ILC2s in Virus-induced Asthma Exacerbations: A Starring or Supportive Role?

Perhaps there is no greater proof of the contribution of viral infections to asthma exacerbation than the significant reductions in healthcare utilization by patients with asthma since the coronavirus disease (COVID-19) pandemic began in March 2020. With the introduction of masking and social distancing, seasonal respiratory viruses—rhinoviruses, respiratory syncytial virus, influenza, parainfluenza viruses, human metapneumovirus, seasonal coronaviruses (OC43, 229E, NL63, HKU1), enteroviruses, and others—soon disappeared, first in Australia and New Zealand and then Europe and North America. Accordingly, asthma exacerbations requiring steroid treatment, emergency room visits, and hospitalizations fell around the world. Medical utilization rates were especially decreased in children.

The role of viruses in asthma exacerbations has not always been apparent, however. In the 20th century, most reviews of asthma triggers focused on exposure to allergens, such as pets, dust, and mold, as well as exposure to air pollution and tobacco smoke. It was only with the advent of PCR that exacerbations were finally linked with asthma exacerbation. A series of PCR-based studies looking at the prevalence of virus identification among various cohorts of patients with asthma when sick and well consistently showed a higher prevalence of viral infection during exacerbations, with rhinovirus making up at least half of the viruses isolated. Subsequent studies employing experimental infection of humans and mice showed that rhinovirus can infect the lower airways and cause exacerbations of allergic airways disease. But the pathogenesis of viral-induced asthma attacks has been more difficult to determine. Previous studies identified roles for exudative macrophages (1), plasmacytoid dendritic cells (2), and others.

At the same time, pediatricians recognized an association between early life viral infections and asthma. Initial attention focused on infection with respiratory syncytial virus and later rhinovirus. But how do respiratory viral infections, which generally induce a short-lived neutrophilic inflammatory response, cause eosinophilic inflammation and mucus hypersecretion typical of allergic asthma?

It was then of great interest when type 2 innate lymphoid cells (ILC2s), also known as natural helper cells or nuocytes, were identified (3–5). ILC2s, although lymphoid in appearance, do not express cell surface receptors of the T, B, or natural killer cell lineage (thus, they are lineage negative). Stimulated by the epithelial-derived innate cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), ILC2s are capable of generating cytokines typically associated with T-helper 2 (Th2) cells, including IL-5, IL-13, IL-9, and, to a lesser extent, IL-4. Importantly, ILC2 activation does not require antigen presentation by dendritic cells. Thus, mucosal ILC2s, stimulated upon epithelial infection or damage, are optimally situated to mediate viral-induced asthma phenotypes. Finally, additional studies suggest that ILC2s play a crucial role in memory responses, enhancing Th2 cell activation in response to allergens (6).

The stage was now set for an examination of the role of ILC2s in virus-induced asthma exacerbations. In this issue of the *Journal*, Dhariwal and colleagues (pp. 1259–1273) measured nasal and BAL cytokines and ILC2s in control subjects and subjects with asthma before and after nasal challenge with rhinovirus A16 (7). Patients with asthma were required to have a doctor diagnosis, treatment with inhaled corticosteroids, airways hyperresponsiveness by bronchoconstrictor challenge testing, and evidence of atopy on skin prick testing. ILC populations were identified by flow cytometry. In addition to lineage-negative, $CD45^+$ (cluster of differentiation 45 positive), $CD127^+$, and chemoattractant receptor homologous molecule expressed on T helper type 2 cells-positive ($CRTH2^+$) ILC2s, the investigators measured IFN-γ–producing CRTH2-ILC1s. Thus, the ratio of ILC2 to ILC1 cells represents the skewing of the ILC response toward, or away from, a type 2 phenotype. As might be expected, subjects with asthma showed higher baseline levels of nasal IL-5 and BAL ILC2s than control subjects.

Upon rhinovirus infection, both control subjects and subjects with asthma showed increased nasal type 1 cytokines (IFN-γ, C-X-C ligand [CXCL]10, CXCL11, and CXCL12) and BAL ILC1s, consistent with a canonical antiviral response, although the ILC1 response was delayed in subjects with asthma. In contrast, nasal IL-4, IL-5, IL-9, and IL-13 levels were significantly higher during infection in patients with asthma but not control subjects. Both control subjects and those with asthma showed a significant increase in ILC2s with infection, again with a delayed response in subjects with asthma. ILC2/ILC1 ratios were higher in subjects with asthma both before and during infection. Importantly, pulmonary ILC2/ILC1 ratios correlated with type 2 cytokine levels, viral load, and change in FEV_1, consistent with a causal role for ILC2s in exacerbation severity. Together, these results establish ILC2s as a likely mediator of viral-induced exacerbations in patients with allergic asthma. Furthermore, they provide additional evidence of type 2 skewing of the immune system, with depressed antiviral responses, in subjects with allergic asthma. Thus, the ILC2 and its upstream activators should be added to list of cell and molecular targets for asthma therapy. Indeed, antibodies against IL-25, IL-33, and TSLP are under development. Small molecule inhibitors of ROR-α (retinoic acid receptor–related orphan

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receptor-α), a transcription factor required for ILC2 development (8), may also hold promise.

Another important observation was that viral load was significantly higher in subjects with allergic asthma compared with control subjects, consistent with a depressed antiviral response. However, other studies of experimental rhinovirus infection have found no differences in viral load (9, 10), and data from patients with real-life viral infections are lacking.

Although the study by Dhariwal and colleagues (7) represents an advance in our standing of virus-induced asthma exacerbations, the picture remains incomplete. Recent studies have illustrated the heterogeneity of ILC2s. Natural ILC2 reside in the lung and expand modestly in response to IL-33, consistent with the results of this study (11). In contrast, inflammatory ILC2s can be detected in the bone marrow and peripheral blood and appear rapidly and in large numbers after IL-25 administration. In addition, the plasticity of ILCs has been emphasized. For example, it is possible that ILC2 expansion in subjects with asthma was limited by conversion of ILC2s into ILC1s through exposure to IL-1β and IL-12 (12, 13), both of which are induced by viral infection (7, 14). IL-1β and IFN-γ have also been shown to attenuate ILC2 maturation and function (15, 16). Thus, just as Th1 cell–derived IFN-γ antagonizes Th2 cellular differentiation, counterregulation of ILC2 responses also exists.

The observed correlations between asthma exacerbation severity and ILC2/ILC1 ratio do not exclude a causal role for other innate immune cells, including exudative macrophages and plasmacytoid dendritic cells. Although naïve T cells would not be expected to play a significant role in the early response to a new viral infection, Th2 cells present in the allergic airway may also play a role. Furthermore, the response of patients with severe, nonallergic asthma to respiratory viral infections has not been studied and may be different for patients with allergic asthma, as it is against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (17). Finally, other respiratory viruses, and even other rhinovirus species, may have qualitatively disparate effects on airway inflammation. For example, we have recently shown that rhinovirus C induces an exaggerated ILC2 response, which is permitted by reduced inflammasome/IL-1β activation (18).

Dharwial and colleagues have cast ILC2s as an actor in viral-induced asthma exacerbation. But will it be a leading or supporting role? We look forward to further studies examining ILCs in asthma pathogenesis. ■

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Marc B. Hershenson, M.D.
Department of Pediatrics
and
Department of Molecular and Integrative Physiology
University of Michigan Medical School
Ann Arbor, Michigan

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