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# Hurdles in Vaccine Development against Respiratory Syncytial Virus

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## Abstract

Respiratory syncytial virus (RSV) infection is a major cause of severe respiratory disease in infants and young children worldwide and also forms a serious threat for the elderly. Vaccination could significantly relieve the burden of the RSV disease. However, unfortunately there is no licensed vaccine available so far. This is partly due to disastrous outcome of a clinical trial of formalin-inactivated RSV (FI-RSV) in children in 1960s; leading to enhanced respiratory disease upon natural infection. These findings contributed significantly to the delay of RSV vaccine development. Other key obstacles in development of RSV vaccine such as a peak of severe disease at 2–3 months of age, challenging biochemical behavior of key vaccine antigens and dependence on animal models that may not truly reflect human disease processes. These challenges could be overcome through maternal immunization, structure-based engineering of vaccine antigens, the design of a novel platform for safe infant immunization, and the development of improved animal models. Currently, several vaccine candidates are in pre-clinical and clinical trials targeting the diverse age groups; young children or older adults from the infection or can reduce incidence, mortality and morbidity among the RSV infected individuals.

**Keywords:** respiratory syncytial virus, vaccines, adaptive immune response, adjuvants, animal models, infants, elderly, enhanced respiratory disease, innate immune response

## 1. Introduction

Respiratory syncytial virus (RSV) infection is a major cause of lower respiratory tract diseases among infants, young children and immune-compromised individuals. RSV infection provides partial immunity and reinfection may occur often throughout life. Therefore, RSV infection forms a severe threat in chronically ill adults and the elderly [1]. Current studies have demonstrated that RSV also a main cause of mortality among the elderly, indeed to similar extents as does influenza [2]. Presently, the only approved medication against RSV infection is a prophylactic monoclonal antibody, i.e., Palivizumab, which is given as a prophylaxis to high-risk

Target groups	Considerations	Vaccines approaches
Infants (<6 months)	<b>Goal:</b> Prevent severe complications <b>Challenges:</b> Less developed immune system; more susceptible to disease; FI-RSV enhanced respiratory disease history, maternal Abs present	1. Live-attenuated vaccines 2. Gene based vectors 3. Virus chimeric vectors
Children (6–24 months old)	<b>Goal:</b> Prevent severe complications <b>Challenges:</b> To achieve the clinical end point is not easy; FI-RSV enhanced respiratory disease history	4. Gene based vectors 5. Live-attenuated vaccines 6. Virus chimeric vectors
Elderly people (>65 years)	<b>Goal:</b> Po provide protection from infections and complications <b>Challenges:</b> Lot of previous infections can decrease response to vaccine; necessary to boost up protection provided by natural infection; absence of indicators for severity of disease, diagnosis difficult	7. Subunit proteins having adjuvant 8. Gene based vectors having subunit proteins 9. Vaccines including virus like particles with adjuvants
Pregnant women	<b>Goal:</b> To prevent transmission from mother to infants, maximize the protection of infants <b>Challenges:</b> Lot of previous infections can decrease response to vaccine, necessary to boost up the antibody level for protection of infants	10. Subunit proteins in combination with standard adjuvants 11. Vaccines having virus like particles with adjuvants

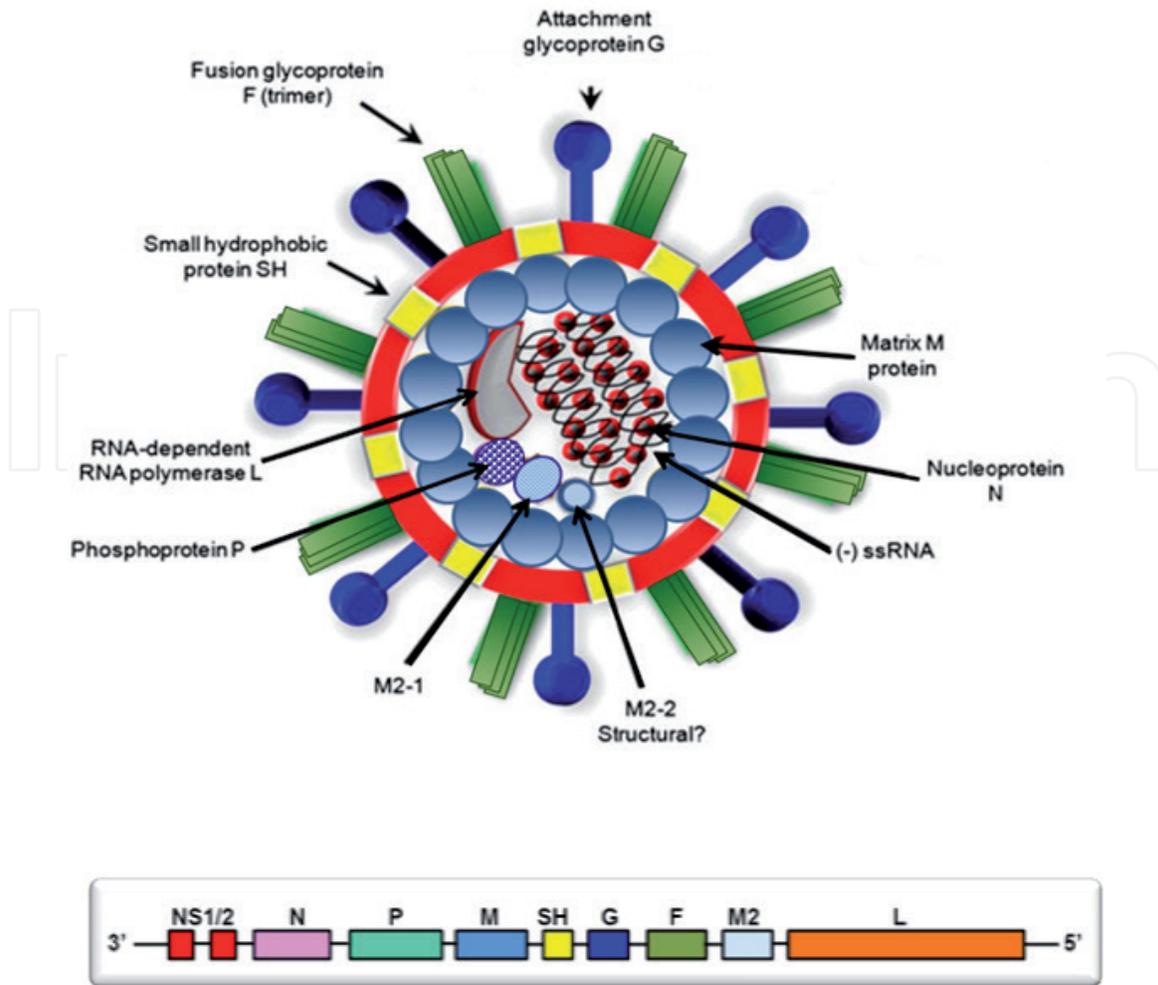
**Table 1.**  
Key target groups for vaccine candidates.

infants [3]. Despite the isolation and characterization of the virus in 1956, efforts to develop a safe vaccine have been unsuccessful so far. In a clinical trial conducted in young children in the 1960s, a formalin-inactivated RSV (FI-RSV) vaccine did not protect against infection rather led to enhanced respiratory disease (ERD) upon subsequent exposure of the vaccinees to the natural virus. These findings that inactivated RSV vaccines may prime for ERD has contributed significantly to the delay of vaccine development. Other major challenges for development of RSV vaccine are a disease severity at 2–3 months of age, challenging biochemical behavior of key vaccine antigens and dependence on animal models that may not exactly mimic human disease processes. These challenges could be overcome through maternal immunization, structure-based engineering of vaccine antigens, the design of a novel strategy for safe immunization of infants, and the development of better animal models (Table 1).

## 2. Respiratory syncytial virus

RSV is an enveloped non-segmented negative-sense single-stranded (ss) RNA virus belonging to the *Orthopneumovirus* genus and *Pneumoviridae* family [4]. Two serotypes of RSV have been recognized i.e., RSV A and RSV B [4].

The RSV genome comprises 10 genes of 15.2 kb nucleotides encodes 11 proteins [5]. RSV comprises of a nucleocapsid enclosed by a lipid envelope with a diameter of 150–300 nm (Figure 1: RSV particle and RSV-genome). RSV expresses two non-structural proteins such as NS1 and NS2. These are detected only in RSV-infected cells and are not packaged into the virion. They mainly serve to inhibit type I interferon responses [7]. Eight RSV proteins are present in the virion particles. Among these structural proteins, three are membrane proteins: the attachment protein G, the fusion protein F and the small hydrophobic protein (SH). The heavily



**Figure 1.**  
 Schematic representation of the RSV virion and its genome composition (adapted and modified with permission from [6]).

glycosylated G protein is responsible for viral attachment to the cell. The F protein not only contributes to binding of the virus to cells, but also plays a crucial part during entry of virus by mediating fusion of the viral envelope with the cell membrane, thereby allowing deposition of the viral genome into the cytosol [8]. Besides this, F protein is a mediator of syncytium formation [5]. The function of the SH protein, which is mostly found in the infected-cell membrane, is unknown [9]. Other viral structural proteins are the nucleocapsid protein (N), the matrix protein (M), the phosphoprotein (P), the RNA-dependent RNA polymerase (L) and the M2 gene product M2-1: all these proteins are located inside the viral particle. Whereas the M2-2 gene product is packaged in the virion is currently unknown [10]. The function of the matrix (M) protein is to connect the viral nucleocapsid with the lipid envelopes and it is also responsible for viral particle assembly. The M2-2 protein is involved in regulation of viral transcription [11]. M2-1 functions as transcription-elongation factor [12]. The phosphoprotein (P) and nucleocapsid protein (N) are essential for transcriptional activity, while the L protein has RNA polymerase activity.

### 3. Epidemiology

RSV infections have a distribution worldwide. These infections are more common during the winter season in temperate climates. However, RSV infections may occur throughout the year in tropical climates, but can be more frequent in the

monsoon season in some areas [13–15]. Although infection can be established in several laboratory animals, however, natural infection with RSV appears to be limited to apes and humans [16]. RSV transmission occurs via direct contact or contact with contaminated surfaces that harbor respiratory secretions. The virus can survive for many hours on toys or other substances, which explains the high rate of nosocomial RSV infections particularly in pediatric wards. The incubation period for RSV infection ranges between 2 and 7 days [17]. Almost 70% of newborns are infected in the first year of their life. By the age of 2 years, almost all children have been infected and over 50% will have been infected twice [18]. RSV infections are common in the population and re-infections probably occur frequently. In a study conducted by Hall et al., 1991, almost 25% of adult volunteers could be re-infected with RSV of the same group, 2 months after a natural infection [19].

RSV infection is the major source of severe respiratory illness in infants and young children and is the most frequent cause of hospitalization of infants and young children in industrialized countries [20]. RSV infections differ in disease severity, from a mild cold to bronchiolitis or pneumonia. Almost 3% of infants infected with RSV need hospitalization due to respiratory failure and feeding problems [21]. Among the hospitalized infants, 20% need mechanical ventilation [22]. The highest morbidity of RSV disease is seen in children under the age of 6 months [23] and in children with associated risk factors such as prematurity, broncho-pulmonary dysplasia, congenital heart disease with increased pulmonary circulation or immune deficiency [24–27]. According to WHO estimates, RSV disease burden is ~64 million cases and 160,000 deaths per year worldwide. In USA, about 85,000–144,000 infants are admitted to hospitals with RSV infection annually which corresponds to 20–25% of pneumonia cases and about 70% bronchiolitis cases in the hospital [28, 29]. The elderly people are also at risk for extreme RSV disease and almost 14,000–62,000 RSV-associated hospitalizations of the elderly occur in USA with an approximate annual cost of RSV pneumonia-related hospitalizations of \$150–680 million [30, 31].

#### **4. Pathogenesis**

After RSV infection, virus primarily multiplies in the epithelial cells of the nasopharynx [32]. The exact mechanism by which RSV spreads to the lower respiratory tract is not clear yet. Currently, it is not known why the disease progression is mild in most children, but severe in a small subgroup. Different studies have described associations between disease severity and genes involved in allergic responses, like IL-4 and IL-4 receptor genes, and genes for inflammatory cytokines, e.g., IL-6 and IL-8 [33]. Furthermore, up-regulation of chemokines during RSV infection is associated with disease severity. For example, CCL11 (eotaxin), RANTES (CCL5) and MIP1 $\alpha$  have been found in higher levels in cases with more severe RSV infection and ERD [34, 35].

Several other factors could be associated with disease severity, for example, environmental factors, patient intrinsic factors, virus strain and viral load. Environmental factors like a high number of siblings, attendance of day-care centers and socio-economic status can enhance the chance of early exposure and may increase the risk of developing lower respiratory tract disease [36]. Other factors like geographical area, parental smoking and the use of wood-burning stoves have also been linked to an enhanced risk of severe RSV infections [37–40]. Patient-intrinsic factors like a compromised respiratory function, e.g., bronchopulmonary dysplasia (BPD) [25], or congenital heart disease with increased pulmonary circulation may significantly enhance the risk to develop severe RSV infection [41]. It is reported

in some studies that RSV-strain A is responsible for more severe disease [42], while other studies report no difference between RSV A and B strains [43, 44]. Furthermore, the course of lower respiratory disease was found to be associated with a high viral load [45]. Finally, RSV-specific immunity induced by vaccination may also be involved in immunopathological mechanisms leading to enhanced disease. This hypothesis is mainly based on experimental animal data [46], and on observations from a clinical trial where, as indicated above, infants were vaccinated with FI-RSV vaccine, which resulted in enhanced respiratory disease (ERD) upon natural infection [47–49]. The notion that inactivated-RSV preparations can prime for ERD is one of the factors that has delayed the development of an effective RSV vaccine.

## 5. Therapeutic approaches against RSV

Only supportive treatment is available. In supportive treatment we can use corticosteroids, bronchodilator and oxygen supplement. These are effective to some extent [50]. It is viral infection, so the use of antibiotics is not recommended, but according to some studies antibiotics can be used to some extent but not regularly to prevent the secondary bacterial infection such as urinary tract infections [51]. Corticosteroids also cannot be used routinely because they are the immunosuppressors [52]. The only recommended antiviral RSV treatment at clinical level is ribavirin. Studies are present which indicate the conflicting results of ribavirin use. It is also less effective and very costly. Due to the conflicting results of ribavirin, American Academy of Science recommendation is that ribavirin should not be routinely used in children having the symptoms of bronchiolitis [53]. Ribavirin completes its function by preventing the polymerase of virus. So ribavirin can inhibit both the DNA and RNA viruses. Ribavirin action may result in anemia and other adverse reactions such as hypersensitivity. According to few studies, ophthalmologic disorders also have been noted after the use of ribavirin. All these side-effects lead towards the limited use of ribavirin in RSV treatment [54]. A study indicated that ribavirin might be used in target groups such as children having RSV infection with comorbid immunosuppression, but it is necessary to investigate and verify more data about its recommendation [55, 56]. It has also been noted that once the disease has occurred, no effective treatment is available for preventing the disease. Another study explained its failure in treatment describing that inhibiting the replication of virus alone is not enough to block the virus mediated pathogenesis in host. So, due to limited treatment options and high disease burden, it is necessary to discover the new treatment as well as prophylactic policies.

Now there is focus on F protein for the development of anti-RSV drugs as well as vaccines. Researches are being conducted for the development of numerous antiviral drugs and antibodies that are in preclinical development stage. Some of the new vaccine and drugs are in evaluation stage. Experiments were conducted on cotton rats and mice in which RSV F specific nano-bodies and immunoglobulin were administered by intranasal route. This led to reduction in lung inflammation and also decreased the virus replication after RSV infection [57]. The RSV G protein is also being targeted for the development of drugs, prophylactic agents and vaccines. The RSV G protein consists of CX3C motif and is homologous in structure with CX3CL1 [58]. RSV G protein increases the infection rate by binding the receptor CX3CR1. Experiments have shown that anti- RSV G monoclonal antibodies have the ability to block the interaction between RSV G CX3C-CX3CR1. This interaction inhibition decreased the lung inflammation. Experiments conducted on rats also have been shown that RSV G monoclonal antibodies have the greater ability to decrease the pulmonary inflammation when compared with anti-F monoclonal antibodies [59–61].

## **6. Current status of RSV vaccine**

At present, there is no approved vaccine is present in the market which can protect from RSV infection. Due to increased burden of disease, it is essential to develop a vaccine that can give protection against the disease [47]. Recently, a lot of RSV vaccine candidates have been emerged using a variety of advanced technologies. About 60 RSV vaccines candidates targeting the pediatric and older populations are in development stage and some are also in preclinical stage [62]. According to a study, 16 RSV vaccine candidates are in clinical development stage [62].

### **6.1 Live attenuated vaccine**

During 1960s, after the failure of formalin inactivated RSV vaccine (FI-RSV), struggles were started to develop live-attenuated vaccines candidates. By serial passaging of RSV A2 strain at lower temperature, live attenuated vaccines were produced; however, it was hard to achieve the balance between immunogenicity and safety [62].

Today, a lot of the cold passage (cp) and temperature sensitive (ts) vaccines have been produced. Evaluation of one cpts-248/404 was done in 1–2 month old aged infants. But it led to the problem such as congestion of upper respiratory tract and so, it was not followed for more investigations. After this struggles were done to attenuate the cpts-248/404 strain and many mutants were produced. After evaluation these generated mutants were found to be over or under attenuated [63].

There is another live-attenuated type RSV vaccine which includes M-2 gene deletion is being tested on nonhuman primates. The M-2 gene regulates the transition from transcription to RNA replication. Studies have shown that by the deletion of M-2 gene, viral RNA replication is decreased but at the same time G and F protein expression is increased through transcription which means that virus is attenuated at adequate level and may lead to neutralizing antibody response [64].

NS2 is another target gene for producing live attenuated vaccine RSV NS2. gene increases the shedding of epithelial cells and reduces or inhibits the antiviral cellular type 1 IFN induction and IFN response of the host. Vaccines which include the deletion of NS2 gene are stable genetically and sensitive to temperature to some extent [65, 66]. SH gene also has been considered for deletion for the live attenuated vaccines. It is believed that SH gene is involved in viral fusion. According to few studies, it is involved in the inhibition of apoptosis by blocking the TNF- $\alpha$  pathway. To obtain the sufficient attenuation level to get the safety is the major continuing problem for live attenuated RSV vaccine [65–67].

### **6.2 Subunit vaccines**

RSV G and F glycoproteins lead to the induction of neutralizing antibodies. These have been evaluated as potential vaccine candidate [68]. Subunit vaccines have the potential to be used for maternal immunization. They are also useful candidates for elderly immunization. A number of subunit vaccines have been evaluated recently. The vaccines which are in clinical trials are co-purified G, F and M proteins; purified F glycoprotein (PFP-1, PFP-2 and PFP-3); and BBG2NA etc. [69–76].

RSV PFP-1, PFP-2 and PFP-3 are the candidates which have been evaluated in children of >12 months of age and also in elderly target populations. These vaccine candidates consist of purified glycoprotein which are adsorbed to Al(OH)<sub>3</sub> (PFP-1 and PFP-2) or AlPO<sub>4</sub> (PFP-3). These candidates were sufficiently tolerated by the target populations but acute reactions were also observed up-to minimum level. There was no observation of enhanced disease occurrence [70–77].

## **7. Challenges to RSV vaccine development**

### **7.1 Early age when immature immune system of neonates**

The most noteworthy risk group for extreme RSV infection is infants under a half year of age [78, 79]. Practically speaking, first dose should be administered at the age of 2 months. Full term newborn children obtain maternal antibodies during the latter 50% of gestation and levels of antibody remain moderately high for a half year after the birth [80]. This would interfere when RSV vaccine would be done [81]. So there is need of an ideal vaccine which will not interfere with the maternal antibodies and will give protection in the presence of maternal antibodies. A few investigations show that newborn children under the age of 8 months have a less serum counter acting agent (antibody) response to characteristic RSV disease as compared to elder ones [82]. A less developed immune system may be the reason of this reduced immunity level, but maternal antibodies may also suppress the immune response [83].

Recent schedule for hepatitis B, diphtheria, rotavirus, pneumococcus, pertussis, tetanus, *Haemophilus influenzae* type b and poliovirus show that vaccine for these infectious diseases will be done ideally after birth at 2, 4 and 6 months of age. Vaccination for RSV should be ideally administered at 6 months of age, so it is necessary and important to make sure that RSV vaccine should not interfere the working and efficacy of other routinely used vaccines during the childhood [84].

### **7.2 Induction of low affinity neutralizing antibodies**

RSV vaccine was developed shortly after it was isolated. In 1960, FI-RSV vaccine was injected by intramuscular route in 2–7 months old infants and children. Instead of providing protection against wild type RSV infection, FI-RSV enhanced the respiratory disease development following wild type RSV infection during the subsequent RSV season. Lungs of children and infants with enhanced disease were rich with large numbers of eosinophils and this was not found in patients of natural infection with RSV. After this disastrous outcome, there was need to develop a safe RSV vaccine including the evaluation of enhanced disease [47–49, 85, 86].

These different immunopathology aspects which were seen in humans after FI-RSV vaccine and enhanced disease were later studied in non-human primates. In newborn macaques which were FI-RSV vaccinated and then infected with RSV virus, enhanced disease with increased level of eosinophils and neutrophils were seen [87–91]. FI-RSV produced the increased level of ELISA titer RSV antibody because it was highly immunogenic, but the provoked antibodies were non-neutralizing. Antibodies produced did not provide the protection against virus because it could not prevent the fusion of virus [92, 93]. FI-RSV induced resulted RSV antibodies were also known to be of decreased avidity and this may be the result of having lack of maturation [94–97].

### **7.3 Lack of appropriate animal models**

No ideal animal model for RSV vaccine is present which can be used for its evaluation. African green monkey kidney cells (Vero cells) were used for production of RSV. High titers of RSV were observed on Vero cell line. Similar results were obtained when grown on human cells (HEp-2). On both these cell lines, RSV infection led to syncytia formation. These cell lines were used extensively to characterize the live attenuated RSV vaccines. In recent studies, there are reports that NHBE

(normal human bronchial epithelial) and HAE (human airway epithelial) cells are used to create model human nasopharyngeal mucosa. RSV infection did not show any pathological sign and also not led to syncytia formation on NHBE and HAE cell lines [98, 99].

Experiments to study attenuation of live attenuated RSV vaccine were also conducted on BALB/c mice, found permissive to infection to some extent. Advantages for mouse studies are that reagents are readily available which can be used for measurement of infection immune correlates [99–104]. Several non-human primates act as host for RSV. RSV can replicate in the nasopharyngeal tract of their host. Macaques, African green monkeys, Chimpanzees and bonnet monkeys have been used to model RSV infection [105–112]. Relative viral titers of live attenuated RSV vaccines compared to wild type RSV disease can also be measured. Chimpanzees are the only non-human hosts which develop and show the clinical sign and symptoms of coryza following RSV infection. They are much permissive to RSV infection. So they are used for evaluation of comparative level of attenuation among vaccines which are candidates in humans. But it has been shown by the recent studies that chimpanzee is not completely predictive of attenuation in young newborns. They are also scarce and expensive. Study conducted by Karron et al. showed that those RSV vaccines sensitive to temperature and also had high degree attenuation in chimpanzee, were able to produce infection in lower respiratory tract in children [112].

#### **7.4 Absence of RSV disease liability data and commercial risk**

It is known that those children and persons primed with RSV are not at risk to RSV enhanced illness, but the absence of enhanced disease illness in RSV primed persons does not support the prediction that it will not be present in RSV naïve population. So it is very difficult to build up safety data which can support and be used for testation of novel RSV vaccine in newborns having age <6 month which is primary target population [113, 114]. There is absence of information (data) on RSV related mortality. This has prevented exact appraisal of the expenses and advantages of RSV vaccines and prioritization of vaccines for various target populaces [49]. Lack of information on disease liability data is a big problem in less developed countries where mortality cases are concentrated [115].

#### **7.5 Limited resources**

Clinical investigations of applicant vaccines in the target populace are fundamental to figure out which vaccines ought to be created for licensure, yet these examinations are tedious and costly, what's more, assets for these investigations are restricted. Measurement of impact of vaccine on disease in all target populations is very difficult and problematic. It is easy to diagnose RSV infection in infants and children because their respiratory secretions have high titers of RSV and so are easy to detect. Titers of virus in adults are low and sensitive RT-PCR assay is used for detection purpose. If there is a decrease in severity of disease, it is a good indication of vaccine being effective. Measures done for disease severity are not accurate at all ages of target populations. So there is need that larger and most costly studies should be performed [116].

#### **7.6 Emerging RSV variants/mutation in RSV genome**

RSV is divided antigenically into two groups which are RSV-A and RSV-B. These groups are further divided into genotypes as well as variants. It has been

investigated that different viruses belong to these different groups; genotypes as well as variants co-circulate in epidemics. So it is very difficult to develop an effective vaccine due to the presence of virus antigenic diversity as well as variability. Like other RNA viruses, RSV has high nucleotide substitution rate ( $10^{-3}$ – $10^{-4}$ ). Spontaneous type of deletions of G and SH genes have been studied *in vitro*. RSV genome encodes 11 proteins; one of them is G protein which is most variable having 2 hypervariable regions. G protein has been investigated to accumulate amino acid changes periodically. RSV genotypes having the amino acids duplications in G proteins also have been isolated.

### **7.7 Disruption of antigenic epitopes**

Researches had shown that formalin inactivation caused the alterations in the epitopes of the G and F proteins and as a result non-neutralizing antibodies were developed which led to formation of immune complex in the lungs [57, 69]. Recent studies have shown that changes in the properties of F protein occur during interaction of virus and host cell. The pre fusion (pre-F) which is highly energetic, transitions irreversibly into post fusion (post-F) form which is low energetic and stable, this occurs during insertion process of virus into host cell membrane. By this process, fusion of virus to host occurs. Although pre and post F are not structurally similar, they share 2 antigenic regions. Neutralizing antibodies target these antigenic regions. Pre F also has 3 other antigenic sites not present in post-F. These sites are neutralization sensitive [68–72]. It is investigated that pre-F conformation changes to post-F conformation during the mechanism of formalin as well as heat inactivation and this change is irreversible. As a result of this change, complete loss of epitopes occurs. So this process explains the one of the reasons for failure of FI-RSV [69, 71].

### **7.8 Older age when immune-senescence of the elderly people**

Elder target population group possess a considerable disease burden. The elder group has preexisting immunity, which makes it inconvenient to increase the existing immunity. Furthermore immune-senescence may lead to decrease in the efficacy of vaccine [116]. Immune-senescence is a challenge for proper vaccination in older target populations. RSV disease burden increases in elderly people in presence of underlying diseases such as cardiac and pulmonary conditions. Live attenuated vaccines are found not to be immunogenic in elderly people. So now focus is on subunit vaccines for this target group [115, 117].

## **8. Future horizons in RSV vaccine and RSV therapeutics**

- Continuous struggles are going on for the development of effective and safe RSV vaccines for each target group (infants, children, elders including pregnant women). Previous struggles made to build up a safe vaccine were failed. High antibody production was seen by the use of FI-RSV vaccine in 1960. However, unfortunately vaccinated children developed a severe disease after administration of FI-RSV vaccine. Difficulties and barriers associated with vaccine development particularly live attenuated vaccine are enhanced respiratory disease, maternal antibodies, nasal congestion, low immunogenicity, genetic variability and instability, immature immune system of infants, vaccine virus transmission and immune-senescence as well as preexisting immunity in elders. However, these problems are being slowly overcome [118].

- Major achievements in last 3 years are that nanoparticle based vaccines and live vector vaccines have been investigated in different phase 1 and phase 2 trials and efficient results obtained. These vaccines step forward into later phase trials for evaluation [119]. So, there is hope that safe and well tolerated vaccine candidates will provide a long lasting immunity to all target groups, may be in our hands with in ~5–10 years.
- *In vitro* tissue culture system has been developed, that are being used for predicting the efficacy and safety of candidate vaccines.
- Palivizumab is only the success which is available to clinicians and is being used to reduce the burden of RSV. Palivizumab has decreased hospitalization; however, its use is limited due to high cost. There is hope that this approved prophylactic approach will be available to everyone and may soon come into extensive and widespread use. Palivizumab is patent of MedImmune and this patent is near to expire. So with expiry of this patent, there is hope that a cost effective palivizumab version will be developed. A recent technology hub has been established by the World Health Organization (WHO), the purpose of this is to increase the production of biosimilar versions [120]. There is hope that these products will be available in the market at low and affordable cost. These good initiatives will greatly reduce the mortality rates caused by RSV in developing world.
- Oral antivirals such as GS-5806 and a nucleoside analogue ALS-008176 have been passed through trials and they significantly decreased the replication of virus in human controlled experiments [121]. Nanobodies (single domain antibodies) has also been developed that protected the mice from infection and now are ready for clinical development. These results renewed the hope that an effective antiviral treatment for different risk populace will be on the horizon in next few years [57].

## 9. Conclusions

One of the most common causes of the respiratory tract diseases is the RSV affecting infants, young children and the elderly people. Only supportive treatment is available such as corticosteroids, bronchodilators, oxygen supplement and ribavirin etc. which may not be occasionally effective. Palivizumab has decreased hospitalization; however, its use is limited due to high cost. Despite it is the era of progress and technology, no RSV licensed vaccine is available in the market to prevent RSV infection. Natural infection also provides partial immunity. A successful vaccine candidate will provide the long term protective immunity and must not lead to induction of enhanced RSV disease. For RSV vaccine development different target groups are being considered such as elders including pregnant women, children and infants. Each of these target groups has different challenges for vaccine development. Maternal antibodies, enhanced disease and immature immune system are the major barriers for vaccine development in the infants. The children >6 months of age have more mature immune system than infants but still can be at the risk of enhanced disease from non-live RSV vaccine. For elderly target population immune-senescence as well as pre-existing immunity is the barrier for vaccine development.

An ideal RSV vaccine should be safe, well tolerated and provide long lasting immunity as compared to natural infection against both RSV strains A and

B. Further, it is recommended that separate vaccines should be developed for each target group. The tools that ought to enable us to build up a sheltered and successful RSV vaccine are accessible and our challenge is to utilize them wisely. We trust the suggestions for vaccine advancement noted above can support researchers, subsidizing offices, and industry center their endeavors and assets most productively and viably.

### **Conflict of interest**

There is no potential conflict of interest among the authors listed in this manuscript.

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