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Epidemiology of Human Metapneumovirus

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INTRODUCTION

Respiratory tract infections are a leading cause of morbidity and mortality worldwide. For children <5 years old, respiratory tract infections are ranked as the second leading cause of death regardless of geographical area (82). Acute respiratory tract infections are the most common illness regardless of age or gender (80). Pneumonia, influenza, and influenza-like illnesses (ILI) are the sixth leading cause of death in the United States, accounting for >45,000 deaths annually (96). Each year in the United States, respiratory tract infections account for 4 million ambulatory care visits and >500,000 hospitalizations, at an annual cost of $14.6 billion (107). In The Netherlands, the overall incidence of acute respiratory tract infection is 5,445 per 10,000 person-years (124).

Although the clinical manifestations of respiratory tract disease are easily recognized, the etiological agent responsible for disease is often not identified. For community-acquired pneumonia, a microbiological diagnosis can be made in <50% of cases (83, 102, 140). In the pediatric population, respiratory syncytial virus (RSV), parainfluenza viruses, and influenza virus are known as the major causes of bronchiolitis and lower respiratory tract infections (LRTI). However, in a third of these cases of LRTI in children, an infectious agent cannot be identified (27, 135). In nearly half of upper respiratory illnesses (URI) in children, an infectious cause cannot be determined (86). The etiology of a majority of LRTI is thought to be viral (39), yet in only 40% of cases can a viral agent be identified, even with use of state-of-the-art genomic amplification methods (70). These observations suggest that previously unknown pathogens may be circulating and may be responsible for a substantial proportion of respiratory tract disease.

DISCOVERY

In 2001, van den Hoogen et al. reported the discovery of a novel virus from children with respiratory tract illness in The Netherlands (120). This agent was detected in respiratory secretions of 28 children that were collected during a 20-year period. This virus was distinct from common respiratory viruses, as immunological assays using virus-specific antibodies and PCR-based methods using virus genome-specific primers failed to identify this agent. The genetic characterization of this agent remained a mystery until the tools of molecular biology were applied to identify portions of the genomic sequence. Using a technique known as randomly primed PCR, these Dutch researchers were able to obtain genomic sequence of this novel pathogen. Based on limited sequence data, this virus appeared to be closely related to the avian pneumovirus, a member of the *Metapneumovirus* genus, and it was called human metapneumovirus (hMPV) (38, 120). Several lines of evidence suggested that hMPV was a common human respiratory pathogen. Seven of 68 respiratory specimens (10%), collected in the winter of 2000, that were screened tested positive for hMPV by reverse transcription-PCR (RT-PCR). Serological studies, based on both immunofluorescence and viral neutralization assays, revealed that by the age of 5 nearly all individuals had evidence of hMPV infection. Furthermore, screening of banked human sera showed that hMPV, or a virus

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Order: Mononegavirales
Family: Paramyxoviridae
Sub-family: Paramyxovirinae
Genus: Respirovirus
Species: Human parainfluenza types 1 and 3
Genus: Rubulavirus
Species: Human parainfluenza types 2 and 4, Mumps
Genus: Morbillivirus
Species: Measles virus
Genus: Henipavirus
Species: Hendra virus, Nipah virus

Sub-family: Pneumovirinae
Genus: Pneumovirus
Species: Respiratory syncytial virus
Subgroup: A and B
Genus: Metapneumovirus
Species: Human metapneumovirus
Subgroup (?serogroup): A and B

FIG. 1. Human pathogens in the family *Paramyxoviridae* (data from reference 38).

closely related to it, had been circulating for at least 50 years, suggesting that the virus did not recently "jump" to the human population from an animal reservoir such as birds (120).

CLASSIFICATION AND GENOMIC STRUCTURE

The complete sequence of the hMPV genome has been elucidated (9, 49, 119). As the preliminary sequence data for the hMPV genome suggested, the virus is a member of the *Metapneumovirus* genus, a branch of the family *Paramyxoviridae*, and is genetically similar to, though distinct from, the avian pneumovirus (previously called turkey rhinotracheitis virus). The family *Paramyxoviridae* is composed of two subfamilies, one of which is *Pneumovirinae*, which includes both the *Pneumovirus* genus and the *Metapneumovirus* genus (Fig. 1) (38).

RSV, a pneumovirus, is the most closely related human pathogen to hMPV, and the genomes of the viruses have similar features as well as distinct differences. The negative-strand RNA genomes of both viruses contain open reading frames (ORFs) that encode three putative viral envelope glycoproteins, the F (fusion), G (attachment), and SH (short hydrophobic) proteins, though the order of these genes differs in the two viruses (23, 119) (Fig. 2). Of these three genes, the F gene is the most highly conserved (33% identity on the amino acid level) between hMPV and RSV (119). hMPV F contains several features that are conserved among the F proteins of paramyxoviruses, which includes a characteristic distribution of cysteine residues, a putative cleavage site (a distinguishing feature of a fusion proteins), fusion domains, and anchor sequences (119).

The amino acid identity between the G and SH proteins of hMPV and RSV could not be determined because the predicted amino acid sequences of the corresponding proteins of the two viruses could not be aligned (119). The putative G protein of hMPV is considerably smaller than the G protein of RSV (236 versus 299 amino acids). Unlike that in RSV, the region of the hMPV genome that encodes the putative G protein also carries an ORF immediately downstream of the G gene (in the same reading frame) and ORFs in the two other reading frames. It is unknown whether these accessory ORFs are expressed as separate proteins or are transcribed as part of the G protein through some RNA-editing event (119). The predicted amino acid sequences of the G gene of both hMPV and RSV indicate that these genes encode anchored type II glycoproteins (119, 127). The intracellular amino-terminal cytoplasmic domain of the G protein of both hMPV and RSV is relatively short (~30 amino acids) and is adjacent to the hydrophobic transmembrane domain. RSV G is heavily glycosylated with both O- and N-linked sugars (24), and the predicted amino acid sequence suggests that the same is true for hMPV. Among isolates of RSV, the G gene is the most variable of all RSV genes, and this appears to be the case for the G gene of hMPV (55). The reasons for the genetic variability of the G gene are not clear, but it may have to do with evasion of the host's immune system by the virus. The SH gene of hMPV encodes a protein (180 amino acids) that is substantially longer than the corresponding protein of RSV (64 amino acids). The function of the SH proteins of both viruses remains unknown.

Other than the gene order, the major differences between the genomes of hMPV and RSV are the lack of two nonstructural genes, NS-1 and NS-2, at the 3′ end of the hMPV genome (Fig. 2). Because of the gradient of transcription along the RSV genome, NS-1 and NS-2 are transcribed in the greatest molar amounts of all the genes the of the RSV genome. These RSV genes encode an anti-interferon activity (15). The signif-

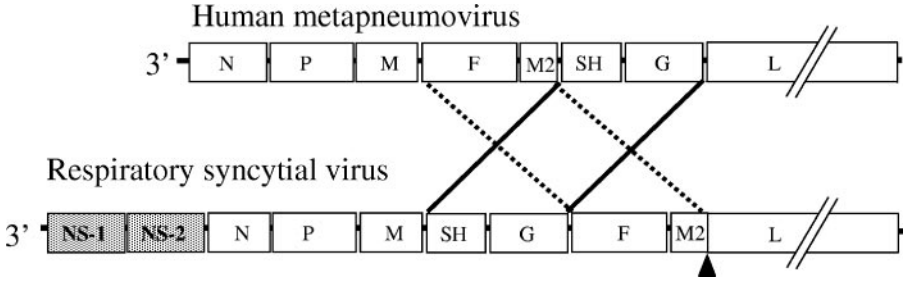

FIG. 2. Genomic maps of the *Pneumovirinae*. Genomic maps of the negative-sense, single-stranded RNA genomes of hMPV and RSV are displayed in the 3′-to-5′ orientation. In hMPV, the F and M2 genes are 3′ to the SH and G genes, whereas in RSV, the order of these genes is reversed. The RSV genome encodes two nonstructural proteins, NS-1 and NS-2 (shaded), that are not present in the hMPV genome. The M2 genes of both viruses carry two ORFs (M2-1 and M2-2) (not shown). The M2 and L genes overlap in the RSV genome (triangle). The L gene of each virus, encoding the viral RNA-dependent RNA polymerase, comprises two-thirds of the viral genome and is shortened for figure clarity. The genomes are not drawn to scale.

icance of the lack of NS-1 and NS-2 in the pathogenesis of hMPV is unknown.

Reverse genetics, developed over the last decade, allows for the recovery of recombinant negative-strand RNA viruses from cloned DNA (101). Manipulation of the hMPV genome by using the reverse genetics systems has begun to yield clues about the features of the viral genome (10, 49). In the relatively short time since the recovery of hMPV from cloned DNA, it is now clear that several hMPV genes are not required for viral propagation in cell culture. Like for RSV, both the G and SH genes can be deleted from the viral genome without eliminating the ability of the virus to replicate in vitro or in vivo (8, 58). The replication of hMPV lacking SH in the African green monkey model is comparable to that of wild-type virus, whereas the replication of hMPV lacking G is reduced sixfold compared to that of wild-type virus (8). These findings suggests that the F proteins of both RSV and hMPV contain an attachment and fusion activity and raise questions about whether the G and SH proteins play an essential role in interactions with the infected cell or immune system of the human host that have not yet been appreciated.

The reverse genetics system has also generated insights into the control of gene expression. The M2 gene of hMPV carries two overlapping ORFs, designated M2-1 and M2-2, and both are expressed during viral infection (17). Similar to the case for RSV, the M2-2 gene appears to control the switch of the viral RNA polymerase from viral gene transcription to viral genome replication. During infection with a recombinant hMPV lacking M2-2 or with a recombinant RSV lacking M2-2, there is accumulation of viral mRNA compared with viral genome template (7, 17). These findings may have implications for vaccine development. A recombinant hMPV lacking the M2-2 gene was found to be attenuated in viral replication but retained adequate gene expression capabilities to induce protective immunity (see "VACCINE CANDIDATES AND ANIMAL MODELS" below) (17).

CLINICAL MANIFESTATIONS OF hMPV INFECTION

Lower Respiratory Tract Disease

Evidence from many studies has demonstrated that hMPV is responsible for a substantial proportion of LRTI in infants and young children and is second only to RSV as a cause of bronchiolitis in early childhood (13, 22, 40, 65, 81, 106, 123, 125, 129). Williams et al. screened respiratory specimens from >2,000 children <5 years old, collected during a 20-year period at Vanderbilt University Medical Center, for hMPV (129). These children were followed as part of the Vanderbilt University Vaccine Clinic center in Nashville. In 20% of cases of LRTI (characterized by wheezing, rales, tachypnea, and dyspnea) which could not be attributed to a culturable respiratory virus, hMPV was detected. The incidence of hMPV-associated LRTI in young children varies with geographical location and time of year. The incidence estimates range from 5 to 15% in most studies (6, 13, 34, 79, 93, 122, 123, 129), but higher rates have been reported in other studies (28, 75).

The clinical manifestations of hMPV infection in young children are indistinguishable from the clinical manifestations of RSV infection. Features of hMPV infection included tachypnea, fever, cough, hypoxia, and changes on chest radiographs such as infiltrates, hyperinflation, and peribronchial cuffing (6, 13, 34, 79, 93, 122, 123, 129). Asymptomatic infection with hMPV in young children appears to be uncommon. In the study by Williams et al., only 1 of 86 asymptomatic children (1.2%) tested positive for hMPV, indicating a causal role of hMPV in LRTI (129).

LRTI associated with hMPV in infants and young children is a frequent cause of hospitalization. hMPV likely accounts for ~10% of LRTI hospitalizations and is second to RSV as a cause of LRTI requiring hospitalization (6, 13, 16, 33, 34, 40, 81, 84, 89, 123, 129). The most common diagnoses in hospitalized children who test positive for hMPV are bronchiolitis, pneumonia, and bronchitis. There are no manifestations of infection that are specific for hMPV-associated disease. In the limited number of population-based studies performed, children <2 years old appear to be the most likely to be hospitalized due to hMPV-associated LRTI (81, 84). hMPV has been associated with severe LRTI requiring intensive care, in one case requiring extracorporeal membrane oxygenation (118), though data from some studies suggest that, in general, the severity of disease associated with hMPV may be less than that observed with RSV (13, 125). Although the mean age for hMPV-associated LRTI is slight greater than the mean age for RSV-associated LRTI in some studies (81), but not in others (129), it is now clear that infants and young children are the groups in the pediatric population that are most susceptible to severe disease caused by either RSV or hMPV. Effective therapies and vaccine strategies will need to target this patient population. Risk factors for severe RSV disease, such as a history of premature birth, underlying heart or lung disease, and a compromised immune system, are likely risk factors for severe hMPV disease (12, 34, 90, 123).

Upper Respiratory Tract Infection

Like other common human respiratory viruses, hMPV is also associated with URI. While the definition of URI varies among studies, hMPV may be responsible for 5 to 15% of cases of URI in children (129, 133). In a recent study, Williams et al. screened respiratory specimens collected over a 20-year period from children <5 years old with URI, characterized by coryza, conjunctivitis, pharyngitis, otitis media (OM), or stomatitis. These children did not have a prior virological diagnosis. Of the >2,000 specimens screened by real-time RT-PCR, the percentage of URIs attributed to hMPV was 1 to 5%. This varied from year to year. Overall, the percentage of URIs associated with hMPV was lower than that observed with influenza virus, parainfluenza viruses, adenovirus, and RSV (133).

Respiratory viruses have been implicated in the pathogenesis of OM (48, 103). Therefore, it was not surprising that hMPV has been identified in children with acute OM (109, 116, 132). In one study, 50% of the children with hMPV-associated URI were diagnosed with otitis media (133). In another study, one-third of children with hMPV-associated LRTI were diagnosed with concomitant acute OM (129). hMPV was detected in the nasal washes of 6% of children who presented with acute OM (132), suggesting that hMPV may be responsible for a small but significant percentage of cases of acute OM. The detection of RSV in the middle ear fluid of

children with OM suggests a role of the virus in the pathogenesis of OM (48). Of eight children with OM whose nasal wash specimens tested positive for hMPV, only one had middle ear fluid that also tested positive for hMPV (132). Similar findings were observed elsewhere (116). These findings suggest that the inflammatory reaction to the virus, leading to incomplete or complete blockage of the eustachian tubes and subsequent invasion by bacteria, is a likely mechanism for virus-induced OM.

Wheezing and Asthma

A causative role of respiratory viruses in the initiation and progression of asthma remains a controversial issue. However, it is fairly well established that common respiratory viruses, such as rhinoviruses, can cause wheezing in some children and that infection with these viruses may induce exacerbations of reactive airways disease or asthma (56). hMPV was detected in 8% of children who presented to a Finnish hospital with exacerbation of wheezing. These hMPV-infected children also had increased levels of interleukin-8 in respiratory secretion (54). Several studies have reported the association of hMPV and asthma exacerbations (123, 126, 129, 131), while this association was not observed in another study (98). In addition, wheezing is a common symptom observed in children with hMPV-associated LRTI (33, 34, 89, 123, 126, 128, 129). hMPV has also been associated with acute asthma exacerbations requiring hospitalizations in adults (128). Investigation of the inflammatory mediators and cytokines induced by hMPV during infection should shed light on the mechanisms of hMPV-induced wheezing. Whether these potential mechanisms of wheezing are specific for hMPV or a more general response to a wide variety of respiratory pathogens remains to be determined.

hMPV in the Adult Population

hMPV has been associated with ILI, bronchitis, pneumonia, and exacerbations of both asthma and chronic obstructive pulmonary disease (COPD) in adults (37). The overall rates of hMPV disease in adults are likely lower than those observed in children. hMPV-associated disease occurs in adults of all ages (36). Falsey et al. detected hMPV in 3.4% of adults with respiratory tract illness. None of the 158 controls, who had no respiratory complaints, tested positive for hMPV (35). In previously healthy adults with acute respiratory infections screened during a 3-month period in San Francisco, Calif., hMPV was infrequently detected, though this may be due to the limited period of time of the study (70). In a study of patients who presented to general practitioners with ILI, 2.2% of individuals who tested negative for both RSV and influenza virus tested positive for hMPV (114). The major risk factor for hMPV-associated respiratory tract disease in adults without COPD is advanced age and underlying cardiopulmonary disease (12, 36, 47, 50). Dyspnea was more likely in elderly adults than in young adults infected with hMPV (36). The role of hMPV in exacerbations of COPD is less clear. hMPV has been detected in individuals with acute exacerbations of COPD (47, 76, 100). However, the duration of viral shedding in individuals with COPD is not established, and therefore a causal role may be difficult to define. Further investigations are needed to define the role of hMPV in COPD.

Infection in the Immunocompromised Host

hMPV can cause prolonged and serious infections in the immunocompromised host. Several case reports and small case series have described the detection of hMPV in immunocompromised individuals with severe lung disease (18, 34, 67, 78, 90, 130). In one study of lung transplant recipients, 9 of 25 individuals who were screened during a 1-year period tested positive for hMPV at least one time. Several fatalities were reported. It is difficult to determine the clinical features of hMPV disease in these individuals, as many of them had concurrent bacterial or fungal infections (67). In a prospective 4-year study of adults with hematological malignancies and respiratory tract infection, 9% of episodes of respiratory tract infections tested positive for hMPV. Sixteen of the 22 individuals who tested positive for hMPV (73%) were hematopoietic stem cell transplant recipients. Twenty hMPV-positive individuals had URI symptoms, but eight of these subsequently progressed to LRTI. Three of the patients with hMPV-associated LRTI died, two of whom had concomitant bacterial infection. hMPV was the only pathogen identified in one patient who died, and death occurred within a month after the onset of LRTI (130). hMPV was reported in an immunocompromised child, who had two hMPV infections with two different strains of the virus during a 10-month period (90). hMPV has been detected in children with human immunodeficiency virus (HIV) infection, though because of the small numbers of children in the study, it is difficult to determine whether hMPV-associated disease was more or less severe in HIV-infected children than in non-HIV-infected children (73).

Coinfection with hMPV and Other Respiratory Pathogens

Because the seasonal distributions of hMPV and RSV, two common pathogens, overlap, the potential for dual infection exists. Indeed, several studies have found a coinfection rate of $<10\%$ (34, 125, 129, 138). However, Greensill et al. reported that 70% of RSV-infected children who required intensive care in Liverpool, United Kingdom, were coinfected with hMPV, suggesting that the disease caused by RSV may be augmented by a concurrent hMPV infection, particularly in otherwise healthy children (44). In another study from the United Kingdom, hMPV and RSV coinfection conferred an increased risk of admission to the pediatric intensive care unit (112). However, population-based and case-control studies of hospitalized children have found that hMPV-RSV coinfections are uncommon. Of 668 hospital admissions screened by Mullins et al., none of the children tested positive for both hMPV and RSV (81). Lazar et al., using a case-control method, screened children with severe RSV disease (cases, defined as children who required admission to a pediatric intensive care unit) and children with mild RSV disease (controls) during a period in which hMPV was circulating in the community. Overall, none of the cases and none of the controls tested positive for hMPV (68).

In 2002, a new respiratory illness, severe acute respiratory syndrome (SARS), emerged in southeast Asia and was eventually attributed to a novel coronavirus (SARS coronavirus)

(29, 62). In some locations in China and Canada, though not in others, a substantial proportion of individuals with SARS were also infected with hMPV (20, 21, 63, 97). It is unclear whether dual infection resulted in greater severity of illness. The significance of this dual infection is not known.

Bacterial pneumonia is frequent complication of influenza virus infection but not RSV infection (45). The current data suggest that bacterial infection complicating hMPV infection is uncommon. However, the proper studies to investigate this issue have not been conducted.

Other Potential Manifestations of hMPV Disease

The current understanding of hMPV is that during infection both the virus and the disease caused by the virus are limited to the respiratory tract. This may be due, in part, to the relative lack of published studies that have specific screened for hMPV in other organ systems or bodily fluids. In a case report on a 14-month-old, previously healthy child who died of encephalitis, hMPV RNA was detected by RT-PCR in both the brain and lung tissues, though hMPV antigens were not detected in either tissue by immune staining (111). In a 1-year study of hMPV in children with respiratory tract disease in Japan, 1 child of 29 children who tested positive for hMPV presented with encephalitis. hMPV was detected in respiratory secretions, though cerebrospinal fluid from this patient was apparently not tested for hMPV (57). These findings suggest that hMPV may cause disseminated infection in some individuals. Replication of RSV is also thought to be limited to the respiratory tract, though the detection of RSV sequences in blood has been reported (139). These findings have not been substantiated and remain controversial. Whether hMPV spreads beyond the respiratory system during infection remains to be determined.

EPIDEMIOLOGY

Geographical and Seasonal Distribution and Molecular Epidemiology of hMPV

hMPV has a worldwide distribution and has been identified on every continent (2, 12, 14, 26, 28, 30, 33, 40, 41, 43, 60, 71, 75, 85, 87, 89, 99, 104, 120). In temperate climates, hMPV circulates predominately in the late winter and spring, and the peak of activity at any given location often coincides with or follows the peak of RSV activity (1, 13, 16, 22, 34, 57, 61, 75, 81, 84, 88, 89, 105) (Fig. 3). In many communities, hMPV has been detected throughout the year, albeit at lower levels during the late spring, summer, and fall (34, 81, 88, 123, 129). Based on genomic sequencing and phylogenetic analysis, there are two major genotypes of hMPV, designated A and B (9, 22, 34, 93, 123, 129). These analyses are based on sequencing of the N, M, F, G, or L gene, and the genotype groupings are concordant regardless of which gene is studied. Whether these two genotypes represent distinct serogroups remains controversial (see "SEROLOGICAL PROPERTIES OF hMPV GENOTYPES" below). Each genotype appears to have at least two distinct subgroups (5, 14, 72, 122).

The genomic organization of the two viral genotypes is identical (9). The major differences between the A and B genotypes are nucleotide polymorphisms, and the G and SH proteins contain the highest concentration of these polymorphisms (9). Like the G gene of RSV (94), the G gene of hMPV displays significant strain-to-strain variability (9, 52, 92). This nucleotide variability results in significant amino acid variability, insertions that retain the reading frame, and the use of alternate transcriptional termination codons (4). Most of the amino acid variability is observed in the putative extracellular domain of the protein. Overall, there is 32 to 37% amino acid identity of the G protein between the A and B genotypes of hMPV (1, 4, 92). This is analogous to that observed with G genes of RSV subgroups A and B (77, 94, 115).

Phylogenetic analysis of strains of hMPV reveals that the epidemiology of hMPV is complex and dynamic. Unlike influenza virus, where two or three strains spread across the globe each year, outbreaks of hMPV appear to be a local phenomenon. Strains of hMPV differ from community to community, and strains identified in one location may be quite similar to strains identified in other locations in different years. For example, the prototype strain identified in The Netherlands is genetically similar to strains identified in Australia; New Haven, Conn.; and Quebec, Canada in different years (34) (Fig. 4). Based on F gene sequences, viruses isolated in Australia (2001), France (2000 and 2002), Canada (1999, 2000, 2001, and 2002), Israel (2002), and The Netherlands (2001) were closely related, with few polymorphisms in the F gene (14). In any given year, viruses of both genotypes (and both subgroups in each genotype) can circulate (1, 14, 19, 34, 57, 93, 121). In St. Louis, Missouri, the predominant genotype of hMPV switched in consecutive years from genotype A to genotype B (1). Similar phenomena have been observed elsewhere (42). In the study from St. Louis, the severity of illness associated with the two genotypes did not differ. The observed epidemiological features of hMPV are similar to those of RSV, where viruses of both A and B subgroups cocirculate each year and the predominant strains vary from location to location and from year to year (94).

In 2004, a variant of hMPV, isolated from a 6 1/2-year-old girl with an acute exacerbation of asthma, was found to be genetically distinct from viruses of the four lineages of hMPV (110). This strain was detected only with primers that targeted the N gene. Whether this strain represents a distinct genotype of hMPV needs to be confirmed with further sequence analysis. Diagnostic genomic amplification methods may have to be modified if there is widespread circulation of this variant. A recent study found that viruses in the A genotype may be more divergent than previously appreciated (51).

Seroepidemiology

Infection with hMPV appears to be common in childhood. Several methods have been used to detect hMPV-specific antibodies in serum, including immunofluorescence using hMPV-infected cells as the antigen (31, 120, 134), enzyme-linked immunosorbent assays using recombinant hMPV proteins (46, 53, 69), and virus neutralization assays (120). Enzyme-linked immunosorbent assays based on recombinant hMPV F or recombinant hMPV N protein are both sensitive and specific for the detection of hMPV-specific antibodies (46, 53, 69). Independent of methodology, several studies have demonstrated that hMPV infection occurs early in childhood (31, 69, 120,

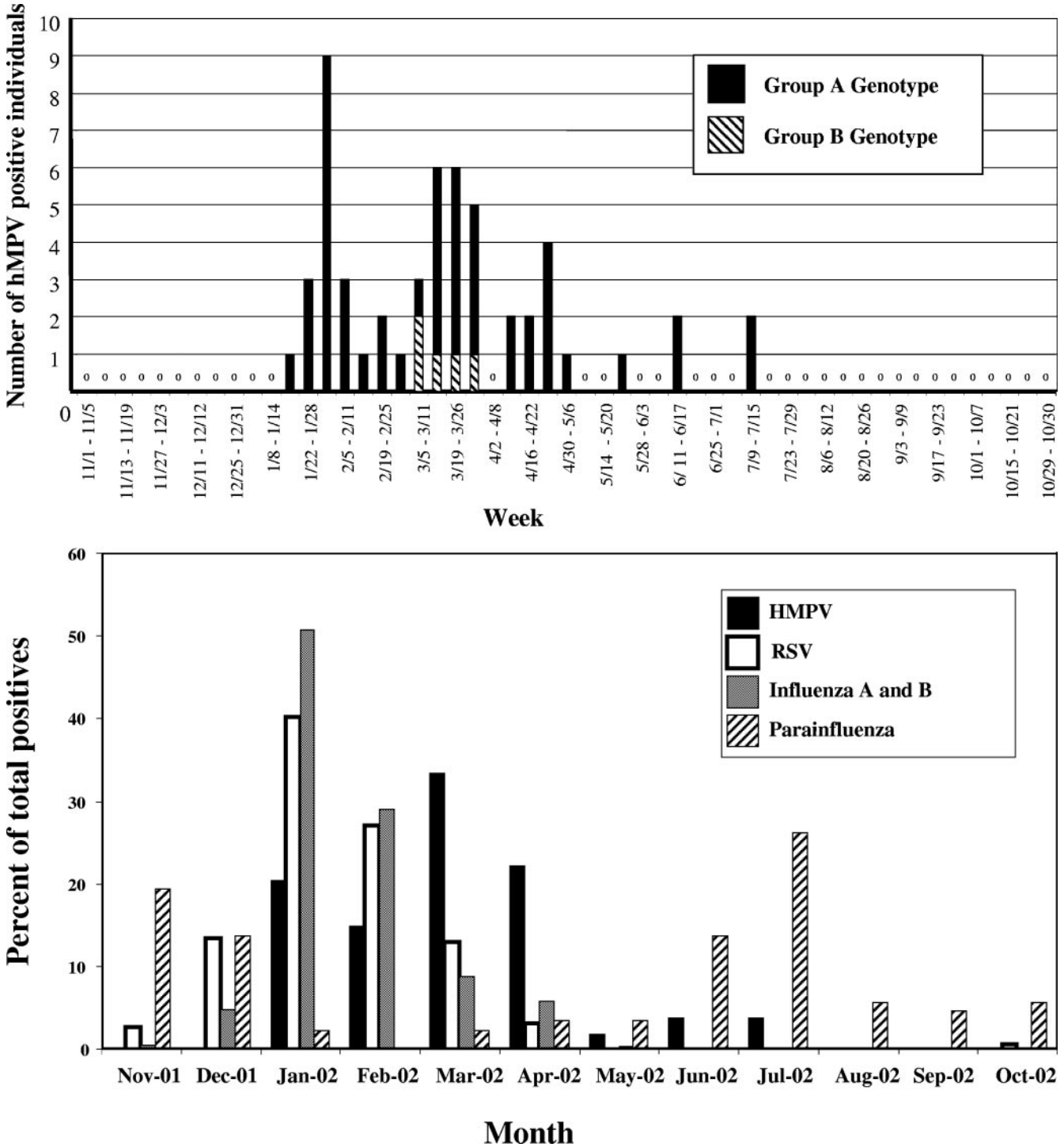

FIG. 3. Distribution of hMPV and other respiratory viruses in New Haven, Connecticut. (Top panel) Weekly distribution of hMPV genotypes from 1 November 2001 to 31 October 2002. Genotype assignments are indicated. Every other week is marked for figure clarity. There are a total of 54 hMPV-positive samples. (Bottom panel) Monthly distribution of hMPV, RSV, influenza A and B viruses, and human parainfluenza viruses 1 to 3. The percentage of total positive specimens for each virus during the 1-year period is indicated. (Reprinted from reference 34 with permission. © 2004 by the Infectious Diseases Society of America. All rights reserved.)

134). By the age of 5 years, >90% of individuals screened have evidence of hMPV infection. The seroprevalence of hMPV-specific antibody in adults is nearly 100% (69, 120). The seroprevalence of hMPV-specific antibody in infants <3 months of age is >90%, indicating that maternally derived antibodies are present in young children (69) (Fig. 5). Whether this hMPV-specific antibody protects against infection or lessens the severity of illness remains to be determined.

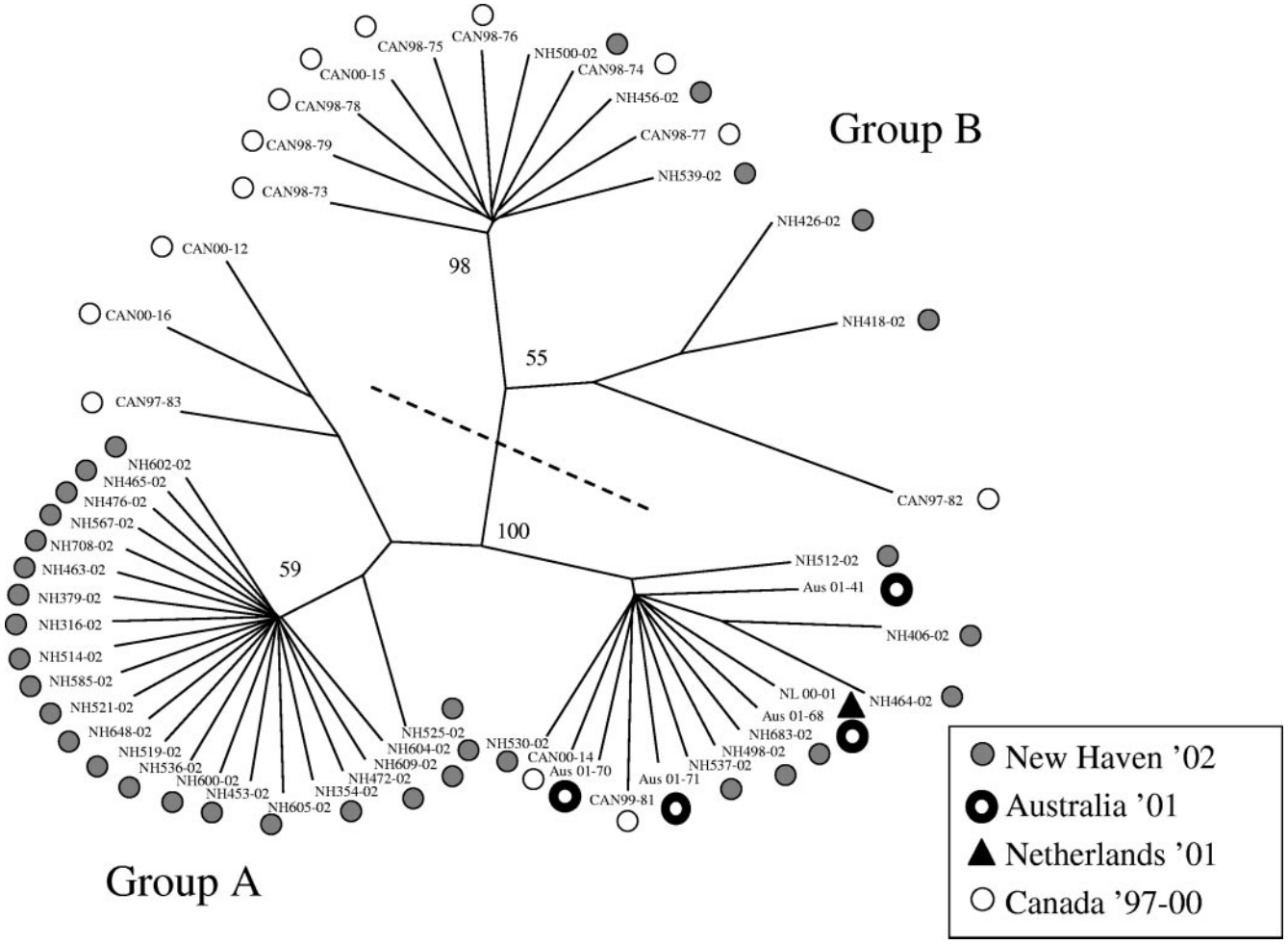

FIG. 4. Phylogenetic analysis of hMPV. Sequences of the hMPV F genes from isolates originating from Connecticut, The Netherlands, Australia, and Canada were used to construct a phylogenetic tree. Bootstrap values are displayed at major branch points. A representative set of Connecticut sequences is displayed. Genotypes are labeled group A and group B as originally proposed in reference 121. NL/1/00 is a prototype group A strain, and CAN 98-75 is a prototype B strain. The dashed line separates group A and B viruses. (Modified [to reflect the current terminology] from reference 34 with permission. © 2004 by the Infectious Diseases Society of America. All rights reserved.)

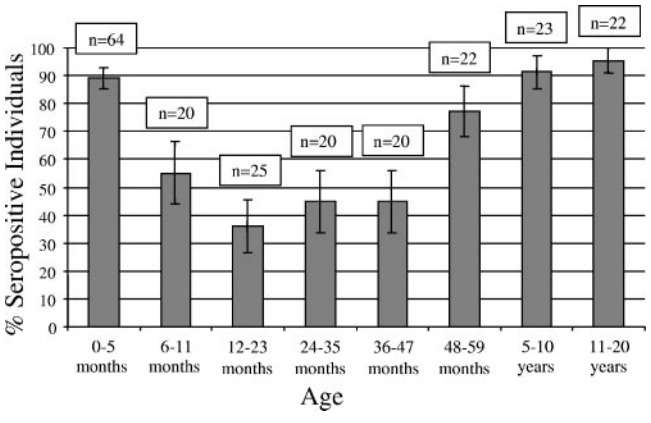

FIG. 5. Seroepidemiology of hMPV. The percentage of hMPV seropositivity and the number of serum samples (*n*) for each age group are indicated. Error bars represent 95% confidence intervals. (Reprinted from reference 69.)

SEROLOGICAL PROPERTIES OF hMPV GENOTYPES

The observation that the greatest genetic diversity between the two genotypes of hMPV occurs in the structural genes (G > SH ≫ F) suggested that the two genotypes may represent distinct serotypes. To address this issue, animal models have been used. Skiadopoulos et al. used hMPV strains CAN98-75 and CAN97-83, which represent the two genotypes of hMPV, in rodent and nonhuman primate models. Infection with a virus of one hMPV genotype protected animals against infection with a virus of the heterologous genotype. Protection was defined as the inhibition of hMPV replication in nasal turbinates or lungs in infected animals that were previously infected with a strain of hMPV (113). Reciprocal cross-neutralization assays with postinfection serum demonstrated that each strain induced a high level of neutralizing antibody to homologous and heterologous strains. Furthermore, immunization with a recombinant human parainfluenza virus expressing the F protein of CAN97-83 induced antibodies that neutralized viruses from both lineages and protected animals from

challenge with either hMPV strain. Taken together, these data suggest that cross-protective immunity occurs after infection with a virus of one genotype and that the F protein is the major antigenic determinant on the virus. Therefore, the two genotypes may not represent distinct serotypes. This is analogous to the case for RSV, in which infection with a virus of either the A or B subgroup elicits an antibody response that is specific for viruses of both the homologous and heteologous subgroups (23). However, van den Hoogen et al., using a ferret model, demonstrated that serum obtained from an animal infected with a representative virus of one genotype could not neutralize a virus of the heterologous genotype in vitro, suggesting that the two genotypes were, in fact, distinct serotypes (121). The antigenicity of hMPV in the natural human host remains to be determined. Does hMPV infection in humans elicit a protective antibody- or cell-mediated response to viruses of the homologous group or the heterologous group? Clearly, the answer to this question will have significant implications for vaccine development.

DIAGNOSIS

hMPV grows poorly in cell culture. The replication of hMPV in vitro is restricted to a limited number of cell lines and requires supplementation of the medium with trypsin for propagation (120). These properties may explain why the virus was not identified until recently. Nonetheless, the sensitivity of cell culture techniques for the detection of hMPV in secretions from the respiratory tract is poor. Currently, RT-PCR is the most common method used to detect hMPV. Several genes of hMPV have been primer targets for genomic amplification. Cote et al. reported that primers that bind regions of the N and L genes are highly sensitive for the detection of hMPV strains of both genotypes (25). Real-time RT-PCR is another powerful method to detect hMPV and other respiratory viruses. Several genes have been targeted for the real-time assays, and certain primers can detect viruses of both subgroups of both genotypes (64, 72, 74, 108).

Immunofluorescence, using virus-specific antibodies, is a rapid method used to detect respiratory viruses and is commonly used in diagnostic laboratories. hMPV-specific antibodies have been developed for immunofluorescence assays, though this method may not be as sensitive as RT-PCR for the detection of hMPV (32, 66, 91). hMPV-specific antibodies for immunofluorescence are available from commercial sources.

VACCINE CANDIDATES AND ANIMAL MODELS

The development of a safe and effective vaccine to protect against hMPV is a reasonable goal. Several promising vaccine candidates have been tested in animal models. A live recombinant human parainfluenza virus that contains the hMPV F gene has been shown to induce hMPV-specific antibodies and to protect experimental animals from hMPV challenge (113). A chimeric bovine/human parainfluenza virus 3 expressing the hMPV F elicits neutralizing antibodies against both parainfluenza virus and hMPV (117). Recombinant hMPVs lacking the G, SH, or M2 gene or both G and SH are attenuated in cell culture, though they retain immunogenicity and elicit protective immunity in experimental animals (8, 11). Antibodies specific for the hMPV F protein are likely induced by these viruses. However, the results of these animal challenge studies should be interpreted cautiously. There are many limitations of the small-animal model for testing potential hMPV vaccines, not the least of which is that the pathogen is highly host restricted.

As stated earlier, hMPV lacking the M2-2 gene is attenuated, though immunogenic, in experimental animals (17). The approach of using live, recombinant, gene-deleted hMPVs as vaccines is based on experience with similar recombinant viruses for the prevention of RSV infection. Live recombinant RSV vaccine candidates containing attenuating mutations and lacking the SH gene are currently in clinical trials (59). An RSV recombinant lacking the G and SH genes has also been studied in human volunteers (58). The preliminary experience with these recombinant RSV vaccines is that they are either overattenuated or underattenuated. Therefore, the safety and efficacy of a recombinant hMPV vaccine may be difficult to predict. Chimeric hMPV/avian pneumovirus recombinant viruses in which the N or P gene of hMPV was replaced with the corresponding gene from avian pneumovirus are attenuated in African green monkeys (the P gene chimera is 10- to 1,000-fold more attenuated than the N chimera), though both are as immunogenic as wild-type hMPV (95). This represents another strategy for the development of recombinant attenuated live hMPV vaccines.

ANTIVIRALS

Other than influenza virus, antiviral therapy for respiratory viruses has not shown tremendous potential. The effectiveness of ribavirin therapy for RSV is limited, at best, and remains a controversial issue (3). The relative ineffectiveness of antivirals for RSV may be due, in part, to the inflammatory reaction elicited in the host, which in all likelihood would not be squelched by antiviral compounds. It is unknown what role the host's inflammatory response plays during hMPV disease. Nonetheless, antiviral compounds have been tested with hMPV. The antiviral activity of ribavirin to inhibit the replication of hMPV is equivalent to that observed with RSV (136). Other compounds, such as NMSO3, a sulfated sialyl lipid that has been shown to have potent antiviral activity against RSV in tissue culture cells, have been shown to have anti-hMPV activity in vitro (137). It is likely that an hMPV-neutralizing monoclonal antibody for prophylaxis of high-risk infants (similar to the anti-RSV F humanized monoclonal antibody currently used for prevention of severe RSV disease) will be developed and tested. The progress towards an effective antiviral strategy for hMPV is currently limited by the scant data on pathogenesis of the virus in the natural host.

CONCLUSIONS

Since the discovery of hMPV in 2001, the virus has been identified worldwide. hMPV is a common respiratory pathogen, particularly in infants and young children. The virus is associated with both upper and lower respiratory tract infections and may be a trigger for asthma. At least two major genotype of hMPV circulate during community outbreaks. Whether these genotypes represent distinct serotypes re-

mains controversial. The major challenges faced by the medical and scientific communities are the understanding of the pathogenesis of hMPV disease and the development of a safe and effective vaccine to protect against infection and disease caused by this newly recognized respiratory virus.

REFERENCES

1. **Agapov, E., K. C. Sumino, M. Gaudreault-Keener, G. A. Storch, and M. J. Holtzman.** 2006. Genetic variability of human metapneumovirus infection: evidence of a shift in viral genotype without a change in illness. J. Infect. Dis. **193:**396–403.
2. **Al-Sonboli, N., C. A. Hart, A. Al-Aeryani, S. M. Banajeh, N. Al-Aghbari, W. Dove, and L. E. Cuevas.** 2005. Respiratory syncytial virus and human metapneumovirus in children with acute respiratory infections in Yemen. Pediatr. Infect. Dis. J. **24:**734–736.
3. **American Academy of Pediatrics Committee on Infectious Diseases.** 1996. Reassessment of the indications for ribavirin therapy in respiratory syncytial virus infections. Pediatrics **97:**137–140.
4. **Bastien, N., L. Liu, D. Ward, T. Taylor, and Y. Li.** 2004. Genetic variability of the G glycoprotein gene of human metapneumovirus. J. Clin. Microbiol. **42:**3532–3537.
5. **Bastien, N., S. Normand, T. Taylor, D. Ward, T. C. Peret, G. Boivin, L. J. Anderson, and Y. Li.** 2003. Sequence analysis of the N, P, M and F genes of Canadian human metapneumovirus strains. Virus Res. **93:**51–62.
6. **Bastien, N., D. Ward, P. Van Caeseele, K. Brandt, S. H. Lee, G. McNabb, B. Klisko, E. Chan, and Y. Li.** 2003. Human metapneumovirus infection in the Canadian population. J. Clin. Microbiol. **41:**4642–4646.
7. **Bermingham, A., and P. L. Collins.** 1999. The M2-2 protein of human respiratory syncytial virus is a regulatory factor involved in the balance between RNA replication and transcription. Proc. Natl. Acad. Sci. USA **96:**11259–11264.
8. **Biacchesi, S., Q. N. Pham, M. H. Skiadopoulos, B. R. Murphy, P. L. Collins, and U. J. Buchholz.** 2005. Infection of nonhuman primates with recombinant human metapneumovirus lacking the SH, G, or M2-2 protein categorizes each as a nonessential accessory protein and identifies vaccine candidates. J. Virol. **79:**12608–12613.
9. **Biacchesi, S., M. H. Skiadopoulos, G. Boivin, C. T. Hanson, B. R. Murphy, P. L. Collins, and U. J. Buchholz.** 2003. Genetic diversity between human metapneumovirus subgroups. Virology **315:**1–9.
10. **Biacchesi, S., M. H. Skiadopoulos, K. C. Tran, B. R. Murphy, P. L. Collins, and U. J. Buchholz.** 2004. Recovery of human metapneumovirus from cDNA: optimization of growth in vitro and expression of additional genes. Virology **321:**247–259.
11. **Biacchesi, S., M. H. Skiadopoulos, L. Yang, E. W. Lamirande, K. C. Tran, B. R. Murphy, P. L. Collins, and U. J. Buchholz.** 2004. Recombinant human metapneumovirus lacking the small hydrophobic SH and/or attachment G glycoprotein: deletion of G yields a promising vaccine candidate. J. Virol. **78:**12877–12887.
12. **Boivin, G., Y. Abed, G. Pelletier, L. Ruel, D. Moisan, S. Cote, T. C. Peret, D. D. Erdman, and L. J. Anderson.** 2002. Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. J. Infect. Dis. **186:**1330–1334.
13. **Boivin, G., G. De Serres, S. Cote, R. Gilca, Y. Abed, L. Rochette, M. G. Bergeron, and P. Dery.** 2003. Human metapneumovirus infections in hospitalized children. Emerg. Infect. Dis. **9:**634–640.
14. **Boivin, G., I. Mackay, T. P. Sloots, S. Madhi, F. Freymuth, D. Wolf, Y. Shemer-Avni, H. Ludewick, G. C. Gray, and E. LeBlanc.** 2004. Global genetic diversity of human metapneumovirus fusion gene. Emerg. Infect. Dis. **10:**1154–1157.
15. **Bossert, B., and K. K. Conzelmann.** 2002. Respiratory syncytial virus (RSV) nonstructural (NS) proteins as host range determinants: a chimeric bovine RSV with NS genes from human RSV is attenuated in interferon-competent bovine cells. J. Virol. **76:**4287–4293.
16. **Bouscambert-Duchamp, M., B. Lina, A. Trompette, H. Moret, J. Motte, and L. Andreoletti.** 2005. Detection of human metapneumovirus RNA sequences in nasopharyngeal aspirates of young French children with acute bronchiolitis by real-time reverse transcriptase PCR and phylogenetic analysis. J. Clin. Microbiol. **43:**1411–1414.
17. **Buchholz, U. J., S. Biacchesi, Q. N. Pham, K. C. Tran, L. Yang, C. L. Luongo, M. H. Skiadopoulos, B. R. Murphy, and P. L. Collins.** 2005. Deletion of M2 gene open reading frames 1 and 2 of human metapneumovirus: effects on RNA synthesis, attenuation, and immunogenicity. J. Virol. **79:**6588–6597.
18. **Cane, P. A., B. G. van den Hoogen, S. Chakrabarti, C. D. Fegan, and A. D. Osterhaus.** 2003. Human metapneumovirus in a haematopoietic stem cell transplant recipient with fatal lower respiratory tract disease. Bone Marrow Transplant. **31:**309–310.
19. **Carr, M. J., G. P. McCormack, and B. Crowley.** 2005. Human metapneumovirus-associated respiratory tract infections in the Republic of Ireland during the influenza season of 2003–2004. Clin. Microbiol. Infect. **11:**366–371.
20. **Chan, P. K., J. S. Tam, C. W. Lam, E. Chan, A. Wu, C. K. Li, T. A. Buckley, K. C. Ng, G. M. Joynt, F. W. Cheng, K. F. To, N. Lee, D. S. Hui, J. L. Cheung, I. Chu, E. Liu, S. S. Chung, and J. J. Sung.** 2003. Human metapneumovirus detection in patients with severe acute respiratory syndrome. Emerg. Infect. Dis. **9:**1058–1063.
21. **Chan, P. K., K. F. To, A. Wu, G. M. Tse, K. F. Chan, S. F. Lui, J. J. Sung, J. S. Tam, and B. Tomlinson.** 2004. Human metapneumovirus-associated atypical pneumonia and SARS. Emerg. Infect. Dis. **10:**497–500.
22. **Chano, F., C. Rousseau, C. Laferriere, M. Couillard, and H. Charest.** 2005. Epidemiological survey of human metapneumovirus infection in a large pediatric tertiary care center. J. Clin. Microbiol. **43:**5520–5525.
23. **Collins, P. L., K. McIntosh, and R. M. Chanock.** 1996. Respiratory syncytial virus, p. 1313–1352. *In* B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), Fields virology, vol. 1. Lippincott-Raven Publishers, Philadelphia, Pa.
24. **Collins, P. L., and G. Mottet.** 1992. Oligomerization and post-translational processing of glycoprotein G of human respiratory syncytial virus: altered O-glycosylation in the presence of brefeldin A. J. Gen. Virol. **73:**849–863.
25. **Cote, S., Y. Abed, and G. Boivin.** 2003. Comparative evaluation of real-time PCR assays for detection of the human metapneumovirus. J. Clin. Microbiol. **41:**3631–3635.
26. **Cuevas, L. E., A. M. Nasser, W. Dove, R. Q. Gurgel, J. Greensill, and C. A. Hart.** 2003. Human metapneumovirus and respiratory syncytial virus, Brazil. Emerg. Infect. Dis. **9:**1626–1628.
27. **Davies, H. D., A. Matlow, M. Petric, R. Glazier, and E. E. Wang.** 1996. Prospective comparative study of viral, bacterial and atypical organisms identified in pneumonia and bronchiolitis in hospitalized Canadian infants. Pediatr. Infect. Dis. J. **15:**371–375.
28. **Dollner, H., K. Risnes, A. Radtke, and S. A. Nordbo.** 2004. Outbreak of human metapneumovirus infection in Norwegian children. Pediatr. Infect. Dis. J. **23:**436–440.
29. **Drosten, C., S. Gunther, W. Preiser, S. van der Werf, H. R. Brodt, S. Becker, H. Rabenau, M. Panning, L. Kolesnikova, R. A. Fouchier, A. Berger, A. M. Burguiere, J. Cinatl, M. Eickmann, N. Escriou, K. Grywna, S. Kramme, J. C. Manuguerra, S. Muller, V. Rickerts, M. Sturmer, S. Vieth, H. D. Klenk, A. D. Osterhaus, H. Schmitz, and H. W. Doerr.** 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N. Engl. J. Med. **348:**1967–1976.
30. **Ebihara, T., R. Endo, H. Kikuta, N. Ishiguro, H. Ishiko, M. Hara, Y. Takahashi, and K. Kobayashi.** 2004. Human metapneumovirus infection in Japanese children. J. Clin. Microbiol. **42:**126–132.
31. **Ebihara, T., R. Endo, H. Kikuta, N. Ishiguro, M. Yoshioka, X. Ma, and K. Kobayashi.** 2003. Seroprevalence of human metapneumovirus in Japan. J. Med. Virol. **70:**281–283.
32. **Ebihara, T., R. Endo, X. Ma, N. Ishiguro, and H. Kikuta.** 2005. Detection of human metapneumovirus antigens in nasopharyngeal secretions by an immunofluorescent-antibody test. J. Clin. Microbiol. **43:**1138–1141.
33. **Esper, F., D. Boucher, C. Weibel, R. A. Martinello, and J. S. Kahn.** 2003. Human metapneumovirus infection in the United States: clinical manifestations associated with a newly emerging respiratory infection in children. Pediatrics **111:**1407–1410.
34. **Esper, F., R. A. Martinello, D. Boucher, C. Weibel, D. Ferguson, M. L. Landry, and J. S. Kahn.** 2004. A 1-year experience with human metapneumovirus in children aged <5 years. J. Infect. Dis. **189:**1388–1396.
35. **Falsey, A. R., M. C. Criddle, and E. E. Walsh.** 2006. Detection of respiratory syncytial virus and human metapneumovirus by reverse transcription polymerase chain reaction in adults with and without respiratory illness. J. Clin. Virol. **35:**46–50.
36. **Falsey, A. R., D. Erdman, L. J. Anderson, and E. E. Walsh.** 2003. Human metapneumovirus infections in young and elderly adults. J. Infect. Dis. **187:**785–790.
37. **Falsey, A. R., and E. E. Walsh.** 2006. Viral pneumonia in older adults. Clin. Infect. Dis. **42:**518–524.
38. **Fauquet, C. M., et al. (ed.).** 2006. Virus taxonomy: classification and nomenclature of viruses. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, Oxford, United Kingdom. [Online.] http://www.ictvdb.rothamsted.ac.uk/Ictv/ICTV8thReport%20Master%20species%20list.htm.
39. **File, T. M.** 2003. Community-acquired pneumonia. Lancet **362:**1991–2001.
40. **Freymouth, F., A. Vabret, L. Legrand, N. Eterradossi, F. Lafay-Delaire, J. Brouard, and B. Guillois.** 2003. Presence of the new human metapneumovirus in French children with bronchiolitis. Pediatr. Infect. Dis. J. **22:**92–94.
41. **Garcia-Garcia, M. L., C. Calvo, F. Martin, P. Perez-Brena, B. Acosta, and I. Casas.** 2006. Human metapneumovirus infections in hospitalized infants in Spain. Arch. Dis. Child. **91:**290–295.
42. **Gerna, G., G. Campanini, F. Rovida, A. Sarasini, D. Lilleri, S. Paolucci, A. Marchi, F. Baldanti, and M. G. Revello.** 2005. Changing circulation rate of human metapneumovirus strains and types among hospitalized pediatric patients during three consecutive winter-spring seasons. Arch. Virol. **150:**2365–2375.

43. **Gray, G. C.** 2006. Human metapneumovirus, Peru. Emerg. Infect. Dis. **12:**347–350.
44. **Greensill, J., P. S. McNamara, W. Dove, B. Flanagan, R. L. Smyth, and C. A. Hart.** 2003. Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. Emerg. Infect. Dis. **9:**372–375.
45. **Hall, C. B., K. R. Powell, K. C. Schnabel, C. L. Gala, and P. H. Pincus.** 1988. Risk of secondary bacterial infection in infants hospitalized with respiratory syncytial viral infection. J. Pediatr. **113:**266–271.
46. **Hamelin, M. E., and G. Boivin.** 2005. Development and validation of an enzyme-linked immunosorbent assay for human metapneumovirus serology based on a recombinant viral protein. Clin. Diagn. Lab. Immunol. **12:**249–253.
47. **Hamelin, M. E., S. Cote, J. Laforge, N. Lampron, J. Bourbeau, K. Weiss, R. Gilca, G. DeSerres, and G. Boivin.** 2005. Human metapneumovirus infection in adults with community-acquired pneumonia and exacerbation of chronic obstructive pulmonary disease. Clin. Infect. Dis. **41:**498–502.
48. **Heikkinen, T., M. Thint, and T. Chonmaitree.** 1999. Prevalence of various respiratory viruses in the middle ear during acute otitis media. N. Engl. J. Med. **340:**260–264.
49. **Herfst, S., M. de Graaf, J. H. Schickli, R. S. Tang, J. Kaur, C. F. Yang, R. R. Spaete, A. A. Haller, B. G. van den Hoogen, A. D. Osterhaus, and R. A. Fouchier.** 2004. Recovery of human metapneumovirus genetic lineages A and B from cloned cDNA. J. Virol. **78:**8264–8270.
50. **Honda, H., J. Iwahashi, T. Kashiwagi, Y. Imamura, N. Hamada, T. Anraku, S. Ueda, T. Kanda, T. Takahashi, and S. Morimoto.** 2006. Outbreak of human metapneumovirus infection in elderly inpatients in Japan. J. Am. Geriatr. Soc. **54:**177–180.
51. **Huck, B., G. Scharf, D. Neumann-Heifelin, W. Puppe, J. Weigl, and V. Falcone.** 2006. Novel human metapneumovirus sublineage. Emerg. Infect. Dis. **12:**147–150.
52. **Ishiguro, N., T. Ebihara, R. Endo, X. Ma, H. Kikuta, H. Ishiko, and K. Kobayashi.** 2004. High genetic diversity of the attachment (G) protein of human metapneumovirus. J. Clin. Microbiol. **42:**3406–3414.
53. **Ishiguro, N., T. Ebihara, R. Endo, X. Ma, R. Shirotsuki, S. Ochiai, H. Ishiko, and H. Kikuta.** 2005. Immunofluorescence assay for detection of human metapneumovirus-specific antibodies by use of baculovirus-expressed fusion protein. Clin. Diagn. Lab. Immunol. **12:**202–205.
54. **Jartti, T., B. van den Hoogen, R. P. Garofalo, A. D. Osterhaus, and O. Ruuskanen.** 2002. Metapneumovirus and acute wheezing in children. Lancet **360:**1393–1394.
55. **Johnson, P. R., M. K. Spriggs, R. A. Olmsted, and P. L. Collins.** 1987. The G glycoprotein of human respiratory syncytial viruses of subgroups A and B: extensive sequence divergence between antigenically related proteins. Proc. Natl. Acad. Sci. USA **84:**5625–5629.
56. **Johnston, S. L., P. K. Pattemore, G. Sanderson, S. Smith, M. J. Campbell, L. K. Josephs, A. Cunningham, B. S. Robinson, S. H. Myint, M. E. Ward, D. A. Tyrrell, and S. T. Holgate.** 1996. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. Am. J. Respir. Crit. Care Med. **154:**654–660.
57. **Kaida, A., N. Iritani, H. Kubo, M. Shiomi, U. Kohdera, and T. Murakami.** 2006. Seasonal distribution and phylogenetic analysis of human metapneumovirus among children in Osaka City, Japan. J. Clin. Virol. **35:**394–399.
58. **Karron, R. A., D. A. Buonagurio, A. F. Georgiu, S. S. Whitehead, J. E. Adamus, M. L. Clements-Mann, D. O. Harris, V. B. Randolph, S. A. Udem, B. R. Murphy, and M. S. Sidhu.** 1997. Respiratory syncytial virus (RSV) SH and G proteins are not essential for viral replication in vitro: clinical evaluation and molecular characterization of a cold-passaged, attenuated RSV subgroup B mutant. Proc. Natl. Acad. Sci. USA **94:**13961–13966.
59. **Karron, R. A., P. F. Wright, R. B. Belshe, B. Thumar, R. Casey, F. Newman, F. P. Polack, V. B. Randolph, A. Deatly, J. Hackell, W. Gruber, B. R. Murphy, and P. L. Collins.** 2005. Identification of a recombinant live attenuated respiratory syncytial virus vaccine candidate that is highly attenuated in infants. J. Infect. Dis. **191:**1093–1104.
60. **Kim, Y. K., and H. J. Lee.** 2005. Human metapneumovirus-associated lower respiratory tract infections in korean infants and young children. Pediatr. Infect. Dis. J. **24:**1111–1112.
61. **Konig, B., W. Konig, R. Arnold, H. Werchau, G. Ihorst, and J. Forster.** 2004. Prospective study of human metapneumovirus infection in children less than 3 years of age. J. Clin. Microbiol. **42:**4632–4635.
62. **Ksiazek, T. G., D. Erdman, C. S. Goldsmith, S. R. Zaki, T. Peret, S. Emery, S. Tong, C. Urbani, J. A. Comer, W. Lim, P. E. Rollin, S. F. Dowell, A. E. Ling, C. D. Humphrey, W. J. Shieh, J. Guarner, C. D. Paddock, P. Rota, B. Fields, J. DeRisi, J. Y. Yang, N. Cox, J. M. Hughes, J. W. LeDuc, W. J. Bellini, and L. J. Anderson.** 2003. A novel coronavirus associated with severe acute respiratory syndrome. N. Engl. J. Med. **348:**1953–1966.
63. **Kuiken, T., R. A. Fouchier, M. Schutten, G. F. Rimmelzwaan, G. van Amerongen, D. van Riel, J. D. Laman, T. de Jong, G. van Doornum, W. Lim, A. E. Ling, P. K. Chan, J. S. Tam, M. C. Zambon, R. Gopal, C. Drosten, S. van der Werf, N. Escriou, J. C. Manuguerra, K. Stohr, J. S. Peiris, and A. D. Osterhaus.** 2003. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet **362:**263–270.
64. **Kuypers, J., N. Wright, L. Corey, and R. Morrow.** 2005. Detection and quantification of human metapneumovirus in pediatric specimens by real-time RT-PCR. J. Clin. Virol. **33:**299–305.
65. **Laham, F. R., V. Israele, J. M. Casellas, A. M. Garcia, C. M. Lac Prugent, S. J. Hoffman, D. Hauer, B. Thumar, M. I. Name, A. Pascual, N. Taratutto, M. T. Ishida, M. Balduzzi, M. Maccarone, S. Jackli, R. Passarino, R. A. Gaivironsky, R. A. Karron, N. R. Polack, and F. P. Polack.** 2004. Differential production of inflammatory cytokines in primary infection with human metapneumovirus and with other common respiratory viruses of infancy. J. Infect. Dis. **189:**2047–2056.
66. **Landry, M. L., D. Ferguson, S. Cohen, T. C. Peret, and D. D. Erdman.** 2005. Detection of human metapneumovirus in clinical samples by immunofluorescence staining of shell vial centrifugation cultures prepared from three different cell lines. J. Clin. Microbiol. **43:**1950–1952.
67. **Larcher, C., C. Geltner, H. Fischer, D. Nachbaur, L. C. Muller, and H. P. Huemer.** 2005. Human metapneumovirus infection in lung transplant recipients: clinical presentation and epidemiology. J. Heart Lung Transplant. **24:**1891–1901.
68. **Lazar, I., C. Weibel, J. Dziura, D. Ferguson, M. L. Landry, and J. S. Kahn.** 2004. Human metapneumovirus and severity of respiratory syncytial virus disease. Emerg. Infect. Dis. **10:**1318–1320.
69. **Leung, J., F. Esper, C. Weibel, and J. S. Kahn.** 2005. Seroepidemiology of human metapneumovirus (hMPV) on the basis of a novel enzyme-linked immunosorbent assay utilizing hMPV fusion protein expressed in recombinant vesicular stomatitis virus. J. Clin. Microbiol. **43:**1213–1219.
70. **Louie, J. K., J. K. Hacker, R. Gonzales, J. Mark, J. H. Maselli, S. Yagi, and W. L. Drew.** 2005. Characterization of viral agents causing acute respiratory infection in a San Francisco University Medical Center clinic during the influenza season. Clin. Infect. Dis. **41:**822–828.
71. **Ludewick, H. P., Y. Abed, N. van Niekerk, G. Boivin, K. P. Klugman, and S. A. Madhi.** 2005. Human metapneumovirus genetic variability, South Africa. Emerg. Infect. Dis. **11:**1074–1078.
72. **Mackay, I. M., S. Bialasiewicz, Z. Waliuzzaman, G. R. Chidlow, D. C. Fegredo, S. Laingam, P. Adamson, G. B. Harnett, W. Rawlinson, M. D. Nissen, and T. P. Sloots.** 2004. Use of the P gene to genotype human metapneumovirus identifies 4 viral subtypes. J. Infect. Dis. **190:**1913–1918.
73. **Madhi, S. A., H. Ludewick, Y. Abed, K. P. Klugman, and G. Boivin.** 2003. Human metapneumovirus-associated lower respiratory tract infections among hospitalized human immunodeficiency virus type 1 (HIV-1)-infected and HIV-1-uninfected African infants. Clin. Infect. Dis. **37:**1705–1710.
74. **Maertzdorf, J., C. K. Wang, J. B. Brown, J. D. Quinto, M. Chu, M. de Graaf, B. G. van den Hoogen, R. Spaete, A. D. Osterhaus, and R. A. Fouchier.** 2004. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. J. Clin. Microbiol. **42:**981–986.
75. **Maggi, F., M. Pifferi, M. Vatteroni, C. Fornai, E. Tempestini, S. Anzilotti, L. Lanini, E. Andreoli, V. Ragazzo, M. Pistello, S. Specter, and M. Bendinelli.** 2003. Human metapneumovirus associated with respiratory tract infections in a 3-year study of nasal swabs from infants in Italy. J. Clin. Microbiol. **41:**2987–2991.
76. **Martinello, R. A., F. Esper, C. Weibel, D. Ferguson, M. L. Landry, and J. S. Kahn.** 10 January 2006. Human metapneumovirus and exacerbations of chronic obstructive pulmonary disease. J. Infect. [Epub ahead of print.]
77. **Martinez, I., O. Valdes, A. Delfraro, J. Arbiza, J. Russi, and J. A. Melero.** 1999. Evolutionary pattern of the G glycoprotein of human respiratory syncytial viruses from antigenic group B: the use of alternative termination codons and lineage diversification. J. Gen. Virol. **80:**125–130.
78. **Martino, R., R. P. Porras, N. Rabella, J. V. Williams, E. Ramila, N. Margall, R. Labeaga, J. E. Crowe, Jr., P. Coll, and J. Sierra.** 2005. Prospective study of the incidence, clinical features, and outcome of symptomatic upper and lower respiratory tract infections by respiratory viruses in adult recipients of hematopoietic stem cell transplants for hematologic malignancies. Biol. Blood Marrow Transplant. **11:**781–796.
79. **McAdam, A. J., M. E. Hasenbein, H. A. Feldman, S. E. Cole, J. T. Offermann, A. M. Riley, and T. A. Lieu.** 2004. Human metapneumovirus in children tested at a tertiary-care hospital. J. Infect. Dis. **190:**20–26.
80. **Monto, A. S.** 2002. Epidemiology of viral respiratory infections. Am. J. Med. **112**(Suppl. 6A)**:**4S–12S.
81. **Mullins, J. A., D. D. Erdman, G. A. Weinberg, K. Edwards, C. B. Hall, F. J. Walker, M. Iwane, and L. J. Anderson.** 2004. Human metapneumovirus infection among children hospitalized with acute respiratory illness. Emerg. Infect. Dis. **10:**700–705.
82. **Murray, C., A. Lopez, C. Mathers, and C. Stein.** 2001. The Global Burden of Disease 2000 project: aims, methods and data sources. *In* Global programme on evidence for health policy. World Health Organization, Geneva, Switzerland.
83. **Nicholson, K. G., J. Kent, V. Hammersley, and E. Cancio.** 1997. Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden. BMJ **315:**1060–1064.
84. **Nicholson, K. G., T. McNally, M. Silverman, P. Simons, J. D. Stockton, and M. C. Zambon.** 2006. Rates of hospitalisation for influenza, respiratory

syncytial virus and human metapneumovirus among infants and young children. Vaccine **24:**102–108.
85. **Nissen, M. D., D. J. Siebert, I. M. Mackay, T. P. Sloots, and S. J. Withers.** 2002. Evidence of human metapneumovirus in Australian children. Med. J. Aust. **176:**188.
86. **Nokso-Koivisto, J., A. Pitkaranta, S. Blomqvist, J. Jokinen, M. Kleemola, A. Takala, T. Kilpi, and T. Hovi.** 2002. Viral etiology of frequently recurring respiratory tract infections in children. Clin. Infect. Dis. **35:**540–546.
87. **Noyola, D. E., A. G. Alpuche-Solis, A. Herrera-Diaz, R. E. Soria-Guerra, J. Sanchez-Alvarado, and R. Lopez-Revilla.** 2005. Human metapneumovirus infections in Mexico: epidemiological and clinical characteristics. J. Med. Microbiol. **54:**969–974.
88. **Osterhaus, A., and R. Fouchier.** 2003. Human metapneumovirus in the community. Lancet **361:**890–891.
89. **Peiris, J. S., W. H. Tang, K. H. Chan, P. L. Khong, Y. Guan, Y. L. Lau, and S. S. Chiu.** 2003. Children with respiratory disease associated with metapneumovirus in Hong Kong. Emerg. Infect. Dis. **9:**628–633.
90. **Pelletier, G., P. Dery, Y. Abed, and G. Boivin.** 2002. Respiratory tract reinfections by the new human metapneumovirus in an immunocompromised child. Emerg. Infect. Dis. **8:**976–978.
91. **Percivalle, E., A. Sarasini, L. Visai, M. G. Revello, and G. Gerna.** 2005. Rapid detection of human metapneumovirus strains in nasopharyngeal aspirates and shell vial cultures by monoclonal antibodies. J. Clin. Microbiol. **43:**3443–3446.
92. **Peret, T. C., Y. Abed, L. J. Anderson, D. D. Erdman, and G. Boivin.** 2004. Sequence polymorphism of the predicted human metapneumovirus G glycoprotein. J. Gen. Virol. **85:**679–686.
93. **Peret, T. C., G. Boivin, Y. Li, M. Couillard, C. Humphrey, A. D. Osterhaus, D. D. Erdman, and L. J. Anderson.** 2002. Characterization of human metapneumoviruses isolated from patients in North America. J. Infect. Dis. **185:**1660–1663.
94. **Peret, T. C., C. B. Hall, K. C. Schnabel, J. A. Golub, and L. J. Anderson.** 1998. Circulation patterns of genetically distinct group A and B strains of human respiratory syncytial virus in a community. J. Gen. Virol. **79:**2221–2229.
95. **Pham, Q. N., S. Biacchesi, M. H. Skiadopoulos, B. R. Murphy, P. L. Collins, and U. J. Buchholz.** 2005. Chimeric recombinant human metapneumoviruses with the nucleoprotein or phosphoprotein open reading frame replaced by that of avian metapneumovirus exhibit improved growth in vitro and attenuation in vivo. J. Virol. **79:**15114–15122.
96. **Pinner, R. W., S. M. Teutsch, L. Simonsen, L. A. Klug, J. M. Graber, M. J. Clarke, and R. L. Berkelman.** 1996. Trends in infectious diseases mortality in the United States. JAMA **275:**189–193.
97. **Poutanen, S. M., D. E. Low, B. Henry, S. Finkelstein, D. Rose, K. Green, R. Tellier, R. Draker, D. Adachi, M. Ayers, A. K. Chan, D. M. Skowronski, I. Salit, A. E. Simor, A. S. Slutsky, P. W. Doyle, M. Krajden, M. Petric, R. C. Brunham, and A. J. McGeer.** 2003. Identification of severe acute respiratory syndrome in Canada. N. Engl. J. Med. **348:**1995–2005.
98. **Rawlinson, W. D., Z. Waliuzzaman, I. W. Carter, Y. C. Belessis, K. M. Gilbert, and J. R. Morton.** 2003. Asthma exacerbations in children associated with rhinovirus but not human metapneumovirus infection. J. Infect. Dis. **187:**1314–1318.
99. **Robinson, J. L., B. E. Lee, N. Bastien, and Y. Li.** 2005. Seasonality and clinical features of human metapneumovirus infection in children in Northern Alberta. J. Med. Virol. **76:**98–105.
100. **Rohde, G., I. Borg, U. Arinir, J. Kronsbein, R. Rausse, T. T. Bauer, A. Bufe, and G. Schultze-Werninghaus.** 2005. Relevance of human metapneumovirus in exacerbations of COPD. Respir. Res. **6:**150.
101. **Rose, J. K.** 1996. Positive strands to the rescue again: a segmented negative-strand RNA virus derived from cloned cDNAs. Proc. Natl. Acad. Sci. USA **93:**14998–15000.
102. **Ruiz, M., S. Ewig, M. A. Marcos, J. A. Martinez, F. Arancibia, J. Mensa, and A. Torres.** 1999. Etiology of community-acquired pneumonia: impact of age, comorbidity, and severity. Am. J. Respir. Crit. Care Med. **160:**397–405.
103. **Ruuskanen, O., M. Arola, T. Heikkinen, and T. Ziegler.** 1991. Viruses in acute otitis media: increasing evidence for clinical significance. Pediatr. Infect. Dis. J. **10:**425–427.
104. **Samransamruajkit, R., W. Thanasugarn, N. Prapphal, A. Theamboonlers, and Y. Poovorawan.** 2005. Human metapneumovirus in infants and young children in Thailand with lower respiratory tract infections; molecular characteristics and clinical presentations. J. Infect. **52:**254–263.
105. **Sarasini, A., E. Percivalle, F. Rovida, G. Campanini, E. Genini, M. Torsellini, S. Paolucci, F. Baldanti, A. Marchi, M. Grazia Revello, and G. Gerna.** 2006. Detection and pathogenicity of human metapneumovirus respiratory infection in pediatric Italian patients during a winter-spring season. J. Clin. Virol. **35:**59–68.
106. **Sasaki, A., H. Suzuki, R. Saito, M. Sato, I. Sato, Y. Sano, and M. Uchiyama.** 2005. Prevalence of human metapneumovirus and influenza virus infections among Japanese children during two successive winters. Pediatr. Infect. Dis. J. **24:**905–908.
107. **Schappert, S.** 1999. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 1997, p. 1–39. *In* Vital and health statistics, series 13. Data from the National Health Survey. Centers for Disease Control and Prevention, Atlanta, Ga.
108. **Scheltinga, S. A., K. E. Templeton, M. F. Beersma, and E. C. Claas.** 2005. Diagnosis of human metapneumovirus and rhinovirus in patients with respiratory tract infections by an internally controlled multiplex real-time RNA PCR. J. Clin. Virol. **33:**306–311.
109. **Schildgen, O., T. Geikowski, T. Glatzel, J. Schuster, and A. Simon.** 2005. Frequency of human metapneumovirus in the upper respiratory tract of children with symptoms of an acute otitis media. Eur. J. Pediatr. **164:**400–401.
110. **Schildgen, O., T. Geikowski, T. Glatzel, A. Simon, A. Wilkesmann, M. Roggendorf, S. Viazov, and B. Matz.** 2004. New variant of the human metapneumovirus (HMPV) associated with an acute and severe exacerbation of asthma bronchiale. J. Clin. Virol. **31:**283–288.
111. **Schildgen, O., T. Glatzel, T. Geikowski, B. Scheibner, B. Matz, L. Bindl, M. Born, S. Viazov, A. Wilkesmann, G. Knopfle, M. Roggendorf, and A. Simon.** 2005. Human metapneumovirus RNA in encephalitis patient. Emerg. Infect. Dis. **11:**467–470.
112. **Semple, M. G., A. Cowell, W. Dove, J. Greensill, P. S. McNamara, C. Halfhide, P. Shears, R. L. Smyth, and C. A. Hart.** 2005. Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. J. Infect. Dis. **191:**382–386.
113. **Skiadopoulos, M. H., S. Biacchesi, U. J. Buchholz, J. M. Riggs, S. R. Surman, E. Amaro-Carambot, J. M. McAuliffe, W. R. Elkins, M. St Claire, P. L. Collins, and B. R. Murphy.** 2004. The two major human metapneumovirus genetic lineages are highly related antigenically, and the fusion (F) protein is a major contributor to this antigenic relatedness. J. Virol. **78:**6927–6937.
114. **Stockton, J., I. Stephenson, D. Fleming, and M. Zambon.** 2002. Human metapneumovirus as a cause of community-acquired respiratory illness. Emerg. Infect. Dis. **8:**897–901.
115. **Sullender, W. M., M. A. Mufson, L. J. Anderson, and G. W. Wertz.** 1991. Genetic diversity of the attachment protein of subgroup B respiratory syncytial viruses. J. Virol. **65:**5425–5434.
116. **Suzuki, A., O. Watanabe, M. Okamoto, H. Endo, H. Yano, M. Suetake, and H. Nishimura.** 2005. Detection of human metapneumovirus from children with acute otitis media. Pediatr. Infect. Dis. J. **24:**655–657.
117. **Tang, R. S., K. Mahmood, M. Macphail, J. M. Guzzetta, A. A. Haller, H. Liu, J. Kaur, H. A. Lawlor, E. A. Stillman, J. H. Schickli, R. A. Fouchier, A. D. Osterhaus, and R. R. Spaete.** 2005. A host-range restricted parainfluenza virus type 3 (PIV3) expressing the human metapneumovirus (hMPV) fusion protein elicits protective immunity in African green monkeys. Vaccine **23:**1657–1667.
118. **Ulloa-Gutierrez, R., P. Skippen, A. Synnes, M. Seear, N. Bastien, Y. Li, and J. C. Forbes.** 2004. Life-threatening human metapneumovirus pneumonia requiring extracorporeal membrane oxygenation in a preterm infant. Pediatrics **114:**e517–e519.
119. **van den Hoogen, B. G., T. M. Bestebroer, A. D. Osterhaus, and R. A. Fouchier.** 2002. Analysis of the genomic sequence of a human metapneumovirus. Virology **295:**119–132.
120. **van den Hoogen, B. G., J. C. de Jong, J. Groen, T. Kuiken, R. de Groot, R. A. Fouchier, and A. D. Osterhaus.** 2001. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat. Med. **7:**719–724.
121. **van den Hoogen, B. G., S. Herfst, L. Sprong, P. A. Cane, E. Forleo-Neto, R. L. de Swart, A. D. Osterhaus, and R. A. Fouchier.** 2004. Antigenic and genetic variability of human metapneumoviruses. Emerg. Infect. Dis. **10:**658–666.
122. **van den Hoogen, B. G., D. M. Osterhaus, and R. A. Fouchier.** 2004. Clinical impact and diagnosis of human metapneumovirus infection. Pediatr. Infect. Dis. J. **23:**S25–32.
123. **van den Hoogen, B. G., G. J. van Doornum, J. C. Fockens, J. J. Cornelissen, W. E. Beyer, R. de Groot, A. D. Osterhaus, and R. A. Fouchier.** 2003. Prevalence and clinical symptoms of human metapneumovirus infection in hospitalized patients. J. Infect. Dis. **188:**1571–1577.
124. **van Gageldonk-Lafeber, A. B., M. L. Heijnen, A. I. Bartelds, M. F. Peters, S. M. van der Plas, and B. Wilbrink.** 2005. A case-control study of acute respiratory tract infection in general practice patients in The Netherlands. Clin. Infect. Dis. **41:**490–497.
125. **Viazov, S., F. Ratjen, R. Scheidhauer, M. Fiedler, and M. Roggendorf.** 2003. High prevalence of human metapneumovirus infection in young children and genetic heterogeneity of the viral isolates. J. Clin. Microbiol. **41:**3043–3045.
126. **von Linstow, M. L., H. Henrik Larsen, J. Eugen-Olsen, A. Koch, T. Nordmann Winther, A. M. Meyer, H. Westh, B. Lundgren, M. Melbye, and B. Hogh.** 2004. Human metapneumovirus and respiratory syncytial virus in hospitalized Danish children with acute respiratory tract infection. Scand. J. Infect. Dis. **36:**578–584.
127. **Wertz, G. W., P. L. Collins, Y. Huang, C. Gruber, S. Levine, and L. A. Ball.** 1985. Nucleotide sequence of the G protein gene of human respiratory syncytial virus reveals an unusual type of viral membrane protein. Proc. Natl. Acad. Sci. USA **82:**4075–4079.

128. **Williams, J. V., J. E. Crowe, Jr., R. Enriquez, P. Minton, R. S. Peebles, Jr., R. G. Hamilton, S. Higgins, M. Griffin, and T. V. Hartert.** 2005. Human metapneumovirus infection plays an etiologic role in acute asthma exacerbations requiring hospitalization in adults. J. Infect. Dis. **192:**1149–1153.
129. **Williams, J. V., P. A. Harris, S. J. Tollefson, L. L. Halburnt-Rush, J. M. Pingsterhaus, K. M. Edwards, P. F. Wright, and J. E. Crowe, Jr.** 2004. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. N. Engl. J. Med. **350:**443–450.
130. **Williams, J. V., R. Martino, N. Rabella, M. Otegui, R. Parody, J. M. Heck, and J. E. Crowe, Jr.** 2005. A prospective study comparing human metapneumovirus with other respiratory viruses in adults with hematologic malignancies and respiratory tract infections. J. Infect. Dis. **192:**1061–1065.
131. **Williams, J. V., S. J. Tollefson, P. W. Heymann, H. T. Carper, J. Patrie, and J. E. Crowe.** 2005. Human metapneumovirus infection in children hospitalized for wheezing. J. Allergy Clin. Immunol. **115:**1311–1312.
132. **Williams, J. V., S. J. Tollefson, S. Nair, and T. Chonmaitree.** 2006. Association of human metapneumovirus with acute otitis media. Int. J. Pediatr. Otorhinolaryngol. **70:**1189–1193.
133. **Williams, J. V., C. K. Wang, C. F. Yang, S. J. Tollefson, F. S. House, J. M. Heck, M. Chu, J. B. Brown, L. D. Lintao, J. D. Quinto, D. Chu, R. R. Spaete, K. M. Edwards, P. F. Wright, and J. E. Crowe, Jr.** 2006. The role of human metapneumovirus in upper respiratory tract infections in children: a 20-year experience. J. Infect. Dis. **193:**387–395.
134. **Wolf, D. G., Z. Zakay-Rones, A. Fadeela, D. Greenberg, and R. Dagan.** 2003. High seroprevalence of human metapneumovirus among young children in Israel. J. Infect. Dis. **188:**1865–1867.
135. **Wright, A. L., L. M. Taussig, C. G. Ray, H. R. Harrison, and C. J. Holberg.** 1989. The Tucson Children's Respiratory Study. II. Lower respiratory tract illness in the first year of life. Am. J. Epidemiol. **129:**1232–1246.
136. **Wyde, P. R., S. N. Chetty, A. M. Jewell, G. Boivin, and P. A. Piedra.** 2003. Comparison of the inhibition of human metapneumovirus and respiratory syncytial virus by ribavirin and immune serum globulin in vitro. Antiviral Res. **60:**51–59.
137. **Wyde, P. R., E. H. Moylett, S. N. Chetty, A. Jewell, T. L. Bowlin, and P. A. Piedra.** 2004. Comparison of the inhibition of human metapneumovirus and respiratory syncytial virus by NMSO3 in tissue culture assays. Antiviral Res. **63:**51–59.
138. **Xepapadaki, P., S. Psarras, A. Bossios, M. Tsolia, D. Gourgiotis, G. Liapi-Adamidou, A. G. Constantopoulos, D. Kafetzis, and N. G. Papadopoulos.** 2004. Human metapneumovirus as a causative agent of acute bronchiolitis in infants. J. Clin. Virol. **30:**267–270.
139. **Yui, I., A. Hoshi, Y. Shigeta, T. Takami, and T. Nakayama.** 2003. Detection of human respiratory syncytial virus sequences in peripheral blood mononuclear cells. J. Med. Virol. **70:**481–489.
140. **Zambon, M. C., J. D. Stockton, J. P. Clewley, and D. M. Fleming.** 2001. Contribution of influenza and respiratory syncytial virus to community cases of influenza-like illness: an observational study. Lancet **358:**1410–1416.