Progress and Challenges in RSV Prophylaxis and Vaccine Development

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Respiratory syncytial virus (RSV) is a respiratory tract pathogen that causes significant morbidity and mortality in children aged <5 years (most disease occurs at age <1 year) and is a major public health burden worldwide. More than 90% of children are infected at least once with RSV before the age of 2 years [1–3]. RSV accounts for approximately 70% of hospitalizations due to bronchiolitis [1, 4]. In the United States alone, the estimated healthcare costs associated with RSV hospitalizations exceed $950 million, making it a significant economic burden [5]. Further, the RSV burden is disproportionately greater in children aged <5 years living in developing countries [3]. RSV infection does not confer long-term protection, as reinfections occur throughout life, which poses a significant disease risk in individuals with cardiopulmonary disease, immunocompromised patients, and the elderly [7]. In the elderly, complications of RSV infection often result from exacerbation of underlying pulmonary and cardiac disease. [7].

A member of the Paramyxoviridae family, Pneumovirus genus, RSV exists as an enveloped virus containing a negative-sense, single-stranded RNA genome. The genome encodes for the following 11 proteins: nonstructural proteins (NS1, NS2, and M2-2), the viral nucleocapsid protein (N), phosphoprotein (P), matrix (M), RNA-dependent RNA polymerase (L), M2-1, and 3 surface glycoproteins (G [attachment], F [fusion], and SH [small hydrophobic]). There are 2 RSV major groups, A and B, and multiple genotypes within each group. The protective immune response to RSV infection is primarily directed against the 2 major surface viral glycoproteins, F and G. The F glycoprotein seems most important for induction of protective immunity and is associated with a high serum neutralizing antibody response as well as activation of CD14 and Toll-like receptor-4 (TLR-4) [8]. RSV F protein undergoes structural rearrangement during the fusion process. Antigenic sites on the postfusion form of the protein have been associated with a range of neutralizing activity. However, recent evidence suggests that most of the F protein–specific neutralizing antibodies in human sera are directed against the prefusion form of the RSV F protein. Thus, the antigenic nature of the RSV F protein may have important implications for prophylaxis and vaccine development [9]. The RSV G protein has been implicated in the pathogenesis of disease after primary infection and formalin-inactivated (FI-RSV)–enhanced disease [6]. The highest degree of antigenic diversity in RSV is found in the RSV G protein. This diversity may play an important role in viral pathogenesis by facilitating immune evasion. The ability of this protein to evade or inhibit the host immune response may complicate vaccine development. RSV binds to several surface ligands, including cellular glycosaminoglycans, CX3CR1 [10], ICAM-I [11], Annexin-II [12], and nucleolin [13].

PROGRESS IN PROPHYLAXIS

Prophylaxis is limited to passive immunization with palivizumab, a humanized anti-F glycoprotein monoclonal antibody. Palivizumab is effective in decreasing hospitalizations and preventing serious lower respiratory tract disease in young children at high risk for RSV infection [14]; it is ineffective as a treatment for established infection. Palivizumab is expensive (approximately $6000 per child per season), and its use is only recommended for high-risk infants and young children.
developing countries, the high cost has made palivizumab immune prophylaxis impractical. Motavizumab, the next-generation affinity mature variant of palivizumab, was evaluated but not approved for licensure due to concerns related to hypersensitivity [15]. Although motavizumab is not being further developed for prophylaxis of severe RSV disease, its therapeutic potential in young children was under evaluation (ClinicalTrials.gov; identifier# NCT00435227). MEDI-557, a high-affinity anti-F monoclonal antibody with an extended half-life, recently completed phase 1 clinical therapeutic evaluation (ClinicalTrials.gov; identifier# NCT01562938).

**PROGRESS IN TREATMENT**

Current therapeutic options are limited. Treatment for RSV infection is primarily supportive. Bronchodilators and inhaled and systemic corticosteroids are minimally effective [16–19]. The nucleoside analog ribavirin is the only clinically approved treatment for RSV infection. Studies have shown that it has limited efficacy and is seldom used because of toxicity and administration concerns [19]. No approved RSV therapy is clearly effective in treating disease once infection is established. One possible explanation for treatment failure is that stopping virus replication alone is not sufficient to prevent the virus-induced host contribution to disease pathogenesis. A combined antiviral and antiinflammatory treatment may be an effective approach. The limited treatment options emphasize the need for new prophylactic and therapeutic strategies.

The F protein has been the focus for most efforts to develop RSV antiviral drugs, immune prophylaxis, and vaccines. Several new antibodies, antivirals, and antiinflammatory agents are either in preclinical development or clinical evaluation. Prophylactic and therapeutic intranasal administration of RSV F-specific immunoglobulin single-variant domains or nanobodies reduced virus replication and pulmonary inflammation after RSV infection in mice and cotton rats [20]. This novel therapeutic agent completed a phase 1 clinical trial during 2012 (ClinicalTrials.gov; identifier# NCT01483911). The RSV G protein is another target for treatment and prevention strategies. This protein contains a CX3C chemokine motif and has structural homology with the CX3CL1 chemokine (fractalkine), a leukocyte chemotactant [10]. RSV G CX3C can bind to the fractalkine receptor CX3CR1 in order to facilitate infection [10]. Prophylactic and therapeutic treatment with murine anti-RSV G monoclonal antibodies that block the RSV G CX3C-CX3CR1 interaction have been shown to significantly reduce pulmonary inflammation in mice compared to a palivizumab-like anti-F monoclonal antibody [21, 22] or an anti-RSV G monoclonal antibody that does not block RSV G CX3C-CX3CR1 interaction [23]. High-affinity human anti-RSV G monoclonal antibodies that are directed at the central conserved region of the RSV G protein have also shown therapeutic efficacy in mice; however, the utility of these antibodies as a human therapy requires clinical testing [22].

**PROGRESS IN VACCINE DEVELOPMENT**

Currently, there is no licensed vaccine to prevent RSV infection. The burden of RSV disease is great and warrants substantial effort to develop a vaccine to prevent disease. The first clinical trial using a FI-RSV vaccine yielded disastrous results in young vaccinees upon subsequent natural infection; many vaccinees developed enhanced pulmonary disease and required hospitalization, and 2 deaths occurred [24]. The cause of FI-RSV vaccine failure remains uncertain. Studies suggest the following scenarios are likely contributors: formalin inactivation disrupted key epitopes on the virus necessary for the development of an effective neutralizing antibody response and cytotoxic T lymphocyte responses; poor TLR stimulation may have led to a lack of virus-specific antibody maturation; low antibody avidity may have contributed to disease severity due to immune complex formation in the lungs; and an imbalance in the T-helper cell response likely predisposed vaccinees to an allergic asthma response upon natural infection [24–27]. RSV vaccine development has been hindered by the need for a strong and robust protective immune response as well as risk of vaccine-enhanced disease. In development of future vaccines, better understanding of the pathogenesis of FI-RSV and the immune correlates of protection from RSV remain important. Because of the experience with FI-RSV, it has not, to date, been possible to advance development of nonlive virus vaccines in RSV-naive children.

Despite these challenges, a number of candidate vaccines have been developed by groups in academia, government, and industry. These include pursuit of a broad range of approaches to induce a safe and protective immune response. Several live-attenuated, genetically engineered virus and subunit-based vaccines are undergoing clinical evaluation (Table 1), and many more vaccine candidates are in preclinical development, several of which are listed in Table 2. The following discussion highlights a portion of vaccine candidates currently under investigation [16, 37].

**VACCINES IN THE CLINICAL DEVELOPMENT STAGE**

**Live-Attenuated/Genetically Engineered Virus Vaccines**

A number of cold-passaged (cp), temperature-sensitive (ts) RSV vaccine candidates have been developed. One, cpts-248/404, was evaluated in infants aged 1–2 months, the target age group for an RSV vaccine. However, the vaccine caused upper respiratory tract congestion and was felt to be insufficiently attenuated and not pursued for further development [44].
Table 1. Respiratory Syncytial Virus Vaccines Under Development: Undergoing Clinical Evaluation

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Manufacturer/Institution</th>
<th>Experimental Approach</th>
<th>Clinical Evaluation Status</th>
<th>Target Population</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDI-559</td>
<td>Medimmune LLC</td>
<td>Live attenuated/genetically engineered</td>
<td>Phase 1/2a (completed August 2012)</td>
<td>Seronegative pediatric populations</td>
<td>[44, 46]</td>
</tr>
<tr>
<td>MEDI-534</td>
<td>Medimmune LLC</td>
<td>Viral vector based (RSV/PIV-3)</td>
<td>Phase 1/2 (completed October 2012)</td>
<td>Seronegative pediatric populations</td>
<td>NCT00686075</td>
</tr>
<tr>
<td>MEDI-ΔM2-2</td>
<td>NIAID</td>
<td>Live attenuated</td>
<td>Phase 1</td>
<td>Seronegative pediatric populations</td>
<td>[47] NCT01459198</td>
</tr>
<tr>
<td>RSV F nanoparticle vaccine</td>
<td>Novavax</td>
<td>Subunit vaccine</td>
<td>Phase 2 (young women, phase 1 elderly)</td>
<td>Women of childbearing age (18–35 years), elderly, adults</td>
<td>[48–50] NCT01704365; NCT01709019</td>
</tr>
</tbody>
</table>

Researchers later used reverse genetics to further attenuate the cpts-248/404 strain. Several mutants were generated, evaluated, and determined to be either over- or underattenuated [44]. One recombinant strain, rA2cp248/404/1030ΔSH, carried 5 independent attenuating elements, including a deletion of the SH gene [33, 34, 45], and appeared to be adequately attenuated [46]. During initial clinical studies, rA2cp248/404/1030ΔSH demonstrated immunogenicity in seronegative children, an acceptable safety profile in infants aged 1–2 months, and was protective after a second dose of the vaccine [46]. Postvaccination nasal washes showed that more than 30% of recovered isolates exhibited a partial loss of the temperature-sensitive phenotype that was largely associated with a tyrosine-to-asparagine substitution in the polymerase L protein at position 1321 [46]. Notably, no vaccinees presented with vaccine-enhanced disease.

Later, rA2cp248/404/1030ΔSH vaccine was modified, by MedImmune, to include 39 silent nucleotide substitutions [45]. This modified version of rA2cp248/404/1030ΔSH is designated MEDI-559 and biologically indistinguishable from the original virus [34]. A phase 1/2a multicenter trial to evaluate safety, tolerability, viral shedding, immunogenicity, and genotype and phenotype stability of MEDI-559 in healthy RSV-seronegative children aged 5–23 months was completed in late 2012 (Clinical Trials.gov; identifier NCT00767416). The genetic and phenotypic instabilities of MEDI-559 and rA2cp248/404/1030ΔSH necessitate further improvement of the attenuated phenotype and may pose a challenge to further development of this vaccine.

Another live-attenuated RSV vaccine candidate that includes an M2-2 deletion (RSV MEDIΔM2-2) has been tested in nonhuman primates [47] and is now undergoing further testing in a phase 1 clinical trial to evaluate safety and immunogenicity in adults (aged 18–49 years), RSV-seropositive children (aged 12–59 months), and RSV-seronegative infants and children (aged 6–24 months; ClinicalTrials.gov, identifier NCT01459198). A continuing challenge for live-attenuated RSV vaccines is to achieve sufficient attenuation for safety while having sufficient immunogenicity to be effective.

Viral Vector-based Vaccine

The RSV/parainfluenza virus type 3 (PIV-3) vaccine candidate (MEDI-534) is based on the bovine PIV3 backbone with substituted human PIV3 fusion and hemagglutinin-neuraminidase surface glycoproteins and engineered to express the RSV F protein. In phase 1 studies in adults, RSV-PIV-3–seropositive children (aged 1–9 years), and RSV-PIV-3–seronegative young children (aged 6 to <24 months), MEDI-534 demonstrated acceptable safety, virus shedding, and immunogenicity profiles at 10^4, 10^5, and 10^6 50% tissue culture infectious dose. A phase 1/2a study in RSV-PIV-3–seronegative young children (aged 6 months to <24 months) and in 2-month-old infants was completed in late 2012 (ClinicalTrials.gov, identifier # NCT00686075). No results have been published to date.

Subunit Vaccine

Novavax has developed a recombinant nanoparticle vaccine that comprises an oligomeric form of a modified full-length RSV F protein. The RSV F protein was expressed using an SI9 insect cell/baculovirus expression system; purified protein was then assembled into nanoparticles. Two intramuscular doses of the RSV F nanoparticles induced a relatively high palivizumab-like neutralizing antibody response in cotton rats and conferred protection against lung infection following RSV challenge without immunopathology [48]. In a phase 1 safety and immunogenicity clinical trial in young adults, the RSV F nanoparticle vaccine candidate was well tolerated without dose-related adverse events. Using both traditional and novel measures to evaluate immunogenicity, the vaccine has been shown to induce neutralizing antibodies that can competitively inhibit palivizumab binding to RSV F, a finding that should be studied further [49]. Novavax has a clinical development partnership with the Program for Appropriate Technology in Health to develop its RSV F vaccine for maternal immunization in low-resource countries [50]. Under this partnership, Novavax is currently conducting a phase 2 clinical study in women of childbearing age (18–35 years) to evaluate the RSV F nanoparticle vaccine immunogenicity and safety.
VACCINES IN THE PRECLINICAL DEVELOPMENT STAGE

Live-Attenuated Vaccines

To address the issue of genetic and phenotypic instability, researchers at the National Institutes of Health recently created a more genetically stable version of rA2cp248/404/1030ΔSH, called cps2, by stabilizing the position 1321 with an alternative attenuating amino acid [33, 34]. When evaluated in nonhuman primates, cps2 remained reasonably attenuated. However, clinical studies will be necessary to further evaluate the virus stability.

Inactivated Virus/Particle-based Vaccines

Nanoemulsion-based mucosal vaccination is a vaccine approach being evaluated for a number of pathogens [28, 29, 51], including RSV [35]. In a mouse model, an intranasal nanoeulsion-based inactivated RSV vaccine induced RSV-specific immune responses and enhanced virus clearance upon virus challenge [35]. Vaccination did not lead to immunopathology or a Th2-type bias.

Virosomes or reconstituted RSV viral envelopes with incorporated TLR4 ligand, monophosphoryl lipid A (MPLA), are one approach to enhance immunogenicity and skew the immune response toward a balanced Th1/Th2 type response [30, 36]. MPLA is currently being used as an adjuvant in a number of licensed vaccines [31]. The advantage of virosomes is that the process to inactive RSV does not involve any cross-linking chemicals; the virosomes maintain the RSV G and F proteins in their native conformations and are fully replication incompetent. Immunization of RSV virosomes in mice protected against virus challenge without producing enhanced immunopathology [36].

Vector-based Vaccines

Viruses such as Sendai virus [32], measles virus [41], adenovirus [42], and alphaviruses [43] have been used as virus vectors with encouraging animal model results. Vaccine delivery systems based on bacteria, rather than viruses, also have been used to develop RSV vaccines. Nonliving bacterium-like particles (BLP) that contain stable native trimeric F protein have been evaluated as an intranasal vaccine in both mice and cotton rats [40]. This RSV F-BLP elicited local antigen-specific immunoglobulin-A, a balanced Th1/Th2 type response, and protected from RSV challenge.

Table 2. Respiratory Syncytial Virus Vaccines Under Development: Preclinical Evaluation

<table>
<thead>
<tr>
<th>Vaccine Description</th>
<th>Experimental Approach</th>
<th>Animal Model Evaluated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>cps2 (rA2cp248/404/1030ΔSH stabilized 1030 mutation)</td>
<td>Live attenuated</td>
<td>Nonhuman primates</td>
<td>[33]</td>
</tr>
<tr>
<td>ΔNS2/ΔA131/1314L</td>
<td>Live attenuated</td>
<td>Nonhuman primates</td>
<td>[34]</td>
</tr>
<tr>
<td>Nanoeulsion adjuvanted inactivated RSV virosome</td>
<td>Inactivated</td>
<td>Mouse</td>
<td>[35]</td>
</tr>
<tr>
<td>MPLA adjuvanted RSV virosome</td>
<td>Subunit</td>
<td>Mouse, cotton rat</td>
<td>[36]</td>
</tr>
<tr>
<td>Post-fusion RSV F trimers</td>
<td>Subunit</td>
<td>Mouse, cotton rat</td>
<td>[16, 37]</td>
</tr>
<tr>
<td>Subnucleocapsid nanoparticle</td>
<td>Subunit</td>
<td>Mouse</td>
<td>[16, 37]</td>
</tr>
<tr>
<td>Truncated, secreted F (ΔF) + CpG and IDR microparticles</td>
<td>Subunit</td>
<td>Mouse, cotton rat</td>
<td>[38]</td>
</tr>
<tr>
<td>RSV G-derived immunogenic peptides</td>
<td>Subunit</td>
<td>Mouse</td>
<td>[39]</td>
</tr>
<tr>
<td>RSV F and/or RSV G plant-based</td>
<td>Subunit</td>
<td>Mouse</td>
<td>[16, 37]</td>
</tr>
<tr>
<td>Bacterium-like particles expressing trimeric RSV F</td>
<td>Particle based</td>
<td>Mouse, cotton rat</td>
<td>[40]</td>
</tr>
<tr>
<td>RSV virosome +Pam3CSK4</td>
<td>Particle based</td>
<td>Mouse</td>
<td>[30]</td>
</tr>
<tr>
<td>MVA-BN RSV F DNA</td>
<td>Gene-based vector</td>
<td>Nonhuman primates</td>
<td>[16, 37]</td>
</tr>
<tr>
<td>Measles virus-based RSV F</td>
<td>Gene-based vector</td>
<td>Cotton rat, nonhuman primates</td>
<td>[41]</td>
</tr>
<tr>
<td>Sendai virus vector full-length RSV F</td>
<td>Gene-based vector</td>
<td>Cotton rat, nonhuman primates</td>
<td>[32]</td>
</tr>
<tr>
<td>Adenovirus-based RSV F</td>
<td>Gene-based vector</td>
<td>Mouse</td>
<td>[42]</td>
</tr>
<tr>
<td>Alphavirus: SFV and VEE replicon particles encoding RSV F</td>
<td>Gene-based vector</td>
<td>Mouse</td>
<td>[16, 37, 43]</td>
</tr>
<tr>
<td>NDV-VLP RSV F and RSV G</td>
<td>Gene-based vector</td>
<td>Mouse</td>
<td>[37]</td>
</tr>
<tr>
<td>DNA plasmid vaccines RSV G, RSV F or RSV N</td>
<td>Nucleic acid</td>
<td>Mouse</td>
<td>[37]</td>
</tr>
</tbody>
</table>

Abbreviations: IDR, innate defense regulator peptide; MPLA, monophosphoryl lipid A; MVA, modified vaccinia virus Ankara; NDV-VLP, Newcastle disease virus-like particles; P3CSK4, synthetic bacterial lipopeptide; RSV, respiratory syncytial virus; SFV, Semilike forest virus; VEE, Venezuelan equine encephalitis virus.

This is a partial list of current RSV vaccines under preclinical evaluation and the vaccine approaches and animal model(s) used for evaluation.

(ClinicalTrials.gov, identifier NCT01704365). Maternal immunization has the potential to prevent RSV disease in young infants when vaccination is most challenging. Maternal immunization is currently practiced against influenza, pertussis, and other pathogens. Also, a phase 1 clinical study to evaluate the immunogenicity and safety of the RSV F nanoparticle vaccine coadministered with a licensed inactivated influenza vaccine in elderly adults is ongoing (ClinicalTrials.gov, identifier NCT01709019).
Subunit-based Vaccines

Researchers have incorporated a truncated secreted form of RSV F formulated with a TLR agonist (CpG ODN) and host-defense peptide (innate defense regulator) into microparticles [38]. Mice and cotton rats developed robust immune responses and were protected from RSV challenge after either intramuscular or intranasal vaccination. Stable postfusion RSV F trimers elicited high neutralizing antibody titers in cotton rats and provided protection from RSV challenge. These results highlight how structural-based antigen design can be used to develop an RSV vaccine.

Mice immunized with peptides from the central conserved region of RSV G protein [52] or microparticle vaccines containing these peptides developed antibodies [39] that inhibited the RSV G CX3C-CX3CR1 interaction and protected from disease. Importantly, RSV G peptide vaccination was not associated with pulmonary eosinophil infiltration or immunopathology from RSV challenge. These studies suggest that the RSV G protein nanoparticle approach may provide a new direction for developing novel RSV vaccines that are effective and prevent RSV G protein-mediated immune modulation and disease pathogenesis.

The N protein is critical for viral replication. A recombinant N protein mucosal vaccine composed of several N subunits adjuvanted with bacterial RNA protected against virus replication upon RSV challenge in adult mice. However, it did not prevent pulmonary inflammation and other signs associated with severe disease when tested in BALB/c neonates [53]. The study results suggest that safety and efficacy of RSV vaccine candidates should be tested not only in the adult mouse model but also in a more sensitive neonatal model.

FINAL REMARKS

Despite recent progress, several challenges in developing an RSV vaccine exist. Natural infection induces only partial protective immunity. A successful vaccine candidate will need to induce a more robust and long-lasting protective immune response than that induced by natural infection [54]. Also, the vaccine must avoid induction of T-cell–mediated enhanced disease. Several target populations are being considered for RSV vaccination, including RSV naive infants, older children (aged >6 months), pregnant women, and the elderly. Each target population poses a unique challenge for vaccine development.

Immunological immaturity, maternal antibody interference, risk of enhanced disease, and susceptibility to severe complications from infection are challenges to vaccine development in the RSV naive infant. While a successful vaccine candidate would ideally induce a better immune response than natural infection, for infants a vaccine that induces immunity equivalent to natural infection may be effective in reducing disease burden. The older child, aged >6 months, has a more mature immune system, has less interference by maternal antibody, and is less susceptible but still is at risk from enhanced disease from nonlive virus vaccines. Live-attenuated vaccines will likely be more suitable for young children with no previous RSV immunity. Vaccines for pregnant women face the challenge of inducing a robust immune response in the face of immunity induced by multiple previous natural infections and general concern regarding vaccinating the pregnant women. Immunosenescence and preexisting immunity are challenges for RSV vaccination in the elderly population. Numerous studies have demonstrated multiple defects in the innate and adaptive immunity as individuals age [55].

An ideal RSV vaccine would be immunogenic, safe, well tolerated, and target both A and B strains of RSV. Vaccine candidates for the prevention of RSV would benefit public health by not only providing protection for those immunized but possibly also by decreasing spread to at-risk populations by improving herd immunity. Current advances in therapeutic and vaccine development could open new opportunities in control of RSV disease.

Notes

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed. The results shown in this paper have not been presented in any meetings.

References


