



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 lymphoid cells (ILC2s).

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Current Opinion in Immunology 2017, **48**:31–37

This review comes from a themed issue on **Allergy and hypersensitivity**

Edited by **Cezmi Akdis**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 14th August 2017

<http://dx.doi.org/10.1016/j.coi.2017.07.017>

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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- λ s, 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 dysregulation 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 TLR9 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- λ 1, and IFN- λ 2/3 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.

Table 1**Expression of pattern recognition receptors and downstream signalling molecules in asthma**

Study	Asthma severity	Cell type	Molecules assayed	Findings in asthma
Shikhagaie 2014	Moderate-to-severe	Bronchial mucosa	TLR5	↓
Hatchwell 2015	Moderate-to-severe	Bronchial mucosa	TLR7	↓
Rupani 2016	Severe	Bronchoalveolar lavage cells (86% macrophages)	TLR7	↓ in eosinophilic asthma
Sykes 2012	Mild-to-moderate	Bronchoalveolar lavage cells	13 in total including TLR3, TLR7, TLR8, RIG-I, MDA5, IRF3, IRF7	↔
Parsons 2014	Moderate-to-severe	Bronchial epithelial cells	TLR3, MDA5, RIG-I	↔
Pritchard 2014	Mild-to-moderate	PBMCs	TLR7, IRF1, IRF7, STAT1	↓ after rhinovirus
			TLR8, IRF5, IFNAR	↔ vs controls

TLR, toll-like receptor; RIG-I, retinoic inducible-gene I; MDA5, melanoma differentiation-associated protein 5; IRF, interferon regulatory factor; STAT1, signal transducer and activator of transcription 1; IFNAR, interferon- α/β receptor; PBMCs, peripheral blood mononuclear cells.

Epithelial susceptibility to viral infection in asthma

Airway epithelium forms a protective barrier and is, for most respiratory viruses, the primary site of replication. Allergen exposure decreases airway epithelial tight junction proteins [26], facilitating virus and allergen penetration. Consistent with this, mouse-specific and house dust mite-specific serum IgE levels are positively correlated with virus-induced exacerbation severity in children [27]. Viral infection also compromises epithelial integrity [28], compounding exposure to allergens. Taken together with IgE-mediated impairment of pDC IFN production, this suggests asthmatics with seasonal allergies, which includes house dust mite [29], are more vulnerable to virus-induced exacerbations in the autumn and spring, corresponding to spikes in rhinovirus infection that are also associated with school terms. This also potentially explains why not all respiratory virus infections lead to asthma exacerbations.

Other changes in asthma also facilitate epithelial viral infection. For example, expression of the receptor for major group rhinoviruses, intercellular adhesion molecule (ICAM)-1, is increased on airway epithelia in asthma [30]. *In vitro*, exposure to IL-4 and IL-13 upregulates ICAM-1 expression [31] providing a plausible mechanism.

Epithelial-derived cytokines IL-33, IL-25 and TSLP

As well as being a source of antiviral IFN, epithelial cells produce other cytokines including three that may be pivotal in promoting type 2 inflammation: IL-33, IL-25 and thymic stromal lymphopoietin (TSLP). Each of these can initiate a cascade of type 2 inflammation, activating group 2 innate lymphoid cells (ILC2s) and T helper 2 (Th2) cells to release IL-4, IL-5 and IL-13. *In vitro*, there appears to be a hierarchy with IL-33 the most potent activator [32].

Human rhinovirus challenge studies support the role of IL-25 and IL-33 in the asthmatic response to virus. IL-25 is increased in asthma at baseline, and following rhinovirus infection both IL-25 and IL-33 rise to a greater extent in subjects with asthma [6,33], with levels of IL-33 correlated with viral load, IL-5 and IL-13. BECs from subjects with asthma infected with rhinovirus *ex vivo* exhibit increased capacity for IL-25 production, while IL-25 receptor blockade restricts type 2 immunopathology and mucus production in a mouse model of virus-induced asthma exacerbation [33]. Similarly, supernatants from *ex vivo*-infected BECs induce IL-4, IL-5 and IL-13 production by human T cells, and IL-5 and IL-13 production by human ILC2s, in an IL-33-dependent manner [6].

Less is known about TSLP, with much knowledge coming from animal models. The recruitment of ILC2s, a potent source of type 2 cytokines, following Respiratory Syncytial Virus (RSV) infection in mice is dependent on TSLP and IL-33 [34]. TSLP and IL-33 have also been implicated in the development of Th2 cells at the expense of regulatory T cells (Treg). Simultaneous exposure to rhinovirus and ovalbumin prevents the development of ovalbumin tolerance in mice and is associated with a shift in the balance of Treg to Th2 cells, but TSLP receptor-deficient mice and mice treated with an IL-33 receptor-blocking antibody have tolerance restored [35]. TSLP may be important in steroid-resistant asthma, as levels are increased in BAL fluid of people with asthma and this correlates with an inability of dexamethasone to inhibit type 2 cytokine production by ILC2s purified from BAL [36]. This study additionally identified novel therapeutic targets in the form of molecules upregulated by TSLP that are not inhibited by dexamethasone.

IL-33 may modulate IFN responses in addition to promoting type 2 inflammation. In mice co-exposed to CRE and PVM (as described earlier), IL-33 receptor blockade

or neutralizing anti-IL-33 antibodies reversed the deficient IFN- α and IFN- λ responses to PVM, as well as the airway hyper-responsiveness and type 2 inflammation [2^{*}]. Further experiments with pDCs revealed that IL-33 mediates its effects by downregulating signalling molecules downstream of TLR7, interferon regulatory factor 7 (IRF7) and interleukin-1 receptor-associated kinase 1 (IRAK-1), leading to TLR7 hypo-responsiveness and deficient IFN- α [2^{*}]. This was corroborated in human samples by demonstrating that anti-IL-33 increased IFN- λ 2 and decreased viral load in BECs from donors with and without asthma infected with rhinovirus *ex vivo* [4]. This supports a direct effect of IL-33, as type 2 cytokines are absent in a BEC monoculture system. One would therefore predict therapies targeting IL-33 to have anti-viral effects analogous to anti-IgE treatment.

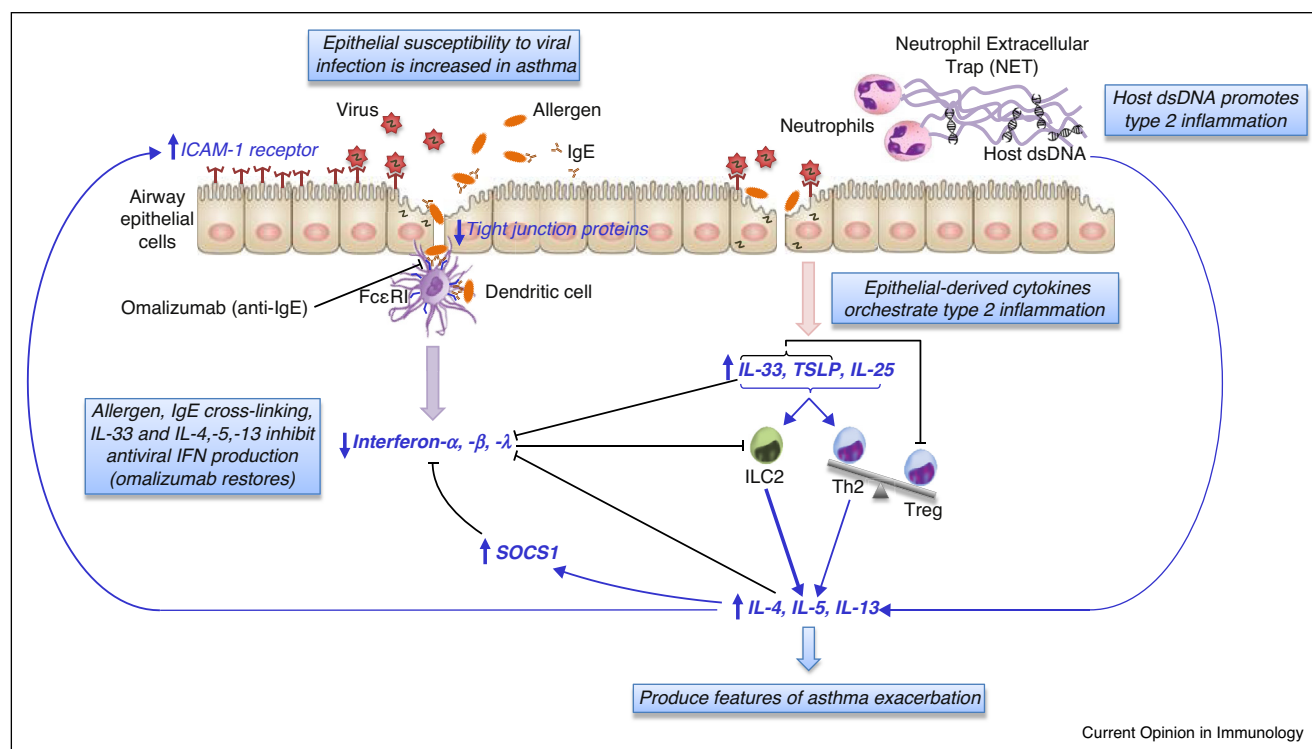
Innate immune cells

The peak in symptoms and viral titres following experimental rhinovirus infection occurs within a few days —

too rapidly for a *de novo* acquired immune response (although a common epitope may induce activation of pre-existing memory T cells [37]). Most research has focused on epithelial cells as the primary site of infection, but innate immune cells are also resident in the airways (e.g. alveolar macrophages, mast cells, dendritic cells) and able to initiate type 2 immunopathology.

These include the relatively recently discovered ILC2s, which are present in elevated numbers in asthmatic airways, particularly in subjects with severe asthma in whom they are the predominant source of IL-5 and IL-13 despite being less abundant than CD4⁺ T cells [38]. In a murine model of chronic asthma, ILC2s were necessary for the persistence of airway inflammation and hyper-responsiveness, whereas antigen-specific T cells were not, as shown by bone marrow chimeras using Rag1^{-/-} (deficient in T and B cells) and Rag2^{-/-} γ c^{-/-} mice (additionally deficient in ILCs) [39]. ILC2s are regulated by IL-33 [39] and negatively regulated by type I IFNs

Figure 1



Immune response to respiratory viruses in asthma. Virus and allergen both act to reduce epithelial integrity, for example, via a decrease in epithelial tight junction proteins. Host factors in asthma also facilitate viral infection, such as upregulation of the ICAM-1 receptor to which most rhinoviruses bind. Subsequent epithelial infection and damage triggers the production of the cytokines IL-33, IL-25 and TSLP. These promote recruitment of type 2 immune cells and their release of type 2 cytokines (IL-4, IL-5 and IL-13), which produce the cardinal features of asthma exacerbations. New evidence suggests neutrophils might also initiate type 2 immunopathology, as host dsDNA trapped in NETs stimulates type 2 cytokine release. This pattern of inflammation inhibits the induction of antiviral interferons. Specifically, IL-33 and type 2 cytokines can, either directly or indirectly via SOCS1, inhibit interferon responses — as can allergen and IgE cross-linking, providing a mechanism by which anti-IgE therapy (omalizumab) might reduce asthma exacerbations. dsDNA, double stranded DNA; ICAM-1, intercellular adhesion molecule 1; IgE, immunoglobulin E; IFN, interferon; IL, interleukin; SOCS1, suppressor of cytokine signalling 1; TSLP, thymic stromal lymphopoietin.

and IL-27 [40]. The absence of robust antiviral interferon induction therefore enables unchecked ILC2 expansion and activation.

Neutrophils are particularly increased in the airways following rhinovirus infection [41], and a recent study suggests they may also contribute to type 2 inflammation through the formation of neutrophil extracellular traps (NETs) [42^{••}]. Rhinovirus infection of mice sensitised to house dust mite (HDM) causes release of host double-stranded DNA (dsDNA), as well as producing the typical features of an asthma exacerbation. NETs contain dsDNA, and degrading NETs with DNase or preventing their formation by inhibiting neutrophil elastase reduces type 2 inflammation, whereas injection of dsDNA alone (in place of virus infection) in allergic mice is sufficient to enhance type 2 inflammation. In human subjects experimentally infected with rhinovirus, dsDNA in nasal lavage samples is induced to greater levels in asthma, and correlates with type 2 cytokine levels and exacerbation severity [42^{••}].

Non-allergic asthma

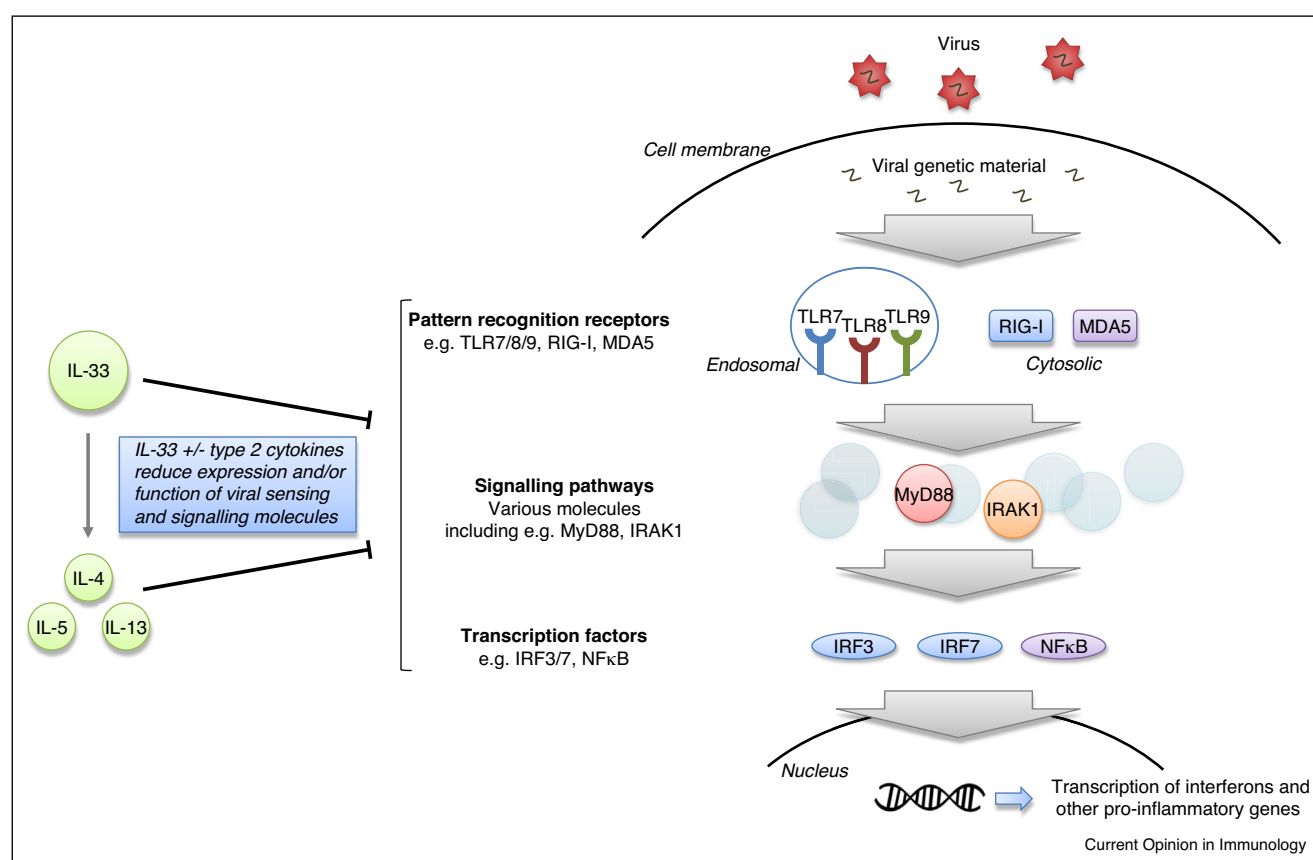
Whilst the majority of asthma sufferers have allergic disease, the remainder are a heterogeneous group that has been relatively neglected, although interest in these patients is growing. For example it has been shown that, in severe asthma, sputum neutrophilia and high dose inhaled corticosteroids are independent predictors of impaired IFN- α induction following *ex vivo* infection of PBMCs with rhinovirus [43].

Another evolving story is that of IL-6, which was elevated in the sputum of two cohorts of asthma patients of varying severity and, in the severe asthma cohort, was associated with reduced lung function and more frequent exacerbations [44]. Whether defects in antiviral immunity underlie the frequency of exacerbations is unknown.

Conclusion

The immunopathology of virus infections in asthma is complex. Aberrant viral sensing and intracellular signalling in asthma, potentially following exposure to allergen

Figure 2



Modulation of cellular viral sensing and signalling pathways by IL-33 and type 2 cytokines. Numerous studies have shown that IL-33 and type 2 cytokines (IL-4, IL-5, IL-13) affect cellular signalling pathways upstream of antiviral interferons, with some finding reduced expression of viral sensing and/or signalling molecules, for example, TLR7. The overall effect is of deficient interferon responses to respiratory viruses. TLR, toll-like receptor; RIG-I, retinoic inducible-gene I; MDA5, melanoma differentiation-associated protein 5; MyD88, myeloid differentiation primary response gene 88; IRAK1, interleukin-1 receptor-associated kinase 1; IRF, interferon regulatory factor; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells.

and type 2 inflammatory proteins, results in deficient IFN responses to virus (Figure 2). Epithelial-derived cytokines promote skewing towards a type 2 inflammatory response, particularly IL-33 which activates ILC2s, unopposed by type I IFNs (Figure 1). Further investigation of exacerbations in non-type 2 asthma is also warranted, as the immunology may differ from that seen in exacerbations in type 2 asthma.

Conflicts of interest

None.

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