Common Emergence of Amantadine- and Rimantadine-Resistant Influenza A Viruses in Symptomatic Immunocompromised Adults

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The importance and significance of amantadine- or rimantadine-resistant influenza viruses in immunocompromised patients was studied in a population of adult bone marrow transplant (BMT) recipients and patients with leukemia prospectively cultured for respiratory viruses. Influenza A viruses were isolated from 29 patients with acute respiratory illness (14 BMT recipients and 15 patients with leukemia). Fifteen patients (52%) received amantadine (n = 4) or rimantadine (n = 11) therapy. All influenza isolates recovered from six patients shedding virus for ≥3 days were screened for antiviral susceptibility; resistant isolates were further genetically characterized. Initial influenza isolates were susceptible to amantadine or rimantadine, but subsequent isolates from five of six patients were resistant. Influenza-associated mortality was similar among patients with and without documented antiviral resistance (2 of 5 vs. 5 of 24). We conclude that development of antiviral resistance in immunocompromised individuals should be considered when they have been treated with antivirals and have shed influenza virus for a prolonged period. Isolation procedures should be instituted for all immunocompromised patients with influenza, both during and after therapy with amantadine or rimantadine.

Although influenza A viruses may cause serious disease in immunocompromised patients [1–8], few data exist on the efficacy of prophylaxis or treatment with amantadine or rimantadine in these patients. Amantadine and rimantadine (Flumadine; Forest Laboratories, St. Louis) have been shown to be efficacious for the prevention and treatment of infections with influenza A virus in studies of young adults, children, families, and the elderly [9–13], and amantadine or rimantadine has therefore been recommended as prophylaxis and treatment for immunocompromised patients [14]. The need for antiviral prophylaxis and chemotherapy against influenza A is important in immunologically impaired individuals, such as those undergoing bone marrow transplantation or intensive chemotherapy, because influenza vaccine administered to these patients is often poorly immunogenic and unlikely to be fully protective [15].

In the laboratory, resistance to either amantadine or rimantadine has been shown to develop rapidly when influenza A viruses are grown in the presence of these drugs. Resistance to one compound confers resistance to the other [16]. The development of influenza viruses resistant to amantadine or rimantadine during or following antiviral therapy has been well documented in studies of humans [11, 17–23]. Apparent transmission of resistant viruses to family members or close contacts who were receiving antiviral prophylaxis also has been documented [20–23]. Although several cases of long-term shedding of drug-resistant influenza virus in amantadine- or rimantadine-treated immunocompromised patients have been reported previously [24, 25], the frequency and importance of drug-resistant influenza virus in immunocompromised patients remains unclear.

Severely immunocompromised patients are an important population to study since they may shed viruses for prolonged periods, with or without the use of antiviral therapy, and are at risk for serious complications [24–28]. Prolonged shedding in the setting of antiviral therapy could potentially lead to increased rates of development of resistance and nosocomial spread of resistant virus. In this article we describe a prospective study of severely immunocompromised patients who presented with respiratory symptoms during an influenza epidemic period.

Methods

Study Design

Between 1 November 1993 and 31 January 1994, hospitalized adults receiving bone marrow transplants (BMTs), BMT recipients who were followed as outpatients, and hospitalized adults with leukemia at M.D. Anderson Cancer Center (Houston) who had signs and symptoms of an acute respiratory illness
were prospectively evaluated [4–7]. An acute respiratory illness was defined as the recent onset (≤14 days) of rhinorrhea, nasal or sinus congestion, pharyngitis, coryza, sinusitis, otitis media, cough (with or without expectoration), and/or a new radiographic infiltrate. Fever alone was not considered an indication for obtaining a respiratory specimen for viral culture. Pneumonia was defined as an acute respiratory illness occurring in association with a new radiographically evident pulmonary infiltrate.

Antiviral therapy (with amantadine, rimantadine, or ribavirin) was prescribed at the discretion of each patient’s attending physician. During this study period, prophylaxis with amantadine or rimantadine was seldom administered in the patient population under study. Treatment with these drugs was initiated during the epidemic period on the basis of clinical symptoms and often before the results of viral culture became available. To the best of our knowledge, influenza vaccine had not been administered to any of these patients, many of whom presented with acute disease at or around the time of transplantation.

### Viral Surveillance

Hospitalized patients were screened daily for signs and symptoms of acute respiratory illness by health care personnel. A team physician collected respiratory secretions for viral culture [6, 7]. A nasopharyngeal wash, along with a throat swab specimen, was obtained from cooperative adults. From patients requiring ventilatory support, endotracheal tube secretions were obtained for culture. Bronchoalveolar lavage fluid samples were cultured when available. Asymptomatic patients did not routinely have cultures performed. Cultures were repeated at the request of either the physician caring for the patient or a study physician. The clinical course of many of these patients has been previously described [6, 7].

Respiratory specimens were inoculated into viral transport media containingveal infusion broth and antibiotics (streptomycin and gentamicin) and were transported on ice to the viral diagnostic laboratory. Within 4 hours of collection, the specimens were inoculated into cell culture lines, including Madin-Darby canine kidney (MDCK), continuous rhesus monkey kidney (LLC-MK), human embryonic lung fibroblast (WI-38), and human epidermoid carcinoma (HEp-2) lines. Hemadsorption was performed on the 3rd, 5th, 10th, and 21st days after collection. Influenza infection was confirmed by ELISA and by influenza subtyping determined by PCR [29].

### Characterization of Viral Resistance

Early-passage frozen viral isolates from patients who had influenza virus recovered on two or more occasions from specimens obtained ≥3 days apart were tested for rimantadine susceptibility with two different bioassays. In initial testing, a two-step yield-reduction assay was conducted [27] with use of 96-well plates containing MDCK cells. In this assay, viral isolates were added to wells containing varying concentrations of rimantadine in minimal essential medium containing trypsin (2 µg/mL) and were allowed to incubate for 24 hours at 36°C. At the end of this incubation, the contents of each well were transferred to secondary plates containing MDCK cells (but not rimantadine) and were serially diluted. These plates were incubated for up to 21 days.

Presence or absence of influenza virus in each well was determined by the addition of a 0.5% suspension of chicken erythrocytes and assessment for hemadsorption. Resistance was defined as viral replication in the presence of ≥1-µg/mL concentration of rimantadine. A second bioassay with an EIA format was performed by measurement of the nucleoprotein synthesis in MDCK cells in the presence of rimantadine (1 µg/mL [20, 24]. In this confirmatory bioassay, the virus was considered resistant if nucleoprotein synthesis was inhibited by <50%.

The yield-reduction assay was performed to permit near-real-time identification of resistant virus, and the nasopharyngeal ELISA was utilized as a confirmatory bioassay on the batched specimens. Correlation between the two assays in this study was 100%, except for three viral isolates that could not be grown to a titer sufficient for quantitation by yield reduction.

PCR-restriction analysis was performed to confirm the bioassay results; reverse transcription–PCR amplification of the M2 region of RNA segment 7 was followed by endonuclease digestion with restriction enzymes that differentiate between nucleotide sequences typical of susceptible and resistant strains [24]. Correlation of this method with direct sequence analysis has been previously demonstrated [24].

### Results

Infection with influenza A(H3N2) viruses was documented by isolation of virus from 14 BMT recipients and 15 patients with leukemia. Initial influenza isolates were cultured from combined nasal wash/throat swab specimens (n = 24), bronchoalveolar lavage aliquots (n = 3), and endotracheal tube aspirates (n = 2). Fifteen (52%) of the 29 patients with documented influenza infection received amantadine or rimantadine therapy: 4 received amantadine at a dosage of 100 mg b.i.d., 10 received rimantadine at a dosage of 100 mg b.i.d., and 1 received both rimantadine and aerosolized ribavirin (6 g/300 mL, aerosolized over 18 h/d). One additional patient received aerosolized ribavirin only (table 1).

Two or more specimens were obtained from 13 (45%) of the 29 infected patients 2–21 days after the original sampling, and influenza virus was again isolated from 6 of these patients. The median time between the first and last influenza isolation was 7 days (range, 3–44 days). In four of six patients, viral shedding was documented for ≤8 days. Prolonged viral shedding in a BMT recipient (44 days) and a patient undergoing
amantadine or rimantadine was documented in subsequent virus 15 patients with leukemia vs. 2 of 14 BMT recipients; to antiviral therapy and no isolates thereafter; both virus isolates were seen. Although median ages were not significantly differ-
multiple viral isolates was susceptible to amantadine or riman-
ting was not routinely assessed. With regard to the interval
between appearance of the first signs or symptoms of an upper
respiratory tract infection and the initiation of amantadine or
rimantadine treatment, no difference was noted between pa-
tients infected with resistant virus and those infected with non-
resistant virus, in both the BMT recipient group and the leuke-
mia group. Furthermore, no differences in outcome (as assessed
by mortality or development of resistance) were noted between
patients undergoing bone marrow transplantation and those un-
dergoing chemotherapy for leukemia.

Characteristics of Patients

The characteristics and clinical symptoms of patients in-
ferred with amantadine- or rimantadine-resistant influenza
t were similar to those of patients in whom resistance was not
identified (table 2). A comparison of the duration of viral
shedding in the two groups cannot be made because viral shed-
ing was not routinely assessed. With regard to the interval
between appearance of the first signs or symptoms of an upper
respiratory tract infection and the initiation of amantadine or
rimantadine treatment, no difference was noted between pa-
tients infected with resistant virus and those infected with non-
resistant virus, in both the BMT recipient group and the leuke-
mia group. Furthermore, no differences in outcome (as assessed
by mortality or development of resistance) were noted between
patients undergoing bone marrow transplantation and those un-
dergoing chemotherapy for leukemia.

Although no significant differences in clinical presentation or
outcome were noted between the BMT recipients and patients
with leukemia, some differences between the two populations
were seen. Although median ages were not significantly differ-
et, more patients with leukemia were older than 50 years (8 of
15 patients with leukemia vs. 2 of 14 BMT recipients; \( P < .05 \)).

The first viruses resistant to amantadine or rimantadine were
isolated between 2 and 15 days following initiation of therapy.
Two patients had resistant viruses isolated following <4 days
of antiviral therapy. The most common amino acid change
occurred at position 31 in the transmembrane region of the M2
protein, with a substitution of serine for asparagine (\( n = 4 \)).
A change at position 30 was documented in two patients; in
one of these, a virus bearing this mutation was isolated 6 days
after isolation of virus with a mutation at codon 31.

All five patients with documented antiviral resistance were
symptomatic at the time of admission to the hospital or at the
clinic visit and likely acquired their infection in the community.
Resistant virus was found in four of these five patients after
documented exposure to antiviral therapy. Four patients had
isolates with similar amino acid substitutions at position 31,
but these changes were not seen in patients on the same ward
at similar times.

One BMT recipient (figure 1, patient 4) was an outpatient
who was never hospitalized during this period, in contrast to
the other BMT recipient with resistant influenza. The two pa-
tients with leukemia whose resistant viral isolates had similar
amino acid substitutions were not hospitalized at the same time.
The single patient who had a resistant virus isolated before
documented antiviral therapy (patient 3) was hospitalized dur-
ing the same time and in the same area as another patient with
resistant influenza (patient 2), but the resistant isolates from
these two patients were genetically distinct.

Table 1. Description of antiviral therapy for immunocompromised
patients and viral cultures for respiratory tract disease from November
1993 through February 1994 at M.D. Anderson Cancer Center (Hous-
ton).

<table>
<thead>
<tr>
<th>Variable</th>
<th>BMT recipients</th>
<th>Patients with leukemia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients with influenza</td>
<td>14</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>No. of influenza isolates</td>
<td>24</td>
<td>27</td>
<td>51</td>
</tr>
<tr>
<td>No. (%) of patients from whom &gt;2 isolates were recovered, &gt;3 d apart</td>
<td>2 (14.3)</td>
<td>4 (26.7)</td>
<td>6 (20.7)</td>
</tr>
<tr>
<td>No. (%) of patients receiving anti-influenza therapy</td>
<td>7 (50)</td>
<td>9 (60)</td>
<td>16 (53.8)</td>
</tr>
<tr>
<td>Amanantadine only</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Rimantadine only</td>
<td>6</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Rimantadine + ribavirin</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ribavirin only</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No. of patients with influenza resistant to amantadine or rimantadine</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>After amantadine therapy</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>After rimantadine therapy</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>With no documented exposure to rimantadine or amantadine</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No. (%) of treated patients with resistance documented</td>
<td>2/7 (28.6)</td>
<td>3/8 (37.5)</td>
<td>5/15 (33.3)</td>
</tr>
</tbody>
</table>

NOTE. BMT = bone marrow transplant.

Viral Analysis

The first influenza isolate from each of the six patients with
multiple viral isolates was susceptible to amantadine or riman-
tadine. One patient had two influenza A isolates obtained prior
to antiviral therapy and no isolates thereafter; both virus isolates
were susceptible to amantadine or rimantadine. Resistance to
amantadine or rimantadine was documented in subsequent virus
isolates from the remaining five patients. Thus, resistant viruses
were isolated from 5 of 6 patients who shed influenza virus for
at least 3 days and from 5 of 16 treated patients (31%).

The first viruses resistant to amantadine or rimantadine were
isolated between 2 and 15 days following initiation of therapy.
Two patients had resistant viruses isolated following <4 days
of antiviral therapy. The most common amino acid change
occurred at position 31 in the transmembrane region of the M2
protein, with a substitution of serine for asparagine (\( n = 4 \)).
A change at position 30 was documented in two patients; in
one of these, a virus bearing this mutation was isolated 6 days
after isolation of virus with a mutation at codon 31.
Figure 1. Timeline (24 December 1993 through 28 February 1994) depicting emergence of resistant influenza A/H3N2 virus in 5 adult immunocompromised patients (3 patients [numbers 1–3] with leukemia and 2 bone marrow transplant recipients [numbers 4 and 5]). The genotypes of the M2 protein of the resistant viruses are presented in the right-hand column, with amino acid substitutions listed by date when more than one genotype was documented. Symbols used in the figure include the following: ○ = isolation of influenza virus susceptible to amantadine or rimantadine; ● = isolation of influenza virus resistant to amantadine or rimantadine; ▼ = no growth of influenza virus in cell culture; ↓ = onset of respiratory symptoms; — — = admission and hospitalization without antiviral therapy; — — = administration of antiviral therapy (Aman = amantadine; Riman = rimantadine; Rib = ribavirin); and † = death.

Pneumonia by chest radiography were associated with subsequent morbidity and death, regardless of the drug susceptibility of the influenza isolate. No clinical or laboratory factor other than the isolation of a virus during or following the use of amantadine or rimantadine could be related to viral resistance. Overall, mortality rates were relatively high, both in patients who did and in those who did not have resistant influenza isolates documented (2 of 5 and 5 of 24, respectively).

Table 2. Characteristics of immunocompromised patients with documented influenza virus infection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>BMT recipients</th>
<th>Patients with leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Median age, in y (range)</td>
<td>30 (28–31)</td>
<td>42 (42–70)</td>
</tr>
<tr>
<td>Median time (d) from onset of symptoms until treatment (range)</td>
<td>4*</td>
<td>3 (0–6)</td>
</tr>
<tr>
<td>Median duration (d) of respiratory symptoms (range)†</td>
<td>39*</td>
<td>12 (7–43)</td>
</tr>
<tr>
<td>Pneumonia initially radiographically evident‡: no. positive per no. tested</td>
<td>0/2</td>
<td>2/3</td>
</tr>
<tr>
<td>No. of deaths associated with influenza, per total deaths in group</td>
<td>0/2</td>
<td>2/3‡</td>
</tr>
</tbody>
</table>

* Data available on only one of two cases.
† Duration of respiratory symptoms may have been related to influenza as well as other underlying conditions or acquired infections.
‡ Significantly fewer BMT recipients than patients with leukemia had radiologic evidence of pneumonia (P = .005, Fisher’s exact test, two-sided).
§ One death in each of these two groups was associated with dual infection with influenza A/H3N2 and respiratory syncytial virus.
Both patients with fatal influenza infection who were shedding resistant virus were undergoing induction chemotherapy for acute myelocytic leukemia. From one patient (figure 1, patient 1), amantadine-resistant influenza virus was repeatedly isolated, from 13 days following the initiation of rimantadine therapy until the day before death. No other pathogens were isolated, and no autopsy was performed. From the second patient (patient 3), amantadine-resistant influenza virus was isolated 2 days before the documented initiation of antiviral therapy. Ribavirin therapy was added to the regimen because of clinical deterioration. Over the following 2 weeks, multiple respiratory specimens were cultured and did not yield influenza virus.

Discussion

The frequency of the development of amantadine/rimantadine-resistant influenza virus infection in immunocompromised patients is documented for the first time in this study. All influenza isolates from patients with prolonged shedding were initially susceptible to amantadine or rimantadine, as anticipated. Overall, 4 (27%) of 15 severely immunocompromised patients with influenza who were treated with amantadine or rimantadine subsequently shed resistant influenza virus, and 5 (83%) of 6 patients with symptomatic disease who shed influenza virus for ≥3 days had drug-resistant influenza. Similar, serious consequences of influenza infection occurred in these severely immunocompromised patients whether or not persistent shedding with resistant virus was documented.

Although the incidence of drug-resistant influenza in immunocompromised patients has not been previously documented, the incidence of viral resistance developing during or after rimantadine/amantadine therapy has been shown to be ≈30% in healthy children [11, 18]. Generally, resistant viruses have been isolated 4–6 days following initiation of therapy in pediatric [11] and family studies [20], and often they were isolated from minimally symptomatic individuals who appeared to be recovering from infection.

In ferret [30] and avian [31] models of influenza virus infection, drug-resistant viral infection has been relatively rapidly induced, and the virus has been shown to be genetically stable, be transmissible, and cause disease similar to that due to wild-type influenza virus. The mutations responsible for the drug-resistant phenotype in this study were the same single amino acid changes in the transmembrane portion of the M2 protein previously noted in studies of animal models and humans [16, 32].

Although one case of fatal influenza associated with a probably nosocomially transmitted rimantadine-resistant virus in a 72-year old resident of a nursing facility has been described [22], no fatal cases of rimantadine or amantadine-resistant influenza involving immunocompromised adults have been reported. It is interesting that three immunocompromised patients infected with resistant virus in this study had continued clinical symptoms compatible with ongoing viral infection and disease for a period of at least 3 weeks. Two immunosuppressed patients with ongoing symptomatic disease have also been described: one symptomatic child with severe combined immunodeficiency who received a BMT shed resistant virus for 5 weeks, and one adult BMT recipient was shown to shed resistant virus for 9 days [24]. Both of these patients survived. It is not clear if the detection of resistant isolates is linked to prolonged clinical symptoms in treated immunocompetent individuals [33].

Genetic variability was seen in the influenza isolates with documented resistance from one patient. This has been previously reported with regard to a persistently infected, untreated immunodeficient child [26] and two treated immunocompromised patients [24]. Such variability could be due to heterogeneous populations of virus existing in a single patient or even to oscillations of quasispecies [26]. The isolation of amantadine/rimantadine-resistant influenza virus in the absence of exposure to antiviral therapy also has been reported [34]; however, the occurrence of resistance among wild-type influenza strains is quite uncommon. Our single patient infected with resistant virus that was isolated before known drug exposure initially shed influenza virus that was susceptible to amantadine. A spontaneous mutation may have arisen at this codon, but the possibility that this patient was exposed to antiviral therapy on an outpatient basis cannot be excluded.

Prolonged shedding of drug-resistant influenza virus in a hospital setting has important implications for nosocomial control of infection. Evidence of potential spread of resistant virus in nursing homes and among family members has been well documented [20–30]. No evidence of nosocomial spread of drug-resistant influenza virus was documented in this study, but the study was not designed to determine if this actually occurred. However, if amantadine/rimantadine therapy is being routinely utilized for treatment of influenza in patients in a hospital setting and nosocomial transmission does occur, the potential consequences should be recognized. Furthermore, the use of traditional infection control measures—including the vaccination of clinical staff, families of patients, and the patients themselves—continues to be an important measure in the control of influenza [14].

No specific antiviral therapy has been proven to be effective for severe lower respiratory tract disease due to influenza. Primary influenza A viral pneumonia has an associated high mortality in the general population; five of 11 otherwise healthy patients died despite treatment with high-dose oral amantadine (400–500 mg/[kg · d]) in one study [35]. In prospective studies of severely immunocompromised patients, approximately half the patients infected with influenza developed pneumonia [4–8], and fatal outcomes were highly associated with the development of pneumonia. Mortality rates also were high among young pediatric organ transplant recipients infected with influenza who required supplemental oxygen [36]. In our study, the development of lower respiratory tract disease in patients with
influenza infection, whether due to a documented resistant virus or not, was associated with serious morbidity and mortality among immunocompromised patients.

The use of amantadine and rimantadine in immunosuppressed patients is complicated by the rapid development of drug resistance, the lack of parenteral formulation of these compounds, and the potential for dose-related CNS side effects [25]. Ribavirin, a broad-spectrum antiviral agent, has antiviral activity against influenza A and B in vitro and has been used as an aerosolized drug in the treatment of influenza infection in controlled studies of young adults [37, 38] and children [39], with evidence of clinical benefit and antiviral efficacy. The use of aerosolized ribavirin for the treatment of influenza pneumonia in immunocompetent adults [40] and immunocompromised patients [6–8] has been associated with clinical improvement in uncontrolled studies. Intravenous ribavirin given as a continuous infusion has also been administered to one immunocompromised patient with severe disease due to resistant influenza A virus [23]. The development of new anti-influenza agents, such as neuraminidase inhibitors, also offers the opportunity for future studies of combination antiviral therapies.

The potential for development of resistance to amantadine or rimantadine should be recognized in immunocompromised patients who are treated with these drugs for influenza A virus infection. Persistent viral shedding during antiviral therapy is highly associated with the presence of drug-resistant virus. Because of the potential for spreading of influenza viruses—susceptible or resistant—and the high morbidity associated with influenza pneumonia, immunocompromised patients infected with influenza virus should be considered contagious and worthy of isolation from other patients for prolonged periods of time, in both the inpatient and outpatient setting, whether or not they are receiving antiviral therapy. Influenza virus infections remain one of the most common and important respiratory viral infections in humans, but further investigations are needed to define the optimal therapy and prophylaxis for these infections in immunocompromised patients.

References