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Respiratory syncytial virus infections in the adult asthmatic – mechanisms of host susceptibility and viral subversion

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Introduction

Respiratory syncytial virus (RSV), a single-stranded RNA virus of the paramyxovirus family, is a major cause of bronchiolitis in infants and is also conjectured to be an early-life influence on the development of asthma [1,2]. While the data supporting a role for RSV in bronchiolitis in children is robust, and there is evidence to support its role in juvenile asthmatics, RSV's role in asthma pathogenesis in adults is not as clearly defined. Our focus will be to review the literature to further elucidate RSV's impact on adult asthmatics, including its importance as a cause of asthma exacerbations. We will examine the morbidity associated with RSV infection and how the immune response may differ between adults with asthma and those without. We will closely review the responses by specific cell types from adults with asthma that are stimulated by RSV. Additionally, we will consider the role of early-life exposure to RSV and its contribution to asthma in adults. Lastly, we will review the mechanisms by which RSV evades normal host immune responses and subverts these responses to its benefit.

RSV in adults and its impact on asthma exacerbations

How important is RSV as a cause of asthma exacerbations?

While not as settled as the data supporting RSV as a cause of bronchiolitis in young children, there is evidence to support RSV's role as an important cause of acute respiratory illness in adults as well. Compared to healthy adults, patients who are at high risk for complications of respiratory illness, including those with asthma, are more likely to require hospitalization when infected with RSV [3]. In a prospective study of subjects 15 to 56 years of age with atopic asthma who recorded symptom scores and daily peak flow rates, viruses were detected in approximately 60% of asthma exacerbations that were associated with symptoms of a respiratory tract infection, with RSV being the most common virus detected [4]. In this same study, roughly 60% of children with allergic asthma had symptoms of a respiratory infection associated with an asthma exacerbation, and RSV was the most commonly detected virus in this age group as well [4]. Viral assays based on the polymerase chain reaction (PCR) were not available at that time for viral detection and therefore the true incidence of viral etiology

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may have been skewed by the limited diagnostic methods available, as only about 30% of those with exacerbations had detectable virus.

In a prospective study of hospitalized patients over a four-year period performed to determine the frequency of specific viral infections that caused respiratory illness severe enough to require hospitalization in patients with chronic illness, RSV was the most commonly detected virus. In this study, 80% of the infections leading to hospitalization were in children less than five years old, while RSV was detected in 17% of those adults hospitalized for respiratory symptoms [5]. One of the caveats of studying the epidemiology of RSV and its contribution to respiratory diseases such as asthma is that this virus has a narrow seasonality; therefore, if a study is performed in the summer, RSV may be underrepresented. On the other hand, if a study is performed in the winter, RSV could possibly be overrepresented as a cause of respiratory infection when compared to other viruses. Therefore, it is crucial to be observant of the season in which a study was performed to account for these potential limitations. As the authors collected data for four entire years, the potential bias of RSV's seasonality was reduced in this study [5].

Even with some evidence supporting the role of RSV in adult asthma exacerbations, other studies have not as clearly implicated RSV. In a study of adult controls and asthmatics without symptoms of an acute exacerbation, sputum was induced with 3% sodium chloride solution and oropharyngeal specimens were analyzed by polymerase chain reaction (PCR) or reverse transcriptase-polymerase chain reaction (RT-PCR) to assess the role of different bacteria and viruses in stable asthma [6]. In this study, rhinovirus, *Chlamydia pneumoniae* or *Bordetella pertussis* positivity was associated with significant reductions in forced expiratory volume in 1 second (FEV₁), FEV₁/forced vital capacity (FVC) or symptoms, but RSV was not [6].

In a prospective study of 79 adult asthmatics with exacerbations requiring hospitalization over a 12-month period, 37% of exacerbations were thought to have infectious etiology, but only one of the 24 confirmed viral infections implicated RSV [7]. This study used serologic testing and cultures of nasopharyngeal aspirates for detection of virus or bacteria, which may have resulted in lower sensitivity for detecting infections than more sensitive diagnostic tests, such as PCR. Retrospectively, other early studies attempting to define the role of viruses in asthma exacerbations seem to have been limited by the technology available for viral detection [8,9]. One study, in which the incidence of both acute asthma and bronchitis was analyzed over an 11-year period, was able to demonstrate increased asthma rates and exacerbations with increasing circulating virus levels based upon epidemiological viral isolation data, but could not causally implicate RSV or any other virus specifically [10].

A longitudinal study of adult asthmatics over a 22-month period examining both symptomatic colds and asthma exacerbations confirmed RSV positivity in only one of 119 documented upper respiratory tract infections, and only once in the 27 infections associated with an objective exacerbation, diagnosed by both symptoms and decrease in peak flow [8]. This study used serum serologic conversion for detection of most respiratory viruses, including RSV, but used PCR for human rhinovirus, the most commonly detected virus. PCR testing for RSV and many other viruses was not yet available at the time of the study and lack of diagnostic sensitivity likely accounted for the low recognized contribution of these viruses.

Another smaller prospective study, which attempted to delineate the role of viral infections in exacerbations of adult asthmatics, used rapid antigen detection and viral culture in all patients presenting to the emergency department over a seven-month period during fall and winter with symptoms of an exacerbation [9]. Even with the addition of serum antibody titers in about half of patients, this group found no positivity for RSV in the population studied, and furthermore, they found no positivity for many other common respiratory viruses, including influenza A or

B, parainfluenza 1, 2 or 3, adenovirus or rhinovirus [9]. The authors concluded that there may be an overstated importance of respiratory viruses in asthma exacerbations, but the lack of use of polymerase chain reaction calls into question that specific conclusion. Furthermore, it has been shown that viral exacerbation etiologies have varied with subject age and the diagnostic methods used for viral detection [11] with adults producing less respiratory secretions than children, further complicating diagnosis in adults [8,12].

Morbidity associated with RSV infections

RSV is an important cause of morbidity in adults and can result in hospital admission and resource utilization, especially during the winter season when the incidence of viral infections increases [3,5,13–18]. In a study conducted over three years in the United Kingdom, from 10–22% of patients who visited a physician for a winter respiratory illness had an RSV infection detected by RT-PCR [19], and in a prospective viral surveillance study over 20 years during the months in which RSV is known to be active in the community, 7% of adults had RSV infection, of which 84% (177/211) were symptomatic [16]. In a retrospective study of a 10-year period in Ontario, Canada designed to study seasonal and temporal patterns of primary care visits for respiratory illnesses, RSV was demonstrated to have a significant influence on visits related to asthma, chronic obstructive pulmonary disease (COPD) and pneumonia, as well as a seasonal influence on the use of health services, with winter visits threefold higher than summer visits [17]. Furthermore, a retrospective analysis of the viral etiology of patients hospitalized for respiratory illness found that among patients with chronic pulmonary disease, RSV accounted for 10.4% of such hospital admissions and together with influenza and parainfluenza, the three viruses were responsible for 75% of all viral infections requiring hospitalization [5].

Another group evaluated morbidity associated with RSV in healthy adults older than 65 years and adults with chronic heart or lung disease in a prospective surveillance study over four consecutive winters and found that RSV disease burden was similar to that of influenza (3–7% in healthy adults and 4–10% in high-risk adults) [3]. Furthermore, RSV accounted for 10.6% of hospital admissions for pneumonia, 11.4% of admissions for COPD, 5.4% of congestive heart failure (CHF) admissions and 7.2% of asthma admissions [3]. The use of healthcare services related to RSV infection was similar to that of influenza among high risk adults and RSV-related hospitalizations are estimated to cost more than \$1 billion annually [3,14]. A retrospective cohort study of patients with chronic lung disease showed RSV to be implicated in 18 per 1000 hospitalizations and 5 per 1000 deaths in elderly persons each year [15]. The mortality associated with RSV in this large study was 46 per 10,000 persons in those aged greater than 65 years with chronic lung disease and 15.3 per 10,000 in subjects aged 50–64 [15]. Additionally, another estimate using viral surveillance and national databases proposed that RSV was responsible for about 10,000 deaths annually in the United States in those aged greater than 65 years [18]. Moreover, treatment of RSV and influenza accounted for 12–14% of antibiotic use in adults with chronic lung disease [15]. Interestingly, RSV-infected patients have been shown to have physical exam findings (wheezing and rhonchi) and laboratory findings (no leukocytosis) that may distinguish RSV infections from bacterial infections [13]; not considering RSV in the differential for such patients is a major hindrance to making the diagnosis.

RSV may also be important as a potential nosocomial infection, especially in hospitalized patients already suffering from respiratory disease or respiratory failure. In a small cohort of intubated patients over a two-month period, 5 of the 11 adult patients on mechanical ventilation in the MICU (mean age 47.8 $\pm$ 15.0 years) had evidence of RSV infection and the mortality rate in this population was 40% [20]. Analysis of adults admitted to the hospital during the winter of 2007–2008 who had RSV infection identified by quantitative RT-PCR revealed

significantly higher nasal viral loads in patients requiring mechanical ventilation than in nonventilated patients, and these higher viral loads were independently associated with respiratory failure [21]. In this same study, significantly more patients hospitalized with RSV had cardiopulmonary disease and COPD, and there was a trend for asthma as well ($p = 0.06$); but the study was underpowered for this endpoint [21]. Therefore, with the advent of more sensitive assays to detect specific viruses, the importance of RSV as a cause of significant morbidity in adults has become more apparent.

Immune response to RSV infection in nonasthmatic adults

The immune response by humans to RSV infection consists of both humoral and cellular components and related cytokine signaling with an intricate interaction among cell types from both arms of the immune response. After initial stimulation of dendritic cells in the nasal mucosa [22,23], in similar fashion to other viruses, it is generally thought that humoral responses, both serum and secretory, are important for protection from RSV infection, while cellular immunity is likely the most important for viral clearance. However, given that recurrent RSV infection is the rule throughout an individual's life, immunity appears incomplete.

Humoral immunity in host defense

Many studies have examined the role of humoral immunity in host defense to RSV, including which viral proteins serve as antigens recognized for sustained immunity. Early in the study of host immune responses to RSV in children, it was noted that recurrent disease was common and multiple infections were needed before a sustained response could be detected serologically [24]. Nonmaternal antibodies could only be discerned after three months of age; however, no antibody to the surface glycoprotein was detected in children less than a year of age [25]. A broadening of the antibody response occurred with increasing age, including a response to the surface fusion protein (F protein) in both IgM and IgA classes, but only rarely was an inclusive response found to all viral proteins [25]. Furthermore, the initial antibody response in Kenyan infants has been shown to be RSV-genotype specific to the variable region of the RSV G protein, with cross-reactivity developing with subsequent infections [26]. In healthy adults, those with lower serum neutralizing antibodies and serum IgG titers to F protein were at higher risk of infection after inoculation with live RSV [27]. For RSV challenge in young healthy adults, nasal neutralizing IgA correlated more with protection than serum neutralizing antibodies [28]. The protective effect of humoral immunity demonstrated in this challenge study has been duplicated in community dwellers as well [29]. Those naturally infected with RSV had lower RSV-specific nasal IgA, serum IgG to F, G_A and G_B , and neutralizing antibodies to group A and B strains, and multivariate analysis demonstrated that lower serum IgG and nasal IgA levels were independent risk factors for infection [29]. In healthy volunteers, RSV neutralizing antibodies to either F protein or G_A protein were protective, but up to one quarter of the subjects were reinfected regardless of antibody titers and by eight months after initial inoculation, almost two thirds could be re-infected [30]. In another study, adults infected with RSV showed a mean 8-fold increase in antibody titers, but at one year, 75% had at least a 4-fold drop in these titers [26].

Given that RSV infection tends to be most severe in the elderly, the question of the effect of age on antibody production and protection has been examined in multiple studies [31,32]. In one study, older patients had higher mean antibody titers than their younger counterparts at baseline, but did not have increased neutralizing activity [31]. Of those elderly and young infected naturally with RSV during the study, no difference was seen in the immune response, with 79% of older subjects achieving a greater than or equal to fourfold rise in microneutralization assay titers to RSV antigens F, G_A and G_B , whereas only 64% of young subjects had a similar rise [31]. As to why the oldest, frailest subjects in the study had the highest antibody titers, the authors hypothesized that a recent RSV infection may be

responsible, especially given the high rate of acute respiratory illnesses seen in adult daycare centers [33], or that the humoral dysregulation or dysfunction that accompanies increasing age may be at play.

In a separate study, while antibody levels were similar between young and old adults at baseline in their study, older individuals had significantly greater neutralizing antibody production to both strains of RSV, as well as increased responses to specific RSV antigens F, G_A and G_B [32]. In this study, there was a trend toward higher viral titers in seroresponders compared to nonresponders, but the authors also speculated that the boosted humoral response might be a reflection of a shift from a Th1 to a Th2 subtype response, given the evidence of diminished T cell responses to RSV in aged mouse models and older persons [34–37]. Furthermore, the ratio of neutralizing antibodies pre- and post-inoculation were similar between the two groups, suggesting a similar qualitative response between the young and old [32]. While 85% of RSV culture-positive elderly nursing home residents had a greater than fourfold rise in RSV-specific IgG, acute antibody titers were similar to those residents without RSV infection. There was no correlation between antibody titers and severity of illness, causing the authors to question the protective effect of antibodies in either acquiring the illness or modulating its severity [38]. On the other hand, one study did show low serum neutralizing antibody titers to be a significant risk factor for increased severity of disease in healthy elderly adults and high-risk young adults (OR = 5.89) [39]. Furthermore, a more recent study found that serum neutralizing antibodies were much more likely to be found in younger patients than in frail patients (92% vs 36.21%) at baseline [40]. Therefore, the discrepancy in the results suggests that there are additional factors other than age that regulate humoral responses to RSV.

Cellular immunity—While the effectiveness of humoral immunity for protection from RSV infection may still be debatable, cellular immunity clearly plays a role in clearance of RSV-infected cells [41]. The role of cytotoxic T cells (MHC class I-restricted) was examined in RSV-infected adults and it was shown that nine of the ten viral proteins were recognized by cytotoxic T cells when B lymphoblastoid cells were infected with recombinant vaccinia viruses with genes for single RSV proteins, of which the nucleoprotein N and surface proteins SH and F were most strongly recognized [42]. Interestingly, the G protein, an antigen commonly evaluated for the humoral immune response, was rarely recognized (2/9), [42]. Others have attempted to elucidate the balance between proinflammatory cells (CD4, Th1 and CD8) and anti-inflammatory cells (CD4, Th2) in RSV infection by measuring cytokines associated with specific cell types in both healthy elderly and healthy young adults [43]. To this end, elderly subjects had a decreased IL-10/interferon (IFN)- γ ratio (CD4 cells only) compared to young adults, indicating perhaps a predominance of proinflammatory T cells in the elderly that might account for the more severe clinical syndrome seen in this population [43]. There was no difference in the number of IL-10- or IFN- γ -producing cells between young and old, but RSV-induced IFN- γ responses were stronger for CD4 cells than CD8 cells, leading the authors to speculate that increased disease severity in the elderly may be related to older subjects' inability to rapidly expand CD8 replication in the setting of infection [43,44]. In fact, one study was able to show that memory T cell populations for RSV are lower for both elderly patients and those with COPD, and they required in vitro expansion to detect RSV-specific CD8 memory cells [45].

Cytokines and virus infection

Cytokines and other signaling molecules produced by immune cells during an RSV infection are a reflection of the cell types that predominant in clearing the virus. Just as nasal IgA has been demonstrated to have a protective effect against RSV infection [28,29], the cytokines and chemokines found in the nasal secretions of patients with RSV infection act as chemoattractants for the immune cells in response to the virus. Peripheral blood mononuclear cells of adults and

children previously infected with RSV showed a RSV-specific response with Th1-type cytokines IFN- γ and IL-2, without similar responses in IL-4 or IL-5 [46]. In healthy, nonsmoking adults inoculated with live RSV, there was increased IL-8 in nasal lavage specimens during and following infection, along with increased levels of regulated upon activation, normal T-cell expressed, and secreted (RANTES), macrophage inhibitory protein (MIP)-1 α and monocyte chemotactic protein (MCP)-1 at the time of viral shedding [47]. Additionally, it has been shown that through up-regulation of toll-like receptor 3 and protein kinase R, RSV increases nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), and thus, IL-8 production [48].

As was seen with both humoral and cellular immunity with RSV infection, the cytokine response differs in healthy populations between younger and older subjects. When cultured peripheral blood mononuclear cells were presented with RSV by autologous dendritic cells, older subjects' PBMCs produced significantly fewer IFN- γ ELISPOTS than younger subjects' PBMCs [35]. The authors suggest that this may be due to an aging defect in T cell response to RSV as previously described [34,36,49], especially in the face of using dendritic cells to maximize costimulation versus using peripheral blood mononuclear cells alone [35].

Association of host cytokine genotype with RSV-induced immune responses

Because of the influence of cytokines on the immune response and the severity of illness in viral respiratory infections, a few studies have been conducted to evaluate the potential impact of gene polymorphisms on cytokine responses to RSV in both adults and children [50,51]. Using DNA extracted from adults experimentally inoculated with RSV, one group was able to genotype the cytokine response using PCR-sequence-specific primer technology [51]. They found that IFN- γ genotype could predict IL-1 levels, with low IFN- γ producers (genotype A/A) making more IL-1 and high IFN- γ producers (genotype T/T or T/A) making less IL-1; furthermore, they showed that TNF α genotype could predict IL-6 and IL-8 protein levels, again in an inverse fashion, with low TNF α producers (genotype G/G) making more IL-6 and IL-8 than high TNF α producers (genotype G/A or A/A) [51]. Additionally, throat and general symptoms correlated with IL-6 protein levels, but not with the IL-6 genotype (44). A similar study in children hospitalized with confirmed RSV infection showed that IFN γ genotypes could predict severity of lower respiratory illness (higher producers, genotype T/T or T/A, were more severe), duration of ICU stay (high producers, genotype T/T or T/A stayed longer) and frequency of acute otitis media (low producers, genotype A/A had more episodes), while the IL-6 genotype inversely correlated with length of oxygen supplementation and hospital stay, with high producers (genotype G/G or G/C) needing less oxygen and shorter stays than low producers (genotype C/C) [50]. High production levels of IFN γ had a protective effect on otitis media, but detrimental effect on the severity of lower respiratory illness and duration of ICU stay, for which the authors speculated that it reflected the degree of infection localization. Interestingly, IL-10 (low producers, ACC/ACC, ACC/ATA or ATA/ATA genotypes) and TGF β 1 (C/C-G/C, C/C-C/C, T/T-C/C or T/C-C/C genotypes) polymorphisms were predictive of likelihood of pneumonia and low oxygen saturations at presentation, respectively, in infants, while these two cytokine genotypes were not predictive of any measures in experimentally inoculated adults [50,52]. Furthermore, in contrast to experimentally-inoculated adults, TNF α genotype was not predictive of any outcomes measured in children [50]. Any discrepancy in outcomes of these two studies regarding the predictability of specific cytokine genotypes may be related to several factors, including the age of patients, natural versus experimental infection, the polymorphisms being examined, severity of illness in individual subjects and the differences in the outcome measures themselves. Furthermore, cytokine responses are influenced not only by genotype, but also by cellular signaling at transcriptional, translational and post-translational levels, all potentially altering cytokine levels.

Other mediators

It has also been suggested that viruses can trigger illness using local inflammatory mediators in addition to the cytokines produced during the systemic immune response. RSV infection and resulting antigen-antibody complexes stimulate a significant thromboxane B₂ response from neutrophils, which may contribute to bronchoconstriction, as well as superoxide, potentially implicating oxidative damage as mediator of pathogenesis [53], all of which may be COX-2 mediated [54]. Healthy adults experimentally inoculated with RSV who develop infection have a significant rise in leukotriene (LT) C₄ and there is an observed temporal relationship between the rising levels and the development of illness [55]. LTC₄ has been implicated as an important mediator in asthma pathogenesis [56,57]. However, while arachidonic acid pathway products have been shown to be upregulated with RSV infection [55], in vitro, prostaglandin E₂ levels were not increased with RSV-infection of human alveolar mononuclear phagocytes [58]. While nitric oxide (NO) has been recognized as an inflammatory mediator in both lower and upper respiratory tract infections [59] and in vitro infection of epithelial cells produced increased nitrites and inducible nitric oxide synthase (iNOS) [60], healthy adults inoculated with RSV who later developed symptoms and had evidence of RSV infection, did not have changes in NO levels [61], which parallels studies done previously with influenza and rhinovirus [62,63].

In vitro epithelial cell responses to RSV

The epithelium of the airway has been demonstrated to be important in immune signaling in RSV infection by releasing chemokines responsible for initial signaling to inflammatory cells. RSV-infected bronchial epithelial cells show enhanced ICAM-1 expression that is thought to be autocrine in nature and mediated by IL-1α [64]. IL-8 has been demonstrated to be constitutively expressed by nasal epithelium, but also upregulated with RSV infection [65]. RSV infection induces activator protein-1 (AP-1), nuclear factor IL-6 (NF-IL6) and NF-κB transcription and translation, which lead to up-regulation of IL-8 production by epithelial cells [66–68]. Early studies of infection of bronchial epithelial cells with RSV showed that IL-8 production was detectable within 4 hours and accumulating in supernatants at 24 hours, while IL-6 and granulocyte macrophage colony-stimulating factor were present by 96 hours after RSV infection, the phase of replication for the virus [69]. RSV infection of well-differentiated respiratory epithelial cells results in significant release of IL-8 and RANTES by 48 hours to the basolateral surface and likely influence the recruitment of leukocytes to sites of infection [70]. These chemokines can be substantially, but not completely, inhibited by administering inhaled corticosteroids [71], possibly explaining the mechanism by which inhaled steroids benefit asthmatics during virus-induced exacerbations. A similar study demonstrated significant increase of RANTES after RSV-inoculation in an infection-dependent manner and a similar effect was seen in vivo when comparing infected children versus both controls and asymptomatic baseline [72].

Immune response to RSV infection in adults with asthma

In examining the immunologic responses of asthmatics to RSV, there were differences when compared to healthy adult responses as described in the previous section. In one study, adults asthmatics had reduced levels of IFNα production by blood cells compared to matched controls when stimulated with RSV [73], which represents an altered systemic response and not just a local cytokine response. Adults with acute asthma exacerbations and viral infection, including RSV, had increased levels of IL-10 compared to both nonasthmatics with viral infections and stable asthmatics without infection [74]. However, while RANTES and MIP-1α mRNA were elevated with viral infection as aforementioned, this response was seen in both asthmatics and nonasthmatics, suggesting that it may be more a reflection of the viral infection and not a response specific to asthmatics [74]. One interpretation of this data is that increased IL-10 gene

expression may be responsible for suppressed eosinophil translocation in acute asthma exacerbations, as IL-10 has previously been shown to have an anti-eosinophilic effect in mouse models [75]. In children and adolescents with asthma, asthma severity has been correlated with IgG₁ and IgG₂ to G protein of RSV [76], suggesting that these antibodies may play a direct role in airway hyperresponsiveness by either activating complement or causing the release of histamine by cross-linking Fc RI receptors on mast cells.

In severe asthma exacerbations that resulted in death, in which viral infection was established by PCR (3 rhinovirus, 2 RSV), higher percentages of activated (CD25+) CD8+ cells were present in postmortem lung samples than in either nonasthmatics or asthmatics whose cause of death was unrelated to asthma [77]. Additionally, perforin expression, a marker of cytotoxicity, was highest in the fatal asthmatic group and there was lower expression of bcl-2, an anti-apoptotic gene, in both groups of asthmatics compared to controls. This may be evidence that the immune system permits apoptosis of virus-infected cells more readily in asthmatics, which may be a viral survival mechanism [77]. Furthermore, CD8+ production of IL-4 and IFN- γ was higher in the fatal asthmatic group than controls, with the IFN- γ /IL-4 ratio half the value found in controls [77], which may support a T-cell switch phenomenon reported in viral infections that results in eosinophilia and impaired viral clearance [78].

Another study demonstrated that the blood eosinophil count was higher in subjects with serologic evidence of current or recent RSV infection further supporting the possible Th1/Th2 switch with RSV infection. Clinically these subjects were more likely to be diagnosed with asthma by a physician or have bronchial hyperresponsiveness on provocative challenge [79]. In subjects with COPD undergoing serial RSV detection sampling, those who are more frequently infected have more rapid decline of lung function, independent of smoking status, frequency of exacerbation, or bacterial load in lower airways. The subjects with COPD also had more airway inflammation, measured by sputum IL-6, IL-8, and myeloperoxidase levels than those less frequently infected [80]. These markers of inflammation were associated with a trend to functional decline in FEV₁, potentially implicating a mechanism by which the virus may cause a decrease in FEV₁, but the authors point out that the persistent inflammation could also predispose to RSV infection as well.

Response of specific cell types from adults with asthma to RSV

Among the different cell types involved in the immune response, it has been postulated that RSV infections in asthmatics may elicit a different response from the same cells than in nonasthmatics infected with RSV [81]. In vitro RSV infection of peripheral blood mononuclear cells revealed that while the cytokines IL-6 and IL-10 that are associated with innate immunity did not differ between asthmatics and nonasthmatics, the adaptive immune response of asthmatics showed a trend to stronger IFN- γ production and significantly decreased IL-13. Neutralizing IL-10 had no impact on the response, supporting the idea that the trend was not due to counter-regulation by IL-10 [81]. Furthermore, blocking toll-like receptor 4, which had been implicated in RSV-mediated cytokine release in mice [82], did not abrogate RSV-stimulated IL-6 or IFN- γ .

Immune responses to other antigens influenced by RSV infection

There has been conjecture and evidence to support the idea that RSV infection may also indirectly contribute to illness in asthmatics and asthma exacerbations by upregulating immune responses to other antigens. For instance, adults experimentally inoculated with RSV have increased wheal and flare responses and new positive allergen responses measurable as late as 21 days after exposure [83]. In seeming contrast to these in vivo findings, peripheral blood mononuclear cells (PBMCs) from atopic adults had increased IL-5 production when exposed to mite allergen, but this was attenuated with live RSV infection, for which these subjects

showed significantly higher IFN- γ production compared to nonatopic subjects [84]. Similarly, the PMBCs from atopic asthmatic subjects had a 6-fold increase in the frequency of IL-4-producing cells to cat and birch allergen compared to nonasthmatics; however, atopic subjects had significantly more IFN- γ -producing cells specific for the RSV F/G proteins than nonatopics [85]. Additionally, inactivated RSV increased IL-10 production in atopic subjects' PBMCs when concurrently exposed to mite allergen, further demonstrating the influence of RSV exposure to immune responses of mite allergen in atopic subjects [84].

Does early-life RSV infection contribute to asthma in adults?

One question that has persisted, regardless of any data supporting RSV's role in morbidity in adult asthmatics, is the existence of a causative role for RSV infection early in life in determining the presence of asthma in adulthood. One longitudinal case-control study of children admitted to the hospital for RSV bronchiolitis in infancy revealed an increased prevalence of asthma up to age 13 compared to controls admitted to hospital for other reasons [1]. Continued follow-up of this Swedish population will be very informative about the long-term risk of severe infant RSV bronchiolitis for adult asthma. In a study in Arizona of children who experienced lower respiratory tract viral infection, yet who were not admitted to hospital, RSV infection in early life was significantly associated with wheezing up to age 11, but not beyond that age [2]. There have been very few studies to date that have followed subjects long enough to be informative about RSV infection in childhood and the subsequent risk for developing asthma. In a long-term follow-up of infants hospitalized with bronchiolitis, a multivariate analysis demonstrated that elevated eosinophils outside of infection predicted recurrent wheezing only until early school years, but not into adulthood [86]. Subjects infected with RSV had lower eosinophil counts during infection than those with non-RSV bronchiolitis, but during the convalescence period there was no difference in eosinophil counts [86]. In a subgroup analysis of these same subjects, non-RSV etiology of bronchiolitis was a significant risk factor for asthma in adulthood compared to those with RSV-associated bronchiolitis as infants, with no significant difference between the two groups when examining lung function, although the power of study was relatively low [87]. A separate analysis of this same cohort showed that while RSV bronchiolitis was an independent risk factor for lung function testing abnormality, it was not associated with either asthma or bronchial reactivity in adulthood when controlling for atopy, diagnosed by at least one positive skin prick test, and atopy was similar between RSV-infected and controls [88]. These results suggest that RSV bronchiolitis in infancy does not directly predispose one to asthma and/or atopy; instead it may represent a marker of common abnormalities seen in both conditions related to immune function [89]. However, the authors do correctly point out that the power of their study is limited by the fact that more subjects in the cohort dropped out than met the diagnostic criteria used for asthma. Therefore, we must await continued follow-up of the cohorts from Sweden and Arizona to more fully determine the relationship between RSV infection in infancy and the development of asthma.

Immune subversion by RSV

RSV has a variety of different mechanisms by which it can suppress the host antiviral immune response and therefore aid its own survival. Two major RSV proteins that antagonize host defense are the nonstructural (NS) proteins, NS1 and NS2 [90]. There are two promoter proximal genes that code for these two proteins which are termed nonstructural because they are synthesized in RSV-infected cells but not packaged in the mature virion structure [90]. The predicted structures of NS1 and NS2 do not share any significant homology with any other protein [90]. Recombinant RSV strains have been created in which either or both NS genes have been deleted to determine how these nonstructural proteins regulate immune evasion. To

fully understand the methods by which the two RSV NS proteins subvert host immunity, a brief review of antiviral host defense is necessary.

RSV binds to the cell membrane through its G protein, which is the attachment protein [90]. The RSV F protein is the fusion protein which allows for the virus to penetrate the cell membrane [90]. Once in the cell, RNA viruses such as RSV can be recognized by RNA helicases that include retinoic acid inducible gene I (RIG-I), melanoma differentiation associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2) [91]. RNA recognition by RIG-I requires the presence of a free 5'-triphosphate structure and this allows for differentiation of nonself viral RNA from self RNA, as host RNA is either capped or post-translationally modified to remove the 5'-triphosphate [92]. Because of this requirement for the 5'-triphosphate structure for RIG-I recognition, viruses that activate RIG-I include RSV and related paramyxoviruses such as human metapneumovirus, as well as influenza A [92]. RIG-I and MDA5 contain two N-terminal caspase activation and recruitment domains (CARDs) which are essential for their signaling activity [91]. After binding nonself ligand, RIG-I and MDA5 interact via these CARD domains with the signaling-adaptor molecule IFN- β promoter stimulator 1 (IPS-1) which is present in the outer mitochondrial membrane [91]. Experiments using an NS2-deletion RSV revealed NS2 inhibited RIG-I-mediated IFN promoter activation by binding to the N-terminal CARD of RIG-I, thus blocking its interaction with the downstream component IPS-1 [93]. IPS-1 recruits a signaling complex to activate transcription factors including interferon regulatory factor (IRF3) and NF- κ B. Activated IRF3 and NF- κ B can translocate to the nucleus, resulting in induced expression of the Type I IFNs, IFN- α and IFN- β , as well as IRF3 target genes and NF- κ B target genes to drive antiviral and inflammatory responses against infection [91].

Other experiments revealed that NS2 is essential for the early inhibition of IFN transcription as infection with the NS2-deletion mutant of RSV resulted in a greater level of IRF3 compared to wild type RSV containing NS2 [93]. Experiments using an RSV deficient in NS1 indicated that this protein also negatively regulated IRF3 activation by reducing IKK ϵ , an important protein kinase that phosphorylates and activates IRF3 [94]. Thus, RSV NS1 prevented IRF3 translocation to the nucleus by inhibiting IRF-3 phosphorylation and activation, blocking Type I IFN production. Other experiments revealed that NS1 and NS2 acted cooperatively to suppress IRF-3 activation, and therefore its nuclear translocation [95]. Type I IFN production can also be blocked further downstream as the NS1/NS2 heterodimeric complex upregulates expression of suppressor of cytokine signaling (SOCS) 1, which also inhibited expression Type I IFN production [96].

After the Type I IFNs are produced by the cell, they can act in either an autocrine or paracrine fashion to stimulate cellular antiviral defense by binding to the same heterodimeric IFN- α/β receptor, resulting in activation of Tyk2 and Jak1 and phosphorylation of the cytoplasmic transcription factors signal transducer and activator of transcription (STAT)1 and STAT2 [92]. IRF9 is recruited to the phosphorylated STAT1/STAT2 heterodimer to form the IFN-stimulated gene factor 3 (ISGF3) heterotrimeric complex which can translocate to the nucleus and induce transcription of the Type I IFN-regulated genes [92]. RSV can interrupt this pathway at several different points. For instance, both NS1 and NS2 degrade STAT2 to inhibit Type I IFN signaling [94,97,98]. The NS1 protein used the elongin-cullin E3 ligase to target proteosomal degradation of STAT2 [99].

Not only do the NS proteins affect production of antiviral IFN proteins, they subvert host defense by other mechanisms as well. Experiments using RSV in which NS1 was deleted resulted in augmented expression of dendritic cell cell surface markers that are characteristic of maturation, in addition to heightened expression of several chemokines and cytokines. While deletion of NS2 by itself did not have an effect on these endpoints, deletion of both NS2 and

NS1 further increased the effects present with NS1 deletion alone [100]. These results suggest that NS1 decreases dendritic cell maturation and cytokine secretion and is aided in this activity by NS2. NS proteins also inhibited cell apoptosis of infected cells by expressing several antiapoptotic factors, allowing for greater viral replication and subsequent release of viral progeny. Experiments using short interfering RNAs (siRNA) for NS1 and NS2 led to a reduction in the expression of antiapoptotic proteins, resulting in early cell death and a significant decrease in RSV growth [101]. Finally, NS2 suppresses cytotoxic T lymphocyte responses. Mouse experiments using an RSV in which NS2 was deleted resulted in a greater RSV-specific cytotoxic T lymphocyte response in the lung, an increase in IFN- γ producing CD8 cells, and cytotoxicity [102].

Summary

Through epidemiologic studies, RSV is increasingly recognized as an important cause of asthma exacerbations in adults, and especially in the elderly. The phenotypic response to RSV infection at the molecular, cellular and the individual subject level can be explained at least in part by the immunologic milieu of the host at the time of infection, which is dependent on host gene-environment interactions and is manifested in the different cytokine and cellular responses between asthmatics and nonasthmatics. The importance of severe early life RSV infection on whether asthma persists into adulthood is unknown, but ongoing longitudinal studies may provide clues as to whether this is the case. Finally, RSV has unique capabilities for subverting host antiviral responses and understanding these mechanisms may provide novel therapeutic targets against this very important pathogen.

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Abbreviations

CARDS	caspase activation and recruitment domains
COPD	chronic obstructive pulmonary disease
CHF	congestive heart failure
FEV₁	forced expiratory volume in 1 second
FVC	forced vital capacity
iNOS	inducible nitric oxide synthase
IFN	interferon
IPS-1	interferon- β promoter stimulator 1
IRF3	interferon regulatory factor
ISGF3	interferon-stimulated gene factor 3
LGP2	laboratory of genetics and physiology 2
LT	leukotriene
MIP	macrophage inhibitory protein
MDA5	melanoma differentiation associated gene 5
MCP	monocyte chemotactic protein
NO	nitric oxide

NS	nonstructural
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NF-IL6	nuclear factor IL-6
PBMCs	peripheral blood mononuclear cells
PCR	polymerase chain reaction
RANTES	regulated upon activation, normal T-cell expressed, and secreted
RSV	respiratory syncytial virus
RIG-I	retinoic acid inducible gene I
RT-PCR	reverse transcriptase polymerase chain reaction
siRNA	short interfering RNAs
STAT	signal transducer and activator of transcription
SOCS	suppressor of cytokine signaling

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