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# Viral infections in allergy and immunology: How allergic inflammation influences viral infections and illness



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**Viral respiratory tract infections are associated with asthma inception in early life and asthma exacerbations in older children and adults. Although how viruses influence asthma inception is poorly understood, much research has focused on the host response to respiratory viruses and how viruses can promote; or how the host response is affected by subsequent allergen sensitization and exposure. This review focuses on the innate interferon-mediated host response to respiratory viruses and discusses and summarizes the available evidence that this response is impaired or suboptimal. In addition, the ability of respiratory viruses to act in a synergistic or additive manner with T<sub>H</sub>2 pathways will be discussed. In this review we argue that these 2 outcomes are likely linked and discuss the available evidence that shows reciprocal negative regulation between innate interferons and T<sub>H</sub>2 mediators. With the renewed interest in anti-T<sub>H</sub>2 biologics, we propose a rationale for why they are particularly successful in controlling asthma exacerbations and suggest ways in which future clinical studies could be used to find direct evidence for this hypothesis. (J Allergy Clin Immunol 2017;140:909-20.)**

**Key words:** Virus, allergic inflammation, interferon, asthma, T<sub>H</sub>2

Viral infections are associated with asthma exacerbations (AEs) in adults and children.<sup>1</sup> Although the mechanisms behind

## Abbreviations used

ACQ: Asthma Control Questionnaire  
 AE: Asthma exacerbation  
 AM: Alveolar macrophage  
 DC: Dendritic cell  
 GWAS: Genome-wide association study  
 HBEC: Primary bronchial epithelial cells  
 IFNAR: IFN- $\alpha$  receptor  
 ILC2: Type 2 innate lymphoid cell  
 IRF: Interferon regulatory factor  
 ISG: Interferon-stimulated gene  
 poly I:C: Polyinosinic:polycytidylic acid  
 PRR: Pattern recognition receptor  
 RSV: Respiratory syncytial virus  
 SOCS: Suppressor of cytokine signaling  
 TLR: Toll-like receptor

this association are incompletely understood, recent evidence has shown that viral infection of epithelial cells can produce cytokines, such as *IL-25* and *IL-33*, that interact with allergic inflammation, inducing T<sub>H</sub>2 pathways (including both innate and antigen-specific T<sub>H</sub>2 cell-related pathways) and resulting in increased T<sub>H</sub>2-related inflammation, eosinophilia, and enhanced

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ST, Davies DE. Interferon-beta for anti-virus therapy for respiratory diseases. European patent no. 1734987 [May 5, 2010]), licensed a patent (Wark PA, Johnston SL, Holgate ST, Davies DE. Anti-virus therapy for respiratory diseases [IFN $\beta$  therapy]. Hong Kong patent no. 1097181 [August 31, 2010]), licensed a patent (Wark PA, Johnston SL, Holgate ST, Davies DE. Anti-virus therapy for respiratory diseases [IFN $\beta$  therapy]. Japanese patent no. 4807526 [August 26, 2011]), licensed a patent (Wark PA, Johnston SL, Holgate ST, Davies DE. Interferon-beta for anti-virus therapy for respiratory diseases. New Hong Kong-Divisional patent application no. 11100187.0 [January 10, 2011]), and licensed a patent (Burdin N, Almond J, Lecouturier V, Girerd-Chambaz Y, Guy B, Bartlett N, et al. Induction of cross-reactive cellular response against rhinovirus antigens. European patent no. 13305152 [April 4, 2013]) pending. The rest of the authors declare that they have no relevant conflicts of interest.

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Terms in boldface and italics are defined in the glossary on page 910.

IL-4, IL-5, IL-13, and mucin production.<sup>2,3</sup> Furthermore, cells or tissues from asthmatic patients can respond to infection with a delayed and suboptimal antiviral response exemplified by delayed and deficient production of the innate interferons.<sup>4-6</sup> Although both processes are undoubtedly important in determining disease outcomes, one idea is that these 2 outcomes are linked and that current anti-T<sub>H</sub>2 biologics can function not only by reducing harmful T<sub>H</sub>2-related inflammation but also by restoring an impaired antiviral response.

This review will examine this hypothesis and discuss the evidence that supports not only impaired interferon production in asthmatic patients but also that impaired interferon production

could be caused at least in part by increased T<sub>H</sub>2 pathways and allergic inflammation. For simplicity, we will group allergic inflammation and T<sub>H</sub>2 pathways together, although we accept that T<sub>H</sub>2 cytokines might be made or might function independently of allergen exposure. This review will be limited to the discussion of impaired innate type I (*IFN- $\alpha$ /IFN- $\beta$* ) and type III (*IFN- $\lambda$ s*) interferons in asthmatic patients. Some studies have shown a defective or impaired type II *IFN- $\gamma$*  response in cells of asthmatic patients or lower T<sub>H</sub>1/T<sub>H</sub>2 ratios in atopic asthmatic patients versus control subjects<sup>7</sup>; however, these studies will not be discussed further. Additionally, this review will focus on discussing the role of innate interferons in patients with established

## GLOSSARY

**CCL5:** A chemokine also known as RANTES, it has been shown to be chemotactic for T cells, eosinophils, and basophils and plays an active role in recruiting leukocytes into inflammatory sites. With the help of particular cytokines that are released by T cells, CCL5 also induces the proliferation and activation of certain natural killer cells to form CC chemokine-activated killer (CHAK) cells. It has also been shown to be an HIV-suppressive factor released from CD8<sup>+</sup> T cells.

**IFN- $\alpha$ :** A type of human type I interferon protein that helps regulate the activity of the immune system. IFN- $\alpha$  is produced by leukocytes and has been shown to be mainly involved in the innate immune response against viral infection.

**IFN- $\beta$ :** A type of human type I interferon protein produced in large quantities by fibroblasts. IFN- $\beta$  displays antiviral activity in the innate immune response.

**IFN- $\gamma$ :** A type II interferon, IFN- $\gamma$  is a cytokine required for innate and adaptive immunity against viral, bacterial, and protozoal infections. IFN- $\gamma$  has been shown to be an important activator of macrophages and inducer of class II major histocompatibility complex molecule expression. IFN- $\gamma$  is produced predominantly by natural killer (NK) and NK T cells as part of the innate immune response and by CD4 T<sub>H</sub>1 and CD8 cytotoxic T-lymphocyte effector T cells once antigen-specific immunity develops.

**IFN- $\lambda$ :** This recently classified type III interferon group consists of 3 molecules called IFN- $\lambda$ 1, IFN- $\lambda$ 2, and IFN- $\lambda$ 3 (also called IL-29, IL-28A and IL-28B, respectively). IL-28 (IFN- $\lambda$ 2/3) is a cytokine that comes in 2 isoforms, IL-28A and IL-28B, which play a role in innate immune defense against viruses. IL-28A and IL-28B are highly similar (in amino acid sequence) to IL-29. Both IL-28A and IL-28B have been shown to exhibit antiviral qualities and inhibit tumor cell proliferation while promoting antitumor immune responses. IL-29 is a potent antiviral cytokine through upregulation of MHC class I expression on the cell surface.

**IL-4:** A cytokine that induces differentiation of naive helper T cells (T<sub>H</sub>0) to T<sub>H</sub>2 cells. IL-4 subsequently produces additional IL-4 in a positive feedback loop. IL-4 is a ligand for the IL-4 receptor that also binds to IL-13, which contributes to many overlapping functions of IL-4 and IL-13.

**IL-5:** A major maturation and differentiation cytokine expressed by T<sub>H</sub>2 cells and eosinophils in mice and human subjects. IL-5 has been shown to play an instrumental role in eosinophilic inflammation in patients with allergic diseases.

**IL-6:** A cytokine also known as IFN- $\beta$ 2 is implicated in a wide variety of inflammation-associated disease states. IL-6 has been associated with maturation of B cells and has been shown to act as an endogenous pyrogen capable of inducing fever in patients with autoimmune diseases or infections.

**IL-13:** A cytokine produced primarily by T<sub>H</sub>2 cells that is involved in several stages of B-cell maturation and differentiation and is critical to the pathogenesis of allergen-induced asthma but operates through mechanisms independent of IgE and eosinophils.

**IL-25:** A proinflammatory cytokine that shares sequence similarity with IL-17 and has been shown to favor the T<sub>H</sub>2-type immune response IL-25 activates dendritic cells and innate lymphoid cells (ILC2s).

**IL-33:** A member of the IL-1 family of cytokines that potently drives production of T<sub>H</sub>2-associated cytokines. IL-33 is produced from epithelial cells and activates T<sub>H</sub>2 cells and ILC2s.

**INTERFERON REGULATORY FACTOR (IRF) 3:** A member of the IRF factor family, which plays an important role in the innate immune system's response to viral infection through induction of type I interferons.

**MELANOMA DIFFERENTIATION FACTOR 5:** A RIG-I-like receptor double-stranded RNA (dsRNA) helicase enzyme that functions as a pattern recognition receptor (recognizing long dsRNA) that is a sensor for viruses.

**MYELOID DIFFERENTIATION PRIMARY RESPONSE 88 (MyD88):** A universal adapter protein that functions as an essential signal transducer in the IL-1 and Toll-like receptor (TLR) signaling pathways (except TLR3). These pathways regulate activation of numerous proinflammatory genes, including the transcription factor nuclear factor  $\kappa$ B.

**RETINOIC ACID-INDUCIBLE GENE (RIG) I:** A RIG-I-like receptor double-stranded RNA (dsRNA) helicase enzyme that functions as a pattern recognition receptor for short dsRNA and 5' phosphorylated single-stranded RNA. RIG-I is a sensor for viruses, specifically influenza A, Sendai virus, and flavivirus, resulting in induction of members of the type I interferon family.

**SUPPRESSOR OF CYTOKINE SIGNALING 1 (SOCS1):** Negative regulators of cytokine signaling that are members of the STAT-induced STAT inhibitor (SSI) family. Expression of this gene has been shown to be induced by a subset of cytokines, including IL-2, IL-3, erythropoietin, CSF2/GM-CSF, type I interferon, and IFN- $\gamma$ .

**SUPPRESSOR OF CYTOKINE SIGNALING 3 (SOCS3):** Negative regulator of cytokines that signal through the Janus kinase/signal transducer and activator of transcription pathway. Expression of the *SOCS3* gene is induced by various cytokines, including IL-6, IL-10, and IFN- $\gamma$ .

**TOLL-LIKE RECEPTOR (TLR) 3:** A member of the TLR family that plays a fundamental role in pathogen recognition and activation of innate immunity. TLR3 recognizes dsRNA associated with viral infection and induces the activation of interferon regulatory factor 3 and nuclear factor  $\kappa$ B.

**TOLL-LIKE RECEPTOR (TLR) 7:** A TLR that recognizes single-stranded RNA (ssRNA) in endosomes, which is a common feature of viral genomes. TLR7 recognizes ssRNA of viruses, such as HIV and hepatitis C virus, and GU-rich ssRNA.

**TOLL-LIKE RECEPTOR (TLR) 8:** Similar to TLR7, TLR8 endosomal receptor that recognizes single-stranded RNA (ssRNA) and can recognize ssRNA viruses, such as influenza, Sendai, and Coxsackie B viruses. TLR8 binding to the viral RNA signals MyD88 and leads to activation of the transcription factor nuclear factor  $\kappa$ B and an antiviral response. TLR8 also recognizes ssRNA of viruses, such as HIV and HCV.

asthma and will not consider the possible role of innate interferon deficiency in asthma inception in any detail.

## VIRAL RESPIRATORY TRACT INFECTIONS ARE ASSOCIATED WITH ASTHMA ONSET AND AEs

The respiratory viruses associated with asthma include the same viruses that cause the common cold, influenza-like illnesses, and wheeze and bronchiolitis in children. Respiratory viruses are the main triggers of AEs in adults and school-aged children, accounting for around 85%.<sup>8-11</sup> Viruses associated with AEs include respiratory syncytial virus (RSV), influenza viruses, and human rhinoviruses. Coronaviruses, parainfluenza viruses, adenoviruses, and the more recently identified metapneumoviruses and bocaviruses are also involved; however, they are less common.<sup>12,13</sup> Both RSV and rhinoviruses have been associated with asthma inception and are strongly associated with wheeze in infants; however, the exact mechanisms remain elusive. There is some argument whether rhinoviruses and RSV cause asthma or might be markers of asthma susceptibility; it is also unclear whether both viruses act in the same manner or are subtly different. In young children RSV bronchiolitis has been linked to increased allergic sensitization<sup>14</sup> and immune skewing to increased T<sub>H</sub>2 responses.<sup>15</sup> The skewing to T<sub>H</sub>2 responses might reflect a way of evading protective T<sub>H</sub>1 responses. Although related, these topics have been thoroughly discussed elsewhere and will not be the main focus of this article; interested readers are directed to other helpful reviews.<sup>1,16,17</sup>

## IMPAIRED INTERFERON EXPRESSION IN CELLS AND TISSUES FROM ASTHMATIC PATIENTS Type I and III interferon system

Respiratory viruses are capable of infecting the bronchial epithelium of the lower airway, resulting in a local inflammatory reaction characterized by airway neutrophilia, attraction of T lymphocytes, activation of macrophages, and damage to the integrity of the bronchial epithelium.<sup>7</sup> In an effort to limit viral spread, host cells also mount a type I (IFN- $\alpha/\beta$ ) and type III (IFN- $\lambda$ ) interferon response. Interferons are antiviral cytokines made by a wide range of cells in response to viral infection and ligation of various pattern recognition receptors (PRRs). Type I IFN- $\alpha$  and IFN- $\beta$  signal through the IFN- $\alpha$  receptor (IFNAR) 1 and IFNAR2 complex, whereas type III IFN- $\lambda$ s (IFN- $\lambda$ 1/IL-29, IFN- $\lambda$ 2/IL-28A, and IFN- $\lambda$ 3/IL-28B in human subjects) act through the IL-10 receptor  $\beta$  chain and IFN- $\lambda$ 1 receptor  $\alpha$  chain.<sup>18</sup> Interferons are the first-line defense against viral infection<sup>19</sup> and induce expression of the distinct genes termed interferon-stimulated genes (ISGs) through signaling through the type I and type III interferon receptor complexes.<sup>20,21</sup> ISGs can be extracellular proteins, such as the T<sub>H</sub>1-attracting chemokines CXCL10 (interferon-inducible protein 10) or CXCL11 (interferon-inducible T-cell alpha chemoattractant [ITAC]), or other interferons, with IFN- $\beta$  inducing downstream IFN- $\alpha$  and IFN- $\lambda$ s.<sup>22</sup> Most ISGs are intracellular proteins that interfere with and thus attempt to contain viral protein trafficking, nucleic acid synthesis, or virion assembly and release. Currently, the number of known ISGs is thought to exceed 380<sup>23</sup>; however, there is the possibility of cell type-specific ISGs that are yet to be identified.

## Impaired innate interferons in asthmatic patients

Rhinovirus infection induces rapid transcription of type I IFN- $\alpha/\beta$  and type III IFN- $\lambda$  mRNAs; however, interferon expression remains in part cell type dependent. The important signaling pathways used by rhinovirus to induce IFN- $\beta$  and IFN- $\lambda$ s have been studied *in vitro* and with mouse models<sup>22,24-26</sup> and require the PRRs *melanoma differentiation factor 5*, *retinoic acid-inducible gene 1*, *Toll-like receptor (TLR) 3*, *TLR7*, and the transcription factor *interferon regulatory factor (IRF) 3*. An increased susceptibility to viral infections in asthmatic patients was first reported by Corne et al.<sup>27</sup> By studying asthmatic and nonasthmatic cohabiting spouse pairs, it was observed that both asthmatic patients and nonasthmatic subjects reported similar incidences of rhinovirus infections; however, asthmatic patients had prolonged lower respiratory tract symptoms and increased symptom severity.

In 2005, Wark et al.<sup>5</sup> cultured primary bronchial epithelial cells (HBECs) from bronchial brushings of allergic asthmatic adults and nonallergic nonasthmatic adults *ex vivo* and infected them with rhinovirus or treated them with the TLR3 ligand polyinosinic:polycytidylic acid (poly I:C). HBECs from asthmatic patients produced lower levels of IFN- $\beta$ , had lower levels of virus-induced apoptosis, and higher rhinovirus loads over time. Proinflammatory cytokine levels (*IL-6* and *CCL5*) between the 2 groups were similar, and the cells from asthmatic patients produced normal levels of rhinovirus replication when pretreated with exogenous IFN- $\beta$ . The authors concluded that asthmatic patients had a specific deficiency in IFN- $\beta$  production.

An earlier study observed impaired IFN- $\alpha$  release in PBMCs from asthmatic patients.<sup>4</sup> This work has since been verified by other studies and expanded to include other cell types, including dendritic cells (DCs),<sup>28,29</sup> bronchoalveolar lavage fluid-derived macrophages,<sup>30</sup> alveolar macrophages (AMs),<sup>31</sup> and further confirmed in PBMCs.<sup>32</sup> Furthermore, *ex vivo* type III IFN- $\lambda$ 1 levels from bronchoalveolar lavage macrophages of asthmatic patients have been shown to negatively correlate with total cold scores, changes in lung function, eosinophilia, and markers of inflammation after rhinovirus infection *in vivo*, strongly suggesting that IFN- $\lambda$ s are important in the pathogenesis of AEs and determining clinical outcomes of rhinovirus infection in asthmatic patients.<sup>6</sup>

More recently, several other reports have confirmed these initial findings,<sup>30,33-35</sup> including impaired interferon expression in children with severe asthma.<sup>36</sup> This work culminated in the first phase II placebo-controlled clinical trial of inhaled IFN- $\beta$  for asthma.<sup>37</sup> This was a study of natural infections with the primary outcome of changes in Asthma Control Questionnaire (ACQ) and FEV<sub>1</sub>. Although the trial found no benefit in the primary outcome in the intent-to-treat population of 72 asthmatic patients receiving active treatment versus 75 receiving placebo, a beneficial effect was observed on peak expiratory flow ( $P = .033$ ), and in a subpopulation composed of patients with more severe asthma ( $n = 27$  active and  $n = 31$  placebo), benefit was observed in ACQ scores ( $P = .004$ ) and peak expiratory flow ( $P = .029$ ). This finding supports the premise that interferon production is indeed impaired in asthmatic patients and that restoring this defect then beneficially influences clinical disease.

The impaired interferon responses seen in patients with atopic asthma to date are likely clinically important for 2 reasons. First, impaired interferon production *in vivo* would not adequately con-

**TABLE I.** Summary of studies showing impaired or defective expression of interferon mRNA or protein in cells from asthmatic patients

Reference	Cell type	Phenotype of asthma and age	Interferons studied	Evidence	Main findings
Bufe et al <sup>4</sup>	PBMCs	Atopic asthmatic children	IFN- $\alpha$	*, †	First report of impaired interferon using NDV
Wark et al <sup>5</sup>	HBECs	Mild atopic asthmatic adults	IFN- $\beta$	*, ‡, §,	Associated with apoptosis and not steroid dependent
Contoli et al <sup>6</sup>	HBECs/BAL cells	Mild atopic asthmatic adults	IFN- $\lambda$ s	*, †	Associated with clinical end points after experimental rhinovirus challenge
Gehlhar et al <sup>32</sup>	PBMCs	Atopic asthmatic adults	IFN- $\alpha$	*	RSV and NDV
Sykes et al <sup>30</sup>	BAL cells	Mild-to-moderate atopic asthmatic patients	IFN- $\alpha$ , IFN- $\beta$	*, †	No impairment of critical signaling molecules, related to atopy
Edwards et al <sup>36</sup>	HBECs	STRA atopic asthmatic children	IFN- $\beta$ , IFN- $\lambda$ s	*, ‡,	Interferon mRNA impaired to rhinovirus and poly I:C
Uller et al <sup>35</sup>	HBECs	Mostly atopic, mixed asthma severity, adults	IFN- $\beta$	*,	TSLP levels greater in asthmatic group
Wark et al <sup>44</sup>	HBECs	Mild persistent atopic asthmatic patients	IFN- $\beta$	*	IFN- $\beta$ protein studied only
Gill et al <sup>28</sup>	Blood pDCs	Atopic asthmatic adults and children	IFN- $\alpha$	*,	Influenza virus, related to IgE
Iikura et al <sup>47</sup>	PBMCs	Atopic mild-to-moderate asthmatic adults and children	IFN- $\alpha$	*	Impaired IFN- $\alpha$ in children only also impaired proinflammatory cytokine
Durrani et al <sup>29</sup>	Blood pDCs	Atopic asthmatic children	IFN- $\alpha$ , IFN- $\lambda$ s	*	Rhinovirus, related to asthma not atopy
Baraldo et al <sup>34</sup>	HBECs	Atopic and nonatopic asthmatic children	IFN- $\beta$ , IFN- $\lambda$ s	*, †, ‡	Associated with increased IL-4 staining, eosinophilia, and IgE in atopic subjects
Wagener et al <sup>46</sup>	NECs/HBECs	Atopic asthmatic patients	IFN- $\beta$ , IFN- $\lambda$ s	*	Poly I:C IFN- $\beta$ and IL-28 mRNA expression in asthmatic patients by microarray
Collison et al <sup>50</sup>	HBECs	Persistent asthmatic adults	IFN- $\lambda$ s	*	No difference in intracellular viral RNA
Spann et al <sup>45</sup>	NECs/TECs	Atopic children with wheeze	IFN- $\beta$ , IFN- $\lambda$ s	*	Impaired IFN- $\beta$ only in NECs with RSV
Hatchwell et al <sup>49</sup>	Bronchial biopsy specimens	Patients with moderate-to-severe eosinophilic asthma	IFN- $\alpha/\beta$ , IFN- $\lambda$ s	*	Impaired IL-28 related to impaired TLR7
Rupani et al <sup>31</sup>	AMs	Severe nonatopic asthma	IFN- $\alpha$ , IFN- $\beta$	*	Identification of mir-150, 152, and 375 as associated with impaired interferons
Kicic et al <sup>33</sup>	HBECs	Atopic asthmatic children	IFN- $\beta$	*, ‡, §,	Related to defective wound repair
Teach et al <sup>51</sup>	PBMCs	Atopic asthmatic children	IFN- $\alpha$	*, †	Restored by anti-IgE therapy
Lin et al <sup>48</sup>	PBMCs	Atopic asthmatic children	IFN- $\alpha$ , IFN- $\beta$	*,	Impaired interferon group identified by using cluster analysis had higher rates of asthma.

BAL, Bronchoalveolar lavage; NDV, Newcastle disease virus; NECs, nasal epithelial cells; pDCs, plasmacytoid dendritic cells; STRA, severe therapy-resistant asthma; TEC, tracheal epithelial cell; TSLP, thymic stromal lymphopoietin.

Level of evidence:

\*Interferon levels in asthmatic donors were significantly lower than those of control subjects.

†Deficient interferon levels were related to clinical disease, such as number of AEs, eosinophilia, lung function, IgE levels, atopy, and T<sub>H</sub>2 markers.

‡Significantly higher viral loads in asthmatic patients or significantly lower interferon levels that were negatively correlated with higher viral loads in asthmatic patients.

§Exogenous IFN- $\beta$  restored the antiviral response.

||Other noninterferon cytokines were not significantly different between asthmatic donors and control subjects.

control viral infection and could lead to greater or prolonged infection, including progression and potentially the establishment of infection in the lower airway and associated virus-induced inflammation. Second, impaired interferon production might not counterregulate ongoing T<sub>H</sub>2-mediated inflammation, thus contributing to established T<sub>H</sub>2-mediated inflammation and thereby increasing disease pathogenesis and increasing risk of hospitalization, which will be discussed at length in the next section.

### Limitations and controversies

Not all studies investigating impaired interferon production in asthmatic patients are in agreement, with several studies failing to show any difference in interferon production between cells from

asthmatic patients and those from control subjects.<sup>38-43</sup> Reasons for this disparity mostly concern studies of primary HBECs, which are cultured *in vitro* for several weeks before infection and study. Differences in the above findings are likely due to several variables, including differences in viral strains used or their preparation,<sup>44</sup> cell-culture methods (eg, different components of medium), or methods of propagation,<sup>39</sup> and also recruitment and phenotype of asthmatic subjects, including the asthma severity, asthma control<sup>43</sup> and atopic status.<sup>40</sup> There also exist differing strengths of evidence for these phenomena between various studies and relevance to clinical findings, such as eosinophilia, atopy, or AE rate. The findings of these studies and a description of the level of supporting evidence are summarized in Table I.<sup>4-6,28-36,44-51</sup> Although the majority of studies use rhinovirus as a test virus, differences in interferon expression using

other viruses have produced mixed results. Newcastle disease virus and RSV have both been used to show differences in interferon levels between groups when studying PBMCs<sup>4,32</sup>; however, a recent side-by-side comparison of interferon responses in asthmatic patients and control subjects with RSV and influenza virus did not show impaired interferon production in HBECs from asthmatic patients.<sup>40</sup> In another study differences in IFN- $\alpha$  responses in plasmacytoid dendritic cells have also been shown with influenza virus.<sup>28</sup> More studies with comparisons of rhinovirus with other respiratory viruses clearly need to be performed to better understand whether the defect in interferon expression is virus specific. Likewise, studies of impaired interferon production in nasal epithelial cells from asthmatic patients have produced mixed results,<sup>41,45,46</sup> suggesting that deficiency might be more profound in lower airway cells, yet deficiency is still observable in peripheral leukocyte populations.<sup>28,32,47</sup> The role of atopy is perplexing; Baraldo et al<sup>34</sup> showed that deficient interferon production was a feature of atopic asthmatic, nonatopic asthmatic, and nonasthmatic atopic children,<sup>34</sup> and studies focusing on allergic rhinitis without asthma have produced mixed results,<sup>4,52</sup> furthering the debate about whether it is asthma or atopy (or both) that drives this defect.

Some argue that there is controversy regarding interferon deficiency in asthmatic patients because studies investigating interferon responses in patients with stable disease<sup>53</sup> or during AEs *in vivo*<sup>54,55</sup> find increased rather than decreased interferon levels in asthmatic patients. However, we would argue that studies of interferon levels *in vivo* will be confounded by differences in viral loads and that if one wishes to investigate the presence of interferon deficiency in a disease state, this can only be done by infecting cells with a standardized quantity of virus *ex vivo* or stimulating cells with a standard quantity of a viral mimic *ex vivo*. However, enhanced rhinovirus replication in asthmatic patients *in vivo* during experimental challenge studies has been observed.<sup>2,7</sup>

Gaps in our knowledge relating to the argument for impaired interferon production in asthmatic patients include (1) a lack of studies testing whether the defect is stable and repeatable in the same samples, (2) few data on asthma phenotypes other than atopic asthma, (3) a paucity of studies with viruses other than rhinovirus or a rhinovirus versus other comparison with other viruses, and (4) a lack of studies relating interferon deficiency to either asthma control at the time samples were taken or underlying asthma severity. Another limitation is that *ex vivo* studies use small patient numbers and a definite study of interferon expression in larger studies, such as birth cohort studies, is only beginning to be applied with impaired antiviral immunity taken into consideration.

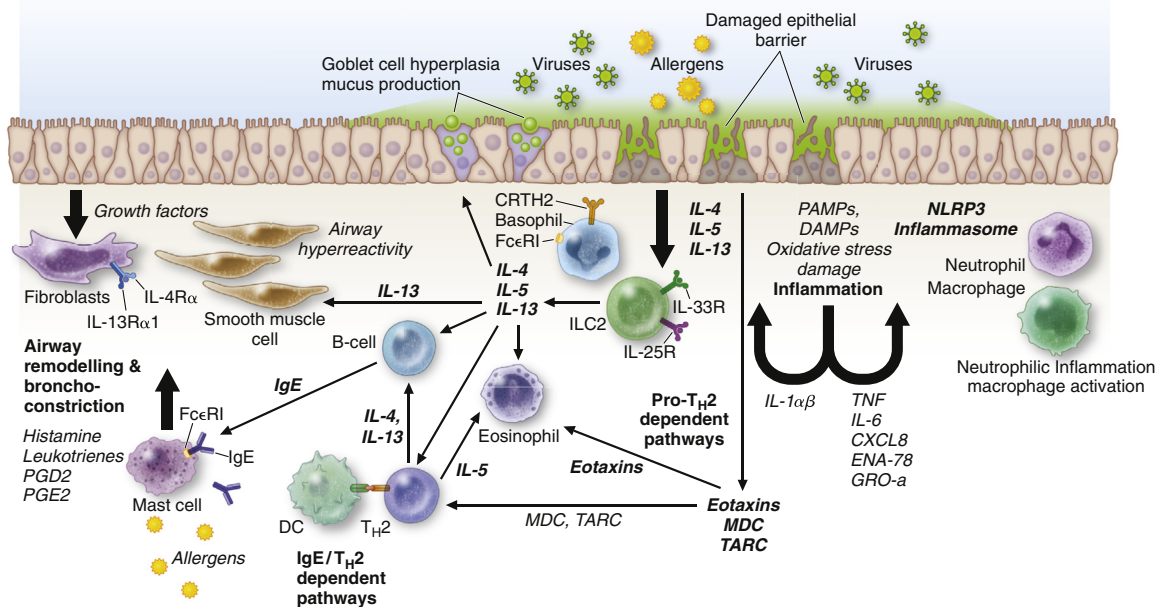
Of note, a recent large birth cohort study identified a population of asthmatic patients with impaired interferon responses to rhinovirus at age 11 years by using cluster analysis.<sup>48</sup> This group had increased wheeze in infancy and asthma at adolescence, increased AE rate, and allergic sensitization in infancy. Conversely, a subgroup of children with very low prevalence of asthma were also identified, and this group had robust interferon responses and antiviral immunity. Overall, the data strongly suggest that at least a subgroup of asthmatic patients exists with impaired interferon and antiviral immunity. However, it is possible, although currently unclear, whether asthmatic patients with impaired interferon production are a new and separate endophenotype or an important subgroup of an existing

endophenotype. Despite the above differences in study design, the conflicting nature of some findings, and the above limitations, the number of studies that support the original work of Bufe et al<sup>4</sup> and Wark et al<sup>5</sup> continue to grow and now number approximately 20 (Table I).

## Search for mechanisms

Our mechanistic understanding of impaired interferon responses in asthmatic patients remains poor, despite much effort. The inability to identify detailed mechanisms likely points to a complex mechanism that is not easily understood. Initially, researchers considered 2 possible explanations. First was that impaired interferon production in asthmatic patients was due to a single gene defect, microRNA, or epigenetic determinant present in asthmatic patients. This hypothesis would describe the defect as static, unchanging, and likely identifiable in genome-wide association studies (GWASs) or perhaps large gene expression data sets from next-generation sequencing approaches or protein activation data sets. The second hypothesis described a more complex gene-environment interaction that could involve a variable or even transient phenotype, immune imprinting, the actions of more than 1 gene, microRNA, or an epigenetic determinant. Thus far, databases of next-generation sequencing<sup>56,57</sup> and microarray data<sup>38,58</sup> have generally all failed to show a reproducible single gene defect that would explain the specific decreased expression of innate interferons observed in asthmatic patients. With the exception of TLR7<sup>59</sup> and the high-affinity receptor for the IgE  $\alpha$  chain (Fc $\epsilon$ RI $\alpha$ ) at 1q23,<sup>60</sup> large published GWAS data sets on asthma again showed no obvious clue that explains these findings.<sup>61</sup> The possibility still exists that a single gene defect resides in a very specific subpopulation of asthmatic patients but has been diluted in the larger studies that define asthma based on the clinical attributes or FEV<sub>1</sub> alone and often involve several hundred donors. Considering the different cell types involved (leukocytes vs structural cells), it is possible that the mechanism responsible for the defect is cell type specific. It is also possible that HBECs, because of the culture associated with their study, are more prone to lose this defect, explaining the conflicting studies that have arisen. Considering the available evidence, coupled with the failure of several HBEC studies to reproduce this defect,<sup>38,39,43</sup> overall, the data argue against the single gene-hypothesis and encourages alternative hypotheses to be considered.

Two studies have identified *suppressor of cytokine signaling (SOCS) 1*, a negative regulator of type I and type II interferon signaling, as potentially important in determining impaired interferons in asthmatic patients.<sup>62,63</sup> SOCS1 expression in T cells and airway smooth muscle had been linked previously to asthma<sup>64,65</sup>; however, studies of airway epithelial cells and effects on viral responses were few.<sup>66</sup> In HBECs and rhinovirus infection, T<sub>H</sub>2 cytokines, and proinflammatory cytokines all induced SOCS1 mRNA and protein in a time-dependent manner. SOCS1, but not the closely related family member *SOCS3*, was observed to be of greater abundance in bronchial biopsy specimens from patients with atopic asthma, and abundance was related to PC<sub>20</sub> data and atopy. In a small cohort of asthmatic children with severe therapy-resistant asthma,<sup>36</sup> levels of *SOCS1* mRNA in unstimulated cells negatively correlated with impaired IFN- $\beta$  and IFN- $\lambda$  expression after rhinovirus infection and poly I:C treatment.<sup>63</sup> SOCS1 can also function as a nuclear protein through a conserved

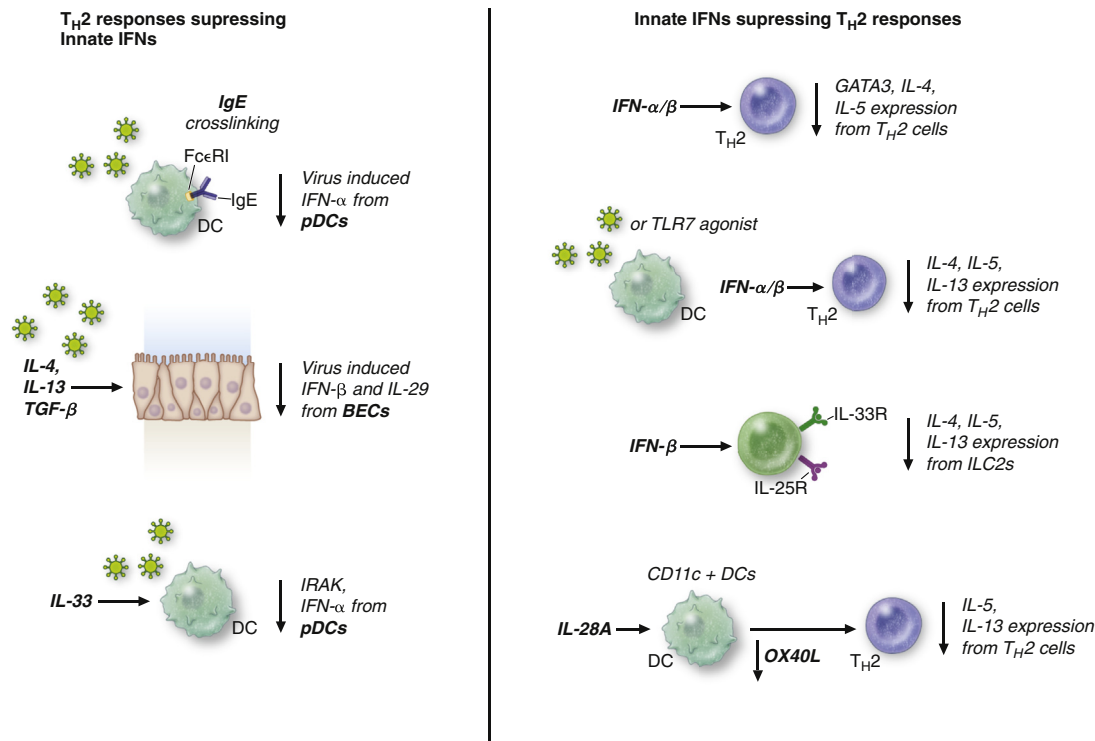


**FIG 1.** Overview of additive or synergistic effects of allergen exposure and viral infection driving both pro- $T_H2$  and IgE/ $T_H2$  inflammation. Viral infection damages the epithelial barrier and results in the pro- $T_H2$  cytokines IL-33, IL-25, and thymic stromal lymphopoietin. These cytokines act on ILC2s, DCs, and  $T_H2$  cells, resulting in production of the  $T_H2$  cytokines IL-13, IL-4, and IL-5. These cytokines have a central role in allergic asthma: IL-4 and IL-13 drive antibody class-switching to IgE in B cells, IL-13 can act also on airway smooth muscle cells, causing bronchoconstriction and contributing to airway remodeling, and IL-5 contributes to eosinophil production. Actions of IL-4 and IL-13 and viruses on airway epithelial cells can induce the eotaxins that attract eosinophils and the chemokines macrophage-derived chemokine (MDC) and thymus and activation-regulated chemokine (TARC), which attract  $T_H2$  cells into the airway. IgE cross-linking by allergen on mast cells produces histamine, leukotrienes, and the prostaglandins  $PGD_2$  and  $PGE_2$ , which further promote bronchoconstriction.  $PGD_2$  binds chemoattractant receptor-homologous molecule expressed on  $T_H2$  cells (CRTH2) and activates basophils,  $T_H2$  cells, and ILC2s. Viruses can also cause oxidative stress, and pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP) production can lead to the proinflammatory cytokines IL- $1\alpha/\beta$ , TNF, and IL-6. This generally results in neutrophilic inflammation and activation of macrophages. Allergen-induced IL- $1\alpha$  can also promote the pro- $T_H2$  response and lead to further IL-33 production and activation of ILC2s. ENA-78, Epithelial-derived neutrophil-activating protein 78; GRO- $\alpha$ , melanoma growth stimulating activity- $\alpha$ ; IL-4R, IL-4 receptor; IL-13R, IL-13 receptor; IL-25R, IL-25 receptor; IL-33R, IL-33 receptor.

nuclear localization sequence and might interfere with gene transcription.<sup>67</sup> In the same bronchial biopsy specimens, SOCS1 nuclear staining was increased in asthmatic patients, and functional studies *in vitro* with mutants lacking the ability to home to the nucleus did not suppress rhinovirus-induced IFN- $\beta$  and IFN- $\lambda 1$  induction.<sup>63</sup> The data strongly suggest that SOCS1, a virus and  $T_H2$  cytokine-inducible negative regulator that is overexpressed in asthmatic patients, might be at least in part responsible for the impaired interferon levels observed. Further studies are required to support these findings, including bigger studies of SOCS1 expression in bronchial epithelium of asthmatic patients. A recent study showed reduced SOCS1 mRNA expression in bronchial biopsy specimens from patients with severe asthma compared with patients with mild-to-moderate asthma; however, immunostaining was not performed.<sup>68</sup> The idea of increased SOCS1 expression in asthmatic patients remains attractive and provides a mechanistic link of how  $T_H2$  pathways can negatively regulate innate interferons.

Studying purified AMs from patients with severe asthma, Rupani et al<sup>31</sup> observed impaired TLR7 expression levels with reduced rhinovirus and TLR7 ligand-induced IFN- $\beta$  and IFN- $\alpha$  mRNA and protein levels; however, expression of other PRRs,

such as TLR8, TLR3, retinoic acid-inducible gene I, and melanoma differentiation factor 5, was not impaired. TLR7 is a single-stranded RNA receptor that uses the adaptor protein *myeloid differentiation primary response 88 (MyD88)* to engage a signaling apparatus leading to IRF3/7 activation and hence IFN- $\alpha$  transcription. Furthermore, TLR7 expression was inversely related to AE rate and ACQ scores, strongly suggesting that TLR7 expression levels are related to clinical asthma outcomes. Studying expression patterns of microRNAs identified several microRNAs that differed between AMs from healthy subjects and asthmatic patients, and 4 of these showed identity to the untranslated region of TLR7. Further expression analysis identified that 3 of these microRNAs, 150, 152, and 375, were significantly up-regulated in AMs from asthmatic patients versus control subjects. Each microRNA was not induced by the  $T_H2$  cytokines IL-4 and IL-13, the proinflammatory cytokine TNF, and also IFN- $\gamma$  and were not affected by steroid treatment. The importance of each microRNA in reducing TLR7 expression was then shown by using TLR7-specific luciferase reporters, and targeting each microRNA resulted in significantly increased interferon levels and ISG induction. Together, the data offer an important mechanistic explanation for impaired interferon in AMs from asthmatic



**FIG 2.** Summary of mechanisms of reciprocal negative regulation of type I and III interferons and  $T_H2$  pathways in asthmatic patients.  $T_H2$  cytokines can negatively regulate virus-induced interferons; cross-linking of cell-bound IgE on DCs prevents virus-induced IFN- $\alpha$  on infection; pretreatment of HBECs with IL-4, IL-13, or TGF- $\beta$  results in low IFN- $\beta$  and IL-29 induction on infection; and IL-33 can act on DCs and reduce IRAK levels and hence IFN- $\alpha$  induction on infection. Interferons can suppress  $T_H2$  responses; pretreatment of T cells with IFN- $\alpha/\beta$  reduces GATA-3 and IL-5 levels in  $T_H2$  cells; viruses or TLR7 agonists produce IFN- $\alpha/\beta$  from DCs, which act on  $T_H2$  cells to reduce level of IL-4, IL-5, and IL-13; and IFN- $\beta$  pretreatment of ILC2s causes a reduction in IL-4, IL-5, and IL-13. Finally, IL-28A (IFN- $\lambda$ ) acts on DCs to reduce OX40 ligand and prevent  $T_H2$  cell expression of IL-5 and IL-13. *BECs*, Bronchial epithelial cell; *pDCs*, plasmacytoid dendritic cells.

**TABLE II.** Summary of anti- $T_H2$  approaches and their effects on AEs and asthma-related outcomes

Target	Agent	Reference	Main findings
IgE	Antibody	Teach et al <sup>51</sup> Busse et al <sup>96</sup>	Reduced AE frequency and in 82 restored IFN- $\alpha$ responses to rhinovirus in PBMCs
IL-5	Antibody	Nair et al <sup>88</sup> Pavord et al <sup>89</sup> Castro et al <sup>91</sup>	Reductions of 52% and 58% in AE rates and increased time to AEs by 8 weeks in patients with eosinophilic asthma
IL-4	Variant protein	Wenzel et al <sup>92</sup>	Improved FEV <sub>1</sub> during allergen challenge in atopic asthmatic patients
IL-13	Antibody	Corren et al <sup>86</sup>	Reduction of 60% in AE rate in $T_H2$ -high asthmatic patients
IL-5R $\alpha$	Antibody	Castro et al <sup>90</sup>	Reduction of 57% in AE rate in patients with eosinophilic asthma
IL-4R $\alpha$	Antibody	Wenzel et al <sup>87</sup>	Reduction of 87% in AE rates in patients with severe eosinophilic asthma
CRTH2	Small molecule	Pettipher et al <sup>93</sup> Gonem et al <sup>94</sup>	Increased FEV <sub>1</sub> and reduced eosinophilic airway inflammation in patients with eosinophilic asthma
GATA-3	DNAzyme	Krug et al <sup>95</sup>	Increased FEV <sub>1</sub> , reduced eosinophil numbers, and reduced IL-5 levels in patients with eosinophilic asthma

CRTH2, Chemoattractant receptor-homologous molecule expressed on  $T_H2$  cells; IL-4R $\alpha$ , IL-4 receptor  $\alpha$ ; IL-5R $\alpha$ , IL-5 receptor  $\alpha$ .

patients in that decreased abundance of TLR7, an important PRR, is due to increased expression of 3 microRNAs that target the mRNA of TLR7. Although the article did not discuss why these microRNAs have increased abundance in asthmatic patients, the 3 microRNAs represent new therapeutic targets aiming to restore defective AM antiviral immunity to rhinovirus.

The significance of TLR7 has been appreciated in other studies also. Hatchwell et al<sup>49</sup> identified reduced TLR7 expression in

asthmatic patients in endobronchial biopsy specimens, which was related to impaired IL-28 (IFN- $\lambda$ 2/3) mRNA levels. Work with a mouse model showed that TLR7 can be downregulated specifically by IL-5, and TLR7 staining in endobronchial biopsy specimens was inversely related to sputum eosinophil levels. Together, these studies suggest that in leukocytes TLR7 expression or function might be a key factor determining impaired interferon production in asthmatic patients. Although these studies



show clearly the important role of virus sensing by TLR7 on AMs, leading to innate interferon induction, the role of T<sub>H</sub>2 pathways on this remain incompletely resolved.

## T<sub>H</sub>2 PATHWAYS NEGATIVELY REGULATE INNATE INTERFERONS

### T<sub>H</sub>2 pathways as targets in patients with atopic asthma and their therapeutic interventions

The T<sub>H</sub>2 cytokines IL-4, IL-5, and IL-13 are well established as effector molecules in patients with atopic asthma. Their roles are well described elsewhere<sup>69</sup> and will only be summarized briefly in this review in Fig 1. Both the actions of allergens and viruses on airway epithelial and immune cells and evocation of T<sub>H</sub>2 responses are summarized in Fig 1.

### Viral infection acts additively with allergen exposure to promote AEs

Evidence for synergy between viral infections and allergen sensitization and exposure was first identified in epidemiologic studies,<sup>70,71</sup> and evidence for this has been extended to both human challenge studies<sup>2,7</sup> and mouse models of rhinovirus.<sup>3,22,49,50,72</sup> For RSV, several studies in mice have shown that prior exposure to RSV G protein leads to a strong T<sub>H</sub>2 response and airway eosinophilia on infection.<sup>73,74</sup> Furthermore, mice sensitized to ovalbumin through the airway route after RSV infection had increased airway responsiveness and lung eosinophilia associated with T<sub>H</sub>2 cytokines, notably IL-5.<sup>75</sup> In asthmatic patients rhinovirus infection of the bronchial epithelium also likely has additive and synergistic effects on allergen sensitization and challenge that are important in AE pathogenesis<sup>72</sup>; however, a detailed mechanistic insight into these processes is not complete. The discovery of important pro-T<sub>H</sub>2 factors (IL-33, IL-25, and thymic stromal lymphopoietin) produced from the epithelium that induce type 2 innate lymphoid cells (ILC2s) now allows a more precise understanding of how respiratory viruses can promote T<sub>H</sub>2 pathways because the mediators are induced by viral infection and their expression is consistent with clinical manifestations of asthma, asthma severity, or features of AE pathogenesis.<sup>2,3</sup> How viruses induced pro-T<sub>H</sub>2 factors from the epithelium and how this relates to establishing T<sub>H</sub>2 immunity are depicted in Fig 1.

### Reciprocal negative regulation between interferon and T<sub>H</sub>2 pathways

No study has yet linked impaired interferon responses to a known asthma phenotype; however, most studies observed these phenomena to date in patients with atopic asthma.<sup>6,28,30,34-36</sup> In the past, sputum eosinophil counts have been related to AE risk<sup>76</sup> and impaired IFN- $\lambda$ s in cultured cells negatively correlated with sputum eosinophilia<sup>6</sup> and both serum IgE and IL-4 staining levels in biopsy specimens.<sup>34</sup> Mechanistically, much evidence shows definitively that type I and III interferons and T<sub>H</sub>2 cytokines can exhibit potent negative regulation on the expression or actions of each other.

IFN- $\alpha$  or TLR7 agonists can suppress T<sub>H</sub>2 cell polarization in pure T-cell and mixed leukocyte culture systems, downregulating levels of GATA-3, IL-4, IL-13, and IL-5.<sup>77-79</sup> In PBMCs from atopic children, IFN- $\alpha$  can downregulate Fc $\epsilon$ RI $\alpha$  mRNA levels

on both unstimulated and IL-4/IL-13-stimulated cells, highlighting a negative regulatory capacity for IFN- $\alpha$  on Fc $\epsilon$ RI expression. In the same experiments IFN- $\alpha$  increased Fc $\epsilon$ RI $\gamma$  chain expression; the  $\gamma$  chain is involved in stabilizing Fc $\epsilon$ RI $\alpha$  on the cell surface and together suggests a more complex role for IFN- $\alpha$  in regulating atopy and IgE-mediated responses.<sup>80</sup> In fact, viral induction of IFN- $\alpha$  in early life is thought by some to enhance Fc $\epsilon$ RI $\alpha$  expression on DCs and other antigen-presenting cells, likely leading to enhancement of allergic sensitization, and has been suggested as another potential mechanism how viruses contribute to the establishment of atopy.<sup>16</sup>

Mouse models have also been able to replicate the negative regulation on T<sub>H</sub>2 responses; IFN- $\lambda$ 2 (IL-28), when administered through the airways, can suppress the generation of allergic airways inflammation in an ovalbumin sensitization and challenge model in mice that is T<sub>H</sub>2 driven.<sup>81</sup> This mechanism involved downregulation of OX40 ligand on DCs, leading to less T<sub>H</sub>2 cell polarization.

Recently, in a mouse model of influenza virus infection, infection of *Ifnar1*<sup>-/-</sup> mice resulted in higher levels of virus-induced lung eosinophilia, ILC2 recruitment, and higher levels of serum IgE.<sup>82</sup> In comparative human studies, ILC2s cultured with IL-33 and IL-17 produced the T<sub>H</sub>2 cytokines IL-4, IL-5, IL-13, and IL-9, which were downregulated in the presence of IFN- $\beta$ .<sup>82</sup> This was the first study to show that, in a similar manner to T<sub>H</sub>2 cells, innate lymphoid cells are also affected by innate interferons and that their type 2 function might be increased in an environment that lacks interferon and hence this important negative regulatory mechanism. These mechanisms are shown in Fig 2.

Conversely, *ex vivo* and mouse model evidence strongly suggests that T<sub>H</sub>2 pathways have potent negative effects on the induction of innate interferons by viruses. For example, cross-linking Fc $\epsilon$ RI on DCs before influenza virus or rhinovirus challenge downregulates IFN- $\alpha$  production.<sup>28,29</sup> Blocking IgE function with omalizumab restored this defect and reduced the rate of AEs, showing for the first time outside an experimental infection setting<sup>6</sup> that *ex vivo* interferon measurements can predict clinical outcomes.<sup>51</sup> In a mouse model of pneumovirus infection, cockroach allergen exposure in early life followed by infection led to an asthma-like phenotype with impaired lung type I and type III interferon production.<sup>83</sup> *Ex vivo*, cultured, plasmacytoid dendritic cells pretreated with IL-33 showed reduced IFN- $\alpha$  levels on infection, along with reduced levels of IRF7, viperin, and IL-1 receptor-associated kinase 1, an important signaling molecule in TLR7 function. These data further support the importance of TLR7 expression or function in impaired interferon production in immune cells.

In HBECs pretreatment with TGF- $\beta$ ,<sup>59,84</sup> IL-4, or IL-13<sup>85</sup> can reduce rhinovirus-induced IFN- $\beta$  and/or IFN- $\lambda$  levels; this suppression is specific for interferons and does not inhibit proinflammatory cytokines, such as IL-8,<sup>85</sup> which is consistent with studies of impaired interferon production in HBECs.<sup>5,36</sup> Additionally, other non-T<sub>H</sub>2 cytokines, such as IL-2, do not have this inhibitory effect on interferons, again highlighting the unique relationship between T<sub>H</sub>2 cytokines and innate interferons, which is not simply an effect of any cytokine impairing the effect of another. Although the mechanism was not studied, it is possibly related to induced SOCS1 because SOCS1 is T<sub>H</sub>2 inducible, its levels are increased in asthmatic patients, and it is a potent negative

regulator of virus-induced interferon production.<sup>63</sup> Therefore establishing how  $T_H2$  cytokines can impair virus-induced interferons is a priority, and the mechanism could be an important therapeutic target. A summary of mechanisms responsible for  $T_H2$  pathway impairment of innate interferon production is shown in Fig 2.

Because the overall evidence strongly underscores many potential mechanisms of counterregulation of  $T_H2$  cytokines and type I and III interferons, it could be proposed that in patients with atopic or eosinophilic asthma, impaired interferon production is due to ongoing and uncontrolled  $T_H2$ -mediated inflammation. The observations of increased SOCS1 levels in biopsy specimens of asthmatic patients and that SOCS1 can directly suppress interferon induction<sup>63</sup> suggest a mechanism explaining why interferon levels *ex vivo* can be inversely associated with markers of  $T_H2$  pathways. However, it is possible that impaired interferon production might not be limited strictly to patients with eosinophilic or  $T_H2$ -high asthma<sup>31</sup> and that other mechanisms could explain impaired interferon expression in asthmatic patients. Considering the evidence that impaired interferon production in asthmatic patients can be associated with atopic asthma and the growing wealth of studies using anti- $T_H2$  biologics, the above idea is a testable hypothesis, and future research should endeavor to examine the effects of  $T_H2$  biologics in clinical trials on interferon expression in asthmatic patients *in vivo*.

### Anti- $T_H2$ biologics prevent AEs

The new generation of anti- $T_H2$  biologics have shown impressive efficacy in reducing the rate of AEs in a number of studies. The different biologics and small molecules currently undergoing clinical trials are summarized in Table II.<sup>51,86-96</sup> The anti-IL-13 targeting mAb lebrikizumab produced improvements in the AE rate in a subset of periostin-high asthmatic patients. In the high- $T_H2$  subgroup, the rate of protocol-defined exacerbations was 60% lower in the lebrikizumab group than in the placebo group ( $P = .03$ ).<sup>86</sup> Dupilumab, an mAb specific for the IL-4 receptor  $\alpha$  chain, reduced AE rates by 87% in a study of patients with moderate-to-severe asthma with eosinophilia.<sup>87</sup> Mepolizumab, an anti-IL-5 antibody, reduced blood and sputum eosinophil numbers in a study focusing on prednisolone-taking asthmatic patients with persistent sputum eosinophilia. Mepolizumab also significantly reduced rates of AEs and prednisolone use and improved the median time to AEs by 8 weeks.<sup>88</sup> In another study of patients with eosinophilic asthma with a history of severe AEs, mepolizumab significantly reduced AE rates versus placebo up to 52%.<sup>89</sup> The anti-IL-5 receptor  $\alpha$  antibody benralizumab significantly reduced rates of AEs up to 57% in asthmatic patients with high baseline levels of eosinophils.<sup>90</sup> Furthermore reslizumab, another anti-IL-5 antibody, showed a 58% reduction in AE rates, which was not significant versus placebo in patients with poorly controlled eosinophilic asthma,<sup>91</sup> and pitrakinra, an IL-4 variant that inhibits IL-4/IL-13 signaling, reduced the decrease in FEV<sub>1</sub> versus placebo during allergen challenge in atopic asthmatic patients.<sup>92</sup> Small molecules targeting chemoattractant receptor-homologous molecule expressed on  $T_H2$  cells have additionally shown promise increasing FEV<sub>1</sub><sup>93</sup> and reducing eosinophilic airways inflammation,<sup>94</sup> and a DNzyme-targeted GATA-3 has improved FEV<sub>1</sub> and reduced sputum eosinophil numbers and plasma IL-5 levels versus placebo in patients with

eosinophilic asthma.<sup>95</sup> These results are encouraging and show proof of concept that targeting  $T_H2$  pathways in atopic asthmatic patients or asthmatic patients with eosinophilia can lead to improvements in AEs.

Targeting IgE has also been shown to be effective. In a study of children and young adults with persistent asthma, omalizumab significantly reduced the proportion of participants who had 1 or more exacerbations by 18% and also reduced the number of days with asthma symptoms.<sup>96</sup> Another study in asthmatic children investigated the effects of omalizumab on AE rates, and omalizumab was found to improve AE rates by approximately 5-fold.<sup>51</sup> IFN- $\alpha$  from rhinovirus-infected PBMC cultures was also sampled before and after omalizumab treatment (4 months). Although there was no difference between omalizumab and placebo before treatment commenced, after randomization, the omalizumab group had improved IFN- $\alpha$  induction after rhinovirus infection, and this increase was related to the AE rate. Based on median IFN- $\alpha$  production in the omalizumab group, those subjects with IFN- $\alpha$  levels of less than the median had an almost 6-fold increase in AE rates compared with those who displayed a higher than median IFN- $\alpha$  response. This observation is of importance because it is the first evidence that measuring IFN- $\alpha$  response *ex vivo* can predict clinical outcomes in a clinical trial and provocatively suggests that a benefit of anti- $T_H2$  pathway treatment might be due to restoring an impaired interferon response and increasing antiviral immunity.

### SUMMARY AND CONCLUSION

Considering the number of studies investigating innate interferon responses in asthmatic patients, interferon therapy for asthma, and the renewed appreciation for anti- $T_H2$  pathway biologics as therapeutic interventions, we have provided a timely summary and offered important insights that might help explain the current state of this field and what will likely follow in future studies. Increased viral replication accompanied by impaired interferon production in lung cells of asthmatic patients was first observed in 2005,<sup>5</sup> and since then, a number of studies have repeated these initial observations, although some have not observed this. This work culminated in the first trial of inhaled IFN- $\beta$  for asthma in 2014,<sup>37</sup> which showed a significant effect in a subpopulation with more severe disease.

Although the importance of impaired interferon production in asthmatic patients remains contentious and the benefit of interferon therapy is in need of confirmation, a number of important discussion points can be made from these studies. The inconsistency of some studies investigating interferon levels in asthmatic patients is likely due to subtle differences in laboratory techniques, including preparation and use of both viruses and cells, as well as patient recruitment and characterization. Furthermore, this phenotype in HBECs might be unstable. There is a paucity of studies measuring interferon levels *ex vivo*, and the study by Teach et al<sup>51</sup> has shown that this is possible and meaningful. A further point is that future investigations of inhaled IFN- $\beta$  could benefit from identifying those with impaired interferon responses as a preferred study population; however, whether this is a stable phenotype remains unproved.

The identification of SOCS1<sup>63</sup> and microRNAs 150, 152 and 375 controlling TLR7 expression<sup>31</sup> remains of interest but requires confirmation. TLR7's role in determining interferon induction in immune cells is also encouraging, tempting speculation

that a dedicated GWAS in poor interferon responders versus good responders might be a worthwhile pursuit. Although the effects of IgE cross-linking on virus-induced interferon<sup>28</sup> and the role of anti-IgE therapy open up exciting possibilities, what determines high FcεRI expression levels and the signaling apparatus that negatively regulates interferon expression is currently not known. Future research should be focused on determining this important mechanism.

Regarding anti-T<sub>H</sub>2 biologics, we have proposed herein that the benefit of these therapies on AEs, which largely have a viral cause, could be through restoration of defective antiviral immunity, thus protecting the host from more severe infections that progress to the lower airway. *Ex vivo* studies, mouse models, and now clinical trials<sup>51</sup> provide conclusive evidence that type I and III innate interferons and T<sub>H</sub>2 pathways have antagonistic effects on each other, providing a mechanism or mechanisms explaining how asthmatic patients might benefit from T<sub>H</sub>2-targeting therapies. The restoration of a defective innate interferon response could be a novel mechanism of action, and future studies of anti-IL-5, anti-IL-4, and anti-IL-13 biologics should endeavor to examine this in detail. This is a testable hypothesis; however, without conclusive human evidence linking interferon responses to AE-related clinical outcomes, this novel hypothesis will remain underappreciated.

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