ABSTRACT  Human metapneumovirus (HMPV), a paramyxovirus identified in 2001, is a leading cause of respiratory tract infections in both children and adults. Seroprevalence studies demonstrate that the primary infection occurs before the age of 5 years, and humans are reinfected throughout life. The four subgroups of HMPV occur with year-to-year variability, and infection with one subgroup confers some serologic cross-protection. Experimental vaccines elicit a humoral response in both animal and human models and have been used to identify antigenic determinants. The main target of protective antibodies is the fusion (F) protein, although many of the remaining eight proteins are immunogenic. Monoclonal antibodies (mAbs) targeting the F protein are both protective and therapeutic in animal models. Most recently, the identification of broadly neutralizing antibodies against HMPV and respiratory syncytial virus demonstrates that common epitopes are present between the two viruses. Broadly neutralizing mAbs have significant clinical implications for prophylaxis and treatment of high-risk hosts as well as vaccine development.

INTRODUCTION  Human metapneumovirus (HMPV), a paramyxovirus first discovered in 2001, is a significant cause of respiratory tract disease in children and adults (1). Humoral immunity plays an important role in HMPV infection, and the study of HMPV antibodies provides important clinical information including the seroprevalence of HMPV, age of primary infection, serological cross-protection between HMPV subgroups, evaluation of vaccine immunogenicity, and strategies for prophylaxis and therapy using monoclonal antibodies (mAbs).

SEROPREVALENCE OF HMPV INFECTION  Primary HMPV infection in children can be determined by seroconversion, which typically occurs within the first 1 to 2 years of life. In an Israeli cohort, 80% of children 2 months of age had evidence of HMPV antibodies, reflective of the broad adult seroprevalence and maternal transmission of immunity. Consistent with waning maternally derived antibodies, only 30% of children at 13 months were seropositive. However, 52% of children had HMPV antibodies at 24 months, suggesting that primary infection had occurred (2). By school age, nearly all children have been infected with HMPV. In a Japanese cohort, 77% of children 2 to 5 years of age were seropositive and 100% of children >10 years of age had HMPV antibodies (3). Reinfection occurs throughout childhood and can be assessed by repeated measurements of serology. In one cohort, HMPV-infected children had both a positive IgM and IgG at the time of acute infection, indicating a history of previous infection. These children had a 4-fold IgG rise in convalescent serology, suggesting that a positive IgG is not entirely protective (4). In a cohort of Thai children, 99.7% had positive HMPV serology. As they were followed longitudinally, 5% had evidence of reinfection, as defined by a 4-fold serology rise, during the 4-year study period (5). HMPV seroconversion typically occurs later than respiratory syncytial virus (RSV). In children aged 4 to 11 months, 48% had positive RSV antibody titers, but only 11% had anti-HMPV

Received: 10 July 2014, Accepted: 15 July 2014, Published: 10 October 2014

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antibodies. In children over the age of 2 years, seroprevalence is similar (6, 7).

Almost all adults have serologic evidence of prior HMPV infection. Older adults are at increased risk of severe disease, which some researchers postulate is due to waning immunity (8). However, in a German cohort, >90% of people 60 to 89 years of age had neutralizing antibodies (9). Similarly, Japanese adults were followed longitudinally to determine whether the presence of HMPV antibody was protective. Nine subjects with baseline positive titers by enzyme-linked immunosorbent assay (ELISA) and neutralization assay became infected with HMPV during the study period. Infection was associated with a rise in antibody titer by both methods, demonstrating that a positive antibody titer is not fully protective (10).

Last, seroconversion can be used to detect asymptomatic HMPV infection in adults. In one study, young adults had the highest rates of asymptomatic infection at 4% (8).

**ANTIBODY SPECIFICITY**

Avian pneumovirus (APV) is the other member of the *Metapneumovirus* genus, and researchers postulate that HMPV diverged from APV type C 200 to 400 years ago (11, 12, 13). The N protein is 80% conserved between APV/A, B, and C, and 100% conserved between APV/C and HMPV. An N-protein polyclonal antibody, which targets a conserved peptide, cross-reacted with all members of APV and HMPV, while an anti-N mAb cross-reacted with both APV/C and HMPV (14). Sera from animals infected with APV/C cross-reacted with HMPV fusion (F) protein, and sera from HMPV-infected animals cross-reacted with APV/C F protein. However, HMPV polyclonal sera did not cross-react with APV/A or B (15), consistent with the closer relationship of HMPV to APV/C. Sera from animals infected with RSV, a member of the same subfamily as HMPV, do not neutralize HMPV in vitro and vice versa (16). Thus, HMPV is serologically distinct from other related pneumoviruses.

**ANTIBODY CROSS-PROTECTION**

Four genetic subgroups of HMPV (A1, A2, B1, and B2) circulate with year-to-year variability (17) (Fig. 1). The subgroups are antigenically related with some evidence of cross-neutralization (18). In hamsters, serum neutralization titers showed a 48% antigenic relatedness between subgroups A and B, and, in nonhuman primates, titers were 64 to 99% related (19). HMPV-infected hamsters were protected against subsequent challenge with a heterologous subgroup virus. Sera from infected hamsters neutralized both homologous and heterologous subgroups in vitro; however, the neutralizing capacity against the heterologous subgroup was reduced by 16-fold. African green monkeys and macaques displayed similar cross-protection (20, 21). In humans, sera from HMPV-infected children cross-neutralized other subgroups with varying degrees of efficacy. For example, sera from an HMPV A2-infected child neutralized A2 and B2 viruses, but had no activity against B1 in vitro (22).

The F protein is highly conserved and immunogenic (13, 18, 23). Antigenic mapping studies using mAbs defined a number of antigenic regions on HMPV F analogous to sites described on RSV F (Fig. 2) (24, 25). Sera from hamsters infected with recombinant parainfluenza 1 (rPIV1) expressing HMPV F neutralized both homologous and heterologous HMPV viruses in vitro. Thus, the F protein is a mediator of antibody cross-protection, and it likely mediates some, although not full, protection against reinfection (19).

Unlike F, the glycoprotein (G) is highly variable and contains only 31 to 35% similarity between the A and B subgroups (12, 26). Convalescent sera from HMPV-infected children reacted with only one G protein, presumably the infecting subgroup. This specificity would suggest that the different HMPV subgroups could be considered serotypes if only categorized by the G protein (27); however, F is thought to be the primary target of neutralizing and protective antibodies. Several different approaches have shown that G antibodies are not neutralizing in vitro or protective in vivo (28, 29, 30).

**ANTIBODY RESPONSE TO INFECTION**

Twenty-one days postinfection, HMPV-infected cotton rats developed a neutralizing antibody response. The average serum-neutralizing titer was 1:180, which correlated with lung protection (31). In macaques infected with wild-type (WT) HMPV, antibody titer and efficacy waned over time, leading to symptomatic reinfection. Repeat inoculations of HMPV boosted neutralizing antibodies, but, despite boosting, antibody titer and efficacy waned over time, leading to symptomatic reinfection. Repeat inoculations of HMPV boosted neutralizing antibodies, but, despite boosting, antibody titer and efficacy waned over time, leading to symptomatic reinfection.
FIGURE 1 Maximum clade credibility tree of HMPV and avian metapneumovirus (AMPV) F nucleotide diversity. Phylogenetic analysis of 85 full-length HMPV F nucleotide sequences from Canada (CAN), Japan (JPS or JPY), Tennessee (TN), or the Netherlands (NL) and 16 AMPV F sequences. The first two digits of the HMPV sequence names indicate the year of the isolate. The names of the AMPV sequences indicate geographic origin (US, United States; UK, United Kingdom; MN, Minnesota) and year. The posterior probability of divergence is indicated at each node. Scale bar represents time in years. Reprinted from reference 13 with permission of the publisher http://creativecommons.org/licenses/by/2.0/legalcode. doi:10.1128/microbiolspec.AID-0020-2014.f1
Interestingly, infection with NL/1/00, a subgroup A virus, induced higher neutralizing titers than NL/1/99, a subgroup B virus (21). Thus, animal studies suggest that, while antibodies can mediate protection, titers wane over time. This phenomenon likely contributes to the capacity of HMPV to reinfect humans throughout life.

AGE-RELATED ANTIBODY DEVELOPMENT

Older adults are an at-risk population for severe respiratory viral infections, including HMPV. One contributing factor is thought to be waning humoral immunity. In a mouse model, both virus-specific and neutralizing antibody responses were higher in younger mice infected with HMPV than in older mice (32). However, in a cohort of nonhospitalized adults infected with HMPV, older adults had a significantly higher acute HMPV titer compared with young adults. Older adults also had a significantly higher convalescent titer contributing to a significantly higher overall rise in titer compared with young adults. Older adults had a trend toward a higher rise in neutralizing antibody, although this was not significant (33).

IMMUNOGLOBULIN CLASSES

IgG and IgA are produced after HMPV infection. In a BALB/c mouse model, IgG1 and IgG2a were detectable 5 days after HMPV infection, and antibody titer peaked at day 8. No IgA or IgE was detected (34). IgG2a antibodies were the dominant immune response in mice immunized with viruslike particles (VLPs) (35).

In an adult cohort, HMPV titers in patients with acute infection were compared with HMPV titers in uninfected patients. Serum IgG titer, but not nasal IgA, was associated with the likelihood of developing HMPV reinfection (11.93 ± 1.25 log2 in infected individuals vs 12.86 ± 1.23 in uninfected individuals, P = 0.001). Although infected adults had lower HMPV IgG titers, the protective level of IgG remains unclear. HMPV-infected
adults had significantly lower neutralizing titers compared with uninfected adults. In a longitudinal study, 71% of adults with a baseline microneutralization assay (MNA) titer of ≤10.5 log₂ were infected compared with 36% infected among adults with a baseline MNA titer >10.5 log₂ (33). These data suggest that there may be a minimum protective threshold of serum-neutralizing antibody titer.

**INDUCTION OF ANTIBODIES BY IMMUNIZATION**

An effective HMPV vaccine will need to induce an antibody response, and the goal is a high neutralizing antibody titer. Potential types of immunizations include live attenuated HMPV strains, inactivated virus, DNA and protein vaccines, and VLPs. DNA and protein vaccines and VLPs will be discussed separately under antigenic proteins.

Live attenuated HMPV vaccines include strains containing gene deletions or other attenuating mutations. Golden Syrian hamsters infected with HMPVΔSH (deleted short hydrophobic protein) mounted a similar neutralizing antibody response to rHMPV; however, the deletion mutant was not attenuated. Hamsters infected with the attenuated viruses HMPVΔG and HMPVΔSH/G mounted a neutralizing antibody response, but at 6-fold lower titer than rHMPV, and the animals were not fully protected from challenge (36). Therefore, the SH protein is not immunogenic and the G protein is weakly immunogenic. Other immunogenic, attenuated viruses include temperature-sensitive HMPV, which was immunogenic and protective in hamsters (37); a virus lacking an N-linked carbohydrate in the F protein, which was immunogenic and protective in mice (38); and viruses with the F protein RGD-binding sequence mutated, which was immunogenic and protective in cotton rats (39). The conserved RGD motif of the F protein serves to bind integrins as receptors for HMPV (40, 41).

In a phase 1 clinical trial, humans with negative HMPV serology were infected with rHMPV-SH (containing a stabilization mutation in the SH protein). Only 15% had a ≥4-fold increase in IgG to HMPV F, 20% had a ≥4-fold increase in serum HMPV F IgA, and 30% had a ≥4-fold rise in nasal wash IgA (42). Thus, the development of a live attenuated vaccine that will produce an effective neutralizing antibody response in previously infected adults may be difficult.

Formalin-inactivated (FI) HMPV is immunogenic in cotton rats. The vaccine induced neutralizing antibodies, and animals were protected against HMPV challenge (43). However, the vaccine was less effective in macaques. One dose of FI-HMPV induced binding antibodies, but 2 doses were needed to yield neutralizing antibodies. Antibody titer rapidly declined 4 weeks later, and animals were not protected against challenge 15 weeks after a third immunization (44). Furthermore, FI-HMPV was associated with enhanced disease upon HMPV challenge in cotton rats and macaques, similar to that observed with FI-RSV vaccine in the 1960s (43, 44).

**ANTIGENIC PROTEINS**

The HMPV genome contains 8 genes that encode 9 proteins (1). The F, G, and SH protein are outer membrane proteins and likely targets for antibodies. To identify antigenic determinants, each of these 3 proteins was inserted into rPIV-1, a model vaccine system. In hamsters, immunization with PIV-1/HMPV F induced an antibody titer similar to WT HMPV and a high level of neutralizing antibodies, which conferred lower airway protection. The G protein, expressed by rPIV-1, was less immunogenic than WT HMPV and did not induce neutralizing antibodies. Last, the SH protein did not induce any binding or neutralizing antibodies. Neither rPIV-1/G nor rPIV-1/SH were associated with significant protection against viral replication upon HMPV challenge. Therefore, the F protein is the main antigenic determinant (28).

In Syrian golden hamsters, chimeric bovine/human PIV3 containing HMPV F induced a neutralizing antibody response equivalent to WT HMPV infection (45). This chimeric virus also was immunogenic in African green monkeys, but neutralizing antibody titers were lower compared with animals infected with WT HMPV (46).

Similarly, soluble F-protein vaccines, generated from both A and B subgroup lineage, are protective in hamsters, and F-specific antibodies were present in all animals. Animals immunized with the A-lineage F protein had higher titers compared with B-lineage F, and an adjuvanted F protein was more immunogenic than an unadjuvanted F protein. Adjuvanted F induced a high neutralizing antibody titer (47). F-protein vaccines were more immunogenic than DNA vaccines in a cotton rat model, and the protein vaccine yielded high neutralizing antibody titers (mean 1:570) (23). VLPs containing the F and matrix (M) proteins are immunogenic and protective in a mouse model. After two immunizations, animals had an F-specific antibody response similar to WT HMPV and were protected against challenge (48).
Although the F protein appears to be the primary driver of humoral immunity, the G protein does induce an antibody response. In a cotton rat model, soluble G protein was immunogenic, but it was not protective (30). Mice immunized with the G protein in a vaccinia virus system developed a G-specific antibody response that neutralized homologous but not heterologous virus (49). However, VLPs expressing the G protein were not immunogenic, unlike VLPs containing both the F and G proteins (35). Although SH is generally not immunogenic, a truncated version of the protein expressed by vaccinia virus induced a modest neutralizing antibody response (49).

In humans, the antibody response is much more diverse. In an Irish cohort, antibodies to HMPV M, nucleoprotein (N), and phosphoprotein (P) were identified (50). Antibody responses to N and M typically occurred only in sera with high neutralizing titers (generally >1:160) (51). Other groups have postulated that many F and G antibodies may be conformation dependent, whereas antibodies to N and P may recognize polypeptides (52). Thus, an effective vaccine strategy might include whole outer membrane proteins in conjunction with immunogenic areas of internal proteins.

**MONOCLONAL ANTIBODIES**

Since the HMPV F protein is highly immunogenic and protective, F-specific mAbs could have clinical use in high-risk hosts, similar to the respiratory syncytial virus anti-F protein mAb, palivizumab (53). Murine mAbs generated against the F protein of HMPV neutralized both A and B subgroups of the virus. Two of these mAbs were effective prophylactically in a hamster model. Although viral replication was minimally decreased in the nasal turbinates, hamsters did not have replicating virus in the lungs at doses of 3 mg/kg. These two mAbs, 234 and 338, bound to the F protein of both the A and B subgroups with nanomolar affinity (54). Mice prophylaxed or treated postinfection with mAb 338 had decreased lung histopathology and airway obstruction compared with mock treated mice (55). DS7, a fully human mAb, neutralized all four subgroups of HMPV in vitro, and intranasal DS7 had therapeutic efficacy against HMPV infection in a cotton rat model. The DS7 mAb also had subnanomolar affinity for the F protein (24).

Monoclonal antibody resistant mutants (MARMs) have identified 5 epitopes on the F protein. Two of these epitopes have analogous epitopes on the RSV F protein (Fig. 2) (25). Crystallization of DS7 with the F protein identified a novel antigenic site on the protein, which is a highly conserved area among HMPV subgroups (56).

The F protein is only 33% conserved between RSV and HMPV, and polyclonal sera against one does not cross-react with the other (1, 16). However, one human mAb isolated from screening 114,000 B cells neutralized both viruses. This mAb, MPE8, is an anti-RSV F mAb in its germ line configuration, but it contains light chain mutations in the variable region, affording it activity against HMPV. MPE8 had prophylactic efficacy against HMPV and RSV and prevented death in a mouse model of pneumonia virus of mice, another paramyxovirus. The epitope is distinct from, but in close proximity to, the epitope recognized by palivizumab (57). Another group reported a human mAb isolated by screening B cells against HMPV F protein by ELISA (58). This mAb, 54G10, exhibited broadly neutralizing activity in vitro against all 4 subgroups of HMPV and bound to recombinant HMPV F protein with subnanomolar affinity. 54G10 provided potent prophylactic efficacy against all 4 HMPV subgroups in a mouse model, as well as therapeutic efficacy (tested only against A2). The generation of MARMs identified the 54G10 epitope as a region relatively conserved in RSV F and other paramyxoviruses (59). Consistent with a shared epitope, 54G10 neutralized RSV in vitro and exhibited both prophylactic and therapeutic efficacy in a mouse model (58). The discovery of mAbs with activity against both HMPV and RSV raises the possibility of not only clinical antibodies that target both viruses, but also epitope-based vaccines that elicit neutralizing antibodies against two distinct viruses.

**CONCLUSIONS**

Humoral immunity is important in HMPV infection. Further studies are needed to elucidate the level of protective antibody, both after infection and after immunization, as well as to explore the utility of monoclonal antibodies for prophylaxis and therapy against HMPV.

**ACKNOWLEDGMENT**

Conflict of interest: We disclose no conflicts.

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Human Metapneumovirus


