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An Overview of Respiratory Syncytial Virus Vaccine Development

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Abstract

Respiratory viral diseases constitute significant global health burdens, bringing about a huge number of hospitalizations every year. Respiratory Syncytial Virus (RSV) is the main cause of severe lower respiratory tract diseases in under-five children and adults above 65 years of age. RSV associated infection can present with symptoms similar to that of the common cold and in severe cases, can present as pneumonia or bronchitis, while in complicated cases, they may lead to extrapulmonary consequences in the brain and other tissues and organs RSV causes a respiratory tract infection that affects 64 million people per year worldwide. It hospitalizes 3 million children under 5 years old and approximately 336,000 older adults annually. Vaccination could significantly relieve the burden of RSV disease. There are no authorized vaccines to forestall RSV diseases, and the main prophylaxis at present is the monoclonal antibody palivizumab. However, its use is restricted to high-risk individuals, it is also very expensive and moderately effective, and hence the need to explore other vaccine options that will be more effective, safe, and affordable and therapeutics for infants and high-risk populations like health care workers. Anatomic understanding of antigenicity is a critical step in the pathway towards the development of a structure-based vaccine; a supersite specific strategy will be advantageous as we can focus on the target with utmost accuracy hence the aim of this review.

Keywords: Respiratory Syncytial Virus, Vaccine, RNA interference

Introduction

Respiratory syncytial infection (RSV) diseases are a significant reason for lower respiratory tract infection among infants, children, and immune-compromised people. RSV diseases give fractional resistance and reinfection might happen frequently all through life. In this manner, RSV constitutes a severe threat to adults and children with chronic diseases (1). Current investigations have shown that RSV is additionally a fundamental cause of mortality among the old, to comparative degrees as does flu (2). Currently, the main approved drug against RSV disease is a prophylactic monoclonal antibody (Palivizumab), which is given to high-risk children (3, 4). Despite engaging many vaccinology approaches such as viral and bacterial vectored vaccines, live attenuated vaccines, and adjuvanted subunit vaccines which were assessed in rodent and primate models, there are still no approved vaccines (5). Developing RSV vaccines are very challenging. Infants less than six months of life is the highest risk group for developing severe RSV disease and the vaccine candidate should preferably be administered as early as possible after birth (6).

In the mid-1960s, a formalin-inactivated RSV vaccine candidate was administered to infants and children. However, rather than protecting them from RSV infection, the children are at increased risk of developing severe respiratory disease and unfortunately lead to the death of two infants (7). Severe RSV-induced disease continues to present a major global health burden in high-risk groups such as preterm infants, newborns, elderly populations, and those with many associated comorbidities. There is no licensed vaccine to prevent RSV infections, and the only prophylaxis currently approved by the Food and Drug Administration is the monoclonal antibody palivizumab. However, its limited use in high-risk groups, as well as the high cost and moderate effectiveness underscore the need for additional options. There remains a critical need to develop safe and effective RSV vaccines and therapeutics to combat RSV disease severity in infants and high-risk populations. This review article examines the structural proteins of the respiratory Syncytial Virus, its vaccine development and the role of interference RNA.

Respiratory Syncytial Virus

Respiratory Syncytial Virus (RSV) is an enveloped, negative-strand, and non-segmented RNA. According to the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), it is reported to be the primary cause of lower respiratory tract infection (LRTI) among infants and children globally (3). During the first year of life, 80% of infants are infected with RSV with a peak incidence between 2 to 6 months of life (8). RSV causes a respiratory tract infection that affects 64 million people per year worldwide. It hospitalizes 3 million children under 5 years old and approximately 336,000 older adults annually with approximately 0.5% to 2.0% of all children are hospitalized with lower respiratory tract

disease, of which 50% to 90% have bronchiolitis and 5% to 40% have pneumonia (9) Fever, cough, coryza, expiratory wheezing, and respiratory distress are characteristic features of RSV that causes upper and lower respiratory tract infections. Severe RSV infections could develop into bronchiolitis and pneumonia which eventually lead to death. Reinfection is normal due to the low level of serum neutralizing antibodies produced in the RSV protection mechanism (10). Direct contact with infected body secretions and fluids is the mode of transmission for the spreading of this infectious virus. Thus, frequent hand washing, isolation of infected patients, and contact precaution remain the best prevention actions (11).

The RNA is associated with viral proteins, consisting of a nucleocapsid core that is wrapped within a lipid envelope. RSV which belongs to the family Paramyxoviridae is classified in the Pneumovirus genus (15). Paramyxoviridae has two subfamilies: Paramyxovirinae, which includes measles, mumps, and the parainfluenza; and Pneumovirinae viruses, which include most of the RSV viruses; Human Respiratory Syncytial Virus (HRSV), Bovine Respiratory Syncytial Virus (BRSV), and Metapneumovirus (16). Second matrix protein (M2) and envelope proteins with no hemagglutinin or neuraminidase activity are distinctive characteristics of genus pneumovirus that differentiate it from other members in Paramyxoviridae (17). The RSV genome consists of approximately 15,200 nucleotides and is transcribed into transcripts encoding 11 distinct proteins; including two non-structural proteins and nine structural proteins (12). RSV has several important proteins that are vital for replication and survival. They are NS1, NS2, N, P, M, SH, G, F, M2, and L as shown in figure 1. Well, known proteins such as G and F are important for viral attachment to the cell and fusion protein, for viral entry, for the development of syncytia formation and infectivity respectively (13, 14).

RSV isolation was first reported in a group of chimpanzees which has been detected in 1956 for having flu (18). “Chimpanzee coryza agent” (CCA) was named after the scientist had recovered a cytopathic agent from the chimpanzee (19). Later, remarkable findings reveal that humans who make direct contact with the chimpanzees were also infected with symptoms of coryza and acute upper respiratory tract illness, which is reported to be less chronic than that discovered in the chimpanzees. Shortly after, the same virus was isolated from children with respiratory illness (20).

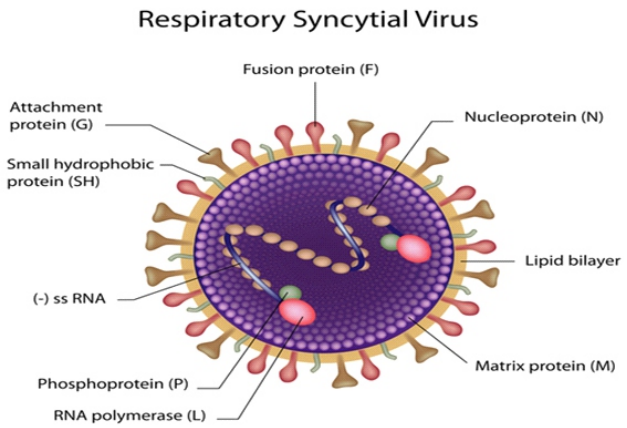

Figure 1. Schematic morphology of RSV with various forms of viral protein. (Source: www.kuleuven.be/rega/mvr/)

RSV has two subtypes; the antigenic subtype A (RSV-A) and the antigenic subtype B (RSV-B) t are differentiated by the genetic molecular difference and the reaction of monoclonal antibodies (mAb) against the G and F glycoproteins (21). In 1997, Walsh and colleagues (22) stated that subtype A strains are more virulent and usually predominant than subtype B strains and both of these viruses are co-circulated. A study by Graham and colleagues (23) suggested that the multiplicity and shifting patterns of circulating RSV strains are crucial to the capability of RSV to constantly cause yearly outbreaks of disease. There is a variety of RSV strain circulation patterns that help them to evade immunity, for example, strain clusters of subtype A and B (24). The variability of this viral strain helps them to alter the pathogenicity and action of the virus which later lead to repeated infection and outbreaks.

The large glycoprotein G (attachment protein) is a disulfide-bonded glycoprotein and the F (fusion protein) is the major antigenic determinant of the virus (26). The G protein is responsible for the attachment of virus while viral penetration and syncytium formation are mediated by F protein where these two genes are the only proteins that induce neutralizing antibodies that cause accumulation of mutations in response to host immunological pressure (27). The SH (small hydrophobic protein), M (matrix protein), and M2 protein are envelope-associated proteins. The N (nucleoprotein), the P (phosphoprotein), and the large L (nucleoprotein) are present in the RSV nucleocapsid. Meanwhile, NS1 and NS2 are non-structural proteins that are found only in an infected cell but not in virion (28, 29). RSV M2-2 protein is a small (90 amino acids for strain A2) protein encoded by a second, downstream ORF in the M2 mRNA that slightly

overlaps the 5'- proximal M2-1 ORF (30). M2-2 protein is responsible for a regulatory 'switch' from transcription to RNA replication, where it provides an initial high level of mRNA synthesis followed by the synthesis of RNA in favour of genomic RNA for virion assembly (31, 32). Bermingham and colleagues (33) found that recombinant RSV lacking M2-2 grows less efficiently than did the wild type.

Respiratory Syncytial Virus Replicative Cycle

Viral entry occurs when the viral envelope fuses with the cell membrane. The initiation interaction occurs when RSV G protein binds to a long polysaccharide of the host cellular matrix that consists of repeating disaccharide subunits called GAGS. In addition to GAGS, several cellular proteins have been implicated in RSV infection in vitro; these include intercellular adhesion molecule (ICAM)-1(34), RhoA, The CX3CR chemokine receptor, and annexin II (35). The F protein then interacts with RhoA to mediate the virus attachment. Viral replication and gene expression occur in the cytoplasm where the nucleocapsid and the genome are released. The M2-2 gene administers the conversion from transcription to the production of genomic RNA. The polymerase then enters the genome at its 3'end and the genes are transcribed into mRNA. A polar transcription gradient leads to more recurrent transcription of promoter starting genes than the genes which are downstream. Replication serves as a template for genome synthesis as it generates an antigenome which is a completely positive sense RNA complement of the genome. For RNA synthesis to occur, both genome and antigenome are coated with the N- protein at all times. Next, the M protein regulates the assembly of RSV by interacting with F, G, and nucleocapsid protein (N, P, and M2-1). An envelope derived from the membrane is required by the newly synthesized protein which leads to a self-assemble and budding process.

The viral mRNAs and proteins can first be detected intracellularly by 4 to 6 hours after infection. The accumulation of mRNA plateaus at 14 to 18 hours. This apparent shut-off of transcription might be due to the M2-2 protein, which appears to shift the balance of RNA synthesis from transcription to RNA replication (36, 37). By this hypothesis, Bermingham and colleagues (33) conclude that M2-2 accumulates during infection increasingly favours RNA replication over transcription. The release of progeny virus begins at 10 to 12 hours post-infection, reaches a peak after 24 hours, and continues until the cells deteriorate by 30 to 48 hours. The overall process of the viral life cycle is shown in Figure 2.

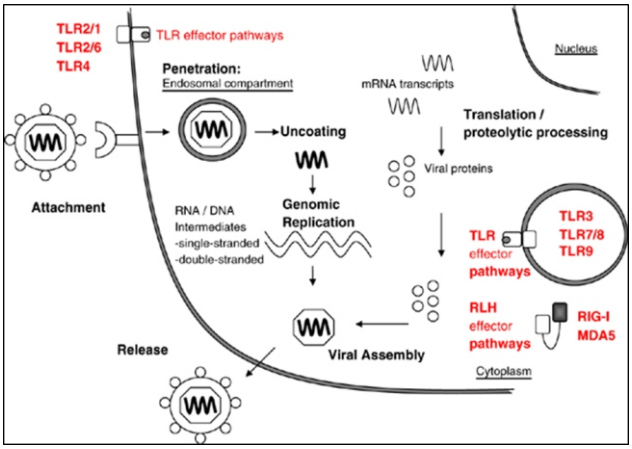

Figure 2. Viral life cycle. Pattern recognition receptors can target multiple steps in the viral life cycle (Source: Malhotra and colleagues (38).

Disease Associated with Respiratory Syncytial Virus

The RSV is recognized to be the primary cause of respiratory tract infections that infect mostly children less than two years old (39, 40). The majority (80-90%) of infants experience at least one RSV infection within the first two years of life (41). Primary RSV infection occurs early in life and most likely affects the lower respiratory tract. Infected body secretions and fluids are the modes of transmission of this infectious virus. Past Infections of RSV will provide immunity for reinfection of the virus within the same subgroup (42, 43). However, protection against natural infection is incomplete and it is correlated with low levels of serum neutralizing antibodies (44, 45). In mild RSV respiratory infections, symptoms are usually similar to the common cold. Meanwhile, severe cases of RSV infection appear with symptoms such as expiratory wheezing, otitis media, bronchiolitis, and pneumonia (46, 47).

RSV is the primary cause of bronchiolitis in young children while pneumonia is the most common feature in infected elderly. Severe symptoms of RSV involving lower respiratory tract infection may be persistent and chronic compared to other viruses (48). RSV is responsible for 50-90% of children who had been hospitalized for bronchiolitis, 5-40% for pneumonia, and 10-30% hospitalizations due to tracheobronchitis respectively. Moreover, children with lung and heart disease are at high risk of having severe RSV infections that could lead to death.

Respiratory Syncytial Virus Vaccines Development

World Health Organization had listed RSV as the main concern in the list of vaccines development due to its high morbidity and mortality worldwide (49). However, the vaccine progress against RSV has been delayed by

several factors. The obstacles faced in developing an RSV vaccine are associated with the nature of RSV disease or vaccine design and its infectivity in the early age of the group. Vaccination is recommended to be given in early life, ideally in one month age of infants or younger as the most infective patients are children below six months old. However, newborns and preterm infants may not respond favourably to immunization as their immune system is poorly developed (50, 51). Frequent re-infections that occur throughout life are another unfavourable observation in RSV infection (52). According to Weisman and colleagues (53), a small number of excellent animal models is one of the factors that contribute to the failure of vaccine development strategy.

Presently, vaccine developments are progressing with several new approaches. A life attenuated RSV vaccine that is given intranasally would have the advantage of being able to give immunity against both the upper and lower respiratory system. However, the disadvantages of this vaccine are that vaccinated children that have sufficient immunity against the virus might eventually cause viral shedding, therefore unvaccinated children are at risk of infection (54). Development of life, genetically engineered RSV vaccine remains a possibility (55).

The research in vaccine development continues with studies in F and G glycoproteins. Subunit vaccines containing purified F and G glycoproteins or novel chimeric substances splicing together F and G glycoproteins are being discovered and may be appropriate for some high-risk populations (56). Research is also being conducted involving the introduction of plasmid cDNA encoding virus genes tagged with reporter genes into mammalian muscle tissue. Li and colleagues (57) revealed that these compounds are present months after their initial introduction into the tissue. This prolonged effect might be useful in allowing an immune response to develop in a newborn as maternal antibody levels decline.

RSV vaccine developments were hindered by several additional issues which include a series of boosters that might be needed in vaccination as natural infection of RSV is not sufficient to protect against reinfection (58, 59). Thus, the main concern of a good vaccine approach is to design a vaccine targeting multiple antigenic subtypes and strains that can trigger immunity before the infection. In addition, designing multiple vaccines that are targeted at a single population size is needed to expand the wide selection of vaccination candidates (60). Profoundly, safe and efficient RSV vaccines with modern technologies approaches need to implement in the new area of vaccination development.

As vaccine development remains a long way to go and the limitation of efficient antiviral agents, looking for the alternative RSV treatment is equally important. The use of RNA interference (RNAi) as one of the strategies to fight against viral infections is a good approach. RNAi is a natural defence of the innate immune system against viruses (65-67). In RNAi, cellular enzymes will degrade complementary viral mRNA, resulting in inhibition of important viral protein expression. During viral infections, the host RNAi system would recognize the double-stranded viral RNA produced during viral replication (68). Dicer, a ribonuclease enzyme cleaves the double-stranded RNA into short oligoribonucleotides in 20 – 30 bases long known as short interfering RNAs (siRNAs). The siRNAs activate the cell RNA cleavage machinery called RNA interference silencing complex (RISC) to cleave the viral RNA (69-72). In RSV, its mRNA that is complemented with the siRNA activates the RISC and destroys the viral message (73, 74). This antiviral approach has been successfully tested in cell culture and animal models against many important human viruses. The mechanism of RNA interference is shown in Figure 3.

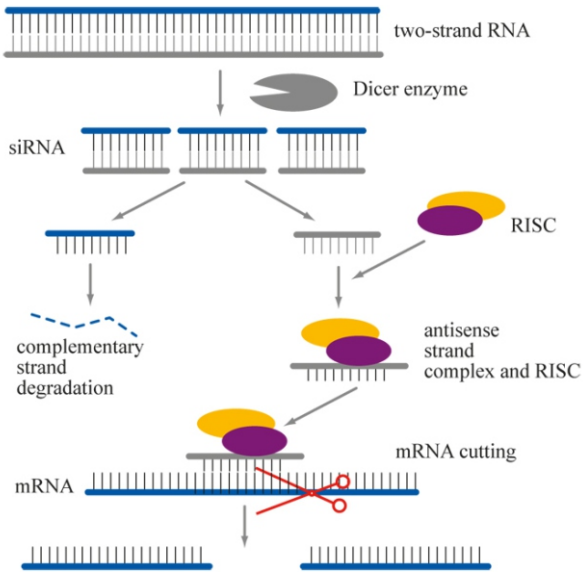

Figure 3. Mechanism of RNA interference in a virus. Source: Fire and colleagues (75).

RNAi has a great impact in treating diseases that contain cells that have RNAi machinery as well as any disease that is caused and exaggerated by the expression of a dominant gene. Most of the study that has been carried out mainly focus on a disease that

had to be cured by current therapy such as cancer, neurodegenerative disease, viral infection, and macular degeneration. Due to the antiviral response by RNAi, viral infection becomes a complex process, especially in plants and lower organisms. Therefore, the potential for a genetic mutation to escape from RNAi needs to be taken into account during viral infection and cancer formation. Several alternatives have been carried out such as targeting multiple genes at one's as well as essential target genes or highly conserved sequence whose mutation would help in viral fitness or tumour cell survival (76).

Conclusion

Scientists worldwide are working faster than ever to develop and produce vaccines that can prevent severe RSV infection. With only one mAb treatment and no market-available vaccine, RSV remains under the WHO's high-priority list of unmet medical needs further research into the progress of the RSV treatment should focus on bringing out the double purpose vaccine candidate, which not only increases immunity and protects from new virus attacks, but also helps in reducing the impact on the lower respiratory tract. Stabilizing the antigen should also be considered during development, so that the main constituents of the vaccine, let it be monoclonal antibodies or structure variants, can have a convincing opportunity to overtake the viral particle.

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