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Challenges and Opportunities for Respiratory Syncytial Virus Vaccines

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Abstract

Reactive airway disease (RAD) is a general term for respiratory illnesses manifested by wheezing. Respiratory syncytial virus (RSV) results in wheezing, either by causing bronchiolitis or by inducing acute exacerbations of asthma. There has been a long standing interest in whether severe RSV bronchiolitis in infancy is a risk factor for the development of asthma later in childhood. While epidemiologic studies have suggested that such a link exists, a very recent study suggests that infants with greater airways responsiveness to methacholine instead have an increased prevalence of severe RSV bronchiolitis. Increased airways responsiveness to methacholine has been implicated as a key factor for loss of lung function in asthmatic subjects, suggesting that instead of being causal, severe RSV infection may instead be a marker of a predisposing factor for asthma. In this chapter, we will explore the evidence that RSV infection leads to RAD in infants and adults, and how these different forms of RAD may be linked.

Keywords

Respiratory syncytial virus (RSV); Reactive Airway Disease (RAD); asthma; wheezing; inflammation; bronchiolitis

5.1 Introduction

The relationship between respiratory syncytial virus (RSV) infection and reactive airway disease (RAD) has been of great interest for decades, mainly because of the question as to whether early life RSV infection predisposes to the development of asthma later in childhood. RAD is a general term for respiratory illnesses manifested by wheezing. Wheezing is produced by turbulent airflow as a result of airway narrowing, either by constriction or obstruction. The wheezing that occurs in asthma is characterized by reversible bronchial reactivity that is predominantly a result of smooth muscle constriction, airway mucus production, and inflammatory cell migration into the airway wall. (NHLBI/WHO workshop report, 1995) It is important to recognize that RAD constitutes

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more than just asthma, and therefore RAD is a broader concept to consider when exploring the relationship of RSV to lung disease. In contrast to asthma, the wheezing that occurs in infants with RSV infection is a manifestation of bronchiolitis.(2006) Bronchiolitis in its simplest definition is inflammation of the bronchioles, which are the smallest air passages in the lung. In this chapter, we will discuss the immunologic and physiologic aspects that lead to both bronchiolitis and asthma, and the supportive, but not definitive, evidence that there is a link between severe bronchiolitis in early life and the later development of asthma.

5.2 RSV Bronchiolitis

5.2.1 Epidemiology of human RSV bronchiolitis

Almost all children are infected with RSV by age 3.(Glezen and Denny, 1973) Most RSV infections in these early years of life are mild and do not require medical attention. However, some infections are more severe and result in outpatient visits to emergency departments, or hospitalization. The percentage of children with mild symptoms versus those with more severe bronchiolitis was reported in infants enrolled in the Tennessee Medicaid program born between 1995 and 2000.(Carroll et al., 2009b) In that study, 82% of infants did not require a health care visit, 9% sought care in an outpatient clinic, 4% required an emergency department visit, and 5% were admitted to the hospital for bronchiolitis. A slightly decreased hospitalization rate of 26 per 1,000 (2.6%) was reported in a retrospective analysis of hospital discharges in the United States from 1997–2006.(Stockman et al., 2012) There are several consistent risk factors that have been identified for severe RSV bronchiolitis. In an Italian study performed over four consecutive RSV seasons from 2000–2004, the seven predictors for hospitalization for RSV infection included the number of children in the family, the chronological age at the onset of the RSV season, birth weight and gestational age, being at least the second baby in the family, daycare attendance, and previous RSV infections.(Rossi et al., 2007) This study suggested that there were specific host/environmental factors that identified children at greatest risk for hospitalization for RSV infection. This was supported by a different study using the Tennessee Medicaid data base previously mentioned in which infants who were 122 days of age (95% CI, 118–126) at the winter virus peak had the highest risk of developing clinically significant bronchiolitis after adjusting for gender, race, number of living siblings, birth weight, gestational age, maternal smoking, marital status, maternal education, region of residence, and season.(Wu et al., 2008) A more recent study in Canadian infants identified age less than 6 months at time of infection, underlying heart disease, and household crowding as risk factors for RSV hospitalization.(Papenburg et al., 2012) Of those hospitalized, age less than 6 months and prematurity were associated with more severe RSV disease. Interestingly, breast-feeding and viral co-infection were protective against hospitalization.(Papenburg et al., 2012) In a study examining infants admitted to the hospital in St. Louis, exposure to post-natal cigarette smoke from the mother, younger age, and Caucasian race in contrast to black race were all predictors of more severe RSV bronchiolitis.(Bradley et al., 2005)

5.2.2 Immunologic determinants of severe RSV bronchiolitis in human infants

RAD and wheezing with RSV-induced bronchiolitis are most likely consequences of physical changes in the airway that are associated with the immune response to infection and

the body's mechanism of clearing the virus. Airway obstruction is a key underlying factor in the severity of RSV infection. The extent of obstruction is determined by the size of the airway, the extent of the inflammatory infiltrate and mucus production, and the dynamic properties of the airway. The pathologic manifestations of bronchiolitis in infants include influx of monocytes, CD3+ double-negative T-cells, CD8+ T-cells, and neutrophils into the airway smooth muscle, epithelium, and lumen.(Johnson et al., 2007) The airway obstruction in RSV-induced bronchiolitis in infants is caused by luminal epithelial and inflammatory cell debris mixed with fibrin, mucus, and edema, in addition to extrinsic airway compression by hyperplastic lymphoid follicles.(Johnson et al., 2007)

Several investigative teams have attempted to determine the immune response profile associated with severe RSV bronchiolitis in order to understand the pathogenic mechanisms leading to morbidity associated with airway obstruction during infection. Bronchoalveolar lavage (BAL) is a technique to sample the alveolar lining fluid and cells in the airway, while nasal lavage can be performed to measure the mediators and cells in the nose as a representation of the upper airway. BAL can be obtained by two methods. First, a bronchoscope is placed into the airway and directed into specific subsegments of the lung, where it is then wedged into a small airway, after which lavage can be performed to obtain samples distal to the location where the bronchoscope is wedged. The second technique is to perform BAL in intubated patients where a suction catheter is advanced until it meets resistance and then lavage fluid is introduced through the catheter and then suctioned. In this situation, the location of the catheter relative to the airways is not known and it is not clear that the catheter is necessarily wedged. Therefore, there may be differences in the results obtained with these techniques.

There are only a few studies in which bronchoscopy and BAL have been performed in infants with bronchiolitis because of the risk associated with hypoxemia during the procedure. One such study compared the BAL findings in 6 healthy children compared to 20 subjects with recurrent wheezing or prolonged wheezing of greater than 2 months over a 6 month period.(Krawiec et al., 2001) In this report, the average age of the healthy children was 23 months compared to 15 months for the wheezing subjects. The wheezing subjects had a significantly greater number of total cells, lymphocytes, epithelial cells, neutrophils, and eosinophils compared to controls. Wheezing subjects also had increased BAL levels of leukotriene (LT)B_4 and LTE_4 than control subjects.(Krawiec et al., 2001) LTB_4 is a neutrophil chemotactic factor.(Wenzel, 1997) LTE_4 is the final metabolite in the cysteinyl leukotriene pathway and has been shown to be an important mucus secretagogue, induce smooth muscle constriction, and be an eosinophil chemotactic factor.(Wenzel, 1997) While mucus is important in clearing foreign particles from the airway, excessive mucus secretion can be an important contributor to airway obstruction. Eosinophils are thought to have an important role in RAD because they produce proteins and enzymes (major basic protein, eosinophil cationic protein, eosinophil peroxidase) that cause desquamation of airway epithelial cells, further contributing to airway obstruction. In addition, eosinophil major basic protein blocks the inhibitory muscarinic M2 receptor on the postganglionic parasympathetic nerve that normally inhibits acetylcholine release.(Jacoby et al., 1993) This inhibition of M2 receptor function leads to increased acetylcholine release and airway smooth muscle contraction.(Jacoby et al., 2001) There was no difference between the

wheezing and healthy children in the BAL concentrations of either PGD_2 or β-tryptase, mediators release predominantly by mast cells, cells which are important in IgE-mediated hypersensitivity reactions that occur in allergic diseases such as asthma.(Krawiec et al., 2001) Bronchoscopy has also been performed to determine if there were differences in mediators and cells present in the lower airway of children with either RSV-induced bronchiolitis (average age 9 months), acute asthma with no identifiable viral infection (average age 5 years), or controls who did not have asthma, but who did have indication for bronchoscopy (average age 6.4 years).(Kim et al., 2003) The subjects with bronchiolitis had significantly greater total cells that the other two groups. Asthmatic subjects had a greater median percentage of BAL eosinophils (3%) than either the bronchiolitis group or control group (both 0%). There was a small subgroup of bronchiolitis subjects with BAL eosinophils (6 subjects with eosinophils and 16 without). Asthmatic subjects and subjects with bronchiolitis and BAL eosinophils had a greater concentration of IL-5 in BAL fluid compared to the subjects with bronchiolitis and no BAL eosinophils or controls. IL-5 is the most important eosinophil growth, differentiation, and survival factor. Interestingly, in this study there was no difference in interferon (IFN)-γ levels in the bronchiolitis, asthma, and control groups. Children with RSV bronchiolitis also had an increased median percentage of BAL neutrophils (37.5%) compared to the asthma group (3.3%) or the controls (2.3%). The increase in total cells and BAL neutrophils in subjects with RSV-induced bronchiolitis was also confirmed by other investigators.(McNamara et al., 2003)

Nasal lavage was used as a means to compare the immune profile of 63 children with RSV bronchiolitis and 22 controls in a study in Texas of children less than 2 years of age.(Bennett et al., 2007) The RSV bronchiolitis group had a significantly greater number of inflammatory cells, as well as median nasal lavage concentrations of IL-8 (269.4 vs 13.8) compared to controls. IL-8 is an important neutrophil survival and chemotactic factor and the increase demonstrated in this study suggests a mechanism for the increased neutrophils seen in the previously mentioned bronchoscopy studies. There were also significant increases in nasal lavage IL-6, GM-CSF, IFN-γ, TNF-α, IL-1β, G-CSF, and MIP-1β concentrations in the RSV group compared to controls. The subjects were stratified based on age at time of infection into four groups, 0≤3 months, 3≤6 months, 6≤12 months, 12≤24 months, and there was no difference between the groups and cytokine levels. Interestingly, there was an inverse correlation between nasal lavage levels of IL-6, IL-8, IL-10, and IFN-γ and the duration of supplemental oxygen necessary to prevent hypoxia in the RSV group. (Bennett et al., 2007) Higher nasal lavage levels of IFN-γ were associated with protection against RSV illness in a British study of 197 infants admitted to the hospital with RSV bronchiolitis.(Semple et al., 2007) Therefore, a deficient IFN-γ response may be a risk factor for more severe RSV disease. IFN-γ is the signature cytokine produced by CD4+ T helper (Th1) cells, and is also produced in abundance by CD8+ cytotoxic T lymphocytes, and Natural Killer (NK) cells which have potent anti-viral activity.

5.2.3 Primary Infection in Mice

Examining host immune response to naturally occurring RSV infection in humans is very complex. First, almost every host has a unique genetic composition. Second, there is little similarity in the environments in which people live or work. Third, there are genetic

differences between RSV strains that may greatly affect the host immune response. These complexities are somewhat simplified by the use of the mouse model of RSV where at least a relatively constant environment can be maintained and utilizing genetically identical mice minimizes differences in host immune responses. However, mice are not perfect models of human disease. Beside the fact that some immune molecules present in humans have no murine counterpart, the airway epithelial desquamation seen in humans is virtually absent in mice. Some of the benefits of using the mouse model are the opportunity to test the inflammatory response of different RSV strains in hosts of the same strain of mouse, or to use mouse strains that vary in only one gene to determine the impact of that gene on the host immune response. (Also see Chapter 18).

The impact on viral genetics on airways responsiveness to methacholine, a surrogate of RAD has been extensively studied between RSV strains A2 and line 19.(Lukacs et al., 2006;Moore et al., 2009) Both A2 and line 19 are RSV group A viruses. There are two major antigenic groups of RSV, A and B, which are classified based on monoclonal antibodies to the major structural glycoproteins G and F.(Anderson et al., 1985) A2 was isolated in Australia in the late 1950s and line 19 is a clinical isolate from the University of Michigan cultured in the 1960s. In BALB/cJ mice, line 19 infection induced lung IL-13 protein expression which was not detected in A2 infected mice.(Lukacs et al., 2006) IL-13 is a central mediator of airway responsiveness, in part as a result of its direct effects on smooth muscle contraction and epithelial cell mucus metaplasia as well as mucus secretion.(Wills-Karp et al., 1998) Indeed, line 19-infected mice had goblet cell metaplasia and hyperplasia, whereas neither occurred following A2 infection.(Lukacs et al., 2006) Line 19 infection induced significant increases in Muc5ac, a mucus gene expressed in the airways, and gob-5, a member of the Ca2+-activated chloride channel family which has selective expression in airway goblet cells. Mice infected with line 19 had significantly increased airways responsiveness to methacholine compared to mice similarly infected with A2. In this technique, mice are anesthetized, intubated, mechanically-ventilated, and challenged with either aerosolized or intravenous methacholine. Methacholine is a synthetic derivative of acetylcholine, which causes airway smooth muscle constriction.(Cockcroft, 2010) The methacholine challenge test is performed in people to help make the diagnosis of asthma when other causes of dyspnea are being considered. People with asthma have increased airways obstruction with concomitant decrease in pulmonary function when they are challenged with aerosolized methacholine in comparison to persons without asthma. The meaning of methacholine reactivity in mice is not as clear as in humans, but the airways responsiveness induced by methacholine in mice is likely a result of a combination of heightened smooth muscle construction, the presence of epithelial mucus metaplasia narrowing the airway lumen, mucus present in the airway, and infiltration of inflammatory cells in the airway wall. In these experiments, the mucus airways responsiveness and mucus seen with line 19 infection was dependent upon the ability of this strain to induce lung IL-13 protein expression, as neither characteristic was present in IL-13 knockout mice. The differences seen between A2 and Line 19 were independent of viral load.(Lukacs et al., 2006)

Further studies have revealed the importance of differences in the F protein between line 19 and A2 in modulating lung IL-13 protein expression, airway epithelial cell mucus

metaplasia, and airways responsiveness. Generation of chimeric RSV strains using a reverse genetics approach where all genes are from the A2 strain with the exception of the line 19 F protein (rA2-line 19F) or an A2 F (rA2-A2F) revealed that rA2-line 19F infection recapitulated the lung IL-13 protein expression, epithelial cell mucus metaplasia, and airways responsiveness seen with line 19 infection, while these were absent from rA2-A2F. Studies are ongoing to determine which amino acids in the F protein regulate these immunologic and physiologic parameters.(Moore et al., 2009)

Mice infected with different low passage clinical isolates also may have pathologic sequelae similar to those seen with line 19 and A2 infection.(Stokes et al., 2011) Nasal secretions obtained from children presenting to the Vanderbilt Vaccine Clinic with RSV group A infection were used to infect HEp-2 cells. The isolates were passaged by limiting dilution and 9 strains were then propagated. BALB/cJ mice infected with one of these strains, RSV A2001/2–20, had lung IL-13 protein expression, epithelial cell mucus metaplasia and increased airway mucus and airway responsiveness. In contrast, mice infected with another strain, RSV A2001/3–12, did not have lung IL-13 protein expression, increase airway responsiveness or mucus production. Interestingly, the child infected with RSV A2001/2–20 had a more severe lower respiratory tract illness than the child infected with RSV A2001/3–12. Studies are ongoing to define the role of RSV strain differences in RSV-induced disease severity and asthma development.(Stokes et al., 2011) (Also see Chapter 3)

5.3 RSV-induced bronchiolitis and asthma later in childhood

In the 1980's several retrospective studies suggested that severe RSV infection in infancy predisposed to the development of asthma later in childhood. However, other retrospective studies refuted these findings, leaving the role of RSV-induced bronchiolitis in asthma development in doubt. Two large prospective longitudinal studies published in the 1990s supported the concept that severe RSV lower respiratory tract infection in early life was an important risk factor for asthma or wheezing-related illness up to adolescence. The first report was a prospective cohort study of 47 Swedish children enrolled before 1 year of age with severe RSV bronchiolitis that was compared to age and gender matched controls in the same location as the cases.(Sigurs et al., 1995) At 3 years of age, the children admitted to the hospital with RSV bronchiolitis had a significant increase in asthma compared to the control group (23% vs 1 %; $p<0.001$). In addition, allergic sensitization as defined by a positive skin test to an aeroallergen was also significantly increased in the RSV bronchiolitis group compared to controls (32% vs 9%; $p=0.002$) In this study, RSV bronchiolitis was the most important risk factor for asthma and allergic sensitization, with a family history for asthma or allergic disease further increasing this risk. This cohort has now been followed to age 18 and the RSV bronchiolitis group continues to have an increased prevalence of asthma (39% vs 9%) and allergic sensitization (41% vs 14%) compared to the control group.(Sigurs et al., 2010) In addition, persistent or relapsing wheezing at age 18 was significantly increased in the group that had RSV bronchiolitis in infancy compared to the controls (30% vs 1%) and the RSV group had decreased lung function compared to controls, independent of a diagnosis of asthma. The second report examined 1,246 newborns were enrolled into the Tucson Children's Respiratory Study between May 1980 and January 1984.(Stein et al., 1999) Lower respiratory tract illnesses (LRTI) suffered by these children in the first three

years of life were assessed for etiologic agents by means of culture and serology and the children were followed to age 13 for determination of wheezing. In this study, RSV LRTI was defined as deep or wet chest cough, wheezing, hoarseness, stridor, shortness of breath. RSV LTRI was associated with an increased risk of infrequent wheezing and frequent wheezing at age 6 and 11, but not at age 13. These results suggested that children might "outgrow" the effect of early life RSV LRTI on wheezing at the start of the teenage years. In this study, RSV LTRI before age 3 was not a risk factor for aeroallergen sensitization as defined by skin testing. A more recent population-based birth cohort study of 90,341 children further suggests a link between the severity of early life RSV infection and the later development of asthma.(Carroll et al., 2009a) The Tennessee Asthma Bronchiolitis Study (TABS) examined the children enrolled in the state Medicaid program from 1995–2000 and found that infants requiring a health care visit for respiratory symptoms during the winter months when RSV is the dominant cause of bronchiolitis were significantly more likely to be diagnosed with asthma between the 4 to 5 ½ years of age (relative risk 1.98). Further investigation of the TABS database revealed that there was a severity-dependent relationship between infant bronchiolitis severity and childhood asthma. Of the children assessed in this study, 11.6% were hospitalized for bronchiolitis, 8.2% had an emergency department visit for bronchiolitis, 13.7% had a clinic visit for bronchiolitis, and 66.5% had no health care visit for bronchiolitis. Infants that had a bronchiolitis hospitalization during infancy had an adjusted odds ratio of 1.51 (1.26–1.80) of an asthma diagnosis at age 4 to 5 ½ years. One limitation of this study was a lack of virologic confirmation of the diagnosis of RSV being the causative agent of bronchiolitis. This is an important concern given the recent recognition that rhinovirus (RV) has also been determined to be a cause of infant bronchiolitis. In one study, wheezing with RV from birth to age 3 was associated with a greater risk of asthma at age 6 (odds ratio 9.8) compared to RSV (odds ratio 2.6), although wheezing from either virus conferred a greater risk of asthma compared to children who did not wheeze.(Jackson et al., 2008)

Other prospective studies support the concept that children who suffer from severe RSV infection in infancy have a predisposition to the later development of asthma and eczema, both diseases associated with allergic diatheses. Two hundred and six infants in St. Louis were enrolled in the RSV Bronchiolitis in Early Life (RBEL) cohort and followed prospectively through 6 years of life.(Castro et al., 2008) Of these children with severe RSV bronchiolitis during infancy, 48% developed asthma and 48% were diagnosed with eczema at age 6. Thirty-two percent had allergic sensitization, as defined by positive skin tests to allergens prevalent in the St. Louis region.(Castro et al., 2008) Independent determinants that were significantly linked to physician-diagnosed asthma at the seventh birthday included the mother having asthma, exposure to high levels of dog allergen, positive aeroallergen skin tests at age 3, recurrent wheezing during the first 3 years of life, and CCL5 (RANTES) expression in nasal epithelia during the acute RSV infection during infancy. Of this cohort, white children and children who attended day care were significantly less likely to have physician-diagnosed asthma at the seventh birthday.(Bacharier et al., 2012) Interestingly, when peripheral blood was drawn from the children diagnosed with asthma at age 6, the isolated T cells had decreased IL-13 expression following phorbol 12-myristate

13-acetate (PMA) and ionomycin stimulation compared to those without asthma.(Castro et al., 2008)

While severe RSV bronchiolitis has been linked to the development of asthma later in life, it is possible that the severe RSV infection is not causative of asthma, but instead a marker of the true risk factor for asthma. This possibility was investigated in the Copenhagen Prospective Studies on Asthma in Childhood ($COPSAC_{2000}$) cohort, a prospective clinical study of a birth cohort of 411 neonates born to mothers with a history of asthma.(Chawes et al., 2012) Methacholine challenge and pulmonary function measurements were performed on 1-month-old infants using the raised volume rapid thoracoabdominal compression technique before these children had experienced a lower respiratory tract infection. The infants were then prospectively followed and closely monitored for bronchiolitis signs and symptoms. Thirty-four subjects (8.5%) were diagnosed with acute severe bronchiolitis before 2 years of age. Twenty-one of these children (62%) required hospitalization and twenty-three of those diagnosed with bronchiolitis had detectable RSV infection. Those children who subsequently experienced severe bronchiolitis had a 2.5-fold increased responsiveness to methacholine at one month of age compared to control subjects. There was no difference in pulmonary function at 1 month between those who developed bronchiolitis and those who did not.(Chawes et al., 2012) This study strongly suggests that baseline bronchial responsiveness to methacholine is an important risk factor for severe bronchiolitis. This study has two significant implications. First, children who are at greatest risk for bronchiolitis can be identified for prophylactic treatment with RSV immune globulin. Second, it is highly likely that these children with increased bronchial responsiveness to methacholine are those that are more likely to develop asthma later in childhood because bronchial responsiveness has long been recognized as the prime factor that determines longitudinal loss of lung function in asthmatic adults.(Peat et al., 1987;Rijcken et al., 1995;Van Schayck et al., 1991)

5.4 RSV as a cause for asthma exacerbations

5.4.1 Identification of RSV during asthma exacerbations in people

Asthma is a disease of intermittent, reversible airway obstruction.(Lemanske, Jr. and Busse, 2010) During exacerbations of asthma, patients experience dyspnea, chest tightness, and wheezing, often associated with cough. Approximately 90% of children with asthma have allergic sensitization defined as positive skin tests to common aeroallergens. Roughly 75% of adults with allergies are allergically sensitized. While exposure to an allergen to which one is sensitive may result in an allergic reaction resulting in increased inflammation and subsequent airflow limitation, in actuality, viral infections are the predominant cause of exacerbations of symptoms and decreased pulmonary function. Approximately 80–85% of children and about 50% of adults with asthma exacerbations have detectable virus by polymerase chain reaction (PCR) in their respiratory tract secretions.(Atmar et al., 1998;Johnston et al., 1995) The virus most commonly identified in asthma exacerbations in both children and adults is rhinovirus; however, RSV has also been attributed to be a cause of acute worsening asthma symptoms. In British children aged 9–11, RSV was detected in 5% of those subjects who were identified as having a viral infection during an asthma

exacerbation.(Johnston et al., 1995) In a study of 49 Australian adults who were enrolled within 4 hours of an emergency department visit for an asthma exacerbation, 19 (39%) had RSV detected by either PCR or direct fluorescent antigen.(Wark et al., 2002) To our knowledge, there are no other studies which report the RSV detection rate in adults with acute asthma exacerbations; however, a 39% detection rate seems unusually high given that only 8% had detection of rhinovirus, which in most studies is by far the most common virus detected in adult asthmatics. In the Australian study, the subjects were enrolled from February 2-December 1, which should have included the peak times of both RSV and rhinovirus infection in the Southern hemisphere. In any case, RSV has been implicated as a cause of acute asthma exacerbation in both children and adults.

5.4.2 Mouse model of RSV-induced asthma exacerbation

A combined mouse model of allergic airways inflammation and RSV infection has to discern the immunologic determinants that result in virally-induced airways responsiveness. In this model, allergic airways inflammation was induced by sensitization and subsequent sequential challenges with ovalbumin, followed by RSV infection during the allergen challenges.(Peebles Jr. et al., 1999) RSV was chosen over rhinovirus as mice lack the receptor for the rhinovirus group, major receptor group, most often associated with asthma exacerbations.(Bartlett et al., 2008) RSV challenged compared to unchallenged mice during ongoing allergic airway inflammation resulted in airway responsiveness and increased airway mucus production, but a decrease in lung levels of the Th2 cytokines IL-5 and IL-13..(Peebles, Jr. et al., 2001b) In contrast to the decrease in Th2 cytokines, RSV infection increased lung protein expression of IL-17A, cytokine that has been associated with airway epithelial mucus metaplasia and inflammation that is resistant to corticosteroid treatment. (Hashimoto et al., 2004) While mucus metaplasia and airway mucus obstruction accounts for some of the airways responsiveness witnessed in this model, there is a strong component of smooth muscle constriction as well as evidenced by the inhibition of airways responsiveness with a Rho kinase inhibitor.(Hashimoto et al., 2002) The timing of RSV infection relative to allergic sensitization and challenge is critical to the inflammatory and physiologic phenotypes that result from this combined model.(Hashimoto et al., 2002;Peebles, Jr. et al., 2001a) RSV infection prior to allergen sensitization and challenge reduced airways responsiveness compared to sensitization and challenge alone, while RSV infection either after sensitization and challenge, or during sensitization and challenge increased airways responsiveness. We are currently in the process of determining the role of IL-17A in RSV-induced airways responsiveness and epithelial cell mucus metaplasia. This model should provide greater insight into other mechanisms by which RSV may exacerbate asthma in humans with underling allergic inflammation.

5.5 Conclusion

In this chapter, we have explored the evidence that RSV causes different forms of RAD, both bronchiolitis and acute asthma exacerbations. However, there are still unanswered questions. First, confirmatory studies need to be performed to determine if increased airways responsiveness to methacholine is truly a risk factor for severe RSV bronchiolitis, and whether this increased methacholine responsiveness is also a risk factor for the later

development of asthma, independent of severe RSV bronchiolitis. Second, once a vaccine against RSV is developed, it will be important to determine if it reduces the prevalence of asthma or wheezing at age 6, since severe RSV bronchiolitis in infancy has been linked to asthma inception. This endpoint may be more difficult to assess in regard to the efficacy of a vaccine which may be targeted at children great than 6 months of age, since infant birth approximately 4 months before the winter virus peak carried the highest risk of developing childhood asthma. Third, more work needs to be performed in the area of RSV-induced acute asthma exacerbations in adults and the elderly and whether specific treatment with cytokine antagonists or prevention with an RSV vaccine can be safely and efficaciously utilized to prevent the sequelae of the symptoms and pulmonary function abnormalities resulting from these exacerbations.

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