Viral infections and asthma-an inflammatory interface?

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Abstract

Asthma is a chronic inflammatory disease of the airways in which the majority of patients respond to treatment with corticosteroids and β_2 adrenoceptor agonists. Acute exacerbations of asthma substantially contribute to disease morbidity, mortality and health care costs, and are not restricted to patients who are noncompliant with their treatment regimens. Given that respiratory viral infections are the principal cause of asthma exacerbations, this review article will explore the relationship between viral infections and asthma, and will put forward hypotheses as to why virus induced exacerbations occur. Potential mechanisms that may explain why current therapeutics do not fully inhibit virus-induced exacerbations, for example β_2 adrenergic desensitisation and corticosteroid insensitivity, are explored, as well as which aspects of virus-induced inflammation are likely to be attenuated by current therapy.

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1. Introduction

Asthma is an intriguing disease that is perhaps best thought of as a series of overlapping aberrant biological processes which ultimately result in airway inflammation and characteristic airway physiology. Whilst inflammation is a hallmark feature of asthma, asthmatic inflammation is not homogeneous. This is exemplified in studies which have measured granulocytes in induced sputum. In such studies asthmatic pathology can be classified as eosinophilic, neutrophilic, mixed or paucigranulocytic (neither) [1]. Altered airways function is manifest in symptoms such as shortness of breath, cough, and wheeze and is characterised physiologically by airways hyperresponsiveness (AHR). AHR represents airways contraction in response to concentrations of agonists which are without effect in the non-asthmatic, together with reversible airflow limitation. Fortunately, even with the heterogeneity which exists in the various clinical and biological phenotypes of asthma,

corticosteroids and β_2 adrenoceptor agonists are clinically useful therapies for the majority of patients. However, even though symptoms can be well controlled for long periods of time by corticosteroids and β_2 agonists, viral respiratory tract infections can cause symptom relapse or exacerbations. The reason(s) why viruses cause exacerbations of asthma is not known- simplistically, viral infection could contribute to asthma symptoms and/or change airways physiology. The purpose of this review is to put forward hypotheses as to why virus induced exacerbations occur, and to discuss potential mechanisms that may explain why current therapeutics do not fully inhibit virus-induced exacerbations.

2. Asthma

Asthma is a chronic lung disease in which corticosteroids are used to control airway inflammation and β_2 agonists provide bronchodilatation. These are the most effective current treatments for asthma and, when taken concomitantly, their efficacy is generally increased. In the absence of any exacerbants (ie a stimulant which causes symptom control to relapse), optimal treatment reduces asthma symptoms, peak expiratory flow variability and indices of inflammation. Exacerbations are characterised by worsening asthma symptoms and a fall in lung function, and contribute substantially to the cost and burden of asthma. For example in New South Wales (a state of Australia with a population of around 7 million) there were 22,942 emergency department visits for asthma, of which 42% were admitted to hospital in 2007 [2]. The annual cost per person for hospital admissions can reach $\approx$ €15,000. In addition to the direct medical costs, the costs of absenteeism from work / school are thought to account for 50% of the total cost of treating asthma. Whilst the majority of asthma exacerbations occur in those who are non-compliant with medication usage, it is important to note that viruses can cause even the well controlled patient to exacerbate [3].

3. Viruses and Asthma

There is overwhelming evidence demonstrating the association of asthma exacerbations with viral infections in the community. Johnston and colleagues were amongst the first to show the extent and ramifications of viral infections in asthmatic school children using polymerase chain reaction (PCR) techniques in combination with other well established viral detection methods. In this seminal study, 108 children aged between 9 and 11 years old were monitored by peak flow measurements, and viral sampling was obtained during symptomatic episodes. Viruses were isolated in 77% of episodes, of which picornavirus was the predominant virus identified (147 episodes, 50%). Coronaviruses were the second most prevalent, being isolated in 38 episodes (13%), parainfluenza and influenza were isolated in 21 episodes (7%) each and respiratory syncytial virus (RSV) was isolated in 12 episodes (4%). Rhinovirus was found to be the predominant virus within the picornavirus group, as it was identified in 84 episodes, whilst relatively few had more than two different viruses detected (5%) [4]. In comparison to children, a similar proportion (80%) of adults has been shown to have a viral infection at the time of episodic asthma worsening [5], however others have found lower detection rates of virus in adults [6, 7]. Several reasons may account for this discrepancy. The time taken for adults to present with symptoms could be longer in comparison to that in children, virus replication may be reduced and/or greater clearance may occur in adults.

Whilst the association of viral infections and exacerbations of asthma is clearly defined, the role of viral infections in the aetiology of asthma itself is more controversial. Several studies have suggested a causal role, as outlined below. However, given the fact that both RSV and rhinovirus are particularly promiscuous in infants, infecting 80-90 % of all children by the time they reach two years of age [8, 9], a simple cause and effect hypothesis does not hold. Two long term follow up studies have both demonstrated sequelae from severe

RSV infection early in life (defined as requiring hospitalisation) at entry to adulthood: Korppi et al demonstrated deficits in lung function following RSV in the first two years of life, whilst Sigurs et al have shown increased rates of asthma (not found in the studies of Korppi et al) in a cohort requiring hospitalisation in the first six months of life [10]. Milder RSV infection up to the age of three in the Tucson birth cohort was associated with increased risk of wheeze up to 11 years of age [11]. The association between RSV infection and the subsequent development of asthma appears to change with age. A recent meta-analysis found that the attributable risk of developing asthma following RSV infection was 13% to 22% in children five and under, 11% to 27% in children aged 5 to 11, and was 32% in children twelve and older [12]. More recent data from the Childhood Origins of ASThma (COAST) cohort suggest a stronger association between rhinovirus and subsequent asthma risk. In this prospective study in a cohort of 259 children, rhinovirus infection was highly associated with the development of wheeze at three years and asthma at six years of age, (odds ratio approximately 10) [13, 14]. When virus -induced wheeze up to age three was correlated to the presence of asthma at six years of age, the odds ratio reported for rhinovirus was almost four times that of RSV (odds ratio 9.8 vs. 2.6), and reached 10 for both viruses taken together. This suggests a more important etiological role for rhinovirus than RSV, and is echoed by other studies describing the impact of rhinovirus in this early childhood setting [12, 15, 16]. Many confounding factors influence the validity of such results. The frequency of asthma in the community is greater in children than in adults but asthma can develop later in life. Therefore when studies are carried out examining effects of viral infection in infancy upon the development of asthma at static time-points, it is plausible that the estimations of causality are inaccurate.

The timing and frequency of viral infections may also be important. Infancy represents a rapid period of growth for both the immune system and lung development.

During this "susceptibility" period, viral infections may have their largest impact [17].

Infants experiencing viral seasons (e.g. RSV) during the first six months of life have a higher

prevalence of asthma [18, 19]. Animal studies have shown that early viral insults can affect

the immune system with long lasting effects on immune and pulmonary function [20, 21].

The importance of frequency of lower respiratory tract infection is suggested by data from

the German Multicentre Allergy Study, which followed 1314 children from birth to 13 years

of age [22]. Children who had more than one viral infection of the upper respiratory tract

(defined by a runny nose) during the first year of life had a decreased likelihood of

developing asthma at age 7, whilst in the same study, children who had two or more

(evidence more powerful for four or more) lower respiratory tract infections during the first 3

years of life were at a greater risk of developing asthma at age 7 [22].

The alternate explanation for such studies is that these early viral infections are

simply the first marker of an underlying predisposition to asthma, due to abnormal lung

function and/or genetic factors, rather than a key insult in the development of asthma [17].

Studies attempting to answer this question have produced conflicting results to date [23, 24],

although a recent analysis of two large separate birth cohorts (COAST study and Copenhagen

perspective study on asthma in childhood) identified a virus specific association between

genetic variation at the 17q21 locus, RV induced wheezing illness and childhood asthma

[25]. To date there is no clear evidence that there is a true increased susceptibility to infection

in these individuals. However, there are several other important factors that appear to modify

subsequent asthma risk in these young children. The German Multicentre Allergy Study

showed that it was the presence of early atopic sensitization in the first few years of life that

influenced subsequent asthma outcomes[26]. Kusel et al demonstrated in a smaller cohort of

almost 200 children, followed from birth to five years, that the presence of sensitisation early

on (at or before the age of two years) appeared to magnify the risk associated with viral infection of subsequent asthma [27].

The current view is that development of asthma in childhood is multifactorial, reflecting both genetic predisposition and multiple environmental exposures occurring at critical time points as the child develops. These include viral respiratory infections [28, 29], delayed immune system maturation [30], and allergic sensitization [27]. The temporal relationship between these factors is unclear. The genetic composition of an individual may also bias such experiments. A maternal history of asthma increases the risk of severe lower respiratory tract infection during the first year of life, independent of the risk of developing asthma. Recent data have shown that better maternal asthma control during pregnancy is associated with a significant decrease in the number of episodes of bronchiolitis occurring during infancy [31]. Similarly, a maternal history of bronchiolitis increases the risk of childhood lower respiratory tract infections [32]. In children who wheeze post bronchiolitis there is increased occurrence of genetic polymorphisms in the interleukin (IL)-8 gene, both in comparison to non-wheezers and the general population [33]. Attempts to investigate the effect of associations between virus infection and the host immune system on subsequent asthma development or exacerbations in those with pre-existing asthma, often focus upon a single viral type, and may possibly overlook the role of multiple concurrent infections. Given that the frequency of two concomitant respiratory viral infections is around 20-30%, [34, 35] it could be this interaction between the different infections and the host immune system which primes, and / or stimulates, the lungs to develop asthma.

4. The difference between poorly controlled asthma and asthma exacerbations

The most commonly used definition of an asthma exacerbation requires not an event of a particular character but the management of it – whether that be hospitalisation, emergency room presentation or a course of oral corticosteroids. Such an exacerbation can be an extension of the pattern of disease in ongoing poor control or an independent event. It is clear that some patients have excellent current control of asthma with minimal or no symptoms and yet have sudden and severe exacerbations. Viral infections have been implicated in such events. At the other extreme, some patients have unrecognised or ineffectively managed asthma and have extreme variability in symptoms and lung function. In these cases, exacerbations may be recorded not necessarily for the deepest fluctuations in lung function, or for specific patterns of asthma worsening, but for those occasions where medical advice was sought and treatment given.

One particularly challenging aspect of asthma exacerbations is the differentiation between inadequate treatment regimens leading to episodic symptomatic asthma and catastrophic failures in asthma control in response to various stimuli i.e. an exacerbation.

It can be argued that when medication regimens are not titrated according to objective features of asthma, insufficient treatment is delivered. Insufficient treatment may provide some benefits to the asthmatic patient in terms of reduced symptoms, however, as inflammation and airway hyperresponsiveness are likely to not be fully controlled, this may allow exacerbations to occur. The difference between poorly controlled asthma and asthma exacerbations is further compounded by the diversity of symptom severity or physiological measurements which are used by researchers to describe an exacerbation, and the fact that patients are often noncompliant with therapeutic regimens. However, in the general

community, asthma treatment guidelines and action plans do provide optimal treatment for asthmatic patients, and in clinical trials, in which medication usage is monitored and adjusted according to symptoms, exacerbations still occur.

5. Can current therapeutic regimens prevent virus induced exacerbations?

There is no doubt that the reductions observed in asthma morbidity and mortality observed over the last 20 years are the result of better therapeutic management, however the real question is are asthma therapeutics effective in virus-induced exacerbations? Large studies have shown that even low dose inhaled corticosteroids (ICS) reduce exacerbations and the risk of death from asthma [36]. For example, in comparison to no treatment, 100ug budesonide twice daily resulted in a 60% reduced risk of having a severe exacerbation in the OPTIMA trial [37]. The addition of long-acting β_2-agonist (LABA) to ICS further reduces the frequency [38] severity and duration [39] of exacerbations. Studies such as these were not designed to identify the cause of the exacerbation, however as viruses are thought to cause at least 50% of all exacerbations, it is reasonable to assume that some reduction in the incidence of virus-induced exacerbations would occur. None of these studies specifically identifies whether improved asthma management does one or more of the following

1. Reduces the rate of respiratory viral illnesses
2. Reduces the rate at which respiratory viral infections trigger a sequence of inflammatory events that will result in an exacerbation
3. Reduces the severity of symptoms or lung function such that the episode does not require exacerbation-defining medical intervention

In a prospective multicenter study of 413 asthmatics, Walter et al aimed to determine factors which would predict loss of asthma control following a cold. In their study there was

no association between the use of ICS with or without β_2-agonists and loss of asthma control [40]. The severity or number of previous colds was also not associated with loss of asthma control, but the severity of the first two days of the current cold could be used to predict loss of asthma control. If we were to assume that all viruses are equal, i.e. have the same pathogenicity, it would be likely that other co-factors would precipitate the exacerbation, however not all viruses are equal. As shown by Wark et al, even serotypes of the same virus can have dramatically different innate immune responses *in-vitro* [41]. There is emerging evidence suggesting that the recently described group of rhinoviruses, rhinovirus C, appears to cause more severe infections *in-vivo*, at least in children [42, 43]. This may not be the case for adults [44]. This suggests that the gene-environment hypothesis (where the role of any given gene is determined by the environment) can now be extended to include the gene virus hypothesis, where the interaction of the specific virus and the host immune response will dictate whether an exacerbation occurs or not.

6. Are corticosteroids effective or ineffective during virus-related infections?

Corticosteroids are known to improve asthma symptoms and decrease exacerbations, however virus-induced exacerbations can occur in ICS-treated asthma in patients who are well controlled [3]. Clearly, a component of specific virus-induced inflammation is not controlled by stable ICS therapy. Whether ICS have no effect on virus-induced asthma deterioration is much less clear. In patients using budesonide/formoterol maintenance and either terbutaline, formoterol or formoterol/budesonide as reliever, the exacerbation rate after onset of reported 'cold' symptoms was reduced by as needed budesonide/formoterol compared to as-needed formoterol reliever [45]. Even in that setting, there is potential confounding by an effect of the additional as needed ICS on residual eosinophilic airway inflammation rather than viral mechanisms.

In-vitro, we and others have found that steroids inhibit rhinovirus [46-48] and respiratory syncytial virus induced cytokine release, supporting the notion that the use of steroids *in-vivo* would suppress virus-induced inflammation. Interestingly, data from *in-vitro* studies have suggested that the timing of administration of steroids has profound effects upon their ability to inhibit virus-induced cytokines. For example, in bronchial epithelial cells ,rhinovirus-induced CXCL-10 was inhibited by budesonide when the drug was given at the start of the infection period [49], but not when applied 24 hours prior to infection [50]. Quite how these *in-vitro* results translate into clinical practice is uncertain. It is clear that patients with asthma who have poor adherence to medication regimens (for a review see [51]) have worse asthma related outcomes, but this is perhaps an extreme example of incorrect medication usage.

Recent *in-vitro* studies have revealed a molecular mechanism of corticosteroid resistance in rhinovirus infected cells [52]. Corticosteroid resistance is often misinterpreted as a complete lack of response to corticosteroids, rather than the correct meaning of reduced efficacy of the corticosteroids. So to achieve a desired level of inhibition in the context of corticosteroids resistance, it is necessary to increase the dose, bearing in mind that maximal effect might never be achieved. With traditional fixed dose treatment regimens, inflammatory states that cause corticosteroid resistance are of particular concern, because if the dose of steroid remains constant, this may be insufficient to control inflammation. In theory, this could be overcome by increasing the dose of steroid as part of an asthma management plan, yet clinical evidence is lacking to support this approach in either children or adults [53]. An age–related effect may also exist, and may reflect the underlying nature of the inflammation. In pre-schoolers with viral-induced wheeze exacerbations, no beneficial effect of routine oral corticosteroid use has been shown, either parent-initiated [54] or physician-initiated once in the emergency department [55]. High dose ICS (750 microgram of

fluticasone propionate twice daily) from the start of a viral illness has, however, been shown to reduce the subsequent need for OCS, but attractiveness of this approach was tempered by a detrimental effect on growth [56]. In older children the beneficial effect of OCS in these settings [57, 58] may in part reflect a greater role for non-viral inflammatory aetiologies (e.g. eosinophilic due to allergic triggers). As required (prn) use of combination inhalers containing a fast onset long acting bronchodilator and steroid are now beginning to used in clinical practice to treat adults with asthma, replacing the combination of short acting bronchodilators and a separate corticosteroid based inhaler, however there is not enough evidence to suggest this is beneficial in comparison to traditional stepwise up-titration of steroid dose [59].

Corticosteroid resistance may occur in the context of viral infections, and may contribute to the occurrence of an exacerbation. In most clinical trials measuring the efficacy of steroids ± bronchodilators, the general observation is that asthma control is increased by the actions of steroids, and is further increased by the addition of bronchodilators. However, even in these carefully carried out and monitored clinical trials, exacerbations still occur. The best example of this phenomenon is the study by Reddel et al [3] previously described. In that study there is clear evidence that despite asthma being well controlled by steroids, exacerbations occur in the context of colds. If we now focus upon experimental infection models in human asthma, no reduction in rhinovirus-induced inflammation has been observed when using a fixed dose of inhaled steroid [60, 61]. Furthermore, even oral prednisone has been shown to be ineffective in controlling rhinovirus-induced asthma symptoms [62], and moreover to increase viral titres [63]

. When the dose of ICS used in asthma is considered, there is no benefit from doubling ICS dose for treatment of exacerbations [64, 65], and this approach is now considered insufficient for the management of asthma [66]. A recent study by Oborne *et al* extended this

finding and evaluated the efficacy of quadrupling the usual dose of ICS at the first symptoms of a cold however this approach did not reduce exacerbations in comparison to placebo [67].

Taken together, these studies suggest that corticosteroids should provide some immunosuppression in the context of respiratory viral infections, but it is likely that they do not fully inhibit virus-induced inflammation or symptoms. They therefore fail to stop the occurrence of virus-induced exacerbations or reduce their severity below the threshold where medical attention is sought and treatment given.

7. Do respiratory viral infections reduce β_2-agonist efficacy?

Reddel et al provided the first objective evidence for loss of β_2-agonist efficacy in asthmatics with a cold [3]. In their study, peak expiratory flow was monitored in asthmatics both prior to and post treatment with the ICS budesonide. Prior to ICS treatment, peak expiratory flow had characteristically high diurnal variation, and, following ICS treatment, good asthma control was reflected by increased peak expiratory flow and decreased variability. In the study volunteers, even though good asthma control had been achieved, 39 exacerbations associated with clinical colds occurred. In these, peak expiratory flow declined and recovered over 7-14 days. Importantly post-β_2-agonist and evening peak flow values were no higher than pre-β_2 agonist and morning values, implying impaired response to β_2-agonist. During these exacerbations, diurnal variability was not significantly different from that observed during good asthma control. This study provides evidence that viral exacerbations have different pathophysiological mechanisms in comparison to untreated or poorly-controlled asthma. One of the proposed mechanisms by which virus infections limit the action of β_2-agonist is via the production of mucus [68]. Whilst it is possible that increased mucus in the airway lumen following virus infection could impair β_2-agonist efficacy by reducing the amount of drug reaching the smooth muscle, β_2-agonists have clinical benefit in respiratory diseases with

abnormal mucus production such as cystic fibrosis [69, 70] and COPD [71]. It is therefore

likely that viral infection of the airway may affect the inherent ability of the airway smooth

muscle to respond to β_2-agonists. A second proposed mechanism to explain the lack of

response to β_2-agonist which occurs during viral infections is the development of tolerance.

The development of tolerance to β_2-agonists can be experimentally demonstrated both *in-vivo*

[72], and *in-vitro [73]*, however in the Reddel study above, β_2-agonist use was lower during

viral exacerbations than during the initial period of poor asthma control, and, as such,

tolerance is unlikely to account for the changes. A third potential mechanism to explain the

reduced efficacy of β_2-agonists during viral infections is the possibility that obstruction

occurs as a direct result of inflammation.   Inflammation is accompanied by increased

microvascular leakage and tissue oedema. Oedema would act to thicken the airway wall,

therefore decreasing lumen size. Increased vascular permeability occurs as asthma control

deteriorates, [74], however oedema is not related to changes in airway hyperresponsiveness

during allergen challenges [75]. This finding is supported by studies in animals which have

found that oedema causes only modest airway narrowing [76]. The exact contribution of

odema to airway obstruction during virus induced exacerbations is not known, but it is

perhaps likely not to be the primary cause of reduced airflow. In an attempt to understand the

mechanisms responsible for the decreased β_2-agonist efficacy observed during viral

infections, we have established an *in-vitro* model [77] to test the hypothesis that virus

infection impairs β_2AR activation. Since it is now accepted that RV reaches, infects and

replicates in lower airway epithelium [78], our model uses a co-culture system in which

epithelial cells are infected with rhinovirus (RV) and conditioned medium is used to treat

airway smooth muscle cells prior to assessing β_2 adrenoceptor function. We chose to use

rhinovirus as the model virus, since it is responsible for at least half of all virus-induced

asthma exacerbations. In our study, we observed that β_2-agonist induced cAMP was reduced

in airway smooth muscle cells that had been treated with conditioned medium from virally infected epithelial cells. We propose that this response is specific to replicating rhinovirus, since no reduction in β_2-agonist induced cAMP occurred in response to conditioned medium from epithelial cells treated with UV inactivated virus, poly I:C (a surrogate for other viruses) or the bacterial endotoxin lipopolysaccharide. Using flow cytometery we were able to assess membrane and total β_2 adrenoceptor levels. Following exposure to rhinovirus conditioned medium, airway smooth muscle cell surface, but not total, β_2 adrenoceptor number was reduced, suggesting that desensitisation of the β_2 adrenoceptor had occurred. In recent studies we have identified the mechanism by which desensitisation occurs [79]. Rhinovirus replication in epithelial cells is not perfect, and in addition to progeny, viral particles and unpackaged viral RNA are also released from infected cells [80, 81]. We took the cell culture supernatant from rhinovirus-infected primary bronchial epithelial cells and were able to detect viral RNA. We purified the viral RNA, and, as a control, used human mRNA, and used both to stimulate airway smooth muscle cells. We found that only viral RNA stimulated prostaglandin release from airway smooth muscle cells. As depicted in figure 1, the prostaglandins acted through an autocrine mechanism to cause β_2 adrenoceptor desensitisation . Reduced total cellular expression of the β_2 adrenoceptor has also been shown to occur in-vitro when airway smooth muscle cells are directly infected with respiratory syncytial virus [82]. This study is interesting but caution has to be observed when examining the direct effect of viral infection upon a cell. Viruses subvert the host machinery in order to manufacture progeny viruses, and, in turn, the cell attempts to apoptose to limit viral spread. As such it would be important to show specific down regulation of a given receptor, rather than a global downregulation prior to apoptosis.

8.   Leukotriene receptor antagonists

For many years leukotrienes have been suggested to be important in the pathology of virus-induced exacerbations, however there is a marked paucity of *in-vivo* and *in-vitro* studies exploring this hypothesis. Respiratory tract viral infection upregulates 5-lipoxygenase in the bronchial mucosa [83], an enzyme needed in the cellular production of leukotrienes. Elevated leukotriene C4 is found in nasal lavage fluid post experimental infection with either rhinovirus, or respiratory syncytial virus, or influenza A virus [84], thus providing some evidence for the potential involvement of leukotrienes in virus-induced exacerbations. Targeted therapy such as the leukotriene receptor antagonist monteleukast, when used as monotherapy, has been shown to be only modestly effective during respiratory tract infection induced wheeze [85], but it is important to note that, in the same study, monteleukast had similar efficacy to ICS. Furthermore, in a randomised double blinded placebo controlled trial, the addition of monteleukast to usual therapy resulted in a 53% reduction in days with worse asthma symptoms compared with placebo and a 78% reduction in unscheduled physician visits for asthma [86]. However the exact nature of the involvement of leukotrienes in the pathogenesis of rhinovirus and other respiratory viral infections is controversial. For example, in an experimental rhinovirus infection model, monteleukast had no effect upon rhinovirus-induced colds or asthma symptoms [87]. Monteleukast has also been found not to affect respiratory symptoms post RSV-bronchiolitis [88, 89], or the incidence of upper respiratory tract infections [90].

9.   Clues from virus-induced inflammation

The immune response to naturally acquired respiratory viral infections is complicated due to the heterogeneity of responses observed between different virus genotypes and serotypes, the effects of concurrent environmental stimuli, and pharmacotherapy. One approach to

overcome such confounders is to experimentally infect volunteers. Experimental rhinovirus infections can induce asthma exacerbations [91], and reduce the peak expiratory flow in both asthmatic [92] and non-asthmatic [93] subjects.

Airway eosinophilia is a characteristic of asthma, and many reports exist showing the relationship between eosinophil activation and recruitment in rhinovirus infected asthmatic volunteers. Given the correlation between increased airway hyper-responsiveness and eosinophil recruitment and activation, for example as assessed by eosinophil cationic protein levels [94], it is tempting to speculate causality. However an increase in eosinophil numbers [94, 95]has not been found in all human experimental rhinovirus infection studies Mouse models of rhinovirus exacerbations against a background of OVA-induced allergic airways disease exhibit increased airways hyperresponsiveness accompanied by increased eosinophil numbers [96]. Interestingly, in such models, the development of airways hyperresponsiveness is inhibited by the administration of anti-eotaxin-1 [97]. However in the context of the murine model of house-dust-mite induced allergic airways disease, rhinovirus infection does not induce eosinophilia [98]. The contrasting results from the two mouse models perhaps tell us more about the utility of the models [99], than the putative role of the eosinophil. In animal models of RSV and Sendai (parainfluenza) infection, eosinophils have been found to be important in viral clearance [100] [101] raising the possibility that eosinophilic inflammation is beneficial under certain circumstances.

The role of the eosinophil in asthma is complex. For example, in people with severe asthma treated with corticosteroids, the presence of eosinophilia is associated with more frequent exacerbations [102]. In such populations treatment with mepolizumab (anti-IL-5) reduces both the number of eosinophils and exacerbations[103] . In steroid responsive asthma, airway eosinophilia is reduced by ICS, and, it is this fact that has enabled eosinophilia to be used as a biomarker to assess optimal ICS treatment [104]

It is our view that virus-induced eosinophilia is unlikely to account for the failure of asthmatic treatment regimens during virus-induced exacerbations.. In contrast, the role of the neutrophil in both the aetiology and pathogenesis of asthma is unclear. Rhinovirus infection increases circulating and bronchial lavage neutrophils in asthmatic volunteers [105], and both neutrophil number and the neutrophil chemokine IL-8 negatively correlate with airway function [106, 107] in experimentally infected volunteers. The relationship between corticosteroid use/efficacy and neutrophilic inflammation is potentially confounded as corticosteroids reduce airway eosinophilia (and therefore increase the proportion of neutrophils) and inhibit neutrophil apoptosis. However in a study of 205 patients, multivariate linear regression has shown no association of airway neutrophilia with corticosteroid use [108], and airway neutrophilia occurs in asthmatic patients who are corticosteroid naïve [109]. In the absence of acute infection, the presence of airway neutrophilia is associated with steroid insensitive asthma [109], therefore virus-induced neutrophilia could represent the steroid insensitive component of virus induced exacerbations. As such, virus-induced neutrophilia deserves further consideration.

The exact role of neutrophils during virus infections of the lung and the ensuing asthma exacerbation is not known, largely because existing therapies for asthma do not directly target neutrophils. PDE4 inhibitors (PDE4i) as used in COPD, and a newer experimental class of therapy CXCR2 (CXCL8 receptor) antagonists, offer some insights. PDE4i are a relatively new class of anti-inflammatory medication and their main mechanism of action is to suppress lung neutrophilia in-vivo (for a review see [110]). In COPD, where 50% of exacerbations are caused by rhinovirus, PDE4i reduced exacerbation frequency [111], suggesting neutrophilia is an important component of exacerbations. PDE4i were developed as a refinement to theophylline, a pan PDE inhibitor, and like theophylline have anti-inflammatory properties. In contrast to theophylline, PDE4i such as roflumilast, do not have

direct bronchodilator activity [112], perhaps because direct bronchodilation is mediated through other PDEs such as PDE3 [113], however the role of other PDEs in direct bronchodilation needs to be verified. We and others have shown that in-vitro PDE4 is the main PDE which degrades β2-agonist-induced cAMP, and furthermore we found the activity of PDE4 to be increased in airway smooth muscle from people with asthma [114]. Whether increased PDE4 in asthma equates to an increased susceptibility to β_2-adrenoceptor desensitisation remains to be determined. Preclinical models have found that bronchodilation induced by low dose salbutamol is enhanced in the presence of PDE4 inhibitors [115], raising the possibility that the reduction in exacerbation frequency observed in patients with COPD treated with roflumilast may in part be mediated by increased or sustained efficacy of β_2-agonists. CXCR2 antagonists are still in development, but a recent publication has shown that one of them-SCH527123 is safe, and importantly reduces exacerbations of asthma [116]. It is likely that these therapies work via suppression of the accumulation of neutrophils, presumably showing that the existing lung neutrophils are sufficient to combat the infection.

We [117] and others have shown that rhinovirus infection induces lung neutrophilia in murine models, but there are no studies which specifically examine the role of neutrophils in the context of an asthma exacerbation. Nagarkar *et al* used a series of knockout mice and elegantly demonstrated that neutrophil derived TNF-α is critical for the development of airways hyper-reactivity [118]. They did not investigate which cells were responsible for neutrophil chemokine production or if inhibition of neutrophil influx affected either the number of infectious virions or the clearance of rhinovirus.

Conventionally, exhaled nitric oxide is thought to reflect airway eosinophilia and is advocated as a biomarker to assess asthma control [119]. However new evidence regarding

the role of nitric oxide in the airways is emerging and it is too simplistic to consider it as a simple marker of inflammation. Exhaled nitric oxide is increased in people without asthma with naturally acquired upper respiratory tract infections [120], and is also elevated following experimental rhinovirus infection [121]. *In-vitro,* nitric oxide inhibits rhinovirus replication and cytokine production [120], and the replication of influenza [122] and respiratory syncytial virus[123], suggesting that it is likely to be important in virus-induced exacerbations. Importantly, in asthmatic volunteers who were experimentally infected with rhinovirus, the production of nitric oxide was negatively correlated with airway hyperresponsiveness to histamine [124], ie the production of nitric oxide was protective. Nitric oxide may represent a paradox in the context of viral infections and treatment with ICS. ICS are well known to inhibit exhaled nitric oxide, however if nitric oxide limits virus replication, and, therefore presumably virus-induced inflammation, reduced nitric oxide by ICS would be detrimental. Further research is needed to elucidate the role of virus-induced nitric oxide *in-vivo.*

10.   The host response to virus infection

The host response to virus infection is complex, and dependent upon a number of variables such as the type and amount of virus, the immunocompetency of the host, and underlying disease pathology.   Given this complexity, and given the importance of rhinovirus infection as a precipitant of asthma exacerbations, the following section discussing the host's response to viral infection is mainly focused upon the response to rhinovirus.

Rhinoviruses are relatively simple viruses. They have a single strand of positive sense RNA which is packaged into an iscohedral capsid, formed by 3 different viral proteins, whilst a fourth viral protein is found on the interior of the capsid and is in contact with the

viral RNA [125]. They are classified according to molecular traits, being defined as species A, B or C. Species A mainly consist of viruses that infect cells via low density lipoprotein (LDL) receptor family, species B via ICAM-1, whilst the receptor for species C is unknown.

The initial innate immune response to rhinovirus infection is considered to occur once the virus RNA has been delivered into the cytoplasm, as part of the infection process, and is recognised by pattern recognition receptors. However, several studies using UV-irradiated virus, which is incapable of replication, have suggested that binding to the receptor alone elicits similar responses to replication competent (live) rhinovirus [126-130]. Interestingly, UV-irradiated rhinovirus has been found to be inert in a number of similar studies [131-134]. There are several potential explanations for the observed differences, and the most likely in our opinion relates to differences in the amount of UV-irradiation. When UV-irradiation is insufficient, virus replication can occur, and when too much UV-irradiation is used, the viral capsid is either partially or totally destroyed, making any interaction with cellular receptors very unlikely.

Rhinovirus is a single stranded RNA virus, and during replication, a double stranded intermediate is formed, which is detected by different intracellular receptor families. Single stranded viral RNA is recognised by both Toll-like receptor (TLR) 7 and 8, whilst double stranded RNA is detected by TLR-3 and the RNA helicases, retinoic acid inducible gene (RIG-I) and melanoma differentiation associated gene-5. Whilst opposing conclusions regarding the importance of the two recognition systems have been found in different studies, it is likely that both receptor systems are important in eliciting the host's response to rhinovirus infection [135, 136].

Regardless of the exact cellular process used to detect a virus, the net result of detection is activation of the innate anti-viral immune response, characterised by the robust

induction of a plethora of pro-inflammatory cytokines, chemokines and anti-viral mediators. This increased inflammation forms the basis of the prevailing hypothesis as to why viral infections cause asthma exacerbations. However, the million-dollar question is why does this increase in inflammation cause some people with asthma to experience an exacerbation, and others not to? One theory that has been put forward is exacerbations occur in those with dysfunctional innate immunity.

No discussion on virus-induced inflammation would be complete without reference to the potential deficits in innate immunity in asthma. In 2005, the first of a series of studies from the UK demonstrating impaired rhinovirus-induced type I interferons in asthmatic epithelial cells *in-vitro* was published [134]. This was followed by a similar study showing deficient rhinovirus-induced type III interferons [137]. As depicted in figure 2, the importance of these findings lies in the fact the type I and III interferons induce apoptosis and therefore limit virus replication. These publications sparked a flurry of research in the area, often with mixed results. Lopez-Souza et al used a more complex *in-vitro* model with differentiated epithelial cells [138]. In their study no differences in the production of rhinovirus induced type I interferon was found. There is no obvious explanation for the discrepancy, and it is likely that differentiated epithelial cells produce different amounts of cytokines in comparison to undifferentiated cells making the differences in the outcomes of the two studies difficult to interpret. However, other researchers have also not found differences in type I and III interferons between asthma and non-asthma in response to rhinovirus infection both in-vitro [139] and in-vivo [140]. Similarly no difference in interferon production has been observed in response to infection with either respiratory syncytial virus or human metapneumovirus [141]. Interestingly, the group which initially reported impaired interferon production in response to rhinovirus infection in asthmatic epithelial cells has recently reported no differences in interferon production [142]. In their

discussion of the potential reasons for the discrepant results, differences in underlying disease severity and or degree of airways inflammation are suggested as potential confounders. The weight of evidence suggests that deficient interferon production from asthmatic bronchial cells as a general concept is unlikely to occur, however it remains to be seen if it is a feature of specific asthma phenotypes.

Irrespective of whether asthmatic cells release fewer type I or III interferons or not, the important question is do ICS affect the production of type I or III interferons? In-vitro, in our laboratory we have found that the corticosteroids dexamethasone and fluticasone do not affect rhinovirus replication in primary lung cells [47], and there is good evidence to suggest that corticosteroids do not affect virus-induced type I interferons [134].

11.   The role of secondary or co-infection in virus induced exacerbations.

Other pathogens have co-evolved with viruses and are able to utilize the window of opportunity created by viral infection and superinfect the host [143]. For example, it is well known that the rate of bacterial infection increases during episodic viral pandemics [144]. One mechanism by which superinfection occurs is by increased binding and retention of bacteria to the respiratory epithelium [145-148]. Furthermore, we have also shown that the innate immune response to bacteria is markedly impaired in virally infected alveolar macrophages [149] . In comparison to other diseases and pathogens, the role of bacterial infections in asthma exacerbations is controversial. However asthmatics have increased susceptibility to invasive bacterial infection [150], and atypical bacterial infection has been reported to be reactivated in virus induced asthma exacerbations [151] and related to exacerbation frequency [152]. There is also evidence that macrolide antibiotics, when used prophylactically[153, 154], or after the occurrence of an acute exacerbation[155] are effective

treatments for asthma If bacteria are co-conspirators in virus-induced exacerbations, it is highly likely that the exacerbation would not respond to ICS and β_2-agonists, as these drugs would not inhibit bacterial growth. Further research is needed in this area to fully describe the role of bacteria in virus induced exacerbations.

12.   A role for allergens in rhinovirus induced exacerbations of asthma

Whilst there is little doubt that both exposure to allergens and viral infections can induce asthma exacerbations, there is surprisingly little information regarding their interaction. It has proven to be very difficult to document personal allergen exposure leading to an exacerbation of asthma except in rare events such as thunderstorms [156][157], and perhaps this is the reason why very few reports document the interaction between naturally acquired viral infections and allergen exposure in asthma exacerbations. Green and others (2002) provide evidence for synergism between virus infections and allergen exposure [158]. In their case controlled study, the risk of being admitted to hospital was considerably increased by exposure to high levels of antigen and concurrent viral infection. This finding is supported by other studies that have measured the amount of IgE, and often specific IgE, to establish if any relationships exist between IgE levels and the likelihood of a virus induced exacerbation. The first of this series of studies found the odds ratio for virus-induced wheeze in children over two attending the emergency department was 4.4 if a rhinovirus was detected. The concomitant presence of specific IgE increased the odds ratio to 17, and was higher if nasal eosinophilia or elevated nasal ECP was present (odds ratios of 21 and 25 respectively) [159]. This relationship may be specific to rhinovirus and not other viruses [160], and furthermore other studies indicate that rhinovirus C may be an important precipitant of exacerbations under such circumstances [161]. Systematic reviews have shown anti-IgE (Omalizumab) to reduce exacerbations in children and adults with asthma [162], and in an elegant study by

Busse et al, omalizumab [163] was shown to reduce exacerbations in spring and autumn further providing evidence for the interaction between IgE and virus-induced asthma exacerbations. Such studies provided the basis for experimental studies in humans. The sequence of events is likely to affect the experimental outcomes, however regardless of the sequence of exposure to allergen and viral infection, both stimuli have been shown to affect the subsequent response to the other. Experimental rhinovirus infection increases eosinophil recruitment into the lower airways, and the occurrence of a late phase response to allergen [164, 165]. Antigen exposure followed by experimental rhinoviral infection one month later also induced a late phase asthmatic response [166]. Whilst changes can be observed in lung function parameters, in contrast, modulation of the immune response in terms of cytokine and chemokine induction is not altered by antigen exposure prior to rhinovirus infection. This is based on the fact that nasal IL-6 and IL-8 levels were not altered by antigen or placebo exposure prior to rhinovirus infection [167]. Similarly, lower airway IL-8 production was not further increased by prior repetitive exposure to low dose house dust mite allergen [92].

13.   How could we stop virus-induced exacerbations

As described above and shown in figure 1, virus-induced asthma exacerbations are likely to involve multiple mechanisms, and, as such, a single treatment modality is unlikely to be effective in all people with asthma. In order to completely stop virus induced exacerbations from occurring, good asthma management would have to be implemented prior to a second prophylactic treatment strategy. Due to the cost and potential side effects of prophylactic treatment, it would be necessary to identify those who are at risk of a virus-induced exacerbation on a background of good asthma control, perhaps by close monitoring of their asthma or through the use of biomarkers. One such potential biomarker is IFN-gamma-induced protein 10 (IP-10). Wark et al have shown that virus-induced exacerbations are associated with approximately 4 fold higher serum IP-10 in comparison to non viral

exacerbations [168]. An alternative strategy would be to predict when virus induced exacerbations are likely to occur, for example at times of the year when children return to school, so prophylactic treatment could be implemented seasonally. Of course, the ideal approach would be to eradicate respiratory viruses, however, realistically, this approach is not feasible. It is possible to immunize against both RSV [169] and influenza [170] but these are likely to provide "immunity" against only certain serotypes of virus, and will not actually stop infection from occurring. Whilst it is not possible to vaccinate against rhinovirus, this approach is being currently evaluated [171]. Anti-rhinovirals are being developed [172] [173], and are available for both RSV and influenza. The efficacy of such antiviral strategies in terms of preventing asthma exacerbations is not known

14.   Perspective

In this century, our understanding of the relationship between virus infections and asthma has greatly expanded but is incomplete. This must be greatly expanded by studies in volunteers using bronchoscopic biopsies and biomarkers[106]. Future studies should evaluate the role of different viruses and virus serotypes of the same virus and how effects are modulated by pre-existing asthma phenotype. Understanding basic mechanisms is necessary so that, armed with this knowledge, we can be aware of which aspects of infection are sensitive and resistant to current treatments. From that position, we can evaluate the effect of novel strategies and pharmacotherapies on virus-induced asthma exacerbations.

Figure 1. Rhinovirus-induced β_2-adrenoceptor desensitisation. A) Inhibition of COX restores β_2-adrenoceptor function in airway smooth muscle cells treated with conditioned medium from rhinovirus infected epithelial cells (data redrawn from 84). B) A cartoon of the proposed mechanism of β_2-adrenoceptor desensitisation: 1 – Rhinovirus infects epithelial cells and replicates releasing progeny virions and (2) viral RNA. The viral RNA is detected by airway smooth muscle cells (3) and stimulates the production of prostaglandins (4). These prostaglandins act in an autocrine manor (5) to cause β_2-adrenoceptor desensitisation (6)

Figure 2. A cartoon illustrating the role of interferons in limiting virus infection.

Figure 3. A cartoon depicting the consequences of viral infection, and an indication as to whether these are likely to respond to current therapy.

15.   References

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