REVIEW

Asthma and respiratory syncytial virus infection in infancy: is there a link?

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Introduction

Respiratory syncytial virus (RSV) is the commonest respiratory pathogen in children. Moreover, at least 50% of infants who have acute viral bronchiolitis due to RSV have subsequent episodes of audible wheezing which are almost certainly asthma [1]. Most recently, it is reported that RSV infection results in airway hyperresponsiveness and enhanced airway sensitization to allergen in animal studies [2]. What, if any, is the relationship between RSV infection in infancy and asthma in later childhood and adulthood? If there is a relationship, effective prevention of asthma may require intervention in infants with RSV infection.

Clinical features

The infant with bronchiolitis characteristically has symptoms of a viral infection such as mild rhinorrhoea, cough, and sometimes a low grade fever. Within 1 or 2 days, these symptoms are followed by the onset of rapid respiration, chest retraction, and wheezing. The infant may be irritable, may feed poorly, and may vomit [3,4].

On physical examination, the respiratory rate is increased. The pulse rate is usually increased, and body temperature may be normal or elevated. Chest retractions are present. Prolonged expirations are frequently found. Rhonchi and wheezes or rales are usually heard through the lungs. Dehydration and cyanosis may be found in some patients [5]. This clinical phenotype of respiratory obstruction is similar to that seen in asthma.

The radiographic manifestations of bronchiolitis are non-specific and include diffuse hyperinflation of the lungs with flapping of the diaphragms, prominence of the retrosternal space, and bulging of the intercostal space [4].

Epidemiology

RSV infection

RSV repeatedly infects and causes disease throughout life, with the most serious disease occurring in the very young, the very old, or immunosuppressed patients of any age [6]. It infects almost all children during the first 2 years of life [7]. The peak incidence of RSV infection is in infants aged between 6 weeks and 6 months of age [8]. RSV is responsible for most cases of bronchiolitis [9]. RSV is also the most frequent cause of bronchiolitis and pneumonia in infants requiring hospitalization [10].

Evidence for an association between viral infections and asthma exacerbations

An association between viral respiratory infections and asthma attacks has been acknowledged for several decades [11,12]. Patients suffering from an acute attack of asthma very often give a history of acute viral respiratory infection in the days preceding the onset of the exacerbation [13].

There are several lines of evidence which associate virus infections with asthma exacerbations. Firstly, using highly sensitive techniques such as the polymerase chain reaction, the identification rate of viruses during exacerbations of asthma in children is as high as 80–85% [14]. Older reports have given lower rates, probably due to less sensitive detection methods [13]. The identification rate of viruses is only about 3% in asthmatic patients when free of symptoms [15–17].

Secondly, there is a close temporal relationship between virus infections and asthma exacerbations, both at the individual and at the population level [13]. A parallel between seasonal variations in wheezing episodes among asthmatic children and identification peaks of RSV and parainfluenza viruses has been noted [18,19]. In another study over 11 years of 6165 lower respiratory illnesses

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(1851 with wheezing) occurring in children, striking parallels between yearly peaks in wheezing-associated respiratory illness and RSV outbreaks were documented [20].

Prospective studies have also indicated that asthma attacks and reductions of peak flow are associated with respiratory viral infection in as many as 80–89% of cases in adults [21]. Most recently, it is reported that upper respiratory viral infections are strongly associated in time with hospital admissions for asthma in children (87%) and adults (80%) [22]. The time course of wheezing associated with infection has a characteristic pattern: the wheezing starts a mean (SD) of 42 (7) h after the first symptoms of respiratory infection and lasts for 3.8 (4.2) days in patients with positive virology [23]. This pattern is consistent with observations in clinical practice, and does not resemble the pattern of acute allergen challenge.

Numerous studies have also documented an association between the severity of the wheezing illness and the viral identification rate [13]. Viruses were isolated in 49% of all episodes of wheezing bronchitis in children, and in 64% of severe episodes requiring corticosteroids [24]. Another study, with children who had attacks of wheezing bronchitis or asthma, showed that more severe blood gas abnormalities in those with positive viral findings than in those with negative findings [25].

Viruses identified in exacerbations of asthma and wheezing

When the identified individual viruses are examined, weighted averages indicate rhinoviruses, RSV, parainfluenza viruses and coronaviruses to be the predominant viruses [13,14], although all common cold viruses may lead to wheezing in some cases. All the studies found RSV and parainfluenza viruses, and all but one found rhinoviruses and adenoviruses [13]. RSV is one of the most important viruses to aggravate asthma or wheezing [26]. RSV infection has the highest rate of associated wheezing (77%) [27].

Links between infant bronchiolitis and asthmatic symptoms in later childhood

Studies indicate there is a strong link between infant bronchiolitis and asthmatic symptoms in later childhood [28–32]. In one uncontrolled prospective study of 48 children with bronchiolitis, 92% were diagnosed as asthmatic at some time within the ensuing 5 years [33]. In another study, 73 children admitted to hospital with infant bronchiolitis were followed for an average of 5.5 years; 42.5% reported wheezing within the final year compared with 15.1% of controls [34].

An analysis of risk factors for the development of asthma and IgE antibodies on a group of 140 children showed that RSV bronchiolitis is the most important risk factor, and a family history of atopy or asthma further increased the risk

[28]. Some studies suggest that hyperresponsiveness to inhaled histamine may persist for 10 years or more after acute bronchiolitis [35], and might even predispose to pulmonary disease in adult life [36,37].

However, other studies show no evidence that an atopic predisposition is required for bronchial hyperreactivity to develop [30,31,33,38]. In a recent prospective study, the factors affecting wheezing before the age of 3 years and their relation to wheezing at 6 years of age were investigated in 1246 newborns [39]. This study suggests that the majority of infants with wheezing have transient conditions associated with diminished airway function at birth and do not have an increased risk of asthma or allergies later in life; in a minority of infants, however, wheezing episodes are probably related to a predisposition of asthma. Most recently, another report showed that there was no correlation between asthma at 10 year and initial RSV infection [40]. The results of this report fit better with the view that RSV infection triggers severe wheezing bronchitis in the predisposed child [41] than with the hypothesis that the RSV infection as such induces a process leading to persistent asthma [28].

Although there are conflicting views, the associations listed above of viral infection and bronchiolitis with exacerbations and onset of asthma, argue strongly from an epidemiological perspective for a causal link. More evidence in pathophysiology and immunology will be helpful to resolve if there is a mechanistic relationship.

Pathology

Inflammation in RSV induced-bronchiolitis typically affects bronchioles of calibre 300 μm down to 75 μm [1]. In the very young and very old, the inflammation may involve the trachea, bronchi and alveoli [42]. The bronchiolar epithelium is colonized by replicating virus. RSV antigen has been demonstrated in autopsy lung tissue from clinical RSV cases [43]. The mucus membrane of the airways was found to be inflamed and swollen, with cellular debris and fibrin forming plugs within the bronchioles. The alveoli usually are normal, except those immediately adjacent to the inflamed bronchioles [4]. In addition to these changes, RSV may cause severe pneumonia with extensive destruction of respiratory epithelium, necrosis of lung parenchyma, and formation of hyaline membranes [44].

In a recent autopsy report of 18 cases, all showed variable amounts of interstitial mononuclear inflammation, pneumocyte injury, with or without hyaline membrane, atelectasis and hyperinflation [43]. In seven cases, there was extensive alveolar and terminal bronchiolar plugging by eosinophilic material admixed with nuclear debris. Four of these cases also had large numbers of syncytial giant cells, usually, but not exclusively, lining alveolar spaces. In five cases, there was less consolidation, and injury was concentrated around

bronchioles and bronchi. In addition to epithelial desquamation, squamous metaplasia, peribronchiolar inflammation, and luminal plugging by mucus mixed with debris and inflammatory cells, airway injury was occasionally characterized by uneven proliferation of epithelial cells with protrusion into bronchial lumina creating a polypoid appearance [43].

In severe cases there is desquamation and neutrophil-rich exudation [9,45,46]. Our recent studies show that neutrophils are the predominant leucocytes in airway secretions in RSV bronchiolitis [47]. An animal study also shows that RSV-inoculated guinea-pigs had significantly increased neutrophil infiltrates [48]. It is reported that transfer of CD8 T cells into RSV-infected mice causes pulmonary neutrophilic lung disease [49]. Our recent studies also show that RSV infection increases neutrophil adherence to respiratory epithelial cells and neutrophils augment RSV-induced damage and detachment of epithelial cells [50]. These studies suggest that neutrophils may contribute to the pathogenesis of RSV infection by inducing epithelial damage and detachment.

Eosinophils are also an important inflammatory cell in RSV bronchiolitis [43]. It has been reported that RSV can activate or prime eosinophils *in vitro* to release various inflammatory mediators [51]. Such virus-induced effects on inflammatory cells may play a role in the pathogenesis of RSV bronchiolitis and may also be critical for the development of persistent airway hyperreactivity after viral infections [51]. Clinical data also suggest that eosinophil degranulation in the respiratory tract occurs during RSV bronchiolitis and may play a significant role in the development of virus-induced airway obstruction [52].

The production and role of inflammatory mediators, especially histamine, in RSV infection was also investigated. Histamine was detectable in nasopharyngeal secretions of some RSV patients with all forms of illness but was detected significantly more often and in higher concentrations in patients with wheezing [53]. Peak titres of RSV-IgE and concentrations of histamine correlated significantly with the degree of hypoxia. These data suggest that the formation of RSV-IgE and release of histamine may adversely affect the outcome of RSV infection [53]. It is also reported that mononuclear leucocytes from normal individuals produce histamine-releasing factor (HRF) in response to exposure to RSV or influenza virus, suggesting that this cytokine, which causes degranulation of basophils and release of histamine, may play a role in the mechanism of virus-induced bronchospasm [54].

Physiology

Associated with the bronchiolar inflammation in RSV bronchiolitis is a marked increase in airway resistance and

a corresponding increase in the work of breathing. In animal studies, it is also shown that RSV infection results in airway hyperresponsiveness and enhanced airway sensitization to allergen [2]. The lungs become overinflated and the resting end-expiratory lung volume (functional residual capacity) is approximately twice normal [4]. As a result the diaphragm is depressed and flattened. In this position it functions least efficiently. Dynamic compliance is decreased, in part because the infant is breathing at a higher lung volume and hence on a stiffer portion of the volume-pressure curve of the lung, and in part because of the uneven distribution of resistances within the lung. The airway obstruction impairs gas exchange with hypoxia and hypercapnia as a result of ventilation perfusion mismatch. Fatigue of the respiratory muscles may be heralded by irregular breathing and can proceed very rapidly [1,9]. These changes are very similar to that in asthma.

Immunology

A challenging unanswered question is why severe lower respiratory disease develops only in certain very young infants. Undoubtedly the airways of such young babies, being narrower than those of older children, are much more readily obstructed by inflammation, oedema, and shedding of necrotic cells into copious mucus. However, this does not explain why only a minority of these small infants develop bronchiolitis. There is evidence that the condition may have an immunological basis [6,7,42].

Limited protective immune response to RSV infection

Natural RSV infection is a poor inducer of immunity. During annual winter epidemics, the majority of children become reinfected [42]. Longitudinal studies of RSV infection in children show they can be reinfected yearly and that each infection provides some, but incomplete, protection from subsequent infection and disease [20]. A study of adults challenged multiple times with RSV highlights the difficulty in inducing protective immunity [55]. Seven of 15 adults challenged with an unattenuated group A virus 2 months after a natural group A infection became infected and shed virus with a pattern similar to natural infection, and five (85% of those infected) developed upper respiratory tract symptoms. With subsequent challenges, fewer (25–30%) became infected and shed virus for shorter times. Each infection appeared to provide some additional protection, but still about 50% of those infected after each challenge were symptomatic [55]. Natural infection therefore induces a very limited protective immune response [6].

Humoral immune response

The best studied component of RSV immunity to natural infection is serum antibody, and serum neutralizing antibody appears to provide some protection from RSV disease. Humans develop antibodies to most RSV proteins. Passive protection with monoclonal antibodies and vaccination with individual RSV proteins clearly show that the F protein is the most effective in inducing a protective immune response, the G protein induces protective immunity that tends to be group specific, and other proteins, N and M2, induce little or no protective immune response in mice [6]. In the adult volunteer study [55], antibodies to the F protein and the homologous G protein and neutralizing antibodies correlated with protection from reinfection, but even at the highest levels of antibodies 25% of subjects could be reinfected. Infants responded to natural infection more slowly than older children and adults. In short, humoral immunity can provide some protection from reinfection, but it is of poor quality and limited duration.

Cell-mediated immune response

Studies of the cell-mediated immune response to RSV in humans have lagged behind those of the humoral immune response [6]. Both major histocompatibility complex (MHC) class I-restricted cytotoxic T cells (CTL) and lymphoproliferative responses are observed after RSV infection. In one study of infants with mild RSV bronchiolitis, four of 22 infants had a CTL response to whole RSV [56]. In a study of nine adults, a CTL response was most commonly seen against the N, F, SH, and M proteins [57]. A recent study of a large group of infants with RSV infection provides further evidence of an immunopathological response to this infection involving lymphocytes and eosinophils [58]. The most convincing evidence that cell-mediated immunity is important in human RSV infection comes from patients with compromised immune systems [59]. Patients with defects in cellular immunity can have prolonged virus carriage and shedding [6]. Those with extensive immune deficiencies, for example, those receiving immuno-suppressive chemotherapy or with congenital immune deficiencies, can have high mortality rates with infection [6].

Even with their limitations, studies of RSV infection in animals provide clearer answers to RSV immunity and pathogenesis of disease. Studies of BALB/c mice depleted of B, CD4, or CD8 cells have shown that CD8 and CD4 cells are important for clearing infection [60,61]. The virus can multiply at high levels and for prolonged periods after depletion of T cells. However, CD4 and CD8 cells also seem to contribute to illness with reinfection. Depletion of CD8 or CD4 cells in mice decreases illness with reinfection, whereas depletion of both eliminates illness [6].

Recently developed approaches to determine cytokine production to subtype (TH₁ and TH₂) memory T cell responses might provide new opportunities to define RSV immunity. As originally described in murine T cell clones [62], a subtype of T helper cells, termed TH₂, which can be distinguished from TH₁ cells by their restricted cytokine pattern: TH₁ cells produce IL-2 and IFN γ (and other lymphokines) but not IL-4, IL-5, IL-6, or IL-10; while TH₂ cells express IL-4, IL-5, IL-6, and IL-10 (and other lymphokines) but not IL-2 or IFN γ [63,64]. A third T helper cell population, which exhibits a mixed pattern of cytokine production, is termed Th₀ [65,66].

One study has shown differences in the subtype of T cell response between different RSV proteins; these differences might explain some of the unique features of RSV disease [67,68]. A recent study shows that RSV infection in infants is associated with a predominant TH₂-like response, which could explain some aspects of the immunopathogenesis of RSV infection and the RSV-specific and non-specific IgE antibody response observed [69]. The reports that the increased levels of IgE and IgG4 in serum and nasopharyngeal secretions in infants are associated with RSV bronchiolitis [70–72] also suggest a TH₂ response in RSV infection [72]. A most recent report shows that bronchiolitis (RSV positive in 57% of the subjects) is followed by activation of cellular immunity, and early wheezing in infants is associated with a TH₂ response [73]. Our recent studies also show similar results [74]. It seems that RSV infection is a TH₂ predominant disease and wheezing in infants with bronchiolitis is also associated with TH₂ response.

Mechanisms for RSV vaccine-augmented disease

Antiviral immunity appears not only to provide partial protection against infection but also to contribute to lung pathology. The first evidence that specific immunity could be harmful came in the 1960s, when children were vaccinated with formalin-inactivated RSV (FI-RSV) [75–78]. Vaccinees developed a strong serological response, but were not protected against further infection. Most vaccine recipients who subsequently became infected with RSV developed severe lower respiratory tract disease and some died [78]. The potential immunological mechanisms of vaccine-augmented pathology have been studied and discussed extensively, with a focus on the role of antibody, CD8⁺ cytotoxic T lymphocytes (CTL), and CD4⁺ T cells [6,7,79–82].

Antibody has been found to be an illness-sparing effector mechanism capable of clearing RSV without enhancing pathology or illness in both infants [83] and animal models [84]. The absence of illness in passive antibody treatment studies [85] makes this an unlikely mechanism of

vaccine-enhanced illness. As CD8⁺ CTL have been demonstrated to play an important role in RSV clearance and illness in primary infection [56], it is also unlikely that CD8⁺ CTL were a major effector mechanism in the vaccine-enhanced illnesses caused by RSV [82].

Knowledge of the role of CD4⁺ T cells in the pathogenesis of vaccine-enhanced pathology is incomplete. However, it has been shown that CD4⁺ T cells are more antiviral and more immunopathogenic on a cell for cell basis than CD8⁺ T cells in RSV infected mice [86]. Selective depletion of CD4 T cells abrogates the histological changes of 'enhanced disease' in the BALB/c mouse [81]. The recent report that enhanced disease might be caused by induction of TH₂ memory cells further supports the importance of the cellular immune response to this phenomenon [87]. Another recent report provided additional support for the role of the TH₂ cell response in enhanced disease [88], which showed that a combination of anti-IL-4 and -10 antibodies, but not individual antibodies, eliminated the enhanced pulmonary pathology usually seen in BALB/c mice. On the other hand, the similarities in the clinical manifestations between RSV bronchiolitis and allergic asthma and the fact that T cells from asthmatic patients stimulated by allergic antigens have a TH₂ phenotype make TH₂ cells attractive as contributors to the pathogenesis of RSV bronchiolitis [6].

TH₂ cells in asthma

Based on the cytokine pattern reported to occur in asthma, the hypothesis has been raised that asthma is characterized by an imbalance of T cells with a predominance of TH₂ cells [65,66,89,90,91,92].

More and more studies show that T cell cytokines IL-4 and IL-5 play a major role in allergic asthma [65,66,91,93,94,95] and non-atopic asthma [93,94]. The functional relevance of IL-4 and IL-5 to clinical asthma has been studied in human and animal *in vitro* and animal *in vivo*.

IL-4 has been identified as an essential factor required for B cells to switch to produce IgE in the murine system *in vitro* [96] and using either IL-4 neutralizing antibodies [97] or IL-4 knockout mice [98], IL-4 has been shown to play a key role in initiating and maintaining an IgE response *in vivo*. IL-4 has also been shown to be obligatory for class switching to IgE production by activated B lymphocytes in humans [99,100], while IFN γ inhibits this effect of IL-4 [101]. Anti-IL-4 inhibits IgE production in a murine model of atopic asthma [102], and IL-4 inhibits the secretion of IFN γ at the transcriptional level [103]. In addition to its role in supporting IgE synthesis, IL-4 has been demonstrated to be essential in the commitment of naive CD4 T cells to the TH₂ phenotype *in vitro* [91,104], a function supported *in vivo* by the effect of anti-IL-4 antibody in

inhibiting the development of TH₂ cells [105,106]. These studies indicate that IL-4 plays a pivotal role as the cytokine which initiates the pathological T cell development characteristic of the allergic phenotype as occurs in asthma [66].

IL-5 has been demonstrated to promote growth differentiation, activation and chemotaxis of eosinophils *in vitro* [107,108]. *In vivo*, intratracheal administration of IL-5 induces airway hyperresponsiveness, blood eosinophilia and neutrophilia [109,110], and transgenic mice overexpressing the IL-5 gene develop eosinophilia in peripheral blood, bone marrow, spleen, muscle and liver [111]. Furthermore, IL-5 expression in the lung epithelium of transgenic mice leads to pulmonary pathological changes characteristic of asthma [112]. On the other hand, administration of neutralizing anti-IL-5 antibodies inhibits eosinophilia after antigen challenge in guinea-pigs [66,113–115] and inhibits the airway hyperresponsiveness after antigen challenge and viral infection in guinea-pigs [110,113].

The role of TH₂ cytokines in asthma has been further demonstrated in clinical studies. When the cells from bronchoalveolar lavage (BAL) of atopic asthma were probed for cytokines, IL-5 and IL-4 expression was increased in comparison to IL-2 and IFN γ [89]. This lymphocyte profile is compatible with the TH₂ subpopulation. There is also a significant correlation between the numbers of T cells expressing mRNA for IL-4 and IL-5 in BAL and symptom scores or the degree of airflow obstruction in asthmatics [116]. Furthermore, IL-5 has been detected in the serum during acute exacerbations of asthma [117]. More recently, it is reported that individuals with either atopic or non-atopic asthma show infiltration of the mucosa with cells expressing TH₂ type cytokines (IL-4 and IL-5), providing further evidence for similarities in the immunopathogenesis of these clinically distinct forms of asthma [93,94].

In short, RSV infection is associated with predominant TH₂-like response; and asthma is characterized by an imbalance of T cells with a predominance of TH₂ cells. They have many elements of similarity in their immunopathology.

TH₁ and TH₂ switching

The evidence presented above implicates TH₂ cytokines in both asthma and bronchiolitis. Interestingly, there is evidence in murine [118] and human [119] studies that the placental environment for the fetus is primarily a TH₂-rich milieu. The fetus then is born with a tendency for TH₂ responses. It is believed that early infant infection, particularly bacterial, induces a switch from a substantially TH₂ response to TH₁, and a recent study showed that RSV-primed lymph node cell (LNC) response consists of T helper

cells which are predominantly of the TH₂ subset, secreting IL-4 [120]. These data suggest that RSV infection may perpetuate a 'fetal' TH₂ response, entraining an allergic diathesis in infants in response to infection. If such speculation is correct, then primary prevention of asthma would require immune modulation of early infant infection, such as RSV, which promotes a predominantly TH₂ response [121].

Conclusions

Asthma is a major clinical problem with significant morbidity and mortality. Current therapies assist in control of symptoms of asthma, but do not produce cure.

There is evidence that asthma is likely to be a TH₂ predominant immune response, at least in part, due to virus infection. It is possible that an RSV-induced switch-failure in infancy provides the framework for subsequent TH₂ response to other viruses in older childhood, expressed as asthma.

If such a sequence occurs, reduction in asthma morbidity and mortality may require immune intervention during infant RSV disease.

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