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BASIC AND APPLIED SCIENCE (I LEWKOWICH, SECTION EDITOR)

Exosomes in Allergic Airway Diseases

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Abstract

Purpose of Review This review will cover what is known regarding exosomes and allergy, and furthermore discuss novel mechanism of exosome-mediated immune modulation and metabolic regulation via the transfer of mitochondria.

Recent Findings Exosomes are nano-sized extracellular vesicles (EVs) derived from the endosome that play a direct role in governing physiological and pathological conditions by transferring bioactive cargo such as proteins, enzymes, nucleic acids (miRNA, mRNA, DNA), and metabolites. Recent evidence suggest that exosomes may signal in autocrine but, most importantly, in paracrine and endocrine manner, being taken up by neighboring cells or carried to distant sites. Exosomes also mediate immunogenic responses, such as antigen presentation and inflammation. In asthma and allergy, exosomes facilitate cross-talk between immune and epithelial cells, and drive site-specific inflammation through the generation of pro-inflammatory mediators like leukotrienes. Recent studies suggest that myeloid cell-generated exosomes transfer mitochondria to lymphocytes.

Summary Exosomes are nano-sized mediators of the immune system which can modulate responses through antigen presentation, and the transfer of pro- and anti-inflammatory mediators. In addition to conventional mechanisms of immune modulation, exosomes may act as a novel courier of functional mitochondria that is capable of modulating the recipient cells bioenergetics, resulting in altered cellular responses. The transfer of mitochondria and modulation of bioenergetics may result in immune activation or dampening depending on the context.

Keywords Exosomes · Asthma · Allergy · Extracellular vesicles · Mito-exosomes · MDRCs

Introduction

Allergy is a multifaceted immunologic disease where our innate and adaptive defense mechanisms become activated by what should be a benign signal, resulting in rampant and deregulated immune responses and chronic inflammation [1]. Many different cell types are involved and they each secrete unique soluble mediators of inflammation that drive disease pathology [1, 2]. Some well described cell types include, but are not limited to, $CD4^+$ T cells (Th2, Th17 and hybrid Th2/Th17 subsets) [3, 4], dendritic cells (DCs) [5, 6], macrophages, myeloid-derived regulatory cells (MDRCs) [6–12], natural killer (NK) cells [13–15], and epithelial cells [5, 16, 17]. For an effective immune system, various signaling mechanisms must come to play. The same is true in the case of allergy, where a coordinated, albeit inappropriate, immune signaling cascade results in persistent inflammation that is harmful for the host. Generally, these signaling cascades are mediated by soluble factors, such as cytokines and chemokines, as well as membrane-bound receptors, such as class-II molecules, the CD1 family of receptors, and Fcε receptor [1, 2]. Class-II molecules, such as HLA-DR, are antigen presentation molecules usually found on antigen presenting cells which play an important role in activating $CD4^+$ T cells through the engagement of the T cell receptor (TCR) [18–20]. The CD1 family of receptors is a type of scavenger molecule found on macrophages and dendritic cells that can activate T cells [21–23]. These scavenger receptors recognize foreign lipids, such as of bacterial and fungal origin [23, 24]. Fcε receptor is an important player in allergy and is found on mast cells and basophils [25]. This receptor binds free IgE and activates degranulation of mast cells and basophils. All three of these molecules play an important role in activating the immune system, and have been found on exosomes.

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Extracellular vesicles (EVs), such as exosomes, are essentially couriers of bioactive material, such as nucleotides, proteins, lipids, and metabolites, which have a substantial impact on the phenotype of the recipient cell [26]. In recent years, exosomes, which are secreted by many types of cells, have emerged as key-signaling mediators in various immunologic diseases [27]. The roles of exosomes pertaining to lung pathology are being increasingly described. In particular, exosomes are being appreciated as immunogenic potentiators especially in the context of allergy [28, 29•, 30, 31, 32•]. Many studies have reported a pro-inflammatory role of exosomes in allergy as well as in asthma [29•, 30, 31, 32•]. Exosomes have been described to transfer pro-inflammatory mediators, such as leukotrienes, and process antigens on surface class-II receptors [29•, 30, 32•, 33]. Similarly in allergic skin diseases, exosomes have been shown to transfer antigens that activate immune responses [30, 34]. In addition to host cell-generated exosomes, microbial EVs have also been implicated in immune activation and hypersensitivity [35–37].

In addition to transfer of bioactive materials, recent studies have also described the packaging and transfer of mitochondria via EVs and exosomes [38•, 39••, 40••]. The transfer of mitochondria results in alterations in the host cell bioenergetics and may have lasting consequences on cellular function and tissue homeostasis. For example, our laboratory has observed the transfer of exosomes containing mitochondria by myeloid-derived regulatory cells (MDRCs) and subsequent internalization of these exosomes by $CD4^+$ T lymphocytes [40••]. Together, the discovery of novel exosome-mediated mechanisms in modulating cellular and tissue homeostasis, and the host immune system will help us understand the intricate and complex mechanism of allergic disease pathology, which will indubitably aid in fruitful advances in the research and development of improved therapies. This review intends to explore in detail each of the unique mechanisms by which exosomes modulate immune responses in the context of asthma and other allergic diseases.

Exosome Biogenesis

Exosomes were first described in the calcification of collagen in the extracellular matrix [41]. Since then, various "blebbings" from cells have been described as extracellular vesicles. The classification and nomenclature to describe EVs have been based on mode of biogenesis and biochemical properties [42, 43]. Currently, exosomes are described as vesicles derived from the endosome and released to the extracellular space [44–47, 48••]. Tetraspanins are highly enriched in exosomes and are used as reliable markers of exosomes [49]. Tetraspanins are transmembrane proteins which interact with one another and with other transmembrane proteins, such as integrins and receptors, acting as a scaffold to organize surface proteins and support cellular signaling [50]. When studying exosomes, reliable markers used in the field include CD63, CD81, CD9, tumor susceptibility 101 (TSG101), and ALG-2 interacting protein X (ALIX). Specifically, endosomal markers or markers that are part of the endosomal sorting complexes required for transport (ESCRT) complex (such as TSG101, CD81, and ALIX) are preferred as they indicate an endosomal origin of the extracellular vesicle, which is part of the definition of an exosome [49, 51]. The biogenesis of exosomes starts with the outward invagination of the endosome, resulting in the formation of vesicles within the endosomal body, referred to as a multi-vesicular body (MVB) [44]. Although the biogenesis of exosomes results from the invagination of the endosome, the process is described as an "outward invagination" to clarify that the lipid bilayer topology is maintained throughout the biogenesis and secretion process. The MVB can either merge with a lysosome, resulting in the degradation of its cargo, or it can fuse with the cytoplasmic membrane, causing the release of exosomes into the extracellular space. Exosomes are released from various different cell types, and can be isolated from several sources of biological fluids, such as bronchoalveolar lavage (BAL) fluid, synovial fluid, serum, urine, breast milk, and semen [27, 33, 52–55]. Although the biological functions of these vesicles are still being characterized, and their association with disease being elucidated, exosomes have been thought to be part of a complex intercellular and systemic messaging system, that also play a role in cellular homeostasis via the autophagy pathways [26, 56]. Exosomes impart their effects on recipient cells through receptor interactions or by transfer of bioactive cargo [29•, 30, 57–60]. Studies have shown that in addition to antigen-specific activation, immune cells can use adhesion molecules to "capture" exosomes [61, 62]. We explore the various mechanisms by which exosomes can modulate the immune system in the following sections.

Exosomes and Inflammation

Exosomes have been described as efficient cell-to-cell messengers that can cross biological barriers and modulate the immune system [26, 27, 63–65]. Inflammation can be triggered by many mechanisms, such as antigen presentation, cytokines, chemokines, leukotrienes, and other lipid mediators of inflammation. Exosomes have been characterized with several membrane-associated immunogenic markers found on the surface, such as class-I and class-II major histocompatibility complex (MHC) molecules, co-stimulatory molecules (CD86, CD80, and CD54), and even functional enzymes that produce lipid mediators of inflammation [30, 31, 55, 66–68].

Antigen loaded exosomes have been demonstrated to induce strong antigen-specific immune responses. Specifically, dendritic cells pulsed with antigens produce exosomes that can activate $CD8^+$ T cells in an antigen-specific manner likely through antigen presentation by MHCI-peptide complexes

[69••]. Antigen-loaded exosomes, as well as peptide class-II complexes associated with exosomes, have been found to be attached to the surface of follicular dendritic cells (FDCs). This exosome-mediated transfer of antigens is suggested as a mechanism by which exosomes can promote antigen-specific activation of T and B cells in primary and secondary lymphoid nodes [70]. Furthermore, adhesion of exosomes to the surface of FDCs is through the oligomerization and binding of tetraspanins between the exosomes and FDCs. We speculate that the adhesion of exosomes may be facilitated by adhesion molecules such as CD54 [71]. CD54 (ICAM-1) has been reported by others and our lab to be expressed on exosomes [29•, 33, 72]. Segura et al. have shown that $CD8^+$ dendritic cells use LFA-1 (the ligand for CD54) to capture MHC-peptide complexes from exosomes [61]. Furthermore, Hao et al. have reported that the internalization of exosomes in immune cells may be mediated by CD54/LFA-1 interactions on dendritic cells [73]. Nolte-'t Hoen et al. have also shown that LFA-1 is important for the recruitment of exosomes to T cells and their subsequent activation [62]. Bone marrow-derived mesenchymal stromal cells internalized PC12 pheochromocytoma cell-derived exosomes through clathrin-dependent endocytosis, resulting in delivery of miR-21 [74]. Additionally, endothelial cells have been shown to internalize exosomes via a dynamin-dependent matter through endocytosis [75]. Together, these observations suggest different modes of internalization that may be cell-type specific. Furthermore that the effects imparted by the exosomes are multi-modal (receptor-ligand interaction, or through transfer of cargo).

Exosomes have been found to transfer or even help generate pro-inflammatory lipid mediators. For example, exosomes from human macrophages and dendritic cells contain enzymes for the biosynthesis of leukotrienes and promote migration of granulocytes [68]. Furthermore, pulmonary epithelial cell-derived exosomes metabolize myeloid cell-derived leukotriene C_4 to leukotriene D_4 [76]. In addition to leukotrienes, ceramides and sphingolipids have been found in exosomes and potentially implicated in inflammation [67, 77]. Pro-inflammatory cytokines, such as TNF-α and IFN-γ haven been shown to drive release of ceramides into exosomes, which become mediators of cell death signaling [78]. Additionally, hepatocytes have been shown to also release pro-inflammatory ceramide-enriched extracellular vesicles under stress [79].

Exosomes can package miRNA which have various different functional implications to the target cells which internalize these vesicles, and is often dependent on the context of disease. In one study, serum exosomes from rats treated with zinc oxide nanoparticles were identified with 16 different pro-inflammatory miRNAs [80]. Additionally, miR-155 and miR-146a, two pro-inflammatory miRNAs, were found enriched in exosomes purified from dendritic cells following treatment with endotoxin [60]. From a clinical perspective, a pro-inflammatory miRNA signature was found from serum exosomes isolated from septic patients admitted to the ICU [81]. In asthma, the exosomes from human bronchoalveolar lavage fluid have been found to contain miRNAs with pro-inflammatory signatures [31]. Several miRNA involved in immune modulation, including miR-27 and miR-24, (important for Th2 responses [82]), miR-21 (important for metabolic regulation of pathogenic Th17 [83]), and Let-7c (M2 polarization) were identified in exosomes from asthmatics [84]. This indicates that exosomal transfer of miRNA can modulate gene programing and promote inflammation in an antigen-independent manner.

Novel Mechanisms of Exosome-Mediators

As discussed earlier, exosomes are couriers of various biological cargo with functional effects [26]. In recent years, exosomes and other extracellular vesicles (EVs) have been shown to alter cellular metabolism by transfer of metabolites to recipient cells or by altering regulation of metabolic enzyme pathways [39••, 40••, 85–90]. Metabolism has been appreciated beyond fulfilling cellular energy requirements, and is connected to various cellular processing such as epigenetic control [91, 92] and gene regulation [93, 94]. The cellular changes induced by metabolism then may impact at an organismal level, such as in immune response [95, 96], tissue repair [97, 98], and disease pathology [99–101]. In particular, the transfer of mitochondria from one cell to another has garnered much attention as a novel mechanism of cellular energetic repair [39••, 85–90].

The transfer of mitochondria from one cell to another has been previously described through a structural mechanism called tunneling nanotubes (TNTs) (Fig. 1) [102–106]. TNTs are membrane nanotube protrusions that extend from the

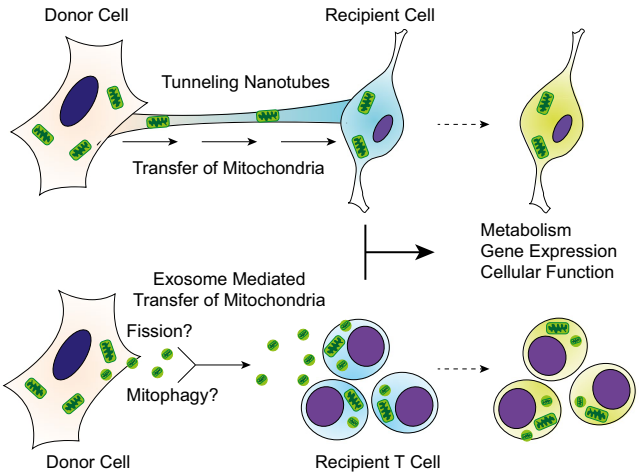

Fig. 1 Mechanism for the transfer of mitochondria between cells. Tunneling nanotubes and extracellular vesicles, such as exosomes, have been shown to carry mitochondria from donor to recipient cells

plasma membrane and bridge the cytoplasm of two cells over a distance [103, 105]. Jackson et al. have shown that TNTs are important for the transfer of functional mitochondria from mesenchymal stem cells (MSCs) to macrophages to promote antimicrobial functions in in vitro and in vivo models of acute respiratory distress syndrome (ARDS) [107]. Their study demonstrates that the transfer of mitochondria from MSCs to macrophages increases their bioenergetics and phagocytic activity. However, inhibition of TNT formation by cytochalasin B did not completely block intercellular transfer of mitochondria, suggesting an alternative cell-contact independent mechanism.

Transfer of mitochondria from MSCs to macrophages can occur in EVs secreted from MSCs [39••]. Morrison et al. demonstrate that the MSC-derived EVs contain mitochondria, and when transferred to macrophages, promote M2 polarization and enhance oxidative phosphorylation. The authors further demonstrate that functional mitochondria are being transferred by MSCs to macrophages by showing that EVs from rhodamine-treated MSCs, which generate dysfunctional mitochondria, have no effect on macrophages. These results are supported by other studies that illustrate MSC-derived EVs can recapitulate the beneficial effects of cell-based MSC therapies [87–90].

The ability of cells to release EVs containing mitochondria has been previously described [38•, 86]. Our lab has also reported that exosomes from bronchoalveolar lavage (BAL) fluid of asthmatics and exosomes derived from myeloid-derived regulatory cells from the airways of asthmatics contain mitochondria, which can be internalized by $CD4^+$ T lymphocytes [40••]. We observe that functional mitochondria that are capable of producing ROS are internalized by $CD4^+$ T cells and merge with the host mitochondrial network. Our studies align with published reports that implicate the importance of mitochondria transfer by EVs and their role in altering cellular function in response to injury and inflammation.

The transfer of healthy mitochondria to cells with damaged mitochondria is an important mechanism for cellular repair. Human mesenchymal stem cells (hMSCs) were shown to package healthy mitochondria inside membrane-bound vesicles that were secreted and subsequently acquired by epithelial cells that were co-cultured in vitro [85]. The study shows that when cultured with A549 ρ° (ρ° phenotype lack mitochondrial DNA) that have defective mitochondria, the transfer of mitochondria by hMSC-derived EVs rescued metabolic activity and aerobic respiration in the A549 ρ° cells.

Functional mitochondrial complex proteins have been reported in exosomes, and viable for the generation of ATP [108•]. Panfoli et al. report that hMSCs from > 37-week old, newborns generated exosomes that contained functional complex proteins that were capable of generating ATP while, hMSC from 28 to 30-week old, newborns generated exosomes that were unable to produce ATP despite having mitochondria complex proteins [108•]. They implicate this difference as potential vulnerability factors between newborn and preterm, such as reduced ability to cope with anoxic environments and repair damaged tissue in preterm.

In addition to the transfer of healthy mitochondria, cells may use EVs to package damaged mitochondria as a danger signal to others as a result of disease pathology, and to maintain mitochondrial quality control [109, 110]. Studies have demonstrated that mitochondria can generate vesicles of various types that are shuttled to the lysosome [111] or peroxisome for degradation [112]. This pathway shares the same pathway as exosome generation—through the late endosome and multivesicular body [111]—and thus would not be alarming if these mitochondrially derived vesicles (MDVs) were secreted. Cells that have damaged mitochondria are undergoing cellular stress that may overwhelm or even shutdown mitophagy and autophagy pathways. The unique coincidence that these pathways are shared with exosome generation may suggest an alternative survival mechanism for cells to shed damaged cellular components extracellular while attempting to regain homeostasis. To support this theory, Davis et al. have shown that damaged mitochondria can be transported to adjacent cells to aid in degradation, which they have coined the term transmitophagy [113].

Conclusions

In allergy, exosomes have been shown to activate T cells in an antigen-specific manner without the need of an APC [30]. The activation is most likely through the engagement of MHCII-peptide complexes on the surface of exosomes with the TCR of $CD4^+$ T cells. The modulation of the immune system by exosomes is not limited to surface receptor interactions. Transfer of RNA by exosomes, such as those produced by mast cells, can alter the transcriptomic landscape of the recipient cell, potentially promoting upregulation of pro-inflammatory genes [28, 59, 60]. Together, exosomes can activate T cells and other immune subsets in a multi-modal manner, such as receptor interaction or fusion and cargo transfer, without the aid of traditional APCs.

Furthermore, we gather that the transfer of mitochondria between cells is not an uncommon occurrence and happens in both healthy and diseases states. Furthermore, new evidence suggests that mitochondria can be packaged into extracellular vesicles, such as exosomes, and transported to recipient cells. Importantly, this transfer has the ability to induce functional changes to the recipient cell, implicating the potential for such transfer to be important in cellular homeostasis and disease pathogenesis. Although the exact pathway by which exosome package mitochondria is still unknown, we speculate that two possible mechanisms exist: 1. mitochondrial fission; and 2. mitophagy. Proteins such as Drp1 induce fission of mitochondria that may promote their packaging into exosomes or other types of EVs. Similarly, the mitophagy

pathway may shuttle mitochondria through pathways that are shared with exosome biogenesis. These pathways need to be studied in the context of exosome biogenesis and mitochondrial packaging to better understand how cellular organelles, such as the mitochondria, can be packaged and delivered.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

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- • Of importance
- •• Of major importance

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