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Review article

Viruses and asthma

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The interrelationship between virus infections and asthma is a large subject. There are several areas of interest, ranging from the epidemiologic approach (association between viruses and the inception of asthma, between bronchiolitis and subsequent asthma, and between viruses and asthma exacerbations) to the cellular and molecular mechanisms involved in these processes. In this review, I will discuss the data available in each of these areas, and the treatment available and in development, in the hope of stimulating further interest in this important subject.

Respiratory viruses

The major respiratory virus types and the diseases they are most associated with are listed in Table 1. Each of the respiratory viruses is capable of causing almost any respiratory disease, from a mild common cold to severe destructive pneumonia, depending on the site and dose of virus inoculated, and the degree of host resistance. The most common respiratory viruses in infancy are respiratory syncytial (RS) viruses – they cause approximately 50% of all wheezing illness, and 80% of cases of bronchiolitis (1). Indeed, 70% of children have been infected with RS virus by 1 year of age, and almost all by 3 years. In older children, rhinoviruses are the major virus type and cause around 60% of acute respiratory illnesses (2). Influenza viruses occur in epidemics, the frequency and severity of which are determined by the degree of antigenic drift, and more rarely in pandemics associated with more major antigenic shift. Illness can vary from mild upper respiratory infection to severe lung infections with high mortality. Parainfluenza viruses infect all age groups and are particularly associated with croup (laryngotracheobronchitis) in young children. Adenoviruses can cause mild colds, but are also associated with severe pneumonia, and coronaviruses cause around 10–15% of colds, although their ability to infect the lower respiratory tract is not well documented.

Virus detection methods

Although a connection between upper respiratory infections (URIs) and asthma has long been recognized, its importance was underestimated in most previous studies due to deficiencies in virus-detection methods or in clinical surveillance of subjects (3). Rhinoviruses and coronaviruses cause around 75% of URIs, but are detected very poorly by standard methods. Neither virus type grows well in the cell-culture systems in use in most diagnostic laboratories, and antibodies are not available for immunofluorescence or serologic techniques. Recent advances in virus-detection methods, particularly for rhinoviruses and coronaviruses, have improved detection rates by around 3–5-fold (4–7).

Association between virus infections and asthma exacerbations

The use of the polymerase chain reaction (PCR) for detection of rhinoviruses has been a major factor in two recent studies documenting the association between virus infections and asthma exacerbations in children and adults. In two community-based studies, it was demonstrated that 85% of asthma attacks in children (8) and 44% in adults (9) were precipitated by URIs.

Table 1. Respiratory virus types and respiratory diseases associated with them

Virus type	Serotypes	Common cold	Asthma exacerbation	Pneumonia	Bronchitis	Bronchiolitis
Rhinovirus	1–100 (plus)	+++	+++	±	+	+
Coronavirus	229E and OC43	++	++			
Influenza	A, B, and C	+	+	++	+	
Parainfluenza	1, 2, 3, and 4	+	+	±	++ (Laryngotracheobronchitis)	+
Respiratory syncytial virus	A and B	+	+	+	+	+++
Adenovirus	1–43	+	+	++	+	+

+++ Major cause; ++ commonly associated; + well recognized; ± occasional/rare; blank – absent.

Rhinoviruses were numerically the most important, accounting for 60% of viruses identified in each of these studies. These studies together have established that respiratory virus infections are associated with most asthma exacerbations occurring in the community; however, neither study investigated whether severe attacks of asthma leading to hospital admission were also precipitated by virus infections.

The last two years have also provided evidence that respiratory viruses can also induce severe asthma. In a time-trend analysis comparing the seasonal patterns of respiratory infections and hospital admissions for asthma in children and adults (10), strong correlations were observed in both groups. The major factor determining pediatric admissions was school attendance – the peaks of both respiratory infections and asthma admissions occurred at the beginning of school terms. These data provide strong support for the hypothesis that virus infections also precipitate asthma exacerbations leading to hospital admissions; however, studies investigating this directly are now required to confirm this hypothesis.

Similar time-trend analysis has been used to investigate the possible contribution of virus infections to asthma mortality (11). Winter peaks in asthma mortality were observed in children under 5 years and in adults over 45 years, suggesting that virus infections also precipitate asthma deaths in these age groups. Interestingly, in asthmatic subjects aged 5–44 years, there was a strong summer peak (July/August). The reasons for this are presently unclear, but may include alterations in use of asthma medication, or in access to medical care during the holiday season. A further possibility is sensitization to mould spores, as levels of these peak at that time of year (12).

Taken together, these data suggest that virus infections are important causes of asthma exacerbations, including attacks severe enough to require hospital admission, in all age groups, but especially in children. Attacks severe enough to lead to death appear to be caused predominantly by virus infections in the young and the old, but less so in the 5–44-year age group. In these subjects, virus infections may not cause sufficiently catastrophic asthma to cause death – this appears to be mainly related to other factors, probably allergen exposure. Further studies directly examining the roles of URIs and other factors such as allergen exposure in precipitating severe asthma attacks are now required to confirm the results of these indirect time-trend studies.

Therapy of virus-induced asthma exacerbations

Inhaled β-agonists and high-dose oral steroids are partially effective at treating virus-induced asthma exacerbations; however in the case of steroids, at the expense of worrying side-effects. Their use in children is undesirable for this reason, and some adults become relatively steroid resistant as their disease progresses. Inhaled steroids are a possible alternative, but intervention with high-dose inhaled steroids is only partially effective (13, 14), and again side-effects are a concern in children. The use of regular low-dose inhaled steroids for prevention is unfortunately ineffective (15). Therefore, there is 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 (16).

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 patient in terms of host responses to the viral infection. A nonasthmatic person 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 in virus-induced asthma can be identified, these factors would represent targets for the development of specific therapy aimed at

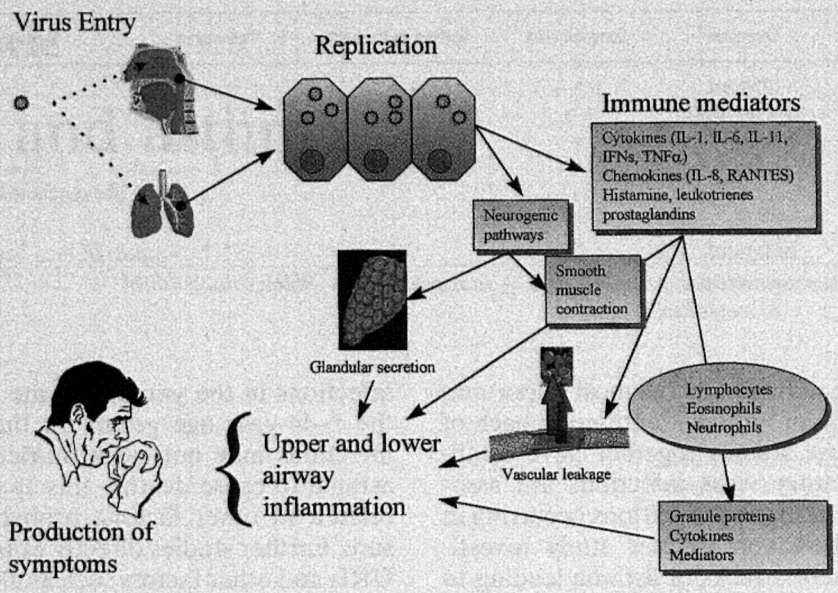

Fig. 1. Diagrammatic representation of some important mechanisms involved in virus-induced respiratory infections.

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

As the mechanisms of virus infections and of asthma itself are both complex, those of virus-induced asthma are therefore necessarily very complex! A summary of some of the important mechanisms thought to be involved is depicted in Fig. 1.

An important question to answer initially is this – do virus infections exacerbate asthma directly by local mechanisms consequent upon lower airway infection, or do they infect only the upper respiratory tract and affect the lower airway indirectly?

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 virus, but the ability of rhinoviruses to infect the lower respiratory tract is controversial. Rhinoviruses have an optimum replication temperature of 33°C, which occurs in the cooler nasal passages. The warmer temperatures (37°C) of the lower respiratory tract are considered less conducive to virus replication; therefore, it has been argued that rhinovirus does not replicate in the lung. However, there is increasing evidence that rhinoviruses can infect the lower respiratory tract.

We and others have demonstrated replication of rhinoviruses in human alveolar and bronchial epithelial cell lines *in vitro* at 37°C (17–20). We have also recently compared the temperature preferences of several rhinovirus serotypes when cultured *in vitro*. Although most rhinoviruses replicated slightly better at 33°C, some replicated better at 37°C, and in all cases were able to reach high titres at 37°C (21). These data suggest that the temperature conditions and cell types present in the lower respiratory tract permit rhinovirus replication under laboratory conditions.

Observations made in our department during bronchoscopy of human volunteers experimentally infected with rhinovirus-16 revealed marked redness of the throat and trachea with patchy erythema around and beyond the carina, suggesting that lower airways infection was likely. In a recent study well designed to control for upper airway contamination, Gern et al. used PCR to assess lower airway rhinovirus load during experimental infections (22). Bronchoalveolar lavage (BAL) cells were positive during the infection in over 80% of their samples, while only 37% of BAL fluid specimens were positive, suggesting that rhinoviruses are able to infect the lower airway, and that rhinovirus RNA is largely cell-associated.

In situ hybridization to detect the rhinovirus genomic RNA can visually distinguish between cellular infection and contamination from the

upper respiratory tract by demonstrating the presence of virus within bronchial epithelial cells *in situ*. In bronchial biopsies from 10 subjects (three asthmatic and seven normal) obtained from a previous virus infection study (23), rhinovirus-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 after 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 rhinovirus infections (24).

Taken together, all these data confirm that all the respiratory viruses (with the exception of coronavirus, for which no data exist) are able to infect the lower respiratory tract. What effects relevant to asthma do they have when they get there?

Inflammatory cell recruitment in virus-induced asthma exacerbations

Many studies have investigated the 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 (Fig. 1).

Neutrophils are involved in the inflammatory response to virus in the upper airways, and the nasal aspirates of children have increased levels of the neutrophil chemotactic factor interleukin (IL)-8 and the neutrophil product myeloperoxidase during virus-induced asthma (25). Increased IL-8 levels have been reported in nasal lavage, and levels correlated with airway hyperreactivity in asthmatic subjects experimentally infected with rhinovirus-16 (26). Replication of rhinovirus in bronchial and alveolar epithelial cells *in vitro* releases IL-8 (17, 20), as does infection of monocytes (27). Gern has shown that rhinoviruses also bind to, but do not infect, pulmonary macrophages, and that tumour necrosis factor-alpha (TNF-α) secretion is induced by rhinovirus (28). Production of IL-8 by macrophages in response to rhinovirus has not yet been studied, but such production seems likely given the above data. Grunberg et al. recently reported increased staining for IL-8 in neutrophils in sputum during experimental rhinovirus infections (29). However, the role of neutrophils in lower respiratory tract virus infection has not been extensively studied, and further research is required. In particular, to date, no studies have compared the importance of neutrophils and factors regulating their recruitment and activation in the normal and in the pathologic 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 rhinovirus infection compared to the uninfected state (30). Eosinophil major basic protein is increased in nasal secretions during rhinovirus infection in asthmatic children (31). Increased intraepithelial eosinophil numbers were observed in bronchial biopsies during experimental rhinovirus infections; interestingly, the eosinophil infiltrate persisted longer in asthmatic subjects than normal subjects (23). Increased eosinophil products have also been observed in induced-sputum supernatants from asthmatic subjects undergoing experimental rhinovirus infections (29). Inflammatory cells such as eosinophils, neutrophils, and lymphocytes, as well as the expression of the intercellular adhesion molecule-1 (ICAM-1), were also found to be significantly increased in atopic in comparison with nonatopic subjects experiencing natural colds (32). These data suggest that eosinophil infiltration is probably a crucial element of the disorder 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, dense $CD3^+$, $CD4^+$, and $CD8^+$ lymphocyte infiltration was also observed in the epithelium and submucosa during acute colds (23). 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 lie in the number of infiltrating cells, but perhaps it may lie in the phenotype of the cells. Respiratory virus infections normally promote $CD4^+$ Th1 and $CD8^+$ Tc1 responses with production of interferon-gamma (IFN-γ) and IL-2, which have antiviral activities via proliferation of natural killer (NK) cells (33). Th2 or Tc2 responses are thought to be important in the pathogenesis of asthma, acting via production of IL-4, which promotes isotype switching to IgE production, and IL-5, which promotes eosinophilic inflammation. There are clear data to demonstrate that such responses can be produced by viral infections in certain conditions in animal models (34), and that $CD8^+$ T cells may be important in regulating this response (35). However, there are few data to corroborate these findings in human virus infections, although in atopic subjects during rhinovirus infections, there is some evidence that a Th2-like response may occur with production

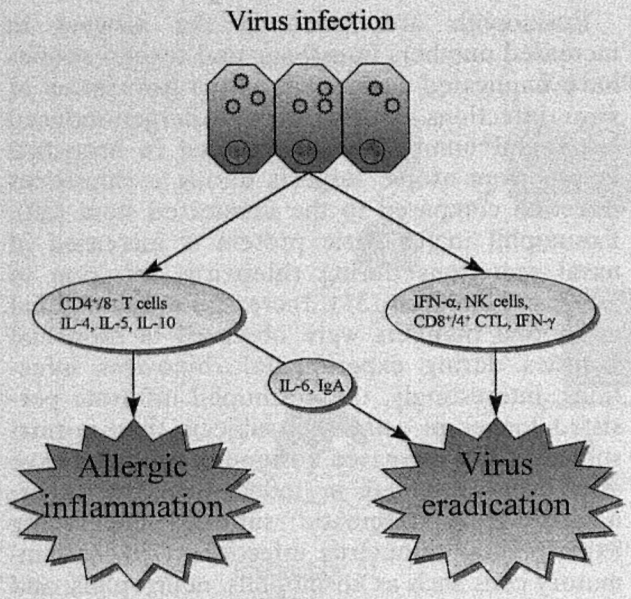

Fig. 2. Diagram of T-cell responses to virus infection involving $CD4^+$ responses providing help for antibody production, but also producing cytokines promoting allergic inflammation, and $CD8^+$ responses involving cytotoxic activity and antiviral cytokine production, but also producing cytokines promoting allergic inflammation. Physiologic balance between these two responses results in effective immunity; imbalance toward type-2 response leads to allergic disease.

of IL-5 (36). There is also evidence that during RS virus bronchiolitis in infants, there is an imbalance in the immune response to a type-2 response (37). The balance between type-1 and type-2 responses to virus infection is depicted in simplified form in Fig. 2. In fact, in reality, a balance between both responses is necessary for effective immune responses to occur. Type-1 responses are important for antiviral cytokine production, NK activity, nd cytotoxic lymphocyte activity, while type-2 responses are important for IL-6 production and isotype-switching to IgA production (the most important immunoglobulin for antiviral activity). Excessive responses of either type are harmful, excessive type-2 responses leading to increased allergic inflammation, and excessive type-1 responses leading to increased inflammation mediated by IFN-γ-positive $CD4^+$ T cells (38). The possible role of Th1/2 and Tc1/2 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 virus-induced 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. *In vitro* studies demonstrate that rhinovirus infection of respiratory epithelial cells induces its own receptor, ICAM-1 (39–41). ICAM-1 is important in the pathogenesis of asthma, and its increased expression by rhinovirus infection may play a role in the retention and activation of intraepithelial lymphocytes and eosinophils. A recent *in vivo* study has confirmed the findings of the *in vitro* studies, by demonstrating increased bronchial epithelial ICAM-1 staining during experimental rhinovirus infections (42). Similar data demonstrated that RS virus infection of bronchial epithelial cells also increased ICAM-1 expression, indicating that this response may be common to many respiratory virus types (43, 44). Clearly, therefore, the factors regulating the induction of ICAM-1 expression by respiratory viruses may represent attractive targets for the development of novel therapeutic interventions.

The eosinophil infiltrate observed in asthmatic subjects during viral infection may also result from epithelial cell production of cytokines/chemokines with direct effect 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 (31). RANTES production is increased *in vitro* in primary bronchial epithelial cells infected by influenza type A, RS virus, and rhinovirus (45, 46, Johnston, unpublished observations), and chemokine receptor expression and the response of the epithelial cells to chemokine stimulation are also both increased (47). Therefore, local autocrine activation of epithelial cells by rhinovirus infection, mediated by chemokines, may play a prominent role in virus-induced asthma exacerbations. 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 (48).

MIP1α is another chemokine of great interest, as it is induced in monocytes exposed to rhinovirus (Johnston, unpublished observations), is found in increased amounts in nasal aspirate samples during wild-type virus infections (31), and is essential for the cellular immune response to respiratory virus infection in animal studies (49).

Induction of bronchial hyperresponsiveness in virus-induced asthma exacerbations

The presence of nonspecific bronchial hyperresponsiveness is an important feature of asthma, and the induction of nonspecific bronchial hyperresponsiveness is a well-documented result of viral infection in normal, allergic, and asthmatic subjects (50–53). Although this induction has not been observed in all studies (54), this discrepancy in observations is probably related to differences in virus dose used and/or in the inoculation method (55). The induction of nonspecific bronchial hyperresponsiveness by respiratory viral infection is a physiologic process that may clearly have relevance to virus-induced asthma exacerbations. However, the mechanisms of this induction are not clear.

Mechanisms of induction of bronchial hyperresponsiveness in asthma exacerbations

One way in which an infection may cause an increase in vagally mediated bronchoconstriction is through a respiratory tract viral infection causing loss of function of the neuronal M_2 muscarinic receptor.

Increased activity of the cholinergic nervous system after a viral infection was first suggested by Empey et al., who showed that during a naturally acquired viral infection normal subjects demonstrated increased reactivity to histamine compared to control noninfected patients (50). Furthermore, this hyperreactivity was prevented when the subjects were pretreated with atropine, indicating that the hyperreactivity was due to increased cholinergic activity. A similar finding has also been reported by Aquilina et al. (51). In animal studies, a number of groups have demonstrated an increase in vagally mediated hyperreactivity after a viral infection (56, 57). Thus, there is evidence in both animal models and in man that a viral infection results in an increase in vagally mediated bronchoconstriction.

It has been shown that infection with parainfluenza virus causes loss of function of pulmonary neuronal M_2 muscarinic receptors in guinea-pig or rat lungs (58, 59). The loss of function is due in part to a directly toxic effect of the virus, especially when there is a heavy infective burden. However, in animals that are less severely infected, the loss of function may be due to an indirect, inflammatory cell-mediated effect (60).

There is now considerable evidence that eosinophils may be pathogenically important in virus-induced exacerbations of asthma, and also a great deal of evidence that eosinophils, particularly eosinophil major basic protein, are responsible for the lost function of the M_2 muscarinic receptor both in antigen-challenged animals and in asthma patients (61–65). Further evidence supporting the possible role of eosinophils in virus-induced asthma exacerbations, particularly vagally mediated bronchial hyperreactivity, is the observation that eosinophils accumulate around airway nerves in patients with fatal asthma (62). Studies focused on the possible role of eosinophil proteins in the loss of function of neuronal M_2 muscarinic receptors in virus-induced asthma exacerbations are now urgently required.

Sensory C fibre stimulation is another mechanism that may be important in virus-induced bronchial hyperresponsiveness. Stimulation of these fibres may result in bronchoconstriction via the brainstem, or by local release of substance P and neurokinin A. These neuropeptides have many properties that make them prominent candidates to be mediators in allergic inflammation, and they are implicated in virus-induced bronchial hyperresponsiveness (66). In addition, their levels may also be increased in virus respiratory infections by reductions in the activity of the enzyme that inactivates them (neutral endopeptidase) consequent upon epithelial cell damage (67).

Nitric oxide (NO) is a potent mediator which has potentially harmful and protective effects in the pathogenesis of asthma. However, recent studies suggest that NO may have a protective effect against virus-induced airway hyperresponsiveness. De Gouw et al. (68) have found a relationship between rhinovirus-induced increases in NO levels and protection from increased bronchial hyperresponsiveness in experimental rhinovirus infections. Folkerts et al. (69) previously showed the same during parainfluenza virus type-3 infections in guinea pigs, and that infusion of a NO donor protected against virus-induced bronchial hyperresponsiveness. Finally, Sanders et al. (70) 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 airway hyperresponsiveness.

As well as its probably important role in the induction of inflammatory cell recruitment and activation, cytokine induction is also likely to play an important role in the induction of bronchial hyperresponsiveness. The proinflammatory cytokine IL-6 was found to be induced by rhinoviruses (18), and IL-6 was also increased in the sputum of rhinovirus-infected asthmatic subjects (29). Another recent, possibly important candidate for a role in the pathogenesis of virus-induced asthma

is the related cytokine IL-11. Several viruses, including RS and parainfluenza viruses and rhinovirus, strongly stimulated production of IL-11 by stromal cells. Furthermore, it was detected in nasal aspirates from children with URI, and its levels correlated with clinically detectable wheezing (71). Since overexpression of IL-11 in airways has been shown to produce airway inflammation, airway wall thickening, and bronchial hyperresponsiveness (72), the induction of this cytokine by virus infections suggests that it may play an important role in virus-induced bronchial hyperresponsiveness and asthma.

Mechanisms of virus-induced cytokine production

The degree of redundancy among cytokine functions may suggest that even if one important molecule's functions can be blocked, another related molecule may fulfil a similar role, thereby rendering a treatment aimed at one specific molecule ineffective. Studies have recently started to examine the mechanisms of rhinovirus induction of proinflammatory cytokines and adhesion molecules, in order to determine whether there is a common mechanism, such as transcription factor activation.

We have demonstrated that induction of IL-8 occurs via activation of the transcription factors AP-1 and NFκB (73), while that of ICAM-1 occurs via NFκB alone (41). Zhu et al. (18) studied the intracellular mechanisms of IL-6 induction – factors binding to the NFκB transcription factor-binding site on the IL-6 promoter were again shown to be important. These *in vitro* data suggest that inhibition of NFκB might suppress rhinovirus induction of a number of proinflammatory cytokines and therefore reduce the severity of rhinovirus-induced asthma exacerbations. Similar data also indicate an important role for NFκB in RS virus induction of IL-1α, IL-6, IL-8, and IL-11 (74, 75). However, other workers have found NFIL-6 to be required as well as NFκB (76, 77). These data suggest that inhibition of more than one transcription factor, or transcription factor family, may be required to downregulate virus-induced cytokine synthesis. Of interest is the fact that aspirin, which is known to inhibit NFκB activation, inhibited RS virus induction of all the above cytokines *in vitro* (74).

The potential roles of these transcription factors have been suggested by *in vitro* studies, but *in vivo* confirmation is required. Furthermore, it is not known yet whether blocking the activity of a single transcription factor is sufficient to inhibit virus induction of proinflammatory cytokines either *in vitro* or *in vivo*, as it is not known what effect aspirin has on other transcription factor activities (78). Nevertheless, activity blocking several of the right transcription factor families (those implicated in disease) might still be a desirable property of a potential treatment. Further studies on the interactions between virus infection and transcription factors and their blockade are clearly required.

Such interesting observations on the pathogenesis of virus-induced asthma exacerbations are beginning to suggest new candidate molecules that might represent targets for novel therapeutic interventions. It is hoped that further advances in our understanding of the cellular and molecular mechanisms involved will lead to clearer identification of such targets, and the development of blocking strategies suitable for testing in the clinic.

Virus infections and allergic sensitization

A further subject attracting considerable recent attention is the interaction between virus infections and the development of allergy or asthma. It is known that simultaneous virus infection and positive specific IgE for inhalant allergens have a much higher odds ratio for the development of wheezing than any of the factors alone (79). Schwarze et al. (80) used a murine model to demonstrate that RS virus infection not only produces airway hyperresponsiveness in the acute phase, but also subsequently enhances allergen sensitization, both phenomena being associated with pulmonary eosinophilic infiltration. A further recent study in mice has demonstrated that acute infection with either RS virus or influenza virus greatly enhances allergic sensitization to inhaled allergen, in association with anaphylaxis and increased specific IgG1 (81). An enhanced reaction to allergen inhalation in allergic patients experimentally infected with rhinovirus has also been demonstrated (30).

These data suggest that concurrent virus infection can increase the airway response to allergens (by increasing penetration of allergens through the damaged epithelium?), but little is known about a possible reverse interaction. Effective virus clearance requires effective Th1-type responses. Asthma is clearly associated with Th2-type responses, and might therefore be expected to be associated with less efficient virus clearance or more severe infections (Fig. 2). Several studies confirm that asthmatic subjects have more severe symptoms during virus infections (3), but it is not currently known whether the virus load or resi-

dence time is greater in asthmatic than normal subjects.

Does RS virus bronchiolitis lead to the development of asthma?

In addition to the above observations indicating that viral infection can increase allergic sensitization, several studies have found an increased incidence of asthma in children with a history of childhood bronchiolitis. A recent study comparing cases of RS virus bronchiolitis in children found a 23% incidence of asthma compared with 1% in matched control cases (82). These data suggest either that the development of bronchiolitis (as opposed to uncomplicated upper respiratory RS virus infection) with early RS virus infection is a marker for the later development of atopy and asthma, or that severe infection with RS virus leading to bronchiolitis actually plays a causal role in redirecting the immune response toward a Th2 phenotype, and contributes directly to the risk of developing asthma later in life. There are some *in vitro* data to support the latter hypothesis, in that responses to certain RS virus proteins can lead to the development of lung eosinophilia and Th2-type cytokine release (83). *In vivo* data also support this hypothesis (37), although the distinction between RS virus bronchiolitis serving as a marker for, or acting as a causative event in the later development of, asthma can be achieved only by the execution of appropriately designed prospective follow-up studies.

The role of respiratory virus infections in the inception of asthma

It is disputed whether exposure to a respiratory viral infection in the first months of life can predispose to or protect against the development of atopy and asthma (84). The above data confirm that concurrent respiratory viral infection and allergen exposure lead to increased allergen sensitization compared with allergen exposure alone. These data and those on the role of RS virus bronchiolitis in the later development of allergy would suggest that childhood respiratory viral infection is likely to increase the risk of development of allergy or asthma.

It has been argued that a decline in recent years in the incidence of serious infectious diseases such as tuberculosis, measles, and whooping cough, combined with a trend toward smaller families, less overcrowding of homes, and improved sanitation, resulting in fewer childhood respiratory viral infections, has, paradoxically, caused an increase in the incidence of atopy and asthma in developed countries (85). West African children infected with wild-type measles virus during measles epidemics (producing severe illness, and therefore, presumably, marked Th1-type response) were less likely to become atopic than those that had been vaccinated with measles vaccine (presumably producing a much more mild immune response) (86). Atopy was also less likely in Italian students seropositive for hepatitis A (87) and in Japanese children with positive delayed-type hypersensitivity responses to *Mycobacterium tuberculosis* (88). The number of older siblings was inversely related to development of atopy, presumably because of the higher number of infections circulated within larger families (85). These studies support the hypothesis that the early exposure of infants to infectious agents stimulates their naïve immune system in some way that reduces the potential development of allergy later in life, possibly by stimulating a Th1-like lymphocyte expansion which suppresses Th2 lymphocyte responses associated with atopy.

Animal studies support this hypothesis in that it has been shown that pulmonary infection with attenuated *M. bovis* given 4 or 12 weeks before allergen challenge reduced allergen-induced airway eosinophilia and T-cell IL-5 release in a murine model of asthma (89). The same effect was not observed in IFN-γ receptor-deficient mice, suggesting that IFN-γ production in response to the mycobacterial infection was an important mechanism in the protective effect.

Similar results have been recently reported with subcutaneous killed *M. vaccae* (90). In this study, bacterial infection was administered after the allergen response was established, but it still inhibited IgE levels and T-cell IL-5 production.

It is likely that the dose and perhaps route and timing of such infections are important in determining the degree of protection against Th2-type responses, as early BCG vaccination offered no protection against the development of asthma (91). These data agree with those of Shirakawa et al., who showed that infection sufficient to produce delayed-type hypersensitivity responses to *M. tuberculosis* protected against the presence of atopy, while BCG vaccination in the absence of delayed-type hypersensitivity responses did not (88).

Concluding remarks

Respiratory viruses, particularly rhinovirus in all age groups, and RS virus in infants and young children, are important triggers of acute exacerbations of wheezing illness or asthma. The mechanisms involved in virus-induced asthma exacerbations are complex and incompletely

understood. The available evidence implicates infiltration of lymphocytes, eosinophils, and neutrophils. The proinflammatory products of these cells and of virus-infected epithelial cells themselves play complex and interacting roles in producing lower airway inflammation. A clearer understanding of the important regulating molecules in this process is likely to lead to identification of targets for development of novel therapeutic interventions for the future. At present, high-dose inhaled or oral steroids are the mainstay of treatment, although response is unsatisfactory.

The role of respiratory virus infections early in life, particularly with rhinovirus and RS virus, is controversial. There is good evidence that infections inducing strong type-1 immune responses (including frequent respiratory virus infections) can lead to reductions in allergic sensitization, and therefore, perhaps, to reductions in asthma. However, there is also direct evidence that virus infection at the time of allergen exposure can increase allergic sensitization, and that RS virus bronchiolitis is at least a marker for the later development of asthma, and may even play a causative/contributory role by inducing type-2 responses in certain circumstances. These data appear to be contradictory, but different responses may easily occur at different times of life, in different environmental circumstances, with different viruses and in different hosts!

I hope that strategies (be they bacterial or viral, infections or vaccinations, or indeed simulations thereof such as the action of IL-12 or IFN-γ) aiming to promote type-1 responses in those with excessive type-2 responses (atopic or asthmatic subjects) can in the future reduce the impact of allergic disease. It is also to be hoped that more effective antiviral or anti-virus-induced inflammation therapy will reduce the impact of acute virus infections on asthma exacerbations and allergic sensitization occurring during the acute respiratory illness.

References

1. Chauhan AJ, Johnson A-WBR, Xie P, et al. Virus infections in infants and children admitted to hospital with acute respiratory illness (submitted).
2. Johnston SL, Holgate ST. Epidemiology of viral respiratory tract infections. In: Myint S, Taylor-Robinson D, editors. Viral and other infections of the human respiratory tract. London: Chapman & Hall, 1996:1-38.
3. Pattemore PK, Johnston SL, Bardin PG. Viruses as precipitants of asthma symptoms. I. Epidemiology. Clin Exp Allergy 1992;22:325-36.
4. Johnston SL, Sanderson G, Pattemore PK, et al. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. J Clin Microbiol 1993;31:111-17.
5. Ireland DC, Kent J, Nicholson KG. Improved detection of rhinovirus in nasal and throat swabs by semi-nested RT-PCR. J Med Virol 1993;40:96-101.
6. Myint S, Johnston S, Sanderson G, Simpson H. Evaluation of nested polymerase chain methods for the detection of human coronaviruses 229E and OC43. Mol Cell Probes 1995;8:357-64.
7. Freymuth F, Vabret A, Galateau-Salle F, et al. Detection of respiratory syncytial virus, parainfluenza virus 3, adenovirus and rhinovirus sequences in respiratory tract of infants by polymerase chain reaction and hybridization. Clin Diagn Virol 1997;8:31-40.
8. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9–11-year-old children. BMJ 1995;310:1225-8.
9. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. BMJ 1993;307:982-6.
10. Johnston SL, Pattemore PK, Sanderson G, et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time trend analysis. Am J Respir Crit Care Med 1996;154:654-60.
11. Campbell MJ, Holgate ST, Johnston SL. Trends in asthma mortality. BMJ 1998;315:1012.
12. Durham S. ABC of allergies. Summer hay fever. BMJ 1998;316:843-5.
13. Wilson NW, Silverman M. Treatment of acute, episodic asthma in pre-schoolchildren using intermittent high dose inhaled steroids at home. Arch Dis Child 1990; 65:407-10.
14. Stick SM, Burton PR, Clough JB, et al. The effects of inhaled beclomethasone dipropionate on lung function and histamine responsiveness in recurrently wheezy infants. Arch Dis Child 1995;73:327-32.
15. Doull IJM, Lampe FC, Smith S, Schreiber J, Freezer NJ, Holgate ST. Effect of inhaled corticosteroids on episodes of wheezing associated with viral infection in school age children: randomised double blind placebo controlled trial. BMJ 1997;315:858-62.
16. Johnston SL. Problems and prospects of developing effective therapy for common cold viruses. Trends Microbiol 1997;5:58-63.
17. Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of human respiratory epithelial cell line with rhinovirus: induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. J Clin Invest 1995;96:549-57.
18. Zhu Z, Tang W, Ray A, et al. Rhinovirus stimulation of interleukin-6 *in vivo* and *in vitro*. J Clin Invest 1996;97:421-30.
19. Bates PJ, Sanderson G, Holgate ST, Johnston SL. A comparison of RT-PCR, *in situ* hybridisation and *in situ* RT-PCR for the detection of rhinovirus infection in paraffin sections. J Virol Methods 1997;67:153-60.
20. Johnston SL, Papi A, Bates PJ, Mastronarde JG, Monick MM, Hunninghake GW. Low grade rhinovirus infection induces a prolonged release of IL-8 in pulmonary epithelium. J Immunol 1998;160:6172-81.
21. Papadopoulos NG, Sanderson G, Hunter J, Johnston SL. Rhinoviruses replicate effectively at lower airway temperatures (submitted).
22. Gern JE, Galagan DM, Jarjour NN, Dick EC, Busse WW. Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. Am J Respir Crit Care Med 1997;155:1159-61.
23. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. Am J Respir Crit Care Med 1995;151:879-86.

24. Bates PJ, Bardin PG, Fraenkel DJ, Sanderson G, Holgate ST, Johnston SL. Localisation of rhinovirus in the bronchial epithelium of experimentally infected human volunteers. Am J Respir Crit Care Med 1998;157:A25.
25. Teran LM, Johnston SL, Schröder J, Church MK, Holgate ST. Role of nasal interleukin-8 in neutrophil recruitment and activation in children with virus-induced asthma. Am J Respir Crit Care Med 1997;155:1362-6.
26. Grunberg K, Timmers MC, Smits HH, et al. Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavage in asthmatic subjects *in vivo*. Clin Exp Allergy 1997;27:36-45.
27. Johnston SL, Papi A, Monick MM, Hunninghake GW. Rhinoviruses induce interleukin-8 mRNA and protein production in human monocytes. J Infect Dis 1997; 175:323-9.
28. Gern JE, Dick EC, Lee WM, et al. Rhinovirus enters but does not replicate inside monocytes and airway macrophages. J Immunol 1996;156:621-7.
29. Grunberg K, Smits HH, Timmers MC, et al. Experimental rhinovirus 16 infection. Effects on cell differentials and soluble markers in sputum of asthmatic subjects. Am J Respir Crit Care Med 1997;156:609-16.
30. Calhoun WJ, Dick EC, Schwartz LB, Busse WW. A common cold virus, rhinovirus 16, potentiates airway inflammation and segmental allergen bronchoprovocation in allergic subjects. J Clin Invest 1994;94:2200-8.
31. Teran L, Seminario MC, Shute JK, et al. RANTES, MIP-1α and the eosinophil product MBP are released into upper respiratory secretions during virus-induced asthma exacerbations in children. J Infect Dis 1998 (in press).
32. Trigg CJ, Nickolson KG, Wang JH, et al. Bronchial inflammation and the common cold: a comparison of atopic and non-atopic individuals. Clin Exp Allergy 1996; 26:665-76.
33. Romagnani S. Induction of Th1 and Th2 responses. Immunol Today 1992;13:379-81.
34. Coyle AJ, Erard F, Bertrand C, Walti S, Pircher H, Le Gros G. Virus-specific CD8+ cells can switch to interleukin 5 production and induce airway eosinophilia. J Exp Med 1995;181:1229-33.
35. Hussell T, Baldwin CJ, O'Garra A, Openshaw PJM. $CD8^+$ T cells control Th2-driven pathology during pulmonary respiratory syncytial virus infection. Eur J Immunol 1997;27:3341-9.
36. Gern JE, Galagan DM, Jarjour NN, Kelly EAB, Busse WW. Cytokine and rhinovirus 16 (RV16) RNA in the lower airway of experimentally infected subjects. Am J Respir Crit Care Med 1996;153:A17.
37. Roman M, Calhoun WJ, Hinton KL, et al. Respiratory syncytial virus infection in infants is associated with predominant Th-2-like response. Am J Respir Crit Care Med 1997;156:190-5.
38. Hussell T, Khan U, Openshaw PJM. IL-12 treatment attenuates Th2 and B cell responses but does not improve vaccine-enhanced lung illness. J Immunol 1997;159:328-34.
39. Terajima M, Yamaha M, Sekizawa K, et al. Rhinovirus infection of primary cultures of human tracheal epithelium: role of ICAM-1 and IL-1β. Am J Physiol 1997;273:L749-59.
40. Sethi SK, Bianco A, Allen JT, Knight RA, Spiteri MA. Interferon-gamma (IFN-γ) down-regulates the rhinovirus-induced expression of intercellular adhesion molecule-1 (ICAM-1) on human airway epithelial cells. Clin Exp Immunol 1997;110:362-9.
41. Papi A, Johnston SL. Rhinovirus infection induces expression of its own receptor ICAM-1 via increased NFκB mediated transcription. 1998 (submitted).
42. Sharon RF, Grunberg K, van Krieken JHJM, Sterk PJ. Rhinovirus (RV-16) infection enhances ICAM-1 expression in bronchial mucosal biopsies of mildly asthmatic subjects, regardless of inhaled steroid treatment. Am J Respir Crit Care Med 1998;157:A22.
43. Stark JM, Godding V, Sedgewick JB, Busse WW. Respiratory syncytial virus infection enhances neutrophil and eosinophil adhesion to cultured respiratory epithelial cells. Roles of CD18 and intercellular adhesion molecule-1. J Immunol 1996;156:4774-82.
44. Arnold R, Konig W. ICAM-1 expression and low-molecular-weight G-protein activation of human bronchial epithelial cells (A549) infected with RSV. J Leukoc Biol 1996;60:766-71.
45. Becker S, Reed W, Henderson FW, Noah TL. RSV infection of human airway epithelial cells causes production of the chemokine RANTES. Am J Physiol 1997; 272:L512-20.
46. Matsukura S, Kokubu F, Tomita T, et al. Expression of RANTES by normal airway epithelial cells after influenza virus A infection. Am J Respir Cell Mol Biol 1998; 18:255-64.
47. Coyle AJ, Tyers M, Church D, et al. Upregulation of chemokine receptors on bronchial epithelial cells *in vitro* following rhinovirus 14 infection. Am J Respir Crit Care Med 1998;157:A200.
48. Stellato C, Collins P, Ponath PD, et al. Production of the novel C-C chemokine MCP-4 by airway cells and comparison of its biological activity to other C-C chemokines. J Clin Invest 1997;99:926-36.
49. Cook DN, Beck MA, Coffman TM, et al. Requirement of MIP1α for an inflammatory response to viral infection. Science 1995;269:1583-5.
50. Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA. Mechanisms of bronchial hyperreactivity in normal subjects following upper respiratory tract infection. Am Rev Respir Dis 1976;113:523-7.
51. Aquilina AT, Hall WJ, Douglas RG, Utell MJ. Airway reactivity in subjects with viral upper respiratory tract infections: the effects of exercise and cold air. Am Rev Respir Dis 1980;122:3-10.
52. Folkerts G, Nijkamp FP. Virus induced airway hyperresponsiveness. Role of inflammatory cells and mediators. Am J Respir Crit Care Med 1995;151:1666-74.
53. Gern JE, Calhoun W, Swenson C, Shen G, Busse WW. Rhinovirus infection preferentially increases lower airway responsiveness in allergic subjects. Am J Respir Crit Care Med 1997;155:1872-6.
54. Skoner DP, Doyle WJ, Seroky J, Fireman P. Lower airway responses to influenza A virus in healthy allergic and nonallergic subjects. Am J Respir Crit Care Med 1996;154:661-4.
55. Johnston SL. Bronchial hyperresponsiveness and cytokines in virus-induced asthma exacerbations. Clin Exp Allergy 1997;27:7-9.
56. Killingsworth CR, Robinson NE, Adams T, Maes RK, Berney C, Rozanski E. Cholinergic reactivity of tracheal smooth muscle after infection with feline herpesvirus-I. J Appl Physiol 1990;69:1953-60.
57. Buckner CK, Songsiridej V, Dick EC, Busse WW. *In vivo* and *in vitro* studies of the use of the guinea pig as a model for virus-provoked airway hyperreactivity. Am Rev Respir Dis 1985;132:305-10.
58. Fryer AD, Jacoby DB. Parainfluenza virus infection damages inhibitory M_2 muscarinic receptors on pulmonary parasympathetic nerves in the guinea-pig. Br J Pharmacol 1991;102:267-71.
59. Sorkness R, Clough J, Castelman W, Lemanske R. Virus induced airway obstruction and parasympathetic hyperre-

sponsiveness in adult rats. Am Rev Respir Dis 1994;150:28-34.
60. Fryer AD, Yarkony KA, Jacoby DB. The effect of leukocyte depletion on pulmonary M_2 muscarinic receptor function in parainfluenza virus-infected guinea-pigs. Br J Pharmacol 1994;112:588-94.
61. Jacoby DB, Gleich GJ, Fryer AD. Human eosinophil major basic protein is an endogenous allosteric antagonist at the inhibitory muscarinic M_2 receptor. J Clin Invest 1993;91: 1314-18.
62. Costello RW, Schofield BH, Kephart GM, Gleich GJ, Jacoby DB, Fryer AD. Localization of eosinophils to airway nerves and effect on neuronal M_2 muscarinic receptor function. Am J Physiol 1997;273:L9344.
64. Evans CM, Fryer AD, Jacoby DB, Gleich GJ, Costello RW. Pretreatment with antibody to eosinophil major basic protein prevents hyperresponsiveness by protecting neuronal M_2 muscarinic receptors in antigen-challenged guinea pigs. J Clin Invest 1997;100:2254-62.
65. Fryer AD, Jacoby DB. Function of pulmonary M_2 muscarinic receptors in antigen challenged guinea-pigs is restored by heparin and poly-L-glutamate. J Clin Invest 1992;90:2292-8.
66. Ladenius ARC, Folkerts G, van der Linde HJ, Nijkamp FP. Potentiation by viral respiratory infection of ovalbumin-induced guinea pig tracheal hyperresponsiveness: role for tachykinins. Br J Pharmacol 1995;115:1048-52.
67. Dusser DJ, Jacoby DB, Djokic TD, Rubinstein I, Borson DB, Nadel JA. Virus induces airway hyperresponsiveness to tachykinins: role of neutral endopeptidase. J Appl Physiol 1989;67:1504-11.
68. De Gouw HWFM, Grunberg K, Schot R, Kroes ACM, Dick EC, Sterk PJ. Relationship between exhaled nitric oxide and airway hyperresponsiveness following experimental rhinovirus infection in asthmatic subjects. Eur Respir J 1998;11:126-32.
69. Folkerts G, van der Linde HJ, Nijkamp FP. Virus-induced airway hyperresponsiveness in guinea pigs is related to a deficiency in nitric oxide. J Clin Invest 1995;95:26-30.
70. Sanders SP, Siekierski ES, Porter JD, Richards SM, Proud D. Nitric oxide inhibits rhinovirus-induced cytokine production and viral replication in a human respiratory epithelial cell line. J Virol 1998;72:934-42.
71. Einarsson O, Geba GP, Zhu Z, Landry M, Elias JA. Interleukin-11: stimulation *in vivo* and *in vitro* by respiratory viruses and induction of airways hyperresponsiveness. J Clin Invest 1996;97:915-24.
72. Tang W, Geba GP, Zheng T, et al. Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling and airways obstruction. J Clin Invest 1996;98:2845-53.
73. Dumitrascu D, Sanderson G, Mukaida N, Matsushima K, Holgate ST, Johnston SL. IL-8 and AP-1 and κB-like sites are necessary for rhinovirus induction of IL-8 promoter activity in A549 cells. J Allergy Clin Immunol 1997;99:S403.
74. Bitko V, Velazquez A, Yang L, Yang Y-C, Barik S. Transcriptional induction of multiple cytokines by human respiratory syncytial virus requires activation of NF-κB and is inhibited by sodium salicylate and aspirin. Virology 1997;232:369-78.
75. Fiedler MA, Wernke-Dollries K, Stark JM. Inhibition of viral replication reverses respiratory syncytial virus-induced NF-κB activation and interleukin-8 gene expression in A549 cells. J Virol 1996;70:9079-82.
76. Jamaluddin M, Garofalo R, Ogra PL, Brasier AR. Inducible transcriptional regulation of the NF-IL6 transcription factor by respiratory syncytial virus infection of pulmonary epithelial cells. J Virol 1996;70:1554-63.
77. Mastronarde JG, He B, Monick MM, Mukaida N, Matsushima K, Hunninghake GW. Induction of interleukin (IL)-8 gene expression by respiratory syncytial virus involves activation of nuclear factor (NF)-κB and NF-IL-6. J Infect Dis 1996;174:262-7.
78. Kopp E, Ghosh S. Inhibition of NF-κB by sodium salicylate and aspirin. Science 1994;265:956-9.
79. Duff AL, Pomeranz ES, Gelber LE, et al. Risk factors for acute wheezing in infants and children: viruses, passive smoke, and IgE antibodies to inhalant allergens. Pediatrics 1993;92:535-40.
80. Schwarze J, Hamelmann E, Bradley KL, Takeda K, Gelfand EW. Respiratory syncytial virus results in airway hyperresponsiveness and enhanced airway sensitization to allergen. J Clin Invest 1997;100:226-33.
81. O'Donnell DR, Openshaw PJM. Anaphylactic sensitization to aeroantigen during respiratory virus infection. Clin Exp Allergy 1998 (in press).
82. Sigurs N, Bjarnason R, Bergsson F, et al. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. Pediatrics 1995;95:500.
83. Alwan WH, Kozlowska WJ, Openshaw PJM. Distinct types of lung disease caused by functional subsets of antiviral T cells. J Exp Med 1994;179:81-9.
84. Shaheen SO. Discovering the causes of atopy. BMJ 1997;314:987-8.
85. Strachan DP. Hay fever, hygiene and household size. BMJ 1989;299:1259-60.
86. Shaheen SO, Aaby P, Hall AJ, et al. Measles and atopy in Guinea-Bissau. Lancet 1996;347:1792-6.
87. Matricardi PM, Rosmini F, Ferrigno L. Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. BMJ 1997;314:999-1003.
88. Shirakawa T, Enomoto T, Shimazu S-I, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. Science 1997;275:77-9.
89. Erb KJ, Holloway JW, Sobeck A, Moll H, Le Gros G. Infection of mice with *Mycobacterium bovis*-Bacillus Calmette-Guérin (BCG) suppresses allergen-induced airway eosinophilia. J Exp Med 1998;187:561-9.
90. Wang CC, Rook GAW. Inhibition of an established response to ovalbumin in BALB/c mice by killed *Mycobacterium vaccae*. Immunology 1998;93:307-13.
91. Alm JS, Lilja G, Pershagen G, Scheynius A. Early BCG vaccination and development of atopy. Lancet 1997; 350:400-3.