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REVIEW

Advances in the treatment of virus-induced asthma

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ABSTRACT

Viral exacerbations continue to represent the major burden in terms of morbidity, mortality and health care costs associated with asthma. Those at greatest risk for acute asthma are those with more severe airways disease and poor asthma control. It is this group with established asthma in whom acute exacerbations triggered by virus infections remain a serious cause of increased morbidity. A range of novel therapies are emerging to treat asthma and in particular target this group with poor disease control, and in most cases their efficacy is now being judged by their ability to reduce the frequency of acute exacerbations. Critical for the development of new treatment approaches is an improved understanding of virus-host interaction in the context of the asthmatic airway. This requires research into the virology of the disease in physiological models in conjunction with detailed phenotypic characterisation of asthma patients to identify targets amenable to therapeutic intervention.

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1. The ongoing problem of acute asthma exacerbations

The Global Initiative for Asthma (GINA) defines asthma as a 'heterogeneous disease usually characterized by chronic airway inflammation'. It is further defined by a 'history of sporadic respiratory symptoms that can vary in severity and are accompanied by variable expiratory airflow limitation' [1]. Exacerbations represent a deterioration of symptoms and lung function from stable status requiring increased medication or an unscheduled hospital visit. The impact of asthma exacerbations is substantial. They account for ~50% of total expenditure on asthma care and remain a substantial ongoing challenge for the clinical management of this disease [2,3]. In developed nations such as Australia, asthma remains the most frequent cause of acute admissions to hospital [4].

2. The old guard – β2 agonists and glucocorticosteroids

Short-acting β2-aderenergic receptor agonists (SABA) reverse acute bronchoconstriction, remain the mainstay asthma therapy and are used to treat acute exacerbations. β2-agonists activate β2-adrenoreceptors on airway smooth muscle causing relaxation and bronchodilation. For control of more severe disease, inhaled corticosteroids (ICS) are remarkably effective anti-inflammatory drugs and have remained the frontline 'preventer' therapy for asthma since the early 1970s [5]. ICS prevent worsening symptoms by controlling inflammation via several mechanisms including glucocorticoid receptor (GR) interaction with negative glucocorticoid response element (GRE) sites. Transrepression also occurs via GR-steroid binding pro-inflammatory transcription factors such as AP-1 and NF-κB. Histone modification is another mechanism by which inflammatory gene expression is suppressed [6]. Glucocorticoids also exhibit systemic side effects and these are often mediated by GR-steroid complexes binding to GRE sites leading to gene transactivation [6].

The effectiveness of ICS-based preventer medications in asthma is not universal and for patients with moderate and severe persistent asthma alternative approaches to treatment are necessary. Rather than increasing the dose of ICS, these patients can be prescribed combination therapy consisting of ICS with a long-acting β2-adrenergic receptor agonist (LABA). This can be administered via a single inhaler which is a preparation that contains ICS and LABA. Combination therapy offers advantages over ICS-only inhaler treatment as they can be used as both a reliever and a preventer. This leads to better symptom control, reduced risk of exacerbation with lower doses of ICS [7,8]. The mechanism of action of combination therapy has been investigated. LABA has been shown to augment the anti-inflammatory and anti-proliferative effects of ICS [9] and synergistically suppress the expression of potentially immuopathogenic chemokines by rhinovirus (RV)-infected bronchial epithelial cells (BECs) [10].

3. The need for treatment alternatives

Short-acting β2-aderenergic receptor agonists (SABA) are typically administered by a dedicated inhaler and used to relieve the acute symptoms caused by bronchospasm. Increased use of reliever SABA is recommended as the

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first-line approach for worsening asthma symptoms with maintenance of usual ICS or ICS/LABA preventer medication. If increased use of reliever fails to reverse symptoms then increased use of preventer therapy is advised. Thus, current management of asthma exacerbations essentially involves increased treatment with the drugs that failed to prevent the exacerbation occurring in the first place [11]. Current preventive and therapeutic strategies such as ICS in combination with LABA are of limited efficacy particularly for children with viral wheeze [12]. In another study of children with acute asthma and symptoms of respiratory virus infection, it was observed that they responded less effectively to β-agonists than during stable disease [13]. So why do current treatment approaches fail to prevent virus-induced asthma? This may be explained in part by interference with anti-viral immunity. Corticosteroid–LABA combination has been reported to inhibit production of pro-inflammatory immune mediators by RV-infected *in vitro*-cultured undifferentiated BECs [10,14]. CXCL10 was one of the chemokines inhibited by ICS/LABA treatment. This interferon-stimulated gene (ISG) is involved in recruitment and activation of NK- and Th1 cells which are involved in the type-1 IFN-mediated anti-viral response to RV [15]. The concept that ICS/LABA can indiscriminately interfere with anti-viral immunity is further supported by studies in human epithelial cells and peripheral blood mono-nuclear cells (PBMCs) [16,17].

Another rationale for advancing therapy options for viral asthma is to reduce the amount of steroid and β2-agonist required to regain control of disease. Corticosteroids target many genes and exert a multitude of undesirable effects on the body. Long-term use from early childhood has significant side effects and has been linked to reduced bone density, mood swings, weight gain, difficulty sleeping, increased risk of cataracts, and increased risk of infection throughout life [18–20]. Whilst specific target options are currently being developed (discussed later), this has also spurred research into new classes of glucocorticoids called dissociated corticosteroids that maintain therapeutic efficacy whilst exhibiting an improved side effect profile [21].

4. Additional therapies for virus-induced asthma

4.1. Leukotriene antagonists

Virus-induced asthma occurs despite optimal use of corticosteroids. This has led to the development of additional therapies that target inflammatory pathways implicated in pulmonary inflammation and bronchoconstriction. These include the 5-lipoxygenase pathway (FLAP) and production of leukotrienes (LT). There are two classes of LT – cysteinyl leukotrienes (CysLT) and LTB_4. They are inflammatory lipid mediators that are generated by the arachidonic acid (AA) pathway in activated immune cells. AA from membrane phospholipids is catalyzed by phospholipase A_2 before conversion to LTA_4 by 5-lipoxygenase (5-LO). Mast cells, macrophages, eosinophils, and basophils convert this molecule to the CysLT LTC_4 which is the precursor for the other cysteinyl leukotrienes (LTD_4 and LTE_4). Although the potency of each can vary in different settings, all three CysLTs can stimulate airway smooth muscle contraction and increase vascular permeability [22].

Neutrophils convert LTA_4 into LTB_4 which can act as a potent neutrophil and monocyte chemoattractant [23]. Increased CysLT production is associated with acute asthma likely reflecting activation of these inflammatory cells that are present in the airways during viral asthma exacerbations [24,25]. There are two classes ($CysLT_1R$ and $CysLT_2R$) of classical G protein-coupled receptors for CysLT. Human $CysLT_1R$ is the predominant CysLT receptor in the airways and is expressed by airway smooth muscle cells, macrophages, and mast cells [26]. LTD_4 is the predominant ligand for this receptor and increases intracellular calcium and smooth muscle contraction. Monteleukast is a $CysLT_1R$ antagonist that has been shown to improve lung function in patients presenting to the emergency department with an exacerbation providing further evidence of the immunopathological role of CysLTs in acute asthma [27]. The enzymes that catalyze production of CysLTs can also be targeted by drugs such as the 5-LO inhibitor zileuton. A number of other FLAP inhibitors are currently being developed [28].

4.2. Anticholinergics

The cholinergic system is involved in bronchial constriction and mucus production via activation of muscarinic receptors by acetylcholine (ACh) which is released by peribronchial parasympathetic nerve fibers [29]. ACh can also be produced by (non-neuronal) airway epithelial cells [30]. Anticholinergics such as the long-acting muscarinic receptor antagonist (LAMA) tiotropium bromide can reduce cholinergic activity impacting on airway smooth muscle tone and mucus hypersecretion by blocking neuronal acetylcholine. Tiotropium has also been reported to reduce non-neuronal ACh augmented IL-13-induced goblet cell metaplasia in air-liquid interface (ALI)-differentiated airway epithelial cell cultures [31]. In human clinical trials the bronchodilatory activity of SABA could be augmented by the use of LAMA to improve treatment outcomes in children and adults with acute, moderate to severe exacerbations [32,33]. For severe, persistent, poorly controlled asthma, the addition of tiotropium to high-dose combination therapy improved lung function over 24 hours [34]. Inhaler tiotropium bromide has demonstrated efficacy for treatment of chronic obstructive pulmonary disease (COPD) exacerbations [35]. In Phase III human clinical trials in asthma, it provided sustained bronchodilation whilst reducing the frequency of exacerbations [36]. Inhaled tiotropium bromide is now licensed for use as an add-on therapy for adults on maintenance combination therapy who have had at least one severe exacerbation in the previous year [37]. A number of other anticholinergic drugs including glycopyrronium (Seebri), aclidinium bromide (Forest Laboratories and Almirall), and umeclidinium bromide (GlaxoSmithKline and Theravance) are also approved for the treatment of COPD and are at various stages of development for the prevention asthma exacerbations [38].

4.3. Theophylline

This xanthine derivative is a non-selective phosphodiesterase inhibitor that has demonstrated therapeutic efficacy in patients who do not respond well to corticosteroids.

Phosphodiesterase inhibition reduces inflammation and bronchoconstriction and can increase the effectiveness of combination therapy [39]. Theophylline may therefore be useful for the treatment of virus-induced asthma. One of its mechanism of action includes up-regulation of histone deacetylase 2 expression, the deficiency of which is thought to underlie steroid resistance in some patients [40]. The use of this drug is limited by its interaction with numerous drugs, narrow therapeutic range, variable pharmacokinetics, and extensive side effects [41].

5. From drugs to biologics and personalized treatment

5.1. Targeting allergy and type-2 immunity

The recognition that asthma is a heterogeneous disease has implications for individualized disease management based on detailed phenotypic characterization derived from immunopathogenic mechanistic insight [1]. There is good evidence that persistent atopic sensitization associated with activated type-2 immunity is a key driver of asthma in children [42,43]. Asthmatic adults represent a far more heterogeneous population. Even so, a Th2-high immune signature has been reported in approximately 50% of adult asthmatics [44]. Type-2 cytokines produced by Th2 cells are critical to many responses that drive allergic airways inflammation and disease in asthma. IL-4 and IL-13 are required for IgE synthesis and IL-5 is required for eosinophil recruitment, maturation, and survival. IL-9 is required for mast cell activation. IL-13 induces airway hyperreactivity (AHR), mucus hypersecretion, and metaplasia of mucus-producing cells.

Innate immune cells, particularly the recently discovered type-2 innate lymphoid cells (ILC2), are now recognized as another important source of type-2 cytokines associated with the pathogenesis of allergic airway disease [45]. ILC2 recruited to the lung during allergen challenge, respond to IL-25 and IL-33 and produce high levels of IL-5 and IL-13 [46,47].

5.2. Synergy with virus

Two seminal studies assessed whether allergen exposure increased the risk of acute asthma in conjunction with viral infection in sensitized asthmatics. Green et al. investigated 60 patients aged 17–50 admitted with acute exacerbations who were assessed for the presence of respiratory infection as well as total and allergen-specific IgE. The combination of sensitization, exposure to high levels of common household, and environmental allergens and viral infection was strongly associated with the risk of hospital admittance with acute asthma [48]. Similar findings in children (aged 3–17 years) were also reported by Murray and co-workers indicating a synergism between sensitization, allergen exposure, and viral infections in inducing asthma exacerbations [49].

Studies in a human experimental RV infection model in allergic asthmatic- and normal-volunteers demonstrated that asthmatics had more severe lower respiratory tract symptoms, reductions in lung function and increases in bronchial hyperreactivity. Exacerbation severity was strongly correlated with BAL Th2 cell cytokine production and viral load [24]. Using direct sampling techniques we could demonstrate increased IL-4, IL-5, and IL-13 in the airway mucosa of asthmatics experimentally infected with RV. Expression of type-2 cytokines in both the upper and lower respiratory tract was associated more severe asthma symptoms [50].

Various promising antibody-based therapies for treatment of virus-induced asthma exacerbations are currently in development and some of which are now licensed for use. This next generation of asthma treatments include monoclonal antibodies that can target specific inflammatory molecules that define asthma phenotypes.

6. Blocking allergic inflammation with anti-IgE

Allergic asthma is the most common form of the disease. IgE plays a central role in allergic inflammation. Activation of FcεR1 on mast cells and basophils by cross-linking of IgE promotes degranulation and release of pro-inflammatory mediator leading to increased inflammation. Omalizumab (Xolair), first approved by the USA in 2003, is a monoclonal antibody that binds and neutralizes IgE thus preventing activation of FcεR1. Omalizumab reduces serum-free IgE levels by binding to the constant region (cε3) which prevents IgE from interacting with its receptor FcεR1. In addition to this, an early study in a group of 15 subjects who were allergic to dust mite showed that treatment with Omalizumab not only reduces serum-free IgE but also decreases FcεR1 expression on circulating basophils from these subjects [51]. Omalizumab has been shown to effectively reduce asthma exacerbations, improve symptom control, and reduce the need for ICS and beta-agonists in Phase II and Phase III trials of severe atopic asthmatics [52]. Recent expert panel guidelines recommend considering Omalizumab as an alternative or in addition to oral corticosteroids in Step V and VI patients with severe allergic asthma. Two studies have examined prophylactic treatment and reported significant reductions for exacerbations (some of which were caused by a viral infection) in the Omalizumab-treated group [53,54]. One of the studies identified an association between increased IFN-α production and fewer exacerbations in the omalizumab treatment group and implicated inhibition of IgεRI cross-linking by IgE as involved in this response [54]. The mechanism linking IgE-receptor binding and regulation of IFN production during viral infection in asthma has been investigated. FcεR1 is on plasmacytoid dendritic cells (pDC), a crucial innate immune cell that produces large amounts of type I IFN in response to viral infection. It was observed that activation of FcεR1 could inhibit the ability of pDC to secrete IFN-α in response to TRL9 stimulation [55]. Gill and colleagues extended these observations and demonstrated deficient influenza-induced IFN induction by pDCs from asthmatic subjects compared to healthy subjects and confirmed that cross-linking of FcεR1 led to profound inhibition of virus induction of IFN-α [56]. Further studies showed that FcεR1 cross-linked PBMCs from children with asthma had deficient RV-induced IFN production [57]. This suggests that omalizumab could also be used to enhance pDC mediated antiviral responses in the treatment of virus-induced asthma exacerbations.

7. Targeting type-2 cytokines

7.1. IL-4 and IL-13

It is well established that type-2 cytokines are the effector molecules that drive allergic inflammation in asthma and biologics that target these cytokines have been the focus of treatment development. IL-4 and IL-13 are type-2 cytokines that are produced by activated mast cells, basophils, eosinophils, dendritic cells, and Th2 cells. These cytokines signal through IL-4Ra/IL-13Ra1 and play a central role in promoting allergic diseases by increasing IgE production, Th2 cell differentiation, mast cell and dendritic cell development, eosinophil recruitment, and AHR.

Animal studies have shown that IL-4 knockout mice were unable to develop eosinophilia making it an attractive target for atopic asthma treatment [58]. Despite the promising results in pre-clinical studies, clinical trials of humanized anti-IL-4 mAb, pascolizumab, showed little efficacy in treating asthma in human clinical trials [59,60]. It appears that anti-IL-4 was only effective at suppressing eosinophil infiltration when administered during allergen challenge while the inhibition of IL-4 before this had a minimal effect in reducing eosinophil infiltration [61]. There is no evidence that blocking IL-4 reduced disease in virus-induced asthma.

A number of humanized anti-IL-13 mAbs have also entered clinical trials. In particular, AstraZeneca's tralokinumab has entered Phase III clinical trials. In a study that included 194 adults with moderate to severe uncontrolled asthma, subcutaneous injection of tralokinumab improved lung function with mean ± SD increases from baseline in FEV_1 of 0.16 ± 0.35 L, 0.21 ± 0.37 L, 0.26 ± 0.41 L in the 150, 300, and 600 mg dose, respectively [62]. Compared with placebo, there was also a greater reduction in the use of β_2-agonist in patients who were treated with tralokinumab. Similarly, lebrikizumab, another humanized anti-IL-13 mAb by Genentech, also showed lung function improvement when used in patients with high levels of the IL-13-induced molecule periostin. In addition, lebrikizumab treatment also reduced asthma exacerbation rate by 60% in periostin-high patients [63].

As both IL-4 and IL-13 signal through IL-4Ra and given the key roles played by IL-4 and IL-13 in the pathogenesis of asthma, a mAb targeting IL-4Ra has been developed. The efficacy of dupilumab was evaluated in asthmatic patients with persistent, moderate to severe eosinophilic disease [64]. In this study, 52 patients were treated with dupilumab 300 mg while the other 52 patients received placebo. Treatments were administered for 12 weeks and patients were instructed to discontinue LABAs and ICS at Week 4 and Week 6 through 9, respectively. The end points measured included asthma exacerbation, lung function, and type-2-associated markers. The results from this trial were promising with the dupilumab-treated patients experiencing an 87% reduction of exacerbations. In addition, dupilumab also improved lung function, asthma control, and reduced expression of biomarkers associated with type-2 inflammation.

In addition to promoting allergic inflammation, type-2 cytokines can interfere with anti-viral immunity. In one study, IL-4 and IL-13 were found to inhibit RV-16-induced interferon production and increased virus replication [65]. Increased RV replication was also observed in mice with type-2-driven allergic airway inflammation [66]. As RV is one the most common triggers of asthma exacerbations, it needs to be determined if blocking IL-4 and IL-13 could be useful in preventing experimental RV-induced exacerbation of asthma [24,50].

7.2. IL-5

IL-5 regulates eosinophil maturation and survival and increased sputum eosinophils correlates with asthma exacerbation severity [67]. Anti-IL-5 mAbs such as mepolizumab (GSK), reslizumab (Teva Pharmaceutical), and anti-IL-5 receptor alpha benralizumab (AstraZeneca) have all entered Phase III clinical trials that treated asthmatics with high eosinophil levels. In a randomized, double-blinded study, patients with eosinophilic asthma and recurrent exacerbations were administered with either 75 mg intravenous dose or 100 mg subcutaneous dose of mepolizumab, or placebo every 4 weeks for 32 weeks. Treatment with mepolizumab decreased blood eosinophil count, halved the exacerbation rates, increased lung function, and improved asthma control [68,69]. Mepolizumab has completed Phase III clinical trials and has been licensed in the USA (trade name Nucala) since November 2015 as add-on maintenance therapy for severe, eosinophilic asthma. Similarly, blocking anti-IL-5 receptor with benralizumab with 20 mg or 100 mg reduced exacerbation rates in adults with uncontrolled eosinophilic asthma [70]. Eosinophils are a prominent inflammatory cell in virus-induced asthma exacerbation. In one study, eosinophilic airway infiltration persisted for up to 8 weeks following infection in asthmatic individuals and correlated with increasing airway hyper-responsiveness [71]. A more recent human experimental RV infection study reported a very substantial increase in BAL eosinophil numbers in asthmatic subjects [50]. Targeting IL-5 to treat virus-induced eosinophilic asthma exacerbations is currently being investigated (e.g. Mepolizumab treatment for rhinovirus-induced asthma exacerbations (clinical trials.gov)).

8. Targeting epithelial responses

Airway epithelial cells possess the capacity to directly influence type-2 immunity by expression of type-2-promoting cytokines such as TSLP, IL-25, and IL-33. We have shown that expression of these cytokines is linked to viral replication in bronchial epithelium, type-2 responses, and inflammation in asthma exacerbations [50,66,72]. From a translational-treatment development perspective, the airway epithelium is an attractive site for drug delivery with inhaled therapies having numerous advantages over systemic delivery approaches. If it can be demonstrated that pulmonary type-2 inflammation can be broadly suppressed by inhaling a drug or mAb that targets a type-2 immune-activating cytokine expressed by bronchial epithelium, then this will be a major advance on current type-2 cytokine-targeting approaches that rely on systemic delivery of large doses of expensive antibodies [64,73].

8.1. IL-25

IL-25 is an IL-17 family member and has been identified as an initiator and regulator of type-2 immunity [74]. Studies have

demonstrated increased IL-25 gene expression together with its receptor, IL-17RB, in tissue with type-2-dominated inflammation, while eosinophils, mast cells, and the airway epithelium have been also been reported as sources within the lung. Blocking IL-25 in a mouse model prior to antigen sensitization and/or challenge caused a striking reduction in Th2 cell responses and type-2 cytokine (IL-5 and IL-13) production [74]. IL-25 is a potent activator to type-2 immunity. It can stimulate type-2 cytokine production via activation of IL-17RB-expressing Th2 cells [75] and ILC2 [76]. We have reported that asthmatic BECs express increased levels of IL-25 when RV infected. We further showed that blocking IL-25 receptor with anti-IL-17RB mAb in a mouse model of RV-induced asthma exacerbations prevented virus-induced increased allergic inflammation and type-2 cytokine production [66].

8.2. IL-33

IL-33 signals through an IL-1R-like (IL-1RL1) subunit, also known as ST2 or type 1 ST2 (T1/ST2), and associates with the IL-1R accessory protein (IL-1RAcP). Infection with influenza induces an innate T1/ST2+ population of ILC2, which responded to IL-33 by producing IL-13 [77]. We have recently performed experimental RV infections in atopic asthmatic and healthy human subjects and observed that expression of IL-33 protein in the bronchial mucosal lining fluid was associated with increased type-2 cytokine production and asthma exacerbation symptom severity. *In vitro* blocking IL-33 activity with an anti-ST2 mAb suppressed production of type-2 cytokines by T cells and ILC2 stimulated by medium from RV-infected asthmatic BECs [50].

8.3. TSLP

TSLP was the first epithelial type-2-promoting cytokine observed to be over expressed in asthma [78]. In mice, transgenic overexpression of TSLP in the airways induces type-2 inflammation [79] leading to skewing of naive T cells to become Th2 cells [75]. TLSP activates dendritic cells via a heterodimeric receptor composed of the TSLPR and the IL-7Rα. This leads to priming and recruitment of Th2 cells via production of CCR4-binding chemokines CCL17 and CCL22 [78]. We observed that RV infection increased TSLP expression in the lungs of mice with allergic pulmonary inflammation [72]. An anti-TSLP mAb has demonstrated therapeutic efficacy in a human study of experimental allergen-driven asthma [80]. Blocking studies demonstrating a role for TLSP in amplification of pulmonary type-2 inflammation and airways disease during viral infection in asthma are so far lacking.

9. Viruses: targeting the trigger of asthma exacerbations

Early-life viral wheezing illness is a risk factor for asthma development. Jackson et al. examined the relationship between early-life virus-induced wheeze and asthma development in an at-risk (parents have a history of respiratory allergy/asthma) cohort of children (COAST study [81]). They showed that RV infections conferred the greatest probability (odd ratio = 9.8) for asthma development by age 6 [82]. Respiratory viral infections are also the cause of most asthma exacerbations. The precise mechanisms by which viral infections make asthma worse are still poorly understood. One reason for this is the difficulty inherent in repeatedly sampling the lower airways and accurately measuring the presence of infectious virus during an exacerbation. Nonetheless, inhibiting viral replication and reducing virus-induced inflammation is a sensible approach and treatments that stimulate anti-viral immunity are a potential therapy. Unlike the immune-blocking approaches described earlier, the aim here is to precisely stimulate innate anti-viral immunity thereby limiting replication and inhibiting production of asthmogenic immune mediators. The idea is that Toll-like receptors detect infection and induce expression of innate anti-viral interferons which play a key role in reducing viral load via induction of anti-viral molecules directly and initiation of a type-I immune response that antagonizes type-2 immunity and associated pulmonary allergic inflammation. The major caveat to an immune-stimulatory approach is the potential to promote inflammation and cause worse disease. Clearly, the anti-viral/immune-regulatory versus inflammatory profile of any drug in this category will need to be meticulously assessed pre-clinically before moving into human asthma trials.

10. Toll-like receptor agonists

Toll-like receptors (TLRs) recognize a range of bacterial and viral components and are critical for the detection of pathogens and activation of innate immune cells. Although being important in the clearance of pathogens, activation of TLRs could act as a double-edged sword especially in the setting of chronic lung diseases. For example, activation of TLR3 by viral double-stranded RNA, and TLR4 by bacterial component LPS are linked to increased airways inflammation in a mouse model [83]. Conversely, activation of TLR7 or TLR9 may be protective in asthma.

10.1. TLR7

TLR7 plays an important role in antiviral immunity. TLR7 is predominantly expressed on pDCs and B-cells [84] and is also expressed on airway epithelial cells [85]. Reduced TLR7 function has been associated with asthma [86,87]. In a pre-clinical study, treatment with a TLR7 agonist in a mouse model of allergic asthma prevented development of airway resistance, leukocyte infiltration, and suppressed production of type 2 cytokines [88]. We have shown that Allergic *Tlr7*(-/-) mice displayed impaired IFN release upon RV1B infection, with increased virus replication and eosinophilic inflammation and airways hyper reactivity. Treatment with exogenous IFN or adoptive transfer of TLR7-competent pDCs blocked these exaggerated inflammatory responses. TLR7 expression in the lungs was suppressed by allergic inflammation and by IL-5-induced eosinophilia in the absence of allergy. We then examined endobronchial biopsies from subjects with moderate-to-severe asthma and eosinophilic but not neutrophilic airways inflammation, despite inhaled steroids, showed reduced TLR7 and IFN lambda 2/3 expression. Furthermore, TLR7 expression

inversely correlated with percentage of sputum eosinophils [89]. This implies IL-5-induced airways eosinophilia acts as a negative regulator of TLR7 expression and antiviral responses, and provides a molecular mechanism underpinning the effect of eosinophil-targeting treatments for the prevention of asthma exacerbations. Further, in a chronic allergic asthma model, pre-treatment with a TLR7 agonist before ovalbumin antigen challenge also prevented airway remodeling, goblet cell hyperplasia and increases in airway smooth muscle mass [90]. Similar to the acute allergic asthma model, TLR7 agonist pre-treatment also reduced both type 1 and type 2 cytokines in the chronic model. Apart from its roles in innate immunity, TLR7 can also mediate human airway smooth muscle relaxation in a dose-dependent manner [91]. Given that TLR7 promotes antiviral defense and protects against virus-induced airway dysfunction, TLR7 agonists are a viable therapeutic option for virus-induced asthma exacerbations. This is supported by mouse studies showing TLR7 deficiency leads to a more severe RV-induced airways disease in house dust-mite allergic mice. This effect could be reversed with type I or type III IFN treatment [89]. GSK have developed a selective TLR7 agonist (GSK-2245035) which has been tested in a Phase 2 trial involving patients with allergic rhinitis. Intranasal delivery of <100 ng stimulated type I IFN and ISG expression without causing symptomatic inflammation [92].

10.2. TLR9

TLR9 recognizes both bacterial and viral CpG-DNA. TLR9 is predominantly expressed in airway epithelium, macrophages, neutrophils, pDCs, and B-cells [93–95]. In a murine model of asthma, it was found that TLR9 agonist treatment during allergen challenge markedly reduced lung eosinophilia, type-2 cytokines production, and airway hyperreactivity [96]. Further studies showed that CpG-DNA also suppressed subepithelial fibrosis and goblet cells hyperplasia, which are key features of airway remodeling [97]. It remains to be studied if TLR9 agonists could be useful in treating viral-induced asthma.

11. Innate anti-viral interferons and asthma

Innate anti-viral type I interferons (IFN-α and – β) play an important role in several biological processes as they can have an anti-proliferative, anti-viral, and immunomodulatory activity [98]. Type I IFNs are an important component of innate immune response against virus and can be induced upon stimulation of pattern recognition receptors (PRRs) by bacteria and viral components [99]. Type I IFN receptor consists of two subunits, IFN-α receptor (IFNAR)-1 (α subunit) and IFNAR-2 (β subunit). Upon binding with its ligand, type I IFNs signal through JAK-1 and protein-tyrosine kinase (Tyk)-2, which phosphorylates STAT-1 and STAT2 to form a heterodimer before translocating into the nucleus and binding to promoters of ISGs [98]. Many ISGs are involved in inhibiting viral replication and the importance of ISGs in viral pathogenesis is summarized in a review [100].

There is evidence to suggest that type I IFNβ expression by RV-infected BECs is impaired in asthma, identifying a potentially important protective role for type I IFN signaling in asthma exacerbations [101,102]. In a further study to identify mechanisms of impaired IFN expression, it was shown that suppressor of cytokine signaling 1 (SOCS1), induced by either RV infection or inflammatory cytokines, suppressed IFNβ promoter activation in BECs. In addition, expression of SOCS1 was increased in bronchial biopsy specimens from adults with mild to moderate atopic asthma prompting the conclusion that increased expression of this negative regulator contributes to IFN deficiency in asthma [103].

Type III IFN (IFNλ1, 2, and 3) are another group of innate anti-viral IFNs that signal through a distinct receptor complex composed of IFN-λR1 and IL-10R2 [104]. Analysis of *ex vivo* RV-infected asthmatic BECs and bronchoalveolar lavage (BAL) cells, Contoli et al. demonstrated impaired RV-induced type III IFN correlated with increased symptoms and viral load and decline in lung function during RV infection *in vivo* in the same patients [105]. A subsequent study employed mouse models to demonstrate that exogenous expression of IFNλ could reduce the severity of allergic airways disease via modulation of CD11c+ DC-mediated differentiation of Th1 cells [106]. Type I interferon can also restrict type 2 immunopathology by acting on ILC2. Mice that were deficient in type I IFN signaling demonstrated an increase in ILC2 and type 2 immunopathology following infection by influenza A virus [107]. More recently, two separate studies simultaneously reported that IFNβ negatively regulated activated ILC2, which in turn reduced type-2 inflammation [107,108].

The prevalence of defective IFN expression is not universal with 'normal' IFN expression in asthma observed in some studies [109,110]. There has even be a report of increased IFNλ in children who had wheezed with a viral infection when compared to children whose viral disease was confined to the upper respiratory tract. Expression positively correlated with disease severity and the authors concluded that this was evidence that IFNλ was driving disease. Whilst this is possible, the authors were unable to measure viral load in the lower airways and determine if increased IFNλ was being stimulated by higher viral replication in the wheezing children [111]. The interaction between IFN-expression, viral replication and effect on disease is complex and each can influence the other. Another important factor appears to be disease severity. Discrepancies in identifying IFN deficiency in asthma have been attributed to asthma severity with mild, well-controlled asthma less likely to exhibit this phenotype [112].

Although there is still much to learn about the role of IFNs in viral asthma exacerbations, these molecules are an obvious therapeutic option with the potential to restore deficient IFN expression, improve control of viral infection, and suppress type-2-driven allergic airways disease. A clinical trial investigated the effect of type I interferon therapy on asthmatics. The study recruited participants with a history cold-induced asthma exacerbations. They were treated with either nebulized IFN-β or placebo within 24 hours of the onset of cold symptoms. There was no difference observed between the IFN-β and placebo-treated groups in terms of asthma control questionnaire; however, IFN treatment did enhance morning peak respiratory flow recovery, reduce the need for additional treatment and boosted innate immunity as assessed by blood and sputum biomarkers. It should be noted that intention to

treat analysis of this population revealed that the majority with colds failed to develop significant exacerbations. Further analysis of a subgroup with persistent asthma of moderate or greater severity showed a deterioration in symptoms and peak flow with a cold and treatment with IFN-β significantly improved peak flows and prevented virus-induced asthma symptoms [113]. These results suggest that interferon therapy might be useful in treating a subset of asthmatics with persistent, poor control who, despite treatment, are at greater risk of acute exacerbations. A large Phase III randomized controlled trial is now underway to determine this.

12. Anti-viral approaches for rhinovirus

Almost all acute asthma episodes in children are preceded by a virus-induced cold [114]. In adults, the data is less comprehensive but viruses are still the predominant cause of asthma attacks for this group too [115,116]. Respiratory viruses are an obvious target for advancing treatment of asthma exacerbations. Directly inhibiting viral infection would obviate the need for blocking downstream inflammatory cytokines or boosting anti-viral immunity. This approach comes with its own set of challenges. Not least of which is the number of viruses that can cause an exacerbation. They include RVs, influenza, RSV, human metapneumovirus, parainfluenza virus, adenovirus, and coronavirus. Whilst all of these viruses have been detected, they are not equal in terms of the frequency with which they are associated with acute asthma. Most viral asthma (particularly in children) is caused by RV infection. Blocking infection with this group of viruses would have a substantial impact on virus-induced asthma exacerbations. There are currently no anti-viral drugs available for RV.

12.1. Anti-human ICAM-1 monoclonal antibody

Intercellular adhesion molecule-1 (ICAM-1) is expressed on epithelial cells, endothelial cells, leukocytes, and neutrophils. Expression of ICAM-1 is crucial for the recruitment and activation of cells expressing its natural ligands. These include macrophage adhesion ligand 1 expressed by macrophages and granulocytes, leukocyte function-associated antigen 1 (LFA-1) expressed by T-cells, and extracellular matrix protein. ICAM-1 is also the natural receptor for 90% of species A/B RV and classifies them as major group viruses [117]. Therefore, targeting ICAM-1 could be an alternative in treating viral-induced asthma. Note that infection with 10% of RV-A subtypes and all RV-C species viruses would not be inhibited by anti-ICAM-1. In our previous work we showed that administration of anti-human ICAM-1 antibody, targeting domain 1 of human ICAM-1, was able to block the entry of HRV16 and HRV14 reducing cellular inflammation, pro-inflammatory cytokine production and viral replication in a transgenic mouse expressing a chimeric human-mouse ICAM-1 protein that confers permissiveness to RV-16. Further, administration of anti-human ICAM-1 successfully prevented HRV16-induced exacerbation of allergic airway inflammation and airway hyperresponsiveness in these mice [118]. It remains to be determined if such an approach can be translated into human clinical trials.

12.2. Anti-rhinovirus compounds

Initial interest in treatments for RV infection stemmed from a need to reduce the burden of the common cold [119]. A number of drugs including R61837, WIN54954, and pirodavir were designed to bind to the viral capsid and inhibit attachment, un-coating and productive infection. Results from clinical trials were generally disappointing and development was discontinued [120]. Another anti-rhinoviral compound, pleconaril, is a capsid binder that prevents virion uncoating and this made it to Phase II clinical trials. Subjects were administered pleconaril within 24 hours of a viral cold. Results from this study were promising with a significant reduction in symptom scores. However, the drug was not approved due complications arising from interactions with the oral contraceptive. Another drawback was the emergence of drug-resistant mutants [121]. A company (Merck Sharp & Dohme Corp)-funded Phase II study was conducted in 2007 to assess the effect of pleconaril nasal spray on colds and asthma exacerbations. No reduction in RV-positive asthma exacerbations was reported. There is renewed interest in development of capsid-binding anti-rhinovirals specifically for the treatment of patients with chronic respiratory diseases such as asthma. Biota Pharmaceuticals is currently testing the small molecule enterovirus capsid inhibitor vapendavir in Phase II clinical trials involving approximately 400 asthmatics. The trial is due to be completed in March of this year and frequency of asthma exacerbations will be one of the endpoints assessed.

Rupintrivir (AG7088, Agouron Pharmaceuticals, Inc) is a 3C protease inhibitor that was designed to inhibit 3C-mediated proteolytic cleavage of the viral polyprotein thereby preventing RV replication. Frequent delivery of this drug by nasal spray as a cold treatment did reduce symptoms and nasal viral secretion [122]. There has been interest in rupintrivir as a treatment for enterovirus (coxsackievirus), the causative agent of hand, foot and mouth disease which is a common affliction amongst infants and young children [123]. The efficacy of rupintrivir in the treatment of viral asthma has not been reported.

Resveratrol is a natural phenol (stilbenoid) produced by a number of plants in response to damage or infection. This compound has been reported to inhibit RV replication in nasal epithelia [124] and potentiate glucocorticosteroid activity [125]. So far, studies with this molecule have been limited to *in vitro* and animal models.

13. Rhinovirus vaccine

A vaccine that provides life-long immunological protection is the 'holy grail' of preventative treatment for virus-induced asthma exacerbations. Given the predominance of RV in this disease an effective vaccine for this virus would have an enormous impact. We have successful vaccines for many important viral diseases – so why not RV? One principal barrier to vaccine development is viral diversity. RVs are a group within the *Enterovirus* genus that encompass 3 species (RV-A,

B and C) that cover 150+ genetically distinct subtypes based on capsid protein sequence [126]. RV capsids consist of 4 structural proteins (VP1-4) with VP1 being the largest and primary target for neutralizing antibodies [127]. RV capsid proteins exhibit substantial antigenic diversity and as a result antibody responses to RV are similarly diverse. In fact, species A and B can be divided into 100 immunologically distinct serotypes. Serological responses to the 51 RV-C viruses have not been characterized but a similar level of antigenic diversity is likely to exist for these viruses [128]. The existence of 100+ RV serotypes explains why humans remain susceptible to infection throughout life. Despite antigenic diversity naturally occurring, antibody cross reactivity has been detected in human serum. These antibody responses varied amongst individuals (presumably because the RV infection history of each subject is different) and protection against infection was not assessed [129]. Immunization with peptides derived from RV capsid proteins has also generated cross-reactive antibodies. However, cross-reactivity was limited such that no more than 50% of RV subtypes were neutralized [130]. Thus it is becoming increasingly clear that any completely protective vaccine against RV will need to incorporate neutralizing antibody epitopes from multiple RV subtypes across three viral species which is a major challenge for vaccine design.

Recently, an alternative to generating mucosal neutralizing antibodies has been considered for RV vaccine design: based on generation of protective T cells. The main attraction of exploring this approach is that RV precursor and non-structural protein sequences are more highly conserved [131] and are therefore more likely to generate broadly protective immunity reducing the complexity of vaccine formulation required for broad spectrum protection. We investigated this using an RV infection model [132] in which mice had been immunized with RV-A VP0 (VP4+VP2 precursor). We observed induction of cross-species (RV-A and RV-B) T cell responses. Enhanced production of species B virus-specific neutralizing antibodies following infection of VP0 immunized mice with RV-B virus was also observed [133]. Challenges for a T-cell-based RV vaccine development include the time required to mobilize an effective T cell response against RV and the potential for such a response to enhance lung inflammation and exacerbate asthma symptoms.

14. Expert commentary

Respiratory virus infections are usually confined to the upper respiratory tract where they cause a mild, self-limiting condition commonly referred to as a 'cold'. In susceptible individuals with chronic lower respiratory disease such as asthma infection can exacerbate symptoms requiring increased medication or professional medical intervention. It is now clear that the vast majority of asthma exacerbations are triggered by a viral infection the most common being human RV. This is particularly the case in children, where acute wheezing illnesses remain amongst the most common causes of hospitalization. In those aged six and under the combination of atopy and recurring episodes of wheeze with virus infections, in particular RV, are strongly associated with the development of asthma in later life. Bronchoconstriction and airway inflammation underpin disease in acute viral asthma and current preventive and therapeutic strategies consist of drugs that relax airway smooth muscle to reverse airway constriction and broad spectrum anti-inflammatory corticosteroids that reduce the impact of pathological immune mediators. For many asthmatics this combined approach controls their symptoms and reduces the impact of viral infections. However, there is a sub-population of asthmatics who tend to have more severe disease that is difficult to control with standard treatment. This group is susceptible to viral exacerbations and constitute the biggest burden in terms of morbidity, mortality, and health care costs associated with asthma. To address this need for new treatment options (summarized in Figure 1) much research has focused on the development of monoclonal antibodies (mAbs) that inhibit key immune mediators in asthma. This has been combined with research to define asthma immune phenotypes which has proven critical to the therapeutic efficacy of these new biologics. Anti-IgE (omalizumab, Xolair) is the most advanced and is now recommended for use as add-on therapy for persistent, moderate to severe atopic asthma. Xolair has been shown to reduce the incidence of asthma exacerbations; however, its specific effectiveness for viral asthma exacerbations is not known. MAbs against type-2 cytokines such as mepolizumab have completed Phase III clinical trials and have begun to be licensed for use. The airway epithelium is the site of respiratory virus infection and asthmatic airway epithelial cells can respond aberrantly to infection and produce inflammatory mediators that are potentially instrumental in the initiation of an asthma attack. Strategies being developed to address this include mAbs targeting type-2 promoting cytokines such as TSLP, IL-25, and IL-33 and adjuvanting innate epithelial anti-viral immunity with IFNβ, IFNλ, or TLR agonists. It is now recognized that RV infection is by far the most frequent cause of viral asthma, particularly in children. Directly targeting RVs with anti-virals or generating protective immunity with an anti-RV vaccine are other approaches being considered. Structural/antigenic diversity amongst approximately 150+ RV sub-types presents a substantial challenge for anti-viral drug- and vaccine-development.

15. Five-year view

Much has been achieved with current asthma therapies, in particular the use of moderate dose ICS or ICS/LABA combinations which are enough to improve asthma control and reduce the risk of exacerbations. The problem of acute exacerbations of asthma and in particular the role of virus infection in triggering these events remains an important unmet need. In the next five years, emergent monoclonal antibody therapies that target type-2 airway inflammation; such as those directed against IL-5 and potentially IL-4/IL-13 will continue to become available for clinical use, with the expected benefit of substantial reductions in exacerbation frequency in those patients not controlled with ICS/LABA alone. A greater understanding of the role of other emergent cytokines, in particular IL-25, IL-33, and TSLP in acute asthma and the existence of monoclonal antibodies against them is also likely to see them deployed in the setting of preventing asthma exacerbations and possibly

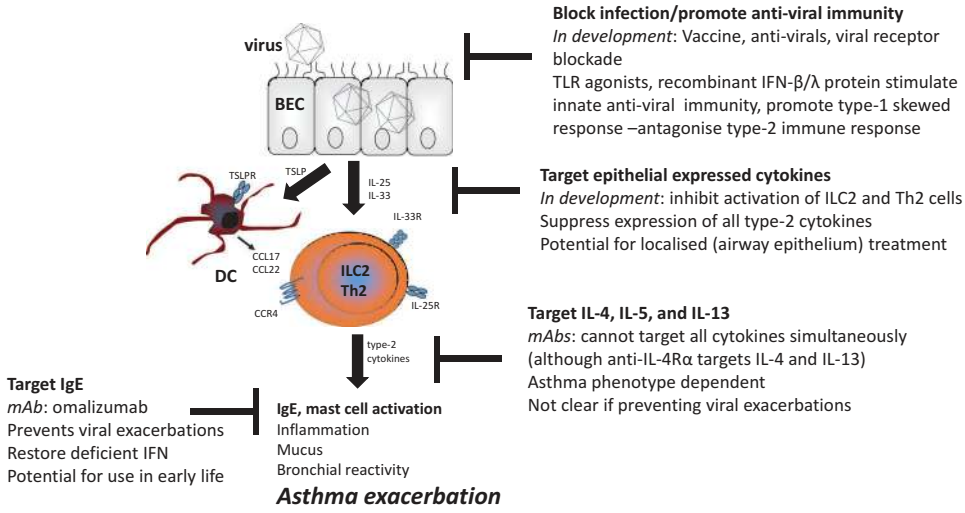

Figure 1. Advances in treatment of virus-induced asthma. Bronchial epithelial cells (BEC) are the site of virus infection in the lung. Preventing or reducing the level of infection using anti-viral approaches that either block infection or promote anti-viral immunity are attractive options but technically challenging. If infection proceeds this causes release of targetable cytokines (IL-25, IL-33 and TSLP) that can activate (either directly or via dendritic cells (DC)) type-2 innate lymphoid cells (ILC2) and Th2 cells which are the primary source of type-2 cytokines. It is the type-2 cytokines that stimulate pathological responses such as IgE production. Monoclonal antibodies (mAbs) targeting type-2 cytokines (mepolizumab, anti-IL-5) and IgE (omalizumab, anti-IgE) are now licenced for use in asthma.

treating them. In regard to specific treatments that target acute viral infection in asthma, the effectiveness of nebulized IFN-β as either an antiviral agent or immune modifier should be known. The application of other direct antiviral agents or vaccination strategies appears unlikely within this timeframe with perhaps the exception of vapendavir which is currently in Phase II clinical testing. What is unclear is whether any of these strategies will impact on the group during the first years of life who experience the most frequent episodes of virus-induced airways disease and in whom this may play a crucial role in the development of adult asthma. If clinical benefit can be demonstrated in young children, which in itself is a challenge due to difficulties in recruiting this age group for regular injections, Omalizumab may hold the most promise of modifying the development of asthma. This is because there exists such a strong association between atopy and virus-induced wheeze in the development of persistent asthma in later years.

16. Key issues

- Standard treatment (β adrenergic receptor agonists and inhaled corticosteroids) has evolved little in recent decades and exacerbations continue to cause a disproportionately large burden of disease.
- Asthma is now recognised as a heterogeneous disease encompassing a range of phenotypes driven by distinct immunopathological mechanisms; this is driving development of novel biologics and personalised treatment.
- Directly targeting viruses to prevent or treat exacerbations is technically very challenging due to viral structural/antigenic diversity; interferon therapy is broad spectrum and has potential to overcome the problems of structural/antigenic diversity.
- Airway epithelium is the site of viral infection and initiates the inflammatory cascade during an exacerbation. Targeting type-2 promoting cytokines at the airway epithelium offers real hope for the development of new treatments.
- Emerging therapies such as type-2 cytokine targeting-mAbs, anti-IgE and type I/III IFN show real promise for improving treatment of exacerbations of established asthma however their therapeutic efficacy in viral wheeze in pre-asthmatic young children is completely unknown.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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•• of considerable interest

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• **Glanville et al 2013. Immunized mice with RV VP0 (VP4+VP2 precursor capsid protein) and demonstrated production of cross-serotype cellular immune responses and neutralising antibody, evidence supporting further research into a RV vaccine.**