

Infections, Immunity & their Effects on Asthma

Introduction

It is increasingly recognised that infection, particularly virus infection but also atypical bacterial infection, is intricately linked with the pathogenesis of asthma from infancy, through childhood and into adulthood. Among viral infections, respiratory syncytial virus (RSV) is prominent as the major cause of bronchiolitis in infancy. Rhinoviruses are the major cause of wheezing at all ages, and influenza is an important cause of wheezing in the annual influenza epidemics. The relationships between the innate and acquired immune responses to infection, and those to allergen driven inflammation, are areas in which significant recent advances have been made, but a great deal remains to be learned. There is also an urgent need for development of experimental models of infection and asthma, to assist in understanding of mechanisms, and to aid development and testing of new treatments.

RSV bronchiolitis

Acute bronchiolitis is the main cause of hospitalisation in infants and has significant mortality. RSV infection is the major cause of bronchiolitis, accounting for 70–80% of cases [1]. RSV bronchiolitis is clearly linked with the later development of wheeze and asthma though its link with later development of atopy is still controversial [2]. An association with the later development of atopic sensitisation has also been reported in studies involving severe bronchiolitis. No association has been observed in less severe bronchiolitis. The mechanisms underlying the association between RSV bronchiolitis and the later development of wheezing are incompletely understood, though there is increasing evidence that impaired antiviral immunity is likely to play an important role in increasing susceptibility to virus infections early in life, as well as perhaps the later development of asthma [3]. Whether RSV bronchiolitis plays a causal role in increasing the risk of asthma development is a subject of great interest though clear evidence for a causal role has not yet been established. We need to understand the mechanisms of association of bronchiolitis with later wheezing and asthma in the hope that we will be able to define potential avenues for development of new therapies.

Lower respiratory tract infections in infancy

In addition to RSV bronchiolitis, lower respiratory tract infection with other virus types in infancy is associated with later development of wheezing and asthma. However, the epidemiological evidence linking other viruses

with later development of wheeze and asthma is less extensive than the literature on RSV. This may be because RSV has a particular tropism for the lower respiratory tract and perhaps other special features such as the ability to induce TH2 biased inflammation or to induce long-lasting changes in local immune responses. However, it may also be a result of relatively poor diagnostic methods for the other virus types and a lack of appreciation of the importance of these viruses resulting in studies not being carried out as frequently or effectively with these viruses as with RSV. There is clearly an urgent need for studies specifically investigating the role of upper and lower respiratory tract virus infections in this context.

Acute asthma exacerbations in children and adults

Virus infections are the major cause of wheezing illness in young infants at a time when clear evidence of allergic sensitisation is frequently not present. The mechanisms explaining this relationship are also to a large degree unknown, though the presence of smoking in the household and of small airway size are known risk factors.

In school age children, virus infections are associated with 80–85% of acute exacerbations of asthma [4]. The role of respiratory virus infections as precipitants of asthma exacerbations in adults is less well established. Studies vary in their detection frequencies from very low percentages to detection rates of 70–76% [5]. All these studies have detected rhinoviruses as the most frequent virus implicated. Rhinoviruses are detected in around two-thirds of virus associated episodes and around 50% of all exacerbations [3,4]. Other respiratory viruses are also implicated – influenza in the annual influenza epidemics and RSV in the annual RSV epidemics.

Although viruses are associated with the majority of acute exacerbations, there is evidence indicating a synergistic interaction between concurrent allergen exposure and virus infection in increasing risk of exacerbation [6,7]. Similarly, exposure to tobacco smoke and indoor and outdoor pollutants also increases the risk of exacerbation with virus infection [8]. The mechanisms of these interactions remain to be explained.

Mechanisms of virus induced asthma can be investigated with difficulty in naturally occurring exacerbations. As a result, both animal and human experimental infection models are needed to try to increase our understanding of the mechanisms involved. Induction of airway hyperreactivity appears to be one important mechanism and induction of lower airway inflammation and neurogenic inflammation have also been implicated [9].

The role of currently available therapies, including inhaled steroids and long acting beta agonists in suppression of virus induced inflammation needs to be better understood so that currently available therapies can be used optimally and targets for development of new therapies identified.

Innate and acquired immunity in susceptibility to respiratory infection in asthma

Clinical studies indicate that people with asthma have increased susceptibility to respiratory virus infection in that they develop lower respiratory symptoms of greater severity and duration when infected with rhinoviruses and that these are accompanied by greater falls in peak flow [10]. Increasing attention is therefore being focused on host responses to virus infection in the belief that impaired innate and/or acquired immune responses may be important in determining the severity of infection and that severity of infection is the main determinant of severity of clinical illness [9].

There is preliminary evidence both in relation to RSV in infancy and virus induced asthma later in life that people with asthma have impaired TH1 acquired antiviral immunity and recent studies suggest impaired innate immune responses in relation to apoptosis and interferon- β production [11]. Serum neutralising antibody is important in protection against virus infection but particularly with RSV, protection against repeated infections is incomplete. The role of cellular immune responses in protection against respiratory virus infection is incompletely understood but CD8 T cell responses, in particular, interferon- γ production are thought to be important [12]. In certain circumstances, disease augmentation can be associated with specific patterns of cellular immune responses [13]. The mechanisms of disease augmentation need to be better understood.

Antigen presenting cells, in particular dendritic cells, and co-stimulatory pathways are important in shaping virus induced immune responses and inflammatory responses [14]. The roles of these pathways in inducing effective antiviral immunity and of promoting effective virus clearance in the absence of excessive inflammation (both TH1 and TH2) are poorly understood.

Finally, recent evidence suggests that people with asthma have increased susceptibility to bacterial infection in that they have an increased risk of invasive pneumococcal disease [15]. Further studies are required to confirm whether increased susceptibility in asthma is limited to *Streptococcus pneumoniae* or whether it includes other bacterial infections as well. If increased susceptibility to bacterial infection is confirmed, the epidemiologic importance and the mechanisms of this susceptibility will need to be defined.

Antiviral therapies and vaccines

The role of therapeutic intervention for virus induced airway disease is a matter of increasing interest and importance. However, little specific therapy is currently available. Palivizumab has been shown to reduce incidence and severity of RSV infection in at risk children. Ribavirin has proved disappointing in the treatment of RSV bronchiolitis, the only other specific antiviral therapies available are against influenza viruses. There are few studies in asthma, but one suggests early intervention can reduce the severity of influenza associated asthma exacerbations. A number of new antiviral therapies are in development for RSV and rhinoviruses. There is an urgent need for antiviral therapies to be developed and studied in the treatment/prevention of virus induced asthma.

Vaccination against influenza currently exists, but it remains controversial whether this protects against influenza induced asthma exacerbations. No approved vaccine exists for any other virus type. Developing safe and effective vaccines against all the respiratory viruses is clearly an important priority.

A typical bacterial and bacterial infection in asthma

There is increasing evidence that chronic infection/colonisation/reactivation of *Chlamydomphila* (previously *Chlamydia*) *pneumoniae* and *Mycoplasma pneumoniae* may play a role in the pathogenesis of both chronic and new onset asthma and also in acute exacerbations [16]. Diagnostic methods for both organisms are sub-optimal and much of the controversy regarding the possible association of infection with asthma pathogenesis results from difficulties in diagnosis.

A recent study has demonstrated that an antibiotic active against these bacteria is effective in therapy of acute exacerbations in adults [17]. However, as these antibacterials also have anti-inflammatory activity, the mechanisms of this therapeutic effect are unknown.

Chronic infections in severe asthma

Severe asthma is a major problem in asthma therapy as it accounts for a major proportion of morbidity, mortality and healthcare costs, but current therapies are relatively ineffective in treatment of severe asthma. The mechanisms of severe asthma are poorly understood, but the relative steroid resistance and prominent neutrophilic inflammation associated with severe asthma suggest the possibility of viral and/or bacterial infection. Recent studies also indicate that in animal models, RSV is able to persist following an acute infection for a number of months [18]. The possibility of chronic infection with atypical bacteria and viruses requires investigation in severe asthma.

Conclusions and recommendations

RSV bronchiolitis

Clinical studies of the impact of therapy for RSV bronchiolitis on downstream protection against developing wheezing, asthma and allergic sensitisation are required. Improved human and animal models of disease are required to increase understanding of mechanisms of disease. Areas that particularly require attention include interplay between the innate and acquired immune responses in host defence; the role of viral persistence in development of wheezing illness and asthma; the roles of antigen presenting cells and other specific cell compartments such as epithelial cells, T cells, neutrophils, eosinophils and non T cell lymphocytes in host protection and disease severity. The role of genetic variation in the host immune response as a determinant of disease severity and of HLA subtype also needs to be determined. The importance of specific cytokines and chemokines in inflammatory responses to virus infection requires investigating as specific antagonists are available for some and in development for others and there is good potential for intervention if individual cytokines or chemokines can be demonstrated as being important in disease pathogenesis. Developing and testing safe and effective antiviral therapies and vaccines against RSV remains an urgent priority. To achieve this will require *in vitro* models, *in vivo* animal models and human experimental challenge models. The role of RSV in impairment of lung growth in infancy and in the initiation of airway remodelling responses should be studied. The relationship between infection and development of immune sensitisation or tolerance to allergens requires investigation.

Lower respiratory tract infections in infancy

Further studies investigating the importance of different virus types in the association between lower respiratory infection in infancy and wheezing illness in asthma are required to clarify the relationships, including rhinovirus, human metapneumoviruses, coronaviruses and bocaviruses. There is an urgent need for studies specifically investigating the role of upper and lower respiratory tract virus infections in the development of asthma. It is also essential that birth cohort studies employ modern day molecular methods to identify all the common respiratory virus types so that this data can be linked to focused hypotheses about gene-environment interactions. These studies need to be linked with concurrent studies of antiviral immunity as infections and immunity are inextricably connected, with immunity determining severity of infection and infections in turn determining immune responses. Developing safe and effective antivirals and vaccines against all the respiratory viruses in addition to

RSV is clearly an important priority, as is developing the models in which to test them.

Acute asthma exacerbations in children and adults

Studies with antiviral agents in virus induced asthma are urgently required, as is development of vaccines and studies with agents aiming to augment various aspects of antiviral immunity in a non virus-specific manner. Studies with anti-inflammatory agents in virus induced asthma are also required, as are studies with combinations of agents. The epidemiology of virus induced asthma in adults requires further investigation, preferably with studies including detection of viruses in lower respiratory tract specimens such as induced sputum and perhaps in exhaled breath condensate. These studies need to include appropriate control samples and virus detection should be with modern molecular methods able to detect all common respiratory viruses. Analysis of control samples will be needed to ensure appropriate sensitivity of these assays. Further studies of interactions between infection and other provoking agents such as air pollution, smoking and allergen exposure are needed to define the interactions and associated risks more clearly. Most important will be studies investigating mechanisms of virus induced asthma in both children and adults. They should include studies investigating mechanisms of induction of airway inflammation in the context of virus infection. Mechanisms of induction of airway hyperresponsiveness and mechanisms of neurogenic inflammation in the context of virus infection require investigation. The role of virus infection in induction of steroid resistance and of currently available therapies, including inhaled steroids and long acting beta agonists in suppression of virus induced inflammation needs to be better understood and targets for development of new therapies identified.

Development and refinement of *in vivo* and *in vitro* models of virus induced inflammation are needed. These will include development of human experimental infection models with rhinovirus, RSV and influenza. Murine models of all these virus infections are required and they should be combined with both acute and chronic models of allergen induced inflammation to develop good models of virus induced exacerbations. *In vitro* studies can yield a great deal of valuable information regarding mechanisms of induction and resolution of virus induced inflammation. These should include models in both undifferentiated and differentiated epithelial cells, macrophages, eosinophils, mast cells and neutrophils. Studies investigating mechanisms of inducing effective antiviral immunity and of promoting effective virus clearance in the absence of excessive inflammation (both TH1 and TH2) are needed. Further studies investigating interactions between exposures such as allergens, tobacco smoke, air pollution and virus infection and the mechanisms of these interactions

are needed. The mechanisms regulating neutrophilic inflammation in the context of virus infection and the role of virus induced neutrophil chemo-attractants and activators need investigation.

Innate and acquired immunity in susceptibility to respiratory virus infection in asthma

Clinical studies in humans as well as human experimental infection studies are required to better define the roles of innate and acquired immune responses and to determine which of these are deficient *in vivo*. Animal models will be required to better define mechanisms of interplay between innate and acquired immune responses, in particular mechanisms of promotion of effective antiviral immunity in the context of TH2 driven allergic airway inflammation. The role of antigen presenting cells, in particular dendritic cells, in this context will be particularly important. The mechanisms of induction of type-1 interferons in response to respiratory virus infection will be important in understanding the molecular mechanisms of deficient interferon responses in asthma.

The availability of quantitative PCRs will permit investigation of the importance of virus load in all these disease contexts. Studies investigating virus load in detail will be required to dissect these mechanisms. In the context of virus induced asthma it would be helpful to dissect mechanisms that promote antiviral immunity while not increasing inflammatory responses or even decreasing inflammatory responses. Detailed investigation of the molecular regulation of antiviral immune responses and pro-inflammatory immune responses will therefore be required and will assist development of immunomodulatory and vaccine approaches, as will the testing of novel immune modulators with selective actions. Investigation of the importance of neutralising antibodies to virus in host protection is required.

Antiviral therapies and vaccines

There is an urgent need for antiviral therapies for RSV and rhinoviruses in particular; to be developed and studied in the treatment/prevention of virus induced asthma. To enable this there is an urgent requirement for development and validation of experimental models of virus infections in which candidate molecules can be tested. Studies are needed to determine whether existing anti-influenza therapies such as antivirals and vaccines can protect against influenza induced asthma exacerbations. Influenza vaccination and antivirals can also clearly be improved, in terms of protective efficacy and duration, protection against different subtypes and ease of delivery. Developing safe and effective vaccines against all the other respiratory viruses is clearly also an important though technically challenging priority.

Atypical bacterial and bacterial infection in asthma

Further studies investigating the epidemiological evidence for association of both *C. pneumoniae* and *M. pneumoniae* with both chronic stable asthma and acute exacerbations are required. In order for these to be successful, studies aimed at improving diagnostic methodology for both organisms are also needed. Animal models of both infections need to be developed and integrated with animal models of allergen induced inflammation so that mechanisms of disease and of host immunity can be developed. The mechanisms of macrolide and ketolide efficacy need further investigation, particularly in relation to virus induced inflammation as well as atypical bacterial inflammation. Studies are needed to determine whether macrolides and/or ketolides have antiviral activity.

Key questions

- What are the roles of and interactions between innate and acquired immune responses in inducing wheezing in response to viral and bacterial respiratory infection in infants who may be predisposed to, or individuals who have developed asthma?
- What are the mechanisms of virus and/or bacteria induced pathology in wheezing illness, both as a result of failure of clearance and of potentially immunopathogenic immune responses?
- How is virus induced inflammation and antiviral immunity regulated at the molecular and cellular level and can targets for the development of new therapies be identified by better understanding these mechanisms?

Studies are required to confirm the reported association between asthma and increased risk of invasive bacterial infection and to investigate whether the risk extends to bacteria other than *S. pneumoniae*.

Long-term infections in severe asthma

Studies are required investigating frequency of detection of respiratory viruses and atypical bacteria in severe asthma (both stable disease and acute exacerbations). Further studies are required to confirm the presence of long term RSV infection in asthma, both in children and in adults. These studies should also include detection of viruses other than RSV and should include assessment of clinical severity as well as immunopathologic markers such as airway inflammation. Relationships between chronic infection and disease severity should be sought.

Addressing the key questions (listed above) will require an integrated approach from epidemiologic studies right down to studies of the molecular regulation of anti-infective and inflammatory responses to infection in wheezing illness. It will also require development and

refinement of *in vitro*, animal and human models of infections and asthma.

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