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Human metapneumovirus (HMPV) was isolated from a 63-year-old multiple myeloma patient who had undergone hematopoietic stem cell transplantation and who presented with lower respiratory tract infection several weeks prior to the diagnosis of lung cancer. The isolate was phylogenetically and biologically characterized and compared to HMPV prototypes and recent pediatric isolates. Remarkably, it belonged to the novel genomic subgroup A2b.

CASE REPORT

A 63-year-old male patient with relapsed multiple myeloma was admitted in early February 2004 because of dyspnea, cough, and fever. The patient had received autologous hematopoietic stem cell transplantation (HSCT) in 1999 but, after relapsing multiple myeloma, he eventually had been treated with high-dose dexamethasone according to the study protocol of the Velcade study, arm B (7). The last application of dexamethasone had been 3 weeks prior to the onset of respiratory symptoms. Four weeks before admission, pulmonary embolism (right lower lobe) had been diagnosed.

Initial clinical examinations revealed ubiquitous sibilant rhonchi and bilateral fine rales upon auscultation. Oxygen saturation under application of 4 liters of oxygen per min was 88%. Values for C-reactive protein were slightly elevated (29 mg/dL). The initial white blood cell count was 4.5 × 10^9/μL. Flow cytometry of whole-blood EDTA samples obtained at day 8 after admission showed severely decreased absolute counts of CD3+ (320/μL) and CD4+ (74/μL) T cells and NK cells (48/μL) but only moderately lowered CD8+ T-cell counts (198/μL) according to age-matched reference values (1). The CD4+/CD8+ ratio (0.28) was inverted. Staining for HLA-DR showed high percentages of activated T cells (16.7% CD8+ HLA-DR+). The normal proliferative response to phytohemagglutinin and gamma interferon production after in vitro phorbol myristate acetate-ionomycin stimulation were also assessed.

A computed tomography (CT) scan of the thorax (Fig. 1) demonstrated diffuse infiltrates in the lower left lobe with pleural effusion, as well as nodules in the upper left lobe and paracardially in the right middle lobe initially thought to represent residual lesions after pulmonary embolism. Bronchoscopy with bronchoalveolar lavage (BAL) was performed, and the patient was treated empirically with broad-spectrum antibiotics and voriconazole. BAL fluid was subjected to virological and microbiological nucleic acid amplification and culture procedures. Only human metapneumovirus (HMPV) was detected in lavage specimens by reverse transcription-PCR.

The patient developed respiratory insufficiency and eventually needed mechanical ventilation but recovered thereafter and remained clinically stable. A chest CT scan obtained 4 weeks after admission showed regression of the diffuse infiltrates in the lower left lobe but persistence of the nodular infiltrates in the upper lobe (data not shown). However, a few weeks later the patient again developed respiratory insufficiency and progressive left-sided pleural effusion. Cytologically, there was a suspicion of bronchial carcinoma cells in a pleural specimen. The clinical condition rapidly deteriorated, and the patient died with signs of left-sided rapidly progressing bronchial carcinoma with effusion. Autopsy confirmed this diagnosis.

An HMPV isolate (D04-299) was recovered from a BAL specimen on LLC-MK2 monkey kidney cells using culture medium supplemented with 5 μg of trypsin/ml. Five days after inoculation, a cytopathic effect was visible. Positive cultures were confirmed by immunofluorescence testing using an anti-HMPV serum (kindly provided by P. L. Collins, National Institutes of Health, Bethesda, MD) and reverse transcription-PCR (4). After three passages on LLC-MK2 cells, the viral isolate was further characterized together with four reference HMPV isolates previously recovered in our lab. These belonged to genotypes A1, A2a, B1, and B2 as proven by phylogenetic analysis (4). Sequencing of the complete open reading frames for nucleoprotein (N), fusion protein (F), and G protein (G) was followed by alignment using the CLUSTAL W algorithm, including prototype HMPV strains from Canada and The Netherlands (GenBank accession numbers AY297749, AY145294, AF371337, AY145295, AY145277, AY525843, AY355335, AY297748, AY355324, and AY574227). Phylogenetic analysis (using the neighbor-joining algorithm within MEGA software and the Kimura two-parameter substitution model) revealed that the newly isolated HMPV strain D04-299 belonged to the viral subtype A but displayed considerable diversity to subgroups A1 and A2. Comparison with sequences of the previously identified genotype A2b (4) showed coclustering of
Interestingly, genotype A2b was detected frequently in children during the respective winter-spring season in Germany. Viral replication capacity of HMPV strain D04-299 was compared to that of the four HMPV reference isolates belonging to genotypes A1, A2a, B1, and B2 by multicycle growth kinetics (multiplicity of infection of 0.1) on monkey kidney cell lines (Vero and LLC-MK2) and two human cell lines (larynx carcinoma [HEp-2] and bronchial epithelium [HBE]). Virus titers were determined by plaque assay in Vero cells under methylcellulose overlay. Infection of LLC-MK2 and Vero cells showed comparable replication of all viral isolates, with higher titers reached in Vero cells. The growth kinetics in LLC-MK2 cells (data not shown) showed peak titers within 32 h postinfection (hpi), whereas in Vero cells the peak was not reached before 90 hpi. Interestingly, and in contrast to the other isolates, no decrease in virus titers was observed for D04-299 and the A1 reference strains after this time point (Fig. 3A). Virus titers in HEp-2 and HBE cells were lower compared to the monkey kidney cells but similar for all isolates (Fig. 3B). Viral spread in HBE cells occurred less rapidly, and titers remained constant for up to 4 days.

In conclusion, the replication kinetics of HMPV strongly depend on the cell lines used, being most efficient in Vero cells. The replication of D04-299 differed only marginally from that of the reference isolates belonging to the four HMPV subgroups described thus far.

Since its initial discovery in 2001, a variety of reports have described the clinical significance of HMPV (5). Being prevalent throughout the world, HMPV was shown to be responsible for a part of the respiratory infections that could not be explained by any of the bacterial or viral pathogens known thus far. Seroprevalence reaches about 100% by the age of 5 years (8). Reinfections with HMPV have been reported in children (6) and could be identified also in adults, especially in the elderly. The clinical presentation of HMPV infection in otherwise-healthy individuals is similar to respiratory syncytial virus-related disease and ranges from upper respiratory infection to pneumonia and bronchiolitis (2). Severe illness in adults has been reported in association with immunosuppression, including HSCT (3, 6, 9). However, further prospective studies are needed to investigate in more detail the incidence of HMPV infection, as well as the HMPV-related morbidity and prognosis in these patients.

We have presented here a case of HMPV infection in an adult patient with relapsed multiple myeloma and rapidly progressing lung cancer after autologous HSCT. Sequencing of three complete genes (F, G, and N) of the D04-299 isolate and phylogenetic analysis has revealed that it belongs to the newly described genomic subgroup A2b (4), which was highly prevalent in the community at the time of the patient’s respiratory infection. When comparing the isolate D04-299 to HMPV reference strains from a pediatric cohort, only marginal differences of in vitro replication could be observed. The patient mounted a weak humoral immune response to HMPV, as determined by indirect immunofluorescence testing 4 weeks after HMPV recovery from BAL (data not shown). Six months earlier, he had been found to be seronegative, which might be explained by loss of antibodies due to his underlying disease and therapeutic measures rather than by recent primary infection.

Thus, the clinical presentation and the laboratory data suggest lower respiratory tract disease due to HMPV reinfection aggravated by immunosuppression. Although the case was complicated by the rapidly progressing bronchial carcinoma with lethal outcome, the association of respiratory symptoms and radiological features with viral isolation strongly supports a key role of HMPV in this acute pulmonary disease. High-dose dexamethasone treatment administered 3 weeks before onset of symptoms and resulting in severe immunosuppression has probably triggered the progression of this community-acquired virus infection. The temporary clinical improvement observed, together with subsequent regression of the diffuse lower respiratory tract disease, suggests a potential role of HMPV in this complex clinical scenario.
FIG. 2. Phylogenetic analysis of human metapneumovirus isolates. Neighbor-joining phylogenetic trees compare the isolate D04-299 to prototype isolates from Canada, Japan, and The Netherlands, as well as our own reference strains. The phylogenetic trees are calculated on complete open reading frames of the 1,206-nucleotide N gene (A), the 651- to 708-nucleotide G gene (B), and the 1,645-nucleotide F gene (C). Bootstrap values are plotted at the main internal branches to show support values.
infiltrates, is compatible with this interpretation and suggests the presence of two unlinked lung pathologies in this case.

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FIG. 3. Replication of isolate D04-299 in Vero cells (A) and HBE cells (B) compared to our reference isolates, representing the four main genetic lineages of HMPV.