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Respiratory Syncytial Virus Vaccine Approaches: a Current Overview

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Abstract

Purpose of Review—Respiratory syncytial virus (RSV) is a global human pathogen responsible for lower respiratory tract infections (LRTI). While RSV infection is innocuous in healthy adults, it is the leading cause of infant hospitalization for respiratory tract infection. Nearly everyone shows evidence of an RSV infection by the age of 3. However, there is still not a vaccine commercially available. This review will provide an update on the clinical and preclinical vaccine studies and different approaches to prevent RSV infection.

Recent Findings—Novel vaccine approaches that induce protection against RSV without enhancement of respiratory tract disease.

Summary—Recent technological approaches have led to generation of different strategies to prevent RSV infection, including live attenuated, chimeric, and subunit vaccines, virus-like particles, and nanoparticles. These vaccine approaches represent promising candidates towards an efficient RSV vaccine that effectively protects infants, children, and adults.

Keywords

Respiratory syncytial virus; Paramyxovirus; Vaccine

Introduction

Human respiratory syncytial virus (RSV) is an enveloped, negative sense, single stranded RNA virus as part of the Paramyxoviridae family [1–3]. Its genome of 15.2 kb, encodes 11 proteins including the attachment (G), small hydrophobic (SH), fusion (F), nucleoprotein (N), phosphoprotein (P), polymerase (L), matrix proteins (M1, M2-1, M2-2), and two non-structural proteins (NS1, NS2) [2, 3] (Fig. 1a, b). RSV was originally isolated in 1956 as chimpanzee coryza agent [4] and it was further identified in infants 1 year later [5]. RSV has been identified as the leading cause of epidemic lower respiratory tract infections (LRTI) in

Compliance with Ethical Standards

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infants and children. RSV is a major cause of bronchiolitis and pneumonia estimated to cause more than 30 million new cases of lower respiratory tract illness in children younger than 5 years, with 3–4 million hospitalizations, and 66,000–200,000 fatal outcomes with more than 95% of these deaths occurring in developing countries [6–8]. By the age of 3, virtually all children have been infected by RSV [9]. Other high-risk populations for RSV infection include the elderly and immunecompromised individuals [7, 10]. Reinfection by the same and different strains of RSV are common throughout the lifetime of adults, but usually asymptomatic [1]. Failure of RSV to stimulate a lasting immune response is still a popular puzzle for basic research, and it makes designing an efficacious vaccine very challenging.

Since its identification, 60 years ago, RSV has been a priority for vaccine research in order to reduce the lower respiratory tract morbidity in the risk populations. The first RSV vaccine tested in a clinical trial, back in 1966, consisted in a formalin inactivated virus (FI-RSV) from the Bernett strain, which was administered in 3 doses to 23 infants between 2 and 7 months of age [11]. Previous potency and safety tests executed in guinea pigs, cynomologous monkeys, rabbits, and mice did produce satisfactory results. However, the FI-RSV vaccine had a disastrous conclusion since it failed to induce an adequate response of neutralizing antibodies and resistance to infection. Furthermore, immunized children developed a more severe pulmonary disease and peribronchiolar infiltration of eosinophils that led to the death of two infants aged 14 and 16 months, following natural infection with RSV [11].

The most common vaccine target for RSV is F protein (Fig. 1a), which is a highly conserved envelope protein across different RSV subgroups A and B [12] (Fig. 1c). Furthermore, F protein has been identified as the primary antigen target of neutralizing antibodies with multiple specific peptide sequences having been identified [13–16]. More recently the structural conformation of F protein has been examined for antigenic peptides. McLellan et al. identified pre-fusion F protein as having the majority of epitopes for neutralizing antibodies [15]. Structural conformation of F protein has started to take a contribution to modern vaccine design, hopefully leading to more efficacious vaccines.

The inability of the virus to trigger a protective memory has also made several vaccines ineffective at preventing disease. Therefore, following the failure of the FI-RSV vaccine trial, numerous new RSV vaccine candidates have been designed. This brief overview highlights the current advances in the different approaches including live attenuated vaccines, chimeric vaccines, subunit vaccines, virus-like particles vaccines, and nanoparticles vaccines, which show promise towards protecting against RSV infection.

Live Attenuated Virus Vaccines

Initial development of RSV live attenuated vaccines included in vitro serial passages at a temperature of 26 °C [17]. Among the advantages found in the live attenuated vaccines is that unlike FI-RSV, they do not cause an enhanced disease severity after exposure to natural RSV infection. In these vaccine candidates, it is critical to have a balance between an attenuated viral replication and an induction of an effective immunity. A recent example of

live attenuated vaccine demonstrated that removing the CX3C motif of G protein can improve vaccine safety by polarizing the T helper response towards a Th1 response and away from a Th2 response associated with enhanced respiratory disease (ERD) [18]. However, there are some concerns about how the genetic stability of genetic attenuation of live vaccines will hold up against strong selective pressure. To test this, phenotypical analysis has been performed in serial passages of codon-deoptimized live attenuated viruses through Vero cells. The results indicate that specific open reading frames (ORFs) are less stable than others [19]. This effect raises concerns about whether the ORF conserves the sequence responsible for attenuation without other random and potentially compensatory mutations emphasizing another aspect of safety testing necessary for live attenuated vaccine candidates. As long as the virus is replication proficient, it is subject to selective pressures. Safety is an especially serious concern in vaccines that target vulnerable populations, including infants. Special consideration was given to this concern in a recently designed severely attenuated live vaccine candidate, OE5. It used a strategy of codon deoptimization of NS1, NS2, and G proteins and completely removed SH protein [20]. This strategy severely limits the replication of the virus while maintaining the F protein intact, the strongest target protein for neutralizing antibodies. The OE5 vaccine stimulated a protective antibody response up to 100 days post infection as demonstrated in BALB/c mice and cotton rats without triggering ERD [20]. Thus, OE5 represents a promising safe and effective vaccine candidate.

Chimeric Vaccines

One of the challenges of a live attenuated vaccine is the inability of natural RSV infection to create a lasting protective memory response. There are multiple epitopes particularly on the F and G proteins capable of being recognized by the adaptive immune response, but protection wanes after viral clearance as infants can be sequentially infected by the same subgroup during a single RSV season. To surmount this obstacle, several research groups created recombinant chimeras expressing immunogenic RSV proteins. A recent example of this is the RSV N gene expressing Bacillus Calmette-Guerin (BCG) vaccine [21]. The BCG vaccine is a live bacteria adapted from *Mycobacterium bovis* to prevent tuberculosis. Unlike RSV, BCG is capable of triggering a lasting immunity, particularly a T cell response, opposed to a B cell response. The study demonstrates that attaching RSV N gene to BCG was effective at stimulating a lasting CD4+ and CD8+ T cell response and was protective against RSV infection in mice [21]. Concerns exist because BCG is a live vaccine and not approved for immunecompromised individuals, one of the main populations at serious risk of major disease from RSV infection. Another recent example of a chimeric RSV vaccine is a Sendai virus (SeV) chimera. SeV is a member of the paramyxoviridae family that primarily infects mice and other rodents. Wiegand et al. used a replication deficient SeV as the backbone of their vaccine. Then they removed the F protein of SeV and replaced it with the F protein of RSV [22]. The replication deficient SeV provides a safe vector that carries RSV F protein as an essential structural component. The vaccine stimulates a T cell and antibody response through both an intranasal and an intramuscular immunization [22].

Potentially, the most promising chimeric vaccines are parainfluenza 5 virus (PIV5) and RSV chimeras. Parainfluenza virus (PIV) is the second most common cause of respiratory tract

infection hospitalization in children. Two PIV5 chimeras expressing RSV F or RSV G protein were used to inoculate cotton rats and African green monkeys [23]. Although both chimeras fully protected naive animals from RSV infection, the RSV F protein chimera (PIV5/F) stimulated a higher antibody titer in African Green monkeys than RSV G protein (PIV5/G) [23]. Also, they both boosted antibody levels in RSV pre-exposed African Green monkeys, suggesting that the PIV5/F vaccine could be helpful to naïve babies and pre-exposed elderly people [23]. Furthermore, genetic stability tests demonstrated that while random mutations were present in both in vivo (African green monkeys) and in vitro (Vero cells) passages, none of those mutations abolished antigen expression [24]. While genetic instability is possibly the biggest concern in using RNA viruses as chimeras, this insertion of RSV F protein in between PIV5 HN and L genes appears to be stable. A further improved chimeric RSV vaccine candidate included a pre-fusion F protein in a PIV5 backbone. The vaccine was protective against RSV in mice and cotton rats [25]. Using a similar approach, a parainfluenza 1 (PIV1) vectored vaccine was designed using F protein stabilized in its pre-conformation structure either with RSV F protein or with RSV F protein with the transmembrane and cytoplasmic tail domains replaced by PIV1 analogs [26]. This vaccine was able to elicit neutralizing antibodies similar to wild-type RSV infection and conferred protection against RSV challenge [26]. Similarly, a chimeric vaccine developed from recombinant human and bovine parainfluenza 3 (PIV3) with RSV F protein stabilized in its pre-fusion conformation was codon optimized to increase immunogenicity in hamsters and in vivo replication to increase neutralizing antibody titers [27].

Subunit Vaccines

Subunit vaccines are inoculations that include only a single protein, protein complex, or even an isolated peptide with or without an adjuvant. The goal of this vaccine approach is to promote a memory response to the viral protein without the danger of introducing a live pathogen or the risk of enhanced respiratory disease, most commonly associated with inactivated virus (FI-RSV). Using a cotton rat experimental model, a recent study found that at high doses, the RSV F protein subunit vaccine did not cause ERD. However, low doses caused ERD with and without an adjuvant [28], raising concerns about the dose and safety in vaccinated individuals.

Because of safety concerns using the whole protein in this type of immunization, subunit vaccines targeting specific peptides have become a new area of development with mixed response. An F protein peptide adjuvanted with poly I:C, a synthetic analog of double stranded RNA, was successful at conferring complete long-term (5 months) protection in mice after a single i.n. vaccination [29]. In comparison with that success, a lipid core peptide vaccine created a very strong antibody response, but those antibodies failed to bind natural F proteins or offer any protection against RSV challenge [30]. These two cases highlight the importance of testing vaccines against challenge protection rather than measuring antibody titers, particularly in the case of peptide subunit vaccines.

The RSV attachment glycoprotein (G) is another typical candidate to induce protective immunity against RSV. A successful preclinical study demonstrated that intramuscular administration of unglycosylated G protein was not associated with ERD and created a

protective immune response to RSV [31]. However, another concern with developing a RSV vaccine is strain differences. Most laboratories use RSV A2 laboratory-adapted strain, but infants can be exposed to more than one RSV strain. Thus, an effective vaccine will need to provide protection against a broad spectrum of RSV strains across both A and B subtypes. By creating a recombinant G protein to combine A and B RSV subtypes (GcfAB), Lee J-Y and colleagues show that mice vaccinated via intranasal and sublingual routes induced a strong humoral response against both RSV subtypes but enhanced lung pathology in the RSV-infected animals [32].

Virus-Like Particles (VLP)

Virus-like particles (VLP) are small replication deficient groups of assembled viral proteins without any genetic material. A recent example of this approach is a construction of recombinant matrix protein (M) and fusion glycoprotein (F) [33, 34]. Cai et al. generated VLPs with influenza virus (IFV) M1 matrix protein and RSV F or RSV G protein. Both the F and G vaccines were effective at creating specific antibodies against RSV and protected mice against RSV challenge [35]. There is some debate over using either pre- or post-fusion F protein conformation. Several recent VLP approaches included the matrix (M) protein from RSV [33], HMPV [34], and NDV [36] and both pre- and post-fusion RSV F protein [33, 34, 36]. In all cases, the VLPs were immunogenic and triggered a desirable Th1 adaptive immune response [33, 34, 36]. Although pre-fusion RSV F protein had a higher neutralizing antibody response than post-fusion RSV F protein, a combination of pre- and post-fusion F proteins produced the strongest Th1 response with the highest proportion of IgG2 antibodies [34].

VLPs are also able to polarize the immune response towards a Th1 response after a booster vaccination with formalin inactivated RSV, suggesting that VLPs can be effective vaccines, despite previous damaging vaccination history [37]. During a challenge with live RSV (A2 strain), the VLP vaccinated mice were more protected than unvaccinated mice, but not more than mice previously infected with live RSV [37]. Future work will need to explore improving the level of protection provided by the VLP vaccine so that exceeds the inadequate protection of natural RSV infection.

Micro and Nanoparticles

The size of a micro or nanoparticle impacts how the antigen is transported to the draining lymph node. Small nanoparticles (2–200 nm) can freely drain to the lymph nodes. Larger nanoparticles (> 500 nm) will need to be phagocytosed inside a dendritic cell. The benefit of larger nanoparticles is that they stay in the lymph nodes longer. Being phagocytosed inside an antigen presenting cell allows the larger nanoparticles to remain in the lymph nodes longer than the smaller nanoparticles (reviewed in [38]). A recent preclinical trial targeted the N protein of RSV, loaded with nanorings and delivered by a transdermal vaccine delivery approach [39]. The patches were loaded with RSV N-nanorings (Viaskin®-N). The epicutaneous delivery of the RSV N-nanorings was successful at stimulating a protective $CD4^+$ T cell response in both mice and piglets [39]. RSV N gene is an uncommon target

protein, but a potential target depending on the epitope variability with other RSV subtypes and if it can cause ERD.

Another example of RSV nanoparticle vaccine (RSV F vaccine) has progressed to a phase II clinical trial when given to healthy adult women at different dosages, number of doses, and with variable amounts of adjuvant [40]. The vaccinated women were 50% more protected from RSV infections than placebos [40]. The vaccine looks very promising; however, it still needs to be tested on the target population. It is intended for women in their third trimester of pregnancy to bolster antibody levels to cross the placenta to the fetus. Potentially vaccinating mother can protect the infants postpartum for the first month of life. Additionally, mothers who contract RSV have a 57% chance of passing it on to the fetus [41]. Vaccinating expectant mothers can help protect their infants through maternal antibodies. A phase III clinical trial for women in the third trimester of pregnancy is currently ongoing.

One of the particularly interesting aspects of RSV is its inability to create a lasting immunity causing frequent reinfection even by the same strain within a single season. This means that the same RSV vaccine might still be effective across age groups and naiveté. The aforementioned RSV F nanoparticle vaccine for pregnant women [40] has been tested in a phase I clinical trial in elderly people. The trial did not reveal any safety concerns [42]. Subjects also had significantly higher antibody titer 12 months post inoculation, suggesting potential protection [42]. A phase II trial is necessary to discern if the antibody response is protective in elderly people.

Conclusion

The search for an RSV vaccine has been high on the agenda for several decades, due to the high rate of infections in infants, but also other vulnerable populations like immunocompromised adults and elderly people, where RSV poses a significant health risk. Previous vaccine attempts have been hindered by the inability of natural RSV infection to create a lasting memory protection against conserved immunogen epitopes in addition to extremely cautious efforts towards clinical trial after the first disastrous attempt and enhanced respiratory disease after inoculations with some vaccine candidates.

Recent technological advancements have opened doors to new vaccine approaches that will hopefully revolutionize RSV vaccine development (Fig. 1c). Improvements to the precision of gene editing have allowed for more specific live attenuated vaccines, more possible recombinant proteins for subunit vaccines, and an ample variety of possible chimeric vaccines. The development of micro and nanoparticles open up more doors to specific manipulation of the type of adaptive immune response desired. Even varied inoculation routes allow researchers to tailor the desired response to their vaccine [43]. These new vaccine approaches will contribute to finding an effective solution to prevent RSV infection in infants and other target populations.

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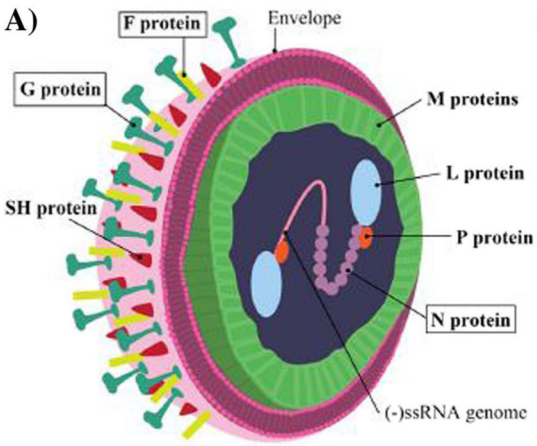

C)

	Protein	Protein Name	Vaccine Target (references)
vaccine target	F	fusion	20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 33, 34, 35, 36, 37, 40, 42
vaccine target	G	attachment	18, 23, 24, 31, 32, 35
vaccine target	N	nucleocapsid	21, 39
	SH	small hydrophobic	
	M	matrix	
	P	phosphoprotein	
	L	large protein-RNA polymerase	
	NS1	non-structural protein 1	
	NS2	non-structural protein 2	
	M2-1	transcription factor	
	M2-2	viral replication	

B)

Fig. 1.

RSV proteins, structure, and genome sequence. **a** A representation of RSV structure. Boxes indicate those major vaccine targets. **b** RSV genome sequence. **c** Summary of RSV proteins, their names, and the corresponding references