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Mucosal vaccines against respiratory syncytial virus

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Respiratory syncytial virus (RSV) is a leading cause of severe respiratory disease in infants, young children, immune-compromised and elderly populations worldwide. Natural RSV infection in young children does not elicit long-lasting immunity and individuals remain susceptible to repeated RSV infections throughout life. Because RSV infection is restricted to the respiratory tract, an RSV vaccine should elicit mucosal immunity at upper and lower respiratory tracts in order to most effectively prevent RSV reinfection. Although there is no safe and effective RSV vaccine available, significant progress has been recently made in basic RSV research and vaccine development. This review will discuss recent advances in the identification of a new neutralizing antigenic site within the RSV fusion (F) protein, understanding the importance of mucosal immune responses against RSV infection, and the development of novel mucosal vaccination strategies.

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Introduction

Human respiratory syncytial virus (RSV) is a leading cause for bronchiolitis and severe respiratory disease in infants, young children, immune-compromised and elderly populations [1–3]. RSV is responsible for an estimated 160,000 deaths worldwide annually. RSV has a linear single-stranded RNA genome with 10 genes encoding 11 proteins, including non-structural proteins (NS1 and NS2), large polymerase (L), phosphoprotein (P), nucleocapsid (N), matrix protein (M1), envelope glycoproteins (SH, G and F), a transcription factor (M2-1) and an accessory protein (M2-2). The attachment (G) and fusion (F) surface glycoproteins have been considered as the two major protective antigens for eliciting neutralizing antibodies. The G protein is heavily glycosylated and involved in viral attachment to host cells. The F protein mediates cell fusion allowing entry of the virus into the cell cytoplasm and formation of syncytia.

Although RSV vaccine development has been conducted since the 1960s, there is still no safe and effective vaccine available. A formalin-inactivated RSV (FI-RSV) vaccine, tested in infants a half century ago, resulted in enhanced morbidity and two deaths after a subsequent exposure to a natural RSV infection [4,5]. The infants and children that received the FI-RSV vaccine exhibited a lower level of neutralizing antibodies following a natural infection. It is likely that the process of formalin inactivation may have altered the structure of the F and G glycoproteins, resulting in altered protein processing and the induction of a largely nonfunctional (i.e. non-neutralizing) antibody response [6].

There are currently no effective treatments for an ongoing RSV infection. A humanized monoclonal antibody specific to the F protein (Palivizumab) administered as monthly injections during RSV season can prevent lower respiratory infection and severe disease in infected infants. However, it does not prevent infection of the upper respiratory system and is not recommended for use in healthy infants [7,8]. In addition, due to the high costs, Palivizumab is not extensively used worldwide. Therefore, a safe and effective RSV vaccine is still a high priority.

Significant progress has been made recently in both basic RSV research and vaccine development. Work in animal models and results from human vaccine trials has led to a greater understanding of RSV pathogenesis and the correlates of protective immunity [3,8,9]. Recent advances in RSV research has created new opportunities and renewed hope, despite the sophisticated nature and significant challenges posed by RSV vaccine development. Since RSV F protein is a very important neutralizing antigen to potentially induce mucosal immunity, this review will focus on discussing firstly, a newly identified neutralizing antigenic site located within the RSV prefusion (F) protein conformation; secondly, the importance of mucosal immunity against RSV infection; and finally, mucosal vaccination strategies in current development.

RSV fusion protein and identification of a new antigenic site in its prefusion state

The RSV F protein is a type I integral membrane protein and serves as an important target antigen for neutralizing antibodies and antiviral T cell responses [10]. To become biologically active and functional, the RSV F glycoprotein

(F_0) after synthesis releases pep27 (a length of peptide of 27 amino acids), following proteolytic digestion by the enzyme furin at the two cleavage sites RKRR136 and RAR/KR109. This generates the F2 and F1 subunits, which are linked via a disulfide bond, and exposes the hydrophobic fusion peptide at the newly created N-terminus of F1 subunit [11,12]. The F protein usually exists in a metastable, pretriggered form on the surface of the virion in order to mediate membrane fusion and viral entry. Once triggered, RSV F undergoes a dramatic conformational extension that leads to the insertion of its hydrophobic fusion peptide into the target cell membrane ultimately folding back on itself to bring membranes together resulting in virus–host cell fusion [13]. Upon triggering, the postfusion F becomes stable and forms 'hat-pin'-shaped molecules that aggregate as rosettes [13]. The RSV F2 subunit, not the attachment G protein, determines the specificity of RSV infection [14]. Therefore, F is a very important protein target for vaccine development. The wild-type RSV F gene cannot be efficiently expressed without the application of codon optimization and deletion of premature polyadenylation signals [15]. Successful expression and immunization with the F protein was shown to induce neutralizing antibody and antiviral T cell responses. Furthermore, broad cross-serotype protection was elicited, likely due to immune responses against highly conserved F protein sequences among RSV strains [16,17,18•,19].

As compared to the immunogenic full length RSV F protein with the transmembrane domain and cytoplasmic tail, the ectodomain of the F protein (i.e. truncated F by removing the transmembrane domain and cytoplasmic tail) also contains the necessary amino acid sequence for multiple neutralizing epitopes. Deletion of the transmembrane domain and the fusion peptide makes the truncated F protein soluble and prevents aggregation [20]. By doing so, Swanson *et al.* engineered a stable, immunogenic postfusion truncated F protein that was capable of eliciting a high level of neutralizing antibodies and significantly protected cotton rats from RSV challenge [21]. In addition, McLellan *et al.* also determined that a similar truncated trimeric F protein missing residues 137–146 contains the critical neutralizing sites (i.e. I, II and IV) in the stabilized postfusion F protein [22].

With regard to the antigenicity, early protein structure data obtained via electron microscopy suggested that prefusion and postfusion F may be antigenically distinct [23]. To prevent RSV infection of the upper respiratory tract, the local neutralizing antibody should presumably bind the prefusion F instead of the postfusion F antigen. However, it has been a significant challenge to produce a stabilized prefusion F, due to its metastable nature. A recent exciting breakthrough has been the identification of the antigenic site ø (zero) within the prefusion F protein. This was discovered through multiple mutations of S190F-V207L to fill up the hydrophobic cavity and creation of disulfide-links S155C-S290C to improve the stability of the prefusion F protein [24••,25••]. The S155C-S290C mutation is critical as it locks the fusion peptide in the central cavity without distortion of the rest of the protein structure. A neutralizing antibody specific to this new antigenic site was found to recognize the prefusion F protein, but not the postfusion F protein. This may explain why highly neutralizing antibodies in human serum cannot be fully absorbed by the postfusion F protein [26]. The stabilized prefusion F protein contains all four neutralizing antigen sites (i.e. ø, I, II and IV) and can elicit potent neutralizing antibody responses up to eight-fold higher than postfusion F protein. In addition, this level of neutralizing antibody was 20–40 times higher than the protective threshold believed to be required in mice and macaques [24••,25••].

The antigenicity of the RSV F protein is dependent on the stability of the protein structure. To form a stable trimer structure for the truncated F protein, it is necessary to add a trimeric motif, such as the T4 phage fibritin trimerization domain to the C-terminus of the ectodomain of the F protein [22,27]. However, the transmembrane domain of the F protein is critical to form stable and soluble postfusion F rosettes after deletion of 10 amino acids from the fusion peptide at the N terminus of F1 subunit [9,28]. On the basis of the recent identification of the very potent neutralizing antigen site ø in the prefusion F, the next generation of RSV vaccine candidates should include the F protein expressed in the prefusion form.

Importance of mucosal immunity against RSV infection

Many pathogens including RSV access the body through mucosal sites. Therefore, effective vaccines that protect at the mucosal port of entry are much needed [29,30]. The efficient induction of mucosal immune responses requires appropriate administration routes and specific adjuvants and/or delivery systems. In contrast to the parenteral route of immunization, mucosal vaccination is usually required to efficiently elicit protective immune responses at mucosal sites. Intranasal delivery is the most effective route to induce potent and broad mucosal immune responses at multiple mucosal sites as compared to other mucosal delivery routes [31••,32].

The four main categories of RSV vaccines include inactivated, live-attenuated, gene-based vectors, and subunit [33]. Live-attenuated RSV vaccines [34•] administered intranasally, and a subunit RSV postfusion F protein vaccine adjuvanted with alum and delivered intramuscularly [28,35] have been extensively evaluated in a number of clinical trials in recent years. The live-attenuated RSV vaccine administered intranasally has the potential to induce a mucosal immune response. However, the response may be weaker in magnitude than that of natural