Review

Recent vaccine development for human metapneumovirus

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Human metapneumovirus (hMPV) and respiratory syncytial virus, its close family member, are two major causes of lower respiratory tract infection in the pediatric population. hMPV is also a common cause of worldwide morbidity and mortality in 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), a negative-sense single-stranded RNA virus, belongs to the Paramyxoviridae family that also includes respiratory syncytial virus (RSV) and parainfluenza virus (van den Hoogen et al., 2001). Soon after its discovery in 2001, hMPV was quickly recognized as a frequent cause for lower respiratory tract infections in young children, immunocompromised patients and older adults (Edwards et al., 2013; Englund et al., 2006; Esper et al., 2004; Falsey et al., 2003). Although hMPV is a clinically important pathogen, no vaccine is currently available.

hMPV comprises two genetic groups, A and B. Each group has two subgenetic classes, i.e. A1 and A2; B1 and B2. Currently, there are five contemporary circulating clades of hMPV, which have existed for decades (Gaunt et al., 2011; Papenburg et al., 2013). The antigenome of hMPV contains nine open reading frames for viral protein expression: 3'-N–P–M–F–M2-1–M2-2–SH–G–L-5'. Among them, the nucleoprotein N, phosphoprotein P, and large protein L are essential for RNA synthesis, which comprises two independent events: viral replication and gene transcription. Upon entry, the viral RNA-dependent RNA polymerase (RdRp), formed by the L and P proteins, binds the N-encapsidated genome at the leader region, then sequentially transcribes each gene by recognizing the start and stop signals flanking the viral genes. mRNAs are capped and polyadenylated during synthesis. Replication presumably starts when enough nucleoprotein is present to encapsidate neo-synthesized antigeneomes and genomes. 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 (Bermingham & Collins, 1999; Schildgen et al., 2011; van den Hoogen et al., 2002). In addition to P and L, the M2-2 protein is also important for viral RNA synthesis. Whether hMPV M2-2 is a key regulatory factor involved in the switch of the viral RNA polymerase from viral gene transcription to viral genome replication, as with the RSV M2-2 protein, is still controversial (Buchholz et al., 2005; Kitagawa et al., 2010; Ren et al., 2012). The phosphoprotein P, glycoprotein G, small hydrophobic protein SH and M2-2 protein have all been shown to modulate host immune responses (Bao et al., 2008a, b; Goutagny et al., 2010; Ren et al., 2012, 2014). Recombinant hMPVs, lacking G, SH and M-2, individually or in combination, or having P replaced with avian P, are attenuated (Biacchesi et al., 2005; Pham et al., 2005). Fusion protein F is essential for hMPV entry and also induces a strong humoral immune response (Cox et al., 2014). In this review, we will discuss the recent status and efforts for hMPV vaccine development based on the functions of these proteins. We hope all these studies will eventually decrease the medical burden caused by this clinically important pathogen.

Inactivated vaccines

Formalin-inactivated influenza is commonly used for mass immunization because it has good stability, is easy to manufacture and is biologically safe due to the absence of viral replication (http://www.cdc.gov/vaccines/hcp/vis/vis-statements/flu.html). However, the vaccination with a formalin-inactivated human RSV vaccine (FI-hRSV) led to enhanced disease upon natural infection (Kapikian et al., 1969; Kim et al., 1969). Enhanced disease probably resulted from (1) Th2-biased T-cell-memory responses (Boelen et al., 2000; Russell et al., 1997; Openshaw et al., 1992), (2) formaldehyde hypersensitivity (Moghaddam et al., 2006)
and/or (3) immature antibody production and its associated weak recognition of hRSV epitopes from natural infections (Delgado et al., 2009). Recently, a decrease in FI-hRSV enhanced disease by RSV G glycoprotein peptide was reported, suggesting the antibody specific to RSV G is critical for RSV pathogenesis control (Rey et al., 2013). Similarly, vaccine-enhanced pulmonary disease and Th2 response following hMPV challenge were also observed in animals vaccinated with formalin-inactivated hMPV (Hamelin et al., 2007; Yim et al., 2007), suggesting that formalin-inactivated hMPV may not be a suitable vaccine candidate.

Other inactivation methods are also being investigated for safe vaccine development. For example, a nanoemulsion-adjuvanted inactive RSV has been demonstrated to induce durable RSV-specific humoral responses, decrease mucus production and increase viral clearance without evidence of Th2 immune-mediated pathology (Lindell et al., 2011). Meanwhile, vaccinated mice exhibited an enhanced Th1/Th2 response. Although the safety of nanoemulsion-adjuvanted inactive RSV vaccine candidates needs to be further investigated (Mukherjee et al., 2011), nanoemulsion inactivation could be applied to hMPV if nanoemulsion inactivated hMPV is immunogenic and protective and has balanced immune responses.

**Viral protein-based vaccines**

Subunit vaccines are purified or expressed full-length or partial viral proteins. The expressed proteins are usually in a form of virus-like particles (VLPs), nanoparticles or with immune-enhancing adjuvants (Anderson et al., 2013). The most immunogenic protein in paramyxoviruses is mainly the fusion protein F. For RSV, a close family member of hMPV, its F in a form of nanoparticles is being evaluated in a phase II clinical trial by Novavax (Driscol, 2013).

Several animal studies using hMPV proteins as subunit vaccine candidates have been conducted recently. 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 (Lévy et al., 2013). Similar results were observed using an alphavirus replicon-based or a parainfluenza virus type 3 (PIV3)-based hMPV F vaccine (Mok et al., 2008; Tang et al., 2005).

It has also been demonstrated that animals vaccinated by intramuscular injection of adjuvanted soluble hMPV F proteins develop humoral immune responses. However, such responses diminished rapidly over time (Herfst et al., 2008b). Recently, Dr Williams’s group demonstrated that hMPV VLPs obtained by expressing matrix (M) and F protein in suspension-adapted human embryonic kidney epithelial (293-F) cells provide immune protection against hMPV replication in the lungs of mice and are not associated with a Th2-skewed cytokine response. Mice immunized with F-M-VLPs mounted an F-specific antibody response and generated CD8+ T cells recognizing an F-protein derived epitope. VLP immunization also induced a neutralizing antibody response, which was enhanced by the addition of either TiterMax Gold or an α-galactosylceramide adjuvant. These observations suggested that a fusion protein-based vaccine is a potential candidate for hMPV vaccine development (Cox et al., 2014).

Other hMPV proteins which have been used for protein-based vaccine development include the P and G proteins (Lévy et al., 2013; Palavecino et al., 2014). A recombinant bacillus Calmette–Gue´rin (a carrier to promote an 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 the hMPV G-based subunit did not develop protective antibodies, suggesting hMPV G is not important for immunogenicity (Lévy et al., 2013; Ryder et al., 2010; Skiadopoulos et al., 2006). Interestingly, studies using recombinant hMPV lacking G protein (rhMPV-ΔG) suggested that G protein plays an important role in inducing protective immune responses (Biacchesi et al., 2004b). Although the results on the role of G in immunogenicity are still controversial, there are several possibilities that may contribute to unsuccessful immunogenicity of G during the single protein immunization process. One possibility is that hMPV G undergoes certain modifications at the gene and/or protein level during single protein immunization, similar to what has been described for the RSV F protein (Yang et al., 2013). Another possibility is that the same carriers may have reduced abilities to incorporate G than F (Lévy et al., 2013). Overall, whether G is important in immunogenicity still needs to be clarified.

Immunization using hMPV F-based subunit vaccines is promising, but more experiments are needed to determine the combination of inoculation routes, carrier forms and the length of F required 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 effects 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 or deletions during viral passages in cells with or without experimental stresses such as chemical mutagenesis and cold passage (Crowe, et al., 1995; Juhasz et al., 1997; Whitehead et al., 1998). The major risk of non-recombinant live attenuated vaccines is 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 (Wright et al., 2000). Temperature-sensitive hMPV strains have recently been generated, and immunized hamsters showed protective immunity (Herfst et al., 2008a).

Recombinant live attenuated viruses are generated from the cells transfected with the hMPV cDNA genome, with or without gene modification or deletion, with plasmids encoding individual proteins essential for forming the RdPp complex (Bao et al., 2008b; Biacchesi et al., 2004a; Ren et al., 2012). 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) (Biacchesi et al., 2004b, 2005; Buchholz et al., 2005). 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 tracts, respectively, compared to wild-type rhMPV. However, in a 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 a higher level than that of rhMPV-ΔG and had induced comparable immunogenicity and protective efficiency (Biacchesi et al., 2005). There is another attenuated recombinant hMPV whose P protein was replaced with avian MPV P protein (rhMPV-Pavian). Recently, a wild-type recombinant hMPV has been approved to be a suitable parent virus for the development of live attenuated hMPV vaccine candidates in experimental human infection trials (Talaat et al., 2013). Although rhMPV-Pavian was found to be poorly infectious in healthy adults (Schmidt, 2011), the mechanisms associated with poor infectivity of rhMPV-Pavian, and whether other attenuated recombinant viruses have similar human infectivity issues, need to be investigated in the future.

Other factors should be considered in designing future vaccines

Although the F protein is believed to be a major factor determining the immunogenicity of hMPV, the identification of viral antigens that activate both protective cytotoxic T-lymphocytes (CTL) and humoral responses is 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 164VGALIFTK172 from N for H-2b mice, 56CYLENIIEI64 from the M2-2 protein for H-2d mice, 81KLIALLTL41 from the SH protein and 32SLILIGITTL41 from the 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, resulting in reduced viral titres and disease in mouse models (Herd et al., 2006). Recently, dominant and subdominant hMPV H-2d epitopes were screened and identified (Melendi et al., 2007). Among 12 selected predicted epitopes, 81GYIDDQNSI89 of M2-1 and 38SPKAGL515 of N were identified to be dominant and subdominant H-2d epitopes during primary infection. In addition, a wide diversification of H-2d-restricted epitopes in the memory CTL response after secondary and third infection were also identified, including a subdominant role for the memory CTL response against 56CYLENIIEI64 of M2-2. Given the importance of CTL epitopes in immunogenicity, the deficiency of such epitope(s) by complete gene deletion in live attenuated rhMPV may lead to the reduced ability of rhMPV to induce immunogenicity. To prolong the 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.

Since the epitopes identified in the N and M2-2 proteins are completely conserved, the protection afforded by a CTL epitope vaccine is expected to extend to both hMPV strains. Identifying viral proteins which are important for antiviral signalling regulation is also critical in vaccine design. Recently, we identified that some viral proteins, such as G and M2-2, play significant roles in suppressing hMPV-induced host innate immunity (Bao et al., 2008b, 2013; Kolli et al., 2011; Ren et al., 2012). For M2-2 protein, we and others found that it is a protein with multiple functions. It not only regulates viral gene transcription and viral RNA replication (Buchholz et al., 2005; Ren et al., 2012), but also contains a CTL epitope and targets central adaptors for retinoic acid-inducible gene I (RIG-I) and Toll-like receptors (TLRs) (Herd et al., 2006; Kitagawa et al., 2010; Ren et al., 2012, 2014). 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., 2014). The multi-functions of viral protein(s) raise the need to identify the domains 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 (Ren et al., 2012). We also identified M2-2 motifs which are responsible for their inhibition on antiviral signalling (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 purposes and (2) protein interactive motifs to abolish M2-2’s suppression on antiviral signalling, 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 minimize frequent mutations of other viral proteins (Schickli et al., 2008), prevent a skewed Th1/Th2 balance (Becker, 2006) and maintain all naïve CTL epitopes for immunogenicity purposes (Herd et al., 2006).
Overall, dissecting the functional domains of viral proteins is highly desirable for vaccine development.

Discussion

An efficient vaccine candidate should ideally be safe, and more immunogenic and protective than natural hMPV infection, which only launches incomplete immune protection. Studies in cotton rats revealed that immunization with Fl-hMPV enhanced pathology in the lungs of animals after subsequent infection with hMPV (Yim et al., 2007), excluding it as a promising candidate. Subunit vaccines seem to induce short protective responses to primary infection (Herfst et al., 2008b). However, they may be useful to boost the immune response in individuals who have been previously exposed to hMPV. In addition, subunit vaccines are promising and safe, especially in the form of non-infectious carriers, to provide a certain duration of protection for risk groups such as premature infants, immunocompromised individuals and the elderly. Current live attenuated hMPV vaccines are promising, especially for infants and young children. 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 of identifying the major immune regulatory protein(s) and associated functional motifs with an aim to developing vaccine candidates carrying decent attenuation and immunogenicity. Overall, a variety of vaccination strategies have been explored and tested in rodent models and non-human primate models. However, none has yet been tested in human volunteers. Recent experimental infection of adults with recombinant wild-type hMPV have been performed, demonstrating the possibility of testing the immune efficiency of live attenuated recombinant hMPV in humans in the future.

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