Review Article

Recent Vaccine Development for Human Metapneumovirus

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Abstract

Recently identified human metapneumovirus (hMPV) and its close family member respiratory syncytial virus (RSV) are two major causes of lower respiratory tract infection in the pediatrics population. hMPV is also a leading cause of morbidity and mortality worldwide in the immunocompromised patients and older adults. Repeated infections occur often demonstrating a heavy medical burden. However, there is currently no hMPV-specific prevention treatment. This review focuses on the current literature on hMPV vaccine development. We believe that a better understanding of the role(s) of viral proteins in host responses might lead to efficient prophylactic vaccine development.

Introduction

Human metapneumovirus (hMPV) is a recently identified virus belonging to the Paramyxoviridae family that also includes respiratory syncytial virus (RSV) and parainfluenza virus [1]. Soon after its discovery, hMPV has been commonly recognized as a leading cause for lower respiratory tract infections in young children, the immunocompromised patients and older adults [2-5].

hMPV is a negative sense single-stranded RNA virus. Its RNA accumulation is believed to be similar to that of RSV. RSV RNA synthesis is comprised of two independent events: viral replication and gene transcription. Both events are tightly regulated by RNA-dependent RNA polymerase (RdRp) complex of viruses. Upon entry, the viral genome is used as a template for gene transcription, with each gene transcribed individually along a gradient, then poly A-tailed. The negative-sense genome is replicated into a positive-sense antigenome, which serves as a template for replication of many copies of the viral genome [6]. hMPV antigenome contains nine open reading frames for hMPV protein expression: 3'-N-P-M-F-M2-1-M2-2-SH-G-L-5'. Although hMPV is a clinical important pathogen, no vaccine is currently available. In this review, we will discuss the recent efforts for hMPV vaccine development.

Inactivated vaccines

Inactivated influenza is commonly used for mass immunization because it is in good stability, easy for manufacturing, and biologically safe due to the absence of viral replication, (http://www.cdc.gov/vaccines/hcp/vis/vis-statements/flu.html). However, the vaccination of a formalin-inactivated human RSV vaccine (FI-hRSV) led to enhanced disease upon natural infection [7,8], which probably resulted from a Th2-biased T cell-memory responses [9-11], formaldehyde hypersensitivity [12], and/or immature antibody production and its associated weak recognition of hRSV epitopes from natural infections [13]. Recently, decrease in FI-hRSV enhanced disease by RSV G glycoprotein peptide was recently reported, suggesting the antibody specific to RSV G is critical for RSV pathogenesis control [14]. Similarly, vaccine-enhanced pulmonary disease and Th2 response following hMPV challenge were also observed in animals vaccinated with formalin-inactivated Hmpv [15,16], suggesting that formalin-inactivated hMPV may not be a suitable vaccine candidate.

Recently, a nanoemulsion-adjuvanted inactive RSV has been shown to be able to induce durable RSV-specific humoral responses, decrease mucus production and increase viral clearance, without evidence of Th2 immune mediated pathology [17]. However, vaccinated mice exhibited an enhanced Th1/Th17 response. Since IL-17 has been shown to induce pulmonary pathogenesis during respiratory viral infection and exacerbate associated allergic disease [18], the safety of nanoemulsion-adjuvanted inactive RSV vaccine candidate needs to be carefully investigated. Whether hMPV with nanoemulsion inactivation is immunogenic and protective, and launches balanced Th1/Th2/Th17 immune responses needs to be determined.

Viral protein-based vaccines

Subunit vaccines are purified or expressed viral proteins, full-length or partial. The expressed proteins are usually in a form of virus-like particles (VLPs), nanoparticles, or with immune-enhancing adjuvants [19]. The most immunogenic protein among paramyxoviruses is mainly the fusion protein F. In terms of RSV, a close family member of hMPV, its F in a form of nanoparticle is being evaluated in a phase II clinical trial by Novavax [20].

Several animal studies using hMPV proteins as subunit vaccine candidates have been recently conducted. By using retroviral core particles as a carrier, intraperitoneal injection of hMPV F induces a strong humoral immune response against both homologous and heterologous strains. Moreover, the induced neutralizing antibodies prevented mortality upon subsequent infection of the lungs with both homologous and heterologous viruses, while hMPV glycoprotein G vaccination did not induce neutralizing activity [21]. Similar results were observed using an alphavirus replicon- or parainfluenza virus type 3 (PIV3)-based hMPV F vaccine [22,23]. It has been also recently demonstrated that animals vaccinated by intramuscular injection of adjuvanted soluble hMPV F proteins develop humoral immune response. However, such response diminished rapidly over time [24]. Recently, research from Dr. Williams' group demonstrated that hMPV

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VLPs obtained by expressing matrix (M) and F protein in suspension-adapted human embryonic kidney epithelial (293-F) cells provide protection against hMPV replication in the lungs of mice, and are not associated with a Th2-skewed cytokine response, suggesting non-replicating VLPs are a promising vaccine candidate for hMPV [25].

Other hMPV proteins which have been used for protein-based vaccine development includes P and G proteins [21,26]. A recombinant bacillus *Calmette-Guerin* (BSG, a carrier to promote immune response against antigens from other bacterial, parasitic, and viral pathogens) expressing hMPV P protein is able to confer strong effector phenotypes to both CD4+ and CD8+ T cells, which showed protective hMPV immunity equivalent to actively immunized animals. However several groups have suggested that hMPV G-based subunit did not develop protective antibodies, suggesting hMPV G is not important for immunogenicity [21,27,28]. Interestingly, studies using recombinant hMPV lacking G protein (rhMPV-ΔG) suggested that G protein plays an important role in inducing protective immune responses [29]. Although the results on the role of G in immunogenicity are still controversial, there are several possibilities may contribute to unsuccessful immunogenicity of G during the single protein immunization process. One possibility is that hMPV G undergoes certain modification on the level of gene and/or protein during the single protein immunization, similar to what has been described for RSV F protein [30]. Another possibility is that same carriers may have reduced ability to incorporate G than F [21]. Overall, whether G is important in immunogenicity still needs to be clarified.

Overall, the immunization using hMPV F-based subunit vaccine is promising; but more experiments are needed to determine the combination of inoculation routes, carrier forms, and length of F to induce the best immunogenicity efficacy and duration. Since other hMPV proteins are also important for immunogenicity and immune balance, subunit immunization requires more investigation on the effect of immunization on Th1/Th2/Th17 balance.

Live attenuated vaccines

Live attenuated vaccines can be divided into two groups: non-recombinant and recombinant. Non-recombinant live attenuated viruses are usually generated by natural mutations/deletions during viral passages in cells with or without experimental stresses such as chemical mutagenesis and clod passage [31-33]. The major risk of non-recombinant live attenuated vaccine is it's *in vivo* reversion and recovery of viral pathogenicity and subsequent disease development. Some non-recombinant live-attenuated RSV vaccines have been evaluated in clinical trials, but showed some side effects and also insufficient attenuation [34]. Temperature-sensitive hMPV strains have been generated recently by the group of Drs. Fouchier and Osterhaus. Immunized hamsters showed protective immunity [35].

The recombinant live-attenuated viruses are generated from the cells transfected with hMPV cDNA genome, with/without gene modification/deletion, along with plasmids encoding individual proteins essential for forming RNA-dependent RNA polymerase (RdPp) complex [36-38]. Recently, a wild type recombinant hMPV, with the codon optimization in SH, has been approved to be a suitable parent virus for development of live-attenuated HMPV vaccine candidates in experimental human infection trial [39]. The attenuation of recombinant hMPV has been achieved by the deletion of certain accessory genes. They are recombinant hMPV lacking G (rhMPV-ΔG), G and SH (rhMPV-ΔG/SH), and M2-2 (rhMPV-ΔM2-2) [29,40,41]. In infected hamsters, rhMPV-ΔG and rhMPV-ΔG/SH were at least 40-fold and 600-fold restricted in replication in the lower and upper respiratory

tract, respectively, compared to wild-type rhMPV. However, in rodent model, rhMPV lacking SH alone (rhMPV-ΔSH) replicated somewhat more efficiently in hamster lungs when compared to wild-type(WT)-rhMPV, indicating that SH is completely dispensable *in vivo* and that its deletion does not confer an attenuating effect.. In infected African green monkeys, the attenuation of rhMPV-ΔM2-2 reached higher level than that of rhMPV-ΔG, and had induce comparable immunogenicity and protective efficiency [41]. There is another attenuated recombinant hMPV whose P protein was replaced with avian MPV P protein. Although it is well attenuated, it was found to be poorly infectious in healthy adults [42].

Other factors should be considered in designing future vaccines

Although F protein is believed to be a major factor determining the immunogenicity of hMPV, identification of viral antigens that activate both protective cytotoxic T-lymphocyte (CTL) and humoral responses are still necessary to develop a successful vaccination strategy. Indeed, several CTL peptides have been proved to be important for CD8+ CTL responses to hMPV challenge. These peptides are [164]VGALIFTKL[172] from N for H-2[b] mice, [56]CYLENIEII[64] from M2-2 protein for H-2[d] mice, and [35]KLILALLTFL[44] from SH protein and [32]SLILIGITTL[41] from G protein for HLA-A*0201 transgenic mice. Vaccination with these hMPV CTL epitopes upregulates expression of Th1-type cytokines in the lungs and peribronchial lymph nodes of hMPV-challenged mice, resulted in reduced viral titers and disease in mouse models [43]. Given the importance of CTL epitopes in the immunogenicity, the deficiency of such epitope(s) by complete gene deletion in live attenuated rhMPV may contribute to the reduced ability of rhMPV to induce the immunogenicity. To prolong immunogenicity of F protein-based vaccination or to enhance the immunogenicity of deletion mutants of rhMPV, co-immunizing the host with peptides containing CTL epitopes may be a good option.

Identifying viral proteins which are important for antiviral signaling regulation is also critical in vaccine design. Recently, we identified that some viral proteins, such as G and M2-2, play a significant role in suppressing hMPV-induced host innate immunity [37,38,44,45]. Regarding M2-2 protein, we and others found that it is a protein with multiple functions. It not only regulates the viral gene transcription and viral RNA replication [37,40], but also contains a CTL epitope and targets central adaptors for RIG-I and TLRs [37,43,46,47]. In addition, M2-2 also plays a significant role in regulating the expression of miRNAs, some of which are important for the expression of immune related genes (Deng *et al.*, Data will be separately published soon). The multi-functions of viral protein(s) raise the need to identify the domains respectively responsible for their function, as it is important for rational design of live attenuated recombinant virus. Recently, we identified that the regulatory domains of M2-2 for viral gene and genome replication are different [37]. We also identified M2-2 motifs which are responsible for their inhibition on antiviral signaling (manuscript in preparation). All these pieces of information on M2-2 might provide a foundation to design M2-2-based live attenuated vaccine candidates. For example mutants containing mutations on 1) M2-2's viral replication domain for replication attenuation purpose, and 2) protein interactive motifs to abolish M2-2's suppression on antiviral signaling for immunogenicity enhancement. On the other hand, the domains which are important for the transcription of viral genes should not be modified in order to 1) minimize frequent mutations of other viral proteins [48], 2) prevent skewed Th1/Th2 balance [49], and 3) main all naïve CTL epitopes

for immunogenicity purpose [43], Overall, dissecting the functional domains of viral protein is essential for vaccine development.

Discussion

Overall, a variety of vaccination strategies have been explored to protect different groups from hMPV-induced respiratory illness. An efficient vaccine candidate should ideally be more immunogenic and protective than natural hMPV infection, which only launches incomplete immune protection. Studies in cotton rats revealed that immunization with FI-hMPV-induced enhanced pathology in the lungs of animals after subsequent infection with hMPV [16], excluding it as a promising candidate. Subunit vaccines are promising and safe, especially in the form of non-infectious carrier, for the risk groups such as immunocompromised individuals and the elderly. However, they seem to induce short protective immunity [24]. Current live attenuated hMPV vaccine is promising, as well. However, the balance between a satisfactory degree of attenuation and a satisfactory level of immunogenicity may be difficult to obtain. We are currently exploring the possibilities to identify major immune regulatory protein(s) and associated functional motifs with an aim to develop vaccine candidates with decent attenuation and less inhibition on host antiviral systems.

Acknowledgements

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