Editorial

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 et al and reported in this issue of the *Journal of Allergy and Clinical Immunology*[1] establishes that exosomes activate (and are uptaken by) monocyte-derived, inflammatory dendritic cells (DCs) through contactin-1 and Notch2 activation, which in turn promotes T_H2 cell– and T_H17 cell–polarized immune activation along with 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 that they polarize (and activate) naive T cells into T_H2 cells. Those alarmins consist of thymic stromal lymphopoietin (TSLP), IL-33, IL-25, GM-CSF, and other mediators such as serum amyloid A protein.[4] Those cytokines (in particular, TSLP and IL-33) may also activate type 2 innate lymphoid cells. It has been shown that in addition to soluble mediators, protein crystals made of galectin-10 and forming so-called Charcot-Leyden crystals may also activate type 2 immunity.[5]

In a pilot series of experiments reported by Zhang et al,[1] it was shown for the first time that airway epithelial cells release increased numbers of $CD63^+$ and $CD81^+$ exosomes following stimulation by house dust mite (HDM), a major allergen in allergic airway diseases such as rhinitis and asthma, and that those exosomes are also released in the bronchoalveolar lavage fluid from HDM-exposed mice. To address the role of these exosomes in the pathophysiology of asthma, Zhang et al[1] blocked exosomes' release by using GW4869, a noncompetitive inhibitor blocking exosome synthesis and/or release, which resulted in reduced recruitment of eosinophils in the bronchoalveolar lavage fluid from HDM-exposed and HDM-challenged mice. Airway tissue infiltration by inflammatory cells, mucus production, and levels of type 2 cytokines (IL-4, IL-5, and IL-13), as well as IFN-γ levels but not IL-17A levels, were also downregulated. In addition, granulocyte influx by eosinophils and neutrophils was observed following transfer to mice of exosomes released by HDM-activated airway epithelial cells, as compared with exosomes from resting cells. Both T_H2 cytokines and T_H17 cytokines were upregulated in this system, as was 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.

To identify the cells through which exosomes could mediate such proinflammatory effects in the lungs, Zhang et al[1] traced exosomes by labeling them with PKH26 and showed that up to 50% of "activated" exosomes (released by HDM-activated airway epithelial cells) are uptaken by DCs. In addition, exosome-treated mice displayed increased numbers of $CD11b^+$ DCs, the levels of which were consistently reduced in HDM-treated mice following GW4869 pretreatment. In addition, another set of transfer experiments was 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 eosinophil recruitment and T_H2 cell and/or T_H17 cell responses, as well as mucus production and airway infiltrates.

To go deeper into the mechanism by which activated exosomes promote allergic airway inflammation, an unbiased proteomic analysis of epithelial-derived exosomes was carried out, revealing 27 proteins that were overexpressed (by at least 2-fold) in activated exosomes (released by HDM-activated epithelial cells versus those released by PBS-treated cells). Following heatmap and cluster analysis, 4 proteins were mostly enriched; among these, contactin-1 (CNTN1) was further explored. First, CNTN1 was upregulated in HDM-treated airway epithelial cells and in lungs from HDM-treated mice. When Zhang et al[1] injected a silencing CNTN1 RNA into mice before HDM treatment, there was a decrease in the numbers of monocyte-derived DCs (whereas their numbers increased by more than 2-fold following treatment with exogenous CNTN1, which induced increased migration of EGFP-labeled DCs toward mediastinal lymph nodes), along with decreases in the numbers of T_H2 cells and T_H17 cells, mucus production, and airway hyperresponsiveness to methacholine. Furthermore, Zhang et al[1] showed that the only known receptor for CNTN1 that was upregulated in DCs treated with CNTN1 was Notch2, the binding of which was confirmed by coimmunoprecipitation. When DCs were treated with γ-secretase inhibitors to inhibit Notch signaling, reduced responses of DCs to CNTN1 were observed in terms of CD40 and IL-6 expression (whereas other cytokines or HDM uptake were unaffected), and *in vivo*, γ-secretase inhibitor pretreatment of DCs before the transfer could partly alleviate the induction of allergic airway inflammation.

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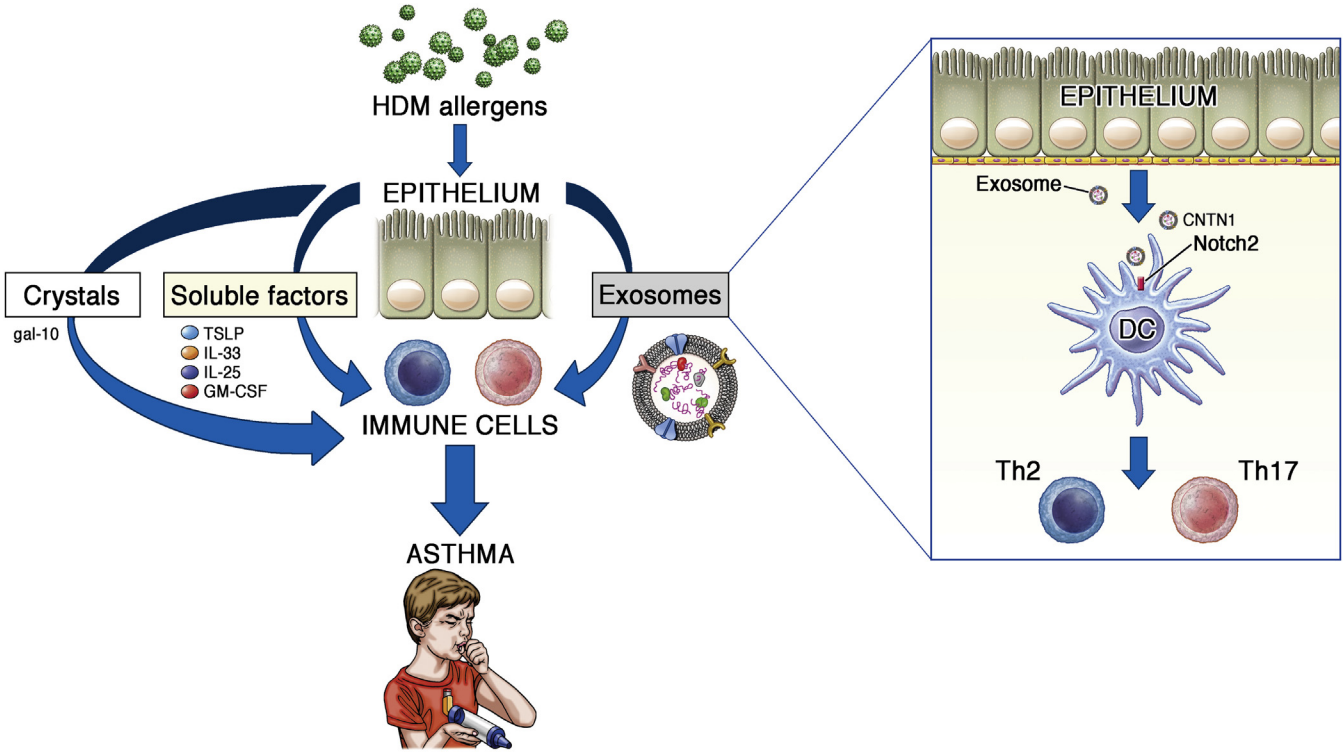

FIG 1. Epithelium-derived signaling to immune cells that instructs type 2 immunity in asthma. The airway epithelium from susceptible individuals may, following activation by inhaled allergens such as HDM, release different sets of molecules that will drive type 2 immune responses, which include cytokines, Charcot-Leyden crystals, and exosomes. *Inset,* Those exomes provide DCs with pro–type 2 signals through interactions between contactin-1 (CNTN1) and Notch2.

Finally, the study reported by Zhang et al[1] shows that CNTN1 is upregulated in the serum (both in exosomes and in exosome-free serum) of patients with HDM-allergic asthma, as compared with in the serum of controls or patients with allergic bronchopulmonary aspergillosis. CNTN1 staining was also upregulated in bronchial biopsy samples from patients with asthma, whereas serum CNTN1 level was correlated with the levels of type 2 biomarkers such as total IgE and fractional exhaled nitric oxide.

Accumulating evidence shows that exosomes are involved in several biologic processes, notably, in the lungs and in relation to aging, cancer, dysimmunity (such as allergic asthma), fibrosis, and regeneration (stemness) and are candidate and innovative tools for diagnosis, prediction, or therapy.[4] It was shown that the levels of epithelial- (but not macrophage)-derived exosomes are increased in asthma[6] and could contribute to airway inflammation, as suggested by the effects of GW4869, which inhibits exosome production and results 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 DCs, notably, following activation by TSLP,[7] which promoted OX40L expression and thereby T_H2 cell differentiation, or release by fibroblasts or eosinophils.[8] The study by Zhang et al[1] enriches the paradigm that structural cells, and in particular, the epithelium, which is in a pivotal location to sense inhaled allergens, can initiate type 2 immune responses in the airways by releasing exosomes that display particular features such as increased CNTN1 content (Fig 1). In addition, a recent study revealed that T_H2 and T_H17 cytokines may conversely affect the content of airway epithelium-derived exosomes, notably promoting granulocyte chemotaxis.

Biologics have revolutionized the care of patients with severe asthma, with anti-IgE, anti–IL-5 receptor, and anti–IL-4 receptor mAbs. It is expected that anti-TSLP will represent the next wave of antibodies, targeting upstream, epithelial-derived "alarmins." The study by Zhang et al,[1] which shows 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 prove beneficial in severe asthma. Although this possibility is beyond its scope, the study by Zhang et al[1] may warrant some speculation on asthma therapy. Targeting eosinophils has proved highly effective in patients with the most severe asthma with eosinophilic inflammation, and even in those patients with the most severe corticodependent asthma, but this approach does not display disease-modifying effects (ie, sustained effects following withdrawal). In this regard, the TSLP/TSLP receptor pathway has been implicated in the persistence of disease in patients with occupational asthma.[9] It could be speculated that epithelium-derived pathways acting far upstream of type 2 immune pathways, such as soluble TSLP or eventually exosomes, may prove effective, with the promise 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 promising.

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