencing of short RNAs reveals novel microRNAs in minor salivary glands of patients with Sjogren's syndrome. *Oral Dis*. 2012;18(2):127–31.
74. Orlova IA, Alexander GM, Qureshi RA, et al. MicroRNA modulation in complex regional pain syndrome. *J Transl Med*. 2011;9(1):195.
75. Skriner K, Adolph K, Jungblut PR, Burmester GR. Association of citrullinated proteins with synovial exosomes. *Arthritis Rheum*. 2006;54(12):3809–14.

76. Leoni G, Neumann PA, Kamaly N, et al. Annexin A1-containing extracellular vesicles and polymeric nanoparticles promote epithelial wound repair. *J Clin Invest*. 2015;125(3):1215–27.
77. Marcus ME, Leonard JN. FedExosomes: engineering therapeutic biological nanoparticles that truly deliver. *Pharmaceuticals (Basel)*. 2013;6(5):659–80.
78. Properzi F, Logozzi M, Fais S. Exosomes: the future of biomarkers in medicine. *Biomark Med*. 2013;7(5):769–78.
79. Xu R, Greening DW, Zhu HJ, Takahashi N, Simpson RJ. Extracellular vesicle isolation and characterization: toward clinical application. *J Clin Invest*. 2016;126(4):1152–62.
80. Witwer KW, Buzás EI, Bemis LT, et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *J Extracell Vesicles*. 2013;2. http://www.journalofextracellularvesicles.net/index.php/jev/rt/captureCite/20360/0/CbeCitationPlugin. doi: 10.3402/jev.v2i0.20360. eCollection 2013.
81. Witwer KW. Circulating MicroRNA biomarker studies: pitfalls and potential solutions. *Clin Chem*. 2015;61(1):56–63.
82. Song J, Kim D, Han J, Kim Y, Lee M, Jin EJ. PBMC and exosome-derived Hotair is a critical regulator and potent marker for rheumatoid arthritis. *Clin Exp Med*. 2015;15(1):121–6.
83. Momen-Heravi F, Saha B, Kodys K, Catalano D, Satishchandran A, Szabo G. Increased number of circulating exosomes and their microRNA cargos are potential novel biomarkers in alcoholic hepatitis. *J Transl Med*. 2015;13:261.
84. Wong WY, Lee MM, Chan BD, et al. Proteomic profiling of dextran sulfate sodium induced acute ulcerative colitis mice serum exosomes and their immunomodulatory impact on macrophages. *Proteomics*. 2016;16(7):1131–45.
85. Liu Z, Zhang X, Yu Q, He JJ. Exosome-associated hepatitis C virus in cell cultures and patient plasma. *Biochem Biophys Res Commun*. 2014;455(3–4):218–22.
86. Wu SC, Yang JC, Rau CS, et al. Profiling circulating microRNA expression in experimental sepsis using cecal ligation and puncture. *PLoS One*. 2013; 8(10):e77936.