ANTIRETROVIRAL-DRUG RESISTANCE AMONG PATIENTS RECENTLY INFECTED WITH HIV

Susan J. Little, M.D., Sarah Holte, Ph.D., Jean-Pierre Routy, M.D., Eric S. Daar, M.D., Marty Markowitz, M.D., Ann C. Collier, M.D., Richard A. Koup, M.D., John W. Mellors, M.D., Elizabeth Connick, M.D., Brian Conway, M.D., Michael Kilby, M.D., Lei Wang, Ph.D., Jeannette M. Whitcomb, Ph.D., Nicholas S. Hellmann, M.D., and Douglas D. Richman, M.D.

ABSTRACT

Background   Among persons in North America who are newly infected with the human immunodeficiency virus (HIV), the prevalence of transmitted resistance to antiretroviral drugs has been estimated at 1 to 11 percent.

Methods   We performed a retrospective analysis of susceptibility to antiretroviral drugs before treatment and drug-resistance mutations in HIV in plasma samples from 377 subjects with primary HIV infection who had not yet received treatment and who were identified between May 1995 and June 2000 in 10 North American cities. Responses to treatment could be evaluated in 202 subjects.

Results   Over the five-year period, the frequency of transmitted drug resistance increased significantly. The frequency of high-level resistance to one or more drugs (indicated by a value of more than 10 for the ratio of the 50 percent inhibitory concentration [IC_{50}] for the subject's virus to the IC_{50} for a drug-sensitive reference virus) increased from 3.4 percent during the period from 1995 to 1998 to 12.4 percent during the period from 1999 to 2000 (P=0.002), and the frequency of multidrug resistance increased from 1.1 percent to 6.2 percent (P=0.01). The frequency of resistance mutations detected by sequence analysis increased from 8.0 percent to 22.7 percent (P<0.001), and the frequency of multidrug resistance detected by sequence analysis increased from 3.8 percent to 10.2 percent (P=0.05). Among subjects infected with drug-resistant virus, the time to viral suppression after the initiation of antiretroviral therapy was longer (P=0.05), and the time to virologic failure was shorter (P=0.05).

Conclusions   The proportion of new HIV infections that involve drug-resistant virus is increasing in North America. Initial antiretroviral therapy is more likely to fail in patients who are infected with drug-resistant virus. Testing for resistance to drugs before therapy begins is now indicated even for recently infected patients. (N Engl J Med 2002;347:385-94.)

THE use of potent antiretroviral therapy in patients infected with human immunodeficiency virus type 1 (HIV-1) reduces morbidity and mortality[1-3] and often results in substantial recovery of impaired immunologic function.[4,5] Resistance to antiretroviral drugs often develops in patients with incomplete viral suppression and may limit both the magnitude and the duration of the response to treatment.[6-8] Transmission of drug-resistant virus has been recognized for more than a decade, although the trends with respect to transmitted drug resistance and its effect on responses to treatment have not been defined.

We determined the prevalence of transmitted drug-resistant virus in a cohort of subjects with primary HIV infection from 10 North American cities who had not received treatment. We also evaluated the association between base-line susceptibility to anti-HIV drugs and virologic response to therapy among those who began to receive potent antiretroviral therapy.

From the Departments of Medicine (S.J.L., D.D.R.) and Pathology (D.D.R.), University of California–San Diego, San Diego; the Statistical Center for HIV/AIDS Research and Prevention at the Fred Hutchinson Cancer Research Center (S.H., L.W.) and the Department of Medicine (A.C.C.), University of Washington, Seattle; the Department of Medicine, McGill University Health Center, Montreal (J.-P.R.); the Department of Medicine, Harbor–UCLA Medical Center, Torrance, Calif. (E.S.D.); the Aaron Diamond AIDS Research Center, New York (M.M.); the Vaccine Research Center, National Institutes of Health, Bethesda, Md. (R.A.K.); the Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh (J.W.M.); the Department of Medicine, University of Colorado Health Sciences Center, Denver (E.C.); the Department of Pharmacology and Medical Therapeutics, University of British Columbia, Vancouver (B.C.); the Department of Medicine, University of Alabama, Birmingham (M.K.); ViroLogic, South San Francisco, Calif. (J.M.W., N.S.H.); and the Department of Veterans Affairs San Diego Healthcare System, San Diego, Calif. (D.D.R.). Address reprint requests to Dr. Little at the UCSD Antiviral Research Center, 150 W. Washington St., Ste. 100, San Diego, CA 92103, or at slittle@ucsd.edu.

METHODS

Study Subjects

Between May 1995 and June 2000, subjects with signs or symptoms of an acute HIV seroconversion syndrome or evidence of recent HIV infection were referred to one of seven participating Acute Infection and Early Disease Research Programs[9] in 10 North American cities (Table 1). Study participants signed an informed-consent form that had been approved by the local institutional review board and underwent laboratory screening to determine whether they met criteria for acute or early HIV infection.

The criteria for study enrollment included documented HIV seroconversion within the previous 12 months or evidence of acute or early HIV infection. Acute HIV infection was defined by detectable HIV RNA or p24 antigen, in the absence of HIV antibody detectable by enzyme immunoassay (EIA), with subsequently documented HIV seroconversion. Early HIV infection was defined by a positive EIA for HIV and a documented negative serologic test within the past 12 months or a result on a reduced-sensitivity ("detuned") EIA that was consistent with recent infection with HIV.[10] According to the clinical judgment of study investigators, 13 percent of the study subjects had recently acquired HIV infection. These subjects typically had documented levels of HIV RNA and an evolving result on Western blotting within 90 days after the onset of an acute retroviral syndrome (i.e., viral symptoms consistent with acute HIV infection), following high-risk sexual activity or needle use. For the remaining 87 percent of study participants, the date of HIV infection was estimated as follows: if applicable, we used the date 30 days before an indeterminate result on Western blotting; if that method was not applicable, we used the date 65 days before a result on a detuned EIA of less than 1.0; if neither of the above was applicable, we used the midpoint between the last documented negative EIA for HIV and the first positive EIA or the first detection of HIV RNA or p24 antigen. Subjects were excluded if they had previously received antiretroviral therapy for more than seven days or if they had a plasma HIV RNA level of less than 400 copies per milliliter at the time of their base-line evaluation. A total of 377 subjects met the entry criteria and were included in the analysis; of these subjects, 169 have been described previously.[11,12]

Study Design

For all subjects in this retrospective cohort study, information on demographic characteristics and risk factors for exposure to HIV were recorded at base line, and a pretreatment plasma sample was obtained and stored at −70°C. Drug-resistance tests were not performed prospectively; the choice of initial regimen was based on the protocols of clinical studies or the standard of care. Plasma HIV RNA levels and CD4 lymphocyte subgroups were monitored at least every six weeks during follow-up.

Response to Treatment

The response to treatment was evaluated in the 202 subjects who began receiving a potent antiretroviral regimen during follow-up and who had adequate data for analysis (an HIV RNA measurement at least every six weeks); in 95 percent of these subjects (191 of 202), this evaluation was performed within the first year after the estimated date of infection. Potent antiretroviral therapy was defined according to consensus guidelines.[13] The time to viral suppression was defined by the first of two consecutive plasma values of less than 500 HIV RNA copies per milliliter measured in plasma samples collected at least 14 days apart. An interruption of treatment for more than 14 days was considered a discontinuation of therapy. The time to virologic failure was defined as the time from the first achievement of viral suppression to the first value for plasma RNA that was more than 500 copies per milliliter while the subject was receiving potent antiretroviral therapy.

TABLE 1. BASE-LINE CHARACTERISTICS OF THE SUBJECTS.*

CHARACTERISTIC	PATIENTS (N=377)
Age — yr	
Mean	35
Range	16–62
Sex — no. (%)	
Male	343 (91)
Female	34 (9)
Race or ethnic group — no. (%)†	
Non-Hispanic white	208 (72)
Non-Hispanic black	45 (16)
Hispanic	28 (10)
Other	7 (2)
Risk factor for HIV — no. (%)‡	
Sexual activity	184 (77)
Injection-drug use	29 (12)
Sexual activity and injection-drug use	25 (11)
Geographic location of subjects — no. (%)	
Baltimore	29 (8)
Birmingham, Ala.	2 (1)
Dallas	19 (5)
Denver	14 (4)
Los Angeles	55 (15)
Montreal	87 (23)
New York	46 (12)
San Diego, Calif.	74 (20)
Seattle	47 (12)
Vancouver, Canada	4 (1)
Elapsed time — days	
From HIV infection to drug-resistance testing§	
Median	71
Range	0–453
From HIV infection to start of treatment	
Median	97
Range	7–923
Laboratory variables	
Plasma HIV RNA — log copies/ml	4.8±0.93
CD4 count — cells/mm³	
Median	489
Range	47–9166

*The plus–minus value is the mean ±SD.

†Data on race or ethnic group were not available from the 87 subjects in Montreal and from 1 patient each from Seattle and Los Angeles.

‡Data on risk factors for HIV infection were available for only 238 of the study subjects.

§In two subjects, base-line resistance testing was performed more than 365 days after the estimated date of HIV infection.

Susceptibility Testing

At base line, plasma samples were analyzed for drug susceptibility by a rapid recombinant virus assay (PhenoSense HIV, ViroLogic), which uses test vectors derived from the amplified product of the HIV-1 protease and reverse-transcriptase gene sequences from the patient's plasma.[14] Drug susceptibility is measured by determining the ratio of the concentration required for 50 percent inhibition [IC_{50}] of the subject's virus to the IC_{50} for a drug-sensitive reference virus (NL4-3) for each antiretroviral drug tested. An IC_{50} for a subject's virus that was higher than that for the drug-sensitive reference virus by more than a factor of 1.5 to 2.5 (the exact number varies according to the particular drug) was indicative of reduced susceptibility to that drug.[14] Plasma samples were tested at base line for susceptibility to abacavir, didanosine, lamivudine, stavudine, zalcitabine, zidovudine, delavirdine, efavirenz, nevirapine, amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir. In the ab-

sence of precise thresholds defining clinically relevant reductions in susceptibility for many drugs, a value greater than 10 for the ratio of the IC_{50} for the subject's virus to the IC_{50} for the reference virus was considered to indicate clinical resistance to each drug. Multidrug resistance was defined by either an IC_{50} ratio of more than 10 for two or more classes of drugs or the identification of major mutations conferring resistance to drugs in two or more classes.

Sequence Analysis

Population-based nucleotide-sequence analysis of the HIV *pol* gene was performed locally or centrally (ViroLogic) for 301 of 377 base-line plasma samples (80 percent). All samples with evidence of reduced susceptibility to one or more drugs were successfully genotyped. Consensus guidelines for testing for resistance to antiretroviral drugs[15] were used to define well-characterized drug-resistance mutations. Nucleotide substitutions at position 215 that resulted in the expression of aspartate (D), asparagine (N), serine (S), cysteine (C), or glutamate (E) instead of threonine (T) were included as major drug-resistance mutations. These mutations represent changes of a single nucleotide from the well-characterized T215Y mutation that confers resistance to nucleoside reverse-transcriptase inhibitors and are the result of spontaneous reversion of this mutation in the absence of zidovudine treatment.[16,17] Strains with genetic mixtures of wild-type and mutant sequences at amino acid sites that code for major drug resistance were considered to be drug-resistant.

Statistical Analysis

Changes in the percentage of strains that were resistant (according to the year of infection) were analyzed by means of a test for linear trend. Differences in categorical variables were assessed by means of the Mantel–Haenszel chi-square test whenever there were at least five observations for each resistance classification and time interval, or Fisher's exact test when there were fewer than five observations. For time-to-event analyses, the log-rank statistic was used in order to derive P values for the differences in the survival curves.

RESULTS

Characteristics of the Subjects

Study subjects were predominantly non-Hispanic white men whose risk factor for HIV infection was having had sex with men (Table 1). A total of 76 percent of the subjects were referred to participating study centers with an acute retroviral syndrome; symptoms occurred after an identified episode of high-risk sexual exposure (in 77 percent of the 238 subjects with data on risk factors), needle use (in 12 percent), or both (in 11 percent). The median base-line CD4 cell count was 489 cells per cubic millimeter; the mean plasma RNA level was 4.8 log copies per milliliter. Base-line antiretroviral-susceptibility testing was performed before treatment began, a median of 71 days after the estimated date of HIV infection (interquartile range, 35 to 140 days). A total of 202 subjects began receiving a potent antiretroviral regimen a median of 97 days after the estimated date of HIV infection and had adequate follow-up data for analysis.

Phenotypic Analysis of Drug Susceptibility

Since the drug-susceptibility thresholds associated with reduced responses to treatment are not clearly defined for all drugs, three different thresholds of sus-

ceptibility were evaluated in the analysis of the prevalence of drug resistance (Fig. 1A). The proportion of subjects with an IC_{50} more than 2.5 times that for the drug-susceptible reference virus did not change significantly during the study period (P=0.65). In contrast, the proportion of subjects with an IC_{50} more than 5 times that for the reference virus or more than 10 times that for the reference virus increased, primarily between 1998 and 1999. An IC_{50} ratio of 10 was used as the resistance threshold for subsequent analyses to provide a conservative estimate of the prevalence of clinically relevant drug resistance (i.e., high-level drug resistance).

Six percent of the subjects (23 of 377) were infected by a virus with an IC_{50} ratio of more than 10 for one or more drugs. Within each class of antiretroviral drugs, the proportion of subjects with an IC_{50} ratio of more than 10 (Fig. 1B and Table 2) increased significantly for both protease inhibitors (P<0.001) and nonnucleoside reverse-transcriptase inhibitors (P=0.03) and showed a trend toward an increase for nucleoside reverse-transcriptase inhibitors (P=0.07). When the patients were divided into two groups according to the date of diagnosis (the earlier or later era of potent antiretroviral therapy), the prevalence of transmitted high-level drug resistance was shown to have increased significantly from the 1995–1998 period to the 1999–2000 period. The percentage of subjects with an IC_{50} ratio of more than 10 for one or more antiretroviral drugs increased from 3.4 percent during the earlier period to 12.4 percent during the later period (P=0.002). The prevalence of multidrug resistance also increased from the earlier period to the later period, from 1.1 percent to 6.2 percent (P=0.01).

Among subjects with multidrug resistance, none identified before 1999 had an IC_{50} ratio of more than 10 for one or more drugs in all three antiretroviral classes, whereas 75 percent of subjects with multidrug resistance identified in 1999 or 2000 had high-level resistance to all three classes (data not shown). A total of 74 subjects (20 percent) were identified who had an IC_{50} ratio between 2.5 and 10 for one or more drugs, possibly indicating transmitted drug resistance, but they were not classified as having transmitted drug resistance on the basis of our most stringent criterion of an IC_{50} ratio of more than 10 (Table 3).

Genotypic Analysis of Drug Resistance

The percentage of subjects with one or more major drug-resistance mutations was 12 percent (37 of 301) and increased from 8.0 percent during the period from 1995 to 1998 to 22.7 percent during the period from 1999 to 2000 (P<0.001) (Table 2). According to the genotypic analysis, the prevalence of resistance to multiple classes of drugs increased from 3.8 percent (in

1995 to 1998) to 10.2 percent (in 1999 to 2000; P=0.05). Resistance mutations at codons 118, 184, 215, and 103 of the reverse-transcriptase gene and codons 82 and 90 of the protease gene were the most prevalent, each occurring in 2 percent or more of the study subjects (Table 3).

Geographic variability of transmitted resistance was evaluated according to region: East (Baltimore, Birmingham, Montreal, and New York), Midwest (Dallas and Denver), and West (Los Angeles, San Diego, Seattle, and Vancouver). After adjustment for region, a test for the prevalence of resistance still showed a sig-
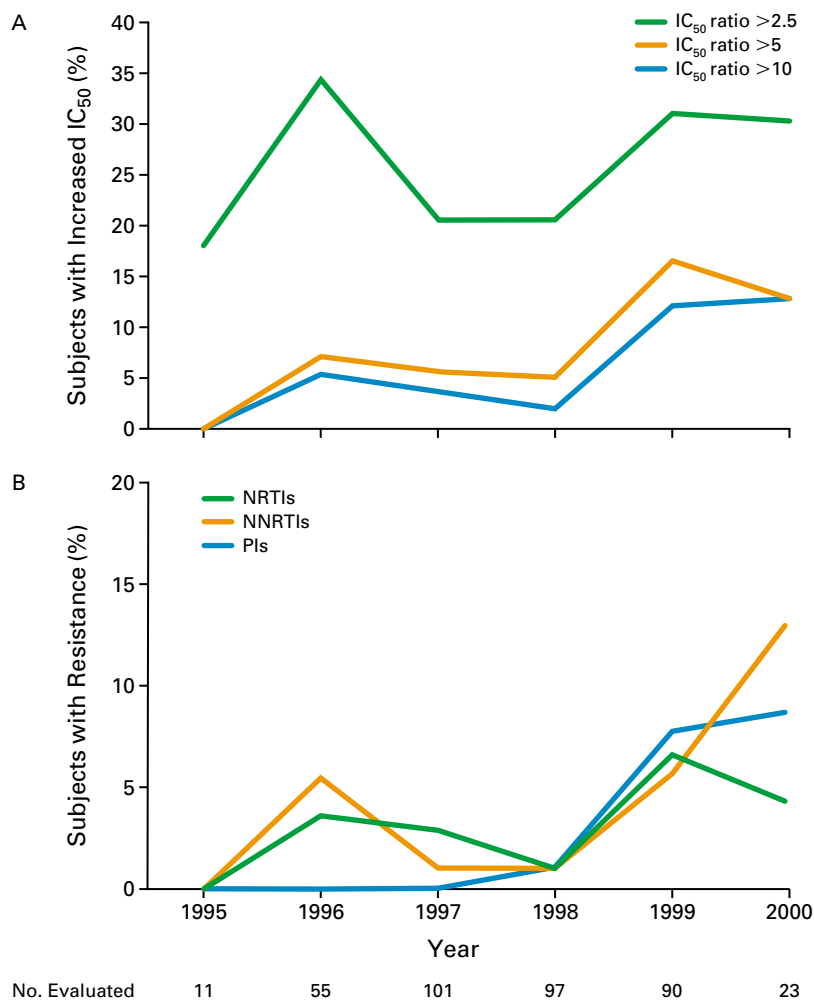
Figure 1. Changes in the Prevalence of Reduced Drug Susceptibility over Time and According to Drug Class.

The 50 percent inhibitory concentration (IC_{50}) ratio is the ratio of the IC_{50} for the subject's virus to the IC_{50} for a drug-sensitive reference virus. Panel A shows the percentages of subjects identified each year with an IC_{50} ratio of more than 2.5, more than 5, and more than 10 for one or more drugs. Panel B shows the percentages of subjects identified each year with an IC_{50} ratio of more than 10 for one or more nucleoside reverse-transcriptase inhibitors (NRTIs), nonnucleoside reverse-transcriptase inhibitors (NNRTIs), or protease inhibitors (PIs). The Mantel–Haenszel chi-square test was used to evaluate the probability that observed changes were significant during the period of study.

TABLE 2. TEMPORAL CHANGES IN THE PREVALENCE OF DRUG RESISTANCE AT BASE LINE.*

VARIABLE	SUBJECTS IDENTIFIED WITH DRUG-RESISTANT VIRUS		
	1995–1998	1999–2000	P VALUE†
	no./no. of samples analyzed (%)		
High-level drug resistance (phenotype assay)‡			
Any antiretroviral drug	9/264 (3.4)	14/113 (12.4)	0.002
NRTIs	6/264 (2.3)	7/113 (6.2)	0.07
NNRTIs	5/264 (1.9)	8/113 (7.1)	0.03
PIs	1/264 (0.4)	9/113 (8.0)	<0.001
Multidrug resistance	3/264 (1.1)	7/113 (6.2)	0.01
Major drug-resistance mutations (genotype assay)			
Any antiretroviral drug	17/213 (8.0)	20/88 (22.7)	<0.001
NRTIs	15/176 (8.5)	13/82 (15.9)	0.09
NNRTIs	3/176 (1.7)	6/82 (7.3)	0.03
PIs	2/213 (0.9)	8/88 (9.1)	0.001
Multidrug resistance§	8/213 (3.8)	9/88 (10.2)	0.05

*Both resistance assays were performed at ViroLogic. NRTI denotes nucleoside reverse-transcriptase inhibitor, NNRTI nonnucleoside reverse-transcriptase inhibitor, and PI protease inhibitor.

†P values are two-sided and were determined by Fisher's exact test.

‡Data are numbers (and percentages) of samples containing virus with a 50 percent inhibitory concentration (IC_{50}) that was more than 10 times that of a reference virus.

§These results did not change when T215D, T215N, T215S, T215C, and T215E mutations were excluded from the analysis (i.e., all subjects with a revertant mutation detected at position 215 had at least one additional major drug-resistance mutation).

nificant increase in the percentage of subjects with an IC_{50} ratio of more than 10 for one or more drugs (P= 0.03) or with drug-resistance mutations (P=0.001). Two of 301 viral isolates (1 percent) were HIV subtype C; the remainder of isolates were subtype B, suggesting that the majority of infections were acquired in North America.

Response to Treatment

Seventy-four percent of the subjects who received treatment received a regimen containing a protease inhibitor, 20 percent a regimen containing a nonnucleoside reverse-transcriptase inhibitor, and 6 percent a regimen containing both a protease inhibitor and a nonnucleoside reverse-transcriptase inhibitor. The time to viral suppression was significantly shorter among subjects with fully susceptible virus at base line than among those with an IC_{50} ratio of more than 10 for one or more drugs (P=0.05) (Fig. 2A) or with a major drug-resistance mutation at base line (P=0.03, data not shown). Although viral suppression was demonstrated by week 24 of therapy in all but one patient with adequate follow-up data, the median time to suppression was 56 days for those with susceptible virus (an IC_{50} ratio of less than 2.5), 55 days for those with an IC_{50} ratio between 2.5 and 10, and 88 days for those with an IC_{50} ratio of more than 10. No significant differences were seen among these three groups in the mean base-line plasma HIV RNA level — 4.78, 4.76, and 4.64 log copies per milliliter, respectively.

Among subjects who had a relapse of viremia while receiving treatment after viral suppression had been attained, the time to virologic failure was significantly shorter among those with an IC_{50} ratio of more than 10 than among those with completely drug-susceptible virus at base line (P=0.05) (Fig. 2B).

Since subjects were treated before data on resistance became available, it is difficult to assess whether the response rates are related to the acquisition of resistant virus or to poor selection of drugs. To assess these relations further, we determined the number of active drugs in each treatment regimen; active drugs were defined as those of which the IC_{50} for the subject's virus was no more than 10 times that for the reference virus. According to a comparison between subjects receiving two or fewer active drugs and those receiving three or more active drugs, the number of active drugs in the treatment regimen did not significantly affect the time to viral suppression (P=0.17, data not shown).

DISCUSSION

The transmission of virus with antiretroviral resistance has been reported in cases of sexual,[16,18-22] parenteral,[23-25] and vertical[26-29] transmission of HIV. Other recent reports describe the transmission of virus with resistance to drugs from one or more classes (multidrug resistance).[11,12,30-33] Cohort studies have identified drug-resistant variants in 1 to 11 percent of persons newly infected with HIV in North America[11,12,34]

**TABLE 3.** SPECIFIC CHANGES ASSOCIATED WITH DRUG RESISTANCE AT BASE LINE.*

VARIABLE	SPECIMENS FROM STUDY SUBJECTS			
	WITH DRUG-RESISTANCE MUTATION	IC_{50} RATIO <2.5	IC_{50} RATIO 2.5–10	IC_{50} RATIO >10
	no./total no. (%)			
Reverse-transcriptase mutation conferring resistance to NRTIs				
E44D	4/258 (2)			
K65R	0/258			
T69D	1/258 (<1)			
K70R	2/258 (1)			
L74V	0/258			
V75T	0/258			
V118I	5/258 (2)			
M184V/I	5/258 (2)			
T215Y/F/D/N/S/C/E†	16/258 (6)			
Q151M	0/258			
T69S/A	3/258 (1)			
NRTI susceptibility				
Abacavir		266/279 (95)	12/279 (4)	1/279 (<1)
Didanosine		371/373 (99)	2/373 (1)	0/373
Lamivudine		365/376 (97)	5/376 (1)	6/376 (2)
Stavudine		373/377 (99)	4/377 (1)	0/377
Zalcitabine		374/375 (99)	1/375 (<1)	0/375
Zidovudine		367/376 (98)	1/376 (<1)	8/376 (2)
Reverse-transcriptase mutation conferring resistance to NNRTIs				
K103N	6/258 (2)			
V106A	1/258 (<1)			
V108I	1/258 (<1)			
Y181C/I	2/258 (1)			
Y188C/L/H	0/258			
G190S/A	0/258			
NNRTI susceptibility				
Delavirdine		312/374 (83)	49/374 (13)	13/374 (3)
Efavirenz		360/375 (96)	6/375 (2)	9/375 (2)
Nevirapine		339/376 (90)	27/376 (7)	10/376 (3)
Protease mutation conferring resistance to protease inhibitors				
D30N	0/301			
M46I	2/301 (1)			
G48V	2/301 (1)			
I50V	0/301			
V82A/F/T/S	6/301 (2)			
I84V	3/301 (1)			
L90M	7/301 (2)			
Protease inhibitor susceptibility				
Amprenavir		275/278 (99)	3/278 (1)	0/278
Indinavir		363/377 (96)	11/377 (3)	<3/377 (<1)
Lopinavir		29/29 (100)	0/29	0/29
Nelfinavir		344/376 (91)	26/376 (7)	6/376 (2)
Ritonavir		355/373 (95)	12/373 (3)	6/373 (2)
Saquinavir		365/373 (98)	4/373 (1)	4/373 (1)
Any major drug-resistance mutation	37/301 (12)			
Susceptibility to any retroviral drug		280/377 (74)	74/377 (20)	23/377 (6)

*Denominators indicate the total number of samples analyzed. The NL4-3 consensus sequence was used for comparison. The 50 percent inhibitory concentration (IC_{50}) ratio is the ratio of the IC_{50} for the subject's virus to the IC_{50} for the reference virus. Isolates with an IC_{50} of no more than 2.5 times that for the NL4-3 reference strain were considered to be drug-susceptible. NRTI denotes nucleoside reverse-transcriptase inhibitor, and NNRTI nonnucleoside reverse-transcriptase inhibitor.

†Of the 17 mutations at position 215, 7 were T215Y, 1 was T215F, 5 were T215D, 2 were T215S, and 2 were T215E; none of the isolates had a T215N mutation or a T215C mutation.
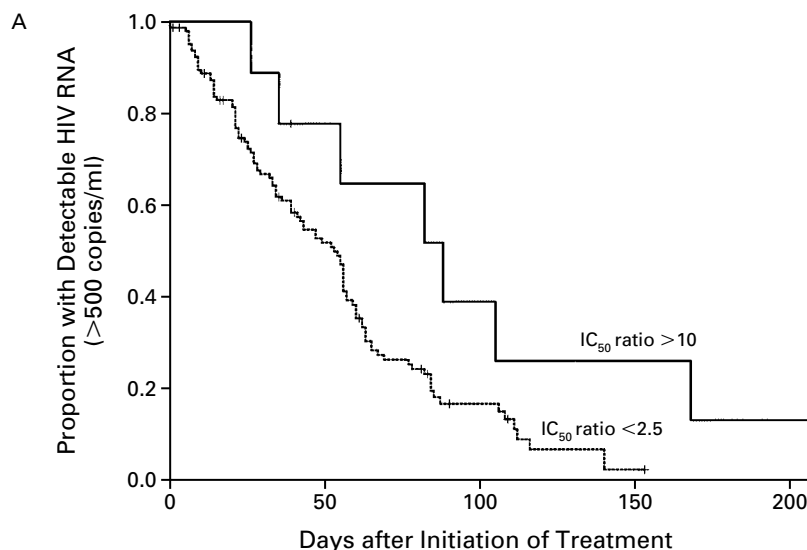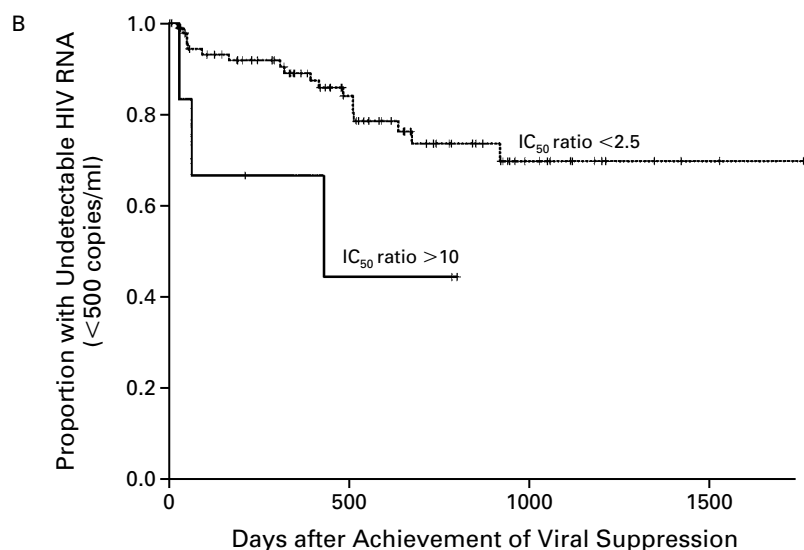
Figure 2. Kaplan-Meier Estimates of the Time to Viral Suppression and Subsequent Virologic Failure Relative to Base-Line Drug Susceptibility.

The time from the initiation of antiretroviral therapy to viral suppression was shorter for subjects with drug-susceptible viral isolates at base line (a value <2.5 for the ratio of the 50 percent inhibitory concentration [IC_{50}] for the subject's virus to the IC_{50} for a reference virus) than for subjects with an IC_{50} ratio of more than 10 for one or more antiretroviral drugs (P=0.05) (Panel A). Among patients in whom viral suppression was achieved by therapy (HIV RNA, <500 copies per milliliter), the time from the initial achievement of suppression to the first virologic failure was shorter among subjects with an IC_{50} more than 10 times that of the reference virus at base line than among those with drug-susceptible virus (IC_{50} ratio, <2.5; P=0.05) (Panel B). Subjects' data were censored (tick marks) if the plasma RNA was measured at intervals of more than 6 weeks or if potent treatment was interrupted for any reason for more than 14 days.

and 9 to 21 percent in Europe.[35-37] Differences in the methods of testing for drug resistance, geographic variability in patterns of antiretroviral-drug use, and variations in the risk factors for exposure to HIV may explain the differences in prevalence among these reports. Increases in the prevalence of drug-resistant virus among patients with established HIV infection may be associated with more frequent transmission of drug-resistant virus to newly infected persons in their community. We avoided some limitations of earlier studies by using standardized methods of identifying subjects, collecting data, and testing for drug resistance in order to assess the longitudinal patterns of transmission of drug-resistant virus at 10 sites in North America.

When we defined drug resistance by the conservative threshold of 10 for the ratio of the IC_{50} for the subject's virus to the IC_{50} for a reference virus, we found that the prevalence of transmitted drug resistance increased from 3.4 percent in the period from 1995 to 1998 to 12.4 percent in the period from 1999 to 2000 (P=0.002). Precise criteria for resistance, defined as a value predictive of an impaired response to treatment, have not been defined or standardized for most drugs. Therefore, we used a variety of criteria to ensure that the observed increases in rates of transmission of resistant virus were not an artifact of a single definition of resistance. When the thresholds of drug susceptibility are defined more rigorously, the estimates of the transmission of drug resistance derived from these data will probably be higher than our current estimates. The most notable increase in the prevalence of drug resistance occurred between 1998 and 1999, perhaps as a result of more widespread access to potent combination therapies during the preceding months.

Only mutations that confer resistance and are not natural polymorphisms can be used as evidence of a transmitted drug-resistant variant.[38] In this study, the prevalence of mutations associated with resistance increased from 8.0 to 22.7 percent (P<0.001). Since many individual resistance mutations will not be associated with clinically significant drug resistance, these numbers may overestimate the true prevalence of the transmission of drug-resistant virus. Estimates of drug resistance based on genotypic testing differ in magnitude from those based on phenotypic testing, but both estimates indicate a significant increase in the prevalence of transmitted drug resistance.

The increasing prevalence of single-drug–resistant and multidrug-resistant HIV has crucial implications for our current prevention and treatment strategies. HIV with drug resistance is most prevalent among men who have sex with men, a risk group that has historically had greater access to HIV care than other groups.[39,40] Reports of increasing rates of unsafe sex and associated increases in the incidence of sexually transmitted diseases, including HIV disease, especially among men who have sex with men, suggest that risky behavior may result in both an increased incidence of HIV infection and an increased frequency of drug resistance in these communities.[41-44] Although some transmitted drug-resistance mutations may persist for months,[17,45,46] such mutations may, in the absence of selection pressure exerted by drugs, revert to a more replication-competent variant. When such reversion occurs, the transmitted mutations are no longer routinely detectable. Thus, the rates of transmitted resistance observed in this study may underestimate the true rates that could be determined by resistance testing performed at the onset of infection. Potential bias in these data could have been introduced by variations within the study group in adherence to treatment, access to and quality of medical care, and demographic characteristics.

Although the prevalence of detected resistance should diminish as the time from transmission to resistance testing increases, we observed no differences in the proportion of subjects identified with drug-resistant HIV according to the time elapsed from the estimated date of HIV infection (data not shown). Even if it is no longer detectable, the transmitted drug-resistant variant persists in the reservoir of latently infected CD4+ memory T cells[47,48] and may rapidly reemerge under the selective pressure provided by antiretroviral treatment. Thus, identification of patients early after the acquisition of HIV infection may facilitate the identification of transmitted drug resistance and improve the selection of more effective first-line treatment regimens.

The increasing rates of transmission of drug-resistant virus observed in this study, coupled with the poorer response to treatment in patients with drug-resistant virus, suggest that resistance tests should be recommended routinely for patients with new infection. In subjects with primary HIV infection who had acquired drug-resistant virus, plasma HIV RNA was not suppressed as readily by potent antiretroviral therapy. The slower response to treatment and the more limited viral suppression permit additional rounds of viral replication in the presence of antiretroviral drugs, which may, in turn, facilitate the selection of variants with greater drug resistance.

The prevalence of transmitted drug-resistant virus, especially multidrug-resistant HIV, has important implications for the use and management of antiretroviral drugs among patients with HIV infection. The existence of fewer options for initial treatment and suboptimal responses to treatment among recently infected patients may seriously limit the expected reduction in the rate of disease progression and increase secondary transmission of drug-resistant variants.[49] With

one in five patients infected with virus bearing a major drug-resistant mutation, guidelines for empirical treatment can no longer be relied on for recently infected patients. In both the developed and developing worlds, the treatment strategies for patients newly infected with HIV should take into account the prevalence of transmitted drug resistance.

REFERENCES

1. Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 1998;338:853-60.
2. Jacobson MA, French M. Altered natural history of AIDS-related opportunistic infections in the era of potent combination antiretroviral therapy. AIDS 1998;12:Suppl A:S157-S163.
3. Murphy EL, Collier AC, Kalish LA, et al. Highly active antiretroviral therapy decreases mortality and morbidity in patients with advanced HIV disease. Ann Intern Med 2001;135:17-26.
4. Autran B, Carcelain G, Li TS, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 1997;277:112-6.
5. Lederman MM, Connick E, Landay A, et al. Immunologic responses associated with 12 weeks of combination antiretroviral therapy consisting of zidovudine, lamivudine, and ritonavir: results of AIDS Clinical Trials Group protocol 315. J Infect Dis 1998;178:70-9.
6. Lorenzi P, Opravil M, Hirschel B, et al. Impact of drug resistance mutations on virologic response to salvage therapy: Swiss HIV Cohort Study. AIDS 1999;13:F17-F21.
7. D'Aquila RT, Johnson VA, Welles SL, et al. Zidovudine resistance and HIV-1 disease progression during antiretroviral therapy. Ann Intern Med 1995;122:401-8.
8. Zolopa AR, Shafer RW, Warford A, et al. HIV-1 genotypic resistance patterns predict response to saquinavir-ritonavir therapy in patients in whom previous protease inhibitor therapy had failed. Ann Intern Med 1999;131:813-21.
9. Acute HIV Infection and Early Disease Research Program (AIEDRP). Seattle: Statistical Center for HIV/AIDS Research and Prevention (SCHARP), 2002. (Accessed July 15, 2002, at http://aiedrp.fhcrc.org.)
10. Janssen RS, Satten GA, Stramer SL, et al. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. JAMA 1998;280:42-8. [Erratum, JAMA 1999;281:1893.]
11. Little SJ, Daar ES, D'Aquila RT, et al. Reduced antiretroviral drug susceptibility among patients with primary HIV infection. JAMA 1999;282: 1142-9.
12. Boden D, Hurley A, Zhang L, et al. HIV-1 drug resistance in newly infected individuals. JAMA 1999;282:1135-41.
13. Carpenter CC, Cooper DA, Fischl MA, et al. Antiretroviral therapy in adults: updated recommendations of the International AIDS Society-USA Panel. JAMA 2000;283:381-90.
14. Petropoulos CJ, Parkin NT, Limoli KL, et al. A novel phenotypic drug susceptibility assay for human immunodeficiency virus type 1. Antimicrob Agents Chemother 2000;44:920-8.
15. Hirsch MS, Brun-Vézinet F, D'Aquila RT, et al. Antiretroviral drug resistance testing in adult HIV-1 infection: recommendations of an International AIDS Society-USA Panel. JAMA 2000;283:2417-26.
16. Rubio A, Leal M, Pineda JA, et al. Increase in the frequency of mutation at codon 215 associated with zidovudine resistance in HIV-1-infected antiviral-naive patients from 1989 to 1996. AIDS 1997;11:1184-6.
17. Garcia-Lerma JG, Nidtha S, Blumoff K, Weinstock H, Heneine W. Increased ability for selection of zidovudine resistance in a distinct class of wild-type HIV-1 from drug-naive persons. Proc Natl Acad Sci U S A 2001; 98:13907-12.
18. Erice A, Mayers DL, Strike DG, et al. Primary infection with zidovudine-resistant human immunodeficiency virus type 1. N Engl J Med 1993; 328:1163-5.
19. Conlon CP, Klenerman P, Edwards A, Larder BA, Phillips RE. Heterosexual transmission of human immunodeficiency virus type 1 variants associated with zidovudine resistance. J Infect Dis 1994;169:411-5.
20. Mayers DL, Yerly S, Perrin L, et al. Prevalence and clinical course of persons seroconverting with AZT-resistant (AZTᴿ) HIV-1 in Switzerland, Australia, and the United States between 1988 and 1994. AIDS Res Hum Retroviruses 1995;11:Suppl:S162. abstract.
21. Quigg M, Rebus S, France AJ, McMenamin J, Darby G, Leigh Brown AJ. Mutations associated with zidovudine resistance in HIV-1 among recent seroconvertors. AIDS 1997;11:835-6.
22. Conway B, Montessori V, Rouleau D, et al. Primary lamivudine resistance in acute/early human immunodeficiency virus infection. Clin Infect Dis 1999;28:910-1.
23. de Ronde A, Schuurman R, Goudsmit J, van den Hoek A, Boucher C. First case of new infection with zidovudine-resistant HIV-1 among prospectively studied intravenous drug users and homosexual men in Amsterdam, the Netherlands. AIDS 1996;10:231-2.
24. Veenstra J, Schuurman R, Cornelissen M, et al. Transmission of zidovudine-resistant human immunodeficiency virus type 1 variants following deliberate injection of blood from a patient with AIDS: characteristics and natural history of the virus. Clin Infect Dis 1995;21:556-60.
25. Fitzgibbon JE, Gaur S, Frenkel LD, Laraque F, Edlin BR, Dubin DT. Transmission from one child to another of human immunodeficiency virus type 1 with a zidovudine-resistance mutation. N Engl J Med 1993;329: 1835-41.
26. Kamkamidze G, Sullivan T, Charbonneau T. Occurrence of HIV-1 reverse transcriptase gene mutation at codon 215 in HIV-infected infants. J Clin Virol 2001;22:143-8.
27. Johnson VA, Petropoulos CJ, Woods CR, et al. Vertical transmission of multidrug-resistant human immunodeficiency virus type 1 (HIV-1) and continued evolution of drug resistance in an HIV-1-infected infant. J Infect Dis 2001;183:1688-93.
28. Colgrove RC, Pitt J, Chung PH, Welles SL, Japour AJ. Selective vertical transmission of HIV-1 antiretroviral resistance mutations. AIDS 1998; 12:2281-8.
29. Masquelier B, Chaix ML, Burgard M, et al. Zidovudine genotypic resistance in HIV-1-infected newborns in the French perinatal cohort. J Acquir Immune Defic Syndr 2001;27:99-104.
30. Imrie A, Beveridge A, Genn W, Vizzard J, Cooper DA. Transmission of human immunodeficiency virus type 1 resistant to nevirapine and zidovudine. J Infect Dis 1997;175:1502-6.
31. Hecht FM, Grant RM, Petropoulos CJ, et al. Sexual transmission of an HIV-1 variant resistant to multiple reverse-transcriptase and protease inhibitors. N Engl J Med 1998;339:307-11.
32. Yerly S, Kaiser L, Race E, Bru JP, Clavel F, Perrin L. Transmission of antiretroviral-drug-resistant HIV-1 variants. Lancet 1999;354:729-33.
33. Brodine SK, Shaffer RA, Starkey MJ, et al. Drug resistance patterns, genetic subtypes, clinical features, and risk factors in military personnel with HIV-1 seroconversion. Ann Intern Med 1999;131:502-6.
34. Salomon H, Wainberg MA, Brenner B, et al. Prevalence of HIV-1 resistant to antiretroviral drugs in 81 individuals newly infected by sexual contact or injecting drug use. AIDS 2000;14:F17-F23.

35. Yerly S, Vora S, Rizzardi P, et al. Acute HIV infection: impact on the spread of HIV and transmission of drug resistance. AIDS 2001;15:2287-92.

36. Balotta C, Berlusconi A, Pan A, et al. Prevalence of transmitted nucleoside analogue-resistant HIV-1 strains and pre-existing mutations in pol reverse transcriptase and protease region: outcome after treatment in recently infected individuals. Antivir Ther 2000;5:7-14.

37. UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance. Analysis of prevalence of HIV-1 drug resistance in primary infections in the United Kingdom. BMJ 2001;322:1087-8.

38. Leigh Brown AJ, Precious HM, Whitcomb JM, et al. Reduced susceptibility of human immunodeficiency virus type 1 (HIV-1) from patients with primary HIV infection to nonnucleoside reverse transcriptase inhibitors is associated with variation at novel amino acid sites. J Virol 2000;74:10269-73.

39. Bozzette SA, Berry SH, Duan N, et al. The care of HIV-infected adults in the United States. N Engl J Med 1998;339:1897-904.

40. Richman DD, Bozzette SA, Morton SC, et al. The prevalence of antiretroviral drug resistance in the US. In: Program and abstracts of the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, December 16–19, 2001. Washington, D.C.: American Society for Microbiology, 2001:LB-17. abstract.

41. HIV incidence among young men who have sex with men — seven U.S. cities, 1994–2000. MMWR Morb Mortal Wkly Rep 2001;50:440-4.

42. Outbreak of syphilis among men who have sex with men — southern California, 2000. MMWR Morb Mortal Wkly Rep 2001;50:117-20.

43. Increases in unsafe sex and rectal gonorrhea among men who have sex with men — San Francisco, California, 1994–1997. MMWR Morb Mortal Wkly Rep 1999;48:45-8.

44. Kellogg T, McFarland W, Katz M. Recent increases in HIV seroconversion among repeat anonymous testers in San Francisco. AIDS 1999;13:2303-4.

45. Little SJ, Holte S, Routy JP, et al. Antiretroviral resistance and response to initial therapy among recently HIV-infected subjects in North America. Antivir Ther 2001;6:Suppl 1:21. abstract.

46. Little SJ, Daar ES, Holte S, et al. Persistence of transmitted drug resistance among subjects with primary HIV infection not receiving antiretroviral therapy. Presented at the 9th Conference on Retroviruses and Opportunistic Infections, Seattle, February 24–28, 2002. abstract.

47. Wong JK, Hezareh M, Günthard HF, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 1997;278:1291-5.

48. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 1997;278:1295-300.

49. Leigh Brown AJ, Frost SDW, Mathews WC, et al. Will transmission of drug resistant HIV be driven by individuals infected with drug resistant strains? Presented at the 9th Conference on Retroviruses and Opportunistic Infections, Seattle, February 24–28, 2002. abstract.

FULL TEXT OF ALL *JOURNAL* ARTICLES ON THE WORLD WIDE WEB

Access to the complete text of the *Journal* on the Internet is free to all subscribers. To use this Web site, subscribers should go to the *Journal*'s home page (http://www.nejm.org) and register by entering their names and subscriber numbers as they appear on their mailing labels. After this one-time registration, subscribers can use their passwords to log on for electronic access to the entire *Journal* from any computer that is connected to the Internet. Features include a library of all issues since January 1993 and abstracts since January 1975, a full-text search capacity, and a personal archive for saving articles and search results of interest. All articles can be printed in a format that is virtually identical to that of the typeset pages. Beginning six months after publication the full text of all original articles and special articles is available free to nonsubscribers who have completed a brief registration.