

Epidemiologic, Experimental, and Clinical Links between Respiratory Syncytial Virus Infection and Asthma

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INTRODUCTION

Respiratory syncytial virus (RSV), the most common cause of severe lower respiratory tract disease among infants and young children, typically infects persons by 2 years of age and can cause subsequent infections throughout life (122). By 2 years of age, almost all children have been infected with RSV, and over half have been infected twice (38). In Spain, the annual hospitalization rate is 37 out of 1,000 infants under 6 months and out of 1,000 among those under 1 year of age (137). Persons at increased risk for severe disease or death related to RSV include premature infants, elderly adults, and persons of any age with compromised respiratory, cardiac, or immune systems (27, 139). RSV is transmitted from person to person via close contact, droplets, or fomites. In temperate climates, peak RSV activity typically occurs during the winter. However, year-to-year national and regional variability in the RSV season onset and offset occurs in the United States (87). RSV circulations also differ by geographic location; for example, Florida has an earlier season onset and a longer season than the rest of the United States (16). A case-control study was undertaken in southern Israel, and the authors examined the possible association between birth season (date of birth) and future development of asthma in children. Infants who were exposed to RSV infection at a very young age were better protected against the development of asthma, although those born at the end of winter and in early spring and who were exposed to RSV infection during the last quarter of their first year of life might be a higher risk for future development of asthma (36).

About 30% of children hospitalized for acute bronchiolitis will have recurrent respiratory symptoms in the years following

their having the disease (124, 125, 127). This observation raises the possibility of a relationship between acute bronchiolitis and the subsequent development of bronchial hyperreactivity. A number of authors have studied a possible role of this virus in the development of asthma, although the nature of the association between bronchiolitis and asthma is not completely understood. Asthma could be a direct consequence of RSV infection itself, or the virus may trigger changes in pulmonary physiology in patients who are especially predisposed to asthma (117). RSV infection could increase susceptibility to asthma by acting on the immune response, genetic factors, or neural control of the respiratory tract (82). Experimental models have also shown long-term persistence of RSV in respiratory epithelial cells (121). The elucidation of the relationship between RSV infection and the development of asthma, as well as a better understanding of the nature of their association, would have important implications for prevention and treatment. However, the studies so far have been observational, and they differ widely with regard to population, design, methodology, and length of follow-up. A critical appraisal of the epidemiologic, experimental, and clinical links should enable us to better understand the relation between RSV infection and the development of asthma.

PATHOBIOLOGY OF RSV INFECTION

The RSV genome is composed of single-stranded negative-sense RNA. The mature, infectious virus particle consists of ribonucleoprotein (RNP) formed by the interaction of the viral genomic RNA with the nucleocapsid (N) protein, the phosphoprotein (P), and the large (L) protein. The M2-1 protein is a virus protein required for efficient transcription of the virus genome by the polymerase complex (20, 28, 45, 53, 142). The RNP is surrounded by two layers (the matrix [M] protein and a lipid envelope derived from host cell); three virus-encoded proteins (the attachment [G], fusion [F], and small hydrophobic [SH] proteins) are embedded within the envelope (3, 5, 15, 89, 96, 114). The two nonstructural proteins, NS1 and NS2, are

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not readily detectable in virions but are found in RSV-infected cells (26, 59).

The receptor(s) for RSV has not been unequivocally identified, but *in vitro* data provide some clues about the mechanisms of RSV entry. Heparin-like glycosaminoglycans, unbranched polysaccharide chains on the surface of most mammalian cells, have been implicated as receptors or coreceptors for RSV (78) or in playing some role in virus entry (52). Although glycosaminoglycans are important for RSV G protein interaction with target cells, the precise cellular receptor of RSV remains unknown. The viral F protein, which mediates viral penetration by fusion of the viral envelope with the host cell plasma membrane, has been shown to bind to intercellular adhesion molecule-1 (ICAM-1) (8, 29, 72, 75). Studies using both light and electron microscopy techniques have shown that RSV matures predominantly as filamentous structures on the surface of infected cells. Moreover, lipid rafts have been implicated in the assembly process of the virus particles (13–15, 62, 80).

The airway epithelium is the main target of RSV infection. After infection, RSV replicates in the respiratory mucosa, leading to epithelial damage and perivascular mononuclear infiltration (4, 30). When infected by virus, epithelial cells respond by producing a number of potent immunomodulatory and inflammatory mediators, including cytokines and chemokines (7, 31, 33, 88, 144). RSV infection activates signaling pathways in airway epithelial cells through the surface molecule toll-like receptor 4 (72). It was shown previously that RSV replication activates various transcription factors, including NF- κ B, a central mediator of RSV-induced airway inflammation (33, 48, 61, 135, 144). The cytokines induced by viral infection are also known to activate the JAK/STAT (signal transducer and activator of transcription) signal transduction pathway, which might regulate the subsequent adaptive immune response (130). Subsequent to the acute infection, epithelial cells are potentially capable of presenting viral antigens to lymphoid cells (102, 103). Viral clearance involves the induction of both cellular and humoral immunity; however, immunity is considered incomplete, as the virus can reinfect the host (40, 54). Natural immunity to RSV appears to be minimal, and annual reinfections are frequent during the first years of life (57).

Autopsy studies of children who died of RSV-induced bronchiolitis revealed the degree of inflammation generated by the immune response and the subsequent airway obstruction. The infection results in the loss of cilia, sloughing of epithelial cells into the airway, collection of desquamated epithelial cells, polymorphonuclear leukocytes, fibrin, lymphocytes, and mucus within the airway, and edema around the airway. The degree of epithelial damage has been correlated with the magnitude of inflammation and airway hyperreactivity (AHR) (47, 54, 79, 105). Lower respiratory tract RSV infection in infancy significantly increased the odds of having wheezing fits up to 11 years of age in an outpatient population and more than quintupled the risk of asthma/recurrent wheeze at age 13 for infants who had been hospitalized with RSV bronchiolitis, in comparison to control subjects without infection (43% versus 8%) (127, 131).

EPIDEMIOLOGY OF RSV

RSV is the leading cause of bronchiolitis in infants worldwide. In temperate climates, most RSV infections occur between November and May, whereas in tropical climates, RSV infections occur year-round (12). In the United States, RSV-related disease is a yearly epidemic that peaks in January or February in most parts of the country and slightly earlier in the southeast (17). During the winter epidemics, RSV can infect up to 100% of the children in day care centers (56). The age related to the highest morbidity risk of RSV infection is 50, 95). RSV is also a major cause of respiratory illness in the elderly and high-risk adults. It has been estimated that more than 120,000 infants in the United States will be hospitalized annually with RSV infection, with more than 200 deaths occurring as a result of this illness (122, 123). About 50% of infants hospitalized for lower respiratory tract RSV infection have subsequent episodes of wheezing that in some cases can persist to 11 years of age or later ages (51, 129). There is also evidence that congenital vulnerability and host-dependent genetic heterogeneity are involved in the long-term effects of the infection (76, 77). Some studies have linked severe early RSV infection with allergic sensitization leading to asthma (127). Table 1 presents a list of previously conducted studies showing the increased risk of recurrent wheezing, bronchial hyperreactivity, or asthma in children after RSV-induced bronchiolitis in infancy.

In a population-based birth cohort study conducted by Henderson et al. (58), a total of 150 infants (1.1% of the cohort) were admitted to the hospital within 12 months of birth with RSV-induced bronchiolitis. The prevalences of wheezing were 28.1% in the RSV group and 13.1% in the controls at 30 to 42 months and 22.6% in the RSV group and 9.6% in the controls at 69 to 81 months. The cumulative prevalences of asthma were 38.4% in the RSV group and 20.1% in controls at 91 months (58). After a study conducted in Norway, the researchers concluded that children hospitalized for early-life bronchiolitis are susceptible to recurrent wheezing and reduced pulmonary function by 7 years of age compared to age-matched children not hospitalized for early-life bronchiolitis (32). In a different study, conducted by Schauer et al. (118), a positive test for immunoglobulin E (IgE) antibodies was noted in 33% of RSV-infected children as opposed to 2.3% of children in control group. They concluded that severe RSV-induced bronchiolitis during the first year of life is an important risk factor for the development of wheezing and sensitization to common allergens during the subsequent year (118). Sigurs et al. (127) studied the outcome for 13-year-olds (46 children with RSV and 92 control subjects). They reported 43% prevalence of asthma and recurrent wheezing and 39% prevalence of allergic rhinoconjunctivitis among the RSV-infected group as opposed to 8% and 15% prevalences among the control subjects, respectively (127). Overall, the epidemiological studies have shown that RSV infection is a significant risk factor for future development of asthma, particularly in individuals who have genetic predisposition for allergic disease.

TABLE 1. Clinical and epidemiologic studies linking RSV infection to asthma

Researchers and yr (reference no.)	Location	No. of human subjects	No. of yrs after admission to hospital with bronchiolitis	Outcome/comment
Rooney and Williams, 1971 (115)	Australia	62 (2–7 yrs of age)		A significant association of asthma in 56% of children who subsequently experience wheeze
Gurwitz et al., 1981 (46)	Canada	48	9–10	Incidence of bronchial hyperreactivity is 57%
Hall et al., 1984 (49)	United States	29	8	An association between RSV infection and chronic abnormalities of pulmonary function
Mok and Simpson, 1984 (85, 86)	United Kingdom	200	7	Atopy and bronchial hyperreactivity independently contribute to augmented response to RSV postinfection
Welliver and Duffy, 1993 (141)	United States	43	7	Decreased pulmonary function following bronchiolitis is related to atopy
Sigurs et al., 1995 (126)	Sweden	47	2	RSV infection during first yr is an important risk for asthma and allergy in the subsequent 2 yrs, especially in genetically predisposed children
Stein et al., 1999 (131)	United States	>180	13	Lower respiratory tract RSV infections are associated with increased risk of frequent wheeze by age 6; risk decreased markedly with age and was not significant by age 13
Sigurs et al., 2000 (125)	Sweden	47	7	RSV-induced bronchiolitis severe enough to cause hospitalization is highly associated with the development of asthma and allergic sensitization at age 7.5 yrs

CLINICAL STUDIES

Two very important and pertinent prospective studies in relation to the link between RSV and asthma are noteworthy. The first study included 47 previously healthy infants hospitalized for RSV-induced bronchiolitis during their first year of life and a reference population of 93 infants, matched for age, sex, family history of reactive airway disease (RAD) or atopy, and general living environment and with no history of RSV infection (124). The study design included RAD, wheezing, and recurrent wheezing as the outcome measures. The allergic status of each patient was determined by skin prick testing and titers of serum IgE antibodies. By 7 years of age, 30% of children in the RSV-infected group (versus only 3% of children in the reference group) had experienced RAD. The cumulative prevalence of recurrent wheezing among children in the RSV group was twice that observed for the reference group (68% versus 34%, respectively), and the presence of any wheezing at age 7 years was 38% in the RSV-infected group and 2% in the control group. RSV-induced bronchiolitis was the only significant risk factor for RAD, whereas RSV-induced bronchiolitis, family history of atopy, and male sex were all risk factors for the presence of any wheezing. Multivariate analysis showed that the highest frequency of RAD occurred when both RSV-induced bronchiolitis and a family history of atopy were present as risk factors. That study also found a link between RSV-induced bronchiolitis and atopy; this has not been observed in other studies, possibly due to differences in the severity of RSV-induced disease and/or genetic background among the populations examined.

A second, questionnaire-based study included 519 infants enrolled in the Tucson Children's Respiratory Study who developed mild to severe RSV-induced disease without necessar-

ily requiring hospitalization (131). On each of four occasions, when the children in the study were 6, 8, 11, and 13 years old, their parents were asked to complete a questionnaire about current parental smoking status and the child's history of wheeze. The study prospectively enrolled 1,246 infants born from 1980 to 1984. In this study, lower respiratory tract infection with RSV was associated with increased risks of both infrequent and frequent wheezing (odds ratios of 3.2 and 4.3, respectively) at age 6, but this risk was reduced as children became older, such that it was not significant at age 13. These investigators did not find a relationship between lower respiratory tract RSV infection and skin test reactivity to aeroallergens (131).

CELLULAR AND MOLECULAR LINKS

Epithelial cells are the first line of defense of the airway, and their exposure to either RSV or allergens causes signals the release of several chemokines and cytokines. These inflammatory mediators damage the epithelium and cause loss of epithelial cells, leading to increased airway permeability, mucous plugging, and decreased clearance. The degree of epithelial damage is correlated with the magnitude of inflammation and AHR (47, 54, 79, 105). Epithelial cells also regulate inflammatory events by secreting various cytokines that communicate in a paracrine manner. One of the earliest events in response to RSV infection is the initial innate cytokine response, with a time-dependent increase in expression (21). The early cytokines, including the type I interferons (IFNs), IFN- α and IFN- β , interleukin-12 (IL-12), and IL-18, have been detected in the respiratory secretions of infants with RSV infection (35, 130). Recent studies have shown that in the response to RSV

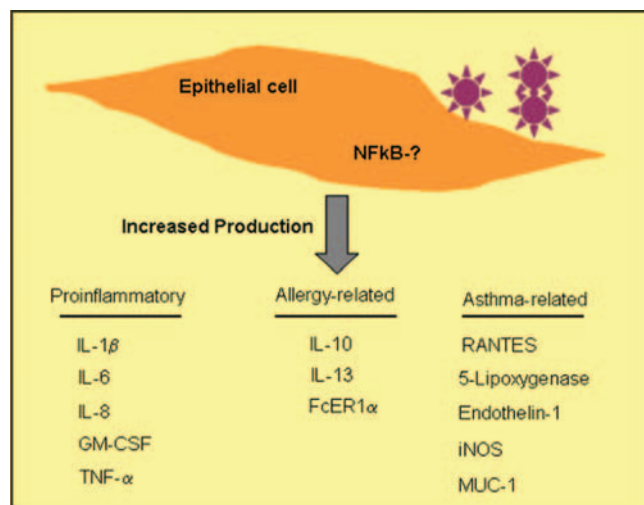


FIG. 1. Epithelial cell as the target of RSV and possible role of NF- κ B (hence the question mark) in mediating RSV-induced activation of genes. RSV infection upregulates the expression of several proinflammatory and allergy- and asthma-related cytokines and chemokines in cultured epithelial cells (1, 2, 25, 134). GM-CSF, granulocyte-monocyte colony-stimulating factor; FcER1 α , Fc fragment of alpha subunit high-affinity (I) IgE receptor; iNOS, inducible nitric oxide synthase; MUC-1, mucin-1.

infection, the airway epithelium also produces chemokines that modulate the influx of inflammatory cells into the infected tissues. These include CC (RANTES [regulated on activation, normal T-cell expressed and secreted], MCP-1 [monocyte chemoattractant protein], MIP1 α [macrophage inflammatory protein 1 α], and MIP1 β), CXC (growth-regulated oncogene alpha [Gro- α], Gro- β , Gro- γ , IL-8, and interferon-inducible T-cell alpha chemoattractant), and CX₃C (fractalkine) subclasses of chemokines in the lower airway epithelial cells (135, 144). Cytokines produced by T cells in the adaptive immune response are thought to play a role in RSV-induced disease pathogenesis. T-helper type 1 (Th1) cells stimulate cell-mediated immunity and produce inflammatory cytokines, such as IFN- γ and tumor necrosis factor alpha (TNF- α). Th2 cells stimulate humoral immune responses and produce cytokines such as IL-4, IL-5, IL-13, and IL-10 (22).

As shown in Fig. 1, RSV infection upregulates the expression of several cytokines and chemokines, such as IL-1 β , IL-6, IL-8, TNF- α , MIP1 α , RANTES, and the adhesion molecule ICAM-1, in cultured epithelial cells (1, 2, 25, 134). During the acute stage of infection, airway epithelial cells can initiate primary local inflammatory responses by directly responding with cytokine secretion to inflammatory stimuli or by amplifying an inflammatory event previously initiated by activated macrophages, eosinophils, or lymphocytes (Table 2) (42, 54, 63). These cell types may have important roles in controlling infection; however, inappropriate expression of inflammatory mediators may be linked to hyperresponsiveness and exacerbation of RSV-associated asthma (24, 120). Acute respiratory viral infections are often accompanied by neutrophilia of upper and lower respiratory secretions, and it is likely that products of neutrophil activation are involved in airway obstruction and lower airway symptoms (37, 81).

Experimental findings suggest that the cellular immune response to RSV, particularly IFN- γ production, clearly influences the clinical outcome of infection. Because the immune response to viral infections appears to have some unique features in persons with asthma, including reduced generation of IFN- γ in peripheral blood cells, asthmatics may suffer more-severe lower respiratory manifestations (93). In addition to the impact on immunogenicity due to the actions of the cytokines and chemokines they produce, bronchial epithelial cells also express adhesion molecules, such as ICAM-1, and major histocompatibility complex class II molecules, which enable them to present antigens directly to T cells. Thus, the role of bronchial epithelial cells in the viral immune response goes beyond simply being a barrier. These cells can influence the extent and potency of the adaptive immune response by means of the cytokines and chemokines they secrete, as well as the major histocompatibility complex class II and ICAM-1 surface molecules. It was shown previously that RSV colocalizes with ICAM-1 on human epithelial HEp-2 cells and that a neutralizing antibody to ICAM-1 significantly reduced RSV infection and secretion of RANTES (8).

The appearance of NF- κ B in the nucleus of a RSV-infected cell coincides with an increase in IL-8 gene transcription. Moreover, RSV-induced IL-8 gene transcription requires viral replication, and the inhibition of viral replication reverses RSV-induced NF- κ B activation and IL-8 transcription (19, 144). It has been proposed that RSV gene products act via the sequestration of protein phosphatases and induction of kinases that affect I κ B kinases, which leads to the persistent activation of NF- κ B and the cellular activation of a whole battery of cytokine and chemokine genes (10). In addition to NF- κ B, the transcription factors CCAAT/enhancer-binding protein (18) and activator protein (AP-1) (70) have also been implicated in postviral replication gene activation events, including expression of the genes encoding ICAM-1 and various cytokines (70). In addition, there is increasing evidence that NF- κ B is involved in chronic inflammatory diseases such as asthma (31).

The involvement of STAT proteins in the regulation of events underlying RSV infection has been implied but not demonstrated. RSV infection can upregulate the expression of IL-6, IL-8, MCP-1, TNF- α , MIP1 α , RANTES, IFNs (2, 25, 134, 136), and ICAM-1, and this is partially controlled by STAT transcription factors (8). RSV increased expression of

TABLE 2. Common inflammatory cells and mediators in the upper and lower airway in RSV infection

Immune response	Immune mechanism(s)
Inflammatory cells	Eosinophils Mast cells Basophils Dendritic cells Neutrophils Th2 lymphocytes
Mediators	Histamine Cysteinyl leukotrienes Prostaglandins Kinins Cytokines Chemokines

IRF1 (interferon-regulatory factor 1), ICAM-1, and RANTES in normal fibroblasts but not in those from STAT1-deficient mice, which indicated a requirement for STAT1 (70). Also, the synergy between IFN- γ and TNF- α in the transcriptional activation of genes such as those encoding IRF1 and ICAM-1 may be mediated by the cooperation of STAT1 and NF- κ B (23, 84). IFN- γ , which acts as an antiviral agent, is a potent inducer of the STAT pathway (74). RSV infection activates STAT1 and STAT3 in the human epithelial carcinoma line A549 and normal human bronchial epithelial cells; however, little is known about the roles of STAT-mediated pathways during RSV infection, especially during early periods prior to RSV replication within host cells (70).

Other pathways are also affected by RSV. During the early events of RSV infection in normal human bronchial epithelial cells, the phosphokinase C- α isozyme translocates to the cell membrane and colocalizes with the viral particles adsorbed to the membrane. Inhibition of phosphokinase C- α prevents viral entry without modifying the adsorption step (116). It has been reported that RSV infection of A549 cells activates the extracellular signal-regulated kinase-1 (ERK-1) and ERK-2 pathways and that inhibition of these ERK pathways significantly decreases RSV infection of these cells compared to the case for controls (69). Blocking ICAM-1 also reduces RSV infection in A549 cells (8).

NEUROLOGICAL LINKS

An animal model for RSV-induced bronchiolitis was developed with Fischer 344 rats (105). These rats build up a strong immune response and are thus able to clear RSV from the lungs in a few weeks. In one of the studies, weanling rats were inoculated with RSV, and the long-term effects of early infection on the nonadrenergic noncholinergic nervous system (NANC) were examined (68). It was found that NANC nerves at the infection site released substance P and other peptide neurotransmitters which play an important role in the initial inflammatory process and in the modulation of the immune response to the virus. Neurogenic inflammatory responses across the respiratory tract were observed as the rats grew from infancy to adulthood.

Recent evidence suggests that RSV infection induces the expression of NK1 neurokinin receptors on T lymphocytes within bronchiole-associated lymphoid tissue (100, 102, 103). These G protein-coupled receptors respond to substance P with activation of the phosphoinositide pathway and production of inositol triphosphate. The authors hypothesized that, following virus infection, NK1 receptor-bearing lymphocytes are deployed into the airways and that subsequent neuropeptide stimulation might be involved in the release of proinflammatory cytokines.

Acute RSV infection is also associated with markedly increased numbers of mast cells in the airway mucosa, which might be involved in the observed massive expression of 5-lipoxygenase, with transient production of cysteinyl leukotrienes (138). It has been suggested that leukotrienes released as a result of the mast cell-nerve interactions during RSV infection could eventually potentiate the inflammatory effects of neuropeptides such as substance P. Based on this model, it was concluded that activation of the NANC by irritants could be

responsible for the recurring airway inflammation which continues after the acute RSV infection has been cleared (103). In the most recent studies, it was shown that RSV infection promotes a large increase in the expression of nerve growth factor and neurotrophin receptors (101). Release of nerve growth factor leads to short- and long-term changes in the distribution and reactivity of sensory nerves across the respiratory tract. These may participate in the exaggerated inflammatory response seen during and after infection. It was postulated that changes in neurotrophin expression in the respiratory tract may represent an important factor in the association between susceptibility to childhood asthma and RSV-induced bronchiolitis in infancy.

RSV-ASTHMA LINK IN EXPERIMENTAL ANIMAL MODELS

Knowledge of the clinical pathogenesis of RSV-induced disease is limited because of the relatively small number of human studies. Animal models of RSV infection utilizing mice, rats, guinea pigs, sheep, cows, and monkeys have been developed to study the disease mechanism in detail, and this has resulted in the realization that RSV causes a multifaceted disease whose clinical manifestations and sequelae depend upon age, genetic makeup, immunologic status, and concurrent disease within the subpopulations. Mice have been extensively used to study the immune mechanisms of RSV-induced disease (97). However, there are significant limitations involved in using murine systems, including a lack of infection in bronchiolar epithelium, failure to spread infection from upper to lower airway, and relatively large amount of inoculum required for initiation of pathology and illness.

In BALB/c mice, a large RSV inoculum (10^7 PFU/ml) administered intranasally induces pneumonia, clinical illness, such as weight loss or ruffled hair, and appreciable pathology in the lung, along with infection of the lung polymorphonuclear cells and macrophages (42, 43, 63, 92). Nevertheless, many elements of the mouse and human responses to RSV infection are similar, particularly in the production of cytokines and chemokines and patterns of lung inflammation (83). The immunohistopathology of RSV infection in mice is well characterized (39–43, 63, 92), and it resembles that of human RSV infection (47). Similar to the case with humans, mice rendered immunocompromised by treatment with cyclophosphamide have increased susceptibility to RSV infection and produce higher viral titers in the lung tissue than untreated mice (132). A study to establish the importance of IFN- γ produced during the primary infection in the protection against the development of airway hyperresponsiveness on reinfection was recently conducted. For this study, both wild-type and IFN- γ -knockout mice were infected with RSV as neonates or weanlings and reinfected 5 weeks later. Airway responses were assessed on day 6 after primary or secondary infection (73). The mouse model has several advantages over models of other species. There is a vast array of inbred, congenic, transgenic, and knockout strains of mice available, and they are cheap to purchase and maintain.

The cotton rat is 100-fold more permissive (per input dose of virus) as well as more responsive immunologically than the mouse (43, 110). Studies with the cotton rat model showed that RSV-neutralizing serum IgG could prevent pulmonary infec-

tion and attenuate nasal infection, and these studies established the rationale for clinical trials of RSV prophylaxis with plasma-derived IgG for high-risk infants (44, 106, 108). Cotton rats also develop a vaccine-enhanced disease similar to that in humans and other primates (107, 109), but the lack of congenic, transgenic, or knockout strains has limited the use of these animals. Thus, most RSV studies will continue to be done with the mouse.

Although chimpanzees have the closest genetic relatedness to humans, practical and biological considerations severely limit the use of chimpanzees in RSV research. Chimpanzees are scarce, extremely expensive, and available only through primate breeding programs. Experimental RSV infections in owl monkeys, rhesus monkeys, African green monkeys, cebus monkeys, squirrel monkeys, bonnet monkeys, and baboons have also been described (9, 64, 111–113, 128). The cost and required maintenance of these species are less than those for chimpanzees, but these species still require specialized housing and handling methods.

Results with animal models have been disappointing in defining mechanisms by which RSV might lead to asthma exacerbations, largely because of the lack of reproducible results among different research groups. For instance, one group found that primary RSV infection in BALB/c mice caused IL-13-mediated increases in airway mucus and airway responsiveness to methacholine compared to the case in sham-challenged mice (133). However, this group found no airway eosinophilia in cases of RSV infection. Another group reported that RSV induced AHR and lung eosinophilia, both of which were mediated by IL-5 in BALB/c mice and not IL-13 in C57BL/6 mice (94, 119). Still another group found that primary RSV infection did not induce AHR, detectable levels of IL-5 or IL-13 mRNA or protein expression, or lung eosinophilia. However, this group found that RSV infection during allergic lung inflammation induced significant AHR and prolonged mucus production (55, 98, 99). AHR in BALB/c mice seems to be linked to both IFN- γ and leukotriene generation (140). It is important to keep in mind the relative strengths and weaknesses of the various animal models in order to better understand the mechanism by which RSV infection predisposes to asthma or exacerbates existing respiratory disease.

PROPHYLAXIS

Despite the considerable impact of RSV on respiratory health during childhood, the treatment options available for the management of lower respiratory tract RSV infections are limited and remain primarily supportive and symptomatic. While bronchodilators are used frequently for infants with acute bronchiolitis, their efficacy remains highly controversial (65, 66, 91). There have been many attempts to produce an effective RSV vaccine or anti-RSV drug but with only limited success. Tests of the first developed vaccine on human children ended with disastrous results when natural RSV infection developed in those children (up to 80% of the children were hospitalized, and two children died) (67).

Passive immunoprophylaxis is currently the only option for avoiding RSV-induced disease. The IMPact-RSV trial was a randomized, double-blind, placebo-controlled trial conducted at 139 sites in the United States, the United Kingdom, and

Canada (60). It evaluated prophylaxis with palivizumab, a humanized monoclonal antibody against the RSV fusion protein, in 1,502 prematurely born children with or without chronic lung disease (CLD) by using reduction in hospitalizations for RSV as the primary datum of this study. Children received an intramuscular injection of either palivizumab (15 mg/kg of body weight) or placebo every month for 5 months. An overall 55% reduction in hospitalizations for RSV (10.6% in the placebo group hospitalized versus 4.8% in the palivizumab group hospitalized) was observed. Specifically, prematurely born children without CLD had a 78% reduction in RSV-related hospitalizations (8.1% versus 1.8%) compared with a 39% hospitalization reduction for children with CLD (12.8% versus 7.9%). A cohort of preterm infants, including 191 who had received palivizumab and were not hospitalized for RSV and 230 who had never received palivizumab, out of which 76 were hospitalized for RSV, were assessed for recurrent wheezing beginning at a mean age of 19 months and continuing for 24 months. The incidence of recurrent wheezing in the palivizumab-treated subjects (13%) was significantly lower than that in the group of untreated subjects (26%) (129). The high cost of palivizumab is the main factor for its restricted use. The cost-effectiveness of the use of palivizumab in children at high risk of hospitalization, preterm infants (≤ 35 weeks gestation), children with bronchopulmonary dysplasia, and children with congenital heart disease was assessed. The United Kingdom National Health Sciences study showed that palivizumab prophylaxis against severe RSV infection in children at high risk is effective in preventing hospitalization and may be cost-effective in comparison to no prophylaxis (90).

These results coupled with epidemiologic evidence of the link between RSV and RAD raise the issue of whether immunoprophylaxis against RSV can reduce the risk for development of RAD. In the Fischer 344 rat model, prophylaxis with palivizumab prevented the development of acute neurogenic inflammatory changes in the lower respiratory tract following inoculation of RSV (104). Palivizumab prophylaxis also appeared to guard against development of long-term vulnerability to neurogenically mediated inflammation.

Another potential area of anti-RSV research has been the investigation of small interfering RNAs (siRNAs) against viral proteins as a means of blocking virus replication (6). Prophylactic intranasal administration of an siRNA formulation specific for RSV P protein is able to significantly reduce the viral load and the disease parameters in RSV-infected BALB/c mice (11). Although intranasal administration of naked siRNA to humans was found to be safe in a phase I study, other studies have shown toxicity for this method.

siRNA specific for the RSV NS1 mRNA also elicited antiviral effects in BALB/c mice (143). Silencing of the NS1 gene attenuated RSV replication and boosted the immune response through increased IFN- γ production (143). The use of silenced-NS1 (siNS1) prophylaxis may be an effective method for preventing RSV-induced bronchiolitis and potentially reducing the later development of asthma associated with severe respiratory infections. The nonstructural proteins of RSV, NS1 and NS2, afford promising targets for siRNA therapy since they are produced early in the infection cycle and are necessary for survival of virus.

In a study conducted with Fischer 344 rats, prophylaxis with

siNS1 significantly reduced lung RSV titers and AHR to methacholine challenge in comparison to its effect on the control group (71). Treatment of rats with siNS1 prior to RSV exposure was effective in reducing virus titers in the lungs and in preventing the inflammation and airway hyperresponsiveness associated with the infection that have been linked to development of asthma (71). A phase I study using nanoparticle-incorporated siNS1 is currently under development, and it may have future implications in prophylaxis/therapy globally.

CONCLUSION

Despite progress in the treatment of asthma, the incidence of asthma appears to be increasing in all age groups, including children. RSV-induced bronchiolitis constitutes a proven risk factor for the development of childhood asthma. The evidence from epidemiologic and clinical studies has been considered in the debate about the role of RSV-induced bronchiolitis in childhood asthma. Some of the variations between results have come from the study design and the geographic regions and the population of subjects being studied. Cellular and molecular studies of RSV infection in human cell lines and neurologic evidence in animal models have demonstrated a possible causal relationship between RSV-induced bronchiolitis during infancy and asthma later in life. Prophylaxis with antibodies or siRNAs in animal models has been shown to prevent acute infections and offer long-term protection from chronic lung diseases. Nonetheless, further studies with humans, particularly children, are needed to verify this. Such studies are important, as they are expected to demonstrate whether achieving successful prophylaxis against RSV infection might reduce the risk for development of asthma in children later in life.

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