

Prophylactic and Therapeutic Approaches Against Respiratory Syncytial Virus

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Abstract: RSV is the main viral respiratory cause of hospitalization in infants and young children in the United States and in the world. This manuscript discusses the different established and experimental approaches to prevention and treatment of respiratory syncytial virus disease. Therapeutic and preventive strategies are examined considering the mechanisms of viral pathogenesis and protection.

INTRODUCTION

Respiratory syncytial virus (RSV) is the leading cause of viral respiratory tract infections in infants and young children in the world. By age 2 years, over 90% of infants and young children have been infected with RSV [1]. In the United States, RSV is responsible for 73,400 to 126,300 hospitalizations annually for bronchiolitis and pneumonia among children younger than 1 year of age [2]. Although mortality is uncommon in healthy infants (< 0.1%), RSV can be devastating in infants born prematurely, those with chronic lung disease (CLD), children with congenital heart disease (CHD) [3], and in immunocompromised children, including pre-engraftment bone marrow transplant recipients, solid organ transplant recipients and lymphopenic patients receiving chemotherapy [4]. Reinfections are frequent during the first few years of life, but often only associated with upper respiratory tract (URT) symptoms. The development of vaccines has been confounded by lack of durable immunity, even after natural infection, and the diversity of populations at risk for infection. This problem may require development of several vaccines to target different populations at risk.

RSV, a member of the *Paramyxoviridae* family, is an enveloped RNA virus of negative polarity. The envelope consists of a lipid bilayer that is derived from the host plasma membrane and contains three virally encoded transmembrane glycoproteins: a SH (small hydrophobic protein), G (attachment) and F (fusion). F elicits protective antibody responses against all RSV serogroups, while protection mediated by G is serogroup specific. There are 2 serologically distinct RSV subgroups, RSV A and B. Most of the variability among RSV strains can be traced to variability within the G protein, as G_A and G_B differ in 47% of the nucleotide sequence and 95% of the amino acid composition [5].

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TRANSMISSION AND IMMUNITY

RSV is highly transmissible and spread by infected respiratory secretions [6-7]. After an incubation period of 4 – 5 days [8-9], RSV replicates in the nasopharyngeal epithelium and can spread to the lower respiratory tract (LRT) within 1 – 3 days [1]. Viral replication is greatest and most prolonged in naïve infants, in particular during primary infection, when 30-70% of cases result in LRT disease. Although LRT disease can occur during the second RSV infection [10], severity of disease decreases with subsequent infections, when it only affects the URT [10]. Protection against LRT disease develops after the first or second infection, presumptively by acquisition of RSV specific neutralizing antibodies (mainly IgG against F and G). The protective role of antibodies against LRT infection during re-exposure is highlighted by the protective effects of monoclonal and polyclonal IgG antibodies against RSV in animal models and children [11]. However, these IgG antibodies cannot protect against URT infection. The mechanisms of protection in the URT are unclear, but a role for IgA has been suggested by studies examining temperature-sensitive live attenuated RSV vaccines [12]. The relative role of cellular immunity in both protection and pathogenesis is not completely elucidated. Depletion of CD4⁺ or CD8⁺ T cells had a small effect on mice previously infected with RSV [13]. The cellular immune response is probably most important in viral clearance, although the exact mechanism of cell mediated clearance is unclear. Cytotoxic T lymphocytes (CTLs) can kill by releasing cytotoxic proteins, *granzyme* (which induces apoptosis in any type of target cell) and *perforin* (which punches holes in the target cell membrane). In addition, CTLs can use a membrane bound molecule, the *Fas ligand*, also capable of inducing apoptosis. Finally, CTLs produce IFN- which is thought to play a role in inhibiting viral replication. Although it appears that CTLs have a pivotal role in modulating the immune response, the mechanisms of control of RSV replication remain uncertain [14-17].

TREATMENT

RSV infection of the respiratory tract in immunocompetent hosts is usually a self limiting disease. In

infants, hospital admission is advised if there is a need for oxygen supplementation. Supportive therapy is required for respiratory failure. Many treatment strategies have been ineffective when examined in rigorous clinical trials.

Ribavirin is a synthetic guanosine analogue with antiviral properties [18]. The drug is administered as an aerosol over several hours a day. Ribavirin is the only antiviral preparation approved for RSV infections. It inhibits the synthesis of viral structural proteins, thereby slowing viral replication [19]. Results from more than 100 studies examining the efficacy of ribavirin are contradictory [20]. The American Academy of Pediatrics recommends that ribavirin may be considered for RSV-infected infants and children with CHD, CLD, immunosuppressive disease, prematurity or severe disease [21]. Guidelines on the use of ribavirin [21] recommend its use at the discretion of the individual physician for children with substantial comorbidities or those with exceptionally severe RSV infection. Its use in clinical practice today is exceptional.

The mechanism of illness in RSV disease is unclear. However, two prevailing theories are widely accepted. The first hypothesis attributes severity of RSV LRT disease to an increase in the inflammatory response associated with activation of Toll-like receptor 4 (TLR4) [22, 23]. The innate immune response is the first line of defense against infectious diseases. A group of proteins that comprise the Toll or Toll-like family of receptors perform this role in vertebrate and invertebrate organisms [24]. TLR4 modulates inflammatory responses to endotoxin and regulates inflammation through NF- κ B, a transcription factor that has a critical role in the induction of pro inflammatory cytokines and certain associated chemokines [25]. The RSV fusion surface glycoprotein (F) interacts with the CD14 monocyte receptor and promotes nuclear translocation of NF- κ B through TLR4 activation [22, 23, 26]. The pulmonary infiltrate during RSV LRT infection is composed overwhelmingly of neutrophils and macrophages attracted by these cytokines and chemokines, including IL-8, IL-1, TNF, MIP-1, MCP-1 and RANTES among others [27, 28]. Damage to the small airways (10-300 microns) affected by the virus may cause debris accumulation in the lumen, inflammation and edema and compromise ventilation.

However, the pathogenic role of innate inflammatory responses during RSV infection has been challenged by recent findings in infants infected with human metapneumovirus (hMPV), another respiratory pathogen. HMPV caused identical clinical signs and symptoms as RSV infection in infants, while producing significantly lower innate inflammatory cytokines in the respiratory tract of infants than RSV. This finding suggests that a mechanism of illness other than production of inflammatory cytokines can elicit symptoms identical to those caused by RSV in infants. Whether hMPV and RSV cause LRT disease by different mechanisms or by a common mechanism of illness, other than innate immunity, is unclear; but both alternatives demonstrate that innate inflammation is not critical for production of respiratory symptoms during viral respiratory diseases [29]. Alternatively, the number of children in this study may have been insufficient to detect subtle differences in severity between RSV and hMPV [30]. **Corticosteroids**

are modulatory agents for inflammation that act reducing the recruitment of neutrophils, monocytes and lymphocytes. Glucocorticoids affect NF- κ B dependent gene induction [31, 32]. The differences in innate inflammatory response between RSV and hMPV described above may explain the poor therapeutic results achieved by the administration of corticosteroids to these patients in the past [33, 34].

A second hypothesis postulates that severe RSV disease is caused by a Th2 polarization of the immune response in the lungs leading to bronchoconstriction. The association of severe RSV disease and Th2 polarization of the pulmonary immune response stems from the clinical similarities of bronchiolitis and an asthmatic exacerbation, a possible association between presence of anti-RSV IgE antibodies and disease severity [35], and an excess in pulmonary eosinophils in the lungs of children affected by enhanced RSV disease in 1967 after immunization with formalin-inactivated RSV vaccine (FIRSV) [36]. Gain-of-function variants of Th2 genes encoding IL-4 and the IL-4 receptor alpha chain have been found more frequently in children with severe RSV LRT illness both in Korea and The Netherlands [37, 38] supporting a role for Th2 polarization in disease severity. This association led to the use of **2-agonist bronchodilators** as a potential therapy for bronchiolitis. However, several trials [39-42] have shown that 2-agonist bronchodilators offer no benefit during RSV bronchiolitis. In the most severely ill patients on mechanical ventilation [43-46], bronchodilator therapy may offer some benefit. **Epinephrine**, because of its α -adrenergic agonist activity and its vasoconstrictive properties, is more effective at decreasing interstitial and mucosal edema and may therefore be more effective at opening small airways than β -adrenergic bronchodilators [47]. Nasopharyngeal suctioning may offer some palliative support for infants with RSV [48, 49].

EXPERIMENTAL THERAPIES

In recent years, several structurally distinct small molecules that appear to interfere with the RSV entry process have emerged from antiviral screens. These include the disulfonated stilbene **CL387626** and **RFI-641** (Wyeth) and the triphenol **VP-14637** (ViroPharma) [78, 79]. Interestingly, although these small molecules show little structural homology with each other, they all appear to inhibit RSV by interfering with virus-cell membrane fusion.

Twenty thousand compounds in a whole-virus cell-based assay were screened to develop new small-molecule inhibitors of RSV. A specific inhibitor of RSV, **CL387626** (Fig. 1c), was identified and subsequently shown to have activity against a wide variety of RSV clinical and laboratory strains [80, 81]. CL387626 showed *in vivo* activity in RSV-infected cotton rats [79]. A second compound, **RFI-641** (Fig. 1b) is the result of a chemical optimization of CL387626 and it was shown to be efficacious by intranasal administration in mice [82]. RFI-641 interacts specifically with the viral F protein and is active against a panel of both type A and B RSV strains when present early during infection, before F mediated fusion [82]. Another fusion inhibitor is **VP-14637** (Fig. 1a) which could act by blocking key protein-protein interactions or conformational changes within the F protein complex [50].

BMS-433771 (Fig. 1d) is a selective inhibitor of RSV *in vitro* with activity against a broad range of laboratory and human clinical isolates of both the A and B subgroups of RSV. BMS-433771 inhibits an early event in the life cycle of RSV and can inhibit F-mediated syncytium formation. Interestingly, single amino acid changes conferring resistance to BMS-433771 were found in the F protein genes of viruses generated by serial passages in the presence of increasing concentrations of compound. All of the amino acid changes occurred within the F1 subunit, which appears to be the molecular target of BMS-433771 [83].

Another potential therapeutic agent for RSV is **RhoA**. RhoA is an essential host cell protein with GTPase activity and is known to influence a variety of signaling pathways and basic cell functions [84]. RhoA activation may play a role in coordinating cellular activities involved in virus replication such as stage of cell cycle and reorganization of the actin cytoskeleton [85]. RhoA is activated during RSV infection and appears to mediate the reorganization of actin

stress fibers that occur during RSV infection [51]. In addition, a peptide derived from the small intracellular GTPase RhoA was effective at reducing the replication of RSV *in vitro* and *in vivo* in a mouse model of RSV disease [86]. Based on truncation studies, a peptide comprising amino acids 80 to 94 of RhoA was optimal for anti-RSV activity. The inhibition of RSV by this peptide is largely due to disruption of viral attachment [87].

PASSIVE PROPHYLAXIS

Premature infants, infants born with CHD and those with CLD constitute high risk groups with increased rates of hospitalization caused by RSV infection. In addition, immunocompromised children and adults, including pre-engraftment bone marrow transplants recipients, solid organ transplant recipients and lymphopenic patients receiving chemotherapy, appear to suffer even higher mortality rates [4, 52].

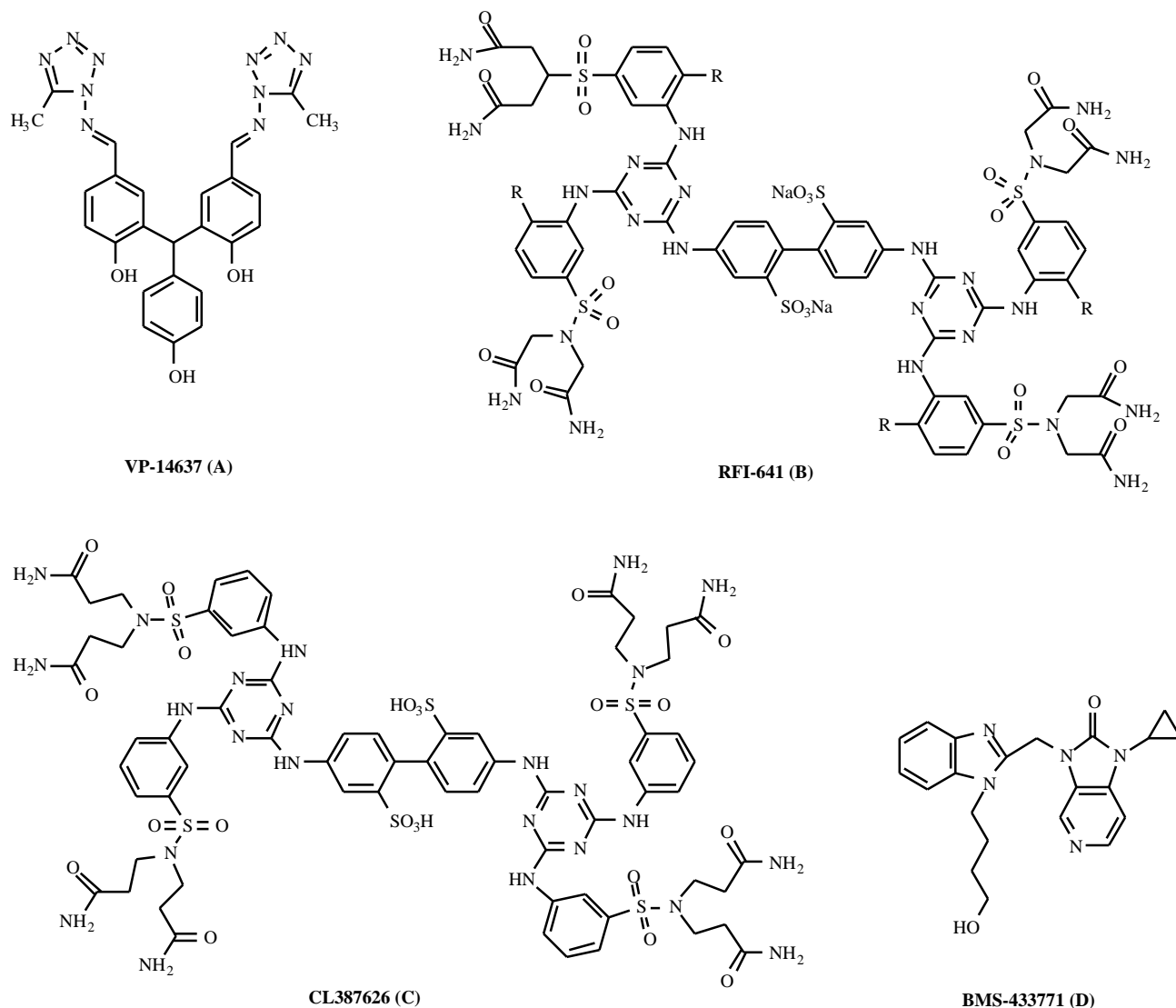


Fig. (1). Chemical structure of RSV inhibitors.

Initially, prophylaxis against RSV in populations at risk was examined using **RSV-IGIV**, a passive immunization with pooled immunoglobulin containing high titers of anti-RSV-F antibodies. RSV-IGIV is administered intravenously in a dose of 750 mg/kg once a month during the RSV season. It was the first prophylactic agent with demonstrated efficacy against RSV, and provided a 41% reduction in RSV hospitalizations [53]. Interestingly, RSV-IGIV prophylaxis provided additional protection against other infections not caused by RSV [53]. RSV-IGIV, as with all blood products, carries the risk of blood borne infection, fever and systemic reactions. In addition, administration of RSV-IGIV is expensive and cumbersome. Therefore, use of RSV-IGIV has been largely replaced by a monoclonal antibody preparation that can be administered intramuscularly.

Palivizumab is a humanized monoclonal antibody (IgG₁) against the F glycoprotein of RSV. It is a composite of human (95%) and murine (5%) antibody sequences [54]. Palivizumab is administered intramuscularly in a dose of 15 mg/kg once a month during the RSV season. Because the monoclonal antibody is not derived from human immune globulin, it is free of potential contamination by infectious agents and can be produced readily in batch lots. Palivizumab reduced 55% RSV hospitalization rates compared with placebo in children with CLD who were younger than 24 months of age and those born prematurely (<35 weeks of gestational age) [55].

RSV prophylaxis should be initiated at the onset and terminated at the end of the RSV season. The American Academy of Pediatrics recommends that Palivizumab and RSV-IGIV prophylaxis be considered for infants and children younger than 2 years of age with CLD who have required medical therapy for their CLD within the 6 months before the anticipated RSV season. Palivizumab is preferred for most high-risk children because of its ease of administration, safety and effectiveness. Infants born at 32 weeks of gestation or earlier without CLD also may benefit from RSV prophylaxis. In these infants, major risk factors to consider are gestational age and chronologic age at the start of the RSV season [53]. Available data indicate that RSV-IGIV should not be used in children with cyanotic CHD [56]. In a recent trial palivizumab was administered to infants and young children with hemodynamically significant CHD and found to have similar adverse effects as placebo recipients. Palivizumab was effective as prophylaxis against RSV in CHD patients, and decreased hospitalizations and oxygen requirement. Evaluation by the FDA is pending [57].

During an RSV outbreak in a high-risk unit, primary emphasis should be placed on infection control practices, as the efficacy of RSV prophylaxis in these situations is unknown. Although specific recommendations for immunocompromised patients are not available, children with severe immunodeficiencies may benefit from prophylaxis. If these infants are receiving standard immunoglobulin intravenous (IGIV) monthly, physicians may consider substituting RSV-IGIV during the RSV season. Unlike RSV-IGIV prophylaxis, which delays immunization with measles-mumps-rubella and varicella

vaccines, Palivizumab does not interfere with the response to vaccines [53, 56].

VACCINES

Development of vaccines to protect infants against RSV disease has been delayed by the enhanced disease (ERD) observed in children exposed to RSV after receiving formalin-inactivated RSV vaccine (FIRSV) in the 1960s. The vaccine was immunogenic, but elicited mainly non-protective antibodies. During the winter of 1966-1967, immunized children exposed to RSV in the community experienced a significant increase in the frequency and severity of bronchoconstriction and pneumonia and greater incidence of hospitalization compared to control children [36, 58-60].

The mechanism of illness in enhanced RSV disease is multifactorial. Complement components activated by immune complexes deposition on sites of RSV replication are critical for bronchoconstriction [61], while CD4⁺ T cells secreting Th2 cytokines are critical for pneumonia [13, 62]. It is noteworthy that both bronchoconstriction and pneumonia appear to result from failure of FIRSV to elicit protective antibodies. Absence of RSV-specific protective antibodies during and immediately after wild-type RSV infection allowed unrestricted RSV replication in the lungs and elicited strong secondary responses from FIRSV-primed T helper cells releasing cytokines and promoting the inflammatory infiltration in the lungs. On the other hand, bronchoconstriction resulted from the presence of RSV binding, non-protective RSV antibodies during RSV replication in the lungs. These antibodies led to immune complexes formation and deposition, and activation of the complement cascade [61].

Protection of infants against RSV may be achieved by one or more different strategies. Administration of non-replicating (inactivated or subunit) vaccines to naïve infants is precluded by the enhanced disease of the 1960s, but may be used to immunize pregnant women and boost transplacental antibody transfer. Replicating vaccines, in particular live-attenuated vaccines, mimic wild-type infection and are the main strategy for immunization of young babies.

In addition to the enhanced disease of 1967, the design of RSV vaccines is hampered by several difficulties. These problems included the incomplete protection induced by wild-type RSV infection, the need to protect against both RSV serogroups A and B, the presence of maternal antibodies and the immaturity of the infant immune system. Controlling reinfections in older and immunocompromised patients may require different vaccines or alternative strategies.

Subunit vaccines are based upon either F protein and/or G protein administration, main targets for the induction of neutralizing antibodies. Since ERD was only seen in infants that were RSV-naïve at the time of FIRSV-immunization, subunit vaccines have been subsequently evaluated in previously primed populations: the elderly, older children at high risk for severe RSV disease and pregnant women. Vaccines recently evaluated in human trials include purified

F glycoprotein vaccines (PFP-1 and PFP-2 with aluminum hydroxide as adjuvant and PFP-3 adsorbed to aluminum phosphate) [63, 64], a co-purified F, G and M protein vaccine using alum₁ or polydicarboxylatophenoxyphosphazene as adjuvant¹, and BBG2Na, a peptide from the G glycoprotein conjugated to the albumin-binding domain of streptococcal protein G, which acts as a carrier protein [65].

Immunization with subunit vaccines may induce neutralizing antibodies, but a class I MHC-restricted cytotoxic T cell response is unlikely [66]. Purified F protein (PFP) vaccines are safe in RSV-seropositive patients, but of moderate efficacy. They do not provide full protection against lower respiratory tract infection and associated disease. However, PFP-2 proved highly immunogenic in pregnant women with >90% 4-fold increase in neutralizing antibody titer [67]. Infants born from vaccine recipients had significantly higher titers of IgG ELISA antibodies against RSV at birth and at 2 and 6 months of life. A second approach in subunit vaccines is the use of BBG2Na, an RSV G_A peptide (amino acids 130 to 230) (G₂Na), bound to the C terminus of an albumin-binding region of the streptococcal G protein (BB) [68]. The BB component is used for immunostimulatory effects.

Much effort is focused on developing **attenuated vaccines**. A live attenuated vaccine may induce an immune response that is more comparable with that elicited by wild type RSV, which is not associated with enhanced disease. In addition, live attenuated vaccines have the potential advantage of intranasal administration and induction of both systemic and mucosal immunity. The first RSV vaccines, consisting of temperature-sensitive (ts) or cold-passaged (cp) mutants, were effective in adults, but insufficiently attenuated in children [69]. Candidates derived from previous cp or ts mutants by repetitive rounds of chemical mutagenesis are attenuated, immunogenic and stable [12, 70, 71].

Recombinant RSV vaccines containing cp and ts mutations and deletions of SH₂ or NS₂ gene are currently being evaluated in clinical trials². Recombinant technology also allows development of chimeric viruses containing the RSV F and G surface glycoproteins, with one or more genes provided by related respiratory viruses [72-74]. Finally, genetically structured or **DNA vaccines** use plasmid DNA containing the gene for the antigen of interest. DNA vaccines are easily manufactured and the antigen can be expressed with no alterations to its structure [75]. Immunomodulating genes might be added to enhance the immune response to the vaccine and aid in improving the safety [76]. DNA immunization induces protective immunity in mice in the presence of maternal antibodies [77], but they have not been used in humans for RSV.

CONCLUSIONS

RSV is the most important viral respiratory cause of hospitalization in infants and young children in the United States and in the world. However, neither therapies nor

vaccines are currently available. A combined approach of prophylactic and therapeutic agents may be necessary to address the diverse clinical manifestations that the virus elicits in different populations. Understanding the mechanisms of protection and pathogenesis will contribute to development of safe treatments and vaccines in the future.

ABBREVIATIONS

RSV	=	Respiratory syncytial virus
CLD	=	Chronic lung disease
CHD	=	Congenital heart disease.
URT	=	Upper respiratory tract
LRT	=	Lower respiratory tract.
SH	=	Small hydrophobic glycoprotein
G	=	RSV attachment glycoprotein
F	=	RSV fusion glycoprotein
TLR4	=	Toll like receptor 4
hMPV	=	Human metapneumovirus
FIRSV	=	Formalin inactivated RSV vaccine
ERD	=	Enhanced RSV disease
PFP	=	Purified F protein
IGIV	=	Immunoglobulin intravenous.

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