Transmission of Human Immunodeficiency Virus Type 1 Resistant to Nevirapine and Zidovudine

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Human immunodeficiency virus type 1 (HIV-1) resistant to the nonnucleoside reverse transcriptase inhibitor nevirapine and to the nucleoside analogue zidovudine was transmitted from a homosexual man to his sex partner. The virus source patient had commenced combination zidovudine and nevirapine therapy 2.5 years prior to his partner’s primary HIV infection. He received both therapies for 7 months, then discontinued nevirapine treatment, continuing to receive zidovudine monotherapy for a further 16 months. He had ceased zidovudine therapy 6 months before the time of his partner’s seroconversion. Analysis of major and minor isolates obtained from both patients soon after onset of the recipient’s primary HIV infection illness confirmed that an HIV-1 variant mutant at codons 70, 98, and 181 of the viral reverse transcriptase was transmitted. This is the first documented case of transmission of HIV-1 resistant to two antiretroviral compounds.

Emergence of drug-resistant human immunodeficiency virus type 1 (HIV-1) was first described in symptomatic and asymptomatic patients receiving prolonged treatment with the nucleoside analogue zidovudine [1]. The zidovudine-resistant phenotype was found to be conferred by amino acid substitutions in the viral reverse transcriptase at codons 41, 67, 70, 215, and 219 [2, 3].

Resistance-conferring mutations in the HIV-1 reverse transcriptase have also been described for other nucleoside analogues and for nonnucleoside reverse transcriptase inhibitors including the dipyridodiazepinone, nevirapine (reviewed in [4]). Substitution of cysteine for tyrosine at codon 181 is most commonly observed in nevirapine monotherapy, although variants of HIV-1 with amino acid changes at positions 98, 100, 103, 106, 108, 188, and 190 have also been isolated from patients receiving nevirapine alone or in combination with other antiretroviral compounds [4, 5].

Zidovudine-resistant variants of HIV-1 may be transmitted sexually [6], but to date, transmission of HIV-1 resistant to other compounds has not been reported. We describe a case of transmission of HIV-1 resistant to nevirapine and zidovudine to the sex partner of a homosexual man who had received combination zidovudine and nevirapine therapy.

Subjects, Materials, and Methods

Virus source patient. The virus transmitter (patient D) is a homosexual man who was 18 years old when he first tested positive for HIV antibody in 1986. In October 1991, his CD4 lymphocyte count was 319/mm³, and his serum was positive for p24 antigen. The following month, he enrolled in a clinical trial evaluating nevirapine and zidovudine alternating and concurrent combination strategies [7]. Patient D received combination zidovudine (600 mg/day) and nevirapine (50 mg/day, increasing to 200 mg/day at week 4) therapy. At that time, oral hairy leukoplakia was patient D’s only HIV-related problem.

Patient D continued both therapies for 7 months, then discontinued nevirapine treatment. He continued to receive zidovudine for a further 16 months, with variable compliance, and had ceased all therapy for 6 months by the time his sex partner seroconverted in April 1994. Patient D was seen in April 1994, at which time his CD4 cell count was 261/mm³ and he remained free of HIV-related opportunistic complications. Kaposi’s sarcoma was diagnosed in June 1995.

Recipient. The recipient (patient R) is a homosexual man who was 37 years old and HIV-seronegative in April 1993. Following onset of fever, lethargy, pharyngitis, and odynophagia in April 1994 and appearance of maculopapular rash 1 week later, patient R was tested for HIV antibody and antigen. Patient R had participated in anal and oral intercourse with his sex partner, patient D, shortly before this time.

Circulating HIV p24 antigen was detected in patient R’s serum on three occasions between day 8 and day 14 following the onset of symptoms, at concentrations ranging from 253 to 386 pg/mL. HIV antibody was first detected by Western immunoblot at day 14 with the appearance of a band at p24; prior to this, patient R was HIV-seronegative. Three weeks after onset of symptoms, HIV antigen was no longer detectable, and additional Western blot bands were visible at gp160 and p55. The full complement of
bands was seen between 3 and 5 months later. Treatment with zidovudine at a dose of 600 mg daily began 2 weeks after the onset of symptoms, continued for ~6 weeks, then recommenced at the same dose after patient R stopped taking the drug for 19 days. Patient R stopped taking zidovudine in September 1994, 5 months after having commenced treatment. At this time, his mean corpuscular volume was 103.3 fL, having increased from a mean value of 85.7 fL (range of three measurements, 85.2–87) in April 1994. Absolute CD4 lymphocyte counts in April and May 1994 ranged from 690 to 814/mm^3 (mean of four measurements, 759/mm^3), increasing to 1152 and 960/mm^3 in July and September, respectively.

**Virus isolation and drug susceptibility assays.** Blood samples were collected from patients D and R soon after onset of symptoms of patient R’s primary HIV infection illness. Peripheral blood mononuclear cells (PBMC) were obtained by ficoll-hypaque density gradient centrifugation and cocultured with phytohemagglutinin (PHA)-stimulated seronegative donor PBMC in RPMI 1640 medium supplemented with 10% (vol/vol) fetal calf serum, interleukin-2, glutamine, and antibiotics (R-10 medium). Half of the medium was exchanged every 3 or 4 days and fresh PHA-stimulated donor PBMC were added weekly. Virus production was monitored by p24 antigen ELISA (Coulter, Hialeah, FL). HIV p24–positive supernatants were used to generate virus stocks as previously described [6, 7]. Stocks were titrated and assayed for zidovudine and nevirapine susceptibility as previously described [6, 7].

**Generation of biologic clones.** Patient PBMC were cocultured with PHA-stimulated seronegative donor cells under limiting dilution conditions [8], and positive cultures were expanded into virus stocks.

**Extraction of viral RNA and generation of cDNA.** Viral RNA was extracted from low-passage, p24-positive culture supernatant, and cDNA was prepared as previously described [6, 7].

**Sequencing of the reverse transcriptase gene.** The viral reverse transcriptase gene was amplified with the primers RT01 (5'-GGATGGACGATTCATG-3') and RT02 (5'-GATCAGTTGAGTCAGATTGG-3') in a first-round polymerase chain reaction (PCR). The reaction proceeded for 35 cycles (95°C for 1 min, 55°C for 2 min, 75°C for 3 min, with a further 10-min extension interval at 75°C) in 50 mM TRIS-HCl, pH 8.3, 30 mM KCl, 1.5 mM MgCl2, and 1.0 mM each dNTP. In a second, nested reaction, a 768-bp segment of DNA encompassing the codons associated with zidovudine and nevirapine resistance was amplified, with the primers A (5'-TTCCATTAGGCCTATT-3') and NE1 (5'-TCACTGAGTCCAAGCCT-3'). The reaction proceeded for 30 cycles (95°C for 1 min, 45°C for 1 min, 75°C for 1 min) in the reaction mixture used previously. PCR products were purified with the Wizard DNA purification system (Promega, Madison, WI) and sequenced by use of the PRISM sequencing kit (Applied Biosystems, Foster City, CA).

**Drug Resistance Phenotype and Genotype**

**Patient D.** HIV-1 from the virus transmitter demonstrated reduced susceptibility to nevirapine (>10 μM) and zidovudine (1.1 μM). Sequence analysis confirmed the presence of substitutions at codons 70 (K→R), 98 (A→G), and 181 (Y→C). Clones isolated at this time point were resistant to nevirapine, displaying IC50 values ranging from 0.53 to 2.73 μM, but were uniformly sensitive to zidovudine (IC50, 0.008–0.09 μM). These clones were shown to be mutant at codons associated with nevirapine resistance, displaying a combination of substitutions at codon 98 (A→G), at codons 98 (A→G) and 181 (Y→C), and at codon 181 (Y→C) only. Arginine was detected at codon 103 instead of lysine, the amino acid normally seen at this position. Since a K103N mutation is normally associated with resistance to the nonnucleoside reverse transcriptase inhibitors, the significance of the K103R substitution is not known. These data are summarized in table 1 and figure 1.

**Patient R.** Isolates obtained from the seroconverter 21, 35, and 84 days following onset of his acute illness were resistant to nevirapine. The earliest isolate was highly resistant, with an IC50 >10 μM. The isolates obtained at days 35 and 84 continued to demonstrate the nevirapine-resistant phenotype, but to a lesser extent (IC50, 2.1 and 1.13 μM, respectively). Although the predominant isolate in the virus donor, patient D, was phenotypically resistant to zidovudine (IC50, 1.1 μM), the earliest isolate obtained from patient R was more sensitive to zidovudine, with an IC50 of 0.2 μM. Although the K70R mutation was detected in the day 35 isolate, the zidovudine IC50 was 0.09 μM, indicating a sensitive phenotype. By day 84, however, HIV-1 with a zidovudine-resistant phenotype (0.54 μM) was isolated. Clones isolated at day 21 and day 35 were resistant to nevirapine (range, 1.35–2.6 μM) and were susceptible to zidovudine (range, 0.01–0.14 μM). These data are summarized in table 1.

The isolate obtained from patient R 21 days after onset of symptoms of primary HIV infection illness was mutant at codons 70 (K→R), 98 (A→G), and 181 (Y→C). The isolates obtained at days 35 and 84 continued to demonstrate the nevirapine-resistant phenotype, but to a lesser extent (IC50, 2.1 and 1.13 μM, respectively). Although the predominant isolate in the virus donor, patient D, was phenotypically resistant to zidovudine (IC50, 1.1 μM), the earliest isolate obtained from patient R was more sensitive to zidovudine, with an IC50 of 0.2 μM. Although the K70R mutation was detected in the day 35 isolate, the zidovudine IC50 was 0.09 μM, indicating a sensitive phenotype. By day 84, however, HIV-1 with a zidovudine-resistant phenotype (0.54 μM) was isolated. Clones isolated at day 21 and day 35 were resistant to nevirapine (range, 1.35–2.6 μM) and were susceptible to zidovudine (range, 0.01–0.14 μM). These data are summarized in table 1.

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**Discussion**

We have identified a case of transmission of HIV-1 resistant to nevirapine and zidovudine. Patients D and R, the virus transmitter and recipient, respectively, were sex partners at the time of patient R’s primary HIV infection illness and subsequent seroconversion in April 1994. Patient D had received nevirapine and zidovudine therapy commencing in November 1991, and variants of HIV-1 demonstrating genotypic and phenotypic

**Results**

**Virus Isolation**

HIV-1 was isolated from the virus transmitter, patient D, 35 days after the onset of symptoms of primary HIV infection in his partner, patient R. Isolates were obtained from the recipient, patient R, on days 21, 35, and 84 following onset of his acute illness.
resistance to these compounds were isolated from patient D within 35 days of onset of symptoms of his partner’s primary infection illness. The recipient, patient R, commenced zidovudine monotherapy 14 days after onset of symptoms of his primary HIV infection illness. Analysis of sequential isolates showed that HIV-1 with the K70R, T215Y, and Y181C genotype predominated at day 84, replacing the Y181C genotype that predominated soon after infection.

Although mutations associated with nevirapine resistance have been observed in isolates obtained from nonnucleoside reverse transcriptase inhibitor–naive patients [9], they occur at low frequency in normal conditions, and it may be assumed that these substitutions do not confer a survival advantage in the absence of drug. The continued presence of HIV-1 with reduced sensitivity to nevirapine almost 2 years after patient D stopped taking the drug was surprising. Nevirapine-resistant mutants also predominated in our patient R soon after transmission. In addition, Richman et al. [5] were able to isolate nevirapine-resistant HIV-1 from 3 patients at least 14 weeks after withdrawal of therapy. These data suggest that certain drug-resistant viruses may possess a survival advantage compared with the wild type form, even when drug selection pressure has been removed. Persistence of resistance-conferring mutations after cessation of therapy has also been described in patients who received long-term zidovudine treatment [10], and we have recently shown that transmitted zidovudine-resistant viruses may persist for up to 1 year following transmission or may revert to the wild type genotype and phenotype [6].

The appearance of zidovudine-resistant HIV-1 mutant at codon 215 within 3 months of primary infection suggests that the T215Y mutation emerged rapidly as a consequence of the large number of replicative events accompanying the acute phase and the concurrent zidovudine selection pressure. Alternatively, resistant variants mutant at codon 215 may have been transmitted and rapidly predominated under the selective pressure of patient R’s zidovudine treatment, despite the fact that we did not detect codon 215 mutants in patient D’s isolates. The major species isolated from patient D was resistant to nevirapine and zidovudine. We did not detect zidovudine resistance–confering mutations in patient D’s isolates other than the K70R mutation as a mixed population, suggesting that minor variants mutant at one or more of the substitutions associated with zidovudine resistance, particularly codon 215, may have been present but were not identified. It is possible that these variants would be selected and amplified in the course of the susceptibility assay, when virus is isolated in the presence of zidovudine, producing the resistant phenotype.

The clinical consequences of primary infection with drug-resistant virus have not been established. In a recent study that compared seroconverters infected with zidovudine-resistant HIV-1 and patients infected with wild type virus, we found no difference in either CD4 cell count 1 year after infection or duration of primary infection illness [6]. Differences in clinical outcomes may become apparent after a longer period of observation, although studies that are able to enroll a greater number of patients will be able to address this question most accurately. When patients are infected with zidovudine-resistant virus, the clinical benefit of zidovudine therapy for primary HIV infection is unclear. In addition to our patient R, a number of patients infected with zidovudine-resistant virus have been treated with drug [6, 11, 12]. Although it may be postulated that administration of zidovudine during the acute phase could

### Table 1. Drug resistance phenotype and genotype of major and minor strains isolated from virus transmitter and virus recipient.

<table>
<thead>
<tr>
<th>Virus source, patient D</th>
<th>Predominant strain</th>
<th>Variant 2 (3)</th>
<th>Variant 3 (1)</th>
<th>Variant 4 (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>70(K→R); 98(A→G); 181(Y→C)</td>
<td>98(A→G)</td>
<td>98(A→G); 181(Y→C)</td>
<td>181(Y→C)</td>
</tr>
<tr>
<td>Virus recipient, patient R</td>
<td>Predominant strain</td>
<td>Variant 2 (1)</td>
<td>Variant 3 (3)</td>
<td>Day 35</td>
</tr>
<tr>
<td></td>
<td>181(Y→C); mixed 70(K→R)</td>
<td>70(K→R); 181(Y→C)</td>
<td>98(A→G); 181(Y→C)</td>
<td>70(K→R); 98(A→G); 181(Y→C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.1</td>
<td>2.1</td>
<td>70(K→R); 181(Y→C); 215(T→Y)</td>
</tr>
</tbody>
</table>

**NOTE.** A, alanine; C, cysteine; G, glycine; K, lysine; R, arginine; T, threonine; Y, tyrosine. IC₅₀ values for wild type viruses = <0.01 for nevirapine and zidovudine; IC₅₀ values shown are of representative isolates. No. of clones isolated at each time point is in parentheses. Predominant strain IC₅₀ was obtained by assaying bulk isolate.
Figure 1. HIV-1 reverse transcriptase nucleotide sequences from virus source (patient D) and recipient (patient R). * Predominant strain sequences are those obtained from bulk isolate and from at least 3 identical clones at same time point, except for recipient day 84 sequence, which constitutes bulk isolate only. R = A or G.

The results indicated that rapid selective outgrowth of resistant viruses and an increased virus burden, no clear association was found between primary infection with resistant virus, zidovudine therapy, and primary infection with drug-resistant HIV-1 can be expected to occur more often as more antiretroviral compounds become available. Although some transmitted resistant viruses can be expected to revert to a sensitive phenotype, resistant mutants...
that persist in the new host may have important implications for patient management: HIV-infected persons who have never been treated with antiretroviral agents and who are assumed to be infected with susceptible virus may in reality harbor resistant forms, and the expected therapeutic outcomes may not occur when antiretroviral treatment is commenced. Similarly, management of occupational exposure may not have the anticipated protective effects if the source patient harbors virus resistant to one or more antiretroviral agents, a situation likely to occur with increasing frequency as these agents are more commonly used [13]. Effective prophylactic regimens may need to combine compounds that act at different sites of the virus’s life cycle or that produce compensatory resistance mutations in vitro. In addition, rapid genotyping of source viruses to identify mutations associated with drug resistance may need to be incorporated into prophylaxis protocols to maximize drug efficacy and to delay or prevent outgrowth of resistant virus.

In this regard, current approaches to the diagnosis and empiric treatment of Mycobacterium tuberculosis infections [14] may serve as a useful paradigm as new approaches to optimal chemotherapy for primary HIV infection are developed.

Zidovudine-resistant HIV-1 has been found in recently infected patients at frequencies between 8.2% and 11.5% [6, 11, 15]. We and others have shown that these variants may persist for months or years, possibly limiting the effectiveness of zidovudine monotherapy in early infection. We have now shown that HIV-1 resistant to another class of antiretroviral compounds, the nonnucleoside reverse transcriptase inhibitors, may be transmitted. Treatment strategies for primary infection need to take into consideration the possibility of primary infection with drug-resistant (and multidrug-resistant) virus. In addition, treatment of primary HIV infection with combinations of antiretroviral compounds, acting at different sites of the virus’s life cycle, needs to be investigated further.

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Study Group Members

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References