Trend of Drug-Resistant HIV Type 1 Emergence among Therapy-Naive Patients in Nagoya, Japan: An 8-Year Surveillance from 1999 to 2006

SHIRO IBE,1 JUNKO HATTORI,1 SEIICHIRO FUJISAKI,1 URARA SHIGEMI,1 SAEKO FUJISAKI,1 KAYOKO SHIMIZU,1 KAZUYO NAKAMURA,1 TAKEJIRO KAZUMI,2 YOSHIYUKI YOKOMAKU,1 NAOTO MAMIYA,1 MOTOHIRO HAMAGUCHI,1 and TSUGUHIRO KANEDA 1

ABSTRACT

We studied the emergence of drug-resistant human immunodeficiency virus type 1 (HIV-1) with major amino acid mutations in 402 therapy-naive patients at Nagoya Medical Center, Japan, between 1999 and 2006. The mean prevalence of drug-resistant HIV-1 was 6.7% (range, 2.3–10.0%; n = 27). HIV-1 variants with protease inhibitor (PI)-resistant mutations alone were most frequently found (3.5%, n = 14), followed by those with nonnucleoside reverse transcriptase inhibitor (NNRTI)-resistant mutations alone (1.7%, n = 7). Variants with nucleoside reverse transcriptase inhibitor (NRTI)-resistant mutations alone were sporadically found (1.0%, n = 4). A variant possessing both NRTI- and PI-resistant mutations was detected in one patient (0.2%) and a variant possessing both NNRTI- and PI-resistant mutations was identified in another patient (0.2%). In addition, another 17 variants (4.2%, n = 17) with only 215-revertant mutations (T215C/D/G/L/S) that can easily reconvert to the nucleoside analogue-associated mutation of T215Y/F were found. The 402 viruses were phylogenetically analyzed, revealing three independent clusters comprising PI-resistant variants with the M46I or L90M mutation, NNRTI-resistant variants with the K103N mutation, and 215-revertant variants. The PI-resistant and 215-revertant strains have been spreading since 2000, and the NNRTI-resistant strain has started spreading since 2003. The nature of the epidemic and information for successfully blocking the spread of drug-resistant HIV-1 were clarified in this study.

INTRODUCTION

Combination therapy with three or more antiretroviral drugs (highly active antiretroviral therapy, HAART) can strongly suppress the replication of human immunodeficiency virus type 1 (HIV-1) and maintain the amount of HIV-1 RNA in plasma (viral load) under detectable levels in many cases.1–5 However, HIV-1 variants with decreased susceptibility to antiretroviral drugs are sometimes found under conditions in which the drug concentration is insufficient to suppress viral replication following poor adherence to treatment regimens.3–9 Such variants might become an origin for HIV-1 transmission, resulting in the finding of drug-resistant HIV-1 in therapy-naive individuals. This represents a serious problem in therapy, as such variants hinder antiretroviral therapy from the first trial.10–16 Determining whether therapy-naive patients are infected by drug-resistant HIV-1 before starting HAART is thus important. The present study studied emergence trends for drug-resistant HIV-1 with major mutations among therapy-naive patients in the Nagoya Medical Center, Japan, between 1999 and 2006. We also studied the emergence of HIV-1 with 215-revertant amino acid mutations in the reverse transcriptase (RT), as 215-revertant variants can easily change to nucleoside RT inhibitor (NRTI)-resistant variants.17–20 The final aim of the study was to understand the epidemiological nature of drug-resistant variants and obtain information to successfully block their spread.

1Clinical Research Center and 2Department of Clinical Research Laboratory, National Hospital Organization Nagoya Medical Center (Tokai Area Central Hospital for AIDS Treatment and Research), Nagoya, Aichi 460-0001, Japan.
MATERIALS AND METHODS

Patients

A total of 441 therapy-naive HIV-1-infected patients underwent their initial consultation at Nagoya Medical Center in Nagoya, Japan, between January 1999 and December 2006. Genotypic drug-resistance testing for HIV-1 was performed on 402 of the 441 patients (91%) after obtaining patient consent. The characteristics of the 402 patients are shown in Table 1.

Genotypic drug-resistance testing for HIV-1

Genotypic drug-resistance testing for HIV-1 was performed as previously reported. HIV-1 RNA was purified from a plasma sample using a QIAamp viral RNA mini kit (QIAGEN, Tokyo, Japan). A single DNA fragment containing both protease (PR) and reverse transcriptase (RT) genes was amplified by reverse transcription-nested polymerase chain reaction (RT-nested PCR) using the Superscript one-step RT-PCR for long templates kit (Invitrogen, Tokyo, Japan) and LA Taq polymerase (Takara, Shiga, Japan). A labeling reaction for DNA sequencing was performed using the BigDye terminator cycle sequencing kit (Applied Biosystems, Tokyo, Japan), and DNA sequences were determined by the direct sequencing method using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). DNA sequences were converted to amino acid sequences, and then amino acid mutations were extracted through comparison with amino acid sequences of the HIV-1 HXB2 strain. Judgment of drug-resistant amino acid mutations was performed according to the latest version of the International AIDS Society USA panel, Fall 2006.

Phylogenetic analysis

Phylogenetic analysis was performed using the nucleotide sequences of HIV-1 obtained from all 402 therapy-naive patients. Nucleotide sequences (1005 bases) containing both PR (codons 1–99) and RT (codons 1–236) genes were used. Multiple sequence alignment was performed using CLUSTAL W, and evolutionary distances were calculated using the Kimura two-parameter model. A phylogenetic tree was constructed by the neighbor-joining method with 1000 bootstrap replicates. These analyses were performed using MEGA software version 3.1. Nucleotide sequences of 32 reference HIV-1 strains were obtained from the HIV sequence database in the Los Alamos National Laboratory. Subtyping of HIV-1 was also performed using the phylogenetic tree.

Measurement of viral load and CD4 cell count

Viral load was measured using an Amplicor HIV-1 monitor v1.5 system (Roche Diagnostics, Tokyo, Japan). CD4 cell counts were measured using a FACSCalibur flow cytometry system (Becton Dickinson, Tokyo, Japan).

Statistics

Multiple logistic regression analysis was performed to assess associations between patient characteristics and infection with drug-resistant or 215-revertant HIV-1 variants. Values of $p < 0.05$ were considered statistically significant. Analyses were performed using SYSTAT version 10.2 software (SYSTAT Software, California, USA).

RESULTS

Emergence trend of drug-resistant HIV-1 in therapy-naive patients

The prevalence of drug-resistant HIV-1 fluctuated between 2.3% and 10.0% through the period from 1999 to 2006 (Fig. 1). The first wave was observed from 2001 to 2003, with prevalence increasing from a trough of 2.3% in 2001 and peaking at 10.0% in 2003. After that, the prevalence dropped to 4.2% in 2004, but increased again to reach 8.8% by 2006. The mean prevalence for the past 8 years was 6.7% (27/402).

Variants with NRTI-resistant mutations were sporadically found (Fig. 2A). Concerning nonnucleoside reverse transcriptase inhibitor (NNRTI)-resistant variants, none was found from 1999 to 2002 (Fig. 2B). However, two variants with the K103N mutation first emerged in 2003, and this type of variant was continuously detected thereafter. Variants with the L90M, L33F, or M46L mutation alone appeared once each in 2001, 2003, and 2006, respectively. A variant possessing

| TABLE 1. CHARACTERISTICS OF 402 THERAPY-NAÏVE HIV-1-INFECTED PATIENTS |
|-----------------|------------------|-----------------|
| Age, years      | Median (IQRa)    | 33 (28–41)      |
| Sex             |                  | Male 362 90.0%  |
|                 |                  | Female 40 10.0% |
| Nationality     |                  | Japanese 335 83.3% |
|                 |                  | Foreign 67 16.7% |
| Risk factor for infection |                  | Homosexual 237 59.0% |
|                 |                  | Heterosexual 87 21.6% |
|                 |                  | Bisexual 32 8.0% |
|                 |                  | Unknown 46 11.4% |
| CD4 cell count, cells/µl | Median (IQRb) | 270 (94–400) |
| Viral load, log10 copies/ml | Median (IQRb) | 4.77 (4.26–5.26) |
| HIV-1 subtype   |                  | B 346 86.1% |
|                 |                  | Non-Bb 56 13.9% |

aIQR, interquartile range. bCRF01_AE, 30; A, 9; C, 8; D, 4; F, 2; G, 2; unclassified, 1.
multiple mutations of V32I, M46I, I47V, and L90M was found very recently.

**Characteristics of drug-resistant HIV-1**

Characteristics of drug-resistant HIV-1 found in our surveillance are shown in Table 2. The most frequently found variant was a PI-resistant virus with the M46I mutation alone (n = 12), followed by an NNRTI-resistant virus with the K103N mutation alone (n = 4). Variants with two-class resistance were found in two cases, one possessing both PI- and NNRTI-resistant mutations, and the other with both PI- and NRTI-resistant mutations. Of note is the fact that no virus with resistance against all three classes was found in our surveillance.

**Emergence trends for HIV-1 variants possessing the 215-revertant amino acid mutation in the reverse transcriptase**

T215A/C/D/E/G/H/I/L/N/S/V amino acid substitutions in the RT represent revertant mutations of the T215Y/F NRTI-resistant mutation. The 215-revertant mutations do not exhibit NRTI resistance by themselves, but most can reconvert to the T215Y/F NRTI-resistant mutation by acquiring a single nucleotide mutation. In other words, most 215-revertant variants can much more easily change to NRTI-resistant variants under the pressure of NRTIs than wild-type HIV-1.[17–20] We feel drug-resistant variants with the T215Y/F mutation are difficult to survive in the drug-free condition, as only one variant with the T215Y mutation has been found during an 8-year surveillance. The results of other researchers support our feelings. Examination of the emergence of the 215-revertant variant in addition to the T215Y/F-possessing resistant variant is thus important. In our surveillance, variants possessing the T215A/C/D/E/G/L/S mutation were found in 21 cases; since T215G/D was found in 2000, such variants have been increasing (Fig. 2D). Among these, 17 cases (81%) can reconvert to the T215Y/F NRTI-resistant mutation by acquiring a single nucleotide mutation.

**Phylogenetic analysis**

This study identified 27 drug-resistant variants from 402 therapy-naive patients. We next performed phylogenetic analysis to clarify whether specific drug-resistant strains were spreading. Three different clusters were identified from 20 of 27 drug-resistant variants (#1–13, #14–18, and #19–20) on a phylogenetic tree (Fig. 3A). All the clusters were consisted of subtype B viruses. The remaining seven variants were dispersed over the tree (Fig. 3A, #21–27). Two out of the seven were non-B viruses, subtype D and CRF01_AE. Detailed divergence of
Emergence trends for drug-resistant HIV-1 and 215-revertant variants. The y-axis shows detected numbers of drug-resistant HIV-1 or 215-revertant variants: NRTI-resistant variants (A), NNRTI-resistant variants (B), PI-resistant variants (C), and 215-revertant mutations (D). Major drug-resistant mutations and 215-revertant mutations are shown in bold and italic characters, respectively. *1, five variants detected in non-Japanese patients. *2, a variant simultaneously possessing M41L, D67N, and T215C mutations in the RT and V32I, M46I, I47V, and L90M mutations in the PR. *3, a variant possessing the K103N mutation in the RT and the M46L mutation in the PR.

FIG. 2.

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FIG. 3. Phylogenetic analysis of HIV-1 strains from 402 therapy-naive patients. (A) A phylogenetic tree was constructed by the neighbor-joining method using nucleotide sequences (1005 bases) containing both the PR (codons 1–99) and RT (codons 1–236) genes. Bootstrap analysis was performed with 1000 replicates, and values greater than 70 were shown as orange dots at the nodes of the tree. The scale bars represent nucleotide substitutions per site. Green closed circles, NRTI-resistant variants; blue closed squares, NNRTI-resistant variants; red closed triangles, PI-resistant variants; brown closed diamonds, two-class-resistant variants. Green open symbols indicate HIV-1 variants with a 215-revertant mutation that can reconvert to the T215Y/F NNRTI-resistant mutation by acquiring a single nucleotide mutation (green open circles) or more than two nucleotide mutations (green open triangles). Black open squares indicate reference HIV-1 strains. Group O_MVP5180 was used as the outgroup. Each cluster containing 13 variants with the M46I or L90M mutation in the PR (closed squares, NNRTI-resistant variants; red closed triangles, PI-resistant variants; brown closed diamonds, two-class-resistant variants) is shown as a solid line. Each cluster containing 5 variants with the K103N mutation in the RT (C), or 19 variants with the 215-revertant mutation in the RT (D) is shown as an enlarged figure. Major drug-resistant mutations and 215-revertant mutations are shown in bold and italics, respectively. PR, protease; RT, reverse transcriptase.

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