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Vaccine Development for Respiratory Syncytial Virus

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REVIEW ON - VACCINE DEVELOPMENT FOR THE RESPIRATORY SYNCYTIAL VIRUS

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ABSTRACT:

The respiratory syncytial virus (RSV) is a human pathogen that causes a lower respiratory infection in infants and healthy adults. The first incidence of RSV was recorded in the 1960s. The greatest success against viruses has always been by increasing immunity through vaccination like in smallpox, measles, influenza, polio. Though RSV spread its roots almost six decades ago, the creation of a vaccine against RSV is still an ongoing challenge. The structural proteins of RSV, mainly F and G, play an essential role in pathogenicity. Structural instability of the F protein is responsible for making the vaccine discovery an uncertain outcome. This review focuses on the details of the vaccine strategies that have been explored so far. It includes an emphasis on the initial formalin-inactivated vaccine, structure-based vaccine, monoclonal antibodies like Palivizumab with a concise portrayal of nanoparticle, chimeric vaccines, and maternal derived immunization. The structure-based vaccine is one of the most reliable strategies to explicate further research. Focusing on the epitopes that monoclonal antibodies can act upon will result in dependable vaccine outcomes.

INTRODUCTION:

Being a member of the *Paramyxoviridae* family, Respiratory Syncytial Virus is a non-segmented, single-stranded RNA genome virus which has been the cause of lower respiratory tract infections. The risk for RSV respiratory illness and its related complications is seen majorly in newborns, preterm infants, and aged populations. The burden of RSV is more severe in infants with critical congenital heart diseases, bronchopulmonary dysplasia, malformations, neuromuscular diseases, and immunological disorders (Domingo et al., 2014). From being identified in chimpanzees with lower respiratory tract infection in 1955 to passing its virulence to humans, affecting the infants

and elderly, this virus unveiled many phases. It was first described over 160 years ago and was considered a severe illness in children in the late 1950s.

Acute bronchiolitis is the main symptom seen in infants suffering from RSV. About 77.5% of the acute bronchiolitis episodes in children younger than two years are RSV related (Calvo et al., 2009). The most common symptoms of patients admitted with RSV are asthma, wheezing, rapid breathing, inflammation of small airways, and in many cases, respiratory compromise is also present, which further debilitates to acute lung function failure (Hall et al., 2009). The respiratory syncytial virus shows its impact on all the major systems of the human body, starting from the cardiovascular system to the immune system. About 9% of RSV patients are found to have cardiovascular complications; it is more devastating to know that this 9% includes infants less than 12 months of age. Elevated troponin levels are an index for myocardial damage, and this was observed in about 35% to 54% of infants with RSV infection that required ventilators (Domingo et al., 2014).

Electrolyte balance is one of the significant vitals that physicians are always concerned about. Complications related to electrolyte balance are seen in around 19% of the cases reported with RSV and associated bronchiolitis infection. A deeper understanding of the electrolyte balance reveals that hyponatremia of less than 130mmol/L, the condition that needs intensive care, is seen in 11% of infant cases (Eisenhut et al., 2006). Seizures also occur, but thankfully, it is manifested in only 1.8% of infant cases. However small the percentage may be, the effect of RSV on the nervous system is unnerving. A few studies have also shown that there are cases of acute otitis media and allergic rhino conjunctivitis associated with RSV. The worldwide estimates in the year 2005 of RSV and associated lower respiratory tract infections, under the age of 5, were thought to be around 66,000 and 199,000. Among this, 99 % of the deaths were seen in developing countries. Even with the high risk of complications, the number of cases that were given necessary treatment was found to be only around 37 per 1000 infected infants. This number does not seem to be a healthy count, and the reason being the necessary hospitalization is always dependent on the intensity of comorbidity (Domingo et al., 2014).

An analysis of pneumothorax risk factors is considered in RSV infections (Geoghegan et al., 2007). It is also essential to know that some of the risk factors include exposure to tobacco, breastfeeding period shorter than two months, school-age siblings. Reinfection of RSV is also seen in infants

and is more frequent when compared with reinfection in the elderly (Hall et al., 1991). Overall, the above statistics reveal that the impact of RSV is beyond the acute episode phase. The need for interventions is highly recommended, and it should also aim to improve awareness about the virus (Mejias at al., 2015).

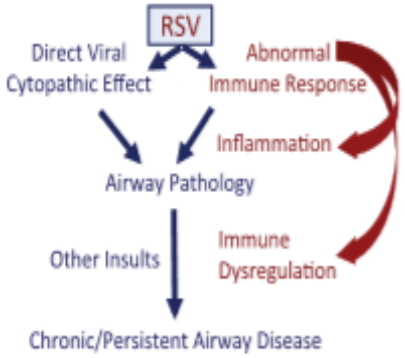

Fig:1(Mejias et al., 2015)

Abnormal immune responses have a significant role to play in the pathogenesis of this infection, which further drives to the signature symptom of RSV that is the lower respiratory tract infections or chronic/ persistent airway disease (see Figure:1). Many studies are henceforth emphasizing the importance of the effective immune system to overcome the pathologic burden caused by RSV. This kind of intervention will be attainable by vaccines (Mejias et al., 2015).

CLASSIFICATION AND THE STRUCTURE OF THE RSV VACCINE:

Respiratory syncytial virus was first isolated in 1955, but it took a long time to focus on the biochemical and molecular characterization of the virus. The virus is tightly associated with the viral protein to form the nucleocapsid. It has a viral envelope that is composed of a plasma membrane-derived lipid bilayer, which holds the virally encoded transmembrane proteins. RSV belongs to the genus *Pneumovirus*, subfamily *Pneumovirinae*, and family *Paramyxoviridae* and order *Mononegavirales*. The RSV genome is said to be composed of about 15,222 nucleotides,

which encode three transmembrane surface proteins (F, G, and SH), two matrix proteins (M1, M2), three nucleocapsid proteins (N, P, L), and two non-structural proteins (NS1, NS2) (Collins et al., 2013). It is vital to know the structure of this virus to optimize the vaccine strategy. The G protein has an epitope in the center conserved domain with neutralization sensitive properties. The F protein is a more potent and cross-protective candidate for the vaccine design.

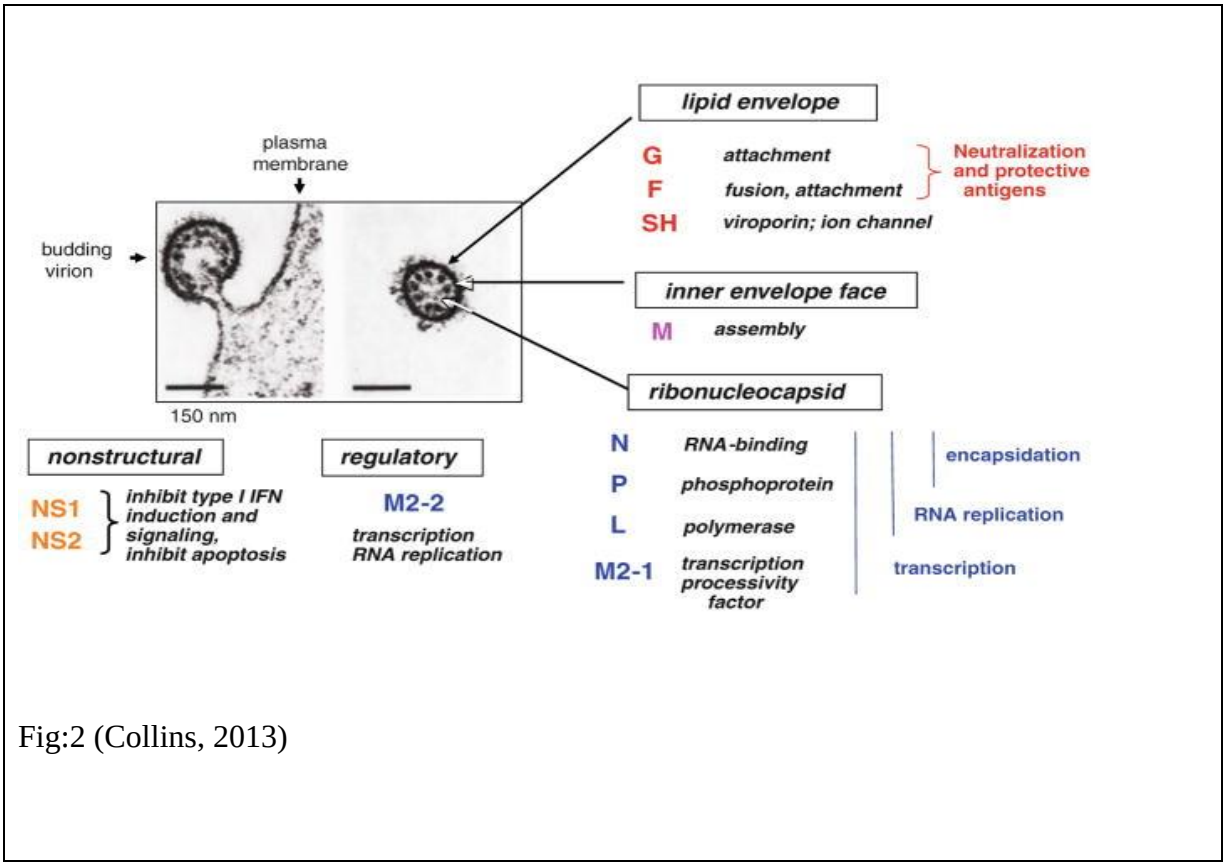

Fig:2 (Collins, 2013)

The virion of RSV consists of a nucleocapsid that is packaged in a lipid envelope, which is derived from the host cell plasma membrane. The envelope, as demonstrated in the Figure:2, contains three viral transmembrane surface glycoproteins. They are the large glycoprotein G, the fusion protein F, and the small hydrophobic SH protein. The non-glycosylated matrix M protein is seen in the inner surface of the envelope. The viral glycoproteins have separate homo-oligomers. These are appreciated as small spikes on the surface. Apart from these structural proteins, there are four nucleocapsid or polymerase proteins, which include Nucleoprotein N, the phosphoprotein P, the transcription processivity factor M2-1, and the large polymerase subunit L (Collins et al., 2013).

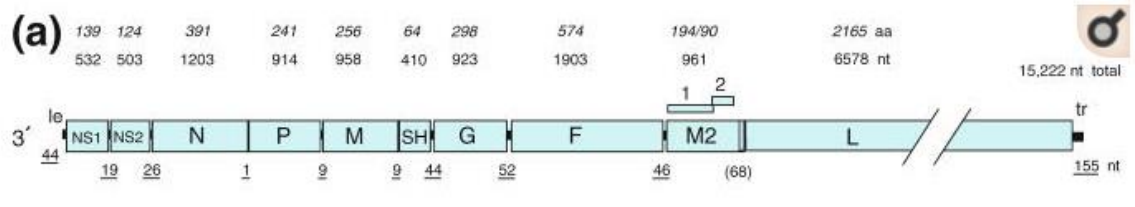

FIG: 3 (Peter L.Collins, 2013)

The genome has ten genes in the order *NS1-NS2-N-P-M-SH-G-F-M2-L* from 3` to 5` direction. Each gene encodes a corresponding mRNA, which is methylated at the 5' and poly-Adenylated at the 3`tails. Except for the M21, each other mRNA encodes singe protein (see Fig:3). The membrane protein M consists of both positively charged and hydrophobic domains. These are responsible for membrane budding and virus particle formation (Collins et al., 2013). When the M gene is null mutated, there are impairments in the long viral filaments. So, studies say that the presence of polymerization of M at the budding state will promote the elongation of viruses (Ghildyal et al., 2005). The *M2* gene encodes two proteins: M2-1 and M2-2. These are functional in genome transcription and replication. M2-1 has two distinct roles. It is a transcription regulator and switches RNA synthesis from protein transcription to genome replication. It also serves a structural function, where it colocalizes with the cytoplasmic inclusions that are present in infected cells (Kiss et al., 2014).

F is a 574 amino acid class 1 fusion protein. It has a trimeric structure, a thermodynamically stable pre-fusion state, numerous intermediate conformational states, and a stable post-fusion state. The viral fusion process starts with this trimeric metastable pre-fusion conformation, which rearranges into a six irreversible helix bundle post-fusion conformation that initiates fusion pore formation between the host cell plasma membrane and the viral membrane (McLellan, 2013). It is essential to consider the role of the pre-fusion F protein in the virus entry process and to maintain F in a pre-fusion state to elicit a high-level immune response by RSV vaccine.

ASSEMBLY:

Studies have shown two essential pathways for viral assembly. The first describes the viral assembly and the maturation occurring at the plasma membrane. The second depicts an alternative method with some steps of the virus assembly in the cytoplasm without reaching the plasma membrane. The Cryo-Electron microscopy studies by Ke et al. (2018) revealed the length of the filament to be 1.5 microns and diameter to be around 130nm. Vaccine antigens that are stabilized in the pre-fusion conformation are linked by the mobile and immobile portions of the F protein through the disulfide bonds, and this might be the reason antibodies are targeting the antigenic site Φ (McLellan et al., 2013).

There is a sequence of steps that lead to the formation of RSV filamentous particles. They are initiation, elongation, and scission. Initiation is characterized by the accumulation of viral components at the plasma membrane but precedes protrusion of the viral filament from the membrane. Elongation occurs when the viral filament has protruded from the membrane and is actively extending. Scission is defined as the narrowing of the RSV filament diameter at the cell proximal end of the filament and is followed by the release of RSV particles from the cell (Ke Z et al., 2014). Three distinct morphological structures have been identified: filamentous, spherical, and structural intermediate (Kiss et al., 2018). Morphological quantification suggested the presence of more of the filamentous structures of the RSV. Electron microscopy findings suggest that RSV F glycoproteins are in the metastable pre-fusion conformation on the filamentous particles and are in the stable post-fusion conformation on the spherical virus particles. The formation of the filamentous networks is important for the cell to cell transmission of the viral particle (Ke et al., 2014).

HISTORY OF THE RSV VACCINE:

FORMALIN INACTIVATED VACCINE:

As soon as the risk of RSV was identified, the National Institute for Allergy and Infectious Disease considered it to be a research priority, and the first vaccine trial was initiated. The primary research for the RSV vaccine was the formalin-inactivated vaccine. It was created in the 1960s and is similar to the inactivated polio vaccine. Dilution of the seed virus was inoculated into monkey kidney

bottle cultures, and the resultant tissue culture fluid harvests were inactivated with formalin. The fluid was centrifuged, and the pellet residues were concentrated by precipitating with alum and other materials. After adding the preservatives, the concentrated final product was called LOT100 (Derscheid et al., 2013). The FI-RSV vaccine proved to be highly immunogenic and showed high titers of RSV antibody. Still, these antibodies were later revealed to be devoid of neutralizing antibodies and fusion inhibiting activity. Infants had high F glycoprotein, and children aged 7 – 40 months had F and G antibody titers. However, in one study, vaccinated children developed a lower level of neutralizing antibodies compared to the control group. Two infants who were initially immunized passed away (Hurwitz, 2011). The FI-RSV vaccine appeared to stimulate an unbalanced immune response in which a large fraction of the induced antibodies targeted protective epitopes instead of functional antibodies that target viral elements (Murphy et al., 1986). Before 1996, the standard treatment option for RSV infection was Ribavirin (Rebetol). It is a nucleoside analog that interferes with viral replication. However, this was not successful because it had a high rate of morbidity, reducing its application for clinical use (Cingoz, 2009).

SUBUNIT VACCINES:

These vaccines are inoculations, as they include a single protein with or without adjuvant. Eliciting the memory response is the main aim of these subunit vaccines, but this memory response is not associated with a live vaccine. RSV attachment glycoprotein is also one of the potential candidates for elucidating humoral immune responses. A concern with the RSV processing is the strain difference. As the laboratory-adapted strain is A2, most of the lab work holds good for this strain, but infants may be affected with more than a single strain (Clark & Guerrero-Plata, 2017).

CHIMERIC VACCINES:

For the live attenuated vaccine, its inability to create a long-lasting protective memory response has been one of its significant disadvantages. Though there are multiple epitopes on F and G proteins that are capable of recognizing the immune response, infants were not successfully protected even after virus clearance because they were infected with another virulent strain. Recombinant chimeras expressing immunogenic RSV proteins are created to avoid the danger of another virus attack (Wiegand et al., 2017). Two PIV5 chimeras expressing RSV F or RSV G protein were used in cotton rats and African monkeys. These boosted antibody levels in RSV pre-

exposed African Green monkeys suggesting that PIV/F could help naïve babies and preexposed older adults (Crank, 2017).

NANOPARTICLE VACCINE:

The size of the particle is crucial as it impacts the antigen transported to the draining lymph node. Nanoparticle vaccines that incorporate antigenic components from RSV have been shown to stimulate a mucosal and systemic immune response (Hurwitz, 2011). A study of an RSV nanoparticle vaccine in phase II clinical trials involved a healthy adult woman who was given different dosages with various adjuvants. This trial was only 50% successful, highlighting the need for future testing. It was intended for women in their third trimester of pregnancy. As vaccinating mothers can protect infants for the first month of life postpartum, this approach stands as a ray of hope in the future (Glenn et al., 2016).

MATERNAL IMMUNIZATION:

The main targets of the RSV are neonates and infants. They have an immature immune system and may not be able to elicit a robust immune response following vaccination. So, the idea of maternal immunization is highlighted. Blancoa et al. (2018) worked on cotton rats to see if the maternal immunization can provide any protection to the infants. They stated that female cotton rats could induce immunity by producing RSV neutralizing antibodies that are actively transferred to their litter. However, similar data of RSV maternal immunization in humans is not available (Blancoa et al., 2015). Transplacental transfer of the antibodies from pregnant women can be efficient but not to the extent of protecting severe disease infants (Chu et al., 2017).

STRUCTURE BASED VACCINE:

The concept of structure-based vaccines will be the spotlight for future research. RSV F mediates membrane fusion and is required for infection. Fusion protein has two different states, a metastable stable state adopted before interacting with the virus, and a steady-state that occurs, after merging with the cell membrane. These states are called pre-fusion and post-fusion, respectively (McLellan et al., 2013). Most of the antigenic sites on the membrane-proximal regions of the pre-F head domain are retained on the post-F molecule after rearrangement. The apex of pre-F contains sites like ∅ and V are highly neutralization sensitive and are present only on pre-F confirmation (Gilman

et al., 2016). So, the focus of the vaccine is going to be the preservation of the pre-F site structure. Neutralizing activity in human sera can be adsorbed by using the pre-F. The neutralizing potency of the monoclonal antibodies concerning the pre-F is minimal compared to the post-F. So, by introducing mutations, the pre-F trimeric subunit protein DS-Cav1 should be stabilized. Talking about DS-Cav1, phase I clinical trials of DS-Cav1 were performed with and without alum, where alum is an adjuvant. The results have shown that neutralizing activity was increased seven-fold after immunization with 50microns of DS-Cav1. The neutralization activity observed here is much higher than the earlier trials. (Crank et al., 2019).

McLellan et al. (2013) conducted a competitive neutralization assay that can accurately identify the pre-F and post-F antibodies. Also, they helped to prove the presence of pre-F exclusive antibodies when vaccinated with DS-Cav1. IgG binding is increased with both pre-F and post-F structures. The increase in neutralizing efficacy because of the DS-Cav1 led scientists to believe that it will improve both vaccine efficacy and reduce the vaccine-associated adverse effects. The main goal is to enhance the stability of the pre-F structure for the F protein neutralizing activity relative to the increase in binding antibodies. This goal is achieved, and there is an increase in antibodies specific to the neutralization sensitive apex of the pre-F molecule. It is done through the competition with biotinylated site ∅ directed by Mab D25 and is significantly increased over baseline (McLellan et al., 2013).

Coming to the antibodies that are attached to the side of the pre-F, a few studies have shown a new structure-based vaccine strategy. The crystal structure that is bound to the F glycoprotein epitope allowed to create epitope scaffolds and this helped to develop a motavizumab epitope on the heterologous proteins (Hurwitz, 2016).

The goals for developing a structure-based vaccine should be:

1. Identify a viral site targeted by multiple antibodies with extremely potent neutralizing activity,

2. To determine the structure of the site in complex with a representative antibody,

3. To engineer the stable presentation of the site in the absence of recognizing antibody and

4. To elicit high titer protective response through immunization with engineered antigens, that stably present the neutralizing sensitive site (McLellan, 2013).

The antigenic site Ø was chosen as the target site, as the RSV neutralizing antibodies will recognize it much better than the other sites. The antigenic structure should involve the T4 phage fibritine trimerization domain to the c terminus of the RSV F ectodomain and binding of the pre-fusion specific D25 antibody. To stabilize the antigenic site in the absence of the D25 antibody, the C terminal trimerization domain should be retained. Combining it with cysteine pairs or cavity filling hydrophobic substitutions could help improve stabilization. Identifying the hydrophobic sites on the distal membrane head of the prefusion structure makes it a well-grounded theory. So, to stabilize this structure, the cavities are filled with hydrophobic substitutions, such as As190f and V207L. These sites adopt prevalent side-chain conformations (McLellan et al., 2013). DS variant was least stable to ph. and temperature variation but more stabilized in the trimeric state. At the same time, a low level of continual conversion from trimer to aggregate was observed for Cav 1 and TriC on size exclusive chromatography (Crank et al., 2019). These observations will point that DS-Cav1, TriC variants displayed a variety of physical and antigenic properties.

When compared with the other variants here, though the DS variant retained features of the pre-fusion state RSVF, including the fusion peptide in the interior of the trimeric cavity, it failed to fix the antigenic site Ø in its D25 – bound conformation. The tetragonal crystal lattice at the C terminus of the F2 is disordered in the D25- bound F structure. Still, here in CAV1, it tunnels into the trimeric cavity alongside the fusion peptide (McLellan et al., 2013). The C terminus ends with Ala 107 and not ARG 109 as expected after cleavage of the furin site. The positive charge of the Arg 106 is offset by an ordered sulfate ion. Biologically, the central position of the F2 C terminus may play a role in triggering the pre-fusion F confirmation. Experiments in mouse models suggested that immunization with the RSV F variants stabilized in the pre-fusion state, especially DS-Cav1 resulted in high titers of an immune response. This can be attributed to the antibodies that targeted antigenic site Ø (Joyce et al., 2016).

Overall, the DS-Cav1 combination appeared to be optimal in terms of trimer yield and physical stability to extremes of temperature. However, the Cav1 structure was more ordered at its distal membrane apex with alpha three-helix, beta 3,4 hairpin, and alpha four helices clearly defined (McLellan et al., 2013). Joyce et al. (2016) examined the relationship between design, physical, and antigenic properties and worked on increasing the physical stability of the antigenic site Ø. By performing iterative cycles of the structure-based vaccine design, a second-generation DS2- F

glycoprotein was also identified. Because of the presence of disulfides, there was almost a four-fold higher neutralizing activity than DS-Cav (Joyce et al., 2016).

CELL MEDIATED IMMUNITY:

As one may expect, the presence of antibodies for the RSV infection does not reduce the chance of reinfection. As mentioned earlier, this is a hallmark of the virus and is the primary reason for remaining without successful treatment after these many years. So, new interventions in immunity have grown to be a ray of hope for possible treatment. Neutralizing antibodies were thought to decrease the severity of RSV associated disease **(**Mucosal Immunology, 2019**).** When antibodies bind to virions, it results in neutralization. This effect of neutralization is seen at various stages before the virus attachment and after cell attachment. By concentrating on the mechanism of antibody-mediated neutralization of RSV, a detailed understanding of the host humoral response against RSV can be obtained. The human polyclonal antisera from RSV infected persons was used to neutralize an RSV infection *in vitro* by Anderson et al. (1994). The neutralization titers from the control sera were one order magnitude lower when compared with RSV convalescent sera. This brought out an exciting fact that there is a high prevalence of anti- RSV antibodies in the general population (Parrott et al., 1995).

To focus further on the specificity binding, Anderson et al. (1994) gave a new approach by using the radiolabeled RSV to adsorb to the monolayer cultures of Hep-2 from insect Sf9 cells. Using the sources from JRH biosciences, it was found Sf9 cells lack the appropriate receptor for RSV. All the sera were assayed for post cell attachment neutralization activity (PAN). The correlation between the neutralization and virus-cell attachment was less reliable. PAN titers and scanning the G band, to calculate the amounts of RSV bound to Hep-2 cells, aided in discovering that neutralization of *in vitro* RSV infection by human serum may be mediated by inhibiting the events that occur following the attachment of the virus to the cell surface. The PAN titer was relatively high initially and decreased with time (Osiowy et al., 1994). This confirmed that the antibody is blocking early events in the RSV replication cycle. Both the F and G proteins for RSV induce neutralizing antibodies (Hall et al., 1990). Circulating antibodies from the previous RSV infection neutralize similarly as that of the antibody-mediated neutralization.

It is known that the CD8 T cells are playing a decent role in protecting against RSV in initial stages. Schmidt et al. (2020) worked on checking the capability of the RSV specific memory CD8T cells to protect against the secondary infections in the absence of RSV specific memory CD4 T cells and antibodies. M2 82 immunodominant T cell epitope was generated and introduced in mice. After an acute RSV attack, these mice exhibited enhanced weight loss and pulmonary dysfunction due to the excessive IFN gamma produced by the CD8 T cells (Schmidt et al., 2020). So monoclonal antibody studies are necessary to determine the target sites on both the RSV G and F proteins (Tang et al., 2019). The F protein exists in a trimer form, as mentioned earlier, a metastable pre-fusion form and a highly stable post-fusion form (McLellan et al., 2013). Antigenic sites I, 11, are binding sites for the monoclonal antibody Palivizumab (Tang et al., 2019). MEDI8897 is an antibody under clinical development for treating the passive immunoprophylaxis for infants and binds to antigenic site Φ (McLellan et al., 2013).

Abortive replication is also one of the important features exhibited by RSV. Boukhvalova et al. (2007) cited that RSV reinfection in animals that are infected 21 days earlier is seen to have an increased expression of viral transcripts and genome replication. However, this replication does not result in the production of detectable progeny virus. This type of replication is termed as abortive replication. Passive administration of antibodies is not sufficient to combat this abortive replication in the virus, which adds to the fact that RSV is a poor inducer of long-term immunological memory. This abortive reinfection results in the altered microenvironment of the lung, which predisposes the host inflammatory allergic disorders (Boukhvalova et al., 2007).

Mucosal IgA titers can also be indicative of susceptibility to RSV infection. Habibi et al. (2015), were the first to try the experimental human infection model. This model helped in predicting the RSV infection risk in seropositive patients. The methodology used here is completely different from the previous study. They used the nasal lavage, which resulted in a more dilute sample and a two-fold lower nasal IgA titer. This helped to prove that unlike other viruses, RSV antibodies were only temporarily boosted and waned to preinfection levels within just 6-12 months (Habibi et al., 2015).

MONOCLONAL ANTIBODIES:

The preclinical characteristics for RBI:

RB1 is the human IgGI monoclonal antibody, and it targets the antigenic site IV of the fusion proteins. This RBI is a parental antibody for the MK-1654, which is currently in clinical development. The focus for MK- 1654 is in providing the intramuscular immunization for the prevention of RSV infection in infants. Tang et al. (2019) measured binding affinities using the pre- and post-fusion conformation ELISAs and surface plasmon resonance. This helped in revealing the preferential binding of the RBI antibody, as it showcased strong pre- and post-fusion glycoprotein binding in ELISA with a preference for the post-fusion. So, the main criteria of the vaccine strategy to have a targeted action on the virions are satisfied with RB1 (Tang et al., 2019).

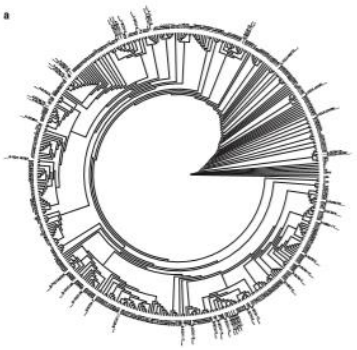

FIG:4 (Tang et al., 2019)

Dendrogram demonstrates the neutralization activity and showcases the sequence diversity (see Figure:4) A phylogenetic tree of 345 Gen Ball sequences is represented in the inner part of the circle, and the fusion protein sequences of about 46/47 RSV A and B clinical isolates are marked as spokes on the outside of the ring. The shift from the prefusion to post-fusion conformation near the epitope can be appreciated by the CA ribbon representation in Figure 5 with one strand spanning the residues 464- 470.

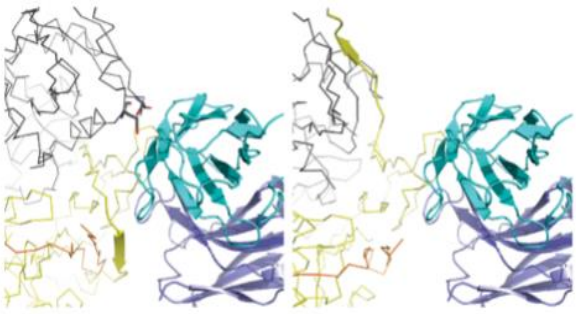

Fig: 5 (Tang et al., 2019)

The neutralization of the various clinical isolates was a significant finding done by Tang et al. (2019). The shift from the pre-fusion to post-fusion is an essential concept of RSV, which has made the vaccine discovery a very rough path for ages (Tang et al., 2019). However, does this RB1 target the RSV virus? As established by Diez-Domingo et al. (2015), site IV spans the residues from 422 to 468, and it is the target of antibodies like Mab19, 101F, and 3M3. This is true for RB1 as well. Tang et al. (2019) used crystallography techniques to show that RB1 binds to an epitope within the antigenic site IV, which was previously cited as C.

PALIVIZUMAB AND MOTAVIZUMAB:

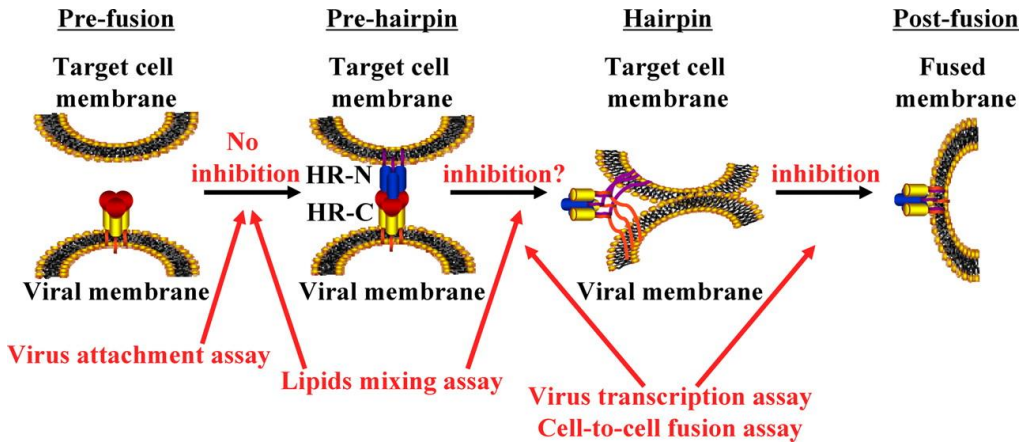

Fig: 6: (Huang K, Wu H. et al., 2014)

Palivizumab and Motavizumab bind to antigenic A site of the F protein, which is found on the surface of RSV. Huang et al. (2010), conducted some assays that focused on individually testing if PalivuzuMab and MotavizuMab inhibit virus attachment like a virus to cell fusion, cell-cell fusion, lipid – mixing assay. (see Figure 6). The combined results of these assays say that the monoclonal antibodies inhibit a step in virus replication, which occurs after the initial F protein-mediated interaction with the host cell membrane and again before virus transcription. With the help of fluorescence microscopic examination, it was found that these monoclonal antibodies can prevent the F mediated fusion. Boukhvalova et al. (2007) and Huang et al. (2010) found that there is a loss of neutralization when the virus is pretreated with the neutralizing amounts of PalivizuMab or MotavizuMab. The concept of pretreating the virus is a new concept that came out with these studies.

Although no published data says, MotavizuMab inhibits this fusion, Huang et al. (2010) say it is conceivable that these antibodies could inhibit budding as both of these monoclonal antibodies bind F protein on the surface of infected cells. By performing immunofluorescence assay and adapting it to ELISA format, Huang et al. (2010) proved that it is possible to inhibit the attachment of the virus-cell that is mediated by F protein. However, the specific attachment could still occur through G protein (Huang et al., 2014).

MotavizuMab is a second-generation monoclonal antibody and is derived from Plaivizumab by using an affinity maturation technique. PalivizuzMab is a humanized monoclonal antibody; it is only approved as an immunoprophylactic measure against RSV infection in high-risk infants. The preclinical studies show that PalivizuMab and MotavizuMab neutralize replication in cell culture when the virus is pretreated with these antibodies. The higher importance of PalivizuMab is because it is proved to be effective even after the commencement of infection. (Wu et al., 2007)

Walsh, Schlesinger, and Brandriss (1984) conducted experiments on cotton rats to identify the level of protection induced by these antibodies. The analysis included three week old outbred cotton rats in groups of five to six. They were injected via an intraperitoneal route with 0.6 to 0.8 ml of mouse ascitic fluid containing monoclonal antibody to either GP90 or VP70. Control animals

were given either saline or monoclonal antibody to RSV nucleocapsid protein. The monoclonal antibodies in this study were targeted against NP44, VP70, and GP90 antigens. All these titers showed a reduction in the lung titers of the virus. Based on this observation, effective paramyxovirus must induce an antibody to both the envelope and glycoproteins. However, this study helped to focus on the fact that circulating antibodies to either of these viral proteins of RSV can be beneficial by liming the viral growth in the upper respiratory tract (Walsh, Schlesinger, & Brandriss, 1984).

Haynes et al. (2009) worked on the RSV G protein to see its effect on the host immune response. This work was to see the impact of anti-RSV G monoclonal antibody 131- 2G by blocking the G protein receptor CX3C, which is associated with the activity of the RSV G protein. Expression results in a lower frequency of interferon-gamma expressing cells and cells with a higher incidence of interleukin 4 (Harcourt et al., 2016). RSV G protein is also associated with increased levels of pulmonary eosinophilia. So, this lies as a foundation for the concept that inducing the anti-RSV G may influence the anti-inflammatory response and reduce the lung pathology.

MAb 131-2G reacts between amino acids 1 and 173. Haynes et al. (2009) worked on mice, which are at the maximum pulmonary inflammation. When the mice were treated with anti-RSV G mAb, they experienced a 60% reduction in pulmonary inflammation. Chemotherapy to combat RSV should always be an antiviral as well as have an anti-inflammatory effect. So, Haynes et al. (2009) state that the combination of anti-RSV G Mab with an antiviral drug or with PalivizuMab antibody, which, as known, works on F protein of the RSV, can make a useful weapon in fighting against RSV. Tachykinin neuropeptide substance P, also known as SP, plays a role in G protein-coupled inflammation. It is also important to note that in BALB/mice which were infected with RSV had high titers of SP levels. This will be an excellent key point to state that increased SP levels actually exacerbate the inflammatory responses and thus play a vital role in the pathogenesis of RSV infection (Tripp, Jones, & Anderson, 2000). Some deep pathology behind these proteins came out through the experimental approach of Haynes et al. (2009). The G protein binds to its CX3CR1 receptor, and the resulting SP expression leads to the lower respiratory rate.

The success of the RSV vaccine can only be declared after the successful completion of clinical trials. Studies have shown many numbers of clinical trials that took place for testing one of two

primary monoclonal antibodies – PalivizuMab. A phase III trial was conducted in 1988, where premature infants were given monthly intramuscular injections of 15mg/kg of PalivizuMab. This turned out to be a decently successful trial, as there was a 55% reduction in RSV hospitalizations (Pediatrics, 1998). The next clinical trial was done in RSV infected infants with intravenous infusion of the PalivizuMab. This trial produced notable decreases in the concentration of RSV in tracheal aspirants. In the case of immunocompromised patients, the specificity of the monoclonal antibodies is thought to be more helpful (Malley et al., 1998).

CONCLUSION:

Structural biology is used in vaccine development to identify critical sites by which a pathogen can be stabilized to pinpoint residue substitutions. Right paths for further research include inactivating the toxoids that maintain the selected conformations of a subunit antigen and to determine antigen-antibody complexes that serve as the basis for epitope-specific strategies of elicitation. Anatomic understanding of antigenicity is a critical step in the pathway towards the development of a structure-based vaccine. For diseases like RSV, where neutralizing antibodies act exclusively through one or two glycoproteins, a supersite specific strategy will be advantageous as we can focus on the target with utmost accuracy.

However, 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. Despite the hurdles the RSV vaccine witnessed in its early days, commendable research has taken place so far in revealing the potential strategies for the vaccine outcome. If further developments in clinical research can occur, and potential vaccine candidates are tested parallelly, there can be a chance to expedite vaccine discovery.

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