The Role of Viruses in the Induction and Progression of Asthma

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Viral respiratory infections have been related to asthma in several ways. It is well established that viral common colds precipitate exacerbations of asthma. Severe bronchiolitis in early life is related to subsequent wheezing and therefore may represent a marker of susceptibility to asthma; alternatively, it could be involved in the initiation of the disease. Finally, it is possible that some infections may protect from the development of asthma and allergies by promoting a type-1 host response. However, whether respiratory or other viruses could mediate such a protective effect is debated. The design and implementation of novel anti- or proviral strategies targeting asthma depends on the resolution of these questions. This review presents current evidence on the epidemiologic correlations and proposed mechanisms for the involvement of viral infections in the development and progression of asthma.

Introduction

There has been a remarkable increase in the prevalence of asthma and other allergic diseases in affluent societies during the past decades [1]. Asthma now affects over 150 million people worldwide and costs more than tuberculosis and AIDS combined [2]! Although there is no doubt that both genetic and environmental factors influence the development of asthma, it is difficult to ascribe such rapid epidemiologic changes to genetic factors; thus much of the increasing prevalence should be attributed to the environment [3].

Among myriad factors included in the term "environment," viral infections are currently attracting much attention as possible inducers or triggers of asthma. The correlation of common colds with asthma episodes is an old one. However, until recently we lacked sensitive methodologies for the detection of the most prevalent respiratory viruses, such as rhinoviruses (RVs) and coronaviruses [4]. Furthermore, some of the most obvious changes in affluent societies, such as family structure, congregation, and hygiene, have implications in the epidemiology of infections, leading to speculation that infection-associated factors may be implicated in the asthma epidemic [5].

With the advent of powerful detection methods, such as the polymerase chain reaction (PCR) [6] and detailed analysis of epidemiologic data [7], our understanding of the implication of viral infections in asthma has increased. Nevertheless, there are still some apparently contradictory effects and many unexplored aspects to be addressed. The mechanisms by which respiratory viruses exacerbate asthma are under scrutiny. Furthermore, the possibility that some viral or intracellular bacterial infections may initiate asthma is strongly debated. Finally, the possible protective role of these infections in the development of allergy and asthma still requires considerable attention. This article reports on the current status and recent developments regarding the involvement of viral infections in the induction and progression of asthma.

Viral Infections and the Development of Allergy and Asthma

To understand the potential implications of viral infections in the development of asthma, one has to remember that asthma has a complex natural history that includes different phenotypes, which may have differences in their pathogenesis. It is well established that the majority of asthma cases start early in life. However, a significant proportion of children that wheeze at a young age, mostly secondary to upper respiratory viral infections (URIs), overcome their problem before school age. These subjects, characterized as transient early wheezers, have reduced airway function at birth; thus it is likely that their disease is at least partly of a mechanical rather than purely of an immunologic nature [8]. Other children start wheezing early and continue to do so, at least until adolescence. These persistent wheezers have an altered immune response with a rise in IgE levels during their first reported URI but no reduction in eosinophil numbers during the acute phase of the URI, in contrast to transient wheezers [9].

The predominant virus during the first years of life is respiratory syncytial virus (RSV). RSV infection occurs in almost all children before their second birthday, and clinical

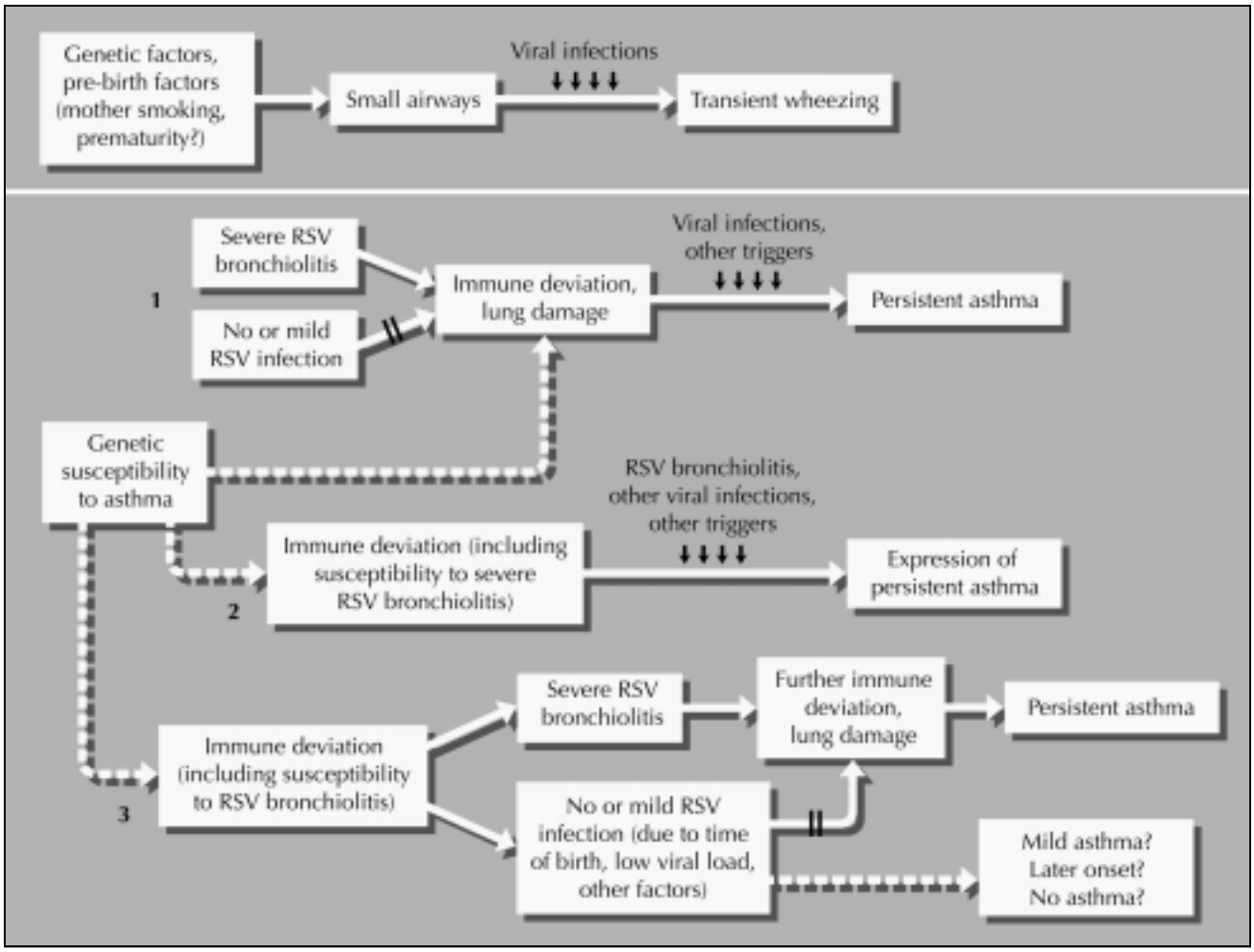

Figure 1. Pathogenesis of asthma in early life. Children with small airways at birth develop a wheezing syndrome triggered by viral infections, which resolves before school age (upper panel). Severe RSV bronchiolitis in early life may lead to immune deviation, lung damage, or both, leading to persistent asthma (1). Alternatively (2), children with genetic susceptibility to asthma may present with severe bronchiolitis and continue to wheeze with various stimuli. These two possibilities are not mutually exclusive (3). A predisposition to RSV infection may result in severe bronchiolitis, further increasing the possibility of persistent asthma. However, because the severity of bronchiolitis might be influenced by other factors, mild RSV disease may delay asthma onset, result in milder symptoms, or even not induce asthma. Double bars across an arrow denote that the circumstance is not thought to lead to the indicated result. RSV—respiratory syncytial virus.

presentations vary from subclinical to severe, life-threatening bronchiolitis [10]. Early studies pointed out that children suffering from bronchiolitis have an increased risk of developing asthma in subsequent years [11]. Whether RSV bronchiolitis represents a marker of susceptibility to wheezing or it can per se divert the immune system or affect the lung and initiate asthma is still debated (Fig. 1).

Respiratory syncytial virus bronchiolitis and asthma

In 1971, Rooney and Williams [11] found that 56% of children hospitalized for bronchiolitis would continue to wheeze 2 to 7 years later, and this has been confirmed in several subsequent studies. However, it has been difficult to ascertain whether this is solely an association or whether causation is also involved, resulting from either direct lung damage or an RSV-mediated immunologic deviation towards type-2 cytokine production [12]. Although pulmonary function is reduced many years later in children with a history of lower respiratory tract infection, it seems that this is a preexisting feature of these children [8,13].

When bronchial responsiveness was assessed, the results were conflicting; either no difference or an increase in bronchial reactivity several years after bronchiolitis has been reported [14]. Equally conflicting are the results relating to potential effects of RSV in allergic sensitization. One group has shown that RSV bronchiolitis is an independent risk factor for the development of asthma and allergic sensitization at age 7 years, in fact a stronger risk factor than is family history of asthma [15•]. In contrast, other studies have failed to establish such an effect [16,17•]. Differences in disease severity and age of evaluation may partly account for this discrepancy. It is conceivable that severe RSV disease may be required for the establishment of long-lasting effects. In another study, the correlation of RSV bronchiolitis with

sensitization that was observed at age 6 years was not present at age 9 to 10 years [18]. Importantly, RSV-related effects on wheezing and asthma decline with age, becoming nonsignificant by adolescence [17•,18,19]. These findings should also be interpreted with caution: asthma symptoms may be undervalued in adolescence or they may relapse later in life [20]; thus, prospective evaluation of the current cohorts is required to evaluate these possibilities.

A type-2/type-1 cytokine imbalance in favor of type-2 responses or with impaired type-1 responses in acute RSV bronchiolitis has been reported in several instances [21,22]. These findings could be explained as either an inherent defect or a direct result of the RSV infection itself. Recently, we also observed a profound imbalance in infants with acute RSV bronchiolitis, with significantly reduced production of the type-1 cytokines interferon (IFN)–γ, interleukin (IL)–12, and IL-18 and increased production of IL-4 (Legg *et al*., unpublished data, 2000). This imbalance was associated with impaired virus clearance, which suggests that this imbalance may be an important mechanism in determining disease severity. In addition, because the imbalance was observed as early as the 1st or 2nd day after initiation of disease, the immune deviation was almost certainly already present in these infants before RSV infection; deviation of the immune response by the virus itself is highly unlikely to have occurred so early in the course of the illness, when virus-specific immunity is only beginning to develop.

From the above, it is obvious that no safe conclusion about whether RSV bronchiolitis may cause or is only associated with asthma in later life (through common causality) can be reached currently (Fig. 1). These two possibilities are not mutually exclusive. Children with a predisposition to asthma may be more prone to develop severe RSV bronchiolitis; however, this infection may also further affect their immune responses or lung structure, leading to the development of asthma symptoms [23]. With the advent of effective RSV prevention modalities it is now possible to design randomized intervention studies. In these studies, confounding factors are ruled out by randomization; thus they may offer more conclusive evidence. In one such study, modest differences in pulmonary function were observed between infants treated with ribavirin versus placebo-treated control infants, but the number or subjects was small [24]. Larger intervention studies are awaited.

Most data relating to acute severe infections early in life and the increased risk of asthma implicate RSV, but most of the studies on which these conclusions are based did not adequately look for other respiratory viruses. Stein *et al*. [17•] reported a fourfold increased risk of asthma later in life in children with RSV infections that were severe enough to lead to a pediatric consultation early in life. However, increased risks of two- to threefold were observed with other respiratory viruses, suggesting that any single acute infection severe enough to lead to a pediatric consultation early in life is also a risk factor for asthma later in life. A likely explanation for this observation is that these infants have impaired type-1 immunity, leading to increased illness with viral infections in infancy and putting the infants at risk for the development of allergic sensitization and asthma later in life.

Could viruses protect against allergy and asthma?

In 1989, Strachan [5] first described an inverse relation between number of siblings and development of allergy in children. It was proposed that infection in early life, transmitted by unhygienic contact with older siblings, might prevent the development of allergic diseases. Consequently, improved hygienic standards in westernized societies, as well as increased use of antibiotics and widespread immunization programs, may facilitate the development of allergic diseases by decreasing the prevalence of infections. In addition to its conceptual attractiveness, this hypothesis fits with our current understanding of the immunologic framework of allergic disease, *ie*, an impaired transition from type-2 immunity in the fetus to the mature type-1 response in normal children and adults, possibly mediated by an antigenically rich (dirty) environment, of which infections are an essential element (Fig. 2) [25]. This is supported by epidemiologic studies that reported an inverse relationship between asthma and the overall burden of respiratory infections in different communities [26–29].

The individual effect of specific viral or bacterial agents has been more difficult to assess. In Japanese children with positive tuberculin responses, the prevalence of asthma and atopy was lower compared with that in negative responders [30]. In contrast, bacille Calmette-Guérin immunization after birth did not affect the prevalence of atopy and asthma [31]. In a study in West Africa, childhood measles seemed to protect against asthma in early adulthood [32]. However, 25% of children aged less than 3 years who contracted measles in that setting died from the disease, suggesting that the protective effect could be due to high mortality of allergy-prone individuals. A contrasting cross-sectional study was recently published, showing increased prevalence of asthma associated with naturally acquired measles [33].

It is possible that the cumulative effect of repeated infections with several different microorganisms rather than with a single microorganism may be responsible for protection or induction of allergy and asthma. In addition, stimuli that are not infective per se but are related to infectious agents (*eg*, endotoxin) may contribute to the deviation of the immune system towards a "normal" type-1 immune response (Fig. 2). It has been recently documented that the protection from allergy and asthma that is conferred on children living on farms can best be explained by close exposure to livestock and poultry, which are rich sources of bacterial stimulation and endotoxin [34]. In support of this concept, a recent study showed that indoor endotoxin exposure early in life may protect against allergen sensitization [35••]. Unfortunately, there are also

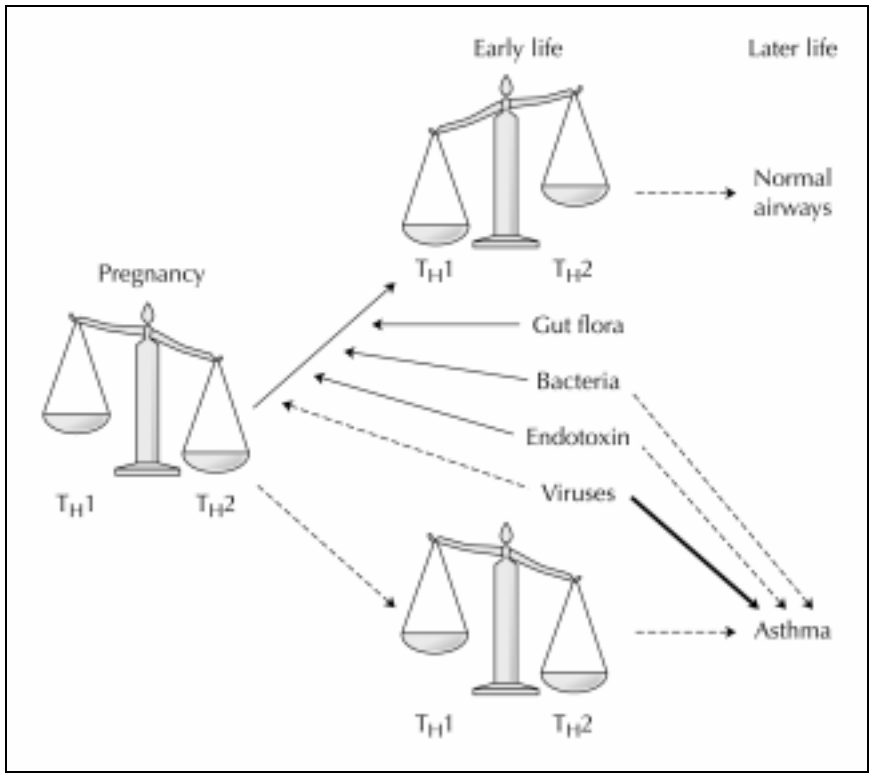

Figure 2. Could infections both protect against and induce asthma? Transition from the normal type-2 fetal immune responses to the normal mature type-1 responses (upper panel) may be affected by several infectious agents that induce the production of interferon-γ, interleukin (IL)–12, and IL-18. If normal immune maturation fails to develop (lower panel), the interaction of some of the same infectious agents with the abnormal host will lead to severe infections early in life (including bronchiolitis) and later development of asthma.

several reports, including very recent ones, demonstrating a positive correlation of bacteria or endotoxin exposure, not necessarily in early life, with increased asthma severity [36].

Another important source of microbial stimulation without infection could be the normal commensals and pathogens of the gut. Although there are significant differences in the composition of the intestinal flora between normal and atopic children [37], further studies are required to assess whether this is a causal association and what its potential effect on asthma may be. Nevertheless, in Italian military recruits, the prevalence of atopy was significantly lower in subjects positive for hepatitis A virus antibodies [38]. More recent data from the same cohort include antibodies to *Toxoplasma gondii* and *Helicobacter pylori* in the "protective" effect [39••]. Measles, mumps, rubella, chickenpox, cytomegalovirus, and herpes simplex virus had no effect in the same study. The authors suggested that the orofecal microbes might be better candidates for a protective effect against atopy than are airborne respiratory viruses. This is an intriguing idea, but further prospective studies are required to evaluate the individual and cumulative effects of different childhood infections on the development of allergy and asthma.

In considering the above studies, it is important to differentiate between studies that examine factors that are likely to represent overall exposure to agents promoting type-1 immunity (*eg*, orofecal bacteria and increased numbers of siblings) and studies that investigate individual infections. The reason being that a high overall exposure to these agents is likely to promote type-1 immunity and provide protection against the development of asthma. In contrast, when examining individual infections such as RSV, subjects developing severe illness are likely to have impaired type-1 immunity, thus putting them at increased risk of developing asthma. Other individual infections—such as measles, mumps, rubella, and chickenpox—infect only once, and almost all people are infected or immunized during childhood. Thus, they are not likely to reflect the overall load of infectious agents and would not be expected to be associated with protection against the development of asthma. In support of this interpretation, Illi *et al*. [40••] recently reported that infants with frequent "sniffles" during the 1st year of life (*ie*, frequent URIs) are protected against the development of asthma later in life. Further such studies will help explain why some individual childhood infections may correlate to an increased rather than a decreased odds ratio for asthma, even though the protective effect of family size (or repeated or frequent infections) remains [41].

Viruses in Asthma Exacerbations

Epidemiology of virus-induced asthma exacerbations

Although the role of viruses in the induction of or protection from allergy and asthma is still inconclusive, the evidence for the participation of these pathogens in asthma exacerbations is much stronger. The observation that asthma attacks often follow URIs (common colds) is old and a daily experience of practicing physicians, especially pediatricians. Early reports showed that viral shedding decreased soon after the cold, before the patient referred to their physician or the hospital, indicating that early sampling was necessary for viral detec-

tion [42]. Furthermore, virus detection rates in these studies fluctuated considerably; this was attributed to difficulties in RV and coronavirus isolation. With the use of PCR-based detection for RVs and prospective designs, the magnitude of the problem was revealed.

In our prospective study in the community, asthmatic children aged 9 to 11 years were followed-up for 1 year and sampled as soon as they reported cold symptoms [43]. The percentage of asthma exacerbations following virologically confirmed colds was 80% to 85%. In a more recent study in children hospitalized with severe asthma exacerbations, the viral detection rate was 82% [44]. In adults, the proportion of virus-attributed asthma exacerbations is generally lower. However, it is possible that viral shedding is less or of a shorter duration in adults than in children. In one of the first community-based prospective studies using PCR detection for RVs, virus detection rates were 44%, although cold symptoms preceded 70% of the episodes [45]. In another study (with a combined longitudinal and cross-sectional design) of inner-city asthmatic adults [46], virus detection was once again 44% in followed-up subjects and 50% to 55% in subjects presenting to the emergency department. In our most recent study, virologic confirmation was achieved in 60% of asthma exacerbations in adults (Corne and Johnston, unpublished data, 2000).

The conclusion from the above studies is that respiratory viruses are the most common triggers of asthma attacks. Moreover, when we performed a time-trend analysis of hospital admissions for asthma compared with virus isolation rates during the same time in our cohort, an impressive correlation was observed, indicating that viruses are able to induce severe asthma requiring hospitalization [47]. Peaks in hospital admissions for asthma and in virus isolation occurred in most instances immediately after school vacations. This pattern of segregation-dependent disease is a characteristic of RV colds, in contrast to other respiratory viruses such as RSV, which have a specific season. Similar seasonal variation was partly observed in asthma mortality, especially among young children and the elderly, who are most susceptible to viral infections [48].

Another important point, on which all of these studies agreed, is that RVs are the most prevalent agents, accounting for 50% to 60% of all detected viruses (Fig. 3). This seems to reflect the prevalence of these viruses in common colds, rather than any specific asthmogenic properties, because there are minor or no differences in symptoms produced by different viruses [43] or in the proportion of asthma episodes resulting from colds by any specific virus [42].

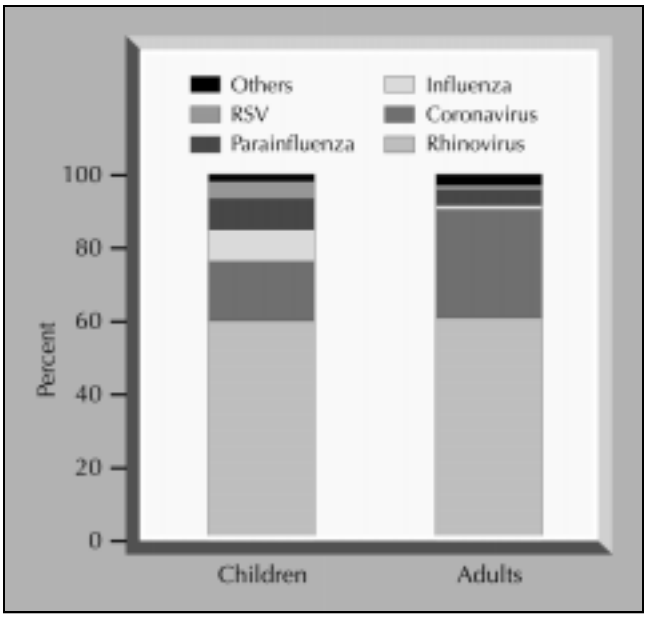

Figure 3. Viral agents implicated in acute asthma exacerbations in school children and adults, as a percentage of total isolated viruses. Rhinoviruses and coronaviruses are responsible for 80% to 90% of cases. RSV— respiratory syncytial virus. *Adapted from* Johnston *et al.* [43] and Nicholson *et al.* [45].

Virus-induced changes in airway reactivity

The above epidemiologic data have raised considerable interest regarding the mechanisms of virus-induced asthma exacerbations, the understanding of which would suggest potential therapeutic targets. Airway hyperresponsiveness is one of the most prominent functional abnormalities in asthma that can be objectively assessed in human and animal models. An increase in airway responsiveness to histamine in normal subjects after URIs, lasting as long as 7 weeks, was observed more than 20 years ago [49]. Although results have varied— probably because of differences in methodology, models, viral strains, and so forth—increased airway reactivity has since been documented after RV, RSV, influenza, parainfluenza, and adenovirus infections, mostly in animal models [50].

Because of the lack of an appropriate animal model, human experimental infections have been used as a model of RV infection. Using this model, the increased airway responsiveness to histamine after RV infection in atopic asthmatic subjects was correlated with the severity of the experimental cold, which was paralleled by an increase in IL-8 in nasal lavage fluid [51]. In addition, when daily forced expiratory volume in 1 second was monitored, a variable airway obstruction was observed [52]. When normal and atopic rhinitic subjects were compared, lower airway responsiveness was more affected in the allergic group [53]. However, in another study, experimental RV infection induced small changes in either upper or lower airway symptoms in normal and asthmatic subjects, with no effect on bronchial reactivity, leading the authors to suggest that RV infection by itself may not be sufficient to provoke clinical worsening of asthma [54]. Exposure to allergen during a respiratory viral infection is the most obvious cofactor, because it is well known that RV experimental infection enhances the responses to inhaled allergen [55] and potentiates inflammation after segmental allergen bronchoprovocation [56].

Most surprisingly, a recent study by Avila *et al.* [57], using the same human model in allergic rhinitis subjects,

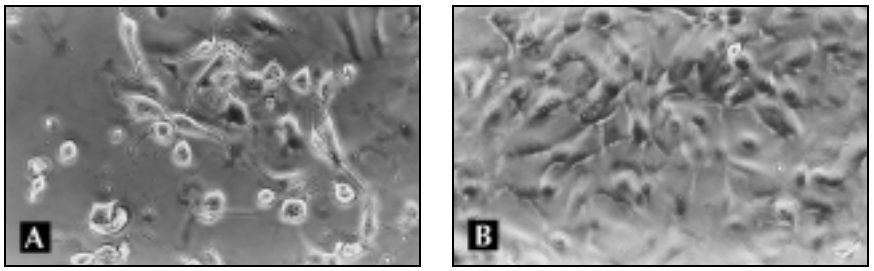

Figure 4. Induction of cytopathic effect to cultured human bronchial epithelium by rhinovirus (A). A normal, confluent monolayer is also shown (B). *See also* Schroth *et al*. [60•] and Papadopoulos *et al*. [61••].

showed that preexposure of the nose to an allergen significantly delayed the onset of cold symptoms, reduced the duration of the illness, and delayed the appearance of proinflammatory cytokines locally. An inverse correlation between nasal eosinophils at the time of inoculation and eventual cold symptoms was also observed, suggesting that an allergic response might protect from RV colds. Although this evidence seems contradictory to most previous findings, it could prove helpful in several ways. First, it suggests that eosinophils may be involved in RV immunity [58]. Furthermore, it indicates that allergen exposure and viral infection do not have a simple additive effect, and timing or dosage may be important.

Mechanisms of virus-induced inflammation

Several characteristics of virus-mediated pathology can also be seen in asthma. Among these, direct virus-mediated damage to lower airway epithelium is a characteristic of several viruses including influenza and RSV. Dead epithelial cells drop into the airway lumen, inducing or increasing airway obstruction. However, the ability of RVs to infect the lower airways or induce cytotoxicity has long been debated. One of the most frequent arguments against this possibility has been the alleged preference of these viruses for a 33°C environment, as in the nasal cavity, and their suboptimal replication at 37°C, as in the lung. However, when we compared the ability of a range of RV serotypes and wild-type strains to replicate at either 33°C or 37°C, we found differences to be relatively small and certainly not prohibitive of RV replication [59].

This was confirmed in another study in which RV replication was also achieved in primary human bronchial epithelial cells (HBEC) [60•]. Very recently, we also reported productive infection of HBEC with different RV serotypes [61••]. Using in situ hybridization, we showed conclusively that RV RNA, of both the genomic and replicative strand, can be found in the bronchial mucosa of subjects with an experimentally induced common cold [61••]. Most interestingly, in both of the above studies the induction of a cytopathic effect of RV in HBEC was shown, indicating that under some conditions [61••], or with some strains [60•], there can be a cytotoxic effect on the epithelium (Fig. 4). Furthermore, infection of HBEC with RV resulted in mRNA expression and production of IL-8, RANTES (regulated upon activation, normal T-cell expressed and secreted), and granulocyte-macrophage colony-stimulating factor [60•] or IL-6, IL-8, RANTES, and IL-16 [61••]. These studies strongly suggest that RVs infect the lower airway and induce a local inflammatory response that could represent the first step in the pathogenesis of an asthma episode, adding to and clarifying previous attempts to prove this notion [62,63]. It is possible that the degree of inflammation, which is similar with other respiratory viruses [64], and not the degree of cytotoxicity is more relevant in the induction of an exacerbation.

Epithelial damage and mediator production after viral infection are only some of the mechanisms that could initiate or sustain an asthma exacerbation. A dysfunction of the inhibitory M_2 muscarinic receptor has been documented after viral infection, which could lead to increased reflex bronchoconstriction [65]. A role for tachykinins has also been suggested, partly explained by reduction of neutral endopeptidase activity, which is the major metabolizing enzyme for substance P and neurokinin A [66]. Most of the above studies have been performed in animal models with cytotoxic viruses such as influenza, parainfluenza, and RSV. A neurally mediated effect does not seem to be a prominent feature of human experimental RV infections [67].

Most puzzling is the potential involvement of the immune response to respiratory viruses in asthma exacerbations. Although the asthmatic phenotype is paradigmatically related to type-2 lymphocyte responses, predominantly IL-4 and IL-5, viral infections induce strong type-1 responses with high levels of IFN-γ that would be expected to downregulate, rather than augment, "allergic" immune responses. Among respiratory viruses, RSV, influenza, and parainfluenza are more extensively studied in animal models. Sensitization of BALB/c mice to the virus attachment protein (G) of RSV, followed by live virus infection, leads to pulmonary eosinophilia and type-2 cytokine production. Although IFN-γ is still the dominant T-cell cytokine, a localized relative reduction of IFN-γ mRNA expression with concomitant increase of IL-4 and IL-5 transcripts has been reported [68]. When *Dermatophagoides farinae*–sensitized mice were repeatedly infected with RSV, an increased production of type-2 cytokines was observed [69]. In the presence of IL-4, virus-specific $CD8^+$ T-cells can switch to IL-5 production and induce airway eosinophilia [70]. Interestingly, IL-4 can also inhibit antiviral immunity, delaying both influenza [71] and RSV [72] clearance.

Unfortunately, no animal model of RV infection currently exists. However, we have recently observed that

RV-infected peripheral blood mononuclear cells from atopic asthmatic subjects produce significantly lower IFN-γ and IL-12 and significantly higher IL-10 and IL-4 than do cells from normal individuals (Papadopoulos and Johnston, unpublished data, 2000). Although IFN-γ remained the dominant T-cell cytokine in this model, a shift towards a type-2 response may be involved in the induction of an asthma exacerbation by mechanisms similar to the ones described above for RSV in the mouse.

Conclusions

Although it is early to reach safe conclusions, there is no doubt that strong associations exist between viral infections and the induction and development of asthma. The most conclusive evidence exists in relation to viral URIs and subsequent asthma exacerbations. However, several questions must be answered before this information becomes clinically useful. It is likely that viral infections interact with allergen exposure in the induction of asthma exacerbations, but the sequence of events and mechanisms are largely unexplored. As new therapeutic modalities against viruses become available, it is imperative to search for windows of opportunity for antiviral treatment in the context of asthma exacerbations.

In contrast, the relevance of infection early in life to the development of asthma is less clear. Individual infections such as RSV are associated with an increased risk of asthma, most likely because of impaired type-1 immunity. Conversely, there is strong evidence that improved hygiene or a reduced overall load of exposure to infectious agents early in life may be responsible for the increase in allergy and the asthma epidemic. Although these data could spontaneously lead to the conclusion that we should change to a more "natural" or "dirty" lifestyle, we should bear in mind that improved hygiene was imposed because of the significant morbidity and mortality due to infectious diseases in previous eras. More detailed studies are thus required to identify the best way to train our immune systems without threatening our health and to identify those in whom such treatment will be of most benefit.

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