Antiretroviral Recommendations May Influence the Rate of Transmission of Drug-Resistant HIV Type 1

Carmen de Mendoza,[1] Carmen Rodriguez,[2] José Maria Eiros,[3] Javier Colomina,[4] Federico Garcia,[5] Pilar Leiva,[6] Julián Torre-Cisneros,[7] Jesús Aguero,[8] José Pedreira,[9] Isabel Viciana,[10] Angélica Corral,[1] Jorge del Romero,[2] Raúl Ortiz de Lejarazu,[3] and Vincent Soriano[1]

[1]Service of Infectious Diseases, Hospital Carlos III, and [2]Centro Sanitario Sandoval, Madrid, [3]Service of Microbiology, Hospital Clínico, Valladolid, [4]Service of Microbiology, Hospital de la Ribera, Valencia, [5]Service of Microbiology, Hospital San Cecilio, Granada, [6]Service of Microbiology, Hospital Central de Asturias, Oviedo, [7]Department of Internal Medicine, Hospital Reina Sofia, Córdoba, [8]Service of Microbiology, Hospital Marques de Valdecilla, Santander, [9]Department of Internal Medicine, Hospital Juan Canalejo, La Coruña, and [10]Service of Microbiology, Hospital Virgen de la Victoria, Malaga, Spain

(See the editorial commentary by Little and Smith on pages 233–5)

Background. Human immunodeficiency virus (HIV) treatment guidelines have evolved, shifting from more-aggressive to more-conservative approaches. The potential impact of these shifts on the transmission of drug-resistant virus is unknown.

Methods. Drug-resistance genotypes were examined in all consecutive patients with recent HIV type 1 (HIV-1) seroconversion (hereafter, "HIV-1 seroconverters") seen at 10 Spanish hospitals since 1997. During the same period, the proportion of patients with chronic HIV-1 infection having undetectable viremia was examined, to estimate the extent and effectiveness of antiretroviral therapy.

Results. A total of 141 recent HIV-1 seroconverters were identified, 67.4% of whom were men who have sex with men. The rate of primary drug-resistance mutations, by year of infection, was 33.3% for 1997, 29.4% for 1998, 20% for 1999, 14.3% for 2000, 3.4% for 2001, 15.4% for 2002, and 10.9% for 2003. On the other hand, the proportion of 8388 persons with chronic HIV-1 carriage who had an undetectable virus load was 33.4% for 1997, 34.6% for 1998, 39.7% for 1999, 47.5% for 2000, 52.9% for 2001, 39.7% for 2002, and 58.1% for 2003. A significant inverse correlation between transmission of drug-resistant HIV-1 and undetectable virus load was found (r = −0.955, by Spearman's test; $P = .001$). The lowest rate of transmission of drug-resistant HIV-1 was seen in 2001, when relatively "aggressive" treatment guidelines were used. Transmission of drug-resistant HIV-1 increased in 2002, in parallel with a reduction in the number of patients with chronic HIV-1 carriage and undetectable virus load, reflecting the popularity of drug holidays or treatment interruptions.

Conclusion. The rate of drug resistance in recent HIV-1 seroconverters inversely correlates with the proportion of chronically HIV-1–infected individuals who have undetectable virus loads in the same region, which indirectly reflects antiretroviral treatment rules at any given time.

Resistance to antiretroviral drugs represents one of the major obstacles for the success of HIV-1 therapy [1]. Viruses that carry drug-resistance mutations can be transmitted to and subsequently compromise the response to antiretroviral therapy in drug-naive individuals [2–4]. In Western countries, where many HIV-1–infected subjects are receiving antiretroviral therapy, an increase in the prevalence of primary drug resistance among patients with new HIV-1 seroconversion (hereafter, "HIV-1 seroconverters") has been noticed in recent years [4–6]. Accordingly, updated guidelines recommend drug resistance testing before antiretroviral treatment is initiated for subjects with recently acquired HIV-1 infection [7, 8].

In Spain, surveillance studies conducted during the 1990s have demonstrated that the rate of genotypic resistance among drug-naive individuals with chronic HIV-1 infection decreased significantly from 1997 to

2000 [9–11]. However, data from HIV-1 seroconverters are scarce, although alarming rates of primary drug resistance were reported in the late 1990s [12, 13]. In parallel, HIV-1 treatment guidelines have evolved over the past few years, shifting from more-aggressive to more-conservative approaches [14, 15].

We were interested to know the extent to which changes in antiretroviral treatment recommendations may have influenced trends in the proportion of drug-resistant viruses in newly infected individuals. For this purpose, and given the strong correlation found between plasma HIV-1 RNA level and infectiousness [16], we assumed that the proportion of patients with chronic HIV-1 infection who have detectable plasma virus loads indirectly reflects the population of potential transmitters. Thereafter, we confronted it with yearly rates of primary drug resistance among recent HIV-1 seroconverters. Specimens and information from a relatively large number of newly infected persons identified in different Spanish cities were assessed.

PATIENTS AND METHODS

Study Population

Recent HIV-1 seroconverters. All consecutive individuals with new HIV-1 infection seen during the period of January 1997 through December 2003 at 10 different hospitals distributed across Spain (A Coruña, Córdoba, Granada, Madrid [2 hospitals], Málaga, Oviedo, Santander, Valencia, and Valladolid) were examined. The eligibility criteria for a given subject to be enrolled in the study were laboratory evidence of acute primary HIV-1 infection (detectable plasma HIV-1 RNA level plus negative or indeterminate HIV-1 antibody test result) or seropositivity for HIV-1 infection (reactive ELISA and Western blot results) and negative results of a previous test performed within the prior 12 months.

Chronic HIV-1 carriers. During the same 7-year period, the proportion of patients with demonstrable HIV-1 infection that had lasted at least 3 years and with undetectable virus loads (<500 or <50 HIV-1 RNA copies/mL) was examined in a total of 8388 individuals. Considering that patients with chronic HIV-1 infection are regularly seen every 3 months in the outpatient clinic, only consecutive samples collected during the first trimester of each year were analyzed. The population included subjects who were drug naive and those who were receiving antiretroviral therapy. We assumed that most patients with undetectable virus loads were receiving successful antiretroviral therapy, reflecting indirectly the extent of therapy in this population.

Laboratory Tests

The measurement of plasma HIV-1 RNA level was performed using a branched DNA assay (Versant, versions 2.0 and 3.0; Bayer), in accordance with the manufacturer's instructions. The lower limits of detection of the assay were 500 HIV-1 RNA copies/

mL during 1997 and 1998 and 50 HIV-1 RNA copies/mL since 1999. The CD4$^+$ T lymphocyte count was determined by flow cytometry (Coulter) using fluorescein-labelled antibodies.

Genetic sequence analyses of both HIV-1 reverse-transcriptase (RT) and protease genes were performed for all plasma specimens obtained from recent HIV-1 seroconverters using an automatic sequencer (ABI Prism 3100; Celera Diagnostics). For the purpose of this study, only major or primary drug-resistance mutations listed in the latest guidelines from the International AIDS Society–USA panel were recorded (http://www.iasusa.org; updated November 2004). HIV-1 subtyping was performed using *pol* sequences by phylogenetic analyses, as previously described [17].

Statistical Analyses

Baseline characteristics of the study population were recorded as percentages or as mean values ± SDs. The proportion of patients with drug-resistance mutations and undetectable virus loads was also recorded as a percentage. Nonparametric tests were used to compare the proportion of patients with drug-resistance mutations at different time points. Spearman's test was used to analyse the correlation between the rate of drug-resistance mutations in recent HIV-1 seroconverters and the proportion of potential transmitters at different time points. All reported *P* values were 2-sided and were considered as significant if less than .05.

RESULTS

A total of 141 recent HIV-1 seroconverters were identified. The median estimated time from initial exposure to the first detection of HIV-1 infection was 7.6 months. Overall, 67.4% had been infected through homosexual sex. The prevalence of genotypes associated with drug resistance in this population was 14.2% (20 of 141 genotypes).

The distribution of resistance genotypes is summarized in table 1. Primary mutations at the RT gene were T215Y (4 subjects), M41L (4), M184V (2), Y181C (2), K103N (2), L210W (2), D67N (1), T69N (1), and K219Q (1). Moreover, 7 subjects presented with revertant forms at position 215 (C/D/L/N/S). At the protease gene, primary resistance mutations were L90M (3 subjects), M46L (2), and V82A (2). Five subjects had resistance mutations to >1 drug family. Two of these subjects presented with mutations associated with resistance to nucleoside RT inhibitors and nonnucleoside RT inhibitors, and another 3 presented with mutations associated with resistance to nucleoside RT inhibitors and protease inhibitors.

The overall proportions of primary drug-resistance mutations, by year of infection, were 33.3% for 1997, 29.4% for 1998, 20% for 1999, 14.3% for 2000, 3.4% for 2001, 15.4% for 2002, and 10.4% for 2003. When we used different periods of time, the proportions were 29% for 1997–1999, 5.6% for 2000–

Table 1. Characteristics of patients with recent HIV-1 seroconversion who harbor drug-resistant virus.

Patient	Year of seroconversion	Estimated duration of HIV infection, months	Risk group	Plasma HIV RNA level, copies/mL	CD4 cell count, cells/μL	Drug-resistance mutations	
						RT	Protease
1	1997	12	MSM	2880	472	T215L, K219Q	...
2	1997	11	MSM	1925	717	T215Y	...
3	1997	6	Heterosexual	500	Unknown	M41L	...
4	1998	12	Heterosexual	8876	545	M41L, L210W, T215D	...
5	1998	12	MSM	Unknown	Unknown	M41L, D67N, L210W, T215Y, Y181C	...
6	1998	12	Heterosexual	13,070	678	M184V	M46L, L90M
7	1998	7	MSM	39,420	689	T215Y	...
8	1998	12	IDU	9330	1302	M41L	...
9	1999	4	Heterosexual	20,401	840	T215N	...
10	2000	9	MSM	500,000	530	...	M46L
11	2001	11	MSM	Unknown	Unknown	M41L, T215	...
12	2002	12	MSM	187,459	736	M41L, T215C	...
13	2002	11	MSM	Unknown	Unknown	M41L, T215S	V82A, L90M
14	2002	6	MSM	2464	1023	Y181C	...
15	2002	3	MSM	500,000	420	T69N	...
16	2003	11	MSM	65,385	656	M41L, T215Y	V82A, L90M
17	2003	3	Heterosexual	500,000	Unknown	T215S	...
18	2003	12	Heterosexual	Unknown	Unknown	M184V, K103N	...
19	2003	9	MSM	123,761	805	M41L	...
20	2003	10	Heterosexual	75,000	257	K103N	...

NOTE. IDU, injection drug user; MSM, men who have sex with men; RT, reverse transcriptase.

2001, and 12.5% for 2002–2003. There was a significant decrease between the first and second periods ($P = .014$), and there was a rebound between the second and third periods, although this did not achieve statistical significance ($P = .211$) (figure 1). All but 2 patients were infected with HIV-1 subtype B viruses. One of the remaining 2 subjects presented with subtype C virus in 2001, and the other presented with a BG recombinant (CRF14_BG) virus in 2003.

At total of 8388 patients with chronic HIV-1 infection seen during 1997–2003 were examined. Only 1 plasma virus load determination from each patient per year was evaluated. With use of a detection limit of 500 HIV-1 RNA copies/mL, undetectable virus loads were found for 44.6% of patients in 1997 and 48.6% in 1998; with use of a lower detection limit of 50 copies/mL, undetectable virus loads were noted for 37.5% of patients in 1999, 47.5% in 2000, 52.9% in 2001, 39.7% in 2002, and 58.1% in 2003.

To make the threshold for viremia uniform, we estimated the virus loads for samples collected in 1997 and 1998 that harbored <50 copies/mL instead of <500 copies/mL. In prior studies [18–20], ~75% of individuals with virus loads of <500 copies/mL in fact had virus loads of <50 copies/mL. With this assumption, the proportions of patients with virus loads of <50 copies/mL in 1997 and 1998 were indirectly estimated to be 33.4% and 34.6%, respectively.

A significant inverse correlation between yearly rates of transmission of drug-resistant viruses and the proportion of patients with chronic HIV-1 carriage and undetectable virus loads was found (r = −0.955, by Spearman's test; $P = .001$). Figure 2 shows the relationship between the proportion of individuals with new HIV-1 infection who had drug-resistance mutations and the effect of antiretroviral therapy on plasma virus load in chronic carriers throughout the study period.

DISCUSSION

The widespread use of HAART has lead to a dramatic reduction in the morbidity and mortality of HIV-1–infected individuals [14]. A reduction in HIV-1 load—often to undetectable levels—is usually seen in patients who are receiving HAART. Given that virus load titers correlate with the transmission risk for HIV-1, either from mother to child [21, 22] or to sex partners [16], it should not be surprising to find lower infectiousness for patients who are receiving HAART [23]. However, virological failure may appear after exposure to suboptimal therapy or as consequence of poor compliance with treatment, allowing drug-resistant viruses to emerge [1]. If individuals who harbor drug-resistant viruses continue to engage in high-risk practices, transmission of drug-resistant strains may occur, as was noticed soon after antiretroviral therapy was first introduced [24].
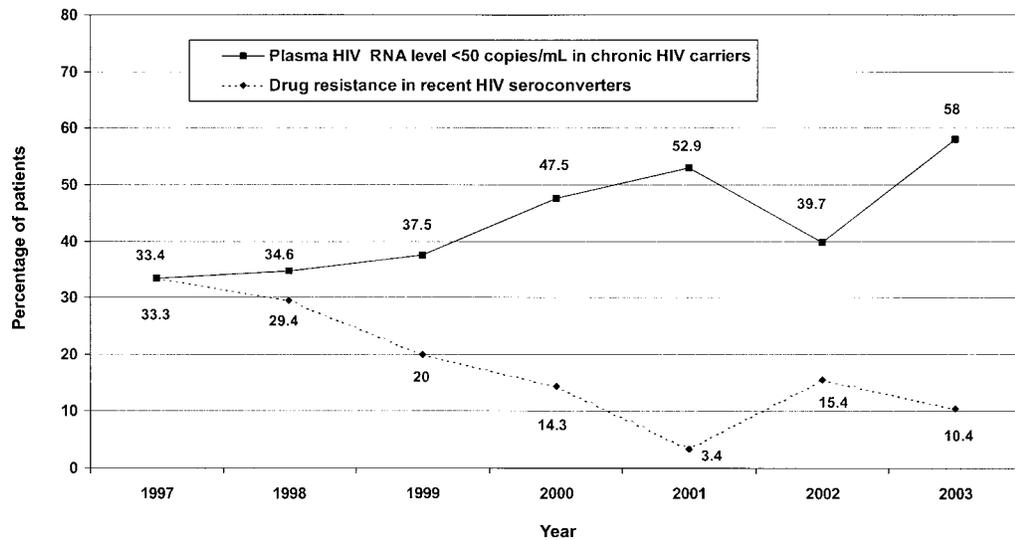
Figure 1. Rate of drug-resistance mutations in patients who had recent HIV-1 seroconversion and the proportion of patients with chronic HIV carriage who had undetectable virus loads, 1997–2003. For 1997 and 1998, the percentage of subjects with an HIV RNA level of <50 copies/mL was estimated.

In our study, >14% of recent HIV-1 seroconverters harbored drug-resistant viruses. Fluctuations over time were noticed, with the highest rate of transmission of drug-resistant strains occurring between 1997 and 1999, followed by a reduction in 2000–2001 and a rebound in 2002–2003. These results are in agreement with trends reported in other western European countries [25–27]. Recent decreases in the rate of transmission of drug-resistant HIV-1 have correlated with increases in the transmission of non-B subtypes in France, Switzerland, and Italy [28–30]. However, this was not observed in our study (we found only 2 subjects who had recently been infected with non-B strains) and has not been documented in other studies from Spain [31] in which subtype B continues to be, by far, the most predominant transmitted HIV-1 variant.

Differences in the proportion of patients with chronic HIV-1 infection for whom HAART failed could explain our findings for recent HIV-1 seroconverters. We analyzed the proportion of patients with chronic HIV-1 carriage who had undetectable virus loads, which mostly reflects the success of HAART in the overall population. By contrary, those with detectable virus loads are the potential transmitters. This group includes either patients who are antiretroviral naive or those for whom therapy is not successful. The latter is a larger population and is more likely to carry drug-resistant strains.

The proportion of HIV-1–infected individuals with undetectable virus loads steadily increased from 1997 to 2001, reflecting more-widespread use of HAART and application of relatively more-aggressive treatment recommendations [32, 33].

The rate of drug resistance among recent HIV-1 seroconverters steadily decreased during that period, suggesting that a growing proportion of new HIV-1 infections involved treatment-naive subjects. In 2002, concerns of drug toxicity led to widespread use of drug holidays, structured treatment interruptions, and more-conservative treatment rules [14, 15, 34]. Together, this has resulted in an increased proportion of patients with chronic HIV-1 infection who have detectable virus loads. Individuals with prior antiretroviral exposure could then become potential transmitters of drug-resistant viruses [35, 36]. In accordance with this hypothesis, we found a rebound in the proportion of drug-resistant strains among recent HIV-1 seroconverters in the year 2002.

In 2003, worries about the use of structured treatment interruptions and/or drug holidays [37], as well as the availability and extensive use of new potent drugs (lopinavir/ritonavir and tenofovir) [38, 39], seemed to revert the situation. Currently more patients with undetectable viremia are seen again among chronic HIV-1 carriers, and lower drug resistance rates are found among HIV-1 seroconverters.

Our study has several limitations. First, the number of HIV-1 seroconverters varied significantly from year to year. This is mainly because of the introduction of new assays for the diagnosis of HIV-1 infection in recent years [40] that have allowed the simultaneous detection of HIV-1 antigen and antibodies, and because of a more active search for HIV-1 seroconverters; in conjunction, these factors resulted in a higher rate of identification of primary HIV-1 infections during recent years. Sec-

ond, the proportion of patients with undetectable virus loads was used as a surrogate marker for the effectiveness of HAART. However, some long-term nonprogressors may also have undetectable virus loads without having received any antiretroviral therapy [41]. In our population of chronic carriers, we identified only 20 individuals who might be considered to belong to this category; therefore, their impact was negligible. Third, the population of potential transmitters we examined was quite heterogeneous. On one hand, it included patients with no prior exposure to therapy, but it also included subjects for whom antiretroviral therapy failed. Only the latter group might harbor drug-resistant strains more frequently. On the other hand, different levels of detectable virus may account for differences of transmission efficiency [42, 43], and this aspect was not assessed in our study. With all of these limitations in mind, however, we feel that our data support the hypothesis that the appropriate use of HAART is associated with a lower rate of transmission of drug-resistant viruses. In this context, although new HIV-1 infections may continue to occur, they are likely to derive from subjects who are antiretroviral naive, who rarely transmit drug-resistant strains.

In summary, higher proportions of virological suppression in chronic HIV-1 carriers associated with the use of HAART correlate with lower rates of transmission of drug-resistant viruses among individuals with new HIV-1 infection in a given community. Antiretroviral therapy has benefits for people other than those who are receiving treatment, as recently highlighted by others [44], and this should be emphasized, particularly in the light of recent increased sexual risk behaviors among high-risk groups [45–47] that result from a misperception that HIV-1 infection is no longer a deadly illness.

Acknowledgments

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