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Ezrin in Asthma: A First Step to Early Biomarkers of Airway Epithelial Dysfunction

The airway epithelium has gained significant traction in recent years as a major site of disease origin, perpetuation, or resolution in lung diseases such as asthma, chronic obstructive pulmonary disease, bronchiolitis obliterans syndrome, or idiopathic pulmonary fibrosis. Previously underappreciated, the diversity of epithelial cell types in the airways significantly contributes to homeostasis of the lung's inner surface (entirely represented by epithelial cells). Airway epithelial cell dysfunction has been detected in most, if not all, chronic lung diseases. Importantly, our knowledge about the lung epithelium continuously increases with the availability of novel technologies, and in particular single-cell RNA sequencing approaches, which have uncovered previously unknown cell types in the lung, such as the recently described ionocyte (1).

Despite this wealth of knowledge on airway epithelial cell phenotypes, we are currently unable to easily detect or monitor airway epithelial cell viability/dysfunction in minimally invasive liquid biopsies, such as exhaled breath condensate (EBC), serum, or urine. It is reasonable to hypothesize that we could intervene in lung disease pathogenesis and progression more efficiently if we were able to detect early biomarkers of epithelial cell dysfunction in the abovementioned biosamples.

This is the truly exciting finding that Jia and colleagues report in this issue of the *Journal* (pp. 496–507) (2). The authors sought to measure ezrin, a member of the ERM (ezrin/radixin/moesin) family of membrane cytoskeletal proteins, in BAL fluid (BALF) in mice, and EBC and serum in humans with asthma. Using a moderately sized asthma single-center cohort ($n = 61$), and an OVA (ovalbumin)-induced animal model of asthma, the authors explore the relationship between ezrin abundance (which the authors suggest is present in airway epithelial cell-derived exosomes) and clinical parameters of asthma severity. Protein measurements of ezrin detected a surprisingly tight correlation between ezrin and lung function, independent of the origin of the liquid biosample used

to measure ezrin (e.g., EBC, serum in humans, or BALF in mice). In other words, the authors were able to detect a physiologically secreted ERM protein, ezrin, in all liquid biosamples investigated. High levels of ezrin in EBC and serum correlated with normal lung function, whereas a decline in lung function (FEV_1 , $FEV_1\%$ predicted, or FEV_1/FVC) correlated with decreased ezrin levels. The authors went on to show that ezrin is contained in exosomes secreted by cultured airway epithelial cells, and that IL-13 is a key factor inhibiting ezrin secretion in a JAK2 (janus kinase 2)-dependent manner. Knockdown of ezrin resulted in increased epithelial permeability, which the authors argue could be an early event in IL-13-driven asthma. Although exosome secretion to EBC and BALF seems logical given ezrin expression in airway epithelial cells, it will be informative to investigate how and why ezrin levels are detected in serum, and whether these are truly derived from airway epithelial cells.

At this point, most readers will ask, What is ezrin, and what is its normal cellular function? Ezrin is a protein with a molecular mass of 80 kD, which was initially identified as a substrate for the epidermal growth factor receptor protein-tyrosine kinase and as a structural component of the microvillus cytoskeleton in chicken intestinal epithelial cells and other actin-containing surface structures (3, 4). Ezrin is a critical member of the ERM family of proteins and acts at the cytoplasmic surface of cellular membranes by linking plasma membrane proteins to the cortical actin cytoskeleton. This function is essential for cell–cell and cell–substrate adhesion, cell morphology, and polarity, as well as the formation and maintenance of actin-based cell surface structures (5, 6). Of note, ezrin remains inactive in the cytoplasm and exerts its crucial regulatory role in plasma membrane-based processes through direct interactions with membrane proteins, such as the hyaluronan receptor CD44 (7) or cytoplasmic adaptor proteins, such as ezrin-binding protein EBP50 (8) via a dynamic phosphorylation and dephosphorylation process.

In 2001, Laoukili and colleagues first reported that IL-13 downregulates the expression of ezrin and interferes with its apical localization to alter epithelial cell polarization and ciliated cell differentiation in human nasal epithelial spheroid cultures (9).

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Similarly, IL-13 treatment results in a loss of ciliated epithelial cells by mislocation of basal bodies and loss of ezrin from the apical cell compartment and an increase in mucus-secreting cells (10, 11). These findings, together with the data reported in this issue of the *Journal*, highlight an important role of ezrin in IL-13-mediated airway epithelial mucociliary differentiation and dysfunction as a new molecular and cellular mechanism leading to airway epithelium damage and obstruction in asthma.

In addition, ezrin functions as an anchoring protein in a complex with the CFTR (cystic fibrosis transmembrane conductance regulator) at the membrane of respiratory epithelial cells, and modulates CFTR trafficking (12, 13), cell surface stabilization (14), tight junction formation (15), and chloride secretion (16). As such, it would be worth determining whether ezrin is involved in the regulation of CFTR signaling in the abovementioned new Foxi1⁺ ionocyte (identified as the major source of CFTR transcripts in the lung), and whether ionocyte-derived exosomes account for the majority of ezrin levels detected by Jia and colleagues (2).

Patients with asthma are also frequently affected by exacerbations resulting from infectious or noninfectious triggers. The airway epithelium is the primary target of inhaled pathogens, such as human rhinovirus (HRV), a common trigger of acute exacerbations of asthma. A series of studies demonstrated that ezrin forms a protein complex with ICAM-1 (intercellular adhesion molecule 1) and Syk (spleen tyrosine kinase) at the plasma membrane, and subsequently activates Syk for HRV internalization, HRV-induced activation of p38 MAP (mitogen-activated protein) kinase and PI3 (phosphatidyl-inositol 3) kinase, and IL-8 expression (17, 18). As such, decreased ezrin expression may impair effective antiviral and host defense and lead to increased HRV-induced asthma exacerbations. It would thus be highly valuable to investigate whether patients with asthma with lower ezrin expression are subject to more frequent or severe exacerbations in at least two independent cohorts in the future.

In summary, airway epithelial cells are significant cellular sources for exosomes in the airways of patients with asthma, and thus respiratory exosome-associated ezrin expression has potential implications for disease pathogenesis and intervention in airway diseases. Whether the downregulation of ezrin is a useful biomarker that can indicate airway epithelial cell injury for asthma needs to be further determined. A comprehensive characterization of ezrin expression and distribution in respiratory exosomes should be performed in isolated exosomes from BALF of patients with asthma, clinically relevant allergic (i.e., house dust mite) mouse model, and well-differentiated human primary airway epithelial cell cultures on IL-13 stimulation. All these further efforts will clarify and extend the exciting observations of ezrin down-regulation and lung function decline in an asthmatic setting. It will be of interest to investigate whether ezrin is similarly regulated in other chronic lung diseases with IL-13 increases (e.g., chronic obstructive pulmonary disease), or whether this effect is simply very asthma-specific. Further mechanistic studies exploring how downregulation of ezrin via airway epithelial cell-derived exosomes causes the damage of airway epithelium will also address whether exosomes containing ezrin may present as a new treatment option in asthma or other lung diseases with permeability defects. The current study by Jia and colleagues (2) has clearly begun to open these doors to a more detailed understanding of airway epithelial cell function in asthma. ■

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Effects of Anti-IL-5 on Virus-induced Exacerbation in Asthma Light and Shadow

Viral infections are among the most frequent causes of asthma exacerbation (1). The prevention and/or treatment of viral-induced asthma exacerbations are still unmet medical needs. Indeed, these events, characterized by a transient impaired responsiveness to corticosteroids (2), occur despite appropriate asthma treatment (3). The identification of novel pharmacological approaches able to affect viral-induced asthma exacerbations has the potential to affect the natural history of the disease.

Treatment with anti-IL-5 is a very effective pharmacological intervention to quench eosinophilic inflammation, with current clinical indications in severe eosinophilic asthma and in eosinophilic granulomatosis with polyangiitis (4, 5).

Eosinophils are also important for innate and adaptive immune responses to pathogens (6, 7). Investigating the effects of mepolizumab on virus-induced immune response and on viral infection outcomes is of major clinical relevance.

In this issue of the *Journal*, Sabogal Piñeros and colleagues (pp. 508–517) report the results of an interesting study in which mild steroid-naïve patients with asthma were randomly assigned to receive (irrespective of the blood eosinophil levels) either 750 mg intravenous mepolizumab or placebo in a double-blind manner (8). Two weeks after the injection, the patients were experimentally infected with rhinovirus 16. The study shows that mepolizumab treatment reduced eosinophil activation but did not prevent activation of the remaining eosinophils in response to a rhinovirus 16 challenge. The lack of effect of anti-IL-5 in mild asthma may relate to the remaining activated eosinophils and the altered humoral and cellular immune response to a viral challenge.

The study has the merit to investigate immunological pathways related to triggers that are relevant in real life, and to provide some previously unexplored *in vivo* effects of mepolizumab on immune response to rhinovirus. Mepolizumab induced the potentiation/activation of key elements of the immune response to viral infection (9, 10), including enhanced systemic NK cells, prevention of rhinovirus 16 depletion of B-lymphocytes in BAL fluid, and enhanced secretory IgA. However, patients receiving mepolizumab showed significant higher viral load in nasal swabs, reflecting either enhanced viral replication rates or reduced viral clearance. These data suggest that, despite the fact that mepolizumab treatment promotes pathways with antiviral/antimicrobial activities, the overall changes in the immunologic response yield to pro-infective results. Possibly, the

anti-IL-5-induced reduction of eosinophils [with their innate immune activities (11)] and the attenuation of neutrophil response to the viral challenge (involved in the inflammatory response to viral infection) have a role in these outcomes.

Interestingly, previous studies have shown a negative interplay between the T2 inflammatory milieu (including eosinophils) and the efficacy of the immune response to infections (12). *In vitro* inhibition of T2 inflammatory mediators resulted in enhanced antiviral/antimicrobial activity (12). *In vivo*, treatment with humanized monoclonal anti-IgE antibodies (omalizumab) in children with mild asthma resulted in a significant reduction of viral-induced exacerbation of asthma (13, 14). This positive clinical effect was paralleled by blood leukocytes-enhanced innate immune response to viral infection and, at variance with the finding reported in study by Sabogal Piñeros and colleagues, decreased viral shedding in nasal mucus samples (8, 13). These data suggest that targeting IgE versus eosinophil may result in different clinical outcomes of the viral infection in asthma.

Modulation of T2 inflammatory mediators (including IL-5, IL-13, and IgE) is the novel forefront of asthma treatment. Further studies are needed to better understand the specific mechanisms of action of the biological treatments targeting different biomarkers. In particular, whether these pharmacological approaches can fill the gap in the prevention and treatment of viral-induced asthma exacerbation must be tested in specifically designed studies.

Inevitably, every light has its shadow. The study by Sabogal Piñeros and colleagues does not inform on the clinical effect of mepolizumab in this setting. Indeed, the study was powered for a primary lung function outcome, and no clinical effect was expected. Mepolizumab did not affect lung function or asthma control scores both at baseline and after the experimental infection. The variability of the markers assessed in the study, with significant changes in sputum eosinophils (their Figure 3) and in blood eosinophil activation (*see* Table E1 in their online supplement) in the placebo group before any intervention, and the very weak eosinophilic response [at variance with previous studies (15)] after rhinovirus 16 challenge, question the design of the study and should prompt stringent control conditions in future studies.

Certainly, proof-of-concept studies similar to the one published in this issue of the *Journal* by Sabogal Piñeros and colleagues are of inestimable value and must not be ignored by the scientific community (8). Indeed, by providing light and shadow, they can stimulate the development of new working hypothesis, and thus favor our understanding of the mechanisms of the disease and promote the progress of asthma management. ■