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Prevalence of Primary Resistance to Zidovudine and Lamivudine in Drug-Naive Human Immunodeficiency Virus Type-1 Infected Patients: High Proportion of Reverse Transcriptase Codon 215 Mutant in Circulating Lymphocytes and Free Virus

Mary-Anne Trabaud,[1]* Karine Leriche-Guerin,[2] Corinne Regis,[1] Isabelle Bordes,[2] Laurent Cotte,[3] Jill Detmer,[4] Janice Kolberg,[4] Jacques Ritter,[1] and Christian Trépo[2,3]

[1]Laboratoire d'Hygiène et d'immunologie, Faculté de Médecine, Lyon, France
[2]Inserm U271, Lyon, France
[3]Departement d'hepatogastroenterologie, Hopital Hotel Dieu, Lyon, France
[4]Bayer Diagnostics, Emeryville, California

The presence of primary zidovudine (AZT)-resistance (mutation T215Y/F) or lamivudine (3TC)-resistance (mutation M184V) was evaluated in 90 drug-naive patients infected with human immunodeficiency virus type-1 (HIV-1) between 1987 and 1997. The proportion of mutant strains in proviral samples or plasma viral samples was determined using a differential hybridization assay. Mutation T215Y/F was found in five (5.6%) patients infected between 1994 and 1997, whereas none of these patients harbored the mutation M184V. The T215Y/F mutation was present in the virus and/or provirus and persisted for at least two years. In one patient, the mutant provirus was associated with only wild-type free virus. Four of these patients were followed, and two were treated subsequently to a regimen containing AZT but with low response. The persistence of primary resistance mutations might depend on the proportion of these mutations at the time of infection, although mutant provirus might not give rise to replicating variants. ***J. Med. Virol. 61:352–359, 2000.***

KEY WORDS: differential hybridization; proviral DNA; transmission; drug-resistance mutations; T215Y/F; M184V

INTRODUCTION

The transmission of zidovudine (AZT)-resistant HIV-1 has been reported in about 5 to 10% of newly-infected individuals [Sönnerburg et al., 1993; de Ronde et al., 1996; García-Lerma et al., 1996; Imrie et al., 1996; Yerly et al., 1996; Quigg et al., 1997]. These transmissions often occurred during the time of suboptimal treatments with AZT monotherapy or with 2-drug combinations. With the availability of more antivirals and different combination regimens for the treatment of HIV-1-infected individuals, better suppression of viral replication is now possible. This has raised new questions concerning the transmission of drug-resistant strains, such as whether there will be an increase in the prevalence rate and number of drugs to which resistant viruses may emerge in the newly-infected population, and whether drug-resistance screening would be useful for guiding the choice of initial therapy.

Although reports on patients infected with AZT-resistant viruses do not suggest a higher virulence of these variants as compared to wild-type [Sönnerburg et al., 1993; Imrie et al., 1996; Vanhems et al., 1997], the impact of these resistant viruses on the evolution of infection and response to therapy in the current therapeutic context is still unclear. Strains with AZT-resistance mutations have been found in some therapy-naive patients up to four years after infection [Williams et al., 1998], whereas reversion to wild-type has been observed in others one year after seroconversion [Imrie et al., 1996]. Some reports have described individuals infected with AZT-resistant strains who do not respond to subsequent AZT treatment [Erice et al., 1993; Fitzgibbon et al., 1993; Veenstra et al., 1995], whereas other studies have found no difference in response to AZT between patients with or without AZT-resistance

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*Correspondence to: Mary-Anne Trabaud, Laboratoire d'Hygiène et d'immunologie, Faculté de Médecine, 8 Avenue Rockefeller, 69008 Lyon, France. E-mail: trabaud@rockefeller.univ-lyon1.fr

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TABLE I. Characteristics of Patients With and Without Baseline Resistance Mutations

	Wild type codon 215 (n = 85)	Mutant codon 215 (n = 5)
Time between diagnosis and analysis (months)[a]		
Median	4	1
Range	0–144	0–18
HIV-1 RNA ($\times 10^3$ copies/ml)		
Median	25.8	64
Range	<0.2–22970	26.5–636
% assayed by bDNA	59	40
CD4 count (cells/μl)		
Median	462	391
Range	12–1521	101–421
CDC stage at time of analysis (number of patients)		
A	62	5
B	16	0
C	5	0
Transmission route (number of patients)		
Sexual contact	80	5
Drug use	3	0
Blood exposure	2	0
Primary infection in the 6 months prior analysis (number of patients)	10	0
Treatment after analysis (number of patients)		
Lost to follow-up	5	1
No treatment	17	2
Dual therapy	9	0
Dual therapy switched to triple therapy after 1–2 months	15	1
Triple therapy	39	1

[a]Delay between the first seropositive test and the samples analyzed in this study.

mutations before treatment [Rubio et al., 1998]. These discrepancies may be explained in part by the fact that different techniques with variable sensitivity and capacity were used to estimate the proportion of mutant strain with regards to wild-type. Although most studies analyzed proviral DNA, some examined viral RNA. The outcome of subsequent drug treatment may be different depending on whether the virus is replicating or is only integrated in infected cells in a defective or latent form, and whether the viral population contains only mutant strains or a mixture of both mutant and wild-type.

This study evaluated the prevalence of AZT and lamivudine (3TC)-resistant strains in HIV-1 infected patients in our French region. Untreated patients were screened for the presence of strains carrying the AZT-resistance mutation at codon 215 and the 3TC-resistance mutation at codon 184 of the reverse transcriptase (RT) gene of HIV-1. Genotypic analysis of HIV-1 was performed using a non isotopic differential hybridization assay [Eastman et al., 1995], that is able to measure the proportion of mutant strains in a viral population. The proportion of mutant strains in circulating viral RNA and cellular proviral DNA were compared and followed over time in two patients.

MATERIALS AND METHODS

Patients

Ninety patients were included in this study. Thirty four patients were selected on the basis of a documented infection, the dates of which ranged from June 1987 to November 1996. The detection of the codon 215 mutation was performed on the plasma samples obtained for the first viral load testing in 1995–96, the delay between seroconversion and analysis varying from between 0 to 102 months, with a median of 14 months. Fifty-six other patients attending our medical center for the first time in 1997 were included. In this latter group the likely period of infection was unknown for some patients, the proof of infection being established by serology testing in our unit. In 22 (39%), however, a positive serology was obtained 4 to 144 months before testing in our unit. Analysis from proviral DNA and circulating viral RNA of these patients was performed within 3 months of the first positive serology for 35 of 56 (63%) cases. The median time between positive serology and analysis was 1 month.

Clinical information for patients with (85) and without (5) baseline resistance mutations is summarized in Table I. At the time of analysis most patients were classified in the 1993 CDC clinical category A (76%) and some were classified in categories B (18%) and C (6%). The route of transmission was through sexual contact for 85 (94%), drug use for 3 (3.3%), and transfusion or accidental blood exposure for 2 (2.2%) of the patients. Clinical signs of primary infection were apparent six months before the initial analysis in 10 (11%) of patients. All 90 patients were drug-naive at the time of the first sample analysis. Subsequently, 65 (70%) of the patients were treated with different regimens. Of the 65 treated patients, 25 (38%) received dual therapy and 40 (62%) were treated with a combi-

nation of two RT inhibitors and one protease inhibitor. Of the 25 patients receiving dual therapy, 16 switched to triple therapy 1 month to 2 years later.

Sample Preparation.

Plasma was separated by centrifugation of EDTA-treated whole blood, and stored at −70°C. Peripheral blood mononuclear cells (PBMCs) were isolated from EDTA-treated whole blood by Ficoll-gradient centrifugation, washed and stored as pellets at −70°C.

Viral Load Determination

Viral load measurements were obtained during routine follow-up of patients. The first tests were carried out early when commercial assays for viral load measurement were first being introduced into the management of HIV-1 infection. HIV-1 RNA titers in samples obtained from patients before treatment were measured using two different commercial assays—the branched DNA (bDNA) assay (Quantiplex HIV-1 RNA version 1.0 before 1997, then version 2.0, Bayer Diagnostics, Eragny, France) and the Amplicor HIV-1 RNA Monitor 1.0 assay (Roche Diagnostics, Neuilly sur Seine, France). The same assay that had been used to measure initial viral load was used to measure HIV-1 RNA titers in samples obtained from patients during treatment. These tests were undertaken according to manufacturers' instructions.

Genotypic Resistance Analysis

Genotypic resistance to AZT or 3TC, either present in infected patients before treatment or occurring during therapy, was evaluated by assessing the presence of mutations at codons 215 or 184 in the proviral DNA from PBMCs or in the viral RNA associated with circulating viral particles. Two assays, selective PCR and differential hybridization, were used for detection of the T215Y/F mutation. Only differential hybridization was used for detection of the M184V mutation.

Selective PCR assay for detection of codon 215 mutations. Details about the selective PCR assay have already been described [Larder et al., 1991; Leriche-Guérin et al., 1997]. Briefly, this technique is based on a nested PCR, in which the second round is carried out in parallel with 2 sets of primers, each containing a common sense primer and a different antisense primer specific either for the wild-type codon or for the mutant codon. The specific band is determined by agarose gel electrophoresis.

Differential hybridization assay for quantification of mutant T215Y/F or M184V strains. The differential hybridization assay developed by Eastman et al. [1995] is a non-isotopic assay for determining the relative amounts of mutant and wild-type RNA and DNA associated with AZT resistance. After a first round of PCR to cover the region where mutations arise, selective hybridization with specific probes is used to evaluate the proportion of mutant strains in a viral population. We performed this assay using proviral DNA or viral RNA.

For PCR of proviral DNA, 1 μg of DNA from lysed PBMCs [Leriche-Guérin et al., 1997] was added to a mix containing 30 pmol biotinylated 3RT antisense and 5RT sense primers [Eastman et al., 1995], 1.25 U of Taq polymerase (Promega, Lyon, France), 2.5 mM $MgCl_2$, 0.2 mM dNTP, in 10 mM Tris-HCl, 50 mM KCl, pH 8.3. For viral RNA, 1 ml of plasma was centrifuged for 1 hr at 4°C and 23,500 × *g*. Part of the supernatant was aspirated, leaving approximately 200 μl. RNA was extracted from the remaining supernatant by a phenol-chloroform technique (TriPure, Boehringer Mannheim, Meylan, France), and precipitated with ethanol. The pellet was resuspended in 40 μl of water containing an RNase inhibitor (RNAsin, Promega). RT-PCR was performed in one step from 20 μl of RNA (equivalent to 0.5 ml of plasma). The PCR mix described above, with 10 U per sample of MMLV reverse transcriptase (Gibco BRL, Gergy Pontoise, France), was added. Amplification consisted of a reverse transcription step when RNA was used for 30 min at 42°C, followed directly by PCR. The PCR protocol used for both types of samples was a denaturation step of 5 min at 95°C followed by 40 cycles of 40 sec at 94°C, 30 sec at 55°C, 1 min at 72°C, and 10 min at 72°C.

Hybridization was carried out as described by Eastman et al. [1995]. Five μl of each biotinylated PCR product diluted in binding buffer was dispensed into duplicate streptavidin-coated wells and incubated for 30 min at 50°C. The bound PCR product was denatured with NaOH, followed by washing. The single stranded DNA was hybridized in parallel with either the 184 wild-type (5′-YCARTACATGGATGAYT-3′; nucleotides 2645–2662) and 184V mutant (5′-YCARTACGTRGATGAYT-3′; nucleotides 2645–2662) sequence-specific alkaline phosphatase-labeled probes or the 215 wild-type (5′-TGGGGRYTTACCACRCCAG-3′; nucleotides 2736–2755) and T215Y/F mutant (5′-TGGGGRYTTTWCACRCCAG-3′; nucleotides 2736–2755) sequence-specific alkaline phosphatase-labeled probes in hybridization solution for 30 min at a specified temperature (45–50°C, depending on the probe set). The wells were washed once with 1× SSC (1× SSC is 0.15 M NaCl + 0.015 M sodium citrate), 0.1% Triton X-100 at a probe specific temperature, and then three times with 1× SSC at room temperature. The hybrid was detected with a chemiluminescent substrate (Lumi-Phos 530, Lumigen, Detroit, MI) at 37°C for 15 min. Hybridization was measured on a luminometer (Bayer Diagnostics, Emeryville, CA). To account for the amount of PCR product, the wells were treated with NaOH to denature the probe hybridization and rehybridized with a probe designed to a highly conserved region (generic probe) of the HIV-1 reverse transcriptase. The data was analyzed as described previously [Eastman et al., 1995]. The data is presented as the percentage of the specific mutant sequences in a total population of wild-type and specific mutant sequences, that is calculated by dividing the normalized value of the mutant-specific probe by the sum of the normalized values of both the mutant and wild-type probes.

TABLE II. Analysis of Patients Harboring the T215Y/F Mutation Prior to Treatment*

Patient	Probable period of infection[a]	Time between diagnosis and analysis (months)[b]	HIV-1 RNA ($\times 10^3$ copies/ml)	CD4 (cells/μl)	T215Y/F			
					Selective PCR		Differential hybridization[d]	
					Proviral DNA	Free virus	Proviral DNA	Free virus
A18	07/94–08/94	18	26.5	391		M		100%
A20	09/94–02/95	13	59	421		ND		100%
A33	11/96	0	636	101		M		93%
B21	07/96–05/97	1	64[c]	413	M	WT	95%	WT
B23	1996–06/97	0	65[c]	337	M	M	100%	100%

*M, mutant; ND, not done; WT, wild-type.
[a]Indicates the date of probable infection or last seronegative sample and the date of the first seropositive sample.
[b]Delay between the first seropositive test and the samples analyzed in this study.
[c]These samples were tested with the bDNA assay. Other samples were tested with the Amplicor Monitor assay.
[d]Percentage indicated are % of mutant virus.

Statistics

Results were analyzed with the non-parametric Mann–Whitney test. The detection limit of the hybridization assay was evaluated with the univariate *t*-test.

RESULTS

Evaluation of the Differential Hybridization Assay

A differential hybridization was used to determine the proportion of mutant and wild-type RNA and DNA associated with AZT resistance in plasma or PBMCs from treatment-naive patients, and in patients undergoing therapy. Interpretable results were obtained with most of the samples tested. Detectable signals were obtained from all the proviral DNA analyzed. Plasma viral RNA samples with undetectable viremia levels were not tested. The genotype at codon 215 was determined by differential hybridization in 62 (91%) of the 68 plasma samples with measurable HIV-1 RNA levels ranging from 0.4 to 23,000 $\times 10^3$ copies/ml. Specifically, the genotype at codon 215 was determined for 100% (52 of 52) of samples with HIV-1 RNA titers greater than 10,000 copies/ml, 75% (9 of 12) of samples with 1,000 to 10,000 copies/ml and 25% (1 of 4) of samples with HIV-1 RNA titers below 1,000 copies/ml. The 6 (9%) of the 68 plasma samples in which the genotype could not be determined had HIV-1 RNA titers ranging from 0.4 to 6.1 $\times 10^3$ copies/ml. In addition, there was a perfect qualitative concordance between the results of the differential hybridization assay and those of the selective PCR assay, regardless of whether plasma viral RNA or proviral DNA was analyzed.

The reproducibility of the differential hybridization assay was evaluated from a single sample. RNA from this sample was extracted in triplicate, and the 3 extracts were amplified 3 times each to yield a total of 9 PCR products. These 9 PCR products were hybridized in triplicate in one experiment. From the resulting 27 values, a mean percent of mutant strains of 30 ± 5 was calculated (coefficient of variation = 17%). The coefficients of variation for the 9 triplicate values obtained in one hybridization reaction ranged from 2 to 10%. The PCR products were rehybridized in 2 separate experiments. The mean percent of mutant strains was calculated for each of the 3 hybridization reactions performed, and the coefficient of variation for the 3 mean values was 14%.

The detection limit of the differential hybridization was assessed by measuring the percentage of mutant from mixtures of wild-type and mutant reference isolates. RNA from culture supernatant of these isolates (obtained from the NIH AIDS Research and Reference Program, catalog number 629) was extracted and quantified. HIV-1 RNA titers were adjusted to the same level for the 2 preparations and then mixed in different proportions. Results of the differential hybridization assay indicated that mixtures (with total HIV-1 RNA levels of 500 to 50,000 copies/ml) containing proportions of mutant strains greater than 5% could be accurately measured because these mixtures gave data statistically different than zero (data not shown).

Prevalence of T215Y/F or M184V Mutant Strains

Of the 90 patients included in this study five (5.6%) were found to be infected with HIV-1 strains carrying the mutation at codon 215, both by selective PCR and differential hybridization (Table II). In Patient A33 seropositivity was detected in 1996, that was concomitant to the mutational analysis. In Patients A18 and A20 seropositivity was detected in 1994 and 1995, respectively, but infection probably occurred in both patients in 1994, that was more than 18 months before the mutational analysis. Seropositivity and the presence of resistant strains were identified in Patients B21 and B23 in 1997. One of these patients (B23) probably was infected in 1996, whereas the other (B21) likely was infected between July 1996 and May 1997.

The relative amounts of mutant and wild-type nucleic acid in the proviral or free viral compartments also was evaluated (Table II). In all 5 patients in which the T215Y/F mutation was detected, the proportion of mutant to wild-type was over 90%, even in the 2 patients infected 2 years before analysis. Patient B23 had the same proportion of mutant virus in both the cellular proviral and free viral compartments, whereas Patient B21 had proviral DNA that almost entirely con-

sisted of the mutant variant, whereas circulating viruses were wild-type. The seroconversion of this patient occurred less than 11 months before the mutational analysis was done.

The presence of the M184V mutation in proviral DNA was tested in 52 patients who had been diagnosed with HIV infection in 1997. All 52 patients were found to be infected with wild-type variants, including 2 patients who harbored a T215Y/F mutation. The other 38 patients were not screened for M184V because these patients were infected before the use of lamivudine therapy and, given the long delay between analysis and diagnosis, the presence of such a variant was considered unlikely in these cases.

The characteristics of patients with and without baseline resistance mutations were compared (Table I). There was no statistically significant difference between patients with wild-type strains as compared to patients infected with T215Y/F mutant virus in the delay between seropositivity and mutational analysis. Also, there was no statistically significant difference in HIV-1 RNA titers when all assay results were considered, even though the median HIV-1 RNA titer of patients with mutant viruses was higher than that of patients with wild-type viruses. Unfortunately, the same assay was not used to measure viral loads for each individual. There also was no statistically significant difference, however, in HIV-1 RNA titer when only results from the same HIV-1 RNA assay were taken into account. Similarly, there was no statistically significant difference in CD4 cell number between individuals with or without mutant strains, although the median CD4 cell count in the five patients with T215Y/F mutant virus was lower. In fact, the values for these markers varied largely in the population with wild-type viruses.

Follow-Up Results of Patients With T215Y/F Mutant Strains

All 5 patients with T215Y/F mutant virus had been infected through homosexual or bisexual intercourse and were asymptomatic at the time of analysis. These 5 patients were monitored for viral load and the proportion of circulating mutant strains from routine plasma samples. One patient (A18) was lost to follow-up. Two other patients (B21 and B23) were not treated, and viral load levels and CD4 cell number remained relatively stable in these 2 patients during the following year. Recall that Patient B21 had over 95% T215Y/F mutant virus in T-cells, whereas circulating viruses were wild-type. In Patient B23, circulating T215Y/F mutant virus still represented about 90% of the viral population 11 months after the first test.

Therapy was initiated in the other two patients (A20 and A33) before the genotype resistance results were known. As shown in Figure 1, patient A20 first began with a dual therapy—AZT plus didanosine (ddI). Viral load decreased by about 1 log but rapidly rebounded. A triple therapy was then initiated with the replacement of AZT by stavudine (D4T), and the addition of a protease inhibitor. With this regimen, viral load decreased to <500 copies/ml and remained so during the last 12 months. During the period in which plasma HIV-1 RNA was detectable, the proportion of T215Y/F mutant in patient A20 remained above 95%. As shown in Figure 1, different combinations containing AZT, lamivudine (3TC) and a protease inhibitor were used to treat patient A33. Viral load decreased in patient A33 without becoming undetectable, and then rose despite the addition of a second protease inhibitor. With the replacement of AZT by D4T the viral load decreased again but still temporarily. During this period the viral population was essentially comprised of 100% T215Y/F mutant strains. In addition, the M184V mutation developed after 3 months of 3TC treatment, achieving a proportion of 100% after 6 months.

DISCUSSION

The presence of variants with AZT or 3TC-resistance mutations in drug-naive individuals was evaluated in patients from our clinical center. The differential hybridization assay used allowed us to quantify the level of mutant and wild type viruses in the 5 patients with AZT-resistant strains, within the cellular proviral or free viral compartments. The differential hybridization assay yielded results that were highly concordant with those of the selective PCR, and was able to reproducibly distinguish mutant and wild-type virus for samples with viral loads greater than 10^3 copies/ml.

The prevalence of infection with T215Y/F mutant of our cohort of patient was 5.6%, that is similar to what has been found elsewhere. Previously reported prevalence rates vary from 1.7 to 22% [Sönnerburg et al., 1993; Mayers et al., 1995; de Ronde et al., 1996; García-Lerma et al., 1996; Imrie et al., 1996; Yerly et al., 1996; Quigg et al., 1997; Rubio et al., 1997; Kozal et al., 1998; Tamalet et al., 1998; Williams et al., 1998], but are more often around 5 to 10%. Patients from these earlier studies generally had been infected during the period 1992–1996. The 5 patients in our study that harbored T215Y/F variants were infected between 1994 and the first semester of 1997.

In contrast, we did not find any patient harboring virus with M184V mutations. In most of these cases, however, the sample tested was taken several months to several years after infection. The presence of M184V mutation has been described in a few drug-naive patients tested at the time of primary infection [Conway et al., 1997; Hecht et al., 1998; Tamalet et al., 1998]. It cannot be excluded that such variant could rapidly be replaced by wild-type viruses in the absence of a drug selective pressure. Consistent with this hypothesis is our observation (not shown) of 3TC-treated patients who, after stopping therapy for as little as two months, harbored only wild-type viruses at codon 184, whereas nearly 100% of their viral population contained the M184V mutation in both previous "on therapy" samples and samples obtained 2 months after resuming 3TC therapy. If this phenomenon is general it would mean that transmission of such a variant can

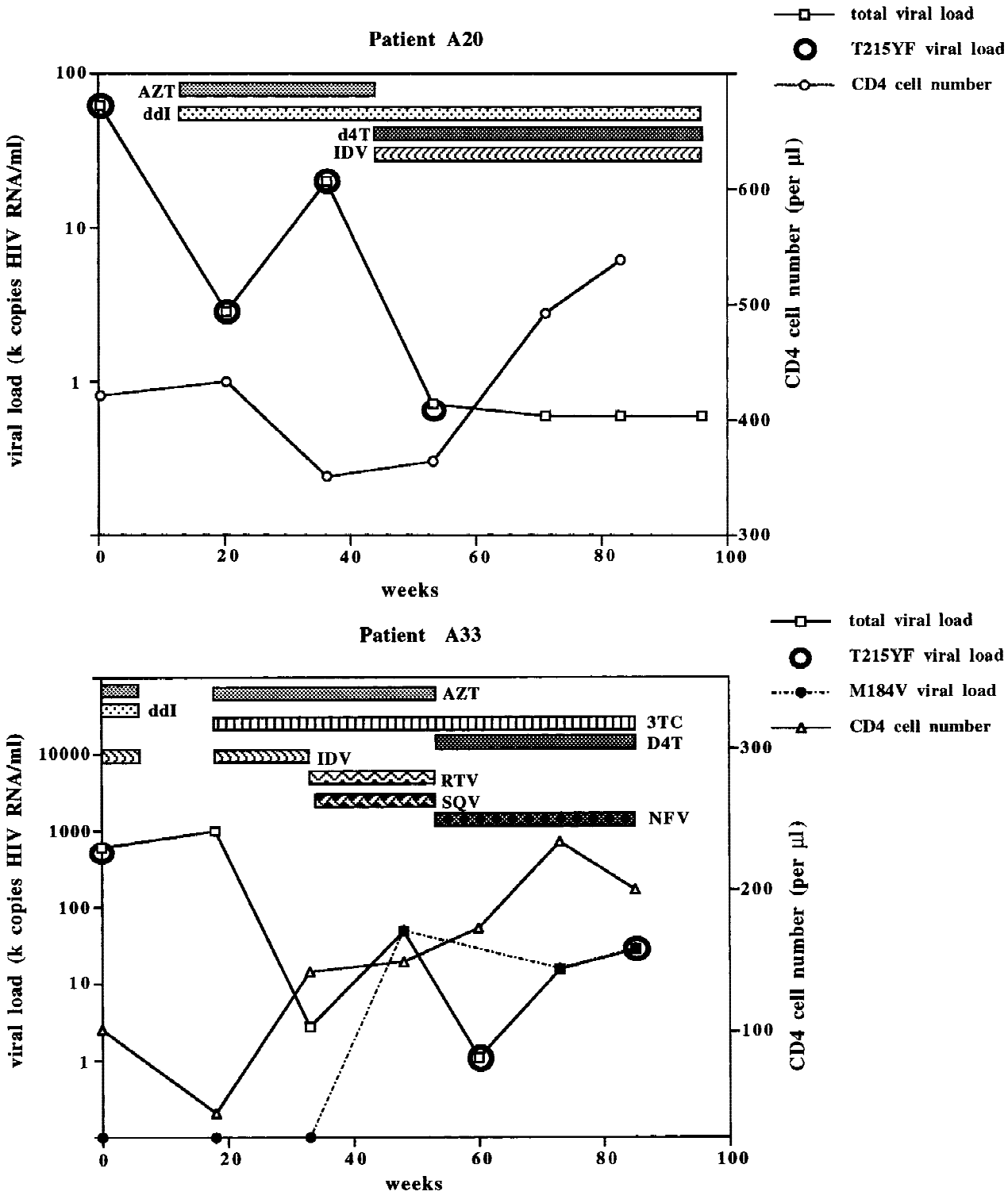

Fig. 1. Evolution of viral load and CD4 cell number in two patients (A20 and A33) infected with T215Y/F variants and subsequently treated. Follow-up began with the initial detection of T215Y/F mutants. T215Y/F and M184V viral loads were calculated from the plasma HIV-1 RNA (total viral load), that was measured by a commercial assay, and the percentage of mutant strains in the plasma HIV-1 RNA at the same time, determined with the differential hybridization assay. Drugs given to the patients and the duration of each drug are shown. AZT, zidovudine; ddI, didanosine; D4T, stavudine; 3TC, lamivudine; IDV, indinavir; RTV, ritonavir; SQV, saquinavir.

only be detected at time of seroconversion and that no information would be obtained by screening for M184V mutations during the chronic phase before therapy.

T215Y/F mutants were still found up to two years after infection in our patients. The persistence of AZT-resistant variants in drug-naive individuals has already been described [Quigg et al., 1997; Williams et al., 1998] but a decrease and reversion to wild-type also has been observed within 6–20 months after infection [Fitzgibbon et al., 1993; de Ronde et al., 1996; Imrie et al., 1996]. What is noticeable here is the persistence of a non-decreasing high proportion of drug-resistant mutant in all the patients to whom such variants have been transmitted, in the proviral and viral compartments. Although the genotype could not be determined from proviral DNA in three patients harboring T215Y/F mutants in their free virus, it is likely that mutant strains would have been found in the lymphocytes of these patients. It can be asked why the proportion of mutant virus was either 0% or close to 100% in all the patients. One hypothesis is that if a mixture of wild-type and mutant virus coexist in the inoculum, infection may be established preferentially with wild-type virus; however, donor-recipient couples in which each individual has a different mixture of wild-type and mutant virus have been described [Angarano et al., 1994; Ippolito et al., 1994]. It is possible that mutants are rapidly eliminated when mixtures are transmitted, because in the studies cited above samples from the recipients were taken soon after primary infection. The delay between infection and analysis in our study may have resulted in our missing the transmission of mixtures. When nearly 100% of mutant strains are transmitted they might disappear much more slowly.

The persistence of the T215Y/F mutation in newly-produced virus particles for several months in the absence of therapy suggests that the replicative capacity of the mutant strain is not impaired as compared to the wild-type virus. In one patient, however, even though the T215Y/F mutant comprised up to 95% of integrated proviral sequences in the circulating lymphocytes, this mutant genome was apparently unable to replicate because no free virus carrying this mutation was detected. Because this patient had not been infected for a longer period of time than the other patients, this phenomenon cannot be explained by a progressive selection against the mutants in the absence of drug pressure. Rather, this observation is consistent with the establishment of a non-replicative infection with these variants early after infection. These findings suggest that the evolution of strains with AZT-resistance mutation may be variable, depending on the proportion of mutant virus to wild-type virus, and the infectious and replicative capacities of the mutant.

It is difficult to attribute any clinical or biological feature to the primary resistance due to the small number of patients involved and the large heterogeneity of the patients infected with wild-type viruses, who represent the majority of our cohort population. We did not find a significant difference in the biological parameters between patients with mutant and wild-type virus. The two non-treated patients with the T215Y/F mutation did not show signs of progression during follow-up, their CD4 cell numbers and viral loads remained relatively stable.

An analysis of the therapeutic response in the study described above is difficult because it concerns only two patients, with certainly distinct initial prognosis and different therapies. These two patients, however, did not respond well to a regimen containing AZT. In patient A20, the initial dual therapy was probably in reality a ddI monotherapy, that would explain the very limited efficiency. The clinical status of patient A33 initially was worse, probably independent of the primary AZT-resistance mutation. It is not known whether an initial triple therapy with D4T instead of AZT would have reduced viral replication in this patient more extensively, given this patient's intolerance to most protease inhibitors. The probability of a prolonged viral suppression has certainly been lowered by the use of a regimen containing AZT before switching to D4T. Nevertheless, it is evident that optimal viral suppression can be obtained in patients with primary AZT resistance with appropriate drug regimen as shown with patient A20 who maintained an undetectable HIV-1 RNA titer during at least one year of efficient therapy without AZT.

In conclusion, the results suggest that the transmission rates of AZT-resistant strains and the persistence of these strains at a detectable level could depend on the proportion of mutant virus in the initial infection. This and other studies, however, leave a number of unanswered questions. For example, does transmission of a mixture of wild-type and mutant virus result in the selection of wild-type over mutant with a rate depending on the proportion of mutant virus, such that low amounts of mutant virus might be transmitted but not detected? Also, is there a difference in the therapeutic response between patients with mixtures of wild-type and mutant virus as compared to patients with only wild-type or only mutant virus? The answers to these questions could be useful for evaluating the prognostic value of screening for drug-resistance before starting treatment. Of course, screening for drug resistance should be extended to other drugs and should take into account the current means of treatment. It might be difficult to answer these questions, given the low number of cases and the higher number of drugs now available and the various combinations in which these drugs are used. Future investigations, however, would need to be able to quantify the proportion of mutant variants in the viral population. In this respect, the differential hybridization assay may be very useful in furthering our understanding of the transmission of mutant viruses in HIV-1 infection.

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