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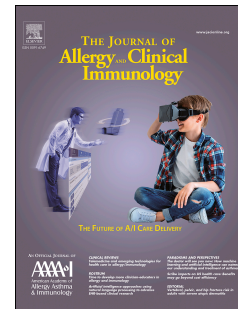
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Weaving innate lymphoid cells (ILC) into the fabric of asthma exacerbations

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Asthma exacerbations are a major cause of morbidity for many patients. Although major progress has been made to establish the mechanisms associated with asthma exacerbations, the complexity and heterogeneity of immune responses during exacerbations remain to be fully elucidated and is an important step towards improved treatment. Dhariwal and colleagues of the MRC-GSK Strategic Alliance consortium focused on a new aspect of these responses, the roles and contributions of innate lymphoid cells (ILCs) in asthma exacerbations.¹ Their findings underscore the contribution of ILC2 cells to airway inflammation, but perhaps more important and interesting is the identification of an altered reciprocal relationship with ILC1 cells that may diminish antiviral activity in asthma.

To frame the contributions of Dhariwal and co-investigators,¹ a brief review of the mechanisms of asthma exacerbation will be helpful. Although multiple environmental factors contribute to asthma exacerbations, rhinovirus (RV) infections are the dominant cause in both children and adults. Investigators in this UK-based consortium have developed an informative translational model of asthma exacerbations with the use of an experimental respiratory infection of asthma patients with RV-A16. Their model has proven to have a high degree of clinical relevance. First, the experimental infections can be performed in asthma patients making observations directly translatable to clinical disease. As the safety aspects of the experimental infection have been verified, this investigative group has inoculated patients with moderate persistent asthma and caused reductions in lung function to parallel changes seen during a natural RV infection. Second, because RV is the experimental inoculum, the infectious organism matches the viral features of clinical events. Finally, by directly sampling the lower airway with bronchoscopy in

relationship to the infection, it becomes possible to more precisely establish patterns of the immune responses associated with the RV illness and changes in pulmonary physiology.

Dhariwal et al.¹ focused on two key elements: 1) RV activation of airway epithelial cells to initiate inflammation and 2) the association of defective antiviral activity in asthma. In patients with asthma, RV infection increases secretion of alarmins, IL-33 and IL-25. Generation of epithelial cell alarmins can activate both adaptive and innate components of airway inflammation, Th2 and ILC2, respectively, to increase secretion of type (T) 2 cytokines, IL-4, IL-5, and IL-13. There may be further amplification of T2 inflammation as RV interact with neutrophils, to cause NETosis with release double-stranded DNA to activate Th2 cells release of T2 cytokines. The T2 inflammatory response that occurs during an RV-infection is characterized by a recruitment of eosinophils to the airway and the development of airflow obstruction. Therefore, a key step in RV-provoked asthma exacerbations is further generation of a T2 inflammatory response in patients with an already established T2 phenotype.

A potential second risk factor for exacerbations is impaired antiviral activity in asthma. Initial studies with cultured bronchial epithelial cells from asthma subjects found diminished interferon β and interferon- λ compared to controls.² Subsequent assessments of RV-induced interferon from *in vitro* and clinical studies were more variable, with reports that asthma was associated with interferon responses that were deficient, normal, or even greater than normal.³⁻⁶ This apparent conflict likely stems from differences in study design and study populations. There is also evidence that asthma is associated with delayed virus-induced interferon responses,⁵ which could influence clinical outcomes. A brisk interferon response at the onset of infection could

limit viral replication and cell-to-cell spread of the infecting virus to reduce the risk of a severe illness. Accordingly, in a study of children with moderate-severe asthma, increased interferon gene expression (a low T2/IFN ratio) in airway epithelial cells sampled at the beginning of the viral respiratory infection season was associated with a reduced risk for wheezing illnesses.⁶ If the initial interferon responses to a virus infection are defective, this may lead to uncontrolled viral replication with a late and excessive interferon response that increases the risk of exacerbation.

In addition, asthma control can influence interferon responses to viral infection. In previous inoculation studies, interferon responses were normal in study subjects with well-controlled asthma but deficient in subjects with mild-moderate persistent allergic asthma (as in the current study by Dhariwal et al.¹). These findings suggest that allergic airway inflammation may impair antiviral responses. Accordingly, Teach et al.⁷ treated children with mild-moderate asthma with omalizumab and tested for its effects on viral infections, illnesses, and immune responses. Omalizumab restored the *ex vivo* IFN α response of RV-stimulated PBMCs, and this restoration in IFN α generation was associated with protection from asthma exacerbations.⁷ These findings demonstrate that T2-mediated inflammation can suppress RV-induced interferon and increase susceptibility to viral wheeze.

Dhariwal and co-workers¹ performed bronchoscopy with lavage to more comprehensively delineate cellular and molecular mechanisms in asthma during a RV illness. By performing bronchoscopy on three occasions: baseline (pre-cold), day 3 of the cold (maximal symptoms), and day 8 (recovery or sustained effects), they were able to define the kinetics of cell recruitment

to the airway as well as ensuing immune and physiological changes. Their focus on ILC participation in the response to RV infection is novel, and sheds new light on how the reciprocal relationships of these two distinct immune cell populations relates to exacerbations.

ILCs are a heterogeneous population of bone marrow derived cells whose roles and contributions to asthma are of escalating interest, importance and complexity. Three ILC subtypes have been identified: ILC1, ILC2, and ILC3. Dhariwal et al.¹ evaluated appearance of ILC1 and ILC2 in the airway during the RV infection and their relationship to the infection and development of an asthma exacerbation. ILC2 cells contribute to T2 inflammation through generation of IL-4, -5, -9 and -13 following activation by epithelial cell alarmins, IL-25, IL-33 and TSLP. ILC1 cells, in contrast, have antiviral and immunoregulatory properties by producing IFN- γ . Until this study, there has been limited information on this cell population in asthma and exacerbations. Dhariwal et al found the reciprocal, or balanced, relationship of these two ILC cells may be shifted in asthma such that the effect is enhanced T2 inflammation and diminished antiviral activity.¹

RV inoculation of 11 patients with moderate persistent asthma caused increased respiratory symptoms and a significant fall in the morning FEV₁. Although RV inoculation increased respiratory symptoms in the control participants (n=12), the symptoms were less severe, and there was no significant change in lung function. The investigators also monitored the upper and lower airways for viral load and generation of cytokines during the experimental cold. Nasal viral load was similar between the two study groups except for a greater increase in viral mRNA at day 4 in asthma.

The investigators used state of the art methods to identify asthma-related differences in nasal and BAL immune responses, including novel observations related to ILC trafficking. There was a skewed T2 immune response (i.e. increased IL-5) in nasal secretions at baseline and following RV infection in asthma. Further, there was an enrichment of ILC2 cells at baseline in asthma, likely serving as a source of T2 cytokines and persistent inflammation. RV infection led to the recruitment of both ILC1 and ILC2 cells in the BAL of both controls and asthma subjects. However, the relative delay in asthma of the appearance of ILC1 cells in the airway during the infection suggests diminished or defective antiviral activity. It is also possible that increased T2 inflammation at baseline suppressed subsequent antiviral IFN responses in asthma. Notably, both ILC1 and ILC2 responses in asthma were delayed and of greater magnitude at the time of the day 8 BAL. These observations suggest that the antiviral response to RV in asthma may be both diminished and delayed.⁵

The authors calculated an ILC2/ILC1 ratio, which was greater in asthma subjects at baseline and after infection, indicating a persistent bias towards T2 inflammation. Overall, the ILC2/ILC1 ratios correlated with virus load, lower respiratory tract symptom scores, and nasal measures of IL-5 protein. One caveat is that the healthy and asthma groups, which differed in the ILC2:ILC1 ratio and several of the dependent variables (e.g. IL-5, viral load, Day 4), were combined in the analysis, which places limits on the interpretation of these correlations. These new data suggest a loss of balance in asthma between protection (ILC1) and inflammation (ILC2) that may contribute to RV illness and exacerbation. We previously noted a similar shift in balance between protection and inflammation in a RV inoculation study, which showed inverse

relationships between the IFN- γ /IL-5 mRNA ratio in sputum cells and both respiratory symptoms and viral clearance.⁸

Entwined in these observations is a central and contributing role by IL-33 (**Figure 4**). IL-33 and IL-25 are generated by RV infection of airway epithelium. The RV-induced increase in IL-33 can activate ILC2s to further promote T2 inflammation, thus contributing to an exacerbation. Further evidence on the importance of IL-33 in asthma has been reported by Wechsler et al⁹ showing that the anti-IL-33 monoclonal antibody, itepekimab, prevented a loss of asthma control when ICS and LABAs were withdrawn.

The clinical relevance of these novel and intriguing findings linking an imbalance in the airway ILC1:ILC2 ratio to facilitate T2- inflammation during virus-induced exacerbations is supported by the efficacy of multiple T2-directed biologics that significantly reduce rates of asthma exacerbations.¹⁰ Although blockage of T2 inflammatory pathways inhibits exacerbations, the preventive response is not complete in all treated patients, suggesting a heterogeneity and complexity in the pathways which lead to asthma exacerbations. The results of Dhariwal et al.¹ suggest that investigations into the pathways that promote ILC1 cells to establish a healthy and normal balance of ILCs in the airway could lead to new strategies to prevent exacerbations, and potentially achieve added benefit (**Figure**).

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Figure Legend

Figure. The roles of innate lymphoid cells in rhinovirus-provoked asthma exacerbations. RV infect airway and stimulate the release of alarmins including IL-33. IL-33 polarizes Th0 cells to Th2 cells and activates ILC2 to collectively generate a T2 pattern of inflammation by IL-4, IL-5, and IL-13. In contrast, ILC1 cells have antiviral activity. Dhariwal et al¹ have found the reciprocal relationship between ILC2 and ILC1 cells is altered in asthma resulting in an increased T2 inflammatory response and diminished ILC1 activity with reduced antiviral action.

