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Extracellular Vesicles and Asthma: A Systematic Review

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Extracellular Vesicles and Asthma: a review of the literature

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Abstract

Asthma is a chronic, recurrent, and incurable allergy-related respiratory disease characterised by inflammation, bronchial hyper-responsiveness and narrowing of the airways. Extracellular vesicles (EVs) are a universal feature of cellular function and can be detected in different bodily fluids. Recent evidence has shown the possibility of using EVs in understanding the pathogenesis of asthma, including their potential as diagnostic and therapeutic tools. Studies have reported that EVs released from key cells involved in asthma can induce priming and activation of other asthma associated cells. A literature review was conducted on all current research regarding the role and function of EVs in the pathogenesis of asthma via the PRISMA statement method. An electronic search was performed using EMBASE and PubMed through to November 2018. The EMBASE search returned 76 papers while the PubMed search returned 211 papers. Following duplicate removal, titles and abstracts were screened for eligibility with a total of 34 studies included in the final qualitative analysis. The review found evidence of association between the presence of EVs and physiological changes characteristic of asthma, suggesting that EVs are involved the pathogenesis, with the weight of evidence presently favouring deleterious effects of EVs in asthma. Numerous studies highlighted differences in exosomal contents between EVs of healthy and asthmatic individuals, which could be employed as potential diagnostic markers. In some circumstances, EVs were also found to be suppressive to disease, but more often promote inflammation and airway remodelling. In conclusion, EVs hold immense potential in understanding the pathophysiology of asthma, and as diagnostic and therapeutic markers. While more research is needed for definitive conclusions and their application in medical practice, the literature presented in this review should encourage further research and discovery within the field of EVs and asthma.

1 INTRODUCTION

Asthma is a chronic, recurrent, and incurable allergy-related respiratory disease characterised by inflammation, bronchial hyper-responsiveness and narrowing of the airways. An estimated 235 million people are afflicted by asthma worldwide, but it is often underdiagnosed and undertreated [1,2]. Both environmental and genetic factors are involved in the pathogenesis of asthma [3,4]. The immunohistopathologic features of asthma include granulocytic and lymphocytic infiltration, mast cell activation, and epithelial cell injury. In chronic cases, persistent changes in airway structure, including sub-basement fibrosis, mucus hypersecretion, injury to epithelial cells, smooth muscle hypertrophy, and angiogenesis, may occur. Interaction between diverse types of cells through mediators often result in airway inflammation and obstruction, as summarised in figure 1 [5-8].

Extracellular vesicles (EVs) are a universal feature of cellular function and can be detected in culture supernatants of different bodily fluids such as serum, broncho-alveolar lavage fluid (BALF), and nasal lavage fluid (NLF) [9]. Due to these characteristics, there has been an increased interest in EVs and their potential applications in understanding the underlying mechanisms of various lung diseases, their use as biomarkers and therapeutic tools in lung inflammation [10]. EVs are classified into three classes based on their biogenesis, secretory components, and size: exosomes, microvesicles (ectosomes), and apoptotic bodies [11].

Exosomes are 50-150 nm EVs of endosomal origin. They contain enriched amounts of certain surface markers including tetraspanins, heat shock proteins, and MHC classes I and II. These components can be transferred to target cells either by direct membrane fusion, endocytosis, phagocytosis, or ligand interaction [12,13]. Microvesicles (MVs) are larger than exosomes (100-2000 nm) and are derived from the plasma membrane of cells through direct outward budding. They contain substantial amounts of phosphatidylserine and membrane components as do their parent cells. Both exosomes and MVs contain elements which allow them to act as intercellular facilitators and release relevant signalling molecules [10,14]. Apoptotic bodies are released from cells that undergo apoptosis and are usually 1-4 μm in diameter. They may contain DNA fragments, non-coding RNAs, and cell organelles [15].

Recent evidence has shown the possibility of using EVs in understanding the pathogenesis of asthma, including their potential as diagnostic and therapeutic tools. Methods such as ultracentrifugation and ExoQuick precipitation allow EVs to be isolated and studied in detail. Studies have reported that EVs released from key cells involved in asthma can induce priming and activation of other asthma associated cells. The production of these EVs can be regulated by pro-inflammatory and oxidative stress stimuli *in vitro* [16-18]. For example, exosomes released from DCs containing costimulatory molecules and MHC class II can activate immune functions of Th2 cells within the lungs [19].

A multitude of research studies have investigated different pharmacological targets in asthma to better understand the pathophysiology of the disease and find novel treatments. In this respect, EVs have great potential, but there is currently insufficient research. In other respiratory diseases, EVs have already shown their potential. For example, microvesicles released from mesenchymal stem cells have been found to be cytoprotective in pulmonary fibrosis, a disease which is currently incurable [20]. DC-derived exosome-based cell-free vaccines have shown success in eradicating established murine tumours [21]. Much ongoing research exploring the potential of exosomes in chronic obstructive pulmonary disease (COPD), lung cancer, cystic fibrosis, primary ciliary dyskinesia, along with many others, is being conducted [22]. This article reviews all available research on EVs in the pathogenesis of asthma to identify existing knowledge and promote further research within the field. Highlighting hypotheses and noteworthy results from current literature also underlines the limitations of past studies, for future improvements. The specific research question we address is whether there is currently sufficient evidence to support pro-inflammatory, airway remodelling and/or protectives roles of cell-derived EVs in asthma.

2 METHODOLOGY

2.1 Sources and Searches

For this review, the PRISMA statement method was implemented. An electronic search was performed using EMBASE and PubMed (results up to November 2018).

The search term for EMBASE was

> ((Exosome* or "Extracellular vesicles" or "extracellular vesicle" or Microvesicle* or ectosome* or "shedding vesicle" or "shedding vesicles" or microparticle) and (asthma* or "type 1 hypersensitivity" or allergy* or bronchospasm* or "status asthmaticus")).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

and the search term for PubMed was

> ((Exosome* or "Extracellular vesicles" or "extracellular vesicle" or Microvesicle* or ectosome* or "shedding vesicle" or "shedding vesicles" or microparticle) and (asthma* or “type 1 hypersensitivity” or allergy* or bronchospasm* or “status asthmaticus”))

2.2 Study Selection

The aim of this review was to combine and interpret all current research on the role and functions of EVs of known cellular origins in the pathogenesis of asthma. Only primary research literature has been included. Research that was either unpublished or published in

non-peer reviewed forms (including abstracts and conference proceedings) were not included. Studies concerning exosomes derived from microorganisms and their effects in asthma were also excluded.

3 FINDINGS

3.1 Results of PRISMA statement Evidence Search and Selection

The **EMBASE** search returned 76 papers, while the **PubMed** search returned 211 papers. Search results were imported using the reference manager, ENDNOTE, to find and exclude duplicates. After duplicates were removed (reducing the number to 260), titles and abstracts were then screened for relevance to the research question: this led to further exclusions due to lack of relevance (e.g. wrong type of 'microparticles') or because publications were reviews rather than primary research (records excluded n = 197).

The remaining 63 studies were eligible for a full text review. A further 29 studies were excluded using inclusion and exclusion criteria (table 1). A total of 34 studies were included in the final qualitative analysis (figure 2). The first study was published in 2001, with numbers of publications increasing yearly until 2018, when nine studies were published (figure 3). There were not enough studies addressing very similar research questions to allow a meta-analysis of data to be conducted.

3.2 Extracellular Vesicles of Known Cellular Origin

Twenty-one studies found that EVs of known cellular origin are associated with asthma and allergic inflammation. Of these, four studies investigated EVs derived from eosinophils, three from mast cells, three from mesenchymal cells, two each from T-cells, DCs and epithelial cells, and one each from myeloid-derived regulatory cells, neutrophils, platelets, B-cells and fibroblasts (figure 4).

3.2.1 Mast cell-derived Extracellular Vesicles (Table 2)

Mast cells release histamine, prostaglandins, leukotrienes, and other inflammatory mediators which are capable of inducing an asthma attack [23]. Three studies have reported that exosomes from mast cells are associated with asthma and the lung inflammatory response. Skokos et al. [24] found that bone marrow-derived mouse mast cells (BMMC) and mast cell lines P815 and MC9-derived exosomes carried immunologically relevant molecules (i.e. MHC class II, LFA-1, ICAM-1 and CD86). The results indicated that these BMMC exosomes can induce splenic B and T cell blast activation without physical contact between the cells. This suggests that exosomes from mast cells may play a role in the recruitment of B and T cells to the lungs [24]. On the other hand, Xie et al. [25] reported that mast cell exosomes

containing high-affinity IgE receptors (FcεRI) can suppress allergic reactions by binding to free IgE. The study demonstrated that exosomes derived from BMMCs, which can bind to free IgE via FcεRI, possess anti-IgE effects, decrease IgE levels and inhibit mast cell activation [25]. The third study by Eldh et al. [26] suggests that mast cell-derived exosomes may have a protective effect on the oxidative stress of the lungs during an inflammatory response. The study established that exosomes released by mouse mast cells exposed to oxidative stress differ in their mRNA content and can influence the response of other cells to oxidative stress by providing the recipient cells with resistance.

3.2.2 Eosinophil-derived Extracellular Vesicles (Table 2)

Eosinophils are often found in severe types of asthma and are amongst the main effector cells in the disease. Elevated levels of eosinophils are found to cause airway inflammation and breathlessness [27,28]. Four trials suggested that exosomes from eosinophils are associated with asthma and the lung inflammatory response (Table 2). Mazzeo et al. [29] investigated exosome secretion by eosinophils. Exosomes were purified from eosinophils in the peripheral blood of asthmatic and healthy subjects. Results confirmed the secretion of exosomes by eosinophils and found that interferon-gamma (IFN-γ) stimulation led to an increase in the production of exosomes. Furthermore, they demonstrated that the production of exosomes was augmented in asthmatic patients [29]. Akuthota et al. [30] also investigated EVs released by eosinophils of allergic and healthy donors. They confirmed that eosinophils produced EVs under physiological conditions, and that exposure of eosinophils to CCL11 (which stimulates IL-4 production) and TNF-α (which stimulates of IL-4, IL-6 and IFN-γ production) increased EV formation.

Canas et al. [31] reported that the amount of exosome production was greater from eosinophils of asthmatic patients compared to healthy individuals. The rate of apoptosis of eosinophils of asthmatic patients was also reduced compared to healthy participants. The exosomes increased eosinophil production of ROS and NO, and promoted chemotaxis and adhesion of eosinophils. This suggests that eosinophil-derived exosomes can promote the inflammatory behaviour of eosinophils that is associated with asthma. Another study by the same group [32] demonstrated that eosinophil-derived exosomes participate in the pathological processes of asthma by activating structural lung epithelial cells and bronchial smooth muscle cells (BMSC). The results indicated that epithelial cell wound healing processes are reduced in the presence of eosinophil-derived exosomes due to increased apoptosis of epithelial cells. Analysis of epithelial cell gene expression also showed increased expression of *TNF*, *CCL26* and *POSTN* (periostin) genes which represent an asthma-specific mRNA signature [33]. Hyperplasia and hypertrophy of bronchial smooth muscle cells (BMSC) is thought to be part of the pathophysiology of the process. Data from the study showed significantly increased

proliferation of BMSCs when co-cultured with exosomes from eosinophils of asthmatic patients, as well as increased gene expression of CCR3 and VEGF A which are associated to angiogenesis and fibrotic processes [32]. In summary, the study shows significant involvement of eosinophil-derived exosomes in the pathophysiology and disease progress of asthma.

3.2.3 Neutrophil-derived Extracellular Vesicles (Table 2)

Although asthma has been classically associated with eosinophils, newer research has found that neutrophils may also play a role in the inflammation and airway remodelling of asthmatics, especially in severe cases [34-37]. Vargas et al. [37] investigated whether exosomes are released by neutrophils and evaluated their abilities in modulating airway smooth muscle cell proliferation in an equine model. The study concluded that exosomes released from activated neutrophils could promote growth of airway smooth muscle cells, indicating the potential for their role in airway remodelling in asthma patients [37].

3.2.4 B cell-derived Extracellular Vesicles (Table 3)

B-cells play a crucial role in the sensitization and adaptive immune response of asthma [38-41]. Admyre et al. [42] concluded that B cell-derived exosomes play a role in stimulating the allergic immune response. Exosomes isolated from B cells can present allergen-derived peptides to induce T-cell proliferation and production of Th2-like cytokines. B cells were isolated and transformed from four donors with a history of hay fever. B cell–derived exosomes were then loaded with biotinylated or un-biotinylated birch peptides, Bet v 1 peptides, Bet v 14-18, and/or Bet v 1142-156. The supernatant was analysed for a variety of inflammatory mediators. The results showed B cell–derived exosomes' ability to bind peptides derived from Bet v 1, induction of Bet v 1 peptides specific T-cell proliferation and induce cytokine production of allergen-specific T cells (IL-4, IL-5, and IL-13 and lower levels of IFN-γ and TNF-α) [43]. The mechanism by which B-cells-derived exosomes activate T-cells is still under debate, but some studies suggest that exosomes can stimulate T cells directly [42,44] whereas others demonstrate that exosomes need the presence of antigen presenting cells to exert their effects [45,46].

3.2.5 T cell-derived Extracellular Vesicles (Table 3)

Th2 cell-derived inflammation, leading to airway responsiveness and tissue remodelling, is one way asthma can be characterized [47-50]. Two studies show that exosomes from T-cells are associated with asthma and the lung inflammatory response. A study by Shefler et al. [51] concluded that T cell EVs carry similar signalling molecules to their cells of

origin and can induce mast cell degranulation and cytokine release. Activated T cells can utilize these EVs to facilitate the activation of mast cells at distant inflammatory sites. The study suggested that blocking the release of these MVs may serve as a therapeutic target. In a more recent study, the same authors [52] reported that T-cell derived MVs carry miR-4443, which might exert heterotypic regulation of protein tyrosine phosphatase receptor type J gene expression in mast cells causing mast cell activation.

3.2.6 Dendritic cell-derived Extracellular Vesicles (Table 3)

Dendritic cells (DCs) play a central role in sensing the presence of foreign antigens and infectious agents and presenting them to T cells. DCs also regulate the activation of allergic pathways in response to potential environmental allergens. Significant increases in the numbers of airway DCs have been reported after exposure to allergen and can lead to an inflammatory response [53]. Two studies have shown that exosomes derived from DCs play a similar role to DCs themselves in promoting asthma. A study by Vallhov et al. [19] explored whether DC-derived exosomes can present the major cat allergen Fel d 1 and contribute to the pathogenesis of allergic disease. Exosomes were isolated from monocyte derived DCs of cat-allergic donors that had been cultured alone, or co-cultured with Fel d 1. It was found that DCs could distribute aeroallergens via exosomes, and that these exosomes could present the allergen and induce Th2-like cytokine production.

A study by Wahlund et al. [54] investigated the immunostimulatory potential of both MVs and exosomes derived from ovalbumin (OVA)-pulsed DCs. Exosomes and MVs were isolated from OVA-pulsed murine bone marrow-derived DCs and were injected into recipient mice. The results indicated that both exosomes and MVs were equally enriched in immunostimulatory molecules, as seen by the surface expressions of MHC class I, class II, and costimulatory molecules CD40, CD80, CD86, and CD54. However, the ability to activate OVA-specific CD8+ T cells was exclusive to exosomes. Exosomes also induced higher levels of OVA-specific IgG antibodies compared to MVs. The study suggested that the superior ability of exosomes to induce OVA-specific T cell responsiveness was because OVA was enriched in exosomes, but could hardly be detected in MVs.

3.2.7 Myeloid-derived regulatory cell Extracellular Vesicles (Table 3)

Free radical generating myeloid-derived regulatory cells (MDRC) are known to regulate activities of T-cells and airway responses in asthma [55,56]. While most previous research has focussed on mechanisms of communication through oxidant pathways and soluble mediators, a study by Hough *et al.* has found that exosome-mediated intercellular transfer could also be a mechanism by which MDRCs in asthmatics can communicate with T cells and signal T-cell proliferation [57]. Since mitochondria are capable of secreting ROS and play a role in the inflammatory response, the study aimed to measure mitochondrial transfer in exosomes derived from MDRCs of asthmatics. The study reported that EVs isolated from airways of

asthmatic patients contain higher levels of mitochondrial DNA. This suggests a potential source of pro-inflammatory signalling by MDRCs of the respiratory system. The study also reported evidence of MDRC-to-T cell transfer of mitochondria packaged within exosomes.

3.2.8 Platelet-derived Extracellular Vesicles (Table 3)

Platelet microparticles (PMP) make up the largest fraction of circulating EVs and are elevated in many systemic diseases such as rheumatoid arthritis, cancer, diabetes, and acute coronary syndrome [58,59]. Furthermore, it has been found that platelets are associated with airway hyper-responsiveness and bronchial remodelling [58-60]. Duarte et al. [61] found that circulating levels of PMP are significantly increased in asthmatic subjects.

3.2.9 Epithelial cell-derived Extracellular Vesicles (Table 4)

Airway epithelial cells play a pivotal role in the pathogenesis of asthma as a primary airway defence against exposure to inflammatory stimuli and antigens. Activation of epithelial Toll-like receptors (TLRs) provides an important link between innate immunity and allergic diseases [62]. Epithelial cells can also promote inflammation by directing DCs to drive a Th2 response [63]. Two studies have addressed the role EVs derived from airway epithelial cells in the pathogenesis of asthma.

A study by Kulshreshtha et al. [64] found that murine epithelial cell–derived exosomes can induce proliferation and infiltration of undifferentiated macrophages into the lungs under the influence of IL-13 released during asthmatic inflammatory conditions. The study established bronchial epithelial cells as a major source of exosomes during acute airway inflammation. Bronchial epithelial cell-derived exosomes were also found to induce proliferation and chemotaxis of undifferentiated macrophages.

Another study conducted by Park et al. [65] aimed to determine potential sources of tissue factor (TF), a primary initiator of blood coagulation, and the mechanisms of its availability in the lung micro-environment. Airway expression of TF can be associated with asthma as the disease is characterized in part by sub-epithelial angiogenesis. The results established that compressive mechanical stress induces TF mRNA expression and intracellular TF protein, as well as TF-bearing exosomes in well-differentiated normal human bronchial epithelial cells. This confirms the ability of exosomes to translocate TF from one cell type to another by vesicle trafficking [65].

3.2.10 Fibroblast-derived Extracellular Vesicles (Table 4)

By regulating the functions of bronchial epithelial cells, bronchial fibroblasts play a key role in the structural changes which occur in asthma [66,67]. A study by Haj-Salem et al. [68] evaluated the role of bronchial fibroblast-derived exosomes on epithelial cell proliferation in severe asthma. Exosomes were purified from fibroblasts of healthy subjects and severe asthmatic subjects and co-cultured with bronchial epithelial cells. The results revealed no difference in exosome release efficiency between fibroblasts of healthy controls and severe eosinophilic asthmatic subjects; however, TGF-β2 levels, which significantly reduced proliferation of epithelial cells, were significantly lower in exosomes of severe asthmatics. Consistent with this, exosomes derived from fibroblasts of the severe asthmatic subjects enhanced epithelial cell proliferation, whereas fibroblast-derived exosomes from healthy controls significantly decreased the proliferation rate.

3.2.11 Mesenchymal stem cell- and Mesenchymal cell-derived Extracellular Vesicles (Table 4)

Mesenchymal stem cells (MSC) are pluripotent stromal cells which differentiate into a variety of mesenchymal cell types including osteocytes, myocytes, and adipocytes. Previous studies have shown that MSCs can reduce asthma airway inflammation by up-regulating the proliferation of Treg cells [69,70]. Three studies have investigated the role of mesenchymal stem cells or stromal cells in airway remodelling in asthma patients. A study by Du et al. [71] found that that MSC-derived exosomes can promote immunosuppression in asthmatics by upregulating IL-10 and TGF-β1, which results in regulatory T-cell (Treg) proliferation. Exosomes from human bone-marrow derived MSCs of healthy adults were co-cultured with peripheral blood mononuclear cells (PBMC) of asthmatic patients. Increased concentrations of IL-10 and TGF-β1 were found in the exosome-PBMC co-cultures, although even higher concentrations were observed in MSC-PBMC co-cultures.

De Castro et al. [72] demonstrated that adipose tissue mesenchymal stromal cells and their EVs reduced eosinophil counts in mouse lung tissue and bronchoalveolar lavage fluid (BALF), as well as modulating airway remodelling. In a third study, Cruz et al. [73] found that systemic administration of EVs from bone marrow-derived mesenchymal stromal cells was as effective as their cells of origin in suppressing Th2/Th17-mediated airway hyper-responsiveness and lung inflammation in a model of Aspergillus-hyphal extract-induced allergic airway inflammation.

3.3 Extracellular vesicles in body fluids

3.3.1 Bronchoalveolar lavage fluid-derived Extracellular Vesicles (Table 5)

Eight studies have investigated the role of exosomes derived from BALF in the pathogenesis of asthma. A study by Hough *et al.* [74] compared the lipid composition and presence of specific lipid mediators in airway EVs purified from the BALF of healthy controls and asthmatic subjects. The results indicated elevated concentrations of EVs in the BALF of asthmatic subjects and that the increase correlated with blood eosinophilia. They also found extensive differences in lipid composition between the EVs of the asthma group and controls. Lipids such as ceramides, ceramide-phosphates, phosphatidylglycerol, and sphingomyelins, which are mediators of inflammation in asthma were found to be increased in EVs derived from asthma patients [74].

A study by Rollet-Cohen et al. [75] used proteomics to compare respiratory exosomal contents isolated from BALF of patients suffering from cystic fibrosis (CF), primary ciliary dyskinesia (PCD) and asthma. Exosomes from asthma patients contained lower amounts of grancalcin and histones, contained Serpin A6, a protein involved in counter regulation of immune proteinases, and APOA4, a protein playing a role in lipid and surfactant metabolism. Most proteins identified are known activators of the NFκB pathway and can contribute to the pro-inflammatory status of these respiratory diseases [75].

Torregrosa Paredes et al. [76] explored the phenotypic and functional characteristics of BALF exosomes in asthma and investigated whether these exosomes have leukotriene biosynthetic capacity. BALF exosomes were collected and purified from healthy individuals and patients with mild allergic asthma to birch pollen before and after birch allergen provocation. The results showed that the BALF exosome profile was altered in asthmatics and had increased expression of HLA-DR. Incubation of BALF exosomes with human bronchial epithelial cells showed that exosomes from asthmatics induced significantly higher levels of LTC4 and IL-8 release compared to exosomes from healthy individuals. The data suggest that exosomes from BALF of asthmatics can induce pro-inflammatory cytokines and LT responses from airway epithelial cells which could potentially contribute to inflammation in asthma.

Levänen et al. [78] found a significant difference in BALF exosomal miRNA between mild asymptomatic asthmatic subjects and healthy subjects. The study found that, at baseline, the expression profile of the altered miRNAs was highly correlated with lung function (FEV1) in the asthma group.

Wan et al. [77] investigated the effects of EVs from healthy mouse lungs in regulation of the immune balance in the respiratory tract. They found that, among the subsets of EVs, CD8α+CD11c+ EVs contain immunosuppressive cytokines TGF-β1 and IL-10. These

cytokines could inhibit T helper cell proliferation via TGF-β1 *in vitro* and relieve asthmatic symptoms in mice. However, even independent of these two cytokines, the EVs were effective at inhibiting OVA peptide–specific CD4+ T cell proliferation. Furthermore, they could also prevent T helper cells from secreting IL-4, IL-9, and IL-17A via IL-10 ex vivo.

A study by Prado et al. [79] demonstrated that BALF-derived exosomes from tolerized mice could prevent an allergic reaction. Female mice were tolerized by respiratory exposure to the olive pollen allergen Ole e 1. Exosomes were then isolated from BALF of these mice as well as naïve mice as controls. The results showed that tolerogenic exosomes inhibited specific IgE and IgG1 in an allergic sensitization model, and that pre-treatment with tolerogenic exosomes inhibited Th2 cytokines, but induced TGF-β. Tolerogenic exosomes were also found to reduce allergen-induced airway inflammation in mice.

A subsequent study by the same authors [80] investigated whether such exosomes generated in response to Ole e 1 could also prevent the sensitization to other unrelated allergens, such as Bet v 1 from birch pollen. Exosomes were isolated from BALF of Ole e 1 tolerized mice using the same methods as in the previous study. The results suggest that pre-treatment with tolerized exosomes specific to Ole e 1 could also block allergic responses to a second unrelated allergen such as Bet v 1.

Gon et al. [81] analysed variations in the production of airway EVs and their miRNA content in a house-dust mite (HDM) allergen-exposed murine asthma model. Airway EVs from BALF of mice were isolated using the ExoQuick Exosome Precipitation Solution. Compared to the usual technique of ultracentrifugation, this provides a higher extraction efficiency but has the risk of protein contamination when used to isolate exosomes in cell culture media [82]. The results showed that the amount of EVs observed increased by 8.9-fold in the BALF of HDM-exposed mice compared to the control group. After exposure to HDM, significant changes in the expression of 139 miRNAs in EVs were also recorded.

Shin et al. [83] evaluated the role of EVs in the development of airway immune dysfunction in response to LPS inhalation. EVs in BALF of mice were isolated after exposure to LPS. The results showed that inhalation of LPS enhanced EV release into the BALF. Furthermore, airway sensitization with allergens and LPS-induced EVs resulted in a mixed Th1/Th17 cell responses and enhanced production of Th1/Th17-polarizing cytokines (IL-12p70 and IL-6) by lung DCs. While not directly correlated to asthma, the study provides evidence that exosomes play a role in immune responses to allergens within the lungs.

3.3.2 Serum-derived Extracellular Vesicles (Table 6)

Two studies have reported that exosomes derived from serum could potentially be involved in the pathogenesis of asthma. Almqvist et al. [84] provided evidence that serum-derived exosomes from OVA-fed mice could prevent allergic sensitization in a model of allergic asthma. The transfer of serum exosomes from OVA-fed mice reduced eosinophil levels and antigen-specific IgE. Gao et al. [85] examined the effects of circulating MVs on airway smooth muscle function and found that exposure to circulating MVs of asthma patients, at a concentration that matched their plasma levels, reduced endothelium-dependent relaxation to bradykinin and increased contraction to acetylcholine in mouse trachea. These results suggest circulating MVs might be involved in the underlying mechanisms of airway smooth muscle dysfunction.

3.3.3 Nasal lavage fluid-derived Extracellular Vesicles (Table 6)

Lässer et al. [86] examined the proteome of NLF-derived exosomes in healthy subjects, as well as its alterations in individuals with asthma and chronic rhinosinusitis. Serum-associated proteins and mucins were increased in the exosomes from individuals with respiratory diseases compared to healthy controls, whereas proteins with antimicrobial functions and barrier-related proteins showed decreased expression.

3.3.4 Exhaled breath condensate-derived Extracellular Vesicles (Table 6)

Sinha et al. [87] demonstrated how miRNAs could be reliably detected in exhaled breath condensate (EBC) using quantitative PCR analysis. RNA was extracted from vacuum-dried and concentrated EBC samples of asthmatic and healthy subjects. It was found that miRNAs present in EBC are mainly associated with exosomes. The EBC miRNA profile of asthmatic patients differed from that of healthy controls, with some of the identified miRNAs known to be associated with asthma, allergy, and inflammatory pathways.

4 DISCUSSION

4.1 Main Findings

The investigation of the biological role of EVs in lung disease has become a rapidly progressing field. The role of EVs as intercellular messengers and the possibility that they could serve as novel diagnostic or pharmacological targets has made this an exciting field. The aim of this review was to combine and interpret all current research on the role and functions of EVs in the pathogenesis of asthma and, in particular, assess the weight of evidence for specific roles of EVs in asthma. Data from the studies included in this review provide evidence that EVs, including exosomes and MVs, may play important roles in the pathogenesis of asthma and airway inflammation.

4.1.1 Findings for Extracellular vesicles of known cellular origins

One objective of the review was to identify all current research on EVs of known cellular origin in asthma. Understanding the effects of EVs and associating them with their cellular origin helps establish specific pathways and cellular networks for EVs within the context of asthma. While the general pathophysiology of asthma is quite well understood, the role of EVs in this complex network between immune and local cells has yet to be clarified. Most research presented in this review concluded that EVs, particularly exosomes, are heavily implicated in the pathogenesis of asthma. This delineates how cells can interact with each other without direct physical contact. Table 7 provides a summary showing that EVs from numerous cell types can stimulate pro-inflammatory responses, a key aspect of the patho-physiology of asthma. Four studies show that EVs derived from eosinophils, neutrophils, epithelial cells and fibroblasts can trigger ASM cell remodelling. In contrast, some other studies demonstrate that EVs from mast cells and MSCs can be used defensively in reducing inflammation and oxidative stress in asthma. Overall, fourteen studies provided evidence that EVs derived from specific cell types may have pro-inflammatory or airway remodelling effects in asthma, whereas just five indicate protective effects. The weight of evidence is therefore in favour of deleterious effects of EVs in asthma, at the present time. However, whilst the studies presented in this review indicate that EVs have a profound impact on the pathogenesis of asthma, the number of studies representing each cell type is limited, and further research is required before firm conclusions can be drawn. This applies particularly to determining whether EVs from particular cell types are primarily deleterious or protective in the pathogenesis of asthma: for example, the current evidence for mast cell-derived EVs is mixed [24-26], whereas the all the current evidence for mesenchymal stem cell-derived EVs indicates that they are protective [71-73].

4.1.2 Findings for Extracellular Vesicles from body fluids

Many of the studies suggest that the composition of EVs and their contents are variable and can be altered depending on specific conditions. EVs of asthma patients often contain pro-inflammatory proteins, lipids and cytokines such as Serpin A6, LTC4, tetraspanins CD81, IL-6, and IL-8, amongst others. In contrast, EVs of healthy individuals obtained from BALF contain immunosuppressive cytokines such as TGF-β1 and IL-10, which are thought to play a role in immune regulation. This suggests that dysfunction within the exosomal system and changes in exosomal contents are associated with the development of asthma. Understanding the differences in exosomal contents between asthmatic and healthy individuals may clarify their role in asthma and their potential as a diagnostic or monitoring tools. Currently, there are insufficient studies to allow firm conclusions to be drawn; there is also the issue of uncertainty in the extent to which findings in animal models may be applicable to asthma in humans.

4.1.3 The therapeutic potential of Extracellular Vesicles

An interesting aspect of exosomes is their potential in inducing tolerance and protection against allergic sensitisation; this provides the possibility for the development of novel vaccines. Three studies demonstrated how mice receiving tolerizing exosomes from other allergen-challenged mice showed reduced specific IgE and IgG1 [79, 80, 84]. Pre-treatment with tolerogenic exosomes also inhibited Th2 cytokine production and induced TGF-β, showing their capacity in limited allergic sensitization. Furthermore, the effects were long lasting, and receiving exosomes tolerized with one allergen, can inhibit the sensitization to other allergens.

Overall, whilst the evidence is not strong currently, nine studies concluded that EVs may be implicated as a therapeutic tool, mostly to reduce inflammation in patients suffering from asthma. EVs isolated from different sources, including mesenchymal cells [71-73], mast cells [25, 26], BALF [79, 80] and serum [84] mitigate allergic airway inflammation through various mechanisms such as reducing eosinophil count, reducing the amount of free IgE, promoting proliferation of Treg cells, and increasing mucosal tolerance to sensitization.

4.1.4 The diagnostic potential of Extracellular Vesicles

Whilst many studies demonstrate measurable differences between the characteristics of EVs in healthy and asthmatic patients, the difficulty of using EVs as diagnostic tools is justifying the use of invasive procedures required to obtain the EVs. Currently, the diagnosis of asthma is clinically based, and does not require invasive procedures such as bronchoalveolar lavage [78] or blood tests [86] to confirm the diagnosis. However, novel non-invasive methods, such as using exhaled breath condensate to obtain EVs [87], may be useful in helping to classify the severity of the disease and predict the prognosis. Clearly, evaluating the potential value of EVs as biomarkers of asthma requires studies involving a much more detailed comparison of EVs with other biomarkers that are already well-characterized and available. In addition, studies are required to investigate the relationship between particular types of EVs and different phenotypes and endotypes of asthma that have been characterized.

4.2 Limitations and future research.

Heterogeneity within the research reviewed here should be considered. The studies adopted various experimental designs from *in vitro* studies of human cells, *in vitro* studies of murine cells, *in vitro* studies of equine cells to *in vivo* studies in mice. Differences in

development, gene regulation and expression, genomics, and epigenetics between each species should be considered before extrapolating the results to humans [88]. Furthermore, the numbers of subjects (patients and controls) is also critical in assessing the confidence with which conclusions can be made: most of the studies discussed in this review employed relatively low numbers of patients or healthy controls (≤ 20), with some having only 3 or 4 subjects. The highest number was in a study employing 58 asthmatic subjects and 16 healthy volunteers to study exosomes derived from eosinophils [31]. Thus more studies with larger numbers of subjects are required in order to consolidate confidence in the findings reported. The heterogeneity of patients in terms of the representation of different phenotypes and endotypes of asthma within the subject populations should also be considered.

Due to the relatively small number of currently available studies in each area of research, the results and findings presented must be interpreted with caution. Thus, further studies are required to consolidate the evidence that is currently available. Every area presented in this review potentially needs more research to be conducted; however, some areas hold more promise than others. For example, additional research on the differences in exosomal contents or levels between asthmatic and healthy subjects could help to develop a novel, non-invasive diagnostic tool for asthma, particularly by investigating EVs in exhaled breath [87]. The role of EVs in preventing allergic sensitisation is also a fascinating area with the potential for developing preventative or prophylactic medicines; however, it is likely to be a significant time before such studies can move from animal models to patients. Because myriads of cells within the body can produce exosomes, each containing distinct constituents, the possibility for further research is considerable.

A further area for research is likely to be the effects of allergen desensitization interventions on the nature and constituents of EVs as this could provide further insights into the relationship between EVs and asthmatic status.

Overall the studies conducted so far on EVs in relation to asthma indicate that they are significant factors in the disease, but much further research is required to consolidate and expand on these findings.

Data sharing Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Conflict of interest The authors have no conflicts of interest to declare.

Reference List

1. World_Health_Organization. Chronic Respiratory Disease: Asthma. 2019; https://www.who.int/respiratory/asthma/en/.
2. National_Asthma_Education_and_Prevention_Program. Third Expert Panel on the Diagnosis and Management of Asthma. Bethesda, MD: National Heart, Lung, and Blood Institute 2007. 2007; https://www.nhlbi.nih.gov/files/docs/guidelines/asthgdln.pdf.
3. Bonnelykke K, Ober C. Leveraging gene-environment interactions and endotypes for asthma gene discovery. *J Allergy Clin Immunol.* 2016;137(3):667-679.
4. Loxham M, Davies DE. Phenotypic and genetic aspects of epithelial barrier function in asthmatic patients. *J Allergy Clin Immunol.* 2017;139(6):1736-1751.
5. Nakayama T. Introduction to "allergic inflammation". *Immunol Rev.* 2017;278(1):5-7.
6. Kubo M. Innate and adaptive type 2 immunity in lung allergic inflammation. *Immunol Rev.* 2017;278(1):162-172.
7. Hirose K, Iwata A, Tamachi T, Nakajima H. Allergic airway inflammation: key players beyond the Th2 cell pathway. *Immunol Rev.* 2017;278(1):145-161.
8. Gelfand EW, Joetham A, Wang M, Takeda K, Schedel M. Spectrum of T-lymphocyte activities regulating allergic lung inflammation. *Immunol Rev.* 2017;278(1):63-86.
9. Simpson RJ, Jensen SS, Lim JW. Proteomic profiling of exosomes: current perspectives. *Proteomics.* 2008;8(19):4083-4099.
10. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol.* 2013;200(4):373-383.
11. S ELA, Mager I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nature reviews Drug discovery.* 2013;12(5):347-357.
12. Escola JM, Kleijmeer MJ, Stoorvogel W, Griffith JM, Yoshie O, Geuze HJ. Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes. *The Journal of biological chemistry.* 1998;273(32):20121-20127.
13. Yoshioka Y, Konishi Y, Kosaka N, Katsuda T, Kato T, Ochiya T. Comparative marker analysis of extracellular vesicles in different human cancer types. *J Extracell Vesicles.* 2013;2.
14. Castellana D, Zobairi F, Martinez MC, et al. Membrane microvesicles as actors in the establishment of a favorable prostatic tumoral niche: a role for activated fibroblasts and CX3CL1-CX3CR1 axis. *Cancer research.* 2009;69(3):785-793.

15. Beyer C, Pisetsky DS. The role of microparticles in the pathogenesis of rheumatic diseases. *Nature reviews Rheumatology.* 2010;6(1):21-29.
16. Fujita Y, Kosaka N, Araya J, Kuwano K, Ochiya T. Extracellular vesicles in lung microenvironment and pathogenesis. *Trends Mol Med.* 2015;21(9):533-542.
17. Minciacchi VR, Freeman MR, Di Vizio D. Extracellular vesicles in cancer: exosomes, microvesicles and the emerging role of large oncosomes. *Semin Cell Dev Biol.* 2015;40:41-51.
18. Loyer X, Vion AC, Tedgui A, Boulanger CM. Microvesicles as cell-cell messengers in cardiovascular diseases. *Circulation research.* 2014;114(2):345-353.
19. Vallhov H, Gutzeit C, Hultenby K, Valenta R, Gronlund H, Scheynius A. Dendritic cell-derived exosomes carry the major cat allergen Fel d 1 and induce an allergic immune response. *Allergy.* 2015;70(12):1651-1655.
20. Choi M, Ban T, Rhim T. Therapeutic use of stem cell transplantation for cell replacement or cytoprotective effect of microvesicle released from mesenchymal stem cell. *Mol Cells.* 2014;37(2):133-139.
21. 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.
22. Hough KP, Chanda D, Duncan SR, Thannickal VJ, Deshane JS. Exosomes in immunoregulation of chronic lung diseases. *Allergy.* 2017;72(4):534-544.
23. Xie H, He SH. Roles of histamine and its receptors in allergic and inflammatory bowel diseases. *World journal of gastroenterology : WJG.* 2005;11(19):2851-2857.
24. Skokos D, Le Panse S, Villa I, et al. Nonspecific B and T cell-stimulatory activity mediated by mast cells is associated with exosomes. *International archives of allergy and immunology.* 2001;124(1-3):133-136.
25. Xie G, Yang H, Peng X, et al. Mast cell exosomes can suppress allergic reactions by binding to IgE. *J Allergy Clin Immunol.* 2018;141(2):788-791.
26. Eldh M, Ekstrom K, Valadi H, et al. Exosomes communicate protective messages during oxidative stress; possible role of exosomal shuttle RNA. *PLoS One.* 2010;5(12):e15353.
27. Burgoyne RD, Morgan A. Secretory granule exocytosis. *Physiol Rev.* 2003;83(2):581-632.
28. Spencer LA, Szela CT, Perez SA, et al. Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially. *Journal of leukocyte biology.* 2009;85(1):117-123.

29. Mazzeo C, Canas JA, Zafra MP, et al. Exosome secretion by eosinophils: A possible role in asthma pathogenesis. *J Allergy Clin Immunol.* 2015;135(6):1603-1613.
30. Akuthota P, Carmo LA, Bonjour K, et al. Extracellular Microvesicle Production by Human Eosinophils Activated by "Inflammatory" Stimuli. *Front Cell Dev Biol.* 2016;4:117.
31. Canas JA, Sastre B, Mazzeo C, et al. Exosomes from eosinophils autoregulate and promote eosinophil functions. *Journal of leukocyte biology.* 2017;101(5):1191-1199.
32. Canas JA, Sastre B, Rodrigo-Munoz JM, et al. Eosinophil-derived exosomes contribute to asthma remodelling by activating structural lung cells. *Clin Exp Allergy.* 2018;48(9):1173-1185.
33. Woodruff PG, Boushey HA, Dolganov GM, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci U S A.* 2007;104(40):15858-15863.
34. Mochimaru T, Ueda S, Suzuki Y, Asano K, Fukunaga K. Neutrophil to lymphocyte ratio is a novel independent predictor of severe exacerbation in asthma patients. *Ann Allergy Asthma Immunol.* 2018.
35. Seys SF, Lokwani R, Simpson JL, Bullens DMA. New insights in neutrophilic asthma. *Curr Opin Pulm Med.* 2019;25(1):113-120.
36. Panettieri RA, Jr. The Role of Neutrophils in Asthma. *Immunology and allergy clinics of North America.* 2018;38(4):629-638.
37. Vargas A, Roux-Dalvai F, Droit A, Lavoie JP. Neutrophil-Derived Exosomes: A New Mechanism Contributing to Airway Smooth Muscle Remodeling. *American journal of respiratory cell and molecular biology.* 2016;55(3):450-461.
38. Wirz OF, Głobińska A, Ochsner U, et al. Comparison of regulatory B cells in asthma and allergic rhinitis. *Allergy.* 2018.
39. Heeringa JJ, Rijvers L, Arends NJ, et al. IgE-expressing memory B cells and plasmablasts are increased in blood of children with asthma, food allergy, and atopic dermatitis. *Allergy.* 2018;73(6):1331-1336.
40. Al-Kufaidy R, Vazquez-Tello A, BaHammam AS, Al-Muhsen S, Hamid Q, Halwani R. IL-17 enhances the migration of B cells during asthma by inducing CXCL13 chemokine production in structural lung cells. *J Allergy Clin Immunol.* 2017;139(2):696-699.e695.
41. Dullaers M, Schuijs MJ, Willart M, et al. House dust mite-driven asthma and allergen-specific T cells depend on B cells when the amount of inhaled allergen is limiting. *J Allergy Clin Immunol.* 2017;140(1):76-88 e77.

42. Admyre C, Johansson SM, Paulie S, Gabrielsson S. Direct exosome stimulation of peripheral human T cells detected by ELISPOT. *Eur J Immunol.* 2006;36(7):1772-1781.
43. Admyre C, Bohle B, Johansson SM, et al. B cell-derived exosomes can present allergen peptides and activate allergen-specific T cells to proliferate and produce TH2-like cytokines. *J Allergy Clin Immunol.* 2007;120(6):1418-1424.
44. Raposo G, Nijman HW, Stoorvogel W, et al. B lymphocytes secrete antigen-presenting vesicles. *J Exp Med.* 1996;183(3):1161-1172.
45. Thery C, Duban L, Segura E, Veron P, Lantz O, Amigorena S. Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes. *Nat Immunol.* 2002;3(12):1156-1162.
46. Vincent-Schneider H, Stumptner-Cuvelette P, Lankar D, et al. Exosomes bearing HLA-DR1 molecules need dendritic cells to efficiently stimulate specific T cells. *International immunology.* 2002;14(7):713-722.
47. Barnes PJ. Th2 cytokines and asthma: an introduction. *Respir Res.* 2001;2(2):64-65.
48. Hoseini-Shahrestanak S, Bazargan N, Rahimian L, Nemati M, Solaymani S, Jafarzadeh A. Imbalanced Expression of Th2 and Treg Cell-related Parameters in Peripheral Blood Mononuclear Cells in Patients with Allergic Asthma. *Tanaffos.* 2018;17(1):1-12.
49. Arae K, Morita H, Unno H, et al. Chitin promotes antigen-specific Th2 cell-mediated murine asthma through induction of IL-33-mediated IL-1β production by DCs. *Sci Rep.* 2018;8(1):11721.
50. Lorentsen KJ, Cho JJ, Luo X, et al. Publisher Correction: Bcl11b is essential for licensing Th2 differentiation during helminth infection and allergic asthma. *Nat Commun.* 2018;9(1):2901.
51. Shefler I, Salamon P, Reshef T, Mor A, Mekori YA. T cell-induced mast cell activation: a role for microparticles released from activated T cells. *J Immunol.* 2010;185(7):4206-4212.
52. Shefler I, Salamon P, Levi-Schaffer F, Mor A, Hershko AY, Mekori YA. MicroRNA-4443 regulates mast cell activation by T cell-derived microvesicles. *J Allergy Clin Immunol.* 2018;141(6):2132-2141 e2134.
53. Gill MA. The role of dendritic cells in asthma. *Journal of Allergy and Clinical Immunology.* 2012;129(4):889-901.
54. Wahlund CJE, Gucluler G, Hiltbrunner S, Veerman RE, Naslund TI, Gabrielsson S. Exosomes from antigen-pulsed dendritic cells induce stronger antigen-specific immune responses than microvesicles in vivo. *Scientific reports.* 2017;7(1):17095.

55. Ray P, Arora M, Poe SL, Ray A. Lung myeloid-derived suppressor cells and regulation of inflammation. *Immunologic research.* 2011;50(2-3):153-158.
56. Arora M, Poe SL, Oriss TB, et al. TLR4/MyD88-induced CD11b+Gr-1 int F4/80+ non-migratory myeloid cells suppress Th2 effector function in the lung. *Mucosal Immunol.* 2010;3(6):578-593.
57. Hough KP, Trevor JL, Strenkowski JG, et al. Exosomal transfer of mitochondria from airway myeloid-derived regulatory cells to T cells. *Redox biology.* 2018;18:54-64.
58. Boilard E, Nigrovic PA, Larabee K, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science.* 2010;327(5965):580-583.
59. George FD. Microparticles in vascular diseases. *Thrombosis research.* 2008;122 Suppl 1:S55-59.
60. Kornerup KN, Page CP. The role of platelets in the pathophysiology of asthma. *Platelets.* 2007;18(5):319-328.
61. Duarte D, Taveira-Gomes T, Sokhatska O, et al. Increased circulating platelet microparticles as a potential biomarker in asthma. *Allergy.* 2013;68(8):1073-1075.
62. Prescott SL. Allergy Takes its Toll: The Role of Toll-like Receptors in Allergy Pathogenesis. *World Allergy Organ J.* 2008;1(1):4-8.
63. Wang Y, Bai C, Li K, Adler KB, Wang X. Role of airway epithelial cells in development of asthma and allergic rhinitis. *Respiratory Medicine.* 2008;102(7):949-955.
64. Kulshreshtha A, Ahmad T, Agrawal A, Ghosh B. Proinflammatory role of epithelial cell-derived exosomes in allergic airway inflammation. *J Allergy Clin Immunol.* 2013;131(4):1194-1203, 1203 e1191-1114.
65. Park JA, Sharif AS, Tschumperlin DJ, et al. Tissue factor-bearing exosome secretion from human mechanically stimulated bronchial epithelial cells in vitro and in vivo. *J Allergy Clin Immunol.* 2012;130(6):1375-1383.
66. Steiling H, Werner S. Fibroblast growth factors: key players in epithelial morphogenesis, repair and cytoprotection. *Curr Opin Biotechnol.* 2003;14(5):533-537.
67. Shao J, Sheng GG, Mifflin RC, Powell DW, Sheng H. Roles of myofibroblasts in prostaglandin E2-stimulated intestinal epithelial proliferation and angiogenesis. *Cancer research.* 2006;66(2):846-855.
68. Haj-Salem I, Plante S, Gounni AS, Rouabhia M, Chakir J. Fibroblast-derived exosomes promote epithelial cell proliferation through TGF-beta2 signalling pathway in severe asthma. *Allergy.* 2018;73(1):178-186.

69. Li JG, Zhuan-sun YX, Wen B, et al. Human mesenchymal stem cells elevate CD4+CD25+CD127low/- regulatory T cells of asthmatic patients via heme oxygenase-1. *Iran J Allergy Asthma Immunol.* 2013;12(3):228-235.

70. Yan Z, Zhuansun Y, Chen R, Li J, Ran P. Immunomodulation of mesenchymal stromal cells on regulatory T cells and its possible mechanism. *Experimental cell research.* 2014;324(1):65-74.

71. Du YM, Zhuansun YX, Chen R, Lin L, Lin Y, Li JG. Mesenchymal stem cell exosomes promote immunosuppression of regulatory T cells in asthma. *Experimental cell research.* 2018;363(1):114-120.

72. de Castro LL, Xisto DG, Kitoko JZ, et al. Human adipose tissue mesenchymal stromal cells and their extracellular vesicles act differentially on lung mechanics and inflammation in experimental allergic asthma. *Stem Cell Res Ther.* 2017;8(1):151.

73. Cruz FF, Borg ZD, Goodwin M, et al. Systemic Administration of Human Bone Marrow-Derived Mesenchymal Stromal Cell Extracellular Vesicles Ameliorates Aspergillus Hyphal Extract-Induced Allergic Airway Inflammation in Immunocompetent Mice. *Stem Cells Transl Med.* 2015;4(11):1302-1316.

74. Hough KP, Wilson LS, Trevor JL, et al. Unique Lipid Signatures of Extracellular Vesicles from the Airways of Asthmatics. *Scientific reports.* 2018;8(1):10340.

75. Rollet-Cohen V, Bourderioux M, Lipecka J, et al. Comparative proteomics of respiratory exosomes in cystic fibrosis, primary ciliary dyskinesia and asthma. *J Proteomics.* 2018;185:1-7.

76. Torregrosa Paredes P, Esser J, Admyre C, et al. Bronchoalveolar lavage fluid exosomes contribute to cytokine and leukotriene production in allergic asthma. *Allergy.* 2012;67(7):911-919.

77. Wan S, Wang S, Weng L, et al. CD8alpha(+)CD11c(+) Extracellular Vesicles in the Lungs Control Immune Homeostasis of the Respiratory Tract via TGF-beta1 and IL-10. *J Immunol.* 2018;200(5):1651-1660.

78. Levanen B, Bhakta NR, Torregrosa Paredes P, et al. Altered microRNA profiles in bronchoalveolar lavage fluid exosomes in asthmatic patients. *J Allergy Clin Immunol.* 2013;131(3):894-903.

79. Prado N, Marazuela EG, Segura E, et al. Exosomes from bronchoalveolar fluid of tolerized mice prevent allergic reaction. *J Immunol.* 2008;181(2):1519-1525.

80. Prado N, Canamero M, Villalba M, Rodriguez R, Batanero E. Bystander suppression to unrelated allergen sensitization through intranasal administration of tolerogenic exosomes in mouse. *Molecular immunology.* 2010;47(11-12):2148-2151.

81. Gon Y, Maruoka S, Inoue T, et al. Selective release of miRNAs via extracellular vesicles is associated with house-dust mite allergen-induced airway inflammation. *Clin Exp Allergy.* 2017;47(12):1586-1598.

82. Tang YT, Huang YY, Zheng L, et al. Comparison of isolation methods of exosomes and exosomal RNA from cell culture medium and serum. *Int J Mol Med.* 2017;40(3):834-844.

83. Shin TS, Kim JH, Kim YS, et al. Extracellular vesicles are key intercellular mediators in the development of immune dysfunction to allergens in the airways. *Allergy.* 2010;65(10):1256-1265.

84. Almqvist N, Lonnqvist A, Hultkrantz S, Rask C, Telemo E. Serum-derived exosomes from antigen-fed mice prevent allergic sensitization in a model of allergic asthma. *Immunology.* 2008;125(1):21-27.

85. Gao J, Xu X, Ying Z, et al. Post-Effect of Air Quality Improvement on Biomarkers for Systemic Inflammation and Microparticles in Asthma Patients After the 2008 Beijing Olympic Games: a Pilot Study. *Inflammation.* 2017;40(4):1214-1224.

86. Lasser C, O'Neil SE, Shelke GV, et al. Exosomes in the nose induce immune cell trafficking and harbour an altered protein cargo in chronic airway inflammation. *J Transl Med.* 2016;14(1):181.

87. Sinha A, Yadav AK, Chakraborty S, et al. Exosome-enclosed microRNAs in exhaled breath hold potential for biomarker discovery in patients with pulmonary diseases. *J Allergy Clin Immunol.* 2013;132(1):219-222.

88. Shanks N, Greek R, Greek J. Are animal models predictive for humans? *Philos Ethics Humanit Med.* 2009;4:2.

Table 1. Inclusion and Exclusion Criteria Used for Study Selection

Inclusion Criteria	Exclusion Criteria
Experiment involving the extraction, identification, or production of extracellular vesicles or their contents (eg. miRNA)	Experiment involving lung disease but not specifically asthma (eg. COPD, lung cancer, bronchitis, primary ciliary dyskinesia)
Experiment involving asthma, inflammatory lung diseases, allergic airway diseases, or any other diseases closely related to asthma within the respiratory tract	Experiment involving allergic inflammation but not in the lungs (eg. eczema)
Full text must be available in English	Non-primary studies (eg. reviews)
Isolation method of EVs must be given if applicable	

Table 2. Summary of studies on exosomes derived from mast cells, eosinophils and neutrophils.

Author, Year [Ref]	Title	Aim	Type	Conclusion
Mast cells				
Skokos *et al.* 2001 [24]	Nonspecific B and T cell-stimulatory activity mediated by mast cells is associated with exosomes	Investigate whether mast cell-derived exosomes can stimulate B and T lymphocytes dependent of the origin cell.	*in vitro*	Exosomes act as complex messengers of mast cells for intercellular communication within the immune system.
Xie *et al.* 2018 [25]	Mast cell exosomes can suppress allergic reactions by binding to IgE	Explore whether extracellular exosomes carrying FcεRI could bind to free IgE.	*in vitro*	By binding to free IgE via FcεRI, mast cell exosomes can regulate multiple physiology of asthma including airway inflammation, airway hyperresponsiveness, and some parts of airway remodeling.
Eldh *et al.* 2010 [26]	Mast cell exosomes produced during oxidative stress provide recipient cells with protection against oxidative stress	Investigate whether mast cell-derived exosomes obtained under variable conditions could initiate cell-to-cell signaling.	*in vitro*	RNA enclosed in exosomes provide recipient cells with protection against external stress stimuli and could be involved in intercellular communication.
Eosinophils				
Mazzeo et al. 2013 [29]	Exosome secretion by eosinophils: A possible role in asthma progression	Characterize eosinophil derived exosomes and investigate their role in asthma.	*in vitro*	MVs and exosomes produced and secreted by eosinophils possibly play an important role in asthma progression.
Akuthota et al. 2016 [30]	Extracellular Microvesicle Production by Human Eosinophils Activated by "Inflammatory" Stimuli	Identify and characterize EVs released from human eosinophils after a culturing period or after isolation and after stimulation with inflammatory stimuli.	*in vitro*	Human eosinophils increase MVs secretion in response to inflammatory stimuli.
Cañas et al. 2017 [31]	Exosomes from eosinophils autoregulate and promote eosinophil functions	Investigate whether eosinophil-derived exosomes can autoregulate and promote eosinophil function.	*in vitro*	Exosomes from the eosinophils of asthmatic patients can modify several specific eosinophil functions related to asthma pathogenesis.
Cañas et al. 2018 [32]	Eosinophil-derived exosomes contribute to asthma remodeling by activating structural lung cells	Investigate whether the activation of structural lung cells leading to asthma pathogenesis can be the attributed to the release of eosinophil-derived exosomes.	*in vitro*	Eosinophil-derived exosomes from asthmatic patients participate actively in the development of the pathological features of asthma via structural lung cells.
Neutrophils				
Vargas et al. 2016 [37]	Neutrophil-Derived Exosomes: A New Mechanism Contributing to Airway Smooth Muscle Remodeling	Determine whether neutrophils release exosomes, and if so identify the proteomic profile and evaluate their capacity to modulate ASM cell proliferation.	*in vitro*	Neutrophil-derived exosomes stimulated by LPS could play an central role in the progression of asthma through inducing the proliferation of ASM cells and promoting airway remodeling in severe and corticosteroid-insensitive asthma patients.

Table 3. Summary of study on exosomes derived from B cells, T cells and dendritic cells, myeloid-derived regulatory cells and platelets.

Author, Year [Ref]	Title	Aim	Type	Conclusion
B cells				
Admyre *et al.* 2007 [43]	B cell-derived exosomes can present allergen peptides and activate allergen-specific T cells to proliferate and produce TH2-like cytokines.	Investigate whether B cell-derived exosomes is involved in allergen presentation and T-cell stimulation.	*in vitro*	B cells exosomes can induce T-cell proliferation and Th2-like cytokine production through presentation of allergen-derived peptides.
T cells				
Shefler et al. 2010 [51]	T cell-induced mast cell activation: a role for microparticles released from activated T cells	Investigate whether T cell MVs can lead to mast cell activation by imitating the functions of their origin cell.	*in vitro*	T cells release MVs containing mast cell-activating factors similar to the origin cell and can convey surface molecules to emulate physical contact leading to mast cell activation.
Shefler et al. 2018 [52]	MicroRNA-4443 regulates mast cell activation by T cell-derived microvesicles	Characterize the contribution of microRNAs delivered by microvesicles to mast cell activation.	*in vitro*	In the context of T cell-mediated inflammation, T cell-derived MVs can carry functional miR-4443 that lead to mast cell activation.
Dendritic Cells				
Vallhov et al. 2015 [19]	Dendritic cell-derived exosomes carry the major cat allergen Fel d 1 and induce an allergic immune response	Explore the possibility of Fel d 1 presentation by DC-derived exosomes and whether this is responsible for the pathogenesis of allergic disease.	*in vitro*	Exosomes are able to present aeroallergens and thereby induce T-cell Th2-like cytokine production in allergic donors.
Wahlund et al. 2017 [54]	Exosomes from antigen-pulsed dendritic cells induce stronger antigen-specific immune responses than microvesicles in vivo	Compare the immunostimulatory potential of OVA-pulsed DC-derived exosomes and MVs.	*in vivo*	While both MVs and exosomes derived from DCs can activate an immunostimulatory response, exosomes are more potent and should be considered and evaluated for immune therapeutic purposes.
Myeloid-derived regulatory cells				
Hough et al. 2018 [57]	Exosomal transfer of mitochondria from airway myeloid-derived regulatory cells to T cells	Investigate the intercellular transfer of bioactive molecules to T cells via exosomes.	*in vitro*	Mitochondria co-localize with the mitochondrial network enclosed in MDRC-derived exosomes support intercellular direct exosome-mediated transfer and can generate ROS within recipient T cells.
Platelets				
Duarte et al. 2013 [61]	Increased circulating platelet microparticles as a potential biomarker in asthma	Determine the levels of PMP and endothelial microparticles in asthma.	*in vivo*	PMPs may play a role in asthma pathophysiology as a possible asthma biomarker.

Table 4. Summary of studies on exosomes derived from epithelial cells, fibroblasts and mesenchymal stem cells.

Author, Year [Ref]	Title	Aim	Type	Conclusion
Epithelial Cells				
Kulshreshtha *et al.* 2013 [64]	Proinflammatory role of epithelial cell–derived exosomes in allergic airway inflammation	Investigate the mechanisms of how structural and immune cells communicate in asthma pathogenesis.	Animal *in vivo* and *in vitro*	During asthmatic inflammation, epithelial cell–derived exosomes stimulated by IL-13 can induce enhanced proliferation and chemotaxis of undifferentiated macrophages in the lungs.
Park *et al.* 2012 [65]	Tissue factor-bearing exosome secretion from human mechanically stimulated bronchial epithelial cells *in vitro* and *in vivo*	Investigate sources of TF within the lungs and mechanisms of its availability.	Animal *in vivo* and *in vitro*	TF mRNA expression and TF-bearing exosomes secreted by mechanically stressed bronchial epithelial cells is a potential key source the TF signal.
Fibroblasts				
Haj-Salem et al. 2018 [68]	Fibroblast-derived exosomes promote epithelial cell proliferation through TGF-β2 signaling pathway in severe asthma	Evaluate the effects of bronchial fibroblast-derived exosomes on epithelial cell proliferation in severe asthma.	in vitro	Through modulation of epithelial cell proliferation, fibroblast-derived exosomes contribute to airway remodelling in severe asthma.
Mesenchymal cells				
Du *et al.* 2018 [71]	Mesenchymal stem cell exosomes promote immunosuppression of regulatory T cells in asthma	Investigated MSC derived exosomes' immunomodulation effect on PBMC of asthmatic patient.	*in vitro*	MSC exosomes upregulated IL-10 and TGF- β1 from PBMCs, thus promoting proliferation and immune-suppression capacity of Tregs.
de Castro *et al.* 2017 [72]	Human adipose tissue mesenchymal stromal cells and their extracellular vesicles act differentially on lung mechanics and inflammation in experimental allergic asthma	Evaluate whether EVs obtained from AD-MSC provide therapeutic effects on established airway remodeling in experimental allergic asthma.	Animal *in vivo* and *in vitro*	Human AD-MSCs and EVs effectively reduced eosinophil counts and modulated airway remodeling, but the effects of EVs on T-cells were not significant.
Cruz *et al.* 2015 [73]	Systemic Administration of Human Bone Marrow-Derived Mesenchymal Stromal Cell Extracellular Vesicles Ameliorates Aspergillus Hyphal Extract-Induced Allergic Airway Inflammation in Immunocompetent Mice	Determine the effects of xenogeneic administration of CM or EVs from human bone marrow-derived MSCs in a model of mixed Th2/Th17 neutrophilic-mediated allergic airway inflammation.	Animal *in vivo* and *in vitro*	Systemic administration of CM and EVs from both hMSCs and mMSCs is more effective than the cells themselves in reducing Th2/Th17-mediated airway hyperresponsiveness and lung inflammation.

Table 5. Summary of studies on exosomes derived from BALF

Author, Year [Ref]	Title	Aim	Type	Conclusion
Hough *et al.* 2018 [74]	Unique Lipid Signatures of Extracellular Vesicles from the Airways of Asthmatics	Determine the lipid composition and presence of specific lipid mediators in airway EVs of healthy and asthmatic subjects.	*in vitro*	Alterations in the composition of lipid mediators driven by EVs may lead to chronic airway inflammation in asthma patients.
Rollet-Cohen *et al.* 2018 [75]	Comparative proteomics of respiratory exosomes in cystic fibrosis, primary ciliary dyskinesia and asthma	Study the similarities and differences in proteomic content of BALF-derived exosomes of CF, PCD, asthma patients.	*in vitro*	Proteomic profiles of exosomes isolated from BALF of CF, PCD and asthmatic patients suggest that they carry different pro-inflammatory proteins, but are involved in the progression of lung damage.
Torregr-osa Paredes *et al.* 2012 [76]	Bronchoalveolar lavage fluid exosomes contribute to cytokine and leukotriene production in allergic asthma	Explore the LT biosynthetic capacity, phenotypic and functional characteristics of BALF-derived exosomes in asthma.	*in vitro*	BALF exosomes from asthmatics increases cytokine and LTC4 generation in airway epithelium and may contribute to subclinical lung inflammation.
Wan *et al.* 2018 [77]	CD8alpha(+) CD11c(+) Extracellular Vesicles in the Lungs Control Immune Homeostasis of the Respiratory Tract via TGF-β1 and IL-10	Investigate the role of EVs in regulating the immune balance of the respiratory tract	*in vitro*	EVs isolated from WT mice BALF contains TGF-β1 and IL-10 which are involved in controlling the immune balance of the airways.
Levänen *et al.* 2013 [78]	Altered microRNA profiles in bronchoalveolar lavage fluid exosomes in asthmatic patients	Investigate whether asthma and following subway air exposure altered BALF exosomal miRNA profiles.	*in vitro*	Healthy subjects and patients with unprovoked, mild, stable asthma showed differences in exosomal miRNA profiles.
Prado *et al.* 2008 [79]	Exosomes from bronchoalveolar fluid of tolerized mice prevent allergic reaction	Determine the effects of allergen-specific exosomes from tolerized mice on the development of allergen-induced allergic response using a mouse model.	mice *in vivo*	Induction of tolerance and protection against allergic sensitization in mice can be attributed to exosomes.
Prado *et al.* 2010 [80]	Bystander suppression to unrelated allergen sensitization through intranasal administration of tolerogenic exosomes in mouse	Investigate whether the preventative effects against sensitization to a specific allergen, Ole e 1, can be transmitted to other unrelated allergens.	mice *in vivo*	Tolerized exosomes specific to Ole e 1 prevented allergic sensitization to an unrelated allergen. This "bystander suppression" may have implications for the treatment of allergy.
Gon *et al.* 2017 [81]	Selective release of miRNAs via extracellular vesicles is associated with house-dust mite allergen-induced airway inflammation	Determine the biological roles of miRNAs in EVs on allergic airway inflammation.	mice *in vivo*	Pathogenesis of allergic airway inflammation following HDM exposure may involve the increase of EVs in the airways, and the selective sorting of its contents.
Shin *et al.* 2010 [83]	Extracellular vesicles are key intercellular mediators in the development of immune dysfunction to allergens in the airways	Investigate whether the development of airway immune dysfunction in response to allergens involved EVs secreted following LPS inhalation.	mice *in vivo* and *in vitro*	As key intercellular communicator, LPS inhalation induced EVs facilitate the development of airway immune dysfunction in response to inhaled LPS-containing allergens.

Table 6. Summary of studies on exosomes derived from serum, nasal lavage fluid and exhaled breath condensate

Author, Year [Ref]	Title	Aim	Type	Conclusion
Serum				
Almqvist *et al.* 2008 [84]	Serum-derived exosomes from antigen-fed mice prevent allergic sensitization in a model of allergic asthma	Investigate whether tolerosomes can protect against allergic sensitization in a mouse model of allergic asthma.	mice *in vivo*	Naïve recipient mice administered intraperitoneally with serum or isolated serum exosomes of OVA-fed mice nullified allergic sensitization in the recipients.
Gao *et al.* 2017 [85]	Post-Effect of Air Quality Improvement on Biomarkers for Systemic Inflammation and Microparticles in Asthma Patients After the 2008 Beijing Olympic Games: a Pilot Study	Investigate whether air quality improved systemic inflammation and affected circulating MVs in asthmatic patients during and after the 2008 Beijing Olympics	*in vitro*	The underlying mechanisms of ASM dysfunction in asthmatics might involve circulating MVs.
Nasal Lavage Fluid				
Lässer *et al.* 2016 [86]	Exosomes in the nose induce immune cell trafficking and harbour an altered protein cargo in chronic airway inflammation	Distinguish the proteome of NLF-derived exosomes from asthmatics/CRS patients and healthy subjects to determine whether they could influence inflammatory cells.	*in vitro*	In subjects with airway diseases, nasal exosomes could contribute to increased risk of infections by stimulating the migration of innate immune cells and decreasing expression in barrier and antimicrobial exosomal proteins.
Exhaled breath condensate				
Sinha *et al.* 2013 [87]	Exosome-enclosed microRNAs in exhaled breath hold potential for biomarker discovery in patients with pulmonary diseases	Determine whether miRNAs contained in EBC-derived exosomes could be an ideal substrate for potential biomarkers.	*in vitro*	The secretion and contents of EBC-derived exosomes are highly regulated and could reflects ongoing biological processes.

Table 7. The role of each EVs separated by cell origin in asthma. ✓ suggests evidence supporting hypothesis. - suggests there is no evidence supporting the hypothesis. Reference to the study is in parenthesis [n].

Cell Origin	Pro-inflammatory	Changes in airway remodelling	Protective effects in asthma
Mast cells	✓ [24]	-	✓ [25, 26]
Eosinophil	✓ [30, 31]	✓ [32]	-
Neutrophils	-	✓ [37]	-
B cells	✓ [43]	-	-
T cells	✓ [51, 52]	-	-
DCs	✓ [19, 54]	-	-
MDRC	✓ [57]	-	-
Epithelial cells	✓ [64]	✓ [65]	-
Fibroblasts	-	✓ [68]	-
MSC	-	-	✓ [71-73]

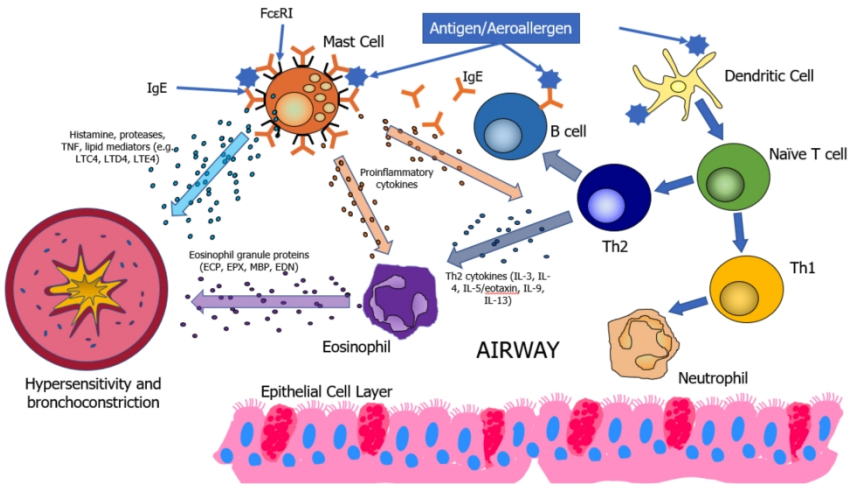
Figure legends

Figure 1. Mechanisms of Airway Inflammation in Asthma. In the sensitisation phase, DCs present antigen to T cells in the lymph nodes causing differentiation of naïve T cells. The Th1 response leads to cell mediated immunity and neutrophilic inflammation. Th2 cytokines cause class switching of B cells to produce IgE (mainly through IL-4), and recruitment of immune cells including eosinophil (mainly through IL-5). In the early asthmatic response, allergen binding to IgE receptors on mast cells causing degranulation and release of histamine, proteases, tumour necrosis factor (TNF), lipid mediators and other proteins. This leads to immediate hypersensitivity and bronchoconstriction. In the late asthmatic response, mast cells release chemokines and cytokines which recruit eosinophil, basophils (not shown), and T helper cells to the local mucosa. Both mast cells and Th2 cytokines leads to eosinophilia. Eosinophils degranulate to release eosinophil granule proteins. These proteins cause bronchoconstriction along with damage towards the epithelial layer. Ongoing inflammatory responses in the airway can lead to smooth muscle dysfunction and airway remodelling.

Figure 2: Summary of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. The process involves identification, screening, eligibility and studies included. Sources were retrieved from two databases (PubMed and EMBASE) with no restrictions to year, status or languages.

Figure 3: The number of studies on EVs of known origin and Asthma Based on Year Published. The first study was published in 2001, with numbers of publications increasing yearly until 2018, when nine studies were published.

Figure 4: The number of studies on EVs and Asthma Based on Origin of EVs. MDRC: Myeloid-derived regulatory cells, BALF: Bronchoalveolar lavage fluid, NLF: Nasal lavage fluid, EBC: Exhaled breath condensate.



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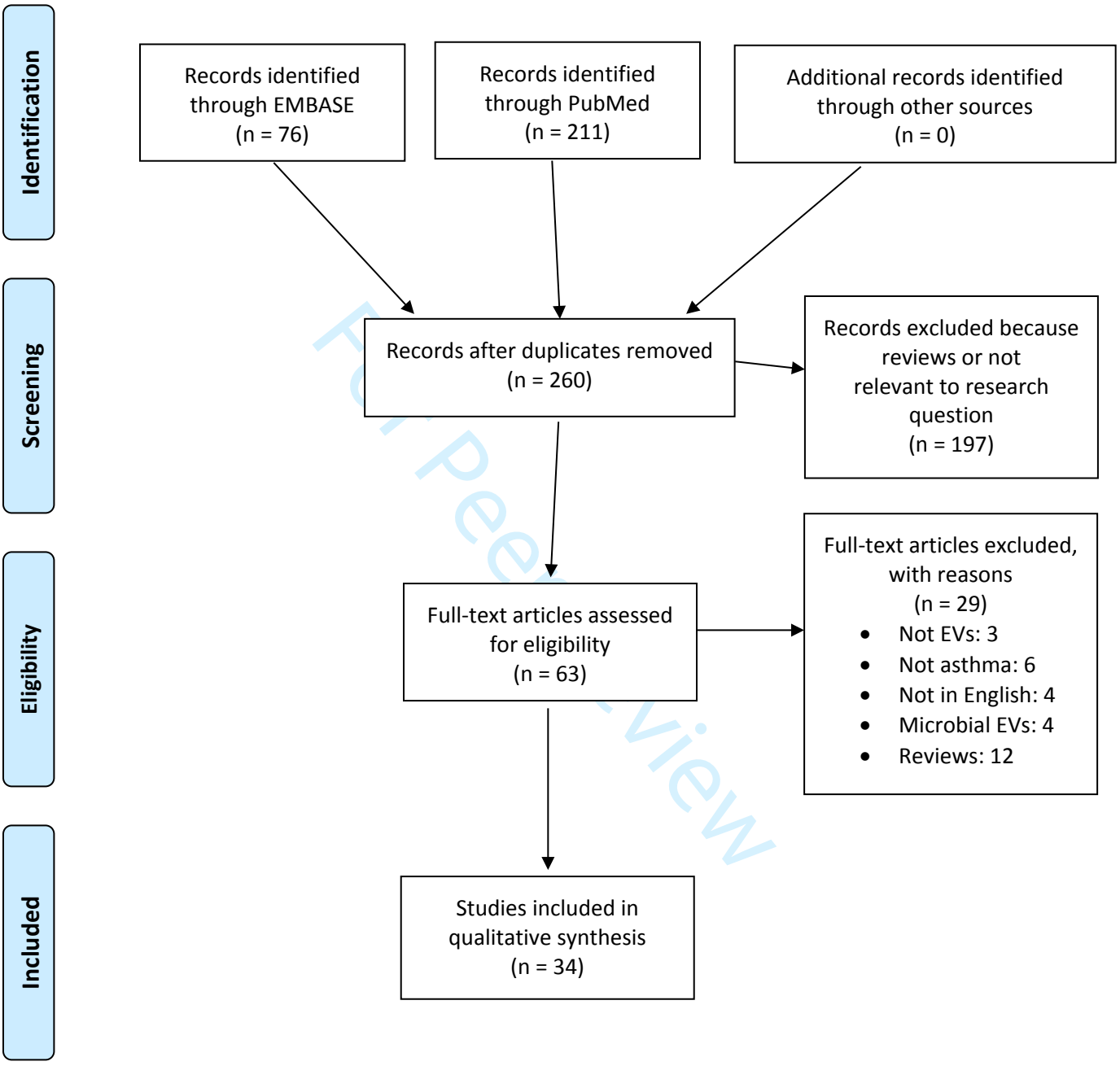

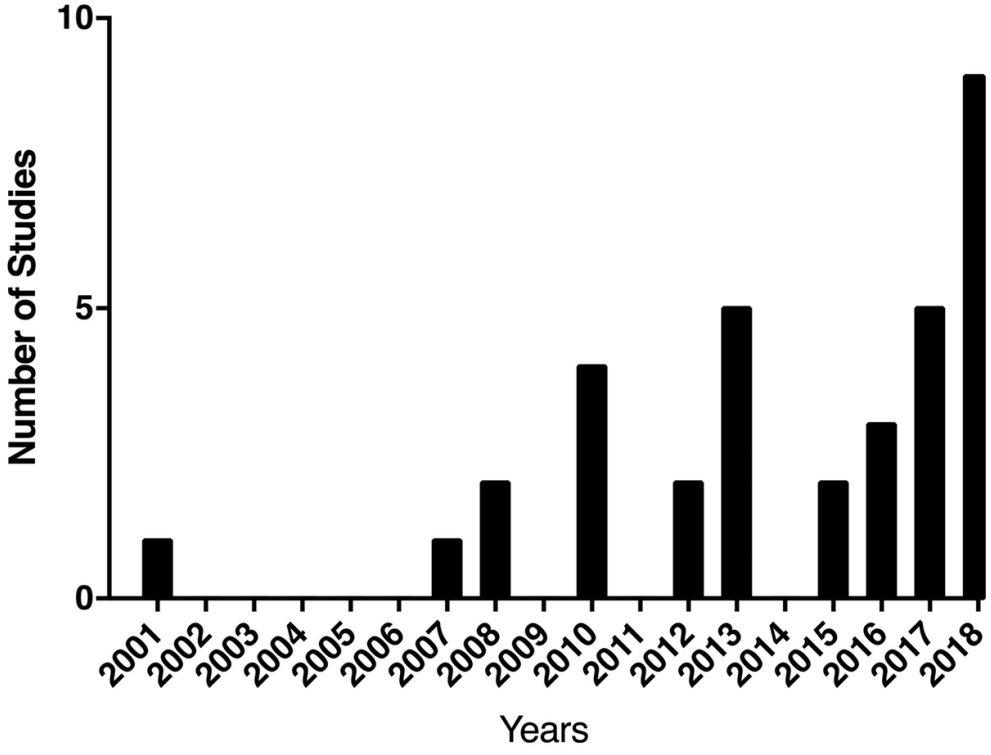

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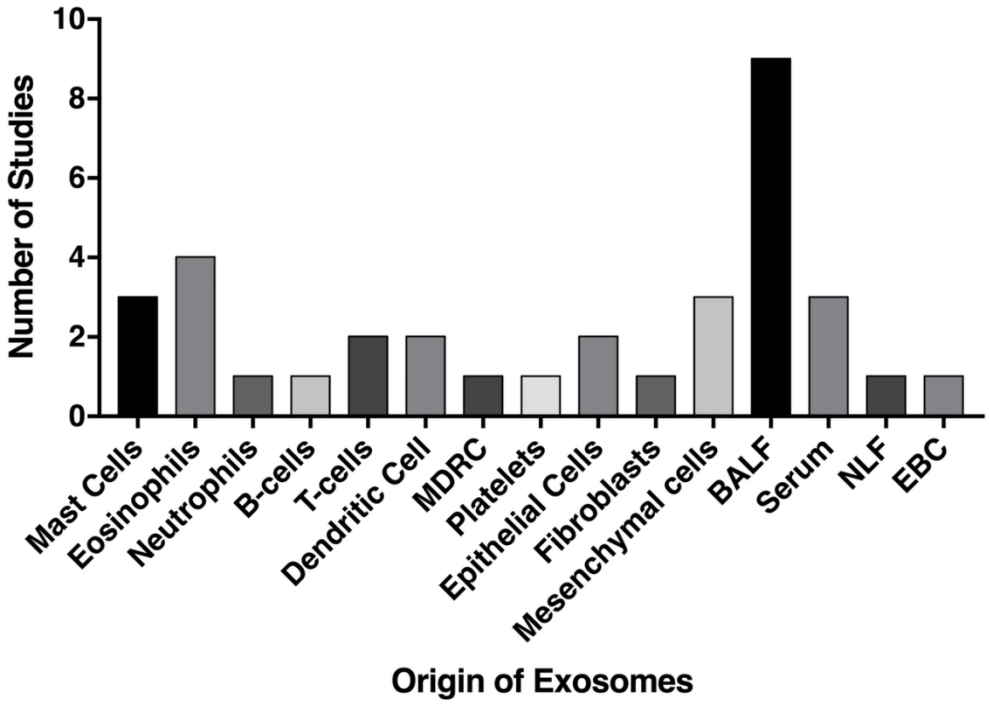

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