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Mechanisms of asthma exacerbation

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Introduction

Asthma exacerbations are the major cause of morbidity and mortality in asthma. Exacerbations are responsible for enormous losses in terms of time off school and work, and enormous costs in terms of medical consultations and hospital admissions. Identifying the causes and mechanisms of asthma exacerbations is therefore a major goal in asthma research, as identifying important mechanisms may lead to development of new therapeutic options in the future.

Epidemiology of exacerbations of asthma

Recent evidence suggests that respiratory virus infection precipitates acute exacerbations of asthma in all age groups. Episodes of acute asthma in schoolchildren were associated with a respiratory virus infection in 80–85% of cases and of viruses detected, 50% were rhinoviruses (RV) [1]. In adult asthma 44% of asthma exacerbations were associated with virus infections – again the majority were rhinoviruses [2]. In addition to these two studies which were both carried out in the community, time-series analysis was used to investigate more severe asthma exacerbations leading to hospital admissions. Virus infection was again strongly implicated in all age groups [3]. Further studies using sensitive methods of virus detection (PCR) for all the common respiratory virus types are underway in adults both in the community and admitted to hospital – preliminary results suggest that virus infection is associated with the majority of asthma exacerbations in both settings.

Studies in children and infants admitted to hospital with wheezing illness have produced similar results, with respiratory syncytial (RS) virus and RV together accounting for 96% of hospital admissions in infants, and RV for 80% of admissions in children aged > 1 year [4]. Finally the role of virus infection in asthma mortality has been studied, and evidence has been found implicating virus infection in the majority of asthma deaths, most notably in the very young and the old [5].

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Virus infections are the major cause of asthma exacerbations in all age groups and are therefore the major cause of morbidity and mortality in asthma. Asthma now affects 20–33% of the population in the UK [6]; the associated health costs in terms of time off school/work, GP consultations, hospital admissions and death are therefore enormous. Furthermore, since frequent episodes of virus-induced lower airways inflammation are likely to lead to enhanced airway wall remodelling and fibrosis, repeated virus-induced asthma exacerbations are likely to be an important contributing factor to the development of irreversible airways narrowing as observed in chronic severe asthma.

Although virus infections are clearly associated with the majority of asthma exacerbations in all age groups, some exacerbations have other causes. Peaks of allergen exposure can clearly cause exacerbations if the levels of allergen are high enough, as in the cat-allergic subject entering a home with cats, and in the well-publicized epidemics associated with soya bean exposure, or thunderstorm asthma. However, in most exacerbations where no virus is identified, the cause of the exacerbation is not clear. Some clues may have emerged from our recent studies on hospital admissions and asthma mortality. In the mortality study we observed a summer peak in asthma mortality in the 5–44 years age group that was unexplained [5]. We hypothesized that this may be related to exposure to fungal allergens, as this has been reported previously in association with near asthma deaths [7]. Interestingly, we observed a similar peak in summer for hospital admissions for asthma in adults aged 16–45 years [8], and are currently in the process of analysing the possible role of fungal allergens in this population.

Therapy of virus-induced asthma exacerbations

Although high-dose oral steroids are partially effective at treating asthma exacerbations, it is at the expense of major side-effects. Their use in children is undesirable for this reason, and many adults become relatively steroid-resistant as their disease progresses. The use of regular low-dose inhaled steroids for prevention is unfortunately ineffective [9]. There is therefore an urgent need for the development of an effective treatment for

virus-induced asthma. Antiviral therapy has been disappointing, as there are major obstacles to the development of effective antiviral therapy, not least, the rapid emergence of resistant strains [10]. An alternative approach to the therapy of virus-induced asthma is to define the factors that lead to the development of an asthma exacerbation in an asthmatic subject in terms of host responses to the viral infection. A non-asthmatic subject undergoing a viral infection will develop principally upper respiratory symptoms, frequently a cough, but little else in the way of lower respiratory symptoms. If the factors leading to the development of lower airways inflammation can be identified, these factors would represent targets for the development of specific therapy aimed at reducing the impact of virus-induced asthma exacerbations. A clear understanding of the mechanisms of virus-induced asthma exacerbations, and the differences from the response of a normal subject to the same infection, will lead to the identification of such targets. Our understanding of both responses is at present very limited.

Mechanisms of virus-induced asthma exacerbations

It is likely that allergen-induced exacerbations have similar mechanisms to those implicated in asthma pathogenesis itself, so this paper will specifically consider mechanisms relating to virus-induced exacerbations, as these may be distinct from asthma pathogenesis.

Lower respiratory tract virus infections

Some types of virus are known to infect and replicate in the lower respiratory tract, particularly adenovirus, RS virus and influenza, but the ability of rhinoviruses to infect the lower respiratory tract is controversial. RV has an optimum replication temperature of 33 °C that occurs in the cooler nasal passages. The warmer temperatures (37 °C) of the lower respiratory tract are considered less conducive to virus replication; it has therefore been argued that RV does not replicate in the lung. However, there is increasing evidence that RV is capable of infecting the lower respiratory tract. We and others have demonstrated replication of RV in human alveolar and bronchial epithelial cell lines *in vitro* at 37 °C [11–13], suggesting that the temperature conditions and cell types present in the lower respiratory tract permit RV replication under laboratory conditions. Observations made in our department during bronchoscopy of human volunteers experimentally infected with RV-16 revealed marked redness of the throat and trachea with patchy erythema around and beyond the carina, suggesting lower airways infection was likely. RV-16 has been detected by RT-PCR in washed bronchoalveolar lavage cell pellets obtained from experimentally infected human volunteers, however, contamination of the samples with RV from the upper airway via the bronchoscope could not be excluded [14].

In situ hybridization (ISH) to detect the RV genomic RNA can visually discriminate between cellular infection and contamination from the upper respiratory tract by demonstrating the presence of virus within bronchial epithelial cell *in situ*. In bronchial biopsies from 10 subjects (three asthmatic and seven normal) obtained from a previous virus infection study (Fraenkel *et al.* 1995), RV-16 was detected in the bronchial epithelium of four subjects (two asthmatic and two normal) when taken at the height of cold symptoms (4 days post infection). No virus was detected in the baseline biopsies taken before the experimental cold, but two biopsies taken 6–8 weeks after infection were found to be positive from naturally occurring RV infections [15].

Taken together, these data strongly suggest that rhinoviruses are indeed capable of replicating in lower airways epithelium, and therefore that infection of the lower respiratory tract by respiratory viruses is likely to play a very important role in the induction of asthma exacerbations in asthmatic subjects.

Inflammatory cell recruitment in virus-induced asthma exacerbations

Many studies have investigated potential mechanisms involved in virus-induced asthma exacerbations, and several inflammatory cell types and factors regulating their recruitment and activation have been proposed as being important.

Neutrophils are involved in the inflammatory response to virus in the upper airways, nasal aspirates of children have increased levels of the neutrophil chemotactic factor IL-8 and neutrophil myeloperoxidase (MPO) during virus-induced asthma [16]. Increased IL-8 levels have been reported in nasal lavage, and levels correlated with airway hyperreactivity in asthmatic subjects infected with RV-16 [17]. Replication of RV in alveolar epithelial cells *in vitro* releases IL-8 [12]. However, the role of neutrophils in lower respiratory tract virus infection is unclear and further research is required. In particular, to date no study has compared the importance of neutrophils in the normal and in the pathological asthmatic responses to viral infection.

Eosinophils are found in the airways in increased numbers in asthma and several studies have implicated eosinophils in the association of viral infections with asthma. Allergen-induced eosinophil numbers were elevated in bronchial lavage from atopic subjects during

a RV infection compared to while uninfected [18]. Eosinophil major basic protein (MBP) is increased in nasal secretions during RV infection in asthmatic children [19]. In the experimental infection study described above, we observed increased intraepithelial eosinophil numbers in bronchial biopsies during experimental RV infection, interestingly, the eosinophil infiltrate persisted in asthmatic subjects compared to normal subjects [20]. Finally, increased levels of the eosinophil product eosinophil cationic protein (ECP) have also been observed in induced sputum in asthmatic subjects undergoing experimental rhinovirus infections [21]. These data suggest that eosinophil infiltration is likely to be a crucial element of the pathology leading to clinical exacerbations of asthma, and that identification of the factors regulating eosinophil infiltration may provide a target for therapy.

In addition to the eosinophil infiltrate observed in the above experimental infection study, we also observed dense $CD3^+$, $CD4^+$ and $CD8^+$ lymphocyte infiltration in the epithelium and submucosa during acute colds [20]. However, there was no difference between normal or asthmatic subjects in the lymphocyte response, suggesting that if lymphocytes are important in differentiating normal from asthmatic responses, then the difference is not likely to be in the number of infiltrating cells, but in the phenotype of the cells. Respiratory virus infections normally promote $CD4^+$ Th1 and $CD8^+$ Tc1 responses with production of IFN-γ and IL-2 which have antiviral activities via proliferation of natural killer cells. Th2 or Tc2 responses are thought to be important in asthma pathogenesis, acting via production of IL-4 promoting isotype switching to IgE production, and IL-5 production and the promotion of eosinophilic inflammation. There are clear data to demonstrate that such responses can be produced by viral infections in certain conditions in animal models [22], and that $CD8^+$ T cells may be important in regulating this response [23]. However, there is little data to corroborate these findings in human virus infections–in atopic subjects during RV infections, there is some evidence that a Th2-like response may occur with production of IL-5 [24]. The possible role of Th2 and Tc2 responses in virus infections in asthmatic and normal subjects clearly requires further investigation, as does the role of the factors regulating Th and Tc responses.

Mechanisms of cellular recruitment in asthma exacerbations

Respiratory epithelial cells are the initial site of virus entry and replication, and have the capacity to produce/express many biologically active molecules implicated in cell recruitment in virus-induced asthma, including many cytokines and adhesion molecules. We have carried out *in vitro* studies to demonstrate that RV infection of respiratory epithelial cells induces its own receptor, ICAM-1 [25]. ICAM-1 is important in the pathogenesis of asthma, and its increased expression by RV infection may play a role in the retention and activation of intraepithelial lymphocytes, and eosinophils. Furthermore, the same studies demonstrated epithelial cell expression of VCAM-1, and its upregulation by RV infection via increased activity of members of the GATA transcription factor family [25]. VCAM-1 is specifically important in eosinophil infiltration by interacting with its ligand VLA-4, and factors regulating its expression may therefore represent targets for therapeutic intervention, however, before firm conclusions about its importance in virus-induced asthma can be drawn, *in vivo* studies are required to confirm the findings of the *in vitro* studies.

The eosinophil infiltrate observed in asthmatic subjects during viral infection may also result from epithelial cell production of cytokines/chemokines with direct actions on eosinophil recruitment and activation. For example, RANTES is a potent eosinophil chemoattractant and activator, and its levels are increased in nasal aspirates from children with virus-induced exacerbations of asthma [26]. RANTES production is increased in RV-infected bronchial epithelial cells *in vitro* [27], and chemokine receptor expression, and the response of the epithelial cells to chemokine stimulation are also both increased [28]. Local autocrine/paracrine activation of epithelial cells by RV infection, mediated by chemokines, may therefore play a prominent role in virus-induced asthma exacerbations. Once again however, these findings need to be confirmed *in vivo*, and their relative importance in asthmatic and normal subjects investigated, before chemokines can be put forward as candidate targets for therapeutic intervention. Furthermore, the above findings relate to a single chemokine, while there are other new chemokines with potent effects on eosinophil chemoattraction and activation, such as eotaxin and MCP-4 [29].

In addition to the candidate molecules discussed above, all of which possess pro-inflammatory properties, it is quite possible, and indeed likely, that virus-induced inflammation in asthmatic airways may be augmented by reduced counter-regulatory mechanisms that normally downregulate inflammation. Little is known in this regard, however, we have recently assayed several cytokines in nasal lavage from normal and atopic subjects during wild type viral infections, and have found significantly reduced induction of IL-10 in the atopic subjects compared with the normal subjects (J. Corne, P. H. Howarth and S. L. Johnston, unpublished data). IL-10 has several anti-inflammatory properties and

deficiency in its production is another potentially important mechanism in virus-induced asthma exacerbations.

Nitric oxide (NO) is a potent mediator that has potential harmful and protective effects in asthma pathogenesis. Recent studies suggest that levels of NO are increased in relation to bronchial hyperresponsiveness (BHR), in response to allergen challenge and are reduced by steroid therapy, suggesting (but not proving) a harmful role for NO in asthma. Recent studies, however, suggest that NO may have a protective role in virus-induced airway hyperresponsiveness. De Gouw *et al.* [30] have found a relationship between rhinovirus-induced increases in NO levels and protection from increased BHR in experimental rhinovirus infections. Folkerts *et al.* [31] previously showed the same during parainfluenza virus type 3 infections in guinea-pigs, and that infusion of a NO donor protected against virus-induced BHR. Finally, Sanders *et al.* [13] have shown that NO donors reduced rhinovirus-induced cytokine release from a bronchial epithelial cell line, and that NO has antirhinoviral activity *in vitro*. These data suggest that NO donors may represent a new therapeutic approach in virus-induced asthma.

Mechanisms of virus-induced cytokine production

The degree of redundancy amongst cytokine functions may suggest that even if one important molecule's functions can be blocked, other related molecules may fulfil a similar role, thereby rendering a treatment aimed at one specific molecule ineffective. We have therefore started to examine the mechanisms of rhinovirus induction of pro-inflammatory cytokines and adhesion molecules, in order to determine if there is a common mechanism such as transcription factor activation. We have demonstrated that rhinovirus-induction of IL-8 occurs via activation of the transcription factors AP-1 and NFκB [32], as does RS virus-induction of IL-8 [33], while that of ICAM-1 occurs via NFκB alone and that of VCAM-1 via NFκB and GATA [25]. Other workers have demonstrated that rhinovirus-induction of IL-6 also occurs via induction of NFκB [34]. These *in vitro* data suggest that inhibition of NFκB might suppress rhinovirus induction of a number of pro-inflammatory cytokines and therefore reduce the severity of rhinovirus induced asthma exacerbations. Once again, the potential roles of these transcription factors has been suggested by *in vitro* studies, but *in vivo* confirmation is required.

Other candidate molecules regulating virus-induced inflammation

It is apparent from the above review of our current state of knowledge, that a large number of candidate molecules exist, that might be of fundamental importance in regulating the inflammatory response to viral infection of the airways. Indeed in addition to those molecules discussed above, several cytokines including IL-1, -6, -11, GM-CSF, TNFα and IFN-α and -γ and transcription factors regulating their synthesis have all been proposed as candidate molecules with potentially important roles in virus-induced asthma exacerbations. The intracellular mechanisms of virus induction of transcription factor activity and cytokine/adhesion molecule expression are completely unknown.

Studies are urgently required to investigate the signal transduction pathways involved in virus-induced pro-inflammatory molecule synthesis, to investigate whether common mechanisms can be identified early in the time course of virus-induced inflammation. Identification of important pathways involved in virus-induced inflammation in asthmatic and normal subjects, may identify a potential target for therapeutic intervention in the future.

Comparative studies in asthmatic and normal subjects

The mechanisms of virus-induced exacerbations of asthma require extensive further investigation. There are very few studies comparing lower respiratory inflammatory responses in normal and asthmatic subjects. One such study, including only six asthmatic subjects, did not induce any changes in lower airway function in the asthmatic subjects and did not address the cytokines/chemokines, adhesion molecules or other biological molecules driving the inflammatory responses [20]. Identification of the important differences between asthmatic and normal subjects responses is of fundamental importance in the search for targets for therapeutic intervention. Studies are urgently required to investigate the potential mechanisms by localizing virus infection in the lower airway in conjunction with implicated biological molecule detection and cell influx using *in-situ* hybridization, immunohistochemistry, PCR and ELISA in bronchial lavage and biopsies from experimentally infected subjects. The results in asthmatic subjects must be compared to those in normal subjects in these studies to identify those factors regulating the altered response in asthma.

Effects of different viruses on lower airway inflammation

Although rhinoviruses are numerically the most important virus type implicated in asthma exacerbations, accounting for around 50% of exacerbations, the remaining 30–50% are caused by RS virus, influenza viruses, parainfluenza viruses, adenoviruses and coro-

naviruses, with the proportions varying depending on season and the age of the patient. Most studies carried out so far have studied the effects of rhinovirus. However, our clinical studies suggest that there is very little difference in the capacity of each of the different virus types to induce asthma exacerbations [1]. It is therefore important to determine whether mechanisms identified as being important for rhinovirus virus-induced asthma exacerbations are also important in exacerbations induced by other viruses such as those listed above. This will have major implications in determining the percentage of asthma exacerbations that could potentially be treated by any therapy stemming from the studies carried out with rhinovirus.

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