Nosocomial Transmission of Respiratory Syncytial Virus in Immunocompromised Adults

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Respiratory syncytial virus (RSV) isolates obtained from nine infected immunocompromised adult patients hospitalized during two consecutive winters (January through April 1987 and 1988) were collected and analyzed against a panel of monoclonal antibodies by an enzyme immunoassay. The history of the patients’ illness, onset of symptoms, and date of initial isolation of virus was correlated with the hospital ward and time of hospitalization. Three patients died of respiratory failure related to RSV infection acquired nosocomially. A cluster of RSV disease in four patients hospitalized simultaneously during the 1987 season was demonstrated to be caused by four antigenically distinct viruses; despite an epidemiologic link among the patients, each had been infected from a different source. The RSV disease in the five immunocompromised adults in 1988 was caused by two distinct strains; three patients were infected with RSV subgroup A/4, and two were infected with RSV subgroup B/2. Combining the epidemiologic and strain characterization studies, none of four patients in 1987 were infected from each other, and two of five patients in 1988 may have been infected, directly or indirectly, from one of the other five. The strain characterization studies demonstrated the potential complexity of RSV nosocomial transmission and the need to consider a number of sources for transmission in developing effective prevention strategies. The three deaths underscore the importance of nosocomial RSV disease and the importance of effective prevention strategies.

MATERIALS AND METHODS

We studied nine immunocompromised adult patients who had undergone bone marrow transplantation 1 to 155 days before illness; one patient (patient 3) had received a cadaveric renal transplant 3 weeks before his illness, and one (patient 8) had been on chemotherapy for 7 months prior to her illness. All nine patients had upper respiratory tract symptoms that included rhinorrhea, sore throat, cough, or sinusitis, and all had radiographic evidence of pneumonia. Three patients, all bone marrow transplant recipients, died as a consequence of nosocomial RSV infection (Fig. 1); these three patients became RSV culture positive less than 15 days following transplantation. RSV was considered the cause of death on the basis of positive viral cultures, clinical findings compatible with RSV at the onset of respiratory distress, lack of isolation of other pathogens, and the presence of compatible findings at autopsy.

Hospital setting. Patients undergoing allogeneic bone marrow transplantation are hospitalized in a 14-bed ward (ward 4A) with single-bed rooms, corridor-to-room airflows, and a high air change rate of HEPA-filtered air. High standards of infection control practices are maintained on this floor. All patients undergoing bone marrow transplantation are confined to their rooms, with visits only from close family members. Patients undergoing renal transplantation, autologous bone marrow transplantation, chemotherapy, or treatment following allogeneic bone marrow transplantation are admitted to single- or double-patient rooms in various wards throughout the 500-bed university hospital. In these wards, isolation techniques are individualized to patients’ disease statuses. Patients in these wards are generally ambulatory and have liberal visitation privileges.

Virus isolation. Specimens for viral cultures, including throat swabs, urine samples, and blood samples, were obtained from each patient at admission, weekly during hospitalization, and when requested by the attending physician. In
addition, nasopharyngeal swabs, nasal washes, and bronchoalveolar lavage specimens were obtained when clinically indicated from symptomatic patients. Specimens were transported within 4 h of collection to the hospital's virology diagnostic laboratory and directly inoculated into human epidermoid, human foreskin fibroblast, and primary rhesus monkey kidney cell cultures as previously described (6, 18).

RSV was identified by its characteristic cytopathic effect in cell culture. The initial isolate from each patient was confirmed as RSV by immunofluorescence staining (Wellcome Laboratories, Greenville, N.C.) or enzyme immunoassay (EIA) (Kallestad, Austin, Tex.) and sent to the Centers for Disease Control, where it was passaged once in HEp-2 cells and characterized by reactions with monoclonal antibodies.

No other respiratory viruses were isolated from any patient during the study period by using the culture system described above. Serological methods were not used to assess viral infection because the patients were immunocompromised and often received so many blood products that seroconversion may not have been detected or been diagnostic of a viral infection.

The duration of viral shedding was difficult to assess in our patients. In four patients, the initial specimen which grew virus was from a bronchoalveolar lavage specimen, which could not be readily obtained again until weeks later from two patients and was not obtained from two other patients. Also, the majority (eight of nine) of patients received ribavirin aerosol therapy, which inhibits the replication of RSV in patients and in tissue culture.

All 78 hospital staff and employees in contact with the index patients in ward 4A in 1987 were screened by the enzyme-linked immunosorbent assay (ELISA) antigen detection method with specimens obtained from nasopharyngeal swabs, and cultures were performed on specimens obtained from symptomatic individuals (6). All cultures and ELISA results were negative. No screening measures were instituted for hospital employees or staff in 1988.

**EIA for strain characterization monoclonal antibodies.** The monoclonal antibodies used for the strain analysis of the clinical viral isolates have been previously described (4, 23) and are listed in Table 2. Reference strains used for analysis included the Long strain (belonging to subgroup A/2; positive against monoclonal antibodies 130-6d, 130-5f, 92-11c, and 131-2a), RSV A2 (belonging to subgroup A/5; positive against monoclonal antibodies 130-6d, 130-5f, 130-2g, 92-11c, and 131-2a), and RSV 18573 (belonging to subgroup B/3; positive against monoclonal antibodies 102-100b and 131-2a and negative or low positive against 130-5f).

EIA. All isolates were reacted against a panel of eight monoclonal antibodies by a biotin-avidin EIA (Table 1). Briefly, column-purified monoclonal antibody was adsorbed to 96-well microtiter plates overnight at 4°C and then reacted against the isolate grown in HEp-2 cells diluted 1:5 in phosphate-buffered saline (PBS) with 0.5% gelatin and 0.15% Tween 20. The plates were washed, biotinylating-detecting monoclonal antibody was added, the plates were incubated for 1 h at 36°C, the plates were washed again, and peroxidase-conjugated streptavidin was added before a further 10-min incubation period. After the plates were washed, the substrate (o-phenylenediamine dihydrochloride in citrate phosphate buffer plus hydrogen peroxide [0.01%]) was added. The reaction was stopped after 45 min, and the A₄₉₀ was read. In this assay, the capture antibody for the biotinylated G protein monoclonal antibodies (130-2g, 130-6d, 143-5a, 130-5f, and 130-9g) was 131-2g; 133-1h was the capture antibody for the biotinylated F protein antibodies (92-11c, 102-10b, and 131-2a) (Table 2). Optimal dilutions for the individual reagents were determined by checkerboard titrations.

Two isolates from 1988 (patients 5 and 6) were further studied with 18 G-protein monoclonal antibodies and 4 F-protein monoclonal antibodies by a previously described tissue culture EIA (1, 3). In this method, the RSV strain was grown in HEp-2 cells and the infected cells were fixed with 80% acetone–20% PBS. The fixed cells were then reacted with the respective monoclonal antibodies, the plates were washed, and peroxidase-conjugated goal anti-mouse IgG antibody was added. The substrate was added after the plates were washed, the resulting reaction was stopped with 3.5 M HCl, and the A₄₉₀ was read.

**RESULTS**

1987 cluster of RSV. Patients 1 and 2 were hospitalized at the same time in the same ward. They became symptomatic and culture positive for RSV more than 10 days after...
admission and presumably acquired their infection while hospitalized (Fig. 1A). Patients 3 and 4 (Fig. 1A) were hospitalized during the same period but in different wards, and they were symptomatic at the time of admission, indicating community acquisition of RSV. The viral isolates from all four patients had distinct reaction patterns against the panel of monoclonal antibodies (Table 2), indicating that each patient had a different source for his infection and that patients 3 and 4 could not have been sources of infection for patients 1 or 2.

1988 cluster of RSV. In 1988, patients 5, 6, and 9 presumably acquired RSV nosocomially because signs of RSV infection and viral isolation were not documented until after more than 10 days of hospitalization (Fig. 1B). Patient 7
became symptomatic 6 days after admission, and RSV was isolated from a specimen obtained 1 day later (Fig. 1B); he could have acquired his infection either in the community or in the hospital. Patient 8 had RSV illness at the time of admission and therefore acquired infection in the community. The strain characterization studies of the viral isolates from these patients demonstrated two patterns (Table 2): one for patients 5, 6, and 7 and a second for patients 8 and 9. Thus, there were two potential clusters of cases. In the first cluster, patients 5 and 6 were hospitalized in the same ward during the same time and could have had a common source of infection, or one could have been the source of infection for the other. Patient 7 was admitted to a separate ward in the hospital after the isolation of RSV from patients 5 and 6. He shared some of the attending and consulting physicians and ancillary hospital support personnel and could have become infected from the same source as patients 5 and 6 or through shared hospital staff. In the second potential cluster, patient 9 was hospitalized 1 day after patient 8’s discharge from the same ward. Patient 9 could have become infected from the same index patient as patient 8 or via intermediary hospital staff.

**DISCUSSION**

The characterization of RSV isolates with monoclonal antibodies proved to be a valuable tool in characterizing two clusters of RSV disease. The pattern of monoclonal reactivity among the various strains identified six distinct strains. Although the clinical significance of these differences is not yet known, they demonstrated that the patients in this study had potentially different routes of nosocomial transmission. Prior to viral strain characterization, we considered all the 1987 cases of RSV in the bone marrow transplant ward to be related and to represent a chain of transmission in which hospital staff either inadvertently transmitted viruses from patient to patient or became infected themselves and then directly infected patients. Strain characterization studies showed that this was not the case. Each patient in 1987 had a RSV isolate with a distinct reaction pattern, suggesting different sources of RSV for each patient.

Similarly, in 1988 we initially suspected that the cluster of RSV cases in 1988 represented either a common source outbreak of RSV or two separate clusters of disease occurring in two separate wards. Again, this was not the case. The reaction pattern of RSV from patient 7 was different from that of viral isolates cultured from patients hospitalized during the same period in the same ward but similar to that of patients hospitalized in another ward. Furthermore, the viral isolate from one patient was similar to that of another patient who had recently been discharged. Thus, the reaction patterns of the RSV isolates suggested two patterns of RSV transmission in 1988: one for patients 5, 6, and 7 and one for patients 8 and 9. These findings suggest that nosocomial acquisition of RSV in immunocompromised patients is more complex than previously believed.

Strain analysis by monoclonal antibody evaluation demonstrated differences in viral strains which indicate different sources of infection. However, identical reaction patterns by monoclonal antibody analysis do not necessarily mean identical routes of transmission. Strains of RSV with the same monoclonal antibody reaction patterns or similar characterization by RNase protection analysis have been identified in different communities during the same or different years (2, 25). Similar RSV strains have been isolated from patients in an outbreak in institutionalized adults by both monoclonal antibody analysis and RNase protection assays (7, 25). Similar strains of RSV could therefore be a result of transmission from one of several sources, as was seen in our 1988 outbreak. Thus, a more precise measure of antigenic or genomic differences is needed to clearly delineate routes of transmission.

A number of potential routes of transmission of RSV among hospitalized pediatric patients have been previously postulated. RSV can be spread by direct contact, fomites, and possibly large-particle aerosol but is unlikely to be spread by small-particle aerosol (11, 12). Nosocomial transmission among pediatric patients appears to involve staff who transmit virus from one patient to another or who become infected themselves and directly infect patients (5, 13). In several outbreaks, transmission may have been increased among patients whose care required oral or tracheal manipulations (21, 26). Many of our patients were critically ill, and viral transmission may have been enhanced in our setting because of the frequency and intensity of supportive care required in these patients. Another source of virus may be visitors. Family members, including young children, were allowed to visit severely immunocompromised patients undergoing transplantation. Finally, in immunocompromised patients, RSV can be shed for prolonged periods of time (15), and viral isolation and clinical disease

### Table 2. Reaction of RSV isolates to MAbs

<table>
<thead>
<tr>
<th>Yr</th>
<th>Patient</th>
<th>Strain</th>
<th>Reactivity (A&lt;sub&gt;490&lt;/sub&gt; — background) with MAb to glycoprotein:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>130-6d</td>
</tr>
<tr>
<td>1987</td>
<td>1</td>
<td>B/3</td>
<td>-0.024</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>A/1</td>
<td>1.268</td>
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<tr>
<td></td>
<td>3</td>
<td>B/4</td>
<td>-0.004</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>B/1</td>
<td>-0.028</td>
</tr>
<tr>
<td>1988</td>
<td>5</td>
<td>A/4</td>
<td>1.323</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>A/4</td>
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<tr>
<td></td>
<td>8</td>
<td>B/2</td>
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<tr>
<td></td>
<td>9</td>
<td>B/2</td>
<td>-0.026</td>
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</tbody>
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*MAb, Monoclonal antibody. MAb 130-2g did not react with any of the isolates; results for this MAb are not included in this table. The patterns of monoclonal reactivity of the strains from patients 1 through 4 are sufficiently unique for these strains to be considered unique. The patterns of monoclonal reactivity of the isolates from patients 5, 6, and 7 are similar, as are the patterns of the isolates from patients 8 and 9.*
could result from a type of persistent infection exacerbated by increased levels of immune suppression (8).

The three RSV-related deaths in our nine patients underscore the risk of nosocomial RSV to the patient and the importance of defining routes of transmission and developing appropriate prevention strategies. Despite the limitations in the understanding of nosocomial transmission of RSV, guidelines for its prevention have been developed (10, 14) and shown to be at least partially effective (19). Two recent studies also suggest that transmission can be further reduced by having staff wear goggles or by strict compliance with hand-washing and gown and glove care techniques (9, 19). Additional study, however, is needed to clarify the importance of different routes of nosocomial RSV transmission and the effectiveness of different prevention strategies and ultimately to determine the most efficient and effective ways to prevent transmission of this serious nosocomial pathogen.

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REFERENCES