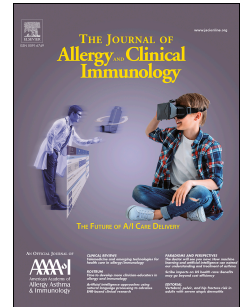


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Role of exosomes in allergic asthma: signaling platforms between the epithelium and type 2 immunity.

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The elegant work carried out by Zhang and Colleagues in this issue (1) establishes that exosomes activate (and are uptaken by) monocyte-derived, inflammatory dendritic cells (DCs) through contactin-1 and Notch2 activation, which in turn promote Th2 as well as Th17-polarized immune activation along mucus production and smooth muscle hyperresponsiveness.

The discovery that the airway epithelium may initiate and shape type 2 adaptive immune responses to allergens has been a major breakthrough in allergy and asthma pathophysiology (2-3). Thus, the airway epithelium releases cytokines that will “alarm” the immune system by activating DCs in such a way they polarize (and activate) naïve T cells into Th2 cells. Those alarmins consist of TSLP, IL-33, IL-25, GM-CSF as well as other mediators such as serum amyloid A protein (5). Those cytokines (in particular TSLP and IL-33) may also activate innate lymphoid cells type 2 (ILC2). In addition to soluble mediators, it has been shown that protein crystals made of galectin-10 and forming so-called Charcot-Leyden crystals may also activate type 2 immunity (4).

In a pilot series of experiments of their paper (1), Zhang M et al. first showed that airway epithelial cells release increased numbers of CD63- and CD81-positive exosomes upon stimulation by house dust mite (HDM), a major allergen in allergic airway disease such as rhinitis and asthma, and that those exosomes are also released in the BALF from HDM-exposed mice. To address their role in the pathophysiology of asthma, they blocked exosomes' release by using GW4869, a non-competitive inhibitor blocking the synthesis and/or release of exosomes, which resulted in reduced recruitment of eosinophils in BAL from HDM-exposed and challenged mice. Airway tissue infiltration by inflammatory cells, mucus production, and levels of type 2 cytokines (IL-4, IL-5, IL-13) as well as of IFN- γ but not of IL-17A, were also downregulated. In addition, granulocyte influx by eosinophils and neutrophils was observed upon transfer to mice of exosomes released by HDM-activated airway epithelial cells, as compared to exosomes from resting cells. Both Th2 and Th17 cytokines were upregulated in this system, as well as mucus production, without difference when mice were pretreated with GW4869 to block endogenous endosome generation. These results collectively indicate that exosomes may contribute, although only partly and to a lower extent than HDM itself, to allergic airway inflammation.

In order to identify through which cells exosomes could mediate such pro-inflammatory effects in the lungs, the Authors have traced exosomes by labelling them with PKH26 and showed that up to 50% of “activated” exosomes (released by HDM-activated airway epithelial cells) are uptake by dendritic cells (DCs). In addition, exosomes-treated mice displayed increased numbers of CD11b⁺ DCs, which were consistently reduced in HDM-treated mice following GW4869 pretreatment. In addition, another set of transfer experiments were performed with

bone marrow-derived DCs stimulated by exosomes released by HDM-activated airway epithelial cells. The transfer of those activated DCs in mice promoted eosinophils recruitment and Th2/Th17 responses as well as mucus production and airway infiltrates.

To go deeper in the mechanism by which “activated” exosomes promote allergic airway inflammation, an unbiased proteomic analysis of epithelial-derived exosomes was carried out, revealing 27 proteins overexpressed (by at least two fold) in “activated” exosomes (released by HDM-activated epithelial cells versus those released by PBS-treated cells). Following heat map and cluster analysis, 4 proteins were mostly enriched among which contactin-1 (CNTN1), which was further explored. First, CNTN1 was upregulated in HDM-treated airway epithelial cells and in the lungs from HDM-treated mice. When the Authors injected a silencing CNTN1-RNA in mice before HDM treatment, monocyte-derived DC numbers were reduced (whereas those cells increased by more than 2-fold upon treatment with exogenous CNTN1, which induced increased migration of EGFP-labelled DCs towards mediastinal lymph nodes), along decreases in Th2 and Th17 cells, mucus production as well as in airway hyperresponsiveness to methacholine. Furthermore, the authors showed that the only known receptor for CNTN1 that was upregulated in DCs treated by CNTN1 was Notch2, which binding was confirmed by co-immunoprecipitation. When DCs were treated with GSI to inhibit Notch signaling, reduced responses of DCs to CNTN1 were observed in terms of CD40 and IL-6 expression (while other cytokines or HDM uptake were unaffected), and, in vivo, GSI pretreatment of DCs before the transfer could alleviate partly the induction of allergic airway inflammation.

Finally, this study shows that CNTN1 is upregulated in the serum (both in exosomes and exosome-free serum) of patients with HDM-allergic asthma, as compared to controls or patients with allergic bronchopulmonary aspergillosis. CNTN1 staining was also upregulated in bronchial biopsies from asthma patients, while serum CNTN1 correlated with type 2 biomarkers such as total IgE and FeNO.

Accumulating evidence shows that exosomes are involved in several biological processes, notably in the lungs and in relation to ageing, cancer, dysimmunity (such as allergic asthma), fibrosis, regeneration (stemness) and are candidate and innovative tools for diagnosis, prediction or therapy (5). It was shown the epithelial (and not macrophage)-derived exosomes are increased in asthma (6) and could contribute to airway inflammation as suggested by effects of GW4869 which inhibits exosome production and resulted in a reduced population of proliferating monocytes and alleviation of various asthmatic features. It is noteworthy that pro-type 2 immunity effects have previously been reported with exosomes released by dendritic cells, notably following activation by TSLP (7) that promoted OX40L expression and thereby Th2 cell differentiation, or release by fibroblasts or eosinophils (8). The study by Zhang enriches the paradigm that structural cells, and in particular the epithelium which is in a pivotal

location to sense inhaled allergens, can initiate T2 immune responses in the airways by releasing exosomes that display particular features such as increased CNTN1 content (Figure 1). In addition, a recent study revealed that T2 and T17 cytokines may conversely affect the content of airway epithelium-derived exosomes, promoting notably granulocyte chemotaxis.

Biologics have revolutionized severe asthma care, with anti-IgE, anti-IL-5(R) and anti-IL-4R monoclonals. It is expected that anti-TSLP will represent the next wave of antibodies, targeting upstream, epithelial-derived “alarmins”. This study showing that the release of exosomes by epithelial cells exposed to HDM allergens represents a novel path contributing to allergic airway inflammation, could constitute the cornerstone to studies further addressing the molecular pathways connected to exposome biology in asthma. It is possible that targeting the epithelium generation of exosomes could reveal beneficial in severe asthma. While this is beyond its scope, the study by Zhang et al. may deserve some speculation on asthma therapy. Targeting eosinophils has revealed highly effective in most severe asthma patients with eosinophilic inflammation, even in most severe, cortico-dependent patients, but this approach does not display disease-modifying effects (i.e. sustained effects following withdrawal). In this regard, the TSLP/TSLP-R pathway has been implicated in the persistence of disease in patients with occupational asthma (9). It could be speculated that epithelium-derived pathways acting very upstream of type 2 immune pathways, such as soluble TSLP or eventually exosomes, may reveal effective with hope of disease-modifying effects and disease remission. Thanks to this kind of basic research, the discovery of further novel therapeutics may be expected in the future and the quest for asthma cure (10) remains hopeful.

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Figure 1 legend. Epithelium-derived signaling to immune cells that instructs type 2 immunity in asthma.

The airway epithelium from susceptible individuals may, following activation by inhaled allergens such as HDM, release different sets of molecules that will drive type 2 immune responses, among which cytokines, Charcot-Leyden crystals as well as exosomes. *Inset*, Those exomes provide DCs with pro-T2 signals through interactions between contactin-1 (CNTN1) and Notch2.

