Adv Ther (2011) 28(2):91-109.
DOI 10.1007/s12325-010-0100-z

REVIEW

Prevention of Serious Respiratory Syncytial Virus-Related Illness. I: Disease Pathogenesis and Early Attempts at Prevention

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Received: September 20, 2010 / Published online: February 4, 2011

ABSTRACT

Respiratory syncytial virus (RSV) was first described 160 years ago but was not officially recognized as a cause of serious illness in children until the late 1950s. It has been estimated that virtually all children have had at least one RSV infection by their second birthday. RSV is responsible for annual disease outbreaks, usually during a defined winter seasonal period that can vary by community and year. RSV is recognized as the leading cause of hospitalization among young children worldwide. Infants of young chronologic age and children with predisposing factors, such as premature birth, pulmonary disease, or congenital heart disease, are most susceptible to serious illness. Unlike other viruses, immunity to RSV infection is incomplete and short lived, and reinfection is common throughout life. Initial attempts to develop a vaccine in the 1960s met with unexpected and tragic results; many children vaccinated with a formalin-inactivated wild-type virus developed serious pulmonary disease upon subsequent natural infection. Numerous other vaccine technologies have since been studied, including vectored approaches, virus-like particles, DNA vaccines, and live attenuated virus vaccine. As of early 2010, only two companies or institutions had RSV vaccine candidates in early clinical trials, and no vaccine is likely to be licensed for marketing in the immediate future.

Keywords: children; history; immunoglobulin; immunoprophylaxis; monoclonal antibody; respiratory syncytial virus; vaccine

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INTRODUCTION

Respiratory syncytial virus (RSV) is the leading cause of serious lower respiratory tract disease requiring hospitalization of infants and children in the United States[1-6] where it accounts for up to 120,000 hospitalizations annually in infants <12 months of age.[4,7,8] On a broader scale, RSV is the most common cause of childhood acute lower respiratory tract infection.[9] Costs associated with RSV-related hospitalization and outpatient visits for bronchiolitis are on

the rise.[10,11] The unique ability of RSV to evade maternal antibodies and infect infants very early in life increases the clinical impact of disease, due in part to narrow airways in infants that are susceptible to obstruction and an immature immune system in these children. In some cases, the clinical consequences of RSV-related illness do not end in childhood. Long-term studies of children hospitalized with RSV-related lower respiratory tract illness before age 24 months who were prospectively followed up for periods ranging from 18 to 25 years indicate that severe illness in early life is an independent risk factor for wheezing throughout childhood.[12-14] Despite the seriousness of RSV illness and its complications, there were few accounts of its existence as a major viral pathogen before the 1950s.

DISCOVERY OF RSV AS A RESPIRATORY PATHOGEN

The initial description of infants with cough, wheezing, and respiratory difficulty resembling asthma 160 years ago is believed to be the first report of disease resembling RSV in young children (Table 1).[15-49] Almost 100 years later, a series of winter epidemics was described in which a severe viral infection associated with cough, dyspnea, fever, bronchiolitis, and pneumonia occurred in newborn infants.[16,17] In October 1955, Blount and colleagues at the Walter Reed Army Institute of Research (WRAIR) noted that several chimpanzees housed for research purposes developed a copious nasal discharge and sneezing.[18] They named the virus that they cultured from the nasal secretions "chimpanzee coryza agent" (CCA) and were successful in inducing the same illness in nonimmune animals. A laboratory worker at WRAIR coincidently developed similar cold symptoms and CCA antibody titers. A year later, Chanock and associates recovered a CCA-like virus in an infant with bronchiolitis and in another infant with pneumonia.[19,20] Laboratory studies indicated that this virus was indistinguishable from the one isolated in the original report by Blount et al.[18] Sera from a group of infants who developed viral lower respiratory tract infections over a 5-month period was subsequently evaluated and indicated that many of the children developed humoral immunity to the CCA-like viruses. Chanock was able to show that infected cells consisted of multinucleated giant cells circumscribed by large syncytia, and proposed "respiratory syncytial virus" as a more suitable name than CCA for this virus. He is credited with the identification of much of the early knowledge about the importance as RSV as a viral pathogen, as well as with the discovery and testing of the RSV formalin-inactivated vaccine.

A number of reports soon surfaced about this newly identified virus and resultant illness in children. Beem and colleagues reviewed the characteristics of acute respiratory disease noted in 41 children infected with the CCA/RSV virus during the winter of 1958-1959 in Chicago.[50] The most frequently observed clinical diagnoses were bronchiolitis, pneumonia, and acute respiratory illness; two deaths were noted in patients who tested positive for the virus. Isolation of RSV from patients occurred between the months of December and April and was detected with highest frequency in January and February. Prospective studies conducted by Chanock's group during the 1959-1960 winter season in Washington, DC, provided further evidence of RSV as a significant cause of lower respiratory illness in young children.[51,52] RSV was recovered from 57% of young infants with bronchiolitis or pneumonia during a 5-month period. Serious illness was most likely during the initial infection, especially if it occurred early in

Table 1. History of respiratory syncytial virus (RSV) and immunoprophylaxis milestones.

Date	Key event	First author, year, reference
1850	First clinical description of an illness in children that resembles what is now termed respiratory syncytial virus disease.	Eberle 1850[15]
January-March 1937, and winter of 1940-1941	Description of two epidemics of bronchiolitis and pneumonia in infants in Minnesota during two winter seasons.	Adams 1941[16] Adams 1942[17]
October 1955 February 1956	Isolation of a virus, termed CCA, from previously healthy chimpanzees housed at Walter Reed Army Institute of Research. Illness reported in a laboratory worker who was working with CCA and who had contact with infected chimpanzees.	Blount 1956[18]
1956	Recovery of CCA-like virus in infants with bronchiolitis and pneumonia; because virus cells exhibited large syncytia, a new name, respiratory syncytial virus, was proposed.	Chanock 1957[19] Chanock 1957[20]
1966-1967 RSV season	Formalin-inactivated RSV vaccine resulted in enhanced disease in vaccinated children.	Chin 1969[21] Fulginiti 1969[22] Kapikian 1969[23] Kim 1969[24]
1971	Cotton rat shown to be a suitable animal model for study of RSV illness.	Dreizin 1971[25]
Late 1970s, early 1980s	Additional studies validate cotton rat model of RSV illness.	Prince 1978[26] Prince 1983[27]
1982	Live attenuated RSV vaccine safe but not effective for prevention of RSV illness.	Belshe 1982[28]
1983	"Baby Moose", Native American infant thought to have B streptococcal disease but who actually was infected with RSV, serendipitously improved when he received IGIV, which prompted study of IGIV for RSV disease.	
Mid 1980s-1990	Studies of standard IGIV for treatment and prevention of RSV illness validated role of antibody in prevention of RSV disease.	Hemming 1987[29] Groothuis 1991[30] Meissner 1993[31]
Early 1990s	Reinitiation of RSV vaccine studies with various subunit varieties. Trials failed to show significant protection from disease.	Belshe 1993[32] Tristram 1993[33] Falsey 1996[34] Groothuis 1998[35] Piedra 1998[36] Power 2001[37] Munoz 2003[38]
Early-mid 1990s	Studies of RSV-enriched IGIV for prevention of RSV illness/hospitalization.	Groothuis 1993[39] PREVENT1997[40] Simoes 1998[41]

(continued on next page)

Table 1. History of respiratory syncytial virus (RSV) and immunoprophylaxis milestones. (*Continued*)

Date	Key event	First author, year, reference
January 1996	Licensure of RespiGam® for prevention of serious lower respiratory tract infection caused by RSV in children younger than 24 months with bronchopulmonary dysplasia (now termed chronic lung disease of prematurity) or a history of premature birth.	
Mid 1990s	Initiation of clinical trials with three distinct monoclonal antibodies (MEDI-493/palivizumab, HNK20, and RSHZ19/SB209763) for prevention of serious lower respiratory disease caused by RSV.	IMpact-RSV Study Group 1998[42] OraVax 1997[43] Meissner 1999[44]
Mid 1990s	Failure of RSV-IGIV treatment of RSV in high-risk hospitalized children.	Rodriguez 1997[45]
1995-1996 RSV season	Positive clinical trial of palivizumab for prevention of serious lower respiratory tract disease caused by RSV in infants with CLD and infants with a history of premature birth (≤35 weeks GA).	IMpact-RSV Study Group 1998[42]
June 1998	Licensure of palivizumab/Synagis® for prevention of serious lower respiratory tract disease caused by RSV in infants with CLD and infants with a history of premature birth (≤35 weeks GA).	
1998-2002 RSV seasons	Positive clinical trial of palivizumab for prevention of serious lower respiratory tract disease caused by RSV in infants with hemodynamically significant CHD.	Feltes 2003[46]
September 2003	Licensure of palivizumab/Synagis® for prevention of serious lower respiratory tract disease caused by RSV in infants with hemodynamically significant CHD.	
Mid-late 2000s	Initiation of clinical trials with motavizumab for prevention of serious lower respiratory disease caused by RSV.	Carbonell-Estrany 2010[47] Feltes 2010[48] Chandran 2008[49]
Early-mid 2000s	Initiation of live attenuated RSV vaccine trials.	
Late 2000s	Initiation of Sendai virus RSV vaccine trials.	

CCA=chimpanzee coryza agent; CHD=congenital heart disease; CLD=chronic lung disease of prematurity; GA=gestational age; IGIV=immune globulin intravenous; RSV-IGIV=RSV immune globulin intravenous (RespiGam).

life, and subsequent infections were common but were usually less severe in the majority of children. Outbreaks of additional cases of RSV illness were soon identified in communities and hospitals between 1959 and 1961 in other parts of the United States and around the world.[53-57] Chanock eloquently summarized the state of knowledge regarding RSV through 1961, and highlighted four key findings that are presently considered hallmarks of the virus and resultant illness: (1) RSV is a prominent cause of bronchiolitis and pneumonia in the young; (2) epidemics appear annually each winter for a period of 3-5 months but those months vary from year to year; (3) most children become infected by age 4 years; and, (4) reinfection can

occur, but is generally associated with a milder form of illness.[58] These early observations paved the way for further study into the epidemiology and prevention of RSV disease.

EPIDEMIOLOGY AND RISK FACTORS FOR SERIOUS ILLNESS

One of the unusual features of RSV is the high frequency of childhood reinfection; initial infection does not protect against subsequent infection, even with the same strain of virus.[59-62] Henderson et al. conducted a 10-year prospective study to examine the characteristics of reinfection with RSV in infants and young children who attended a research daycare program.[62] Repeat infections were associated with less severe disease than the initial occurrence, and an inverse relationship was observed between age and lower respiratory tract involvement. The investigators concluded that future efforts at immunoprophylaxis would likely result in a reduction in the severity of disease rather than prevention of infection. Researchers in Houston conducted a longitudinal study of RSV infection in 125 children followed up from birth to 60 months of age from 1975 to 1980 to quantify the risk of primary infection and reinfection with RSV.[60] Approximately two-thirds (68%) of children experienced their initial infection before their first birthday, and nearly all were infected at least once by 24 months of age. Lower respiratory involvement occurred less often in children who experienced their primary infection after 12 months of age than in their younger counterparts (5.9% versus 21.6%, respectively). Reinfection was common (75.9% in year 2, 45.3% in year 3) and declined with age. It was associated with milder disease and was inversely related to the level of the pre-existing neutralizing antibody titer and number of prior infections. A similar correlation between the level of neutralizing antibodies and RSV disease severity has been reported by others.[59,63-67]

Researchers have identified several medical conditions and associated risk factors that predispose children to severe RSV disease and subsequent hospitalization.[68,69] Preterm births, which have increased more than 20% since 1990, form the largest high-risk special population for severe RSV infection. The largest growth segment within this group consists of the late-preterm infant born between 34 and 36 weeks gestational age (GA).[70] Late-preterm infants, who now account for more than 70% of all premature births, experience greater morbidity and mortality and more frequent rehospitalization after initial discharge and throughout the first year of life than term infants.[71] These children are especially prone to high rates of respiratory disorders, including RSV infection.[72-75]

Children with chronic lung disease of prematurity (CLD; formerly known as bronchopulmonary dysplasia [BPD]) and hemodynamically significant congenital heart disease (CHD) are among those at highest risk for severe RSV illness. Up to 30% of infants born with CLD will eventually be hospitalized with an RSV-related infection during their first 2 years of life.[72,76] Similar hospitalization rates have also been reported for those with CHD.[77] Children with CLD or CHD incur longer RSV-related hospital stays, have greater need for intensive care unit resources, and have higher mortality rates than other high-risk children.[77,78] Other medical conditions that have been reported to predispose children to serious RSV disease include severe neuromuscular disease, congenital abnormalities of the airways, cystic fibrosis, and severe immunodeficiencies.[69,79,80]

A plethora of literature has described nearly two dozen risk factors (social, demographic, and environmental) for severe RSV-related disease and/or bronchiolitis. Age of an infant

at the onset of the RSV season is one of the most important and universally agreed on risk factors for serious RSV illness. Some investigators have found greater risk associated with age <3 months,[81,82] but others have found the risk to be associated with infants aged <6 months.[3,83] Additional risk factors include young siblings in the household,[81,82,84] daycare attendance,[82,84] exposure to environmental tobacco smoke,[81,82,85] low birth weight,[86] family history of wheezing or asthma,[82,84] certain neuromuscular diseases, immunosuppressed states,[87] and multiple births.[87,89] The significance of these risk factors is periodically reviewed by the American Academy of Pediatrics along with recommendations on immunoprophylaxis in select at-risk groups of children.[69]

THE ROLE OF INFLAMMATORY RESPONSES IN DISEASE PATHOGENESIS

The relative contribution of host versus viral factors to the pathogenesis of RSV disease remains controversial and incompletely understood. RSV is highly contagious but is not highly cytopathic or invasive. However, the tropism of RSV for the superficial epithelial cells of the respiratory tract reduces the effectiveness of host immunity. Further, the ability of RSV to infect so early in life causes more frequent and severe disease than most other respiratory viruses. This is due in part to the small size and the narrower and immature airways of the young infant. Further protective immune responses in young infants are neither complete nor long lasting. Commonly proposed immunologic features include exaggerated cytotoxic T-lymphocyte responses, imbalanced T-helper cell (Th)2/Th1 responses, and exaggerated inflammatory responses, coupled with insufficient or altered responses due to young age or as yet unexplained viral factors. Exaggerated inflammatory responses may lead to upregulation in the expression of chemokines and the further activation of nuclear factor kappa B. This in turn leads to the production of inflammatory factors such as neutrophils, which can contribute to tissue damage, and other effects such as hyper-reactivity. Genetic predisposition to severe RSV infection, prematurity, and/or pulmonary, cardiac, and immunologic compromise may also play an important role in disease severity. A better understanding of the viral and host determinants of immunologic responses to RSV will be important for designing vaccines and other preventative agents in the future.[90]

MATERNAL IMMUNOGLOBULIN G AND ROLE OF RSV-SPECIFIC MATERNAL ANTIBODIES IN RSV DISEASE

Maternal immunoglobulin G (IgG) is not efficiently transferred to the fetus until the third trimester of pregnancy, and a linear relationship has been established between the logarithm of the serum IgG levels and GA. Yeung et al. evaluated serum IgG levels of premature and term infants and showed that mean serum concentrations ranged from 180 to 656 mg/dL in infants born at 24.5 to 35.5 weeks GA, to 757 to 1100 mg/dL in infants born at 37 to 40 weeks GA.[91] A high proportion of premature infants remain hypogammaglobulinemic at 6 months of age.[92] Brandenburg and associates measured the decline in maternal RSV-specific antibodies over a 6-month period in 45 healthy term infants.[93] Antibodies were detected in all infants at birth (geometric mean titer [GMT], 301), steadily declined over the first 3 months (GMT, 24), and were undetectable in the majority of infants at age 6 months. The calculated mean half-life of the antibodies was 26 days. Hacimustafaoglu

et al. studied the decline in maternal RSV antibodies in the offspring (84% full term, 10% GA of 35-38 weeks, 6% GA of 32-35 weeks) of 49 pregnant women, 83% of whom tested positive for anti-RSV IgG.[94] At birth, maternal antibodies for RSV were detected in all offspring of RSV antibody-positive women and in similar concentrations. Antibody levels declined steadily over the 6-month observation period and were measurable in 73%, 6%, and 2% of infants at 1, 3, and 6 months, respectively.

Ogilvie et al. prospectively studied 100 infants to determine the role of maternal RSV antibodies on the development of subsequent disease during the first 6 months of life.[66] Infants who did not become infected with RSV had higher mean titers of IgG than infected infants and were born to mothers who had significantly higher maternal RSV-specific IgG antibody levels than the mothers of infants who became infected. Ochola and coworkers characterized the levels of RSV maternal antibodies and their decay rate in 635 newly delivered babies in a rural area of Kenya.[95] Nearly all children (97%) had detectable levels of maternal RSV IgG at birth; levels that declined rapidly thereafter. Infants who subsequently experienced an infection in the first 6 months of life had lower cord titers of anti-RSV antibody than the infants who did not.

The role of maternal antibody on the severity of RSV disease was studied in 15 infants with pneumonia (mean age, 4.9 months) and 19 infants with bronchiolitis (mean age, 4.2 months), all of whom were <9 months of age at the time of admission to hospital.[65] The composite GMT in eight children with RSV illness aged 1 to 2 months (four each with pneumonia and bronchiolitis) was 41, and steadily declined to a level of 10 in four children aged 3 to 4 months with bronchiolitis, and to <8 in 15 children (seven with pneumonia, eight with bronchiolitis) aged 5 to 6 months. Titers of neutralizing antibody were undetectable in the remaining hospitalized children aged 7 to 8 months. In the small sample of infants with pneumonia, linear regression of clinical illness scores on the level of neutralizing antibody showed a significant ($P<0.02$) inverse correlation between the severity of illness and antibody concentrations. No such relationship was observed for bronchiolitis. It was later demonstrated that antibody to the fusion protein is an important correlate of immunity and that children with less severe RSV disease have significantly higher IgG and anti-F titers before infection than children with lower respiratory tract involvement.[64] The results of these studies suggest that maternal RSV IgG antibodies offer protection from severe illness in term infants during the first few months of life and help explain why premature infants are at high risk of serious illness.

HISTORY OF RSV VACCINE DEVELOPMENT

The history of the initial efforts to develop an RSV vaccine began shortly after RSV was first isolated. In 1961, the National Institute for Allergy and Infectious Diseases considered RSV disease a research priority and sponsored the first vaccine trials. Earlier research with formalin as an inactivation agent led to the development of clinically effective vaccines, such as the inactivated polio vaccine, so a similar approach was used to develop a formalin-inactivated (FI) vaccine against RSV. The vaccine was prepared in the department of biologics research at Pfizer from a Bernett strain isolated in 1961 at the US National Institutes of Health (NIH) from a volunteer previously inoculated with RSV virus.[24] The virus was grown in vervet monkey kidney tissue cultures, passaged three additional

Table 2. Formalin-inactivated respiratory syncytial virus (RSV) vaccine studies.

Study location	Patient characteristics	Vaccine dosage schedule	Observations	First author, year, reference
Washington, DC, USA	Primarily black low socioeconomic class children 2-7 months of age.	260 children received 1 dose between December 1965 and December 1966. 125 children received three doses (weeks 0, 4, 12).	80% of vaccinees required hospitalization with more severe disease at time of RSV infection versus 5% for controls. Two children died. Predominant signs of illness were pneumonia, bronchiolitis, and bronchiolitis with pneumonia. Highest incidence of serious illness in children aged <6 months.	Kim 1969[24]
Washington, DC, USA	Otherwise normal homeless children aged 6 months to approximately 4.5 years who resided in a welfare institution.	146 children received three doses (weeks 0, 4, 8), with first dose given February 1966 and last dose given December 1966.	Exaggerated and altered clinical response to infection was noted. 69% (9/13) of vaccinated children aged 6-23 months developed pneumonia versus 9% (4/47) of controls.	Kapikian 1969[23]
Fort Ord, CA, USA	Children of army personnel divided into two groups: children aged 4 months to 9 years; preschool attendees through 9 years of age.	191 children received two doses (weeks 0, 4), and 28 received one dose. Immunization began September 1966, and last dose was given December 1966.	Significantly more vaccine recipients were admitted to the hospital, and had more severe disease, than controls.	Chin 1969[21]
Denver, CO, USA	Children of military personnel at Lowry Air Force Base or Fitzsimmons Hospital between the ages of 6 months to 7 years.	464 children received three doses (weeks 0, 4, 8) beginning in July 1966.	Significantly higher rate for RSV hospitalization (2.4% versus 0.1% to 0.37%) in vaccinated group versus controls. Age-related incidence of hospitalization in vaccinated group: infants aged 6-11 months, 13.7%; children aged 12-36 months, 5%; children aged ≥3 years, <1%.	Fulginiti 1969[22]

times in primary human embryonic kidney cultures and 10 more times in vervet monkey kidney cultures. A dilution of the seed virus was inoculated into vervet monkey kidney bottle cultures, and resultant tissue culture fluid harvests were inactivated with formalin. Fluids were centrifuged and the pellet residues were further concentrated through two additional procedures including one involving precipitation with alum. Preservatives were added, and the final product, which was concentrated 100 times, became widely known as Lot 100.

A series of four field efficacy trials were initiated in December 1965 and conducted through the 1966-1967 RSV season under sponsorship of the NIH (Table 2). Lot 100 was administered in a series of up to three intramuscular injections usually 1 month apart to approximately 1200 children aged 2 months to 9 years. The control groups consisted of either nonvaccinated children or children vaccinated with a FI-parainfluenza vaccine. The FI-RSV vaccine proved to be highly immunogenic and elicited high titers of RSV antibody, but the antibodies were later shown to be devoid of neutralizing and fusion-inhibiting activity.[96-98] Infants aged 2-6 months had high F glycoprotein antibody titers but had low G glycoprotein antibody titers.[97] Children aged 7-40 months had F and G antibody titers comparable with those found in children who were infected with RSV. Both groups of children developed a lower level of neutralizing antibodies than did individuals of comparable age with natural RSV infection.

During the RSV season, significantly more RSV vaccinees were hospitalized with severe, enhanced pulmonary disease upon infection with wild-type virus. In one of the field studies, the hospitalization rate was approximately 80% compared with 5% for RSV unvaccinated controls: two infants who were initially immunized at 5 months or younger subsequently died.[24] Disease experienced in hospitalized children given Lot 100 usually consisted of pneumonia and/or bronchiolitis and hypoxemia, compared with milder disease (rhinitis, pharyngitis, and/or bronchitis) in control children <6 months of age, a group in whom maternal antibody is normally present. The FI-RSV vaccine appeared to stimulate an unbalanced immune response in which a large proportion of the induced antibodies were directed against nonprotective epitopes in place of epitopes that induce functional antibodies.[98]

Given that enhanced disease occurred most often in Lot 100 recipients <6 months of age and in those who had significant levels of serum antibody when illness occurred, it was concluded that serum antibody played a major role in the pathogenesis of serious RSV illness. Subsequent surveillance studies[65-67] and RSV prophylaxis trials of RSV-enriched immune globulin[39] and RSV-specific monoclonal antibodies[42] have long since proven that the initial antibody hypothesis was incorrect. Many theories have emerged since the publication of the results of the Lot 100 studies that offer insight into the potential mechanisms for vaccine-enhanced disease. The most widely propagated theories suggest that the mechanisms responsible for enhanced disease are both complex and multifactorial. Among these include development of poorly neutralizing antibodies, lack of protection due to formalin disruption of key epitopes during vaccine inactivation, exaggerated/biased Th2 response resulting in a strong pulmonary eosinophilic response, and lack of affinity maturation due to deficient Toll-like receptor activation in B cells.[99-108] However, despite 40 years of research, the precise etiology of vaccine-enhanced disease from the failed Lot 100 trials has yet to be conclusively elucidated.

The unfortunate and catastrophic results from the Lot 100 vaccine trials led to a period of inactivity in RSV vaccine research, primarily

Table 3. Characteristics of representative vaccine technologies for prevention of respiratory syncytial virus (RSV) illness.

Vaccine technology	Characteristics
Alphavirus vector	Alphavirus vaccines have shown excellent protection in numerous models for infectious disease and cancer, including influenza, CMV, breast cancer, melanoma, SARS, HPV, HSV, RSV, PIV, Marburg and Ebola viruses, viral encephalitis viruses, and botulinum toxin.
	Safe and highly immunogenic in humans.
	Induces broad and robust humoral and cellular immune responses.
	Capable of expressing a wide array of bacterial, viral, parasitic, and tumor antigens.
	Naturally targets dendritic cells, the most efficient antigen-presenting cell in the body.
	Allows combination products to be produced through multigene or particle mixtures.
DNA vaccine	Antigen can be expressed without alterations to its original structure, and the vaccine can be made optimally attenuated and highly immunogenic.
	May be well suited for maternal immunization; protective immunity has been demonstrated in mice when maternal antibodies were present.
	Initial studies have suggested that DNA vaccines are immunogenic but offer only mildly protective effects in RSV challenge studies.
	Large amounts of DNA are required, although methods to overcome this include novel formulations such as nanoencapsulation and microparticles.
	Other proposed concerns include the risk of inducing autoimmune responses and tolerance to the administered antigen.
Live attenuated virus	Can be administered intranasally and induce a balanced local and systemic immune response.
	Potential efficacy in infants with maternal antibodies.
	Problems may arise concerning over/underattenuation.
	Must be frozen.
SeV vector	Well tolerated in preclinical and phase 1 studies.
	SeV does not infect humans (naturally attenuated).
	SeV confers Jennerian protection against hPIV-1.
	SeV and rSeV induce rapid (by day 7) and long-lasting antibody responses versus hPIV-1 and passenger gene targets.
	rSeV-RSV-F produces RSV-specific antibody and T-cell responses and protects against RSV A and B subtypes.
	Can be formulated for intranasal administration.
	The virus is easy to grow and can be manufactured in large lots.
VSV vector	VSV has been shown to be an effective vaccine vector for use as immunoprophylaxis against numerous viral infections and to induce an immune response against cancer. The following diseases have been studied: hepatitis C, HIV, influenza, HPV, Ebola, RSV.
	VSV is not a human pathogen, and there is little pre-existing immunity that might impede its use in humans.
	It propagates efficiently in cell lines suitable for manufacturing immunogenic compositions.
	VSV infection is an efficient inducer of both cellular and humoral immunity.
	It can be administered intranasally or parenterally.
VLPs	VLP technology serves as the platform used by Merck and GlaxoSmithKline for marketed vaccines for HPV (ie, Gardisil®, Cervarix®) and hepatitis B (ie, Recombivax® HB, Engerix® B), and is currently being used in the development of vaccines for a number of infectious diseases (eg, influenza, norovirus, HIV, herpes zoster, RSV).
	Potent humoral immune response for protection against RSV disease.
	Cell-mediated immune response for clearance of RSV infection.
	Th1-weighted response.
	No replication should result in limited reactogenicity.
	Unlikely to be associated with enhanced disease.
	Potential for multiple routes of administration.
	Ease of production and manufacturing (does not require eggs).

CMV=cytomegalovirus; HIV=human immunodeficiency virus; hPIV-1=human parainfluenza virus 1; HPV=human papillomavirus; HSV=herpes simplex virus; PIV=parainfluenza virus; SARS=severe acute respiratory syndrome; SeV=Sendai virus; VLP=virus-like particle; VSV=vesicular stomatitis virus.

owing to liability concerns faced by researchers and drug manufacturers.[109] Results of a preliminary trial of a live RSV vaccine in the early 1980s revealed that the vaccine was not effective in the children studied.[28] Further trials were initiated in the 1990s with several subunit RSV vaccines composed of either purified F glycoprotein (PFP-1, PFP-2, PFP-3) or BBG2Na, a peptide from the G glycoprotein.[32-36,38] The vaccine population consisted of children >12 months of age, young adults, institutionalized and older adults, and pregnant women. There were some hypersensitivity reactions to the BBG2Na formulation; nevertheless, the vaccines were capable of inducing neutralizing antibodies. However, the observed reduction in incidence of RSV-related lower respiratory tract illness in late-phase studies was not sufficient to warrant further investigation in children younger than 12 months and blunted further interest in additional research with subunit vaccines for RSV prevention.

Numerous other vaccine technologies have since been studied, including vectored approaches (eg, Sendai virus, alpha virus, vesicular stomatitis virus, modified vaccinia Ankara, adenovirus), virus-like particles, DNA vaccines, and live attenuated virus vaccines.[110-113] Each has its own advantages and disadvantages (Table 3). As of early 2010, only two companies or institutions had RSV vaccine candidates in the clinic (live attenuated and Sendai virus vector), and no vaccine is likely to be licensed for marketing for at least 8 years. Maternal immunization with an RSV vaccine has been proposed as an alternative to infant immunization. However, this would not be effective for most premature infants because maternal IgG is not transferred efficiently until the third trimester of pregnancy. In addition, the safety of such an approach remains uncertain.

There are many challenges to overcome before a successful RSV vaccine can be licensed for administration in very young infants.[109,111,114] The most daunting hurdle is that to prevent clinically significant illness and reinfection, the vaccine would have to confer greater immunity than that which occurs from natural infection with wild-type virus. A better understanding of the immune response to RSV infection is clearly necessary to further vaccine research.[115] The primary targets for RSV vaccination are neonates and young infants. They have immature immune systems and may not be able to elicit a robust immune response following vaccination in the presence of maternally acquired antibody. Strategies traditionally employed to overcome this problem involve the administration of more than one vaccine dose and delay in vaccination until after 6 months of age. Neither of these approaches is ideal. In addition, the RSV vaccine must not interfere with the safety and efficacy of other routine childhood vaccines. Assuming all of these obstacles can be overcome, given the history of Lot 100, regulatory bodies may question the safety and efficacy of vaccine candidates for prevention of RSV disease especially if the technology chosen has not been previously validated in young children or if a maternal immunization strategy is considered.

EXPERIMENTAL ANIMAL MODELS OF RSV INFECTION

Although no safety concerns emerged following results of short-term potency and safety tests performed on the initial RSV Lot 100 vaccine in animals according to standard tests at that time, preclinical efficacy studies were not conducted because a reputable animal model for RSV had not yet been identified. Animal models of RSV infection that have been studied include the ferret, cotton rat, primates (eg, chimpanzee,

monkey, baboon), mouse, cow, and pig.[116] Of these, the ferret and cotton rat have proven to be useful and easy-to-study models with RSV infection and IgG antibody response in the cotton rat most closely mimicking RSV infection that occurs in humans.

Ferret

Data from Chanock's research group in the early 1960s in ferrets showed that RSV could be cultured from the nose for 1 week and from the trachea for 4 days following intranasal inoculation.[117] However, no significant pathologic changes were observed in the pulmonary tree. Results from subsequent studies conducted in ferrets of various age groups indicated that RSV was detected in nasal mucosa and replicated in the lungs of infant ferrets, but the latter effects disappeared by age 4 weeks.[118] Minimal or no neutralizing antibody was detected regardless of when inoculation occurred (ie, from birth to age 28 days). These findings indicate that the ferret is not an ideal model for study of RSV because they are only susceptible to the pulmonary effects of disease up to adolescence and do not develop a robust antibody response.

Cotton Rat

In a study conducted in Russia, Dreizin et al. were the first to demonstrate that RSV replicated in high titers throughout the respiratory tract of the common cotton rat (*Sigmodon hispidus*).[25] Maximum viral replication occurred on the fourth day following inoculation, and disease-related effects mainly involved the surface epithelium in the nose, trachea, and lungs. Because findings from this study were published in Russian, it took several years before a series of additional studies, which were conducted by Chanock's group at the NIH, validated and extended Dreizin's findings and confirmed the usefulness of this model.[26,27,119] Viral replication was shown to be restricted to epithelial cells, with high titers noted in the nose and lungs.[26] Animals exhibited exudative rhinitis of moderate severity and mild proliferative bronchiolitis, with serum neutralizing antibody present in all animals between days 5 and 9 postinfection that was not influenced by age at the time of infection. Cotton rats developed complete resistance to pulmonary reinfection, which lasted for at least 18 months, whereas resistance to infection in the nose was of shorter duration and began to diminish at 8 months.[27] A correlation was observed between resistance and serum neutralizing antibody levels.

The quantitative aspects of passive immunity to RSV were further studied to determine the level of neutralizing antibodies in serum required to confer resistance.[119] Infant animals were inoculated with set dilutions of a single pool of sera derived from animals that were convalescent from active infection and then subjected 24 hours later to intranasal inoculation with RSV. A serum neutralizing antibody titer of 1:100 or greater was associated with suppression of virus replication in the respiratory tract with complete/almost complete resistance in the lungs noted at a serum neutralizing antibody titer of ≥1:380. The investigators noted that a less striking protective effect of serum antibody was observed for nasal tissues, a finding that was further explored by Siber and associates a decade later.[120] Siber's group evaluated the effects of low-dose (0.5 g/kg) and high-dose (5.0 g/kg) RSV-enriched immune globulin (IG) and conventional IG with respect to their antibody concentrations to RSV and protective activity against RSV challenge in cotton rats. Serum neutralizing antibody activity was determined 24 hours after intraperitoneal administration of each IG and immediately prior to intranasal

challenge. Mean neutralizing titers following high-dose IG were similar to those produced by low-dose RSV-IGIV, whereas titers following high-dose RSV-IGIV were significantly higher than those after high-dose IG (17,200±1350 MU/mL by microneutralization versus 3800±393 MU/mL, respectively; $P<0.01$). The serum neutralizing antibody titer necessary to lower lung virus concentration by 99% was determined to be 1:390, whereas that required to reduce nasal virus concentrations by 99% was calculated to be 1:3500. Results from this study demonstrated that the in vivo protective activity of RSV-IGIV was significantly greater than that of IG and that RSV-IGIV is approximately 10 times more effective in preventing RSV disease in the lower airways than in the upper respiratory tract. The concentration of serum RSV-neutralizing antibodies necessary to provide resistance to infection in these early cotton rat studies was similar to the level of maternally derived serum antibodies present in human infants <2 months of age. Both of these findings help explain later results obtained during human clinical trials of RSV-IGIV and RSV monoclonal antibodies for prophylaxis against serious lower respiratory tract disease following RSV infection. Results from these early studies provided evidence that the cotton rat is the best animal model of those tested for studying RSV infection and antibody response thus far. Limitations of this model include the fact that cotton rats do not develop measurable clinical disease and cannot, at present, be utilized to model cellular immunity.

CONCLUSION

RSV is unique among respiratory viruses in that it infects infants at an early age, and those affected do not develop sufficient immunity to prevent subsequent reinfection. It is the leading cause of hospitalization in infants younger than 1 year in the United States. There are no vaccines currently available for prevention of serious RSV illness, and none are likely to be licensed in the near future. Immunoprophylactic treatment with polyclonal and monoclonal antibody preparations against RSV has been developed to prevent RSV infection and reduce subsequent morbidity. In the second part of this series we will discuss the clinical history of immunoprophylaxis with antibodies in infants and children at high risk for serious RSV disease.

ACKNOWLEDGMENTS

The authors kindly acknowledge the assistance of Dr. Jay Bauman in the preparation of this manuscript. Dr. Bauman is a consultant to MedImmune and has received compensation for assistance in the preparation of this manuscript.

JRG is the guarantor for this article, and takes responsibility for the integrity of the work as a whole.

REFERENCES

1. Hall CB. Respiratory syncytial virus. In: Feigen RD, Cherry JD, eds. Textbook of Pediatric Infectious Diseases. Philadelphia, PA: WB Saunders; 1987:1653-1675.

2. Hall CB. Respiratory syncytial virus and parainfluenza virus. N Engl J Med. 2001;344:1917-1928.

3. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med. 2009;360:588-598.

4. Leader S, Kohlhase K. Respiratory syncytial virus-coded pediatric hospitalizations, 1997 to 1999. Pediatr Infect Dis J. 2002;21:629-632.

5. Ruuskanen O, Ogra PL. Respiratory syncytial virus. Curr Probl Pediatr. 1993;23:50-79.

6. Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis-associated hospitalizations among US children, 1980-1996. JAMA. 1999;282:1440-1446.

7. Leader S, Kohlhase K. Recent trends in severe respiratory syncytial virus (RSV) among US infants, 1997 to 2000. J Pediatr. 2003;143:S127-132.

8. McLaurin K, Leader S. Growing impact of RSV hospitalizations among infants in the US, 1997-2002. Paper presented at: Pediatric Academic Societies' Meeting; May 14-17, 2005; Washington, DC, USA.

9. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet. 2010;375:1545-1555.

10. Carroll KN, Gebretsadik T, Griffin MR, et al. Increasing burden and risk factors for bronchiolitis-related medical visits in infants enrolled in a state health care insurance plan. Pediatrics. 2008;122:58-64.

11. Forbes ML, Hall CB, Jackson A, Masaquel AS, Mahadevia PJ. Comparative costs of hospitalisation among infants at high risk for respiratory syncytial virus lower respiratory tract infection during the first year of life. J Med Econ. 2010;13:136-141.

12. Ruotsalainen M, Piippo-Savolainen E, Hyvarinen MK, Korppi M. Respiratory morbidity in adulthood after respiratory syncytial virus hospitalization in infancy. Pediatr Infect Dis J. 2010;29:872-874.

13. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet. 1999;354:541-545.

14. Sigurs N, Aljassim F, Kjellman B, et al. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. Thorax. 2010;65:1045-1052.

15. Eberle J. A Treatise on the Diseases and Physical Education of Children. Philadelphia, PA: Lippincott, Grambo and Co.; 1850.

16. Adams JM. Primary virus pneumonitis with cytoplasmic inclusion bodies: study of an epidemic involving thirty-two infants, with nine deaths. JAMA. 1941;116:925-933.

17. Adams JM, Green RG, Evans CA, Northrop B. Primary virus pneumonitis: a comparative study of two epidemics. J Pediatr. 1942;20:405-420.

18. Blount RE Jr, Morris JA, Savage RE. Recovery of cytopathogenic agent from chimpanzees with coryza. Proc Soc Exp Biol Med. 1956;92:544-549.

19. Chanock R, Finberg L. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). II. Epidemiologic aspects of infection in infants and young children. Am J Hyg. 1957;66:291-300.

20. Chanock R, Roizman B, Myers R. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). I. Isolation, properties and characterization. Am J Hyg. 1957;66:281-290.

21. Chin J, Magoffin RL, Shearer LA, Schieble JH, Lennette EH. Field evaluation of a respiratory syncytial virus vaccine and a trivalent parainfluenza virus vaccine in a pediatric population. Am J Epidemiol. 1969;89:449-463.

22. Fulginiti VA, Eller JJ, Sieber OF, Joyner JW, Minamitani M, Meiklejohn G. Respiratory virus immunization. I. A field trial of two inactivated respiratory virus vaccines; an aqueous trivalent parainfluenza virus vaccine and an alum-precipitated respiratory syncytial virus vaccine. Am J Epidemiol. 1969;89:435-448.

23. Kapikian AZ, Mitchell RH, Chanock RM, Shvedoff RA, Stewart CE. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. Am J Epidemiol. 1969;89:405-421.

24. Kim HW, Canchola JG, Brandt CD, et al. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. Am J Epidemiol. 1969;89:422-434.

25. Dreizin RS, Vyshnevetskaia LO, Bagdamian EE, Iankevich OD, Tarasova LB. Experimental RS virus infection of cotton rats. A viral and immunofluorescent study [in Russian]. Vopr Virusol. 1971;16:670-676.

26. Prince GA, Jenson AB, Horswood RL, Camargo E, Chanock RM. The pathogenesis of respiratory syncytial virus infection in cotton rats. Am J Pathol. 1978;93:771-791.

27. Prince GA, Horswood RL, Camargo E, Koenig D, Chanock RM. Mechanisms of immunity to respiratory syncytial virus in cotton rats. Infect Immun. 1983;42:81-87.

28. Belshe RB, Van Voris LP, Mufson MA. Parenteral administration of live respiratory syncytial virus vaccine: results of a field trial. J Infect Dis. 1982;145:311-319.

29. Hemming VG, Rodriguez W, Kim HW, et al. Intravenous immunoglobulin treatment of respiratory syncytial virus infections in infants and young children. Antimicrob Agents Chemother. 1987;31:1882-1886.

30. Groothuis JR, Levin MJ, Rodriguez W, et al. Use of intravenous gamma globulin to passively immunize high-risk children against respiratory syncytial virus: safety and pharmacokinetics. The RSVIG Study Group. Antimicrob Agents Chemother. 1991;35:1469-1473.

31. Meissner HC, Fulton DR, Groothuis JR, et al. Controlled trial to evaluate protection of high-risk infants against respiratory syncytial virus disease by using standard intravenous immune globulin. Antimicrob Agents Chemother. 1993;37:1655-1658.

32. Belshe RB, Anderson EL, Walsh EE. Immunogenicity of purified F glycoprotein of respiratory syncytial virus: clinical and immune responses to subsequent natural infection in children. J Infect Dis. 1993;168:1024-1029.

33. Tristram DA, Welliver RC, Mohar CK, Hogerman DA, Hildreth SW, Paradiso P. Immunogenicity and safety of respiratory syncytial virus subunit vaccine in seropositive children 18-36 months old. J Infect Dis. 1993;167:191-195.

34. Falsey AR, Walsh EE. Safety and immunogenicity of a respiratory syncytial virus subunit vaccine (PFP-2) in ambulatory adults over age 60. Vaccine. 1996;14:1214-1218.

35. Groothuis JR, King SJ, Hogerman DA, Paradiso PR, Simoes EA. Safety and immunogenicity of a purified F protein respiratory syncytial virus (PFP-2) vaccine in seropositive children with bronchopulmonary dysplasia. J Infect Dis. 1998;177:467-469.

36. Piedra PA, Grace S, Jewell A, et al. Sequential annual administration of purified fusion protein vaccine against respiratory syncytial virus in children with cystic fibrosis. Pediatr Infect Dis J. 1998;17:217-224.

37. Power UF, Nguyen TN, Rietveld E, et al. Safety and immunogenicity of a novel recombinant subunit respiratory syncytial virus vaccine (BBG2Na) in healthy young adults. J Infect Dis. 2001;184:1456-1460.

38. Munoz FM, Piedra PA, Glezen WP. Safety and immunogenicity of respiratory syncytial virus purified fusion protein-2 vaccine in pregnant women. Vaccine. 2003;21:3465-3467.

39. Groothuis JR, Simoes EA, Levin MJ, et al. Prophylactic administration of respiratory syncytial virus immune globulin to high-risk infants and young children. The Respiratory Syncytial Virus Immune Globulin Study Group. N Engl J Med. 1993;329:1524-1530.

40. The PREVENT Study Group. Reduction of respiratory syncytial virus hospitalization among premature infants and infants with bronchopulmonary dysplasia using respiratory syncytial virus immune globulin prophylaxis. Pediatrics. 1997;99:93-99.

41. Simoes EA, Sondheimer HM, Top FH Jr, et al. Respiratory syncytial virus immune globulin for prophylaxis against respiratory syncytial virus disease in infants and children with congenital heart disease. The Cardiac Study Group. J Pediatr. 1998;133:492-499.

42. The IMpact-RSV Study Group. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. Pediatrics. 1998;102:531-537.

43. OraVax Reports Results from Phase III Trial of HNK20. Nosedrop for Respiratory Syncytial Virus in Infants [press release]. Cambridge (MA): OraVax; March 19, 1997.

44. Meissner HC, Groothuis JR, Rodriguez WJ, et al. Safety and pharmacokinetics of an intramuscular monoclonal antibody (SB 209763) against respiratory syncytial virus (RSV) in infants and young children at risk for severe RSV disease. Antimicrob Agents Chemother. 1999;43:1183-1188.

45. Rodriguez WJ, Gruber WC, Welliver RC, et al. Respiratory syncytial virus (RSV) immune globulin intravenous therapy for RSV lower respiratory tract infection in infants and young children at high risk for severe RSV infections: Respiratory Syncytial Virus Immune Globulin Study Group. Pediatrics. 1997;99:454-461.

46. Feltes TF, Cabalka AK, Meissner HC, et al. Palivizumab prophylaxis reduces hospitalization due to respiratory syncytial virus in young children with hemodynamically significant congenital heart disease. J Pediatr. 2003;143:532-540.

47. Carbonell-Estrany X, Simoes EA, Dagan R, et al. Motavizumab for prophylaxis of respiratory syncytial virus in high-risk children: a noninferiority trial. Pediatrics. 2010;125:e35-51.

48. Feltes TF, Sondheimer HM, Harris BS, et al. A randomized controlled trial of RSV prophylaxis with motavizumab vs palivizumab in young children with hemodynamically significant congenital heart disease (CHD). Paper presented at: Pediatric Academic Societies Annual Meeting; May 1-4, 2010; Vancouver, BC, Canada.

49. Chandran A, Millar EV, Weatherholtz R, et al. Safety and efficacy of motavizumab in the prevention of RSV disease in healthy infants. Paper presented at: American Pediatric Society/77th Annual Meeting for the Society for Pediatric Research; May 4, 2008; Honolulu, HI, USA.

50. Beem M, Wright FH, Hamre D, Egerer R, Oehme M. Association of the chimpanzee coryza agent with acute respiratory disease in children. N Engl J Med. 1960;263:523-530.

51. Chanock RM, Kim HW, Vargosko AJ, et al. Respiratory syncytial virus. I. Virus recovery and other observations during 1960 outbreak of bronchiolitis, pneumonia, and minor respiratory diseases in children. JAMA. 1961;176:647-653.

52. Parrott RH, Vargosko AJ, Kim HW, et al. Respiratory syncytial virus. II. Serologic studies over a 34-month period of children with bronchiolitis, pneumonia, and minor respiratory diseases. JAMA. 1961;176:653-657.

53. Adams JM, Imagawa DT, Zike K. Epidemic bronchiolitis and pneumonitis related to respiratory syncytial virus. JAMA. 1961;176:1037-1039.

54. Hamparian VV, Ketler A, Hilleman MR, et al. Studies of acute respiratory illnesses caused by respiratory syncytial virus. 1. Laboratory findings in 109 cases. Proc Soc Exp Biol Med. 1961;106:717-722.

55. Kapikian AZ, Bell JA, Mastrota FM, Johnson KM, Huebner RJ, Chanock RM. An outbreak of febrile illness and pneumonia associated with respiratory syncytial virus infection. Am J Hyg. 1961;74:234-248.

56. Peacock DB, Clarke SK. Respiratory syncytial virus in Britain. Lancet. 1961;ii:466.

57. Rowe DS, Michaels RH. Isolation of the respiratory syncytial virus from a patient with pneumonia. Pediatrics. 1960;26:623-629.

58. Chanock RM, Parrott RH, Vargosko AJ, Kapikian AZ, Knight V, Johnson KM. Acute respiratory diseases of viral etiology. IV. Respiratory syncytial virus. Am J Public Health Nations Health. 1962;52:918-925.

59. Beem M. Repeated infections with respiratory syncytial virus. J Immunol. 1967;98:1115-1122.

60. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. Am J Dis Child. 1986;140:543-546.

61. Hall CB, Walsh EE, Long CE, Schnabel KC. Immunity to and frequency of reinfection with respiratory syncytial virus. J Infect Dis. 1991;163:693-698.

62. Henderson FW, Collier AM, Clyde WA Jr, Denny FW. Respiratory-syncytial-virus infections, reinfections and immunity. A prospective, longitudinal study in young children. N Engl J Med. 1979;300:530-534.

63. Bruhn FW, Yeager AS. Respiratory syncytial virus in early infancy. Circulating antibody and the severity of infection. Am J Dis Child. 1977;131:145-148.

64. Kasel JA, Walsh EE, Frank AL, Baxter BD, Taber LH, Glezen WP. Relation of serum antibody to glycoproteins of respiratory syncytial virus with immunity to infection in children. Viral Immunol. 1987;1:199-205.

65. Lamprecht CL, Krause HE, Mufson MA. Role of maternal antibody in pneumonia and bronchiolitis due to respiratory syncytial virus. J Infect Dis. 1976;134:211-217.

66. Ogilvie MM, Vathenen AS, Radford M, Codd J, Key S. Maternal antibody and respiratory syncytial virus infection in infancy. J Med Virol. 1981;7:263-271.

67. Parrott RH, Kim HW, Arrobio JO, et al. Epidemiology of respiratory syncytial virus infection in Washington, D.C. II. Infection and disease with respect to age, immunologic status, race and sex. Am J Epidemiol. 1973;98:289-300.

68. Welliver RC. Review of epidemiology and clinical risk factors for severe respiratory syncytial virus (RSV) infection. J Pediatr. 2003;143:S112-117.

69. Committee on Infectious Diseases. Modified recommendations for use of palivizumab for prevention of respiratory syncytial virus infections. Pediatrics. 2009;124:1694-1701.

70. Makari D, Groothuis J. Health risks of the late-preterm infant. Neonatol Today. 2009;4:1-8.

71. Engle WA, Tomashek KM, Wallman C. "Late-preterm" infants: a population at risk. Pediatrics. 2007;120:1390-1401.

72. Boyce TG, Mellen BG, Mitchel EF Jr, Wright PF, Griffin MR. Rates of hospitalization for respiratory syncytial virus infection among children in Medicaid. J Pediatr. 2000;137:865-870.

73. Colin AA, McEvoy C, Castile R. Respiratory morbidity and lung function in preterm infants of 32 to 36 weeks' gestational age. Pediatrics. 2010;126:115-128.

74. Law BJ, MacDonald N, Langley J, et al. Severe respiratory syncytial virus infection among otherwise healthy prematurely born infants: What are we trying to prevent? Paediatr Child Health. Vol 3; 1998:402-404.

75. Willson DF, Landrigan CP, Horn SD, Smout RJ. Complications in infants hospitalized for bronchiolitis or respiratory syncytial virus pneumonia. J Pediatr. 2003;143:S142-149.

76. Groothuis JR, Gutierrez KM, Lauer BA. Respiratory syncytial virus infection in children with bronchopulmonary dysplasia. Pediatrics. 1988;82:199-203.

77. MacDonald NE, Hall CB, Suffin SC, Alexson C, Harris PJ, Manning JA. Respiratory syncytial viral infection in infants with congenital heart disease. N Engl J Med. 1982;307:397-400.

78. Navas L, Wang E, de Carvalho V, Robinson J. Improved outcome of respiratory syncytial virus infection in a high-risk hospitalized population of Canadian children. Pediatric Investigators Collaborative Network on Infections in Canada. J Pediatr. 1992;121:348-354.

79. Panitch HB. Viral respiratory infections in children with technology dependence and neuromuscular disorders. Pediatr Infect Dis J. 2004;23:S222-227.

80. Wilkesmann A, Ammann RA, Schildgen O, et al. Hospitalized children with respiratory syncytial virus infection and neuromuscular impairment face an increased risk of a complicated course. Pediatr Infect Dis J. 2007;26:485-491.

81. Carbonell-Estrany X, Quero J. Hospitalization rates for respiratory syncytial virus infection in premature infants born during two consecutive seasons. Pediatr Infect Dis J. 2001;20:874-879.

82. Figueras-Aloy J, Carbonell-Estrany X, Quero J. Case-control study of the risk factors linked to respiratory syncytial virus infection requiring hospitalization in premature infants born at a gestational age of 33-35 weeks in Spain. Pediatr Infect Dis J. 2004;23:815-820.

83. Simoes EA. Environmental and demographic risk factors for respiratory syncytial virus lower respiratory tract disease. J Pediatr. 2003;143:S118-126.

84. McConnochie KM, Roghmann KJ. Parental smoking, presence of older siblings, and family history of asthma increase risk of bronchiolitis. Am J Dis Child. 1986;140:806-812.

85. von Linstow ML, Hogh M, Nordbo SA, Eugen-Olsen J, Koch A, Hogh B. A community study of clinical traits and risk factors for human metapneumovirus and respiratory syncytial virus infection during the first year of life. Eur J Pediatr. 2008;167:1125-1133.

86. Lanari M, Giovannini M, Giuffre L, et al. Prevalence of respiratory syncytial virus infection in Italian infants hospitalized for acute lower respiratory tract infections, and association between respiratory syncytial virus infection risk factors and disease severity. Pediatr Pulmonol. 2002;33:458-465.

87. Resch B, Manzoni P, Lanari M. Severe respiratory syncytial virus (RSV) infection in infants with neuromuscular diseases and immune deficiency syndromes. Paediatr Respir Rev. 2009;10:148-153.

88. Resch B, Pasnocht A, Gusenleitner W, Muller W. Rehospitalisations for respiratory disease and respiratory syncytial virus infection in preterm infants of 29-36 weeks gestational age. J Infect. 2005;50:397-403.

89. Simoes EA, King SJ, Lehr MV, Groothuis JR. Preterm twins and triplets. A high-risk group for severe respiratory syncytial virus infection. Am J Dis Child. 1993;147:303-306.

90. Collins PL, Graham BS. Viral and host factors in human respiratory syncytial virus pathogenesis. J Virol. 2008;82:2040-2055.

91. Yeung CY, Hobbs JR. Serum-gamma-G-globulin levels in normal premature, post-mature, and "small-for-dates" newborn babies. Lancet. 1968;i:1167-1170.

92. Ballow M, Cates KL, Rowe JC, Goetz C, Desbonnet C. Development of the immune system in very low birth weight (less than 1500 g) premature infants:

concentrations of plasma immunoglobulins and patterns of infections. Pediatr Res. 1986;20: 899-904.

93. Brandenburg AH, Groen J, van Steensel-Moll HA, et al. Respiratory syncytial virus specific serum antibodies in infants under six months of age: limited serological response upon infection. J Med Virol. 1997;52:97-104.

94. Hacimustafaoglu M, Celebi S, Aynaci E, et al. The progression of maternal RSV antibodies in the offspring. Arch Dis Child. 2004;89:52-53.

95. Ochola R, Sande C, Fegan G, et al. The level and duration of RSV-specific maternal IgG in infants in Kilifi Kenya. PLoS ONE. 2009;4:e8088.

96. Murphy BR, Alling DW, Snyder MH, et al. Effect of age and preexisting antibody on serum antibody response of infants and children to the F and G glycoproteins during respiratory syncytial virus infection. J Clin Microbiol. 1986;24:894-898.

97. Murphy BR, Prince GA, Walsh EE, et al. Dissociation between serum neutralizing and glycoprotein antibody responses of infants and children who received inactivated respiratory syncytial virus vaccine. J Clin Microbiol. 1986;24:197-202.

98. Murphy BR, Walsh EE. Formalin-inactivated respiratory syncytial virus vaccine induces antibodies to the fusion glycoprotein that are deficient in fusion-inhibiting activity. J Clin Microbiol. 1988;26:1595-1597.

99. Blanco JC, Boukhvalova MS, Shirey KA, Prince GA, Vogel SN. New insights for development of a safe and protective RSV vaccine. Hum Vaccin. 2010;6.

100. Castilow EM, Varga SM. Overcoming T cell-mediated immunopathology to achieve safe RSV vaccination. Future Virol. 2008;3:445-454.

101. Delgado MF, Coviello S, Monsalvo AC, et al. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. Nat Med. 2009;15:34-41.

102. Haynes LM, Jones LP, Barskey A, Anderson LJ, Tripp RA. Enhanced disease and pulmonary eosinophilia associated with formalin-inactivated respiratory syncytial virus vaccination are linked to G glycoprotein CX3C-CX3CR1 interaction and expression of substance P. J Virol. 2003;77:9831-9844.

103. Johnson TR, Teng MN, Collins PL, Graham BS. Respiratory syncytial virus (RSV) G glycoprotein is not necessary for vaccine-enhanced disease induced by immunization with formalin-inactivated RSV. J Virol. 2004;78:6024-6032.

104. Ponnuraj EM, Hayward AR, Raj A, Wilson H, Simoes EA. Increased replication of respiratory syncytial virus (RSV) in pulmonary infiltrates is associated with enhanced histopathological disease in bonnet monkeys (*Macaca radiata*) pre-immunized with a formalin-inactivated RSV vaccine. J Gen Virol. 2001;82:2663-2674.

105. Prince GA, Curtis SJ, Yim KC, Porter DD. Vaccine-enhanced respiratory syncytial virus disease in cotton rats following immunization with Lot 100 or a newly prepared reference vaccine. J Gen Virol. 2001;82:2881-2888.

106. Prince GA, Jenson AB, Hemming VG, et al. Enhancement of respiratory syncytial virus pulmonary pathology in cotton rats by prior intramuscular inoculation of formalin-inactivated virus. J Virol. 1986;57:721-728.

107. Sparer TE, Matthews S, Hussell T, et al. Eliminating a region of respiratory syncytial virus attachment protein allows induction of protective immunity without vaccine-enhanced lung eosinophilia. J Exp Med. 1998;187:1921-1926.

108. Stevens WW, Sun J, Castillo JP, Braciale TJ. Pulmonary eosinophilia is attenuated by early responding CD8(+) memory T cells in a murine model of RSV vaccine-enhanced disease. Viral Immunol. 2009;22:243-251.

109. Englund J. In search of a vaccine for respiratory syncytial virus: the saga continues. J Infect Dis. 2005;191:1036-1039.

110. Dudas RA, Karron RA. Respiratory syncytial virus vaccines. Clin Microbiol Rev. 1998;11:430-439.

111. Durbin AP, Karron RA. Progress in the development of respiratory syncytial virus and parainfluenza virus vaccines. Clin Infect Dis. 2003;37:1668-1677.

112. Kneyber MC, Kimpen JL. Advances in respiratory syncytial virus vaccine development. Curr Opin Investig Drugs. 2004;5:163-170.

113. van Drunen Littel-van den Hurk S, Mapletoft JW, Arsic N, Kovacs-Nolan J. Immunopathology of RSV infection: prospects for developing vaccines without this complication. Rev Med Virol. 2007;17:5-34.

114. Schickli JH, Dubovsky F, Tang RS. Challenges in developing a pediatric RSV vaccine. Hum Vaccin. 2009;5:582-591.

115. Power UF. Respiratory syncytial virus (RSV) vaccines--two steps back for one leap forward. J Clin Virol. 2008;41:38-44.

116. Clyde WA Jr. Experimental models for study of common respiratory viruses. Environ Health Perspect. 1980;35:107-112.

117. Coates HV, Chanock RM. Experimental infection with respiratory syncytial virus in several species of animals. Am J Hyg. 1962;76:302-312.

118. Prince GA, Porter DD. The pathogenesis of respiratory syncytial virus infection in infant ferrets. Am J Pathol. 1976;82:339-352.

119. Prince GA, Horswood RL, Chanock RM. Quantitative aspects of passive immunity to respiratory syncytial virus infection in infant cotton rats. J Virol. 1985;55:517-520.

120. Siber GR, Leombruno D, Leszczynski J, et al. Comparison of antibody concentrations and protective activity of respiratory syncytial virus immune globulin and conventional immune globulin. J Infect Dis. 1994;169:1368-1373.