Sequence Note

Presence of Key Drug Resistance Mutations in Isolates from Untreated Patients of Abidjan, Côte d’Ivoire: ANRS 1257 Study

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ABSTRACT

A total of 107 HIV-1 isolates from untreated adult patients recruited in Abidjan, Côte d’Ivoire, in 2001 and 2002 were sequenced in the env, reverse transcriptase (RT), and protease genes. The results show that CRF02_AG is still predominant in this west African population; key mutations of resistance to antiretroviral drugs (NRTI, NNRTI, and PIIs) were detected in 5.6% of the patients. We hypothesize that these resistant mutants have been acquired through horizontal transmission. Compared to a previous study carried out by our group in 1997–2000 in a similar population of Abidjan, it seems that there is a dynamic process of resistance and that a survey will be necessary.

INTRODUCTION

HIV-1 infection is pandemic and is highly prevalent in some developing countries, particularly in Africa. In West Africa, Côte d’Ivoire is one of the most concerned countries with a prevalence of more than 10% in the adult general population.1 HIV infection is a chronic infection, which can be treated with antiretroviral drugs (ARV). The emergence of viral mutants resistant to the different classes of ARV is one of the major causes of treatment failure. These resistant mutants exhibit genotypic mutations in the genes of interest [reverse transcriptase (RT) and protease (prot)]; these mutations are now well characterized in subtype B HIV-1 isolates, which are predominant in developed countries where ARV have been introduced in the late 1980s. ARV are being introduced in developing countries where non-B subtypes and circulating recombinant forms (CRFs) are in the majority.

The aim of the ANRS 1257 project is to analyze the sequences of RT, prot, and env genes of HIV-1 isolates from untreated patients of different countries of Africa (Burkina Faso, Cameroon, Côte d’Ivoire, Sénégal) and Asia (India and Vietnam) to (1) characterize the different polymorphisms and their potential implication in resistance to ARV, (2) search for eventually transmitted key mutations associated with drug resistance, and (3) further determine the local subtypes and CRFs distributions. In a previous study2 we described the subtypes and gene polymorphism of 99 HIV-1 isolates obtained from 1997 to 2000 in a cohort of recently infected individuals (PRIMO-CI) in Abidjan, Côte d’Ivoire. In this study, we present further data on 107 samples from untreated patients who were recruited in Abidjan in 2001 and 2002.

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MATERIALS AND METHODS

Study population

Forty-eight samples were obtained from the PRIMO-CI cohort; the corresponding patients are regular volunteer unpaid blood donors at the National Blood Bank enrolled in the cohort after an HIV-1 seroconversion in the past 3 years. Fifty-nine samples were baseline samples from women enrolled into the DITRAME Plus cohort in 2002; this cohort is a trial of efficacy of AZT/nevirapine (NVP) for the prevention of mother-to-child transmission of the virus. The ANRS 1257 protocol had been accepted by the National Health Authorities and the National Ethics Commissions of Côte d’Ivoire and France; all patients gave an informed consent.

Laboratory investigations

Blood was sampled in ethylenediaminetetraacetic acid (EDTA) tubes; TCD4 lymphocytes were counted by a FAC-Scan flow cytometer (Becton Dickinson, San Jose, CA); plasma was stored at −80°C before being used for sequence studies. Viral RNA was extracted using a High Pure Nucleic Acid Kit from Roche; the RNA was used in reverse transcription polymerase chain reaction (RT-PCR) amplification of RT, prot, and env (C2/V3) genes using two sets of primers in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) thermal cycler. The outer and inner primers for RT, prot, and env regions are described in Table 1. The obtained fragments were sequenced on both strands using a CEQ DTCS Quick Start Kit on an automated sequencer Beckman CEQ 2000 DNA Analysis System. The derived nucleotide sequences of RT, prot, and env C2/V3 regions were aligned by the Clustal W 1.74 multiple sequence alignment program with known reference strains of group M and N pooled from the HIV-1 gene databank (http://hiv-web.lanl.gov/). Phylogenetic trees were inferred using the neighbor-joining method from matrix distances calculated after gapstripping of alignments, with a Kimura two-parameter algorithm. The mutations involved in antiretroviral

<table>
<thead>
<tr>
<th>outer primers</th>
<th>inner primers</th>
</tr>
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<tbody>
<tr>
<td>RT 5’-AGTAGGACCTACACCTGTC AAC-3’ bases 2480–2501</td>
<td>5’TGGTTGCACTTAAATTTTC CCATTAGCTATT-3’ bases 2530–2564</td>
</tr>
<tr>
<td>5’-CTGTTAGTGCCTTGTTCC TCT-3’ bases 3399–3420</td>
<td>5’-CCCTACTAATCTCTGTAGTCA TTGACAGTCCAGCT-3’ bases 3300–3334</td>
</tr>
<tr>
<td>Prot 5’-TAATTTTTTAGGAAGATC TGCCCTTC-3’ bases 2082–2108</td>
<td>5’-TCAGAGCAAGGAGACAGCAACACAGG-3’ bases 2136–2163</td>
</tr>
<tr>
<td>5’-GCAAATACTGGAGTAGATTGTAT GATTTCAGG-3’ bases 2703–2734</td>
<td>5’-AATGCGTTTATTTTTTCTCT GTCAATGCGG-3’ bases 2621–2650</td>
</tr>
<tr>
<td>env C2/V3 5’-CAGTACAATGTACATCTGGG-3’ bases 6955–6973</td>
<td>5’-AATGGCAGTACATCAAGG-3’ bases 7008–7026</td>
</tr>
<tr>
<td>5’-ATGGGAGGGGACATACATTG-3’ bases 7522–7540</td>
<td>5’-TTACAGTAGAAATTTCC</td>
</tr>
</tbody>
</table>

Table 1. Sequences of the ANRS Consensus Primers Used for Amplification of RT, Prot, and env C2/V3 Regions

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PRIMO-CI (n = 48)</th>
<th>DITRAME (n = 59)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years</td>
<td>30</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>Women (%)</td>
<td>20 (41.6)</td>
<td>59 (100)</td>
<td>79</td>
</tr>
<tr>
<td>Men (%)</td>
<td>28 (58.3)</td>
<td>0 (0)</td>
<td>28</td>
</tr>
<tr>
<td>HIV transmission category</td>
<td>Heterosexual</td>
<td>Heterosexual</td>
<td>Heterosexual</td>
</tr>
<tr>
<td>CDC stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1 (%)</td>
<td>17 (35.4)</td>
<td>Not available</td>
<td></td>
</tr>
<tr>
<td>A2 (%)</td>
<td>25 (52.1)</td>
<td>Not available</td>
<td></td>
</tr>
<tr>
<td>A3 (%)</td>
<td>6 (12.5)</td>
<td>Not available</td>
<td></td>
</tr>
<tr>
<td>CD4 cell count, mean cells/μl</td>
<td>460</td>
<td>457</td>
<td>458</td>
</tr>
<tr>
<td>CD4 cell count, median values</td>
<td>393 (190–981)</td>
<td>413 (78–1120)</td>
<td>395 (78–1120)</td>
</tr>
<tr>
<td>Plasma HIV RNA level, mean log_{10} copies/ml</td>
<td>4.5</td>
<td>Not done</td>
<td>—</td>
</tr>
<tr>
<td>Plasma HIV RNA level, median</td>
<td>4.7 (2.6–6.1)</td>
<td>Not done</td>
<td>—</td>
</tr>
</tbody>
</table>

RESULTS

The clinical and biological characteristics of the 107 patients are presented in Table 2; taken together, there were 79 women and 28 men, the mean age was 28 years and the mean TCD4 count was 458/µl; the median count of TCD4 was 395/µl (range: 78–1120); the transmission was considered to be heterosexual. For PRIMO-CI, we got more details: 52.1% of the patients were in the A2 CDC stage, 35.4% and 12.5%, respectively, in the A1 and A3 stages; the mean value of the plasma HIV RNA load was 4.5 log and the median was 4.7 (range: 2.6–6.1). It is clear from Fig. 1 that CRF02-AG is largely predominant in this West African population followed by subtype A; moreover, we could identify CRF06_cpx, CRF04_cpx, one CRF01AE and some intersubtype recombinants (for example, three samples including RT subtype K and one RT subtype D).

The sequence data concerning RT and protease are presented, respectively, in Figs. 2 and 3. In RT, we could identify one K101E, two K103N, and one P236L substitution that are considered to be associated with resistance to NNRTI and one K219Q substitution that is a TAM; the most frequent substitutions related to polymorphism are at codons 30, 35, 36, 39, 49, 60, 122, 123, 135, 137, 173, 174, 177, 178, 200, 207, 211, 214, 243, 245, and 248; among substitutions in codons (106, 135, and 245) that can be involved in low-level resistance to NNRTI, V245Q (59% of AG samples) was the most frequently observed.

In protease, we could observe one key mutation at position 88 (N88D) that is associated with resistance to nevirapine in the French ANRS algorithm; as shown in Fig. 2, the most frequent polymorphisms were recorded at positions 13, 14, 19, 20, 35, 36, 37, 41, 64, 69, 70, and 89; concerning the amino acid changes at positions of minor PI resistance mutations, M36I (94% of CRF02 AG samples) was the most frequently noted.

DISCUSSION

This study is in fact a follow-up of a previous work from our group that included patients recruited in the PRIMO-CI cohort from 1997 to 2000; compared to these published data, we confirm the large predominance of CRF02 AG, the presence of CRF06_cpx, and some intersubtype recombinants. This is in agreement with results from other studies in Côte d’Ivoire and West Africa. The amino acid substitutions observed are similar to those described previously. Concerning the resistance mutations, no mutation had been noted in our first study; in the present work, mutations are observed in RT (one K101E, two K103N, one P236L, and one K219Q) and protease (one N88D); each mutation was observed in separate individuals; we can therefore consider that in this series of 107 untreated patients...
recruited within the past 2 years, 5.6% of them bear resistance mutations against one or several antiretroviral drugs (NRTI, NNRTI, and PIs). The main question we have about the resistance mutations is whether they are related to an inadequate ARV treatment or to transmission of resistant isolates from treated patients as has been described for subtype B isolates in developed countries.\textsuperscript{11} We have no information concerning patients from DITRAME Plus, but we must point out that they

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Amino acid substitutions in the HIV-1 reverse transcriptase of isolates from 107 patients. The gray columns concern significant polymorphism positions and the surrounding columns indicate the positions of resistance mutations.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Amino acid substitutions in the HIV-1 protease of isolates from 107 patients. The gray columns concern significant polymorphism positions and the surrounding columns indicate the positions of resistance mutations.}
\end{figure}
were detected as HIV infected at the time of entrance in the study, were not aware of their infection, and were therefore untreated. Both patients in the PRIMO-CI cohort whose isolates bore mutations at positions 103 and 219 of RT could be further questioned and denied ARV treatment. We can therefore consider that all these patients have probably been infected with resistant HIV-1 strains circulating in the country.

In conclusion, these data show that there is a circulation of HIV-1 isolates containing potential resistance mutations in this studied population of Côte d’Ivoire. Since such isolates were not observed in a previous study 2 years ago, it suggests that there is a dynamic process and that a longitudinal survey of the transmission of resistant strains to untreated recently infected patients will be necessary in the future. The sequences described in this study have been deposited in GenBank with Accession Numbers AY207654 to AY207754 for protease sequences, AY207844 to AY207940 for reverse transcriptase sequences, and AY207755 to AY207843 for envelope sequences.

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