IFN Deficiency in Asthma Attacks

Is Restoring Toll-like Receptor-7 Expression a New Treatment Approach in Severe Asthma?

It is established that respiratory virus infections precipitate the great majority of acute asthma attacks (exacerbations) in all age groups and that although all respiratory viruses can precipitate attacks, rhinoviruses predominate (1). Rhinovirus infections in people with asthma are both more prolonged and more severe than in people without asthma (2–4). In terms of mechanistic explanations for this phenomenon, although not found in all studies (5), there is now abundant evidence that asthma is frequently accompanied by broadly impaired antiviral immunity in both adults (4, 6–10) and children (11, 12) and that deficiencies may relate to markers of severity of allergy and/or asthma.

The greatest burden of morbidity and health care costs in asthma relate to severe asthma; however, the importance of deficient antiviral immunity in severe asthma is less well studied than in milder disease. Edwards and colleagues previously reported profound deficiencies in IFN-β and IFN-λ induction by rhinovirus infection of bronchial epithelial cells from children with severe asthma (bronchial epithelial cells produce no or very little IFN-α) (13). This was accompanied by increased virus replication, and replication correlated strongly with IFN deficiency (13). These data clearly implicate IFN deficiency in the pathogenesis of asthma attacks in children with severe asthma. What about adults? In this issue of the *Journal*, Rupani and colleagues (pp. 26–37) studied 35 adults with severe asthma (a history of recurrent exacerbations and inadequate disease control [median Asthma Control Questionnaire score of 2.9] despite being on step 4/5 of Global Initiative for Asthma management) (14). In the 10 of these 35 subjects in whom IFN induction was compared with healthy control subjects, IFN-α and IFN-β induction in response to rhinovirus infection of bronchoalveolar lavage (BAL)-derived adherent cells (presumed mostly macrophages) was also shown to be profoundly deficient. These are important observations and, to my knowledge, are the first to report deficient type I IFN induction in lung cells in severe asthma in adults. This study therefore fills an important gap in our knowledge. A weakness of the study is the fact that the authors did not study IFN-λ. BAL cells produce IFN-λ, and induction by rhinovirus is deficient in milder asthma (7). Whether induction of IFN-λ is deficient in severe asthma in adults, therefore, is a knowledge gap that remains to be filled.

The mechanisms of deficient innate IFN induction in asthma are of great current interest. Previous studies have strongly implicated IgE binding to its receptor on dendritic cells and peripheral blood mononuclear cells (PBMCs) and on being cross-linked, inhibiting IFN induction by viruses (10, 12). A recent fascinating study has confirmed previous reports that anti-IgE therapy substantially reduced exacerbation frequency, particularly in more severe asthma and in exacerbation-prone asthma in school-aged children (15). Importantly, this study also reported that anti-IgE therapy was able to restore deficient innate antiviral immunity (IFN-α production in response to rhinovirus infection in IgE cross-linked PBMCs) in these children with asthma and that those children with better restoration of antiviral immunity had very substantial reductions in exacerbation frequency compared with children with less good restoration (15). Further research is needed to understand what intracellular mechanisms are involved in suppressing IFN induction after cross-linking of IgE bound to its receptor; at present this is a complete mystery.

Another mechanism implicated in IFN deficiency in asthma is induction of suppressor of cytokine signaling molecule 1 (SOCS1) (16). SOCS1 was induced in bronchial epithelial cells by Th2 cytokines, proinflammatory cytokines, and rhinovirus infection; was up-regulated in bronchial biopsies in mild asthma and in bronchial epithelial cells from children with severe asthma; and correlated with deficient IFN induction and increased virus replication in these bronchial epithelial cells from children with severe asthma. SOCS1 overexpression in bronchial epithelial cells directly inhibited IFN and virus induction of IFN-β and IFN-λ1 promoter activation, and, finally, nuclear localization of SOCS1 was shown to be necessary for this inhibition to occur (16).

This study is consistent with other studies showing that Th2 cytokines and transforming growth factor-β are able to suppress rhinovirus-induced IFN production and augment virus replication in bronchial epithelial cells (17, 18) and fibroblasts (19). These studies did not investigate nuclear SOCS1 expression, so the mechanism(s) through which Th2 cytokines/transforming growth factor-β suppressed IFN induction are not known. The role of nuclear SOCS1 and other possible mechanisms of suppression of innate immune responses by asthma-related cytokines and virus infection clearly require further study.

Viruses and their replication are sensed by pattern recognition receptors (PRRs), which then signal via complex pathways to induce IFNs. Several PRRs and molecules on downstream signaling pathways are also virus inducible. Studies have therefore investigated whether reduced baseline expression of these molecules, or reduced induction by virus, might be implicated in IFN deficiency in asthma. We previously identified deficient IFN-α and IFN-β induction by rhinovirus in BAL cells from people with mild asthma and investigated baseline mRNA expression of the major PRRs (including Toll-like receptor-7 [TLR7]) and downstream adaptor proteins, signaling kinases, and transcription factors (13 molecules in total) and found no differences in expression for any of these molecules between IFN-deficient cells from people with asthma

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and IFN-replete cells from healthy subjects (8). We also investigated induction by virus of eight of these molecules that are virus inducible (including TLR7) and found no differences in induction (8). We concluded that reduced expression/induction of these molecules did not appear to play an important role in IFN deficiency in these cells/subjects.

Recent studies have reported that patients with moderate/severe asthma had decreased bronchial biopsy TLR7 mRNA (20) and protein (21) expression compared with healthy control subjects. Thus, TLR7 expression is decreased in the bronchi in moderate/severe asthma. Roponen and colleagues have also reported that IFN-inducible gene induction by the TLR7 agonist imiquimod was significantly lower in PBMCs from adolescents with asthma than in those from healthy control subjects, indicating that TLR7 function was reduced, although expression of TLR7 itself was not reported (22). They then went on to perform similar studies in PBMCs from adults and reported significantly lower rhinovirus induction of IFN-α, IFN-β, and IFN-stimulated genes in asthma, accompanied by reduced responsiveness to TLR7 activation; however, baseline expression of TLR7 was not reduced (23).

Rupani and colleagues, as well as showing IFN deficiency in severe asthma as discussed above, have also reported that TLR7 mRNA and protein expression is decreased in BAL-derived macrophages in severe asthma, that TLR7 function in response to imiquimod is also similarly reduced, and that TLR7 mRNA expression correlated inversely with number of exacerbations in the previous year and with Asthma Control Questionnaire score (14). The authors propose that TLR7 deficiency and the associated impaired IFN response to virus drives the vulnerability of subjects with severe asthma to recurrent lower respiratory virus infections.

Rupani and colleagues then went on to investigate possible mechanisms of TLR7 deficiency in asthma (14). They investigated expression of 745 microRNAs (miRNAs) in BAL-derived macrophages and identified that 16 were up-regulated in patients with severe asthma (14). Based on *in silico* analysis, 3 of these 16 (miR-150, miR-152, and miR-375) potentially target TLR7. Expression of each of these three was shown by quantitative polymerase chain reaction to be increased in severe asthma, and functional studies with a TLR7-luciferase construct combined with overexpression of the miRNAs showed that all three were able to reduce luciferase activity, and the three combined better than individual miRNAs alone. Finally, Rupani and colleagues designed antagomirs to block the activity of each of these miRNAs. They showed that each antagomir increased TLR7 expression and that this was accompanied by increases in rhinovirus and imiquimod induction of IFN-α and IFN-β. The authors state that their findings provide strong evidence that miR-150, miR-152, and miR-375 mediated deficient TLR7 expression and virus-induced IFN in severe asthma, that these defective IFN responses can be ameliorated by suppressing expression of the three miRNAs, and that successful translation of these findings will provide a novel approach to treat and prevent virus-induced asthma attacks (14).

Are these claims justified by the data? Certainly the findings are very interesting, as they describe a novel mechanism for IFN deficiency in severe asthma; however, the claims must be tempered with some caution. One pivotal finding that antagonizing the miRNAs increased TLR7 expression is based only on quantitative polymerase chain reaction data, not protein; the result shows only a very modest increase of less than 1.5-fold compared with control (scrambled antagomirs); it required 12 experiments to generate a P value < 0.05, again suggesting only a modest/variable effect; we are not told whether the cells being studied came from people with severe asthma or not; and, most importantly, there is no comparison with TLR7 expression levels in cells from healthy control subjects to determine if the antagomirs restored TLR7 expression levels back to normality. Similarly, the other pivotal finding that antagonizing the miRNAs increased IFN responses is based on cells from small numbers of subjects (five, six, or seven); they were from a mix of subjects with severe asthma and healthy donors; the results show only very modest differences compared with control (scrambled antagomirs); P values were all only less than 0.05; and, most importantly, there is no comparison of IFN induction levels in antagomir-treated cells from subjects with severe asthma to levels in untreated cells from healthy control subjects to determine if the antagomirs restored deficient IFN induction levels back to normality.

Further work is certainly needed to address these issues, as well as studies to determine whether similar mechanisms are relevant to the IFN deficiencies observed in bronchial epithelial cells, PBMCs, and dendritic cells in asthma and to understand what process in severe asthma induces these miRNAs. My suspicion is that the described role for TLR7 and for these miRNAs will likely turn out to be only part of the explanation for IFN deficiency in asthma. This is supported by the fact that deficient TLR7 expression was not observed in IFN-deficient BAL cells or PBMCs in milder asthma (8, 23) and by the fact that several other plausible mechanisms for IFN deficiency in asthma have already been reported. Rupani and colleagues are to be congratulated for conducting an ambitious, thoughtful, and difficult-to-execute study; for substantially extending our knowledge in relation to IFN deficiencies in asthma; and for bringing these novel mechanistic insights to the attention of researchers in the field to stimulate further work on this important subject (14). ■

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Association between Impaired Lung Function and Cardiovascular Disease

Cause, Effect, or Force of Circumstance?

Large, population-based cohort studies have consistently shown that lung function, and particularly FEV_1, predicts cardiovascular mortality. For example, in 2005, Sin and colleagues undertook a major systematic review comprising a total of 83,880 participants in 12 studies and reported that pulmonary function predicted cardiovascular mortality (1). The pooled relative risk for the lowest compared with the highest lung function group was 1.99 (95% confidence interval, 1.71–2.29) (1). However, despite the considerable interest surrounding cardiovascular comorbidity in chronic obstructive pulmonary disease (COPD) (2), the mechanisms underlying this doubling in cardiovascular mortality remain uncertain.

For many observers, there are three potential hypotheses linking COPD with cardiovascular morbidity and mortality. First, reduced lung function may cause cardiovascular disease through increased systemic inflammation, promoting atheromatous disease and a prothrombotic state (3). Second, shared avoidable risk factors, such as smoking habit and other environmental factors, may simultaneously and independently cause endothelial dysfunction in both the pulmonary and systemic vasculature, with resultant disease in both organ systems (4). Finally, lung function may, similarly to height, reflect a range of adverse fetal and early life factors, predisposing individuals to increased cardiovascular risk as well as a range of other adverse outcomes (5).

Determining the causal mechanism underlying this well-established association between pulmonary function and cardiovascular disease is the subject of the article published in this issue of the *Journal* by Chandra and colleagues (pp. 38–47) (6). It