

Review article

Respiratory viruses: do they protect from or induce asthma?

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Links between viral respiratory tract infections and asthma have been recognized for over 40 years, but there has been a considerable increase in interest and research into this topic over the last decade. This interest has been fuelled by the significant rise in the prevalence of asthma in many countries and by new insights into interactions between infection and allergy and how this affects the developing immune system. Many studies have been published exploring the associations between asthma and infection often resulting in contradictory findings. On the one hand some studies have suggested that infections in early childhood protect against the later development of allergic diseases (1) such as asthma – the so-called ‘hygiene hypothesis’. On the other hand there are also studies linking early life respiratory tract infections to an increased risk of asthma in later life, suggesting that viral infections may promote allergic sensitization and the development of asthma. The aim of this article therefore is to explore the complex interactions that exist between childhood viral respiratory tract infections and the development of asthma.

Studies linking viral respiratory tract infections with the development of asthma

A number of epidemiological studies have documented a marked rise in the prevalence of asthma and other allergic

diseases in western societies over the past 30–40 years. The International Study of Asthma and Allergies in Childhood has allowed a standardized assessment of the prevalence of asthma and found striking differences in the prevalence rates between countries, with higher rates of disease in Westernised countries (2). Among the factors proposed as being responsible for these differences are increased exposure to indoor allergens and outdoor pollutants, obesity, dietary changes, sedentary lifestyle, alterations in gut flora and changing patterns of infection in childhood. In 1989 Strachan observed that the prevalence of hay fever in a cohort of adults was inversely related to family size, and even more so to the presence of older siblings (3). On the basis of these observations he proposed that infections in early childhood confer a protective effect against the later development of allergy. This premise came to be known as the ‘hygiene hypothesis’.

Since this original observation there have been numerous studies exploring the relationship between early life infections and allergy. These studies have used a number of different methods of assessing infectious episodes in childhood. Some studies have used surrogate markers for increased exposure to infections such as family size, daycare attendance and exposure to farm animals. Others have measured exposure to specific infectious diseases such as hepatitis A, measles and toxoplasma with serology or disease reports. A number of differing

outcome measures as markers of allergic diseases have also been utilized. Among these are asthma, allergic rhinitis, eczema, skin-prick testing and serum IgE levels. However in this article we will focus only on the associations reported between viral respiratory tract infections (or surrogate markers for them) and asthma itself as an outcome measure.

Studies showing a protective effect of respiratory tract infections on asthma

The first studies suggesting an inverse link between respiratory tract infections and asthma were observational studies in two isolated island communities – Tristan da Cunha in the Atlantic and the Western Carolina islands in the Pacific. Because of their isolation respiratory virus infections are rare in these populations, and this is confirmed by serological studies that showed a low prevalence of antibodies to respiratory viruses (4, 5). However these populations were found to have an extremely high prevalence of asthma, with a rate of asthma attacks of 161/1000/year noted in 1971 in Tristan Da Cunha compared to a rate of 25.6/1000/year in the UK (6).

Studies in other populations confirmed this inverse relationship between rates of respiratory tract infections and asthma. In Fiji differing rates of hospital admissions for asthma and pneumonia between the Fijian and Indian populations were noted. The annual hospital admission rate for asthma was three times higher in Indian children compared to the Fijians, but the converse was seen for hospital admission rates for pneumonia, with a higher rate in the indigenous Fijians (7). The same author assessed the community prevalence of asthma, as defined by current wheeze and bronchial hyperreactivity, and the incidence of respiratory tract infections in the two populations (8).

The incidence of infections as defined by productive cough and nasal discharge was 6.0% in Indian children and 15.6% in Fijian children, whereas the prevalence of asthma in the two groups was 11.3% and 4.0%, respectively.

Although these studies are suggestive of a protective effect of respiratory infection on the development on asthma, their weakness is the potential confounding effect of genetic influences. The island populations of Tristan Da Cunha and the Western Carolinas are highly inbred and it has been suggested that a high proportion of the original inhabitants of Tristan Da Cunha were asthmatic. The differences in asthma prevalence between the two ethnic groups on Fiji could also be due to genetic differences between the populations.

The re-unification of Germany in 1990 provided investigators with a unique opportunity to study the prevalence of asthma in genetically similar populations, but with different levels of exposure to various environmental factors, including infections. It was postulated

that children from the former East Germany would have a higher incidence of respiratory tract infections than those in the West, because of the widespread use of childcare at an early age, larger families and overcrowding. The effect of this on the subsequent prevalence of asthma was assessed by von Mutius et al. in 1994 (9). They confirmed a higher rate of doctor-diagnosed bronchitis in East German children compared to those in the former West Germany, whereas the prevalence of current asthma in the two populations showed an inverse relationship at 3.9% and 5.9%, respectively. If one makes the reasonable assumption that the incidence of bronchitis reflects the incidence of respiratory tract infections, these data suggest a protective effect of respiratory tract infections on asthma in a genetically homogeneous population.

Daycare and family size

Other studies have attempted to evaluate the effect of childhood infections on asthma within the same population by defining groups with differing levels of exposure to respiratory tract infections.

Assessing the incidence of childhood respiratory tract infections on a population level is difficult, as these are often mild, self-limiting illnesses for which medical advice is not sought. Therefore investigators have utilized exposures that increase the risk of respiratory infections as surrogate markers of infection rates. The two exposures used most often in these types of studies have been daycare attendance and larger family size.

Children who attend daycare have a higher incidence of infections than those who are cared for at home (10). The infectious diseases found most frequently in children attending daycare are upper and lower respiratory tract infections and otitis media (11). The presence of siblings has also been found to increase the risk of developing lower respiratory tract infections (12). Therefore by comparing children who attend daycare or have multiple siblings with those cared for at home or with no siblings, it is possible to assess the effects of different levels of exposure to respiratory tract pathogens on the later development of asthma.

Daycare

There have been two large studies reporting a protective effect of daycare in infants on the incidence of asthma later in childhood.

The Tucson Children's Respiratory Study is a long-term, prospective longitudinal study of the risk factors for the development of asthma in childhood. Data from this project have been used to study the effects of various factors in infancy on the subsequent development of asthma. In a study from this cohort of children Ball et al. found an inverse relationship between the age of entering

daycare and the incidence of asthma at age 13 (13). This inverse relationship was also present between the number of siblings and asthma. At age 13 the incidence of asthma was 12% among children who had attended daycare and 21% in those who had not.

This finding was confirmed in children in Eastern Germany, in whom the prevalence of asthma at ages 5–14 was related to the age of entry to daycare (14). The authors also assessed the interaction between age of starting daycare and family size.

They found an increased prevalence of doctor-diagnosed asthma among children who entered daycare over the age of 2 years compared to those who entered in the first year of life. This effect was stronger in those families with fewer than four people living together.

Family size

Since the original observation of the inverse link between family size and hay fever, other studies have evaluated the effect of family size on asthma. A retrospective study of children aged 5–11 in the UK using parental questionnaires found that the prevalence of asthma was negatively associated with the number of children in the family (15). Children in families having more than three children were 50% less likely to have asthma than those with no siblings.

This inverse relationship between family size and the prevalence of asthma was confirmed in a cohort of Australian children (16). This study showed a significant protective trend with increasing numbers of siblings but no further protective effect for three or more siblings. The protective effect was greater for the presence of older siblings than for younger siblings and for a short birth interval to the next sibling.

This observation of a protective effect of family size on childhood asthma has been extended to adults. In a cohort of adults aged 20–44, the prevalence of symptoms suggestive of asthma – ‘wheeze with breathlessness’, ‘wheeze without a cold’ and ‘asthma attacks’ – was related to family size (17). As in children, a significant inverse relationship between the two was found.

These findings therefore suggest that factors in early childhood such as daycare and large families that increase exposure to respiratory viruses have a protective effect on the subsequent development of asthma, supporting the hygiene hypothesis.

The evidence for this is based on epidemiological data but studies on the development of the infant immune system have now provided a biological basis for this hypothesis.

Biological basis for a protective effect of early life infections

Our understanding of the regulation of the immune responses involved in allergic disease has been

transformed with the discovery of different subsets of T helper lymphocytes (18). These T lymphocyte subsets were termed T_H1 and T_H2 lymphocytes and they differ in the pattern of cytokines they secrete. Lymphocytes with a T_H1 phenotype produce the cytokines IFN- γ and IL-2, and are involved in host defence against microbial infections including viruses. They contribute to the production of IgG and IgM and the development of cytotoxic T-cell responses. T_H2 lymphocytes on the other hand produce the cytokines IL-4, IL-5 and IL-13. They induce production of IgE and the maturation and recruitment of eosinophils, leading to an allergic inflammatory profile. T_H2 lymphocytes are believed to be intimately involved in the inflammatory cascade that underlies allergic diseases such as asthma and have been demonstrated to be present in the airways of asthmatics (19). This difference in T cell patterns of cytokine production also involves CD8+ (20) lymphocytes, and therefore in relation to the overall balance between the two phenotypes we prefer the term type 1 and type 2 responses rather than T_H1 and T_H2 , a term which should be reserved for the CD4+ subset alone.

Neonates are born with an immune response skewed towards a type 2 profile, manifested as an impaired production of IFN- γ . In infants at high risk of atopy (those with a family history of atopic diseases) this defect in type 1 immunity is even more pronounced (21). Over the first 5 years of life the immune response switches to a predominantly type 1 response, with increased production of IFN- γ over this period (22). In children with atopic disease this maturation occurs to a lesser degree and a consolidation of type 2 responses is seen.

The cytokine interleukin 12 (IL-12) is believed to play a central role in the regulation of immune differentiation of T lymphocytes. IL-12 is produced by antigen-presenting cells (APCs) and its production is enhanced by microbial products such as lipopolysaccharide and viral nucleic acid (23). IL-12 is believed to be the obligatory signal for the differentiation of na T cells into T_H1 cells. It has the effect of promoting type 1 responses by stimulating T cells and NK cells to produce IFN- γ , and suppressing the differentiation of T cells to a T_H2 phenotype.

APC responses are immature in infants and respiratory tract infections stimulate APCs to produce IL-12, also having the effect of increasing the numbers of APCs in the respiratory mucosa (24). It can be postulated therefore that a reduced frequency of infections on a genetic background of impaired type 1 immunity leads to further skewing of the immune system towards a type 2 phenotype, through reduced production of IL-12 (Fig. 1). Via this mechanism a reduction in childhood infectious illnesses, due to improved living standards and smaller family sizes, could lead to an increase in the prevalence of allergic diseases including asthma.

CENTRAL ROLE OF IL-12 IN MATURATION OF TYPE 1 IMMUNITY

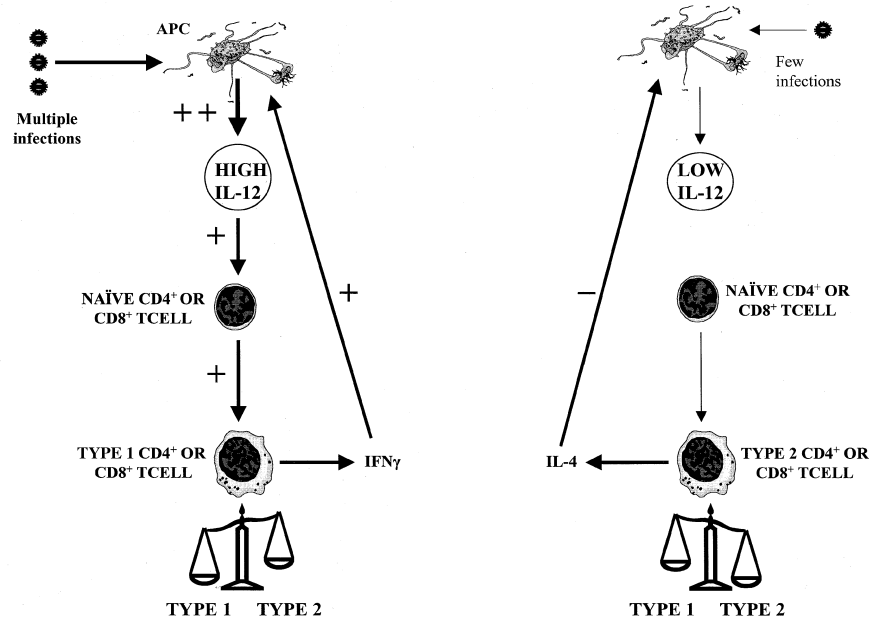


Figure 1. Central role of IL-12 in maturation of type 1 immunity.

Studies suggesting a causative effect of respiratory tract infections on asthma

Other groups have used parental reports of respiratory tract infections in children to measure exposure to respiratory viruses and evaluated this in relation to asthma. Accepting that daycare and the presence of siblings are surrogate markers for increased exposure to respiratory viruses, these studies would be expected to give similar results. However studies using reports of infectious episodes in fact seem to result in the opposite conclusion, i.e., that increased reporting of respiratory tract infections in early life favours the later development of asthma.

In a Norwegian study, Nystad et al. used a retrospective questionnaire survey to assess episodes of respiratory tract infection and the effect of daycare arrangements on asthma (25) in a cohort of school children aged 6–16 years. Asthma diagnosis was used only if it began after the age of 3. The authors found that attendance in full-time daycare before the age of 3 years was associated with an increased relative risk of early infections. A history of parental asthma was also associated with early infections. In addition they found a positive association between both full-time daycare and asthma, and early respiratory tract infections and asthma. The association between early infections and asthma (cOR 5.6 [3.9–8.0]) was stronger than that between daycare and asthma (cOR 1.5 [1.0–2.2]).

When early infections was included as a variable the estimated direct effect of daycare was small and nonsignificant.

Similar results were seen in a Finnish study where an inverse relationship between sibling number and asthma was found but parental reports that their children 'had more respiratory infections on average than other children of the same age' was associated with a greater risk of asthma (26).

An Australian study collected data regarding family size and upper and lower respiratory tract infections in the first year of life in a cohort of children born in 1988 (27). The prevalence of asthma was assessed at the age of 7 years. Two interviews were conducted in the first year of life (median ages 35 and 85 days) and the parents questioned regarding symptoms of upper and lower respiratory tract infections. The authors confirmed an inverse relationship between family size and asthma, but found a positive correlation between both upper and lower respiratory tract infections in the first year of life and asthma at age 7.

In another study from the Tucson group a cohort of children were enrolled at birth and parents asked to report symptoms suggestive of lower respiratory tract infections in the first 3 years of life (28). Children with symptoms were assessed by a paediatrician to confirm the diagnosis of lower respiratory tract infection. Assessments of asthma were made at ages 6 and 11 years and a higher prevalence of asthma was found in children who had had doctor-diagnosed pneumonia or lower respiratory tract infection in early life.

Therefore when daycare and family size are taken as proxy markers of increased exposure to respiratory tract infections a protective effect on subsequent asthma is seen. However when parental reports or doctor diagnosis

of respiratory tract infections are used as a measure of infection rates, a positive relationship results.

Resolving the paradox

The studies that have linked early lower respiratory tract infections to asthma suggest that they have a causative role in the development of the disease. These studies prove an association between the two but not the direction of that association.

Reverse causation is another possible explanation for this effect, i.e., children predisposed to asthma may be more likely to develop lower respiratory tract infections. This effect could be mediated through a common genetic risk factor that results in impaired type 1 immunity. Children with impaired type 1 immune responses would be expected to have both a greater severity of, and therefore a higher incidence of symptomatic or reported viral infections, and a higher prevalence of type 2 mediated diseases such as asthma. There is now evidence that this may explain the positive association between respiratory tract infections and asthma.

The Prevention and Incidence of Asthma and Mite Allergy Study is a prospective birth cohort study assessing the interactions between a parental history of allergic disease, environmental factors and childhood infections (29). It has found that infants with a parental history of allergic disease who had siblings or attended daycare had a higher incidence of symptomatic lower respiratory tract infections, but not upper respiratory tract infections, compared to children with no family history of atopy. The risk increased with exposure to greater numbers of children and if both parents were atopic.

Further evidence for a unique association of lower respiratory tract infections with asthma, compared to other infections comes from a German study that performed a separate analysis of the effects of upper and lower respiratory tract infections. In this study Illi et al. used parental diaries and questionnaires to assess the incidence of childhood infectious diseases, and related them to a doctor's diagnosis of asthma at age 7 (30). They also performed bronchial reactivity testing in a subgroup of the cohort. They found a strong negative correlation between recurrent runny nose in the first year of life and asthma diagnosis, current wheeze and bronchial hyperreactivity at age 7.

However the number of lower respiratory tract infections in the first 3 years of life was positively associated with the same three outcome measures. The study further categorized lower respiratory tract infections as either wheezing or non-wheezing and showed a strong positive association for the former, but a non-significant association for the latter. The frequency of repeated lower respiratory tract infections (≥ 2 infections before age 3) was also significantly higher in those children with a family history of atopy compared to those without.

These studies suggest that children with a family history of atopy have a greater risk of lower respiratory tract infection. Another possibility is that these findings are due to more symptoms in atopic individuals during respiratory viral tract infection, and therefore the seemingly higher incidence of infections is influenced by reporting bias.

A recent study in adults attempted to answer this question by assessing the frequency and severity of rhinovirus infections in cohabiting couples who were discordant for the presence of atopy and asthma (31). In this study regular sampling of the upper respiratory tract was performed, regardless of symptoms, to correct for the possibility of reporting bias. The authors found the same frequency of infections in the partners but that the partners with atopic asthma developed more severe lower respiratory tract symptoms and falls in PEF.

This increased severity of respiratory virus infections in asthmatics may be due to impaired type 1 immune responses. PBMCs from atopic asthmatics exposed *ex vivo* to live rhinovirus produce lower levels of IL-12 and IFN- γ compared to normal controls (32). In a study using experimental rhinovirus infection, the balance of type 1 and type 2 cytokines in sputum was correlated with symptoms and viral clearance. A low IFN- γ /IL-5 ratio (i.e. a type 2 skewed response) resulted in higher peak cold symptoms and slower viral clearance (33).

Whether these findings in adults with established asthma are applicable to children at high risk of developing allergic disease remains to be seen. However there is also evidence of altered immune responses to respiratory viruses in adult atopic subjects without asthma. A study of experimental rhinovirus infection in atopic subjects and normal controls found that those atopics with neutralizing antibodies developed severe colds when infected, whereas normal subjects with antibodies developed mild symptoms only (34). A study using nasal lavage in naturally acquired colds found lower levels of IL-10 and a trend towards lower levels of IFN- γ in the atopic subjects during the acute phase of the cold.

This was associated with higher levels of pro-inflammatory mediators in atopic subjects at convalescence compared to normals, suggesting a prolonged inflammatory response in the atopics (35).

Deficient type 1 immune responses in atopic individuals and a consequent increase in symptoms during viral respiratory tract infections may explain why the results of studies on the effect of respiratory tract infections on asthma differ according to the way the infectious load is measured. Larger family size and daycare are surrogate markers for the overall infectious burden in the first years of life. This probably includes the effect of gastrointestinal infections, which occur with a higher frequency in children attending daycare (36) and that have also been shown to have a protective effect on the development of asthma (37). These studies measure exposure to infections independently of the host response to infection and show

a protective effect consistent with the hygiene hypothesis. However in those studies that use symptomatic lower respiratory tract infections the host response to infection enters the equation as a confounding factor. Studies that rely on symptomatic infections as a measure of infectious load probably select those individuals at high risk of developing asthma as they are more likely to develop symptoms with viral respiratory tract infection. This effect, rather than respiratory infections being a causative factor in asthma, probably explains the positive association found between the two (Fig. 2).

Respiratory syncytial virus and asthma

Perhaps even more controversial is the role of a specific respiratory tract virus – respiratory syncytial virus (RSV) – on the development of asthma. RSV is a single-stranded RNA virus that causes respiratory tract infections in humans. In the majority of cases it causes a self-limiting upper respiratory tract infection, but in infants under the age of 1 year it can cause a more serious infection of the lower respiratory tract – bronchiolitis. It is believed that RSV may have a unique effect on the infant respiratory tract in that it may be capable of inducing allergic sensitization and asthma. For over 40 years studies have linked RSV bronchiolitis with an increased prevalence of asthma in later childhood.

Recurrent wheezing after RSV bronchiolitis was first reported in 1959 (38). Since this initial observation numerous studies have been published reporting a positive association between RSV infection of the lower respiratory tract in infants and subsequent wheezing (39, 40). Comparing results from these studies is difficult due to differences in study design (prospective vs. retrospective), method of diagnosis (clinical vs. virological), severity of illness, study outcomes (asthma, wheeze,

atopy) and length of follow-up. However the majority of these studies have found that children with RSV bronchiolitis in the first year of life have an increased incidence of wheezing in later childhood. Controversy remains however, as to whether RSV plays an active role in inducing asthma or whether it simply identifies those already at risk.

A systematic review of studies of RSV bronchiolitis has been published (41). The authors reviewed 30 publications and identified studies that fulfilled the following criteria:

- 1 hospitalized infants
- 2 age less than 1 year
- 3 a virological confirmation of RSV
- 4 a control group of infants.

Reviewing the data from these studies the authors found a prevalence of wheezing in the first 5 years after the initial episode in the bronchiolitis group of 40% vs. 11% in the control group ($P < 0.001$). The prevalence of wheeze in the bronchiolitis group was double that of the control group (21% vs. 10%) between 5 and 10 years of follow-up, though this difference was not statistically significant. No differences in either a personal or family history of atopy between the two groups were observed.

Further follow-up data from one of the studies included in this review did report a strong association between RSV bronchiolitis and both allergic sensitization and the prevalence of asthma at the age of 7 (39). In this study 47 children who had been hospitalized for RSV bronchiolitis were compared with a matched control group recruited simultaneously. When assessed at age 7 the prevalence of asthma in the RSV group was 30% compared to 3% in the control group.

A multivariate analysis of risk factors for asthma found that RSV bronchiolitis had the highest independent risk ratio for asthma. This study was remarkable for the low

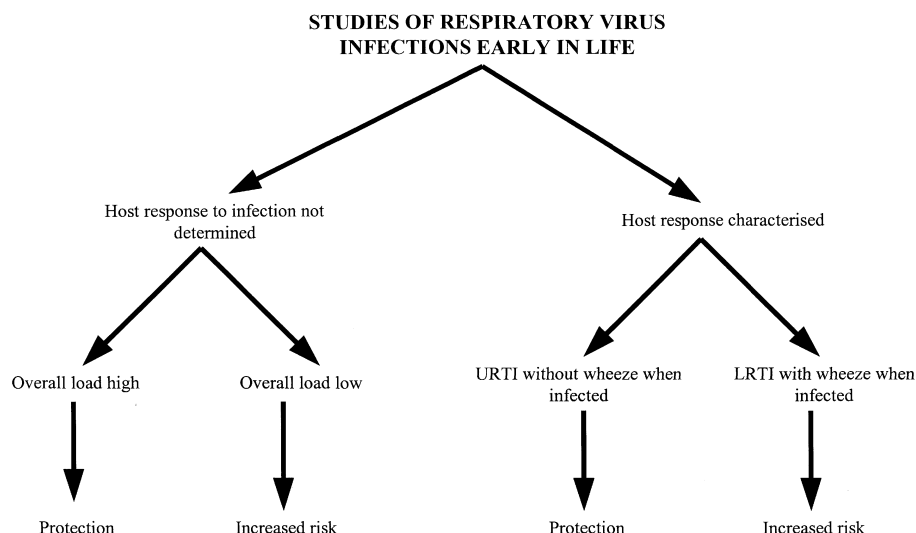


Figure 2. Studies of respiratory virus infections early in life.

prevalence of asthma in the control group of children and especially in those with a positive family history of asthma (prevalence 0%). This suggests that the method of selection of the control group involved negative selection for the risk of asthma. This may have occurred because the controls were age matched and recruited contemporaneously from the same city as the index cases. Thus the control children were probably exposed to RSV but did not develop bronchiolitis when exposed.

Immune responses to RSV in relation to wheezing

Viral infections induce a host response that is characterized by the production of a predominantly type 1 cytokine profile with secretion of IFN- γ and IL-2 and promotion of cell-mediated immunity. Research into the immune response to formalin-inactivated RSV has demonstrated that it can induce type 2 responses in animal models of the disease, and it has been proposed that this is a mechanism by which RSV may induce the development of asthma. However there are no studies with live RSV infection reporting this outcome – all such studies have found predominant type 1 responses, suggesting that the type 2 responses may be a feature unique to formalin-inactivated virus. These studies are obviously pivotally important in relation to RSV vaccine design, but their relevance to RSV bronchiolitis which is a primary infection with live RSV is clearly questionable.

Studies in humans have had mixed results with both type 1 and type 2 responses being demonstrated by different groups. Roman et al. studied the immune responses to RSV by measuring IFN- γ and IL-4 concentrations by ELISA in the supernatants of PBMC cultures from RSV-infected infants and healthy controls (42). They found that in RSV-infected infants IFN- γ production was sub-totally suppressed with IL-4 production suppressed to a lesser degree, resulting in a raised IL-4/IFN- γ ratio.

In a similar study intracellular levels of IL-4 and IFN- γ were measured by flow cytometry in stimulated PBMCs from healthy and RSV-infected infants (43). The infected group were again found to produce higher levels of IL-4 and less IFN- γ compared to the healthy controls.

The finding of a type 2 immune response during RSV infection can have two possible explanations. Firstly it may be the result of RSV inducing a type 2 response and in this way the virus may lead to an increased risk of developing asthma. Alternatively it may reflect an increased susceptibility to develop RSV disease in those infants with a pre-existing type 2 skewed immune response.

Resolving this chicken-and-egg question requires studies in which cytokine profiles are assessed prior to and during RSV infection. There is one such prospective study in which cytokine levels at birth in stored cord blood were correlated with subsequent RSV disease (44). Infants who subsequently developed RSV bronchiolitis were found to

have lower cord blood levels of IL-12 at birth compared to those infants who did not. This would suggest that the finding of a type 2 skewed response in RSV bronchiolitis is due to more severe disease expression in infants with pre-existing impaired type 1 immunity, rather than RSV inducing a type 2 response. Further evidence for this is the finding of a higher prevalence of a family history of atopy in infants hospitalized with bronchiolitis compared to those with mild RSV disease (45). Also studies in which cytokine levels have been correlated with disease severity have shown that infants with severe bronchiolitis had lower levels of the type 1 cytokines IFN- γ (46, 47) and IL-12 (48), and deficient IFN- γ production was still present several months after the infection (49).

A potential mechanism linking the immune response to RSV and the development of asthma is the pattern recognition receptor CD14. CD14 is a receptor for lipopolysaccharide (LPS) and other bacterial wall components and is present in membrane-bound and soluble forms. LPS-CD14 complexes are recognized by antigen-presenting cells which are then stimulated to produce IL-12 resulting in up-regulation of type 1 responses.

CD14 and other pattern recognition receptors are believed play a vital role in linking the innate and adaptive immune responses. A polymorphism in the CD14 gene that is associated with lower levels of soluble CD14 results in higher serum IgE levels and diminished type 1 function (50).

CD14 has also been shown to mediate the innate immune response to the fusion protein (F protein) of RSV in a mouse model (51). In the same study absence of another receptor – TLR4 – resulted in impaired viral clearance. Therefore genetic variations in expression of CD14 and other pattern recognition receptors may influence both the clinical expression of viral disease and the development of allergy.

Does RSV have a unique effect among respiratory viruses?

There is now evidence questioning whether RSV does in fact have a unique effect on the respiratory tract, or whether the association between early life viral infections and wheezing is a non-specific phenomenon also seen with other respiratory viruses. Some of the studies suggesting a link between RSV and wheezing did not confirm the diagnosis virologically but based it on a clinical diagnosis of lower respiratory tract symptoms and signs. A recent report using PCR in infants with a clinical diagnosis of acute bronchiolitis found that in 29% of cases the causative agent was rhinovirus (52).

Therefore as RSV is not the only cause of bronchiolitis in infants this raises the question of whether other respiratory viruses can have the same long-term effects. A study addressing this issue assessed the incidence of subsequent wheezing in children hospitalized during concurrent epidemics of RSV and influenza A lower

respiratory tract infections (53). Sixty percent of the children had two or more episodes of subsequent wheezing, but when the incidence of wheezing was examined in relation to the aetiology of the initial episode there was no difference in the effect of the two viruses.

In the Tucson cohort reported by Stein et al. virological sampling was performed during all reported lower respiratory tract infections in the first 3 years of life (54).

They found an increased point prevalence of frequent and infrequent wheeze in those infected with RSV up until age 11. However there was a progressive decrease in the prevalence of wheeze with increasing age, until at age 13 there was no significant difference between the index and control groups. Similar trends were seen for other respiratory viruses, although these were less consistent than the relationship with RSV.

These findings imply that wheezing following viral respiratory tract infection in infancy is not a phenomenon unique to RSV. The number of publications suggesting this may simply reflect the facts that

- 1 it is the most common viral respiratory tract infection in children of this age group;
- 2 diagnostic methods for other respiratory viruses were insensitive or unavailable at the time these studies were carried out (especially so for rhinoviruses);
- 3 other respiratory viruses were simply not looked for.

Persistent versus transient wheezing and the role of airway size

Another factor that leads to confusing results in studies of the relationship between respiratory viruses and asthma is the heterogeneity of wheezing disorders in childhood. Wheezing in children in response to viral infections is a common event, occurring in around 50% of children under the age of 3 (55). However long-term studies of wheezing children have shown that only around 15% have persistent wheezing in later childhood, while in the majority their wheezing remits. It is now recognized that children who wheeze in response to viral infections are a heterogeneous group and that differing wheezing disorders exist.

Martinez et al. studied 826 children from birth up to 6 years of age, and determined the incidence of wheezing in the first 3 years of life and at age 6 (55). Their findings led them to characterize wheezing children into two groups.

- 1 Children who had wheezing with a lower respiratory tract infection in the first 3 years of life but not at age 6, whom they termed 'transient wheezers'.
- 2 Children who wheezed at both ages whom they termed 'persistent wheezers'.

Assessments of these children were made at birth and at age 6 and a number of differences between the groups were found.

Children with transient wheezing were more likely to have impaired lung function soon after birth and before the first respiratory tract infection (as measured by $V_{\max}$ FRC), a maternal history of smoking, lower levels of IgE at 9 months and a lower prevalence of atopy at 6 years than persistent wheezers. On the other hand the persistent wheezers were significantly more likely to have a maternal history of asthma, normal lung function at birth, higher serum IgE levels and had a higher prevalence of atopy. Other studies have confirmed this association between abnormal pre-morbid lung function and wheezing in response to viral respiratory tract infections (56).

Therefore among children who wheeze during a viral respiratory tract infection there exists a spectrum of wheezing disorders with a similar clinical phenotype, but the underlying mechanisms may have subtle differences. Persistent wheezers are likely to be children with a genetic predisposition to atopy and probably impaired type 1 immunity leading to defective anti-viral responses. In time they may progress to develop classical asthma. Transient wheezers on the other hand may have less skewed immune responses and mechanical factors such as small airway size that contribute to airway obstruction and wheeze during viral infection. As they grow older and their airway calibre increases their wheezing may resolve. In both groups the long-term outcome will also be influenced by levels of postnatal exposure to factors stimulating the development of type 1 immunity (Fig. 3).

Mechanisms of viral-induced wheeze

There have been no prospective studies confirming a greater degree of deficiency in type 1 responses in children with persistent wheeze compared to those with transient wheeze. However there is evidence suggesting that there are differences in the immune responses between these groups.

Studies utilizing a non-bronchoscopic bronchoalveolar lavage procedure on children during elective surgery have assessed the inflammatory cells and mediators in bronchoalveolar fluid in asthmatic children (persistent wheezers) and children with 'viral associated wheeze' (transient wheezers).

In one study differential cell counts were measured in the bronchoalveolar fluid obtained from 52 children with atopic asthma, and 20 children with viral-associated wheeze (57). The group with atopic asthma was found to have significantly elevated eosinophil and mast cell counts compared to those children with transient wheeze. They also demonstrated that levels of the eosinophil activation marker eosinophilic cationic protein (ECP) measured in BAL fluid were elevated in children with atopic asthma, compared to those with viral associated wheeze (58).

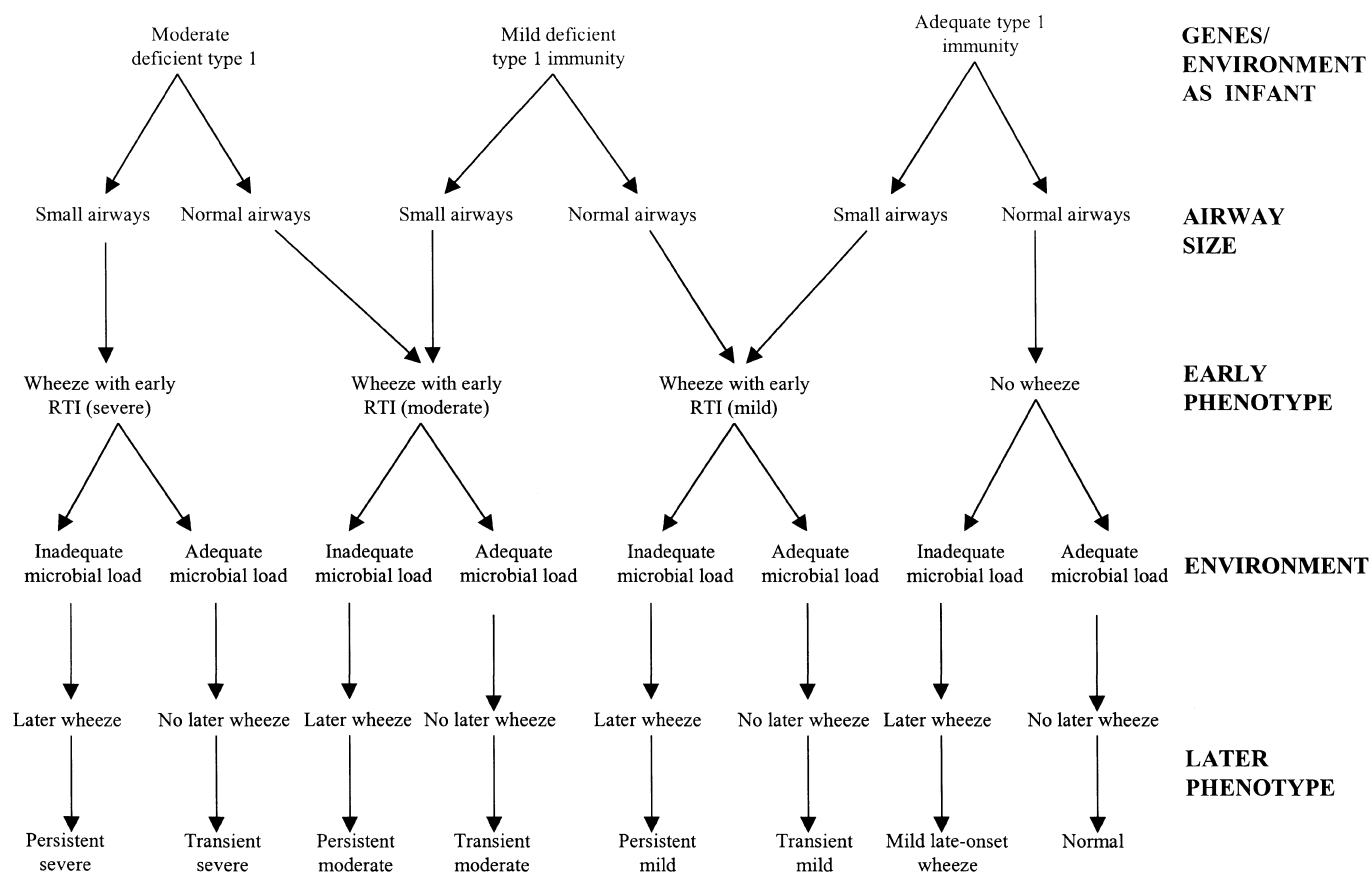


Figure 3. Relation between genes, airway size and environment.

Warner et al. performed bronchoscopies and bronchial biopsies on 27 children aged 1–11 years to investigate recurrent, unexplained respiratory symptoms. When they were re-evaluated 2–6 years later, those children with asthma had higher numbers of activated eosinophils in the lamina propria compared to those who had not developed asthma (59).

A study using exhaled nitric oxide as a marker of airways inflammation has also shown differences between children with persistent and transient wheeze. Levels of exhaled nitric oxide were not significantly different between normal controls and children with viral-associated wheeze but were elevated in persistent wheezers (60).

Differences in immune responses between these two groups of children have also been documented in studies evaluating the peripheral responses to infection. These have showed that the persistent wheezers had higher levels of serum IgE and ECP during viral infections, compared to transient wheezers (61, 62).

These findings of elevated IgE and eosinophil markers in children with persistent wheeze are suggestive of an underlying type 2 skewed immune response in these children. The absence of these in transient wheezers is indicative that their immune responses may be less skewed and other mechanisms also contribute to their wheezing. Identifying those at high risk of developing

asthma among children who wheeze will be crucial in attempts to assess the impact of early interventions on progression of the disease.

Viral infections and allergen sensitization

Early sensitization to aeroallergens is the strongest risk factor for childhood asthma (63). It has been proposed that viral respiratory tract infections may influence the development of asthma by enhancing allergic sensitization in the developing lung. Sensitization to aeroallergens is unlikely to occur through an intact mucosal epithelium because the normal respiratory tract has evolved in close contact with aeroallergens such as grass pollens. Disturbance of the respiratory mucosal surface during viral infection may allow allergens to gain access to the epithelial layer and interact with antigen-presenting cells and inflammatory cells, leading to allergic sensitization.

This possibility was first suggested in a study in 1979 in which 13 children of atopic parents were prospectively assessed for the development of atopic sensitization with skin tests and serum IgE levels (64). Eleven of the children developed allergic sensitization and all of these had an upper respiratory tract infection in the period prior to

becoming sensitized, while 10 of the 11 had serological evidence of a viral infection. These results suggested a temporal relationship between viral respiratory tract infections and the onset of allergic sensitization.

Further evidence for a possible interaction between respiratory viruses and aeroallergens has been obtained from animal models of allergic sensitization. In a canine model of allergic sensitization pups given canine para-influenza virus vaccine intranasally followed by pollen inhalation developed more IgE antibodies than those exposed to pollen alone (65).

Other animal models have confirmed that concomitant viral infection and exposure to allergen leads to enhanced sensitization to the allergen. In a murine model mice were exposed to ovalbumin as an aeroantigen during acute infection with RSV and influenza virus (66). In the uninfected control group ovalbumin sensitization did not occur, but in the infected group of mice subsequent ovalbumin exposure caused anaphylactic collapse. These mice were also found to have developed ovalbumin IgG1 antibodies, which were not present in the uninfected group.

Another study using ovalbumin sensitization and RSV infection in mice found a differential effect depending on the timing of infection.

RSV infection prior to sensitization had a protective effect on allergen-induced airways hyperresponsiveness (AHR) and airway inflammation, whereas RSV infection after ovalbumin exposure enhanced AHR. The authors felt this supported the concept that RSV airway disease occurs in hosts with underlying allergic disease rather than RSV induces allergic sensitization (67).

Despite evidence from animal models suggesting a link between viral infection and allergic sensitization, there is less evidence for such an effect in humans. One study evaluated the effect of RSV infection on sensitization to aeroallergens in the first 2 years of life (68). A cohort of 609 infants was assessed prospectively for evidence of allergic sensitization and RSV infection. Of the children studied, 2.8% were found to be sensitized to at least one aeroallergen at 1 year and 10.5% by age 2. House dust mite sensitization was only seen in RSV seropositive children and both grass pollen and birch sensitization were strongly associated with RSV seropositivity at 1 year of age. However the numbers of children sensitized were small – three for house mites and nine and five for grass pollen and birch, respectively, and no difference was seen between the RSV positive and negative groups by age 2.

There is the possibility therefore that respiratory virus infection has an effect on the developing lung that alters

its response to aeroallergens. However until there are further human studies addressing this question directly, the effect of viral infection in early life on sensitization to aeroallergens in the human lung remains speculative.

Conclusions

The associations between viral respiratory tract infections and the development of asthma are undoubtedly complex. There is strong epidemiological evidence in favour of the hypothesis that increased exposure to microbiological agents in early life, probably including the overall load of respiratory tract viruses, protects against the later development of asthma. This inverse relationship is probably due to the effect of infections on suppressing type 2 immune responses in the developing immune system.

However the studies supporting this hypothesis have relied on surrogate markers for increased exposure to respiratory viruses such as daycare or large family size. There is therefore a need for further studies in which respiratory virus infection is investigated directly.

When the specific effect of individual respiratory tract infections is examined, the relationship between infection and subsequent development of asthma is strongly influenced by host factors determining the response of the host to that infection. The most significant of these is the probability that children with relatively impaired type 1 immunity are more likely to develop both symptomatic viral lower respiratory tract infections early in life and asthma. In such studies it is the nature of the host response to an infection that is likely to explain positive associations reported between individual viral respiratory tract infections and asthma rather than the infection itself having a causative effect. Interpreting these studies is further complicated by the contribution of airway size and other relevant factors such as maternal smoking. Future studies investigating the associations between respiratory virus infections and asthma or allergic sensitization will need to take these complex interactions into account. They should be prospective, long-term, birth cohort studies that include immunological and genetic profiling at birth, combined with virus diagnostic methods capable of detecting all common respiratory viruses. Studies with a less comprehensive study design will continue to throw up conflicting results on the relationship between viral respiratory tract infections and asthma.

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