Research Progress in Respiratory Syncytial Virus Vaccines

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Abstract. In addition to being a substantial source of morbidity and mortality in older persons, respiratory syncytial virus (RSV) is a major contributor to lower respiratory tract infections in newborns and young children. Worldwide, there has been a lot of research done to provide safe and effective vaccinations against this common infection because there isn't an approved RSV vaccine. A succinct summary of current developments in RSV vaccine research is given in this abstract. Many vaccinations have reached the clinical testing stage and shown promising benefits. Live attenuated vaccines, nanoparticle vaccines, and viral vector vaccines are the three primary categories of current vaccination techniques. Additionally, the production of specific RSV antibodies such as MEDI8897 and polyclonal immunoglobulin RI-002 is being evaluated as an important research antibodies for avoiding RSV infection. The continuous dedication to RSV vaccine development gives optimism for the future prevention of RSV-related disorders across all age groups, despite the fact that challenges still exist, particularly ERD (Enhanced respiratory disease).

Keywords: RSV, Disease, Pathogenicity.

1. Introduction

Respiratory syncytial virus (RSV) belongs to the positive lung virus genus of the Pneumoviridae family, and is an enveloped, non-segmented, negative RNA virus. RSV infection can cause seasonal respiratory infections worldwide, with clinical manifestations ranging from mild upper respiratory tract infections to severe pneumonia, with severe cases leading to death. Whether in developing or developed countries, there are a large number of hospitalizations and deaths caused by RSV infection every year, mainly affecting infants and young children, immunocompromised populations, and the elderly. RSV infection is particularly common in children, and RSV is one of the main pathogens causing acute lower respiratory infection (ALRI) in children under 5 years old worldwide [1].

As for the molecular biology characteristics of RSV, the RSV genome size is approximately 15-16kb, encoding 11 proteins, including 8 structural proteins and 3 non-structural proteins (NS1, NS2, and M2-2). The structural proteins include 3 transmembrane surface proteins (G, F, SH), 2 matrix proteins (M and M2-1), and 3 nuclear shell proteins (L, N, and P). The G protein mediates the binding of the virus to the host cell, while the F protein mediates the fusion of the virus and the host cell membrane, allowing the virus to enter the cell. Both are crucial for virus replication and contain B and T cell epitopes. They are the most important viral antigen proteins that stimulate the body to produce humoral and cellular immunity. The coding region of G protein has significant variation, and can be divided into subtypes A and B based on its variation. Neutralizing antibodies induced by G protein have subtype specificity [2]; The coding region of the F protein is highly conserved, and the amino acid sequences of the F protein in subtypes A and B are at least 90% the same. Therefore, neutralizing antibodies induced by the F protein can simultaneously inhibit RSV infection in subtypes A and B. The structure of F protein exhibits dynamic changes, first transcribed and translated into a single inactive peptide (F0) in the host cell; Then, through the first cleavage of the host cell furin protease, a partially cleaved fusion precursor protein (pre-fusogenic protein) is generated; Subsequently, the second cleavage of Flynn protease occurred, generating F2 and F1 subunits, which were linked to a single monomer by two disulfide covalent bonds. Then, the three monomers formed a metastable functional pre-fusion protein trimer; Afterwards, conformational rearrangement can be carried out without further processing to form a thermodynamically stable fusion protein (postfusion protein); The time and cellular location of the two cleavage and conformational rearrangements of Flynn protease are not fully understood, and the conditions for inducing conformational
rearrangements are also unclear. On the surface of F protein, there are mainly four types of antigenic epitopes related to neutralizing activity: II, IV, VIII, and Ø. Among them, epitopes II and IV exist in F protein before and after fusion; VIII and Ø are specific antigen sites for the pre fusion F protein, but the post fusion F protein does not have these two epitopes. Epidemiological studies have shown that RSV neutralizing antibodies can prevent severe RSV-ALRI. The neutralizing activity of epitope A monoclonal antibody is 10-100 times higher than that of epitope II monoclonal antibody, and the neutralizing activity of epitope VIII monoclonal antibody is also high. Therefore, most RSV neutralizing activity in serum only targets the pre fusion F protein antigen site. Inducing high titer neutralizing antibodies is the main goal of developing RSV vaccines, and the fusion pre-F protein with specific antigen epitopes has become the most popular RSV vaccine target. However, the fusion pre-F protein is essentially an unstable protein, and making multiple stability modifications without losing important antigen epitopes is one of the difficulties in RSV vaccine development [3].

2. Immunoprophylaxis for RSV infection

The purpose of WHO's prevention and control of RSV infection is to prevent serious RSV infectious diseases in high-risk groups, and reduce the hospitalization rate and mortality. The most important thing is to protect vulnerable infants and young children. Therefore, WHO has identified two priority research and development directions: (1) prioritizing the development of maternal immune vaccines or long-acting monoclonal antibodies during pregnancy to prevent severe RSV related diseases in newborns and infants within 6 months of age; (2) Priority should be given to developing children's immune vaccines. Once the protection provided by maternal antibodies weakens, infants and young children (over 6 months old) should be vaccinated. The priority development direction includes two measures, namely, active immunization by vaccines, passive immunization by improving the level of maternal antibodies or by giving exogenous antibodies, to focus on protecting infants from serious RSV infectious diseases.

2.1. Active Immune Prevention

The ideal RSV vaccine needs to induce high titer antibodies targeting multiple neutralizing sites and minimize the possibility of inducing mutated strains. It is not yet clear whether the optimal preventive effect requires simultaneously inducing serum IgG, mucosal IgA, and cellular immunity. Safety and immunogenicity are the main challenges faced by RSV vaccine development. Among them, the most serious safety issue is that the inactivated formalin vaccine may lead to enhanced RSV infection related respiratory diseases in RSV serum negative children(Enhanced respiratory disease, ERD), this may be due to the inactivation conditions of formalin not being able to stabilize the F protein in its pre-fusion conformation, resulting in the loss of specific antigen epitopes of the pre-fusion F protein and the inability to induce the production of effective neutralizing antibodies, leading to a series of subsequent reactions that can lead to severe disease. The assessment of immunogenicity is also influenced by factors such as the immune status of the recipient, such as the immature immune system of young infants and the possibility of maternal anti-RSV antibodies inhibiting the body's immune response to the vaccine; Elderly people have low immunity and low immune response to vaccines [4].

2.1.1 Live attenuated vaccine

RSV attenuated live vaccine under development is the use of reverse genetics to insert predetermined mutations (attenuated point mutations or deletions of non-essential genes) into live RSV through cloned cDNA, to obtain attenuated and highly immunogenic live RSV strains. It is generally believed that the virus in attenuated live vaccines can replicate, simulate natural infection, and do not cause ERD, restoring to the wild-type. The possibility of illness is also very low, and the safety is high. In addition, live attenuated vaccines also have other advantages: the nasal drip vaccination method is easily accepted by children and has good tolerance; When maternal antibodies exist, they can still replicate in the upper respiratory tract, and the successful vaccination rate is high
in young infants; It can widely stimulate the inherent immunity, humoral and cellular immunity of the local and systemic respiratory tract, and can prevent upper and lower respiratory tract infections. Therefore, for serum negative children, attenuated live RSV vaccine is the most suitable candidate vaccine. The biggest challenge faced by the development of such vaccines is balancing attenuation and immunogenicity. Live viruses have unstable characteristics and have more complex requirements for vaccine production, storage, and transportation. The two most important methods for constructing attenuated RSV live vaccines are to remove the M2-2 and NS2 genes of the virus. Removing the M2-2 gene can inhibit RSV replication, fully attenuate the virus, and increase the expression of F and G proteins, enhancing immunogenicity. After the NS2 gene deletion, the RSV replication ability decreases, but the stimulation of the body to produce interferon levels increases, resulting in enhanced innate immunity. Candidate vaccine LID/Δ M2-2/1030s not only removed the M2-2 gene, but also inserted a temperature sensitive phenotype point mutation of 1030s. The Phase I clinical trial was conducted in serum negative infants and young children aged 6 to 24 months, and the results showed good genetic stability and could induce the body to produce persistent neutralizing antibodies and memory B cells.

2.1.2 Virus vector vaccine

The viral vector vaccine uses viruses as vectors to express RSV antigens (such as F, G, N, M2-1, etc.), and the adjuvant effect of the vector enhances the immunogenicity of the vaccine. The N and M2-1 proteins are rich in T cell recognition sites, which can enhance T cell mediated immune response. Similar to live attenuated vaccines, viral vector vaccines can stimulate the body to produce humoral, cellular, and innate immunity, including respiratory mucosal IgA antibodies. The most commonly used vector is adenovirus. Inoculate newborn and adult mice with RSV vector vaccines, and no signs of ERD were observed. The virus vector vaccine is also suitable for vaccination in serum negative children over 6 months old. However, the presence of anti-vector immunity in the host body may pose challenges for the clinical application of these vaccines. Ad26. RSV. Pre-F, using human adenovirus 26 as a vector, can express a conformational stable fusion pre-F protein and induce the production of high titer neutralizing antibodies targeting the antigenic epitopes of the fusion preF protein. The target population is the elderly and children. Phase I and Phase II clinical trials have been completed in healthy elderly individuals, and only one case (in Phase II clinical trials) of vaccine related serious adverse reactions (hypertension crisis and bradycardia) was observed, with acceptable safety. A single immunization can cause a continuous 2-year humoral and cellular immune response.

During the Phase II clinical evaluation, there was no mutual interference between the immune responses of the two vaccines when administered in combination with the seasonal influenza vaccine. The effect of Ad26. RSV. PreF vaccination on RSV serum negative children, serum positive children, and healthy adults is yet to be clinically evaluated. Another adenovirus vector vaccine, ChAd155-RSV, uses chimpanzee adenovirus 155 as the vector to express the F, N, and M2-1 proteins of RSV. Phase I clinical trial results showed that when healthy adults were vaccinated for 30 days, the geometric average titer of neutralizing antibodies was 2.6 times higher than before vaccination, and they secreted F protein specific interfering factors γ the median T cell count was 3.1 times higher than before vaccination, and no serious adverse reactions such as termination or death were observed, indicating good immunogenicity and acceptable safety. There are also some virus vector candidates for RSV vaccines using other viruses as vectors [3]. The vaccine MVA-BN-RSV uses an improved Ankara vaccinia virus (MVA) as a vector to express the F, G, N, and M2-1 proteins of RSV, targeting elderly individuals. The results of the Phase II clinical trial showed that the most common adverse reactions of MVA-BN-RSV were local injection pain, no serious adverse reactions, and one dose could induce cellular and humoral immune responses that lasted for at least 6 months [5]; After 12 months of initial immunization, strengthening one dose of vaccination can enhance humoral and cellular immunity, with serum IgA being the most significantly enhanced in humoral immunity. At 2 weeks of booster vaccination, the geometric mean increases (GMFI) of serum IgA increased by 2.1 times compared to before booster vaccination. The immune response of peripheral blood T cells stimulated by M2 protein was the most significantly enhanced in cellular immunity, and the GMFI
increased by 2.8 times compared to before booster vaccination. The vaccine Medi-534 uses bovine/human chimeric parainfluenza virus 3 as a vector to express RSV-F protein, with the expectation of inducing immune responses against both viruses simultaneously.

2.1.3 Nanoparticle vaccine

Nanoparticle vaccines are composed of self-assembled nanoparticle antigens with high copy number RSV antigen proteins on the surface, which have good immunogenicity. Adding adjuvants can further enhance immunogenicity and target antigen presentation. The target population for vaccination includes children over 6 months old, pregnant women, and the elderly. The RSV F nanoparticle vaccine consists of a fusion precursor protein oligomer obtained by introducing a mutation at the cleavage site II of the Flynn protease. Studies have shown that the fusion precursor protein can induce high affinity antibodies targeting four anti in situ sites II, IV, VIII, and Ø, and can resist infection by Pareto strain monoclonal antibody resistant mutant strains [6]. The Phase I and Phase II clinical trials of RSV F nanoparticle vaccine showed that it had good safety and immunogenicity. It was the first to enter the Phase III clinical evaluation for pregnant women and the elderly, but it did not reach the expected goal of reducing the incidence rate of RSV-ALRI. However, this vaccine shows other trends in efficacy: if pregnant women receive the aluminum phosphate adjuvant type RSV F nanoparticles vaccine, their offspring have a reduced hospitalization rate for RSV related ALRI within 90 days of birth, with a vaccine efficacy of 44.4%. The elderly receiving adjuvant free RSV F nanoparticles vaccine reduced the hospitalization rate related to the deterioration of chronic obstructive pulmonary disease by 61%. Therefore, the protective effect of RSV F nanoparticles vaccine still deserves further research. Recent studies have shown that elderly people receiving two doses of adjuvant-containing (aluminum phosphate or Matrix M1) RSVF nanoparticle vaccines can induce a high-intensity and long-lasting immune response, with good tolerance [7]. Another nanoparticle vaccine that has entered clinical evaluation is SynGEM, which uses bacterial like particles (BLP) derived from Lactococcus lactis as adjuvants and carriers to express recombinant fusion pre-F protein and is administered intranasal. The Phase I clinical trial showed good tolerance, with induced humoral immunity lasting for at least 6 months, but no epitope specific antibodies were detected, possibly due to unstable conformation before fusion of F protein [3].

2.2. Passive Immune Prevention

Specific antibodies against RSV are not only the research direction of RSV infection treatment drugs, but also an important research and development strategy for preventing RSV infection, and products for prevention have been launched.

2.2.1 Monoclonal Antibodies

Palizumab targeting the F protein epitope II of RSV is the first specific monoclonal antibody used to prevent RSV related ALRI. It was approved for clinical use by the Food and Drug Administration (FDA) as early as 1998. Generally, starting one month before the outbreak of local RSV infection, Palizumab is administered intramuscularly once a month at a dose of 15mg/kg, up to a maximum of 5 times. Palizumab requires multiple injections, which affects acceptance and is expensive, and is only recommended for use in high-risk infants and children. The use of monoclonal antibodies targeting a single epitope may induce immune escape mutant strains. Mutant strains resistant to Pareto monoclonal antibodies have been detected in countries such as the United States, Japan, and Canada, and there is an increasing trend of prevalence, which undoubtedly adds new difficulties to the prevention of RSV. It should be noted that previous studies have shown that palizumab is ineffective in the treatment of RSV infection [8].

2.2.2 Polyclonal Immunoglobulin

The use of polyclonal immunoglobulins to prevent RSV infection can be traced back to the 1990s. In 48 month old children with high risk factors of RSV infection such as congenital heart disease, bronchopulmonary dysplasia, or premature delivery (≤ 35 weeks), before the RSV epidemic season,
the use of RSV intravenous immunoglobulin (IVIG) [9], 750mg/kg per time, once a month, generally 3-5 times, significantly reduced the incidence rate of RSV-ALRI (8.6% in the treatment group, 22.5% in the control group) and the hospitalization rate (7.4% in the treatment group, 20.2% in the control group). And the severity of RSV-ALRI was reduced. Compared with the control group, the treatment group had a 63% reduction in total hospital stay and a 97% reduction in intensive care days. But with the launch of Palizumab, RSV-IVIG withdrew from the market. In recent years, people have gradually realized the limitations of Pali monoclonal antibodies such as poor acceptance, high price, and induced resistance, and polyclonal immunoglobulins have once again received attention. Polyclonal immunoglobulin RI-002 contains neutralizing antibodies against multiple RSV antigens and other respiratory pathogens, and its neutralizing antibody titers against RSV-F and RSV-G are at least 1.5 times higher than ordinary commercial IVIG [10].

3. Conclusion

RSV infection poses a serious threat to human health, and the development of vaccines and antibody formulations is of great significance in preventing RSV infection and reducing severe and fatal cases. With the deepening understanding of the molecular structure of RSV and the immune response of the body to RSV, the research and development of RSV vaccines is constantly advancing. Currently, more than 30 candidate vaccines have entered the clinical trial stage. Different types of RSV vaccines should be developed for different high-risk infected populations. The four major categories of RSV candidate vaccines currently under development have their own advantages and limitations, and ensuring the safety and immunogenicity of vaccines is the main challenge faced by research and development. One of the vaccines has entered phase III clinical trials, but has not achieved the expected results and further evaluation is needed. The long-term monoclonal antibody MEDI8897, which is more cost-effective than Palizumab, has entered phase III clinical trials and has obtained priority research and development qualifications, with broader application prospects. Polyclonal immunoglobulin RI-002 has shown good preventive effects in immunocompromised populations with low costs and practical significance for development and application.

References