Sequence Note

Presence of CRF09_cpx and Complex CRF02_AG/CRF09_cpx Recombinant HIV Type 1 Strains in Côte d’Ivoire, West Africa

THOMAS TONI,1,2 CHRISTIANE ADJÉ-TOURÉ,3 NICOLE VIDAL,4 ALBERT MINGA,1 CHARLOTTE HUET,1 MARIE-YOLANDE BORGER,5 PATRICIA RECORDON-PINSON,2 BERNARD MASQUELIER,2 MONICA NOLAN,3,5 JOHN NKENGASONG,6 HERVÉ J. FLEURY,2 ERIC DELAPORTE,4 and MARTINE PEETERS 4

ABSTRACT

Based on partial env and pol (protease and RT) subtyping, we recently documented that the majority (>80%) of the HIV-1 strains that circulate in Côte d’Ivoire were CRF02_AG and about 11% were recombinants or could not be clearly assigned to a known subtype or CRF. In order to determine in more detail the precise structure of these viruses we sequenced the full-length genomes for six such strains. Bootscan and phylogenetic tree analysis showed that four strains were complex and unique CRF02_AG/CRF09_cpx recombinants, one was a CRF02_AG/CRF06_cpx recombinant, and one was a pure CRF09_cpx. Reanalysis of the remaining recombinants asserted the predominance of CRF09_cpx within intersubtype recombinants and circulation of CRF09_cpx in Côte d’Ivoire. More detailed analysis of the CRF09_cpx strains revealed also that part of the pol gene belonged to subtype K. This is the first time that such recombinants are described.

One of the major characteristics of HIV is its high genetic diversity. HIV-1 strains can be further subdivided into groups (M, N, and O), subtypes (A–D, F–H, J, and K), sub-subtypes, and circulating recombinant forms (CRFs, CRF01–CRF16). Extensive efforts have been made to collect and characterize HIV isolates from around the world and Africa, and a broad picture of the distribution of HIV strains has emerged. Subtype designations have been powerful molecular epidemiological markers to track the course of the HIV-1 pandemic.1,2 The global subtype distribution is very heterogeneous and the greatest genetic diversity of HIV-1 has been found in Africa. Also within Africa, important differences are observed according to the regions studied, from country to country, and even within a country.3 Overall, in West Africa, CRF02_AG predominates, followed by CRF06_cpx, subtypes A and G. In areas where CRFs have a high prevalence, it is highly probable that like pure subtypes they are also involved in recombination events, as illustrated recently by the emergence of complex CRF02/CRF06 recombinants in certain west african countries.4

Based on partial pol and env sequences, the high prevalence of CRF02_AG (82%) and cocirculation of subtype A (5%), CRF01_AE (1%), CRF06_cpx (4%), and complex intersubtype recombinants (11%)5,6 has been recently documented in Côte d’Ivoire. These intersubtype recombinants had discordant CRF or subtype designations among protease, reverse tran-
scriptase (RT), and C2V3 (env) sequences. To determine in more detail the precise structure of these viruses, we sequenced the full-length genomes for four of them (97IC-PCI3, 01IC-PCI118, 01IC-PCI123, and 01IC-PCI127). The viruses were isolated from patients enrolled in the PRIMO-CI study, which is a cohort of volunteer unpaid regular blood donors who seroconverted in the past 3 years. Sequences from these previously described isolates clustered with different subtypes or CRFs in the different genomic regions studied. 97IC-PCI3 was characterized as CRF02/U/CRF02, 01IC-PCI118...
FIG. 1. For the bootstrap plots, the SimPlot software performed bootscanning on neighbor-joining trees by using SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE from the PHYLIP package for a 400-bp window moving along the alignment in increments of 20 bp. One hundred replicates for each phylogeny were evaluated. The bootstrap values for the studied sequences were plotted at the midpoint of each window. In the left part the bootstrap plots of 97IC-PCI3, 01IC-PCI118, 01IC-PCI123, 01IC-PCI127, 01IC-17395, and 00IC-10029 strains, respectively, are drawn against the consensus (50% threshold) of the nonrecombinant subtype references (A–D, F–H, J, K, and L). The right part shows the corresponding bootstrap plots against the consensus (50% threshold) of the CRF02_AG, CRF06_cpx, and CRF09_cpx references, by using the C subtype as an outgroup. The new sequences were aligned with and compared with the following reference sequences: subtype A (92UG-UGO37, 94ME-ME7523, and 94KE-Q23), subtype B (90US-WEAU160, 86US-JFRL, and 83FR-HXB2), subtype C (86ET-ETH220, 90BW-0502, and 95CN-2006), subtype D (94UG-114, 83CD-NDK, and 99TCMN9836), subtype F2 (95CM-EP266, CM-EP257, and CM53657), subtype G (93SE-SE6165, 93FI-HH7593, and 93DE-DRB11), subtype H (95CM-CRF02, BE.VI995), and BE.VI997, subtype J (93SE-SE92809 and 93SE-SE91733), subtype K (97CD-EQTH11 and 96CM-CRFB), CRF02_AG (IBNG, DJ263, and 98SE-MP1211), CRF06_cpx (BRP90 and 95ML-34), and CRF09 (96GH2911, 95SN1795, and 95SN7808).

as A/K/CRF02, 01IC-PCI123 as CRF06/CRF06/CRF02, and 01IC-PCI127 as CRF02/K/CRF02 in the protease/RT/env C2V3 regions, respectively. The strains were obtained prior to any antiretroviral (ARV) drug and they did not present any major drug resistance mutation.

In addition, between August 1989 and January 2003, as part of an evaluation of the presence of antiretroviral drug resistance

mutations in HIV-1-infected treated patients receiving ARV treatment in the National Drug Access Initiative from Côte d'Ivoire, approved by the national and the CDC ethical committees, we analyzed the pol gene of 42 HIV-1-infected children (0–15 years old). Five samples could not be clearly classified in one of the known HIV-1 subtypes/CRFs and full-length genome sequencing was done on only two such samples (00IC-

FIG. 2. Schematic representation of the mosaic genome structure of the recombinant strains after phylogenetic tree analysis to confirm recombination breakpoints observed on bootscans. In some regions where both CRF02_AG and CRF06_cpx clustered with subtype G and where clear discrimination between CRFs was not possible by phylogenetic tree analysis, subtype G is indicated. U means unclassified; no clear subtype or CRF assignment was possible by phylogenetic tree analysis.
For the six samples, DNA was extracted from primary peripheral blood mononuclear cells (PBMCs) with the QiaAmp DNA blood kit (Qiagen, Courtaboeuf, France). For each strain, overlapping nested polymerase chain reactions (PCRs) were done to obtain the sequence of the entire genome as previously described. For the six samples, DNA was extracted from primary peripheral blood mononuclear cells (PBMCs) with the QiaAmp DNA blood kit (Qiagen, Courtaboeuf, France). Nucleotide sequences were obtained by direct sequencing of the amplified DNA, using the inner primers of each PCR and several primers encompassing the entire fragments. The six HIV-1 genomes were sequenced in their entirety, including the long terminal repeat (LTR) ex- tremities. All reading frames were open and of complete length. None of the genomes had major deletions or rearrangements.

The phylogenetic relationships of the newly derived viruses were estimated from sequence comparisons with previously reported representatives of group M subtypes (A–D, F–H, I, and K) and CRFs documented in West and West Central Africa available from the Los Alamos HIV sequence database (http://hiv-web.lanl.gov/). Nucleotide sequences of the near full-length genomes were aligned using CLUSTAL W with minor man-}

![Phylogenetic tree analysis of 375 unambiguously aligned base pairs from part of the reverse transcriptase, illustrating the presence of subtype K clustering in CRF09_cpx strains.](image)
the mosaic genomic structures of CRF02/CRF09 and CRF02/CRF06 viruses from Côte d'Ivoire. Bootstrap analysis showed in all the CRF02/CRF09 recombinants as well as in the CRF09_cpx strain a fragment in the pol region that was close to subtype K. In addition, we initially classified these recombinants as K in the part of the RT gene that we studied. Subtype K was not described as being involved in the complex recombinant structure of this recently described variant,50 therefore we realigned this part of the genome for the CRF09_cpx prototype strains and our new strains. This additional phylogenetic analysis clearly confirmed the presence of subtype K in CRF09_cpx as shown in Fig. 3. The subtype K fragment corresponds to amino acid positions 115–239 of the RT gene.

We also realigned the remaining 10 strains from the PRIMO-CI cohort that we initially classified as intersubtype recombinants based on the protease, RT, and pol regions. This additional phylogenetic tree analysis was done and included all subtypes and CRFs that were not yet available at the time of the initial analysis. The results are summarized in Table 1 and showed that one strain (DP5) clustered with CRF09 in the three regions. The other isolates were intersubtype recombinants involving CRF02 and CRF09 (PC115, PC141, PC191), or CRF02 and CRF06 (DP43, DP58), or A and CRF09 (DP16).

In this study we have characterized five “second-generation” recombinant forms of HIV-1 in four infected blood donors from Côte d’Ivoire and one seropositive child. Four were CRF02/CRF09 recombinants with different recombination patterns and one was a complex CRF02/CRF06 recombinant virus. We documented that CRF09_cpx, which has recently been described, also circulates in Côte d’Ivoire, that CRF09_cpx is involved in recombination events, and that CRF09_cpx recombinants seem to circulate at higher proportions than the pure prototype CRF09_cpx. Furthermore, based on partial sequences, other strains were shown to cluster with CRF09_cpx and confirms that CRF02/CRF09 recombinants were the most frequent variants involved in recombination in Côte d’Ivoire. Because patient 97IC-PCI3, who is infected with a CRF02/CRF09 recombinant virus, was enrolled since 1997, CRF09_cpx has probably been introduced before this date in this population and even in Côte d’Ivoire. In addition, the pure CRF09_cpx strain was isolated from a 10-year-old child in 2000, who could possibly have been contaminated by vertical transmission 10 years before. Our results may have important implications for the dynamics of HIV subtype diversity in Côte d’Ivoire, and probably West Africa. Further analysis on a larger sample size is needed to better describe the implication of such recombinants on HIV subtype distribution in Côte d’Ivoire.

The emergence of complex recombinants with many breakpoints is not uncommon and secondary recombinants, involving CRFs, have already been described. In Burkina Faso and Niger, two other West African countries close to Côte d’Ivoire, CRF02/CRF06 recombinants represented 10.5% of circulating strains and in China recombination between CRF07 and CRF08 has been reported.11,12 Recombination is the result from coinfection and superinfection occurring in geographic regions where several HIV-1 variants are cocirculating.11,12 Recombination introduces genetic changes that are far greater than those resulting from the steady accumulation of single mutations and possibly also has biological consequences such as increased pathogenicity or acquisition of drug resistance or impairment of vaccine strategy.53,54 In conclusion, HIV-1 subtype distribution is a dynamic process and continuous monitoring of circulating HIV-1 strains in the context of vaccine development and treatment strategies remains necessary. The description of future recombinants should not remain restricted to pure subtypes. Especially in regions where CRFs are predominant, these strains should also be included in the analysis in order to identify the most closely related parental forms and to reflect a reliable picture of the evolution of the epidemic.

SEQUENCE DATA

The new sequences have been deposited in the EMBL Data Library under the following accession numbers: AJ 866553 to AJ 866558.

ACKNOWLEDGMENTS

This work was sponsored by grants from the Agence Nationale de Recherches sur SIDA (ANRS 1257) and by the Centers for Disease Control and Prevention, Atlanta, GA. T. Toni has a doctoral fellowship from ANRS.

REFERENCES


Address reprint requests to:
Martine Peeters
Laboratoire Retrovirus, UMR145
Institut de recherche pour le Developpement (IRD)
and University of Montpellier
911 Avenue Agropolis, BP 64501
34394 Montpellier Cedex 1, France
E-mail: martine.peeters@mpl.ird.fr
This article has been cited by:


