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Immune mechanisms of respiratory viral infections in asthma

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The more severe pathology respiratory viral infections produce in asthma sufferers is a result of a dysregulated immune response. Excess type 2 inflammation is a well-described feature of virally induced asthma exacerbations, with growing evidence that production of antiviral interferons may also be impaired. However, the mechanisms underlying these are little understood. This review summarizes the current understanding and recent discoveries of the cellular and molecular events that follow viral infections in asthma. In particular, we discuss differences in viral sensing and intracellular signalling pathways upstream of interferon induction in asthma, and the role of epithelial-derived cytokines in orchestrating type 2 immunopathology, including type 2 innate lymhpoid cells (ILC2s).

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Introduction

The pathology caused by respiratory viral infections is a product of virus and host factors. Thus influenza viruses cause increased morbidity compared to rhinovirus, the common cold, and the same rhinovirus infection that precipitates a few days of upper respiratory symptoms in healthy individuals triggers lower airways symptoms and airway hyper-responsiveness in those with asthma, lasting up to two weeks. This is underpinned by differences in the immune response to viral infections in asthma, with two dominant themes emerging: an excess of type 2 immunopathology, characterised by the cytokines interleukin (IL)-4, IL-5 and IL-13; and deficient interferon (IFN) responses to virus. Recently attention has focused on the mechanisms underlying these and whether they might be linked, which forms the subject of this review.

Interferon responses

Interferons (IFNs) are a family of cytokines that play a critical role in host defence against virus. Type I (IFN-α and IFN-β) and III (IFN-λs, or IL-28/IL-29) IFNs are produced in response to viral infection and mediate pleiotropic effects via the upregulation and downregulation of hundreds of IFN-stimulated genes (ISGs). These include blocking viral entry into neighbouring cells, cleaving viral nucleic acid, inhibiting viral replication, and inducing apoptosis in infected cells. The type I IFNs and their receptor, IFN-α receptor (IFNAR), are constitutively expressed, although plasmacytoid dendritic cells (pDCs) produce particularly large amounts. Conversely type III IFNs and their receptor are preferentially expressed in the respiratory and gastrointestinal tracts, with epithelial and dendritic cells the principal sources.

A growing number of *in vitro* studies taking different cells (e.g. bronchial epithelial cells (BECs), peripheral blood mononuclear cells (PBMCs), etc.) from asthmatics and healthy controls and infecting them *ex vivo* with respiratory viruses have shown deficient induction of IFNs in asthma (reviewed in [1]). Most but not all studies have demonstrated this, with the finding appearing more robust in subjects with more severe asthma and/or allergies.

A recent *in vivo* study has also identified deficient IFN responses to virus in mice with allergic airway inflammation [2•]. The investigators began from the premise that a 'dual hit' of allergic sensitisation and viral infections in early life leads to asthma, as supported by the observation that the subset of children in a large cohort who were sensitised to aeroallergens and had a history of rhinovirus-induced wheezing had the highest risk of developing asthma [3•]. A sophisticated mouse model was designed to replicate this by exposing mice to cockroach extract (CRE) and pneumonia virus of mice (PVM) in early and later life [2•]. Following PVM infection in later life, the animals developed type 2 immunopathology and deficient IFN-α and IFN-λ induction, associated with increased viral loads. In a follow up preliminary study these animals were infected with rhinovirus four weeks later, mimicking a human asthma exacerbation, and again displayed deficient IFN-α [4].

The same has not been demonstrated in man, possibly because it is hard to disentangle the potentially

confounding effects IFNs and viral loads have on each other — viral replication should be increased in the absence of a robust IFN response, but higher viral loads induce greater IFN production, masking any deficiency. Indeed following experimental infection with rhinovirus, asthma subjects have increased levels of IFN-γ and IFN-λ during exacerbation [5] accompanied by increased viral loads [6]. However, an earlier rhinovirus challenge study showed that deficient IFN induction following *ex vivo* infection of bronchoalveolar lavage (BAL) cells taken at a baseline bronchoscopy correlated with *in vivo* viral load and exacerbation severity when the same subjects were subsequently experimentally infected [7]. This is at odds with the observation of similar viral titres in subjects with and without asthma (the latter should have normal IFN responses) in both naturally occurring and experimentally-induced rhinovirus infections, although the groups were small and interestingly there was a non-significant trend towards reduced IFN-λ [8].

Further evidence in favour of an IFN deficiency in asthma is the demonstration that restoring the IFN responses of PBMCs corresponds to reduced exacerbations *in vivo* [9••]. In a randomized controlled trial, children with a recent history of exacerbations who were treated with omalizumab (anti-IgE) 4–6 weeks before the September rhinovirus epidemic had significantly fewer exacerbations. Experiments on PBMCs taken from a subset of these children before and during treatment showed omalizumab increased the amount of IFN-α produced following *ex vivo* rhinovirus infection in the presence of IgE cross-linking. Moreover those with above average increases in IFN-α *in vitro* had greater reductions in exacerbation frequency *in vivo*. In a trial of inhaled IFN-β, treatment within 24 hours of cold symptoms in moderate-to-severe asthma patients with a history of exacerbations led to reduced symptoms and improved lung function in a subgroup with moderately severe disease (the only subjects who developed a significant increase in asthma symptoms following viral infection) and a trend towards lower sputum rhinovirus virus loads [10•].

How anti-IgE treatment leads to improved IFN-α responses to rhinovirus is unclear. It may be through modification of viral sensing, as an *in vitro* study showing virus-induced IFN-α production by pDCs, the main source of antiviral IFNs, was impaired by cross-linking of the IgE receptor also found downregulation of Toll-like receptor (TLR) 7 on pDCs [11]. TLR7 is one of several pattern recognition receptors (PRRs) that recognise newly synthesised viral nucleic acids, and therefore the first step in the immune response to viruses.

Virus sensing and intracellular signalling in asthma

There have been variable reports of reduced expression of PRRs in asthma, particularly TLR7. TLR7 expression was reduced in the bronchial mucosa of moderate-to-severe asthmatics [12,13•] and in alveolar macrophages from severe asthmatics [14]. Other studies report unchanged expression of various PRRs and signalling molecules, although these analysed different cell types from patients with asthma of varying severity, as defined by the proportion taking inhaled corticosteroids and baseline spirometry (Table 1 [15–17]).

It may be that PRR expression is only reduced in people with asthma with ongoing type 2 inflammation, that is, those with uncontrolled asthma suggestive of active disease or after viral infection when inflammation rises. In support of this, pre-treating BECs from healthy subjects with IL-4 and IL-13 (to model ongoing type 2 inflammation) reduces TLR3 expression [18], and airway eosinophilia indicating continuing inflammation is associated with reduced TLR7 expression [13•]. Co-culturing BECs with eosinophils enhances the effect of transforming growth factor (TGF)-β in suppressing IFN production after *ex vivo* infection [19]. Rhinovirus infection also induces FOXA3, amongst other inflammatory changes, which in turn downregulates expression of several PRRs and their downstream transcription factors [20].

Even in the studies where there were no differences in expression of PRRs or intracellular signalling molecules in asthma, induction of antiviral IFNs was impaired following rhinovirus infection. This implies dysregulaton of signalling pathways downstream of PRRs. Moreover as PRRs and intracellular signalling molecules are themselves ISGs, reduced IFN release ultimately results in deficient induction of this antiviral cellular machinery [21].

In support of this hypothesis, treatment with TLR agonists to overcome dysregulated TLR signalling leads to amelioration of airway inflammation in allergen-sensitised mice (e.g. with resiquimod (a TLR7/8 agonist) [22], TLR7 [23] and TRL9 agonists [24]). It remains to be seen if such an approach is effective in virally induced exacerbations in asthma patients.

Other potential mechanisms of IFN impairment: SOCS1

Suppressor of cytokine signalling (SOCS) 1 is a negative regulator of interferon production. SOCS1 can be induced in BECs by rhinovirus and IL-4 and IL-13. In rhinovirus-infected BECs, levels of SOCS1 are negatively correlated with IFN-β, IFN-λ1, and IFN-λ2/3 mRNA [25•]. Studies using SOCS1$^{-/-}$ mice confirm that SOCS1 directly suppresses virus induction of IFN in BECs as well as IFN-induced amplification of IFN induction *in vivo*. Moreover SOCS1 expression in bronchial biopsies is related to asthma severity and atopic status, providing evidence for this pathway in man.