Distribution of HIV-1 Subtypes Among HIV-Seropositive Patients in the Interior of Côte d’Ivoire

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Summary: Limited data exist on the distribution of HIV-1 subtypes in Côte d’Ivoire. The aim of this study is to describe the distribution of genetic subtypes of HIV-1 strains in six regions of Côte d’Ivoire. In 1997, we consecutively collected blood from 172 HIV-1–infected patients from six regional tuberculosis treatment centers. Peripheral blood mononuclear cells (PBMCs) from these people were analyzed by a restriction fragment-length polymorphism (RFLP) assay that involves a sequential endonuclease digestion of a 297-base pair polymerase chain reaction (PCR) fragment; plasma samples were tested by a V3-loop peptide enzyme immunoassay (PEIA). DNA sequencing of the protease or env genes was performed on all samples discordant in the two assays as well as a random sample of the concordant subtyped samples. Of 172 specimens, 3 were PCR-negative, and 169 were putatively classified as subtype A by RFLP. The 3 PCR-negative samples were unequivocally subtyped A by PEIA. Of the 169 RFLP subtype A samples, 159 (94%) were subtyped A by PEIA. Of the 10 discordant samples, PEIA testing classified 3 as subtype C, 2 as D, and 5 as F. Sequencing of the env gene classified these samples as 1 subtype A, 4 Ds, and 5 Gs. Thus, 163 (95%) of the specimens were subtype A, 3 subtype D, 4 subtype G, 1 A/D, and 1 A/G (IbNG) circulating recombinant forms (CRF). In conclusion, most HIV-1–infected tuberculosis patients throughout the interior of Côte d’Ivoire are infected with HIV-1 subtype A, which are very likely the A/G (IbNG) CRF. The uniform distribution of this subtype makes Côte d’Ivoire a potential site for vaccine trials. Key Words: HIV-1—Genetic subtypes—Côte d’Ivoire, Africa.

In West Africa, Côte d’Ivoire is the country most severely affected by the AIDS epidemic. For instance, in Abidjan, the economic capital, 15% of pregnant women and 45% of tuberculous (TB) patients are infected with HIV-1 (1,2). Several economic and geographic factors help explain this situation. First, because of its economic strength, Côte d’Ivoire attracts many workers, primarily single men, from the interior of Côte d’Ivoire and surrounding countries. Second, being the largest seaport in the region and having a well-developed road system, it is the regional hub for shipping and truck transport. In addition, Abidjan has a large population of female commercial sex workers (CSWs), many of whom come from surrounding countries (3), and contact with these CSWs is a defined risk for HIV infection (3). Finally, the re-
recently ended civil war in Liberia (located west of Côte d’Ivoire) led to an influx of refugees into different regions of the country. These factors that may favor the spread of HIV also might favor the introduction of different subtypes of HIV. We had already established that HIV-1 subtype A is responsible for more than 90% of HIV-1 infections in selected populations in Abidjan (4), but no data exist on the distribution of these subtypes in the interior of Côte d’Ivoire.

On the basis of genetic sequence diversity, HIV is currently classified into two types (HIV-1 and HIV-2); 10 subtypes, designated by letters A through J, constitute the major group of HIV-1 (group M)(5,6). Highly divergent strains of HIV-1 have also been reported and have been classified as group O (7,8). Recently, a third group of viruses, group N, recognized for their similarity to the chimpanzee virus (simian immunodeficiency virus, SIVcm), has been reported (9). Surveillance of HIV-1 subtypes is important for understanding the epidemiology of transmission patterns, vaccine development (planning trials and interpreting the results), and evaluating assays used in diagnostic algorithms. Indeed, the public health significance of divergent strains was highlighted by studies that showed that persons infected with group O viruses were not consistently detected by serologic assays (10,11). Moreover, some non-B HIV-1 subtypes are difficult to quantify by certain commercially available assays (12). In addition, some screening assays have been reported to have significantly lower sensitivity to detect non-B HIV-1 subtypes antibodies during seroconversion (12). Additionally, some non-B HIV-1 subtypes are difficult to quantify by certain commercially available assays for measuring HIV-1 RNA viral load (13–15).

Côte d’Ivoire has received much attention as a potential site for vaccine trials and is currently one of the four sites that the UNAIDS is carrying out feasibility studies to introduce access to antiretroviral therapy in developing countries. Our study was aimed at determining the distribution of genetic subtypes of HIV-1 group M and group O virus strains in different regions of Côte d’Ivoire.

MATERIALS AND METHODS

Patients

Since 1994, HIV counseling and testing has been made available to all TB patients in all TB treatment centers in Côte d’Ivoire. In 1997, we consecutively enrolled HIV-1–infected patients from the six regional TB treatment centers: western region (Man, n = 29), central region (Daloa, n = 24 and Gagnoa, n = 12), eastern region (Abengourou, n = 27), and the northern region (Bouake, n = 49, and Korhogo, n = 31). Blood was drawn into Vacutainer CPT (Becton-Dickinson, Franklin Lakes, NJ, U.S.A.) from a total of 172 consenting HIV-1–infected TB patients from six major cities; plasma and peripheral blood mononuclear cells (PBMCs) were obtained from these people and stored until analysis.

Laboratory Analysis

Serologic Testing

Serologic testing was completed at the Projet RETRO-CI laboratories. We determined HIV-seropositivity by using Genelavia Mixte (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) that has been evaluated in our laboratory and shown to have a very high sensitivity for HIV-1 and HIV-2 viruses (16) and a synthetic peptide-based line immunoassay (Pepfilav 1-2, Sanofi Diagnostics) was used for type-specific serodiagnosis of HIV-1 and HIV-2 (16,17).

HIV-1 Subtyping Algorithm

To determine the subtypes, we simultaneously tested all 172 seropositive people for the V-3 loop synthetic peptide enzyme immunoassay (PEIA) and by restriction fragment-length polymorphism (RFLP) assays. Samples that gave concordant subtype results in the two assays were assigned the HIV-1 subtype, and discordant samples were tested further by DNA sequencing of either the env or protease genes (Fig. 1).

V3-Loop Synthetic Peptides Enzyme Immunoassay

We tested the samples by using a PEIA assay that consisted of eight consensus V3-loop synthetic peptides. The subtype A peptide (DKSVRIGPGQFTFYAT) was derived from previously analyzed HIV-1 strains from Abidjan (4). The remaining seven peptides were derived from HIV-1 group M subtypes (B consensus, DKSFIGPGQAFYAT; C_BR.W, BR025 KSR1RPGQAFYAT; D consensus, DQRTHIGPGQALYTT; E consensus, DTSITIGPGQAFYRT; F consensus, DKSISHPGQAFYAT; G CF.4067, DKSISFGPGQAFYAT, and one HIV-1 group O peptide (O_CM.ANT70, DQEMRIPMAW-YSMG). Each peptide contained 15 amino acids from the principal neutralizing domain of HIV-1 gp120. These peptides were synthesized by a solid-phase method using 9-flouremethoxy-carbonyl chemistry on a Model 432-A Applied Biosystems synthesizer according to the manufacturer’s protocol, and were partially purified by reverse-phase high performance liquid chromatography.

We performed the V3-loop peptide assay as reported previously (18). In brief, the initial PEIA test was performed at a sample dilution of 1:400; if more than one peptide yielded a signal within 90% of the most reactive one, the sample was retested at a dilution of 1:1600. If the same peptide gave the highest reactivity at both dilutions, we assigned a serotype to the specimen on the basis of the most reactive peptide. Otherwise, the sample was classified as multiply reactive and thus could not be typed (NT). If a sample tested below the cutoff (0.3) initially, it was retested at a dilution of 1:100, and either assigned a serotype according to the peptide with greatest reactivity, or if it failed at this dilution, classified as nonreactive (NR). Sera dually reactive to A and C peptides (AC/CAs) were putatively classified as subtype A, on the basis of earlier observations of cross-reactivity (18) and the predominance of HIV-1 subtype A infection in Abidjan (4).

Polymerase Chain Reaction

HIV-specific polymerase chain reaction (PCR) was done on uncultured PBMCs from HIV-1–seropositive samples. Total cellular DNA
was prepared by lysing 4 × 10^6 PBL/ml in lysis buffer (10-mM Tris pH 8.3/0.05% Triton X-100), adding 10-mg proteinase K/ml, incubating at 56°C for 1 hour, followed by 95°C for 10 minutes to inactivate the proteinase K. The final nested-PCR product, a 297 base-pair (bp) fragment that includes the protease (prot) gene was amplified by nested PCR using HIV-1-specific primers. The outer primers were DP10 5’/H11032CAACTCCCTCTCAGAAGCAGGAGCCG3’ (nucleotide positions 2201–2226, according to HIV-1MN) and DP11 5’/H11032CCATTCCTGGCTATAATT TACTGGTA3’ (nucleotide positions 2575–2601), a 401-bp product, and the inner primers were DP16 5’/H11032CCTCAGAT- CACTCTTTGGCAGC3’ (nucleotide positions 2255–2275) and DP17 5’/H11032AAAATTTAAATGCAGCCAA3’ (nucleotide positions 2541–2560). For the C2-V3 region, the outer primers were CL105 5’/H11032CATAATGTTTGGGCCACACATGCCTGTGTACC3’ (nucleotide positions 5997–6029, according to HIV-1LAI) and CL202 5’/H11032TGGTGAATATCCCTGCCTAACTCTATT3’ (nucleotide positions 7933–7959), a 700-bp product, and the inner primers were MK650 5’/H11032FAATGTCAGCACAGTACAATGTACAC3’ (nucleotide positions 6537–6562) and 5’/H11032MK601TCTCCAATTGTCCCTCATT CATATCTCCTCCTCCA3’ (nucleotide positions 7227–7258). The thermal cycler conditions for both PCR systems have been reported previously (4,20).

Restriction Fragment Length Polymorphism

The nested amplified PCR products from the prot gene were analyzed RFLP as described previously (4,19). Briefly, the amplified HIV-1 prot gene was sequenced for RFLP by using restriction enzymes AluI, BclI, and Scal to segregate the HIV-1 viral strains into distinctive subtypes. AluI restriction polymorphism segregates the viral strain into two patterns: subtypes A, C, and F belong to one pattern, and subtypes B and D to another pattern. Subtypes A and C are differentiated from subtype F by BclI restriction polymorphism, and subtype C is distinguished from subtype A by Scal digestion. Hinfl digestion polymorphism is used to discriminate between subtypes B and D.

DNA Sequencing and Phylogenetic Analysis

DNA sequencing was performed as described previously (20). In brief, DNA sequences were obtained for either the env C2-V3 region of gp120 or the prot gene by using an automated sequencer (Applied Biosystems Inc., Foster City, CA, U.S.A.) and customized viral-specific oligonucleotide primers (Tag Dye-Deoxy-Terminator Cycle Sequencing kit, Applied Biosystems) according to the manufacturer’s recommendation. Sequences were aligned with those in the Los Alamos HIV Database by the Lipman/Pearson algorithm (DNASIS Sequencing Analysis Software, Hitachi Software Engineering, Brisbane, CA, U.S.A.). The phylogenetic relationship of specimens was analyzed by the neighbor-joining method in the PHYLIP 3.5c package (21) and then confirmed by the maximum likelihood method using the fastDNaml program (22). The HIV-1 sequences analyzed were aligned with reference subtype sequences. The Ivorian sequences were deposited in the GenBank under accession numbers AF246819 through AF246829 for the env sequences and AF216978 through AF216989 for the protease sequences.

RESULTS

Study Participants

Of the 172 patients that we analyzed, 103 (60%) were men and 69 (40%) were women. Median age was 34 years (interquartile range, 28–42) for men and 29 years (24–37) for women.

Screening Assays

PEIA Testing

All 172 plasma specimens from the patients were tested by PEIA: 114 (66%) were unequivocally classified as subtype A, 1 as subtype C, 1 as subtype D, and 2 as subtype F. There were 23 samples that were dually reactive to A- and C-specific peptides (A/C subtypes), 27 that were multiply reactive, and 4 that were nonreactive. All dual or multiply reactive specimens were retested at a dilution of 1:1600, and a putative subtype was assigned to each specimen according to the most reactive peptide. After this repeated testing, overall distribution of the sub-
types in PEIA was 159 (92%) subtype A, 4 subtype C, 2 subtype D, 5 subtype F, and 2 could not be typed. None of the samples reacted with the HIV-1 group O V3-loop peptide.

**RFLP Testing and Correlation With PEIA Results**

Of the 172 specimens, 3 were PCR-negative and 169 were PCR-positive. Of the PCR-positive samples, 158 (93%) were classified as subtype A by RFLP assay. The remaining 11 specimens were characterized by Alu RFLP as undigested PCR protease products and not initially assigned to a presumptive subtype. These 11 specimens and the 3 PCR-negative samples were subtype A by PEIA. Of the 11 undigested PCR protease products not typed by the RFLP assay, 3 were sequenced and phylogenetically clustered within subtype A viruses (IC0017, IC0024, and IC0028) (Fig. 2). These results were similar to those previously observed among Ivorian samples (4). Therefore, we assigned the remaining 8 specimens with Alu uncut prot pattern to subtype A viruses. Of the 169 RFLP putative subtype A samples, 159 (94%) were concordantly subtyped A by PEIA. Of the remaining 10 discordant samples, PEIA testing classified 3 sera as subtype C, 2 as D, and 5 as F (Fig. 1).

**Phylogenetic Analysis**

The usefulness of the RFLP-based subtyping has been previously demonstrated by comparison with the prot and env sequence analysis (4,19). In addition, in this study we also observed concordance between RFLP, PEIA, and DNA sequence analysis for 10 of 159 concordant samples treated as internal controls: 6 of 10 subtype A strains sequenced are shown in Figure 3 (env: IC0071, IC0095, and IC0123) and Figure 2 (prot: IC0229, IC0245 and IC0275) (Fig. 2). These results were similar to those previously observed among Ivorian samples (4). Therefore, we assigned the remaining 8 specimens with Alu uncut prot pattern to subtype A viruses. Of the 169 RFLP putative subtype A samples, 159 (94%) were concordantly subtyped A by PEIA. Of the remaining 10 discordant samples, PEIA testing classified 3 sera as subtype C, 2 as D, and 5 as F (Fig. 1).

DISCUSSION

HIV-1 diversity affects serologic and virologic diagnostic assays and is an important consideration in the development of HIV-1 vaccine (5,23). Our results, based on a large number of specimens, demonstrate that HIV-1 subtype A strains account for approximately 95% of HIV-1 infections in TB patients in the interior of Côte d’Ivoire; subtypes D and G account for less than 5% of the remaining infections. However, because most of the few subtype A viruses we sequenced clustered with the A/G (IbNG) recombinant virus in both the env and prot phylogenetic tree, it is likely that most viruses we classified as subtype A are actually A/G CRF. Consistent with this assertion, a reanalysis of the sequences we published previously from Abidjan (4) indicates that most of these viruses were A/G recombinants (Ellenberger, personal communication). In addition, our subtype A sequences had a clear separate subcluster from the Ugandan sequences, which is consistent with a recent finding of Carr et al. on distinct clustering of subtype As from East and West Africa (24).

The predominance of these HIV-1 subtype A or presumably A/G (IbNG) recombinants among HIV-1–infected people in the interior of Côte d’Ivoire is strikingly similar to what we and others have observed among female CSWs (25), TB patients, and pregnant...
women in Abidjan (4,26). These findings are consistent with the hypothesis that HIV-1 was introduced into the urban centers and disseminated throughout the country. We found no HIV-1 group O infections at our different sites, corroborating our earlier work that indicated that infection with HIV-1 group O is rare in Côte d’Ivoire (27).

Most non-A subtypes that we identified were from Ivorians and were not clustered in any geographic region, suggesting that these infections do not represent a local outbreak of these subtypes; rather, these people most likely acquired their infections from individuals living in areas outside their region of residence. HIV-1 subtype A viruses are the predominant strains in countries sur-
rounding Côte d’Ivoire, including Mali, Burkina Faso (28), and Ghana (29); subtype G isolates have been reported in these countries as well (28,29). However, available sequence data are insufficient to favor a specific route of introduction for the G strains in Côte d’Ivoire.

Another noteworthy aspect of this study is the potential usefulness of rapid techniques such as PEIA and RFLP for large-scale subtyping for HIV-1 genetic surveys. It will be important to confirm these results in regions with greater genetic diversity of HIV-1 strains, such as Cameroon and Gabon (30,31). Each of these rapid techniques has its strengths and limitations. The RFLP assay is faster than other PCR-based subtyping techniques, such as the heteroduplex mobility assay (32), thus, allowing the analysis of larger numbers of samples. In addition, it has proven useful in documenting coinfections with different HIV-1 subtypes (33). This technique is also useful in subtyping other retroviruses such as HTLV-I (34). However, mutations at the restriction sites may make it difficult for the digestive enzymes to cut the amplified products, thus leading to unclassifiable samples.

The PEIA assay is also a rapid technique that can be used for the large-scale screening of subtypes, especially in areas where resources are limited and in countries where HIV-1 diversity is limited (35). However, the cross-reactivity of sera to different peptides of different subtypes occurs frequently, because of the conservation of common epitopes among different HIV-1 subtypes (35–38). Thus, the choice of V3-loop peptide seems critical in correctly subtyping HIV-1 strains.

In summary, we have shown that three subtypes of HIV-1 circulate in the interior of Côte d’Ivoire, and that subtype A (presumably A/G recombinants) accounts for approximately 95% of the infections. This uniformity in the distribution of subtype A makes Côte d’Ivoire a potential site for vaccine trials.

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REFERENCES


