Outbreak of Human Parainfluenza Virus 3 Infections in a Hematopoietic Stem Cell Transplant Population

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Clinical manifestations and epidemiological features are described for a cluster of 12 cases of human parainfluenza virus 3 (HPIV3) infection that occurred among 64 allogeneic hematopoietic stem cell transplant (SCT) recipients in an 11-week period during spring 2000. Upper respiratory symptoms predominated. Pneumonia occurred in 3 patients and was a contributing factor in the death of 1 patient. Exposure histories and molecular analysis of HPIV3 isolates suggested that both community acquired and nosocomially transmitted infections occurred during this outbreak. A chain of transmission within the outpatient clinic appeared to have occurred in 4 outpatients and to have extended to 2 hospitalized patients. Molecular epidemiology was useful in discerning routes of transmission in this outbreak.

Respiratory viruses, such as respiratory syncytial virus (RSV), influenza virus, human parainfluenza virus (HPIV), and adenoviruses, are important causes of morbidity and occasional mortality in children and immunocompromised patients, especially in those undergoing allogeneic hematopoietic stem cell transplantation (SCT) [1–10]. Tests for these viruses have become available in the diagnostic clinical laboratory of many institutions, including our own [11–13]. Early diagnosis is the key to identifying infected patients and preventing transmission of respiratory pathogens [7, 14–16]. We implemented a prospective study for respiratory viruses in our SCT patient population, to determine the incidence of respiratory viral infections. This surveillance and heightened staff awareness of respiratory viral infections led to the recognition of a cluster of 12 HPIV3 infections that occurred over an 11-week period. This case cluster was recognized first in the outpatient clinic and later in the inpatient units. This report underlines the risk of person-to-person transmission [17] in a clinic where SCT recipients and their family members have frequent contact with one another.

Patients and Methods

Patient population. From 2 May 2000 through 18 July 2000, 12 cases of HPIV3 infection were identified in allogeneic SCT recipients at our institution. No cases occurred in 5.5 subsequent months. The indications for SCT were hematological malignancies (n = 7), renal cell carcinoma (n = 3), severe aplastic anemia (n = 1), and paroxysmal nocturnal hemoglobinuria (n = 1). All 12 patients (11 men and 1 woman) were adults (24–67 years old). Patients were enrolled in clinical trials, undergoing either non-myeloablative (n = 10) or myeloablative allogeneic (n = 2) SCT. Patients requiring inpatient treatment were readmitted to the SCT unit or directly to the intensive care unit. Clinic visits were scheduled once or twice a week for the first 3–4 months after transplantation and at 6, 12, 18, 24, 30, 36, 48, and 60 months after transplantation.

Epidemiological investigation and control measures. After the third HPIV3 case was identified in SCT recipients, it became apparent that an outbreak might be in progress. Information was collected from all patients with positive cultures. Patients were interviewed about symptoms and their exposure to other patients...
and their families. Dates of clinic visits, hospital room location, and identity of other patients in the rooms also were recorded. Charts were reviewed for age, sex, date of specimen collection, specimen type and test method, date of symptom onset and duration, comorbidities, length of stay in hospital, and type and duration of symptoms. More stringent outpatient isolation criteria were implemented at that time. Patients and family members were told to minimize contact with symptomatic patients, to wear masks during such contact, and to use frequent hand washing. Nasopharyngeal washes (NPWs) were performed on symptomatic patients, even those with minimal symptoms, who came to the outpatient clinic. Bronchoalveolar lavages (BALs) often were obtained from patients who developed lower respiratory symptoms.

**Virus isolation and antigen detection.** Respiratory secretions were obtained by either NPW or BAL from patients with respiratory symptoms, as described elsewhere [18]. NPWs also were obtained from asymptomatic patients enrolled in a protocol that required weekly surveillance for RSV. Specimens were transported to the virology laboratory on ice. Each NPW or BAL specimen was inoculated into 96 well plates containing human lung carcinoma (A549; Bio-whittaker) and Rhesus monkey kidney (RHMK; Biowhittaker) cells. Shell vials were tested by immunofluorescence at 24 and 48 h, using respiratory virus monoclonal antibody pool (Chemicon International). If stains were positive, then specific monoclonal antibodies to adenovirus, influenza A and B, HPIV1–3, and RSV were used. The extra A549 and RHMK shell vials for each positive culture were stored at −70°C. For this investigation, the frozen samples from the RHMK shell vials were shipped to the Respiratory and Enteric Viruses Branch at Centers for Disease Control and Prevention (CDC) for molecular characterization.

**Reverse-transcriptase (RT) polymerase chain reaction (PCR) and DNA sequencing.** RT-PCR and DNA sequencing reactions were performed directly on the culture material received by CDC without further passage, as described elsewhere [19], with the following modifications: (1) RNA was extracted from the culture lysate using RNA STAT-50 LS (Tel-Test); (2) cDNA was prepared in a RT reaction using random hexanucleotide primers (Roche Molecular Biochemicals); and (3) the entire hemagglutinin-neuraminidase (HN) gene open-reading frame (1719 nt) was PCR amplified, using a high fidelity DNA polymerase (Herculase Enhanced DNA Polymerase; Stratagene). Reaction products were purified by using the QIAquick gel purification kit (Qiagen), and both DNA strands were sequenced on an ABI 377 Sequencer, using a fluorescent dye-terminator kit (Applied Biosystems).

**Nucleotide sequence analysis.** The HN gene open-reading frame (1719 nt) of 14 HPIV3 isolates, 12 from SCT patients and 2 from hospitalized non-SCT control patients with concurrent HPIV3 infection, were aligned with 14 historical sequences from CDC and GenBank databases, using the program PILEUP (Wisconsin Package, version 10.1; Genetics Computer Group). Phylogenetic analysis was performed by using 3 different methods—maximum parsimony, distance, and maximum likelihood—using the software program PAUP* (version 4.0; Sinauer Associates).

**Results**

**Characteristics of the patients.** In the 77 days, from 2 May through 18 July 2000, 64 allogeneic SCT recipients, at different stages after transplantation, had a median of 6.4 visits to the clinic. Six of 12 HPIV3 cases were beyond day 100 after transplantation, all of whom had graft-versus-host disease (GVHD).

**Clinical manifestations.** Of the 12 patients with HPIV3 infection, 11 had symptoms attributed to the virus, and one was asymptomatic at the time of diagnosis. Symptoms persisted for 2–7 weeks, usually in the absence of fever. Cough was present in 11, coryza in 10, pharyngitis in 6, nasal congestion in 4, and sinusitis in 3. Neither stridor nor wheezing was observed. Pneumonia developed in 3 of the 12 patients, with pulmonary infiltrates on chest x-ray, dyspnea and hypoxia. One of the 3 patients with pneumonia had metastatic renal cell carcinoma and severe (grade 4) acute GVHD. He died of respiratory insufficiency. At autopsy, lung histopathology showed multinucleated type II pneumocytes with intracytoplasmic inclusions, which are consistent with viral pneumonia. Ultrastructural examination of lung tissue showed type II pneumocytes containing lamellar bodies and intranuclear and intracytoplasmic filamentous inclusions suggestive of paramyxoviral nucleocapsids, supporting HPIV3 etiology. Cultures were not done at autopsy. Immunohistochemistry of autopsied lung for HPIV3 was attempted but not confirmatory. The other 2 patients had undergone nonmyeloablative transplantation, were no longer neutropenic, did not have GVHD, and resolved their pneumonia.

**Molecular characterization.** A phylogenetic tree of the HPIV3 HN gene sequences from 12 outbreak cases, 2 hospitalized non-SCT control patients, and 14 diverse HN gene sequences obtained from CDC and GenBank is shown in figure 1. Sequences of isolates from 11 of 12 patients and from the ward control patient were found to be distributed in 3 distinct but related genetic clusters (genetic clusters 1–3); 1 sequence (patient 2) was genetically distant from all other sequences, falling into a different phylogenetic clade. Computation of the genetic distances among all 3 clusters showed an overall range of 0–22 nt differences, with ranges of 8–15 nt differences among clusters and 0–6, 6, and 4 nt differences within clusters 1, 2, and 3, respectively. Clusters 1 and 3 showed the highest statistical support, with bootstrap values of 100%, whereas cluster 2, consisting of patient 1 and ward control patient 2, was only weakly supported (64%). Within cluster 1, a subcluster of sequences from patients 4, 6, and 8 were identical and differed from patients 3 and 10 and ward control patient 1 by only 1 nt. In cluster 3, sequences from patients 9 and 11 also were identical.

**Epidemiologic analysis.** After the index case, all patients had potential exposures to other patients with documented HPIV3 infection in this outbreak (figure 2), with the exception of patients 7 and 12. Patient 1 was the index case who became ill 2 weeks before his diagnosis on 2 May 2000. Patient 7 had been hospitalized in the bone marrow transplant (BMT) unit and then in the ward for 2 weeks before the onset of symptoms and had no documented exposures to other HPIV3-infected patients. Patient 12 was exposed to patient 9 in the clinic 10 days before the onset of symptoms, which is an unusually pro-
Figure 1. Estimated maximum parsimony tree obtained from alignment of human parainfluenza virus 3 hemagglutinin-neuraminidase gene open-reading frame sequences (1719 nt) of 12 hematopoietic stem cell transplantation (SCT) patients (cases), 2 non-SCT control patients, and 14 of 31 diverse sequences (Seq) from Centers for Disease Control and Prevention and GenBank databases. Genetic distances between sequences are shown as the no. of differing nucleotides. Bootstrap values are indicated for selected nodes.

Figure 2. Temporal relationship among the 12 outbreak cases of human parainfluenza virus 3 (HPIV3). Timing is shown for outpatient visits, positive cultures, and hospitalization in the ward or the intensive care unit (ICU).

longed incubation period [20]. Sequence analysis indicated that ≥3 other introductions from the community occurred during this case cluster. Those introductions are represented by patients 2, 3, and 5. Both the exposure history and sequence analysis supported the hypothesis that 2 chains of nosocomial transmission had occurred. Patient 3 initiated a chain of transmissions of HPIV3 to patients 4, 6, and 10 in the clinic and then to patient 8 in the hospital. Patient 8, who died of his infection, was a roommate of patient 10 shortly before the former developed respiratory symptoms. Chronic obstructive pulmonary disease in patient 10 delayed recognition of his viral infection for 3 weeks until the patient suffered acute respiratory decompensation 1 week after transplantation. The other chain of transmission began with patient 5 transmitting to patients 9 and 11. Patient 11 was hospitalized in the SCT ward for a week before the onset of symptoms. Patient 5 visited patient 11 one week after the former was known to have a culture positive for HPIV3. Although patient 11 developed respiratory symptoms a few days after patient 5’s visit, patient 11 remained symptomatic and undiagnosed for several weeks. Patient 9 was exposed in the outpatient waiting room by case patient 5.

In sum, exposure histories and molecular analysis support the possibility that patients 1, 2, 3, 5, 7, and 12 were separate introductions. Patient 7 acquired his infection in the hospital but from an unknown source. Four cases in this outbreak were acquired in the clinic (patients 4, 6, 9, and 10). Two patients acquired their infection in a hospital ward either from a roommate (patient 8) or from a visiting clinic patient (patient 11). No patient appeared to have acquired infection while in a positive-pressure room or in the intensive care unit.

Fraternization between patients and family members com-
Discussion

Respiratory viral infections occur frequently in our SCT patients. In the 12 months before the case cluster occurred, as described above, 25 infections were detected by culture, including 10 HPIV3, 8 RSV, 3 influenza A, 3 adenovirus, and 1 HPIV2. Twenty of these 25 infections occurred during the winter months, November through March 2000. The HPIV3 case cluster we describe here occurred in the spring, which is consistent with the epidemic period for HPIV3 in the continental US since 1977 [21]. Increased HPIV3 circulation in the community increased the likelihood of multiple introductions of the virus that led to misidentification as nosocomial transmission. During the 11 weeks when the HPIV3 case cluster occurred, only 2 other respiratory viral infections were detected in our SCT patients, both due to adenoviruses. Six of 12 HPIV3 cases occurred in the first 100 days after transplantation, a period often considered to have the greatest risk of morbidity from a viral infection. Five of the 6 patients with HPIV3 infection who were beyond 100 days after transplantation had chronic GVHD, and only one had acute grade 4 GVHD.

The presence of HPIV3 in 12 (18.75%) of our 64 SCT patients is higher than the reported incidence of infections with HPIV in SCT populations (5.2% [Houston, TX] and 2.2% [Minneapolis, MN]) [1, 4]. Most reports of HPIV infections in such patients have been with HPIV3, as in our institution. In a retrospective study of 1253 patients at the University of Minnesota, 19 (70%) of 27 patients with HPIV infection were infected with HPIV 3 (i.e., 1.2 cases/year) [4]. Of those 19 patients, 4 developed respiratory failure with a crude mortality rate of 21%. In 1989, a prospective study of respiratory viral infections in 78 immunocompromised patients at the Fred Hutchinson Cancer Research Center (Seattle, WA) detected only 2 patients infected with HPIV3 [6]. Both patients developed pneumonia but recovered. In a publication from the M. D. Anderson Cancer Center (University of Texas, Houston) in 1993, HPIV3 infections were reported in 8 (3%) of 265 adult bone marrow transplant recipients [5]. Of these 8 patients, 5 were allogeneic, and 3 were autologous.

Of these 8 patients, 5 were allogeneic, and 3 were autologous transplant recipients. Six (75%) of 8 patients developed pneumonia, of whom 2 died. In a 1996 report from the same institution, the incidence of HPIV1, HPIV2, and HPIV3 was 61 cases in 1173 adult BMT patients (5.2%) over a span of 3.5 years [2]. Infections were significantly more common among allogeneic than among autologous BMT recipients (8.8% vs. 3.4%, respectively; P < .005). Most HPIV infections were due to HPIV3 (56 [92%] of 61 infections). Almost half (44%) of the patients with HPIV1, HPIV2, and HPIV3 developed pneumonia. Ten (16%) of 61 patients died. These studies established HPIV, particularly HPIV3, as an important cause of morbidity in BMT recipients and an occasional cause of mortality rate in patients in the period immediately after transplantation [8, 10]. In this case cluster, we had a mortality of 8.3% (1/12).

In general, the severity of illness observed in this group of patients was milder than in previous reports. Eighty percent of the patients in this outbreak underwent a nonmyeloablative SCT. This may be associated with more rapid immune reconstitution, compared with conventional myeloablative regimens, thus diminishing the severity of symptoms. The 1 patient who died had acute, severe (grade 4) GVHD, which had been treated with high-dose corticosteroids.

None of the above-cited studies described case clusters or attempted to trace transmission among SCT patients. Transmission of HPIV3 has been thought to occur by person-to-person contact [17]. Tracing chains of transmission of respiratory viruses within an SCT population is complicated by the frequent exposure of these patients to the community outside the hospital, either through personal contact or secondarily through their family. The majority of our SCT patients came from out of town and stayed with accompanying family members, who were more mobile and more likely to interact with the community. Tracing chains of transmission also is complicated by the difficulty in recognizing the onset of new respiratory symptoms in patients with complicating comorbidities.

Two previous studies of HPIV3 case clusters have used nucleotide sequence analysis [7, 14]. One report compared sequences of a 205-nt fragment located 5’ to the HPIV3 F gene [14]. Seventeen cases that occurred during 3 months in a pediatric ward revealed heterogeneity among isolates, although all 5 cases in a pediatric intermediate care ward differed by no more than 2 base differences [14]. A second report analyzed 237 nt from the HPIV3 F gene. Thirteen isolates were obtained from hospitalized patients in a bone marrow transplant ward during 2 case clusters that occurred a year apart. There were no more than 2 base differences among 12 of these isolates, although community isolates from these periods were more heterogeneous. In contrast to these studies of HPIV3 infection, molecular analysis of 7 RSV isolates from a hematologic cancer ward found nucleotide differences sufficient to indicate multiple introductions from the community [22].

Even when strong sequence similarity or identity among iso-
lates is found, this alone cannot establish transmission but is useful in supporting epidemiologic data. In this study, identification of identical or quasi-identical sequences among 1719 nt from the HN gene supported the epidemiologic evidence of HPIV3 transmission in 6 patients in the hospital. However, differences in sequence analysis controverted the epidemiologic evidence for transmission from patient 1 to patients 2 and 3, from patient 3 to patient 5, and from patient 6 to patient 9. One might postulate that even 4–8 nt differences may not represent different lineages of viruses but rather reflect errors introduced during RT-PCR. Because basic procedures were taken in an attempt to minimize the problem (i.e., use of a high fidelity DNA polymerase, reduction of the number of cycles during the RT-PCR reaction, and sequencing of both product strands), the possibility appears remote that the differences among the 4 clusters were due to chance alone.

Confounding morbidities made it difficult to determine morbidity and mortality attributable to HPIV3 infections in our SCT patients. However, it seems likely that HPIV3 infection was a contributing factor to the intensive care management of 3 patients and death in 1 patient. Prevention of transmission of HPIV3 and other respiratory viruses within the hospital is obviously an important part of the management of SCT recipients. Although attention is usually focused on the patients and the medical staff, our observations suggest that family members who come to the clinic and visit the wards need to be considered as sources of infection. Neither this study nor previous reports have evaluated the role of transmission by family members. The camaraderie that develops between patients and family members in SCT clinics, although providing useful psychological support, also may promote transmission of respiratory viruses. Patients and family members should be educated about precautions to be taken when respiratory symptoms occur.

As a result of this outbreak, intense educative efforts have been put in place to alert the SCT recipients, their families, and staff members about the prevention of nosocomially transmitted respiratory pathogens. We believe that early emphasis on infection control measures helped to halt this outbreak.

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