Transmission Fitness of Drug-Resistant Human Immunodeficiency Virus and the Prevalence of Resistance in the Antiretroviral-Treated Population

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Although the prevalence of drug-resistant strains in primary human immunodeficiency virus (HIV) infection in North America has recently increased, their transmission fitness remains unknown. The present study estimated the frequency of transmission of drug-resistant HIV from patients receiving antiretroviral therapy using retrospective surveys of clinic data. It revealed that resistant virus was transmitted only ~20% as frequently as expected from these patients. Individuals with primary resistance may become a significant source of resistant strains.

Antiretroviral therapy (ART) can have a significant impact on the human immunodeficiency virus (HIV) epidemic by reducing the probability of transmission per contact [1]. However, transmission of drug-resistant HIV can reduce the positive impact of therapy. Recently, an increase from 3.8% to 14.1% in the prevalence of drug-resistant virus in primary HIV infection over the course of 4 years was described in a large multicenter study in North America [2]. The increase in transmission rates of drug-resistant virus at a time when death rates from AIDS have decreased substantially [3] is a matter of concern for public health policy.

ART is expected to reduce HIV transmission by lowering HIV loads in plasma and genital secretions, because plasma HIV load is correlated with the risk of sexual transmission [4]. To assess the influence of drug resistance on the HIV epidemic, it is important to evaluate its effect on virus transmission. We have performed a retrospective analysis of clinic data from individuals experiencing virologic failure of ART at an HIV specialty clinic in San Diego, for comparison with genotypic evidence of antiretroviral resistance in acutely HIV-infected individuals in the same city. We conclude that drug-resistant HIV is transmitted substantially less frequently than is wild-type HIV from individuals who have acquired drug-resistant strains while receiving ART.

Methods. The prevalence of transmission of drug-resistant HIV was estimated from genotype data from patients who presented with acute HIV infection in San Diego and Los Angeles; data were used from a subset of the patients for whom phenotypic susceptibility was reported elsewhere [2]. Study participants were predominantly men who reported a history of sex with men. They were enrolled during 2 time periods: 1996–1998 ($n = 65$) and 1999–2000 ($n = 58$). The median time between estimated date of infection and first sample for the entire cohort was 71 days [2]. Amino acid sequences were obtained for the reverse transcriptase (RT) and protease (PR) coding regions from plasma samples, as described elsewhere [5], and were analyzed using StatXact (version 4.0.1; Cytel Software). Amino acid sites associated with antiretroviral resistance were those with primary mutations listed in the consensus guidelines for antiretroviral resistance testing [6].

The prevalence of virologic failure among patients receiving ART was estimated by using quarterly clinic data from the Owen Clinic, a University of California, San Diego (UCSD) HIV specialty clinic serving the city of San Diego. Clinical and laboratory data were stored and accessed using an HIV-specific clinical information system (LabTracker; Ground Zero Software) that documents initial patient evaluation, medications, diagnoses, and laboratory parameters, including longitudinal CD4 cell counts and plasma HIV load measurements. We used data from the fourth quarter of 1997 and the second quarter

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Table 1. Frequencies of genotypically determined antiretroviral resistance in primary human immunodeficiency virus infection in San Diego and Los Angeles, 1996–1998 and 1999–2000.

Years	No. of patients[a]	Drug class						Total, no. (%) of patients
		NRTI						
		Zdv	3TC	ddC	MNR	NNRTI	PI	
1996–1998	65	4[b]	0	0	0	0	1[b]	4 (6)
1999–2000	58	3	1	1	1[c]	3[c]	3[c]	10 (17)

NOTE. Data are no. (%) of patients with genotypic resistance. The difference in percentage of genotypic resistance was close to significance ($P = .056$). 3TC, lamivudine; ddC, zalcitabine; MNR, mutlinucleoside resistant; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; Zdv, zidovudine.

[a] Data are for San Diego and Los Angeles patients as published elsewhere [2].

[b] One patient was infected with a Zdv- and PI-resistant strain.

[c] One patient was infected with an MNR- and NNRTI-resistant strain that was also resistant to PIs.

of 1999, because records for these periods were the most complete and were representative of each year. The probability of transmission of HIV is related to the HIV load of the index patient and is very low when plasma HIV load is <1000 copies/mL [4]. We categorized HIV-infected individuals whose plasma HIV loads were ⩾1000 copies/mL as "potential transmitters" and those whose plasma HIV loads were <1000 copies/mL as "nontransmitters." Among patients receiving ART who had plasma HIV loads ⩾1000 copies/mL in 1997 ("patients experiencing virologic failure"), 66% also had plasma HIV loads ⩾1000 copies/mL in 1999. Although these patients continued to receive therapy, their mean plasma HIV load was almost identical in 1997 and 1999 (4.48 $\log_{10}$ and 4.52 $\log_{10}$ HIV-1 RNA copies/mL, respectively), and there were as many patients with decreasing as increasing plasma HIV loads during this period, with a similar range in each direction. There was no systematic difference in use of any antiretroviral between these 2 periods.

We performed an uncertainty analysis [1] of the estimate of the proportion of transmitters who harbor secondary resistance by using 100,000 random numbers generated from a uniform distribution to generate a range of estimates of the relative number of transmitters of resistant virus in 1997. We assumed that 36%–63% of infected individuals attend a clinic [7] and, on the basis of the clinic data presented here, that 63% of clinic attendees have plasma HIV loads ⩾1000 copies/mL (see Results). We also assumed, on the basis of clinic data, that 86% of individuals attending the clinic who had plasma HIV loads ⩾1000 copies/mL had received some ART and that 70%–80% of these patients harbored resistant virus [8]. Among individuals who did not attend the clinic, 80%–95% were assumed to have plasma HIV loads ⩾1000 copies/mL (a proportion similar to that for individuals attending the clinic but not receiving ART), and 0%–10% of these patients were assumed to harbor transmitted resistant virus.

Results. The prevalence of mutations at amino acid sites associated with antiretroviral resistance among individuals from Los Angeles and San Diego with acute HIV infection in 1996–1998 was 6% (table 1; $n = 65$). A total of 4 individuals had antiretroviral resistance–associated mutations in RT—specifically, T215Y, T215S, T215D, and T215E. T215Y, the prototypic mutation associated with zidovudine (Zdv) resistance, was found in 1 strain, which also demonstrated genotypic evidence of PR inhibitor (PI) resistance (V82T in PR). T215S, T215D, and T215E indicated infection with a 215Y-bearing variant, which subsequently reverted to a phenotypically susceptible genotype either in the subject with primary infection or in the source individual [9, 10].

Among 58 additional individuals identified as having primary HIV infection at the San Diego and Los Angeles clinics during 1999–2000, 10 (17%) showed evidence of infection with a drug-resistant strain (table 1), a substantial increase from the 1996–1998 sample ($P = .056$). Transmitted antiretroviral resistance was no longer predominantly to Zdv. Genotypic resistance to Zdv, zalcitabine, lamivudine, nonnucleoside RT inhibitors (NNRTIs), and PIs was observed, as well as 1 case of multidrug resistance. This case included mutations associated with multinucleoside resistance (69S insertion and T215F), NNRTI resistance (K103N and Y181C), and PI resistance (M46I and L90M in PR).

To assess the probability of transmission of drug-resistant strains, we first estimated the proportion of individuals who were potential HIV transmitters by using retrospective data from the UCSD HIV specialty clinic. In 1997, there were 146 patients (63%) with plasma HIV loads ⩾1000 copies/mL (table 2). However, many HIV transmissions are likely to occur from HIV-infected individuals who are not attending a clinic and, thus, not receiving ART. The proportion of HIV-infected individuals in the United States who are not receiving care has been estimated to be 36%–63% [7]. We therefore assumed that the clinic data reflected ~50% of the HIV-infected population in San Diego; thus, clinic patients who are potential transmitters represent

Table 2. Plasma human immunodeficiency virus type 1 (HIV-1) loads among patients attending a University of California, San Diego, hospital referral clinic in 1997 and 1999.

Year	No. of patients	Never received ART		Ever received ART	
		HIV-1 RNA load <1000 copies/mL	HIV-1 RNA load ⩾1000 copies/mL	HIV-1 RNA load <1000 copies/mL	HIV-1 RNA load ⩾1000 copies/mL
1997	233	5 (2.1)	20 (8.6)	82 (35.2)	126 (54.1)
1999	498	8 (1.6)	47 (9.4)	228 (45.8)	215 (43.2)

NOTE. Data are no. (%) of patients. Data were from the fourth quarter of 1997 and the second quarter of 1999, both of which were considered to be representative of each year. ART, antiretroviral therapy.

63% × 0.5, or ~32%, of the total population of HIV-infected patients. Of 75 untreated patients who attended the clinic in either year, 63 (84%) had plasma HIV loads ⩾1000 copies/mL, and, if the same distribution of plasma HIV load applied to nonattending HIV-infected individuals, 42% (84% × 0.5) were potential transmitters not attending clinics, which indicates that ~74% (32% + 42%) of the total HIV-infected population was at risk of transmitting HIV.

To estimate the proportion of HIV-infected patients who are potential transmitters of drug-resistant virus, we adopted the estimate obtained from a subsample of the cohort recruited through the HIV Costs and Services Utilization Study (HCSUS) [7], a nationally representative cohort of HIV-infected individuals who are receiving care. In this study, 78% of patients for whom ART failed had resistant strains [8]. On the basis of those data, potential transmitters of resistance in 1997 represented 42% (54% × 0.78) of the clinic-attending HIV-infected population, or 21% of the total HIV-infected population. We therefore estimated that ~28% (21%/0.74) of all potential transmitters of HIV are potential transmitters of drug-resistant strains.

A series of assumptions about parameter values were made in deriving this point estimate. We used an uncertainty analysis, based on the 1997 clinic data, to explore model behavior over a range of values (see Methods), which yielded an estimated median (±95% CI) proportion of 30% ± 9%. However, the prevalence of new infections with drug resistant strains was 6% in 1997. Thus, resistant virus was being transmitted ~20% (6%/0.3) as often as expected from the proportion of potential transmitters.

Transmission of drug-resistant virus could be reduced because individuals with drug-resistant virus have lower average plasma HIV loads than do individuals with drug-susceptible virus. To test this hypothesis, we compared plasma HIV loads for 2 groups of potential transmitters: individuals with plasma HIV loads >1000 copies/mL who attended the Owen Clinic and who had never received ART (from both 1997 and 1999) and individuals for whom ART failed in 1997. We observed no difference in the mean plasma HIV load between these groups (mean plasma HIV load for the no antiretroviral group, 4.48 $\log_{10}$ copies/mL [$n = 44$]; mean plasma HIV load for the group in which ART failed, 4.50 $\log_{10}$ copies/mL [$n = 126$]; $P = .78$). In the nationally representative HCSUS cohort [7, 8], we again found no difference. The median plasma HIV load for patients who had received ART and for whom it failed (defined as plasma HIV load ⩾500 copies/mL) and who had phenotypically drug-resistant virus was 4.43 $\log_{10}$ copies/mL ($n = 821$); the median plasma HIV load for individuals with virus load ⩾500 copies/mL who had never received ART was 4.37 $\log_{10}$ copies/mL ($n = 51$). Finally, in the Multicenter AIDS Cohort Study (MACS) cohort of untreated individuals, the median plasma HIV load was 4.05 $\log_{10}$ copies/mL [11], again, no higher than the plasma HIV loads observed in individuals for whom ART failed.

Discussion. We have found that patients who experience treatment failure and who are at risk of transmitting resistance make up 30% ± 9% of all potential HIV transmitters. The prevalence of transmitted resistance was ~20% of this, indicating that resistant strains are transmitted less frequently than expected. HIV load could, on average, be lower in patients for whom ART fails than in untreated individuals, leading to a lower transmission risk; we have shown that, in both the UCSD clinic and the HCSUS, individuals attending clinics who had never received ART had a mean plasma HIV load that was almost identical to that in patients for whom therapy failed. This conclusion, which is surprising, because plasma HIV load generally increases when therapy is stopped and wild-type virus becomes predominant, has also been seen in a third cohort from Scotland [12]. However, since potent therapy has become available, it is possible that patients attending any clinic who are not receiving therapy are predominantly those with lower plasma HIV loads. This is a bias that should not extend to the MACS cohort, which was studied before potent therapy became available, but even in that group, the median plasma HIV load for untreated patients in the MACS cohort was no higher [11], indicating that median plasma HIV load and the consequent probability of HIV transmission from untreated patients and those for whom therapy fails is approximately the same.

A second possible explanation is that the behavior of patients attending clinic and receiving therapy is less "risky" than that of untreated HIV-infected individuals. However, in a recent cohort study of men who have sex with men in 7 US cities, neither plasma HIV load nor ART was associated with the probability of engaging in high-risk behavior [13].

We suggest that transmission of both wild-type and resistant virus occurs from individuals who acquire drug resistance while receiving ART. Either drug-resistant virus is transmitted by only 30% of individuals with acquired resistance, or all individuals with acquired resistance may be able to transmit resistant virus, with a 30% chance per infection event. More than one mechanism could be responsible in either case. Some resistant strains have substantially reduced fitness, which could reduce the prob-

ability of transmission. Viral fitness has been estimated in vivo for V82A in PR and for Y181C and T215Y in RT [9, 14, 15], and the fitness reduction relative to wild-type was no more than 10%. A fitness difference on this scale is sufficient to ensure rapid outgrowth of the fitter strain in a mixed infection but would have little effect on the probability that a resistant strain will establish an infection in a susceptible individual. It has recently been shown experimentally that wild-type strains with a wide range of in vitro replicative capacity can establish an infection [16].

Patients who acquire drug resistance while receiving a failing ART regimen retain wild-type genotypes in latent reservoirs and protected compartments, which becomes predominant on cessation of therapy. Virus populations in the genital and lymphoid compartments are at least partly isolated from each other [17], and, with treatment with some antiretrovirals, the drug concentration in seminal fluid is lower than that in plasma [18]. This could reduce the selective advantage associated with resistance and give rise to a higher average frequency of susceptible strains in this compartment, thus lowering the probability that an individual with secondary resistance will transmit drug-resistant strains.

Transmission of drug resistance increased in San Diego after 1998, as elsewhere in North America [2], despite the estimated low transmission fitness of drug-resistant strains, an increasing use of combination ART (table 2), and an approximate doubling in the proportion of antiretroviral-treated patients who achieved virus suppression. However, although the proportion of patients with virologic failure decreased, the absolute number of patients attending the Owen Clinic for whom ART failed in 1999 was double that in 1997, which increased the risk of transmission of drug-resistant strains. Furthermore, the multiple ways in which transmission fitness of drug-resistant strains can be lower than that of wild-type strains in patients who acquire resistance while receiving ART contrasts with the circumstances of individuals who are themselves infected with resistant strains. These individuals will only be able to transmit drug-resistant virus for as long as that remains the predominant form and may play an important role in the epidemic of transmitted antiretroviral-resistant HIV.

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