Human Metapneumovirus: An Important Cause of Respiratory Disease in Children and Adults

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Human metapneumovirus is a paramyxovirus that was discovered in 2001 in the Netherlands. Epidemiologic studies have shown it to be a major cause of acute respiratory tract disease in normal infants and children worldwide, with a seasonal occurrence and spectrum of clinical illness most similar to the closely related respiratory syncytial virus. The greatest prevalence of severe disease requiring hospitalization in otherwise healthy children appears to be in those aged between 6 and 12 months, older than the peak age of hospitalizations for respiratory syncytial virus. Human metapneumovirus is also a significant cause of acute respiratory disease in adults, particularly the elderly and those with comorbid conditions such as chronic obstructive pulmonary disease, asthma, and cancer. Because there is no rapid diagnostic assay, reverse transcriptase polymerase chain reaction is most widely used. Animal models have been developed, and candidate live-attenuated vaccines are in preclinical trials, offering the potential for future interventions in high-risk groups.

Introduction

Lower respiratory infection (LRI) is a leading cause of morbidity and mortality worldwide, especially in children [1]. Upper respiratory infection (URI), although inherently less serious, nonetheless carries significant societal costs in terms of lost work and school days, and accounts for a large number of health care visits, including unnecessary antibiotic prescriptions. Thus, determining the etiologic agents of these common infections has been the subject of much important research. Decades of epidemiologic studies have established the importance of known viral pathogens such as respiratory syncytial virus (RSV), parainfluenza viruses (PIVs), influenza virus, coronaviruses, and rhinoviruses [2,3]. However, these studies have been unable to identify a specific virus in over 50% of such infections, using traditional methods of viral culture, serology, and newer rapid antigen assays. Previously it was unknown whether these represented infections with known viruses that were not detected by existing assays, or unknown agents. Therefore, the report of a novel paramyxovirus, human metapneumovirus (hMPV), associated with respiratory tract disease by Dutch researchers in 2001 [4••] was a major finding for the field of respiratory virus research. The speed and efficiency with which subsequent studies have elucidated the epidemiology and biology of hMPV illustrate the capabilities of modern medical science.

The Initial Discovery of Human Metapneumovirus

The Dutch group collected a number of unidentified virus isolates, mostly from children, over a 20-year period that grew poorly in cell culture with minimal cytopathic effects (CPEs). The virus isolates could not be identified by hemagglutination and immunofluorescence assays typically used to identify common viruses such as RSV, influenza virus, and PIV. Electron micrograph and biochemical studies of the virus showed that it was pleomorphic with a lipid envelope, consistent with a paramyxovirus. Elegant reverse transcriptase polymerase chain reaction (RT-PCR) experiments yielded extensive genetic sequences from the virus that clearly identified it as a member of the paramyxovirus family, which contains many important human viruses (Table 1). The gene order and putative open reading frames of the new virus were most closely related to avian pneumovirus, the sole known member of the metapneumovirus genus. Avian pneumovirus, discovered in 1979, is a major agricultural pathogen, causing severe respiratory disease in chickens and turkeys, and ensuing economic losses. However, the new hMPV was unable to infect chickens and turkeys, and combined with sequence comparison, this showed that it was a distinct human pathogen. The same group of investigators also conducted serologic assays on 192 archived human sera from 1958 and found that 100% of specimens from patients aged older than 5 years were seropositive for hMPV, suggesting a

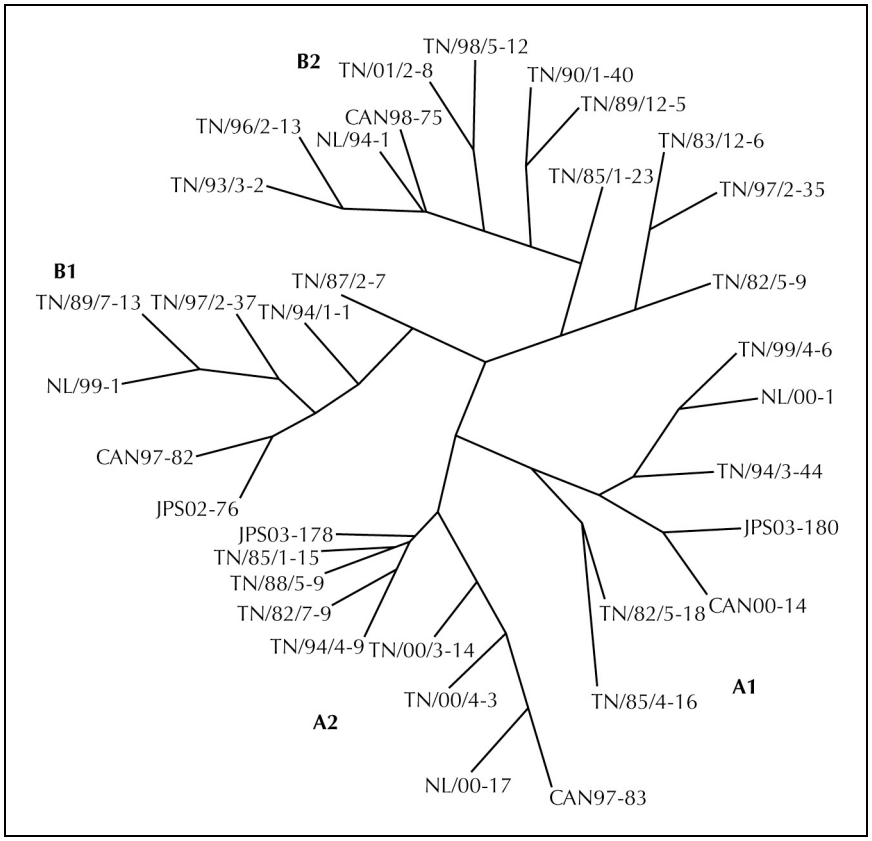

Figure 1. Phylogenetic tree of human metapneumovirus (hMPV) isolates based on fusion (F) gene. Selected full-length sequences of F genes from hMPV strains from Canada (CAN), Japan (JPS), the Netherlands (NL), and Tennessee (TN) were aligned using ClustalW. CAN, JPS, and NL sequences obtained from GenBank (phylogenetic tree was generated by parsimony methods using DNAPARS, and consensus tree was derived using CONSENSE in the Phylip 3.6 package [50]).

high rate of infection early in life, and showing that hMPV had been circulating for at least 50 years. This is in contrast with Nipah virus or the severe acute respiratory syndrome coronavirus, which are thought to be truly novel human pathogens that crossed species from animal origins.

Virology and Genetic Diversity

Human metapneumovirus has a negative-sense, single-stranded RNA genome like other paramyxoviruses, with a lipid envelope containing membrane proteins. The hMPV genome contains open reading frames analogous to all those of the most closely related human virus RSV, except the nonstructural genes NS1 and NS2, which have been shown to modulate host interferon response. The effect of these genes' absence on hMPV pathogenesis is not yet known, but studies will likely also help more fully elucidate their role in RSV pathogenesis. Partial gene sequences for many hMPV strains worldwide are now available, and phylogenetic analysis of these sequences defines two major genetic subgroups of hMPV, A and B, each with two minor subgroups (Fig. 1). These are defined presently as genogroups; it has not been shown in humans that they are antigenically distinct. The fusion (F) protein, the major protective antigen for other paramyxoviruses, is highly conserved in hMPV, with 93% to 95% amino acid identity between major subgroups and approximately 97% amino acid identity within subgroups. In contrast, the glycoprotein (G), another major immune target in other paramyxoviruses, is only 30% to 35% conserved between major subgroups of hMPV, and 70% within subgroups. In one large prospective study, primary infection during infancy was associated with LRI and subsequent URIs, demonstrating that reinfection with hMPV occurs [5••]. It is not clear whether this is entirely attributed to infections with different subgroups that do not induce cross-protective immunity, or more likely, as is the case with RSV, to partial immunity induced by primary infection that protects the lower but not the upper respiratory tract against subsequent infection. There are no definitive data in humans to show that infection with a virus from one subgroup can protect against reinfection with a virus from a different subgroup. However, animal studies of the antigenic diversity between hMPV subgroups suggest that there is cross-protective immunity in hamsters and primates that protects the lungs against reinfection [6,7]. These studies have important implications for the development of candidate vaccines and prophylactic antibodies against hMPV similar to palivizumab, a monoclonal antibody licensed for immunoprophylaxis against RSV for premature infants at high risk for severe disease.

Epidemiology

Initial epidemiologic studies of hMPV were primarily retrospective analyses by RT-PCR of specimens obtained by diagnostic virology laboratories that were previously negative for other viruses [8–10]. In these studies, the

Table 1. Paramyxovirus family

Subfamily Paramyxovirinae
Genus *Respirovirus*
Parainfluenza virus 1, 3
Genus *Rubulavirus*
Mumps
Parainfluenza virus 2, 4
Genus *Morbillivirus*
Measles
Genus *Henipavirus*
Hendra virus
Nipah virus
Subfamily Pneumovirinae
Genus *Pneumovirus*
Respiratory syncytial virus
Genus *Metapneumovirus*
APV (TRTV = APV)
Human metapneumovirus

APV—avian pneumovirus; TRTV—turkey rhinotracheitis virus.

percentage of hMPV detection varied from 6% to 15%, with hospitalization most prominent in infants and the elderly. A prospective study in Hong Kong enrolled 587 children (aged ≤18 years) hospitalized for LRI over a 13-month period and found that 6% of the children tested positive for hMPV, compared with 8% for RSV and 8% for influenza virus [11•]. A Canadian group of investigators detected hMPV in 12 of 208 (6%) children aged younger than 3 years and hospitalized for acute respiratory infection [12]. A large prospective study of over 2000 previously healthy outpatient infants and children in Tennessee found that 12% of all LRI overall in that cohort was attributable to hMPV [5••]. In that longitudinal study conducted over a 25-year period, there was substantial year-to-year variation in the prevalence of hMPV, ranging from none to 31% of otherwise negative samples in a given year. As in the Dutch study, hMPV was rarely detected in nasal washes from asymptomatic patients.

Studies that conduct testing over multiple seasons show that 1) the annual prevalence of hMPV varies widely from year to year, suggesting periodic epidemics, and 2) strains from different genogroups frequently circulate concurrently in a single season. In temperate zones, the seasonal peak of hMPV infections occurs in late winter and spring months, overlapping with (but slightly later than) the peak of RSV infections in most studies.

A number of studies of children hospitalized with acute respiratory disease in many countries have found rates of hMPV associated with between 6% and 40% of acute respiratory illness in a given season [12–24,25•,26,27]. The average prevalence in most pediatric populations with acute respiratory disease studied is approximately 5% to 10% overall, although it may be much higher during the peak months of hMPV circulation. With few exceptions, hMPV ranks after RSV in most studies and has prevalence comparable to that of influenza virus and PIV.

Hospitalization of children for hMPV infection occurs primarily in the first year of life, although a number of studies reported that the peak age of hospitalization for hMPV is from 6 to 12 months [10,11•,12,14–16,21–24,25•,26] and thus significantly later than the 2 month peak age of hospitalization for RSV. Whether this reflects a difference in the decay of maternally acquired antibodies, later acquisition of hMPV infection, or developmental airway physiology is unknown. Males appear to be at greater risk for LRI with hMPV infection compared to females, as has been observed for RSV infections in childhood.

Human metapneumovirus may be more severe in patients with underlying medical conditions. Although most studies of hMPV epidemiology are hospital populations, and thus subject to selection bias, 30% to 85% of children hospitalized with hMPV have chronic conditions, such as asthma, chronic lung disease caused by prematurity, cardiac disease, or cancer. In many studies, the rate of chronic disease was higher in hMPV-infected children than in RSV-infected children [10,12,16,17,22–24,25•]. For example, a large prospective study examined 641 children aged younger than 5 years hospitalized in two US cities during 1 year and found that 54% of children hospitalized with hMPV had underlying conditions, versus 29% of RSV-infected patients ($P < 0.05$) [25•].

Human metapneumovirus infection has been associated with acute respiratory disease in adults as well, although at generally lower rates than in children. In a prospective study of inpatients and outpatients in Rochester, New York, rates of hMPV infection were similar in young healthy adults and older patients, but frail elderly patients infected with hMPV were significantly more likely to seek medical attention [28]. In that study, hMPV accounted for 11% of all hospitalizations for acute respiratory illnesses in older patients, and 85% of the hospitalized adults had chronic heart or lung disease. This observation and results of other adult studies are consistent with the pediatric data cited, suggesting that hospitalizations for LRI attributed to hMPV are most frequently observed in patients with chronic underlying conditions, such as asthma, chronic obstructive pulmonary disease, or cancer [8,28,29].

Clinical Features

Human metapneumovirus has been associated with a variety of respiratory symptoms and diagnoses. Children with hMPV infection typically present with upper respiratory symptoms such as rhinorrhea, cough, and fever. The duration of symptoms before seeking medical attention is usually less than 1 week, and most children shed virus for approximately 2 weeks [5••,10,19]. Nonrespiratory symptoms such as conjunctivitis, vomiting, diarrhea, or rash are occasionally reported but are not prominent in most studies. Only one study in Italy found evidence by RT-PCR of hMPV in patients' serum [14], suggesting that like RSV, hMPV infection is limited to the respiratory tract. The clinical LRI syndromes most

frequently associated with hMPV are bronchiolitis, croup, pneumonia, and asthma exacerbation. In the Tennessee study of previously healthy outpatients, the hMPV-infected children were diagnosed with bronchiolitis (59%), croup (18%), asthma (14%), and pneumonia (8%) [5••]. However, in that prospective study, hMPV was less likely to be associated with croup than was PIV, and less likely to be associated with pneumonia than was influenza virus. A similar spectrum of diagnoses is seen in most studies of LRI associated with hMPV, and hMPV infection overlaps clinically with other common respiratory viruses sufficiently so that reliable distinction cannot be made at the bedside.

In a study of over 2400 distinct episodes of URI in previously healthy outpatient children, hMPV was associated with URI at rates similar to RSV, PIV, and influenza virus (Williams *et al.*, Unpublished data). Fifty percent of the hMPV-infected children were diagnosed with acute otitis media (AOM), suggesting that hMPV is associated with a substantial proportion of AOM in otherwise healthy children. AOM is the most common indication for antibiotic prescriptions in outpatient children, and URI and AOM have significant economic impacts due to time lost from school and work. Thus, hMPV likely has substantial medical and economic effects nationally. The only published study to directly examine the societal impact of hMPV was an Italian study of 42 hMPV-infected children seen in the emergency department in which questionnaires were administered to patients' parents [26]. In 12% of patients' households, other family members had similar illnesses. Parents reported a median of 4 lost working days (range 2–10), and older children reported median 4 lost school days (range 3–15). These rates were similar to those of children infected with RSV or influenza virus in the same study population.

Diagnosis of Human Metapneumovirus

Traditional methods of viral culture are very insensitive for the detection of hMPV. The virus only grows in certain cell lines and requires trypsin for replication in culture, conditions that are not routinely used in diagnostic virology laboratories. These are presumed to be the reasons why hMPV was not detected earlier. Furthermore, even under ideal conditions, CPEs produced by hMPV in cell culture are minimal and do not appear until several weeks of incubation. Unlike RSV and influenza virus, there is no commercially available rapid antigen test for hMPV. Thus, the diagnosis of hMPV currently rests on molecular techniques based on standard or real-time RT-PCR, several of which have been published and are quite sensitive, although labor-intensive and time-consuming [30,31,32•].

Human Metapneumovirus in Special Populations

Given the well-established association between RSV and asthma, several investigators have examined a potential relationship between hMPV infection and asthma. An Australian study of outpatient children with asthma did not find an association between hMPV and asthma exacerbations [33], although a large prospective study of 2000 outpatient children found a highly significant association between hMPV and the diagnosis of acute asthma exacerbation [5••]. A Finnish study found increased interleukin (IL)-8 in nasal secretions from hMPV-infected infants [34], whereas another study from Argentina found decreased IL-8 and other cytokines in nasal washes of hMPV-infected infants, compared with infants infected with RSV [35]. All such studies are complicated by the difficulty of assigning the diagnosis of asthma during infancy, when acute wheezing associated with viral infections is common. Although all open reading frames in the hMPV genome are analogous to those in RSV, two nonstructural RSV proteins that are thought to modulate host immune response are not present in the hMPV genome. This provides a unique opportunity to study the immunopathogenesis of two otherwise closely related viruses, which may elucidate their association with asthma.

There is evidence that hMPV is capable of causing severe and even fatal infections in immunocompromised hosts, a phenomenon that has been well described for influenza virus, RSV, and PIV. There are two reports of fatal infection attributed to hMPV in cancer patients—a 33-year-old woman with leukemia who was 7 days status post-hematopoietic stem cell transplant (HSCT) and a 17-month-old girl with relapsed leukemia [36,37]. The 17-month-old patient had had another unexplained LRI 1 year previously during chemotherapy for leukemia. Postmortem RT-PCR on respiratory samples from both illnesses detected hMPV in both, but from two distinct strains. Studies my colleagues and I are conducting in adult and pediatric patients with cancer, including HSCT recipients, suggest that hMPV is a relatively common cause of acute respiratory infection in these patients, with significant morbidity and mortality (Williams *et al.*, Unpublished data). A report of hMPV infection in African infants found no clinical differences between the three infants who were HIV-infected and the 10 who were not, although the authors cautioned that this may have been attributed to the small number of patients [38]. Further long-term prospective studies are needed to fully characterize the extent and severity of disease caused by hMPV in immunocompromised hosts.

Coinfections with Human Metapneumovirus and Other Viruses

All epidemiologic studies of hMPV that have tested for other viruses have found coinfection rates of 5% to 17%, usually with RSV, and most have not noted more severe disease in these coinfected patients. A few studies of hospitalized patients with hMPV have noted much higher coinfection rates of 30% to 60% [15,34,39,40], with some of these authors suggesting that hMPV infections may be

more severe if another virus is present. One British group addressed this question by using a nested RT-PCR assay to test bronchoalveolar lavage (BAL) fluid from 30 intubated infants with RSV infection and detected hMPV in 21 of 30 patients (70%) [41]. The authors subsequently used the same nested assay to test respiratory specimens from children admitted to the pediatric intensive care unit (PICU) and the general wards. hMPV and RSV coinfection was detected in 18 of 25 (72%) PICU patients and 15 of 171 (9%) general ward patients [42]. The detection rate for hMPV was much higher in BAL than in nasopharyngeal aspirate samples. The authors concluded that dual infection with RSV and hMPV was associated with severe bronchiolitis. However, a Connecticut study of 46 inpatients with mild or severe RSV disease found no coinfections with hMPV [43]. It is unknown whether these conflicting findings are attributed to methodologic differences or geographic variability in circulating virus strains. It is clear that further prospective studies are needed to clarify the nature of disease associated with coinfections.

Animal Models of Human Metapneumovirus Infection

The initial report of hMPV noted that the virus was unable to infect chickens and turkeys, distinct from avian MPV. However, guinea pigs and ferrets, which have been used as models for PIV and influenza virus, respectively, were semipermissive for hMPV replication [4••]. Subsequent reports have shown that hamsters, cynomolgus and rhesus macaques, African green monkeys, and chimpanzees were at least semipermissive hosts [6,7,44]. One study found BALB/c mice to be susceptible to hMPV infection [45], but this was not observed in another report [6] nor in our own studies, which have found hamsters and cotton rats to be permissive hosts (Williams *et al*, Unpublished data). In all of these models, primary infection with an hMPV strain from one subgroup induces protection against subsequent challenge with homologous or heterologous virus, suggesting that the major protective antigens or epitopes are shared between groups. These findings have major implications for vaccine development, which is discussed in the following text.

Therapy and Prevention of Human Metapneumovirus Infection

The only currently licensed antiviral for RSV infection is ribavirin, a nucleoside analog administered by aerosol. Although it has in vitro activity against RSV, it is rarely used because of its lack of convincing efficacy in normal children and difficulty in administration. Ribavirin has been used in severely immunocompromised patients such as HSCT recipients, often in conjunction with RSV-specific immunoglobulin, with some evidence for efficacy [46]. One group recently showed that ribavirin and polyclonal human immunoglobulin possessed in vitro neutralizing activity against hMPV equivalent to their activity against RSV [47]. There are no published animal or human data for these interventions, and although they may be shown to be worthy of consideration in severely immunocompromised hosts with LRI caused by hMPV, it is unlikely they will be used in more routine cases of hMPV infection, for the same reasons they are not used routinely for RSV. Most experts think that a more effective long-term strategy is prevention of severe disease with vaccination. Investigators who have worked for years to develop candidate RSV vaccines are using many of the same methods to approach possible hMPV vaccines.

Live-attenuated virus vaccines have been successfully developed against several viruses, including measles, polio, and influenza. However, the generation of stably attenuated, well-characterized strains of RSV has been difficult. The development of reverse genetic techniques to generate recombinant RSV was a major breakthrough, allowing the incorporation of specific mutations or even entire genes into recombinant viruses. These techniques have now been developed for hMPV. One group developed a recombinant chimeric bovine/human PIV vaccine that expressed hMPV F and was immunogenic and protective against challenge with hMPV in hamsters [48]. The National Institutes of Health group that initially described reverse genetics for RSV has generated recombinant hMPV strains lacking the small hydrophobic (SH) or glycoprotein (G) genes, or both [49]. Strains lacking the G gene were attenuated in hamsters, as measured by significantly lower virus titers in nose and lungs. Viruses lacking G, SH, or both were immunogenic in hamsters, induced high levels of neutralizing antibodies, and protected against challenge with wild-type hMPV. Thus, several potential vaccine candidates are in development within 3 years of the discovery of hMPV—a remarkable achievement.

Conclusions

Human metapneumovirus is a novel paramyxovirus that is an important cause of respiratory infections in adults and children worldwide. Although recently discovered, it clearly has been circulating for at least 50 years and presumably much longer. hMPV is a seasonal virus, with a peak incidence from late winter to early spring in temperate zones. Most infections are common URI, with a high proportion of concomitant otitis media in children. hMPV causes LRI in children and adults, typically bronchiolitis, croup, or pneumonia. LRI associated with hMPV is more common in males, and the peak age of hospitalization is between 6 and 12 months, older than the peak age of hospitalization for RSV. Clinically, hMPV infection most resembles RSV infection, and like RSV, patients can be reinfected with similar or novel strains. Although previously healthy children are hospitalized with hMPV infection, it appears that more severe disease generally occurs in

patients with underlying chronic disease. Fatal infection has occurred in severely immunocompromised patients with cancer. Animal models exist to study pathogenesis, and candidate vaccines are in development.

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