English language literature. Interestingly, these patients had plastic bronchitis, irrespective of the presence or absence of the known underlying diseases for plastic bronchitis; only 1 patient had bronchial asthma which is known to be an underlying cause for plastic bronchitis.[5] The other 2 patients had no obvious history of asthma or allergy, although they sometimes had wheezing when they had had common colds. We suggest that H1N1 influenza can be associated with plastic bronchitis, irrespective of presence or absence of underlying cardiopulmonary diseases. We also suggest that patients who have a history of wheezing episodes may be at higher risk for plastic bronchitis during the course of infection. Patients with wheezing may have similar pathologic conditions in the bronchus to those with asthma that underlie formation of bronchial casts. Those pathologic conditions in the central airway might include temperature, humidity, pressure, viscosity, or amount of fibrin or mucin exudates, degrees of inflammation, or immune response.

While 2009 H1N1 influenza infection induces acute respiratory distress syndrome in adults,[6] it might induce plastic bronchitis preferentially in children. The question arises as to why there have been no reports concerning plastic bronchitis in patients with 2009 H1N1 influenza infection. It is speculative that bronchoscopic examinations might not have been performed in most children who had severe respiratory distress, and repeated bronchoscopy may sometimes be required for detection of bronchial casts, as we experienced in case 1.

The time from symptom onset to admission was 1 to 2 days in our patients. The course is so rapid that anti-influenza virus agents may not be effective for the treatment of plastic bronchitis. Because patients with plastic bronchitis are at high risk for serious complications, admission to an intensive care unit is mandatory. One of the most effective treatments for plastic bronchitis is bronchoscopic removal of bronchial casts.

We conclude that in children with H1N1 influenza virus infection, who develop rapid and progressive respiratory distress with whole lung atelectasis, clinicians should be aware of the possibility of plastic bronchitis and consider bronchoscopic evaluation.

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PERSISTENCE OF HUMAN BOCAVIRUS DNA IN IMMUNOCOMPROMISED CHILDREN

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Abstract: Human bocavirus is frequently detected in immunocompetent as well as in immunocompromised children. However, the course of infection in immunocompromised children is still poorly investigated. In the present study, we describe 4 cases of repeat human bocavirus detection in the presence of severe immunodeficiency. In the view of homologous viral sequences identified in serial samples, possible persistence and reactivation in these patients are discussed.

Key Words: respiratory virus infection, human bocavirus, hematopoietic stem cell transplantation

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Viral acute respiratory infections represent a frequent complication among immunocompromised patients. Virus identification is relevant for preventive and therapeutic measures, including isolation and avoidance of unnecessary antibiotic therapies. The emergence of new respiratory viruses such as human bocavirus (HBoV)[1] in immunocompetent children raises questions regarding the significance of this newly described virus in the immunocompromised host.

In the present study, various respiratory samples were collected from immunocompromised pediatric patients treated at our institution in a 3-year period (2004–2007), whenever new respiratory symptoms occurred. The respiratory samples—together with nonrespiratory specimens (ie, plasma and feces) collected for other medical reasons around the time when respiratory symptoms began—were retrospectively screened by quantitative real-time polymerase chain reaction (PCR) for HBoV DNA.[2] Patients' charts were reviewed for relevant clinical data. Additionally, all respiratory specimens were screened for other respiratory viruses by multiplex PCR, using the ID-TAG Respiratory Viral Panel (TM Bioscience Corporation, Toronto, ON).[3] PCRs were performed prospectively on plasma samples for the detection of Epstein Barr virus (EBV) and cytomegalovirus (CMV). In total, 135 separate respiratory episodes occurring in 69 immunocompromised patients were included. In the present study, we describe 4 cases in which HBoV DNA was repeatedly detected during a prolonged period.

CASE REPORTS

Case 1. A 2-year-old girl with severe combined immunodeficiency syndrome was hospitalized in July 2004 with an EBV-associated B-cell lymphoma and active EBV infection. She presented with fever, lymphadenopathy, and mucosal infiltrates. Her general condition improved with rituximab and cidofovir treatment. Transplantation of allogenic hematopoietic stem cells (HSC) from an EBV-positive donor was planned. A reduced conditioning protocol (thiotepa, 5 mg/kg; fludarabine, 40 mg/m^2; and ATG) was adopted. With this regimen, the EBV DNA load declined from 153,000 to 7000 copies/mL and later was negative. However, the patient was severely granulocytopenic, and developed pulmonary aspergillosis. Pulmonary symptoms continued to deteriorate, despite aggressive antimicrobial and antifungal therapy. At this time, a nasopharyngeal aspirate (NPA), and 2 plasma samples taken 1 day before and 4 days thereafter, tested positive for HBoV (Fig. 1). Coinfection with rhinovirus was assessed by multiplex PCR.[3] A lung biopsy confirmed invasive aspergillosis with focal and intraalveolar bleeding and it also harbored HBoV DNA (Fig. 1). The patient

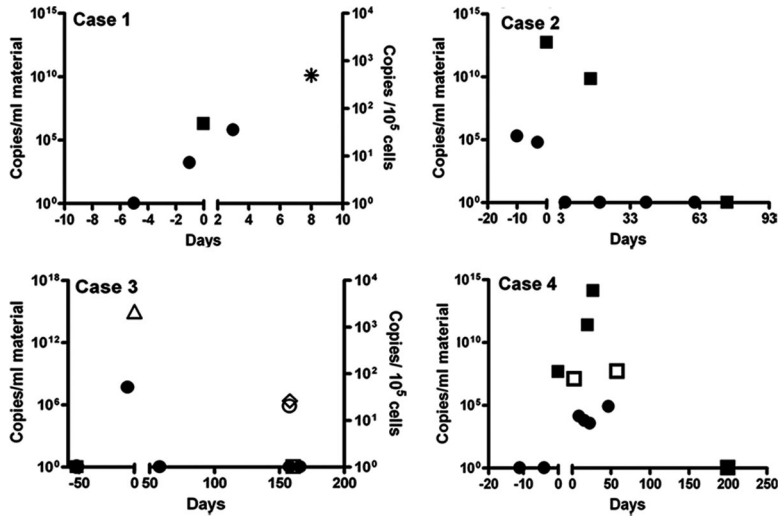

FIGURE 1. HBoV DNA load. HBoV quantification was performed in NPA (■), plasma (●), lung biopsy (*), BAL (Δ), sphenoid sinus biopsy (○), sphenoid sinus secretion (◇), and feces (□). Time point of first HBoV identification in respiratory samples is defined as day 0. Time points before or after day 0 are depicted as negative or positive figures, respectively.

eventually died of acute pulmonary decompensation and generalized capillary leakage.

Case 2. An 8-month-old EBV-positive boy with hemophagocytic lymphohistiocytosis was admitted to the bone marrow transplantation unit in July 2006. Treatment with cyclosporine A and steroids resulted in an increase of EBV DNA load from 518 to 9450 copies/mL in whole blood. The patient was treated with rituximab. At this time point, he also developed respiratory symptoms (tachypnea and supplemental oxygen requirement) with fever. Chest radiography showed lung infiltrates in the basal lower lobes. Two NPAs obtained at the beginning of the respiratory symptoms harbored high load of HBoV DNA (5×10^{12} and 7×10^9 copies/mL). Codetection of rhinovirus and human coronavirus NL63 RNA was assessed by multiplex PCR.[3] Viremia with low HBoV DNA load (1×10^2 and 2.3×10^3 copies /mL) was observed before (ie, day 11 and day 3) but not after HBoV detection in NPA (Fig. 1). The peak of HBoV DNA load in the respiratory tract was concomitant to EBV reactivation. The patient recovered, and subsequent serum samples and NPA were negative for HBoV DNA. Two weeks later, the patient underwent HSC transplantation and died of hepatic veno-occlusive disease.

Case 3. An 18-year-old boy with myelodysplastic syndrome and a history of HSC transplantation, completed 2 years previously, was hospitalized in March 2007 for acute respiratory symptoms. He presented with fever, productive cough, and tachypnea. Peribronchial infiltrates were evident on chest radiograph. The patient was under intensive immunosuppression to treat chronic GvHD of skin and mucosa. A bronchoalveolar lavage (BAL) and a serum sample taken at this time point were retrospectively tested for the presence of HBoV. The samples revealed extremely high DNA load in the BAL ($>10^{15}$ copies/mL) and relatively high load (4.7×10^7 copies /mL) in plasma. Additionally, the BAL sample was positive for PIV-3 in the multiplex PCR.[3] Microbiologic analysis of the BAL revealed the presence of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Aspergillus fumigatus*, as well as *Pneumocystis jirovecii* DNA, although no cysts were detected. Antifungal and antibacterial therapy was started and the patient's general condition improved. Later serum samples as well as an NPA and a stool sample were negative for HBoV (Fig. 1). The patient also had chronic sinusitis, and 5 months later underwent surgery for suspected aspergilloma. However, this diagnosis was not confirmed and microbiologic analysis of the sphenoid sinus biopsy and exudates was negative for the presence of fungi. Analyses of both materials for the presence of respiratory viruses revealed HBoV as the sole agent in the biopsy and HBoV and rhinovirus in the exudate. No NPA was available at the time of testing. Partial sequencing of the VP1 gene of HBoV from BAL, plasma, and sphenoid sinus samples was performed as described,[4] and revealed 100% identity. This finding is in accordance with persistence of the same HBoV strain over a 5-month period.

Case 4. A 4-year-old boy with dyskeratosis congenita developed pneumonia with perihilar infiltrates 17 days after HSCT. Repeat detection of high HBoV DNA load in NPA during a 2-month period with codetection of rhinovirus, which was accompanied by HBoV viremia and prolonged HBoV shedding (3 months) in the feces even after resolution of respiratory symptoms, has already been reported in detail.[5] As described previously, the patient showed a simultaneous CMV reactivation concomitant to the peak in HBoV load in NPS.[5] Partial sequencing of the VP1 gene of HBoV DNA obtained from the different samples was performed as described,[4] and revealed 100% identity, suggesting persistence of the same HBoV strain.

DISCUSSION

The present study shows that HBoV can be detected at moderate to high viral loads in samples from immunocompromised patients with underlying hematologic diseases or primary immunodeficiencies undergoing HSC transplantation. Previous studies have shown that prolonged HBoV shedding can be observed during immunosuppression, which possibly indicate persistence and/or reactivation in these patients.[5,6] A recent work also shows prolonged detection of HBoV in immunocompetent children with respiratory tract disease.[7]

In the 4 cases described in this study, we repeatedly detected HBoV DNA in the respiratory tract and/or in plasma, and in one case also in the gastrointestinal tract, for a period of up to 5 months. In all cases, HBoV replication in the respiratory tract was accompanied by viremia. However, the clinical relevance of prolonged detection of HBoV in respiratory samples and plasma is

unclear. Failure of the immune system in all 4 cases was characterized by severe granulocytopenia (below 500 granulocytes/μL) and/or impairment of both the T-cell and the B-cell compartment with opportunistic infection (aspergillosis) in case 1, EBV reactivation in case 2, aspergillosis and *P. jirovecii* infection in case 3, and CMV reactivation in case 4. This strongly indicates that severe immunodeficiency may lead to high levels of HBoV replication. Detection of HBoV in the lung biopsy of patient 1 should be interpreted with caution, because HBoV DNA was detected at the same time point in plasma, and we cannot exclude blood contamination of lung biopsy. Most intriguing was the detection of HBoV DNA in a sphenoid sinus biopsy 5 months after detection of more than 10^{15} and 10^{7} copies/mL in BAL and in plasma, respectively, in the absence of HBoV DNA in blood. HBoV may have played a role in the pathogenesis of chronic sinusitis in this case; alternatively, the mucosal tissue of nasal sinuses may represent a site of HBoV persistence after high-level replication in the respiratory tract. Similarly, recent findings have suggested that HBoV may establish persistent infections of mucosal lymphocytes and/or contribute to tonsillar hyperplasia in children.[8] Further investigations, with appropriate matched controls, are needed to understand the bystander or causative role of HBoV in chronic sinusitis in immunocompromised and immunocompetent patients.

In cases 1, 2, and 4, HBoV was detected during the late spring and summer months, whereas HBoV infections in immunocompetent patients predominantly have been described to occur during the winter season, and only rarely in spring or summer.[4] This observation, together with the fact that in these cases HBoV was detected after 3 to 5 weeks of strict isolation, further favors the hypothesis of reactivation of persistent HBoV infection during immunosuppression. Although it cannot be excluded, nosocomial infection appears to be less likely because of general prevention measures. The patient described in case 3 was 18 years old. Recent seroepidemiologic data suggest that antibodies to HBoV are present in more than 90% of children at the age of 6 years,[9] which makes primary infection in this patient unlikely. Moreover, sequence identity of HBoV DNA from samples taken 5 months apart was detected by partial sequencing of a region of the VP1 gene (819 nucleotides) known to display the greatest frequency of nucleotide polymorphisms.[4] This finding, further supported by identity of HBoV sequences in subsequent samples from a second patient (case 4), is compatible with persistent infection.

We found coinfections with one or more viruses in all 4 cases. A high frequency of codetection is a significant feature of HBoV and may argue against its causative role in respiratory infections. Moreover, Esposito et al[10] have recently shown that the clinical impact of HBoV in infected children may become significant when it is present together with other viruses. Potentially, HBoV may act as an exacerbating factor increasing the severity of infections caused by other pathogens.

Apart from the unsolved question of pathogenicity, our observations suggest the following 2 possibilities: (i) HBoV may be able to persist at low levels in the setting of an efficient immune system, thus making its detection difficult unless an immunocompromised status and/or coinfection with other viruses and subsequent increased replication occur; (ii) exposure to HBoV during immunosuppression can lead to persistent infection and prolonged viral shedding.

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ADVERSE NEUROLOGIC REACTIONS AFTER BOTH DOSES OF PANDEMIC H1N1 INFLUENZA VACCINE WITH OPTIC NEURITIS AND DEMYELINATION

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Abstract: When a neurologic condition develops after vaccination of a patient, the causal relationship is difficult to determine. We report an unusual case in which neurologic signs occurred in a previously healthy child after both doses of H1N1 2009 influenza vaccine, culminating in bilateral optic neuritis and disseminated encephalomyelitis. A causal association is more likely with repeated injury following influenza vaccination.

Key Words: optic neuritis, acute disseminated encephalomyelitis, post-vaccination, pandemic H1N1 influenza vaccine

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In response to the H1N1 pandemic of 2009, Canada deployed a domestically manufactured H1N1 vaccine adjuvanted with squalene and alpha tocopherol (Arepanrix, adjuvanted, inactivated, monovalent H1N1 vaccine, GSK Laval Quebec). Children, 6 to 35 months old, were recommended to receive 2 doses, at least 21 days apart. Surveillance activities in Canada and elsewhere detected rare instances of various demyelinating neurologic conditions in recently vaccinated persons. However, with vaccination programs occurring in the midst of an influenza pandemic, it was difficult to determine in individual cases whether the cause was infection, vaccination, or another etiology. In the absence of a diagnostic laboratory test for the trigger for demyelination, a useful but rare clinical clue to the cause is recurrence of injury upon re-exposure to the stimulus.[1,2] We report an unusual case in which neurologic