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Interleukin-33: A Potential Link between Rhinovirus Infections and Asthma Exacerbation

Although respiratory virus infections are a common cause for asthma exacerbations (1, 2), the mechanism for these exacerbations in human asthma is not fully established. Respiratory infections in animal models have been shown to provoke a Th2-type inflammatory response; however, until now, such evidence has been lacking in humans. The thymic stromal lymphopoietin (TSLP) has been proposed as the liaison between infection and atopy. Ligands for Toll-like receptors or rhinovirus (RV16) are strong inducers of TSLP by epithelial cells (3–5). TSLP, in turn, can activate dendritic cells to attract or differentiate type 2 $CD4^+$ T lymphocytes (6). In addition to TSLP, IL-25 and IL-33 are also released from viral-infected and injured epithelial cells (7, 8). These three cytokines (TSLP, IL-25, and IL-33) activate the recently identified group 2 innate lymphoid cells (ILC2s). ILC are mononuclear hematopoietic cells that do not express myeloid or lymphoid cell-surface receptors. They can release high levels of cytokines, and their family has newly been reclassified (9). Although ILC1 and ILC3 produce IFN-γ and IL-17/IL-22, respectively, ILC2s produce type 2 cytokines, and in particular, IL-5 and IL-13. IL-33 is the newest member of the IL-1 cytokine family that has gained increased attention because one of its main targets are ILC2s, which are involved in initiating type 2 immune response (10). Coupled with demonstrated generation of IL-33 by viral-activated epithelial cells, the involvement of IL-33 and ILC2s in viral induced asthma has been an area of intense interest. Furthermore, higher levels of IL-33 have been detected in the lung tissue of patients with asthma with a more severe phenotype (11). However, thus far, most of the associations among IL-33, viruses, type 2 immune response, and asthma exacerbations have been demonstrated *in vitro* and in animal models, whereas viral induction of IL-33 in human asthma has not been reported to date.

In this issue of the *Journal*, Jackson and colleagues (pp. 1373–1382) report novel findings in patients with asthma and healthy control patients that provide some of these missing links (12). The authors induced experimental rhinovirus infections in a group of patients with mild and moderate asthma and demonstrated that IL-33 was released *in vivo* in the airways of patients with asthma, whereas only a trend toward its generation was seen in nonatopic healthy individuals. In patients with asthma, airway IL-33 levels correlated with the levels of type 2 cytokines (IL-5 and IL-13), asthma symptom severity, and virus load. To measure cytokines in nasal and bronchial fluids, the authors have used a novel technology that involves the nasosorption and bronchosorption devices (13), which do not require bronchoalveolar lavage or dilution of the mediators present in the airways. The second part of the study, performed *in vitro*, demonstrates that IL-33 released from RV16-infected bronchial epithelial cells (BEC) can initiate a type 2 immune response and activation of ILC2. Although some of these observations had been shown previously, this was done using animal models and viruses different from rhinovirus (14). In the study by Jackson and colleagues, human BEC and lymphocytes were used. $CD4^+CD45RA^+CCR7^+$ undifferentiated T cells (Th0) were isolated from peripheral blood and expanded *in vitro*, and ILC2 (Lineage-$CRTh2^+CD127^+$) were directly isolated from peripheral blood. After the supernatants from rhinovirus-infected BEC were added to Th0 lymphocytes or ILC2, there was a dramatic induction of type 2 cytokines. The production of type 2 cytokines was ST2-dependent, thus confirming the role of IL-33, and just as important, the events were dependent on virus replication in BEC, as ultraviolet-inactivated RV16 did not induce IL-33 in BEC, nor did their supernatants induce type 2 cytokines by lymphocytes. Using bronchoalveolar lavage samples from the same patients who were studied in the current IL-33 article, these investigators have recently shown that rhinovirus infections induced greater expression of IL-25 in patients with asthma compared with healthy control patients (15). These findings lend further support to the hypothesis that RV infections are associated with induction of Th2-type inflammation which is likely to play an important role in promoting asthma exacerbations.

Despite the considerable strengths of this study, there are some limitations and additional questions that would need to be addressed in future studies. For instance, although the reported *in vitro* experiments

involving ILC were very convincing, it would be important to demonstrate the presence of ILC2 *in vivo* after experimental viral infection. Yet we understand the difficulty in identifying ILC2 *in vivo*. In addition, normal individuals produced the same amount of IL-33 after viral infection as the patients with asthma. To examine the influence of disease state, it would be interesting to investigate the difference in IL-33 release by BEC between normal patients and patients with asthma. Similarly, the study did not show why patients with asthma are more responsive to IL-33 with regard to type 2 cytokine production compared with normal individuals. Is there a difference in ST2 expression between normal patients and patients with asthma? Likewise, it would have been informative to show whether, along with the type 2 cytokines, other types of cytokines such as IFN-γ were changed after viral infection, *in vivo*. Furthermore, the study showed that after viral infection, patients with asthma have a higher virus load than normal individuals. This has not been a universal finding, however (16, 17). The authors explain this discrepancy by the use of patients with mild asthma in previous studies. However, their more severe patient group did not show higher virus load compared with patients with mild asthma (Jackson and colleagues' Table E1 in their online supplement). In addition, the increase in bronchoalveolar lavage (BAL) eosinophils after a viral infection has not been previously observed, with many studies showing a minimal increase in total cells and neutrophils. This might be related to the patient population of the timing of BAL fluid collection, as it was done 4 days after inoculation, whereas other studies looked at an earlier time. Finally, as discussed by the authors, from a therapeutic standpoint, it would be important to know whether *in vitro* blockade of IL-33 influences rhinovirus replication in BEC, as previous reports have linked IL-33 with protective immunity against viruses in mice (18). This could have important clinical implications for the prevention of viral-induced asthma exacerbations.

In summary, although additional information and complementary studies are clearly needed, the well-designed study by Jackson and colleagues answered important questions and nicely demonstrated that IL-33 is likely a major cause for viral-induced asthma exacerbations and a potential therapeutic target in asthma. ■

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