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Genetic Subtypes of HIV Type 1 and HIV Type 2 Strains in Commercial Sex Workers from Bamako, Mali

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ABSTRACT

In Africa the highest HIV infection rate has been reported among female commercial sex workers (CSWs) who are at increasing risk of acquiring and transmitting HIV infection. In October 1995, 176 CSWs were studied in Bamako, the capital city of Mali. The ages of the CSWs ranged from 15 to 50 years old (mean, 28.8 years). Only 20.45% of the 176 CSWs were Malian; the majority were from Nigeria (32.9%) and Ghana (31.8%), and the remaining were from other African countries. Forty-one percent were active for less than 1 year as a commercial sex worker, and the length of prostitution for the remaining women ranged from 1 to 15 years (mean, 2.76). A total of 81 (46.02%) of the 176 CSWs were positive for HIV antibodies; 63 (35.8%) were HIV-1 positive, (3.9%) were HIV-2 positive, 11 (6.2%) had antibodies to HIV-1 and HIV-2, and none of them had antibodies to group O viruses. For all HIV antibody-positive samples, PBMCs were separated and genetic subtypes of HIV-1 were determined using the heteroduplex mobility assay (HMA), with ED5-ED12 as outer and ES7-ES8 as inner primers. Among the 66 HIV-1 strains characterized, 53 (80.3%) were subtype A, 2 (3.1%) belonged to subtype C, 1 (1.5%) belonged to subtype D, and 10 (15.1%) were identified as subtype G. Among the 10 subtype G strains, 8 were obtained from women who were very recent CSWs, with an activity of 1 year or less, assuming that there is a high probability that these infections occurred recently. Genetic subtypes of five HIV-2 viruses were determined by sequencing of the *env* and/or *gag* genes followed by phylogenetic analysis, and all of them belonged to subtype A. Comparison of HIV-1 and HIV-2 seroprevalence data from our study with previous data from Mali shows a significant rise in HIV-1 prevalence and a significant decrease in HIV-2 prevalence and confirms similar trends observed in neighboring countries. We have found four different genetic subtypes of HIV-1; however, subtype A is predominant and accounts for 80% of the cases and 15% of the HIV-1 infections were subtype G. It is important to continue the surveillance of subtypes on a systematic basis in order to see to what extent the proportions of the different subtypes will change over time.

INTRODUCTION

THE GENETIC VARIABILITY OF human immune deficiency virus type 1 (HIV-1) is a major challenge in the development of a globally effective vaccine. With the characterization of many isolates of HIV from Africa and from other regions of the world, numerous genetic subtypes of the virus have been identified. Phylogenetic analysis revealed two groups of HIV-1 isolates: group M, the major group with at least 10 different genetic subtypes (A–J), and group O.[1,2] Preliminary data indicate a heterogeneous distribution and dominance of different genetic subtypes depending on the country analyzed. In Africa all the known HIV-1 genetic subtypes, including group O, are present.

This genetic diversity has implications for vaccine development, because vaccine formulations based on only one virus strain (or subtype) would not elicit a broad enough immune response to protect against members of other subtypes.[3,4] The cross-reactivity of cytotoxic T lymphocyte (CTL) epitopes to more conserved regions and to what extent their role is critical for vaccine development must be further studied. The impact of the genetic variation on pathogenesis, disease progression, and virus transmission remains also to be elucidated. A rela-

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tionship between genetic subtype and natural resistance against anti-HIV drugs has been observed; in particular, group O viruses are resistant to non-nucleoside RT inhibitor (TIBO).[5] The genetic subtype can also have an impact on the efficiency of testing for HIV antibodies.[6,7] One of the first major biological differences between group M and group O HIV viruses is that in contrast to HIV group M viruses, the group O viruses do not have to bind and incorporate cyclophilin A to produce infectious virions.[8] Therefore studying HIV-1 genetic variation at the global level is needed, to identify the emergence of subtypes that may be more readily transmitted or have an altered virulence, and to ensure that vaccine antigens are directed against contemporary or older strains of the virus circulating within specific populations.

For all these reasons, it is important to study the geographic distribution of the different HIV-1 genetic subtypes. To date, there have been relatively few systematic large-scale attempts to characterize HIV isolates from different parts of the world, and especially from Africa. The viruses actually characterized from Africa were obtained from convenience samples rather than from random sampling, and so their representativeness is uncertain. Therefore we studied the prevalence of different genetic subtypes of HIV-I and HIV-2 circulating in female commercial sex workers in Bamako, the capital city of Mali.

Mali is a West African country, bordered by Guinea, Senegal, Mauritania, Algeria, Niger, Burkina Faso, and Côte d'Ivoire. It has a strong trading activity and travel links with Côte d'Ivoire and Burkina Faso, where high seroprevalence rates have been reported in high-risk groups.[9,10] Since the emergence of AIDS, data on the HIV situation in Mali are limited; however, both viruses HIV-1 and HIV-2 are present with initially a predominance of HIV-2 in all population groups studied.[11]

Although genetically and biologically related to HIV-1, HIV-2 possesses a number of distinguishing properties. HIV-1 and HIV-2 differ in their natural history of infection and *in vivo* pathogenicity. HIV-2-infected individuals exhibit longer clinical latency periods, progress more slowly after onset of symptoms, and have a lower virus burden than do individuals infected with HIV-1.[12,13] Both vertical and horizontal transmission rates are significantly lower for HIV-2.[14,15] In addition, studies of HIV-2 genetic variation indicate the existence of multiple, highly divergent, evolutionary lineages.[16]

In Africa, the highest HIV infection rate has been reported among female commercial sex workers (CSWs) and in patients with sexually transmitted diseases. Several studies have reported that in areas where the heterosexual transmission mode is prevalent, CSWs are at increasing risk of acquiring and transmitting HIV infection. Therefore we studied the prevalence of different genetic subtypes of HIV-1 and HIV-2 in female commercial sex workers in Bamako, Mali.

PATIENTS AND METHODS

Study population

In October 1995, 176 female CSWs were studied in their work setting in Bamako, Mali. CSWs were recruited in different areas from the city. After giving their informed consent, the women were interviewed according to a standardized questionnaire developed for this study. The questionnaire included demographic characteristics such as age, nationality, and history of travel out of Mali. HIV risk factor information was also included, such as number of sexual partners, duration of prostitution, and history of sexually transmitted disease. A medical visit was included to verify whether symptoms of HIV infection were present. At the end of the interview each subject was given a date for test results, specific counseling, free condoms, and free drugs, depending on the results of the medical visit.

Sample collection and HIV serology

A 10-ml whole-blood sample was collected in EDTA tubes from each individual. The plasma was aliquoted and immediately screened in the national laboratory in Bamako for the presence of HIV antibodies by a rapid test (Multispot HIV 1/2; Diagnostics Pasteur, Marnes la Coquette, France) that differentiates between HIV-1 and HIV-2 infection. For all the samples reactive to HIV antibodies, peripheral blood mononuclear cells were separated from plasma by Ficoll gradient centrifugation. Plasma and cell pellets were stored at −20°C and shipped to Montpellier, France on dry ice. All sera have been retested for HIV antibodies in Montpellier by enzyme-linked immunosorbent assay (ELISA) (Genelavia mixt; Diagnostics Pasteur) and all reactive samples, i.e., samples with a ratio of optical density to cutoff higher than or equal to 1, were confirmed and discriminated by Innolia HIV-1/2 (Innogenetics, Antwerp, Belgium).

Identification of HIV-1 group O antibodies

All HIV-positive samples were screened as previously described[17] by an ELISA in which the solid phase is coated with a combination of V3 peptides from two group O strains (ANT-70 and MVP-5180) to discriminate between group O and M infections (Innogenetics). Sera positive in ELISAs were retested using a Line Immuno Assay (LIA-O), in which different biotinylated V3 peptides from various group M and O strains were applied as a streptavidin complex in parallel lines on a nylon strip (Innogenetics). A polymerase chain reaction (PCR) with discriminatory primers for groups M and O in the *env* and *pol* regions was done on the corresponding samples suspected of group O infection by serology.[18]

Genetic characterization of HIV-1

DNA preparation, PCR, and heteroduplex formation. DNA was extracted from the dry cell pellets using an IsoQuick isolation kit (Microprobe, Garden Cove, CA). A nested PCR technique was used to amplify the viral genomic regions for heteroduplex mobility assay (HMA) and sequencing. For HMA, we used as outer primers ED5-ED12 and as inner primers ES7-ES8, which have been described elsewhere.[19] From 0.5 to 1.0 μg of genomic DNA was used under the following conditions: a first denaturation step for 5 min at 94°C followed by 30 cycles of 94°C for 15 sec, 55°C for 30 sec, and 72°C for 2 min, with a final extension for 7 min at 72°C for the first round. Five

microliters from this amplification was used for the second round with the inner primers using the following cycling conditions for 40 cycles: 94°C for 15 sec, 55°C for 30 sec, 72°C for 1 min. The reaction mixture consists of 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.1% Triton X 100, 1.25 mM $MgCl_2$ for the first round or 1.8 mM $MgCl_2$ for the second round, 10 pmol of each primer, a 0.2 mM concentration of each dNTP, and 2.5 U of *Taq* polymerase.

The PCR amplification products were detected by electrophoresis on a 1% agarose gel and visualized by ethidium bromide staining. To avoid PCR product cross-contamination, pre-PCR and post-PCR manipulations were performed in separate rooms.

Heteroduplex molecules were obtained by mixing 5 μl of two divergent PCR-amplified DNA fragments (the unknown patient strain with a plasmid from typed reference strains) denatured at 94°C for 2 min and renatured by rapid cooling on wet ice. The reference strains used in this study were the following: A1 (RW20, Rwanda), A2 (IC144, Ivory Coast), A3 (SF170, Rwanda), B1 (BR20, Brazil), B2 (TH14, Thailand), B3 (SF162, United States), C1 (MA959, Malawi), C2 (ZM18, Zambia), C3 (IN868, India), C4 (BR25, Brazil), D1 (UG21, Uganda), D2 (UG38, Uganda), D3 (UG46, Uganda), E1 (TH22, Thailand), E2 (TH06, Thailand), E3 (CAR7, Central African Republic), F1 (BZ162, Brazil), F2 (BZ163, Brazil), G1 (RU131, Russia), G2 (LBV21-7, Gabon), G3 (VI525, Gabon), H1 (CA13, Cameroon), H2 (VI557, Zaire), and H3 (VI997, Belgium). The reaction was performed in 100 mM NaCl, 10 mM Tris-HCl (pH 7.8) and 2 mM EDTA in a final volume of 8 μl. The heteroduplex formation was resolved by electrophoresis analysis at 250 V for 3 hr on a nondenaturing 5% polyacrylamide gel in TBE buffer (88 mM Tris borate, 89 mM boric acid, 2 mM EDTA) and was detected after being stained with ethidium bromide. The electrophoretic mobility of the heteroduplexes was inversely proportional to the sequence divergence of the two annealed strands.

DNA sequencing of HIV-1 strains. For sequencing, amplifications were performed on the same starting material with previously described primers: ED31 and ED33 as the outer primers[19] and HE101 and V4 as the inner primers.[20] Cycling conditions were as follows: for the first PCR round 3 cycles of 94°C for 1 min, 37°C for 1 min, and 72°C for 1 min followed by 32 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, with a final extension of 7 min at 72°C. For the second PCR round 25 cycles of 1 min at 94°C, 1 min at 44°C, and 1 min at 72°C with a final extension of 7 min at 72°C. The reaction mixture consists of 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.1% Triton X-100, 1.4 mM $MgCl_2$, 20 pmol of each primer, a 0.2 mM concentration of each dNTP, and 2.5 U of *Taq* polymerase. The PCR amplification products were detected by electrophoresis on a 1% agarose gel and visualized by ethidium bromide staining. Sequencing primers were HE110 (forward) and V4 (reverse). Cycle sequencing was performed using fluorescent dye terminator technology (dye terminator cycle sequencing with AmpliTaq DNA polymerase FS; Perkin-Elmer, Norwalk, CT) according to the instructions of the manufacturer). Electrophoresis and data collection were done on an Applied Biosystems (Foster City, CA) 373A automatic DNA sequencer.

PCR and sequencing for genetic characterization of HIV-2

As for HIV-1, HIV-2 DNA was extracted from the dry cell pellets using the IsoQuick isolation kit (Microprobe). Samples were subjected to nested PCR amplifications in the *env* and *gag* regions with primer pairs previously described by Gao *et al.*[15] PCRs were carried out in a total volume of 50 μl containing 1 μg of genomic DNA, 10 mM Tris-HCl (pH 9), 50 mM KCl, 0.1% Triton X-100, 20 pmol of each primer, a 200 μM concentration of each dNTP, 1.5 mM $MgCl_2$, and 2.5 U of *Taq* polymerase (Promega, Madison, WI). Samples were initially denatured for 3 min at 94°C. The PCR reactions with the *env* primers were done under the following conditions: for the first round 35 cycles of 94° for 90 sec, 48°C for 90 sec, and 55°C for 90 sec and for the second round 45 cycles of 90 sec at 94°C, 90 sec at 41°C, and 90 sec at 55°C. The PCR reactions with the *gag* primers were done under the following conditions: for the first round 35 cycles of 1 min at 94°C, 1 min at 40°C, and 1 min at 72°C and for the second round 35 cycles of 1 min at 94°C, 1 min at 45°C, and 1 min at 72°C.

Second-round PCR products were visualized by agarose gel electrophoresis, isolated from a preparative gel, and purified with a Wizard DNA Clean Up system (Promega). The purified PCR products were directly sequenced or sequenced after cloning into the M13mp18 *Sma*I vector (Amersham, UK). The envelope regions of two samples were sequenced after cloning using the inner PCR primers, and part of the *gag* region of two samples was sequenced after cloning using GAG2-5′ (AGTACAGAGACATCTAGTGGC) and GAG2-3′ (CTTGGCTCTCTAAGCTGCCC). Sequencing was done using fluorescent dye terminator technology (dye terminator cycle sequencing with AmpliTaq DNA polymerase FS; Perkin-Elmer) according to the instructions of the manufacturer. Electrophoresis and data collection were done on an Applied Biosystems 373A automatic DNA sequencer.

Phylogenetic analysis

Nucleotide sequences were aligned by using the Clustal V[21] program with minor adjustments in consideration of their predicted protein sequence. Regions that could not be aligned unambiguously, owing to length or sequence variability, were omitted from the analysis. The newly determined HIV-1 and HIV-2 sequences were aligned with known HIV-1 and HIV-2 sequences representing the different genetic subtypes and the sequence of SIVcpz-gab as outgroup. Evolutionary distances were computed using Kimura's empirical two-parameter method. A distance matrix was then used to estimate phylogenetic relationships by the neighbor-joining method.[22,23] Reliability of the branching orders was confirmed by the bootstrap approach.[24] All analyses were performed using Clustal V. Sequences were submitted to the EMBL Nucleotide Sequence Database and have the following accession numbers: Y14356–Y14364.

Statistical analysis

Statistical comparisons of the means and proportions were performed by the Student's *t* test, using the EPI-INFO software

package (developed by CDC). Differences in trends of prevalences form different subtypes were calculated using the rank test.

RESULTS

Population studied

Table 1 summarizes the demographic data of the population studied. A total of 176 female CSWs participated in the study. Their ages ranged from 15 to 50 years (mean, 28.8 years). Only 36 (20.45%) of the 176 CSWs were Malian; the majority were from Nigeria (58 of 176, 32.95%) and Ghana (56 of 176, 31.81%). The remaining 26 CSWs were from different countries (Senegal [n = 15], Cameroon [n = 1], Côte d'Ivoire [n = 1], Guinea [n = 2], Liberia [n = 2], Niger [n = 1], Togo [n = 1]; for 2 the nationality was not known). Seventy-two (40.9%) were for less than 1 year active as a commercial sex worker, and the length of prostitution for the remaining women ranged from 1 to 15 years (mean, 2.76 years). In general the CSWs from Ghana were older than the Malians or Nigerians, with a mean age of 33.5 versus 25.7 and 25.0 years, respectively. Forty-two (75%) of the 56 Nigerian CSWs were for less than 1 year active, versus 18 of 55 (32.7%) of the Ghanian women and only 3 of 34 (8.8%) of the Malians.

Prevalence of HIV-1, HIV-2, and HIV-1 group O infection

HIV prevalences are shown in Table 1. A total of 81 (46.02%) of the 176 CSWs were positive for HIV antibodies; 63 (35.8%) had antibodies to HIV-1, 7 (3.9%) had antibodies to HIV-2, and 11 (6.2%) had antibodies to HIV-1 and HIV-2. Comparison of HIV prevalences by age groups revealed the lowest prevalence, 27.4%, in the 20 to 29-years group. In the other age groups the prevalences ranged from 60.0 to 66.6%. The variations in HIV-1 prevalences were similar to those observed in the overall HIV prevalence, because HIV-1 mimicks the overall prevalence. Six of the seven HIV-2 cases were detected in women more than 30 years old. HIV prevalence is increasing with length of prostitution; 31.9% of the women who were less than 1 year active in prostitution were HIV positive versus 83.3% of the women active for more than 3 years ($p < 0.0001$). The prevalence of HIV-1 as well as those of HIV-1 + 2 and HIV-2 are increasing with length of prostitution. The HIV prevalence was higher among women with a history of sexually transmitted disease (STDs): 51.4% versus 37.3% in those

TABLE 1. DEMOGRAPHIC DATA AND SEROPOSITIVITY FOR HIV AMONG FEMALE COMMERCIAL SEX WORKERS IN BAMAKO, MALI[a]

Variable	*Tested* (n)	*HIV-1* [n (%)]	*HIV-1 + 2* [n (%)]	*HIV-2* [n (%)]	*HIV* [n (%)]
Total	176	63 (35.8)	11 (6.2)	7 (3.9)	81 (46.0)
Age (years)					
<20	18	11 (61.1)	1 (5.5)	—	12 (66.6)
20–29	84	17 (20.2)	5 (5.9)	1 (1.2)	23 (27.4)
30–39	57	29 (50.8)	3 (5.2)	4 (7.0)	36 (63.1)
>40	15	5 (33.3)	2 (13.3)	2 (13.3)	9 (60.0)
Unknown	1	1	—	—	1
Nationality					
Malian	36	22 (61.1)	1 (2.7)	1 (2.7)	24 (66.6)
Ghanian	56	23 (41.1)	5 (8.9)	6 (10.7)	34 (60.7)
Nigerian	58	10 (17.2)	2 (3.4)	—	12 (20.6)
Other	24	7 (29.2)	2 (8.3)	—	9 (37.5)
Unknown	2	1	1	—	2
Length of prostitution (years)					
<1	72	19 (26.3)	3 (4.2)	1 (1.4)	23 (31.9)
1	38	13 (34.2)	1 (2.6)	1 (2.6)	15 (39.5)
2	21	11 (52.4)	1 (4.7)	—	12 (57.1)
3	14	7 (50.0)	2 (14.3)	1 (7.1)	10 (71.4)
>3	24	13 (54.2)	3 (12.5)	4 (16.6)	20 (83.3)
Unknown	1	—	1	—	1
Number of clients/week					
<10	18	8 (44.4)	1 (5.5)	—	9 (50.0)
10–19	54	23 (42.9)	3 (5.5)	3 (5.5)	29 (53.7)
20–29	36	14 (38.9)	3 (8.3)	1 (2.7)	18 (50.0)
30–39	45	15 (33.3)	2 (4.4)	3 (6.6)	20 (44.4)
>40	14	2 (14.3)	1 (7.1)	—	3 (21.4)
Unknown	2	1	1	—	2
History of STDs					
No	67	18 (26.8)	4 (5.9)	3 (4.5)	25 (37.3)
Yes	107	45 (42.1)	6 (5.6)	4 (3.7)	55 (51.4)
Unknown	1	—	1	—	1

[a]The numbers in parentheses represent the overall seroprevalence of a category.

without a history of STDs; however, these differences were not significant. The highest overall HIV prevalences were observed in Ghanian and Malian women, 66.6 and 60.7%, respectively, and the lowest HIV prevalence was seen in the Nigerians with 20.6%. Six of the seven HIV-2 cases were observed in women from Ghana, and the other case was that of a Malian woman. The seven HIV-2-infected women were older and five of them were active in prostitution for 3 years or more. Five of the 10 dually seropositive women were from Ghana; the others were from Nigeria ($n = 2$), Mali ($n = 1$), Guinea ($n = 1$), and Togo ($n = 1$). All of the sera were screened for antibodies to HIV-1 group O viruses with a specific ELISA, and none of them had antibodies to group O viruses.

Genetic subtyping of HIV-1 group M isolates

The HIV-1 isolates from HIV-1 and HIV-1 + 2 antibody-positive individuals have been analyzed by heteroduplex mobility assay (HMA) or by sequencing of the C2V3 region of the gp120 region of the envelope. Sixty-six HIV-1 strains were genetically characterized: 56 of the 63 HIV-1 strains and 10 HIV-1 strains obtained from the 11 dually reactive individuals. Table 2 summarizes the demographic data and the genetic subtypes of HIV-1 observed in the study population. Among the 66 HIV-1 strains characterized, 53 (80.3%) were subtype A, 2 (3.1%) belonged to subtype C, 1 (1.5%) belonged to subtype D, and 10 (15.1%) were identified as subtype G. For one subtype C strain no epidemiological or demographic data were available, but the other subtype C strain and the only D strain were observed in women from Nigeria, with, respectively, 2 years and less than 1 year of activity in prostitution. Among the 10 subtype G strains, 4 were from Ghanian women, 3 from Nigerians, 2 from Malians, and 1 from a women from Guinea. Eight of these 10 women were very recent commercial sex workers with an activity of 1 year or less. The differences in the trends of prevalence of subtypes A and G according to the duration of prostitution were not significant (rank test, $p < 0.24$).

According to the nationality, the distribution of genetic subtypes differed with more variation among women from Nigeria. Among the 12 HIV-infected women from Nigeria, 7 (58.3%) were infected with a subtype A strain, 1 (8.3%) with subtype C, 1 (8.3%) with subtype D, and 3 (25.0%) with subtype G. Twenty-one (91.3%) of the 23 Malians were infected with subtype A and 2 (8.7%) with subtype G, and for the Ghanian women 18 (81.8%) of the 22 were subtype A and 4 (18.3%) were subtype G. To confirm the HMA assignments, *env*-amplified DNA from nine samples was directly sequenced. The samples chosen for sequencing included seven samples subtyped as G, one subtyped as D, and one sample identified as subtype A, all showing relatively low-migrating heteroduplexes compared with prototype references. In each case, phylogenetic analysis of the sequence data confirmed the subtype obtained by HMA. Figure 1 shows the phylogenetic tree for the nine sequences, with representative sequences from the GenBank database.

Genetic subtyping of HIV-2 isolates

A PCR was done on the 7 HIV-2 samples and the 11 HIV-1 + 2 antibody-positive samples. Only 2 of the 7 HIV-2 and 3 of the 11 HIV-1 + 2 samples were amplifed with the *env* primers. Owing to the cross-reactivity of the *gag* primers for HIV-1 and HIV-2, only the HIV-2 samples were tested with these primers and only two of the seven samples could be amplified. One sample (64) was amplified by both *gag* and *env* primers.

A 349-bp fragment was obtained in the transmembrane region of the envelope, and the *env* sequences were aligned to

TABLE 2. DEMOGRAPHIC DATA AND GENETIC SUBTYPES OF HIV-1 AMONG FEMALE COMMERCIAL SEX WORKERS IN BAMAKO, MALI

		Subtype			
Variable	*Tested (n)*	*A [n (%)]*	*C [n (%)]*	*D [n (%)]*	*G [n (%)]*
Total	66	53 (80.3)	2 (3.1)	1 (1.5)	10 (15.1)
Nationality					
Malian	23	21 (91.3)	—	—	2 (8.7)
Ghanian	22	18 (81.8)	—	—	4 (18.2)
Nigerian	12	7 (58.3)	1 (8.3)	1 (8.3)	3 (25.0)
Other	9	7 (77.7)	1 (11.15)	—	1 (11.15)
Age (years)					
<20	11	8 (72.7)	—	—	3 (27.3)
20–29	20	15 (75.0)	1 (5.0)	1 (5.0)	3 (15.0)
30–39	29	25 (86.2)	—	—	4 (13.8)
>40	5	5 (100)	—	—	—
Unknown	1	—	1	—	—
Length of prostitution (years)					
<1	20	17 (85.0)	—	1 (5.0)	2 (10.0)
1	12	6 (50.0)	—	—	6 (50.0)
2	12	10 (83.4)	1 (8.3)	—	1 (8.3)
3	9	8 (88.8)	—	—	1 (11.2)
>3	12	12 (100)	—	—	—
Unknown	1	—	1	—	—

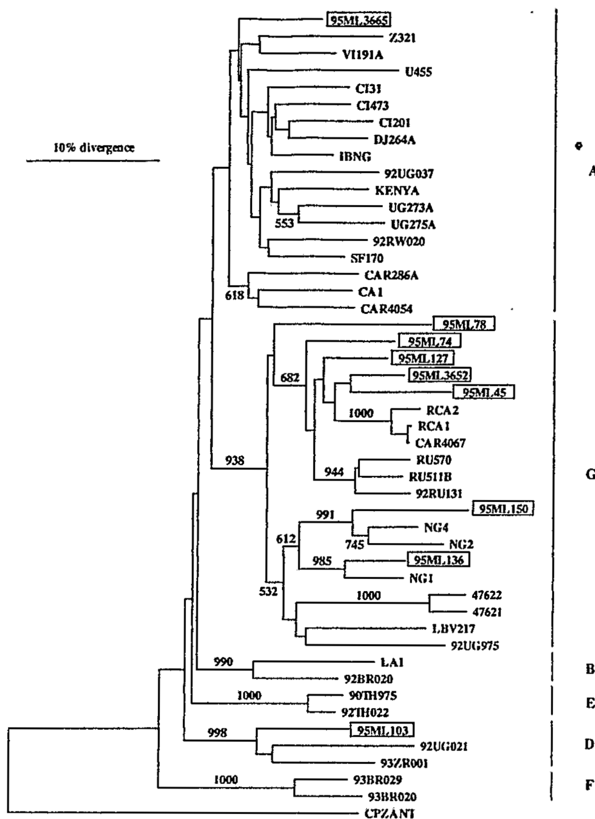

FIG. 1. Phylogenetic tree based on 260 unambiguously aligned positions of the HIV-1 *env* gene C2V3 region. The tree was rooted with a corresponding region of the chimpanzee SIVcpz-ant isolate being used as an outgroup. The analysis was performed as described in Materials and Methods.

previously described HIV-2 envelope sequences. All our strains cluster with subtype A strains, supported by high bootstrap values. The nucleotidic divergence among the HIV-2 *env* sequences from our study ranged from 7 to 12%.

A 746-bp fragment was obtained for two samples (64 and 122) in the *gag* region, covering p16 and p26. Alignment with previously described HIV-2 *gag* sequences showed that the strains from our study clustered with subtype A HIV-2 strains.

DISCUSSION

In Africa, the highest HIV infection rates have been observed in female commercial sex workers (CSWs). This population group is at high risk for acquiring and transmitting HIV infection. The overall HIV prevalence in the group of CSWs that we have studied was 46.02%, which was not significantly higher than the 39.1% seroprevalence rates reported among CSWs from Bamako in 1987.[11] However, the data from 1987 were not analyzed according to age groups and therefore may not be comparable. Interesting to note are the differences in prevalence rates for HIV-1 and HIV-2 in the two studies. In 1987, the prevalence of antibodies to HIV-1, HIV-2, and HIV-1 + 2 was 10, 15, and 13%, respectively, whereas in 1995 the prevalence of antibodies to HIV-1, HIV-2, and HIV-1 + 2 was 35.8, 3.9, and 6.2%, respectively. These data show a significant rise in HIV-1 prevalence (from 10 to 35.8%) and a significant decrease in HIV-2 prevalence (from 15 to 3.9%) and confirm similar trends observed in the neighboring West African countries, where the HIV-2 prevalence is stable or decreasing while the HIV-1 prevalence is increasing.[25] Data on HIV incidence show that HIV-1 infection has a relatively greater incidence than HIV-2 infection. Even in countries where HIV-2 is the predominant virus, including Guinea Bissau, HIV-1 infections appear to be increasing faster than HIV-2. The lower likelihood of sexual transmission of HIV-2 compared with HIV-1 may help to explain why HIV-2 has had a more regional distribution geographically, whereas HIV-1 has spread more dramatically over the same time period.[25]

Genetic diversity of HIV-2 isolates has led to the classification of HIV-2 into five genetic HIV-2 subtypes. The majority of the isolates fall into subtype A, with most of the rest being subtype B HIV-2 viruses. Sporadic strains belonging to subtypes C, D, and E have been described in individuals from Sierra-Leone and Liberia whereas the subtype A group contains viruses originating from diverse locations across West Africa. Subtype B includes viruses from Ghana and Côte d'Ivoire. In our study, all the HIV-2 samples from which an amplification product was obtained belonged to subtype A.[16,26] In general, HIV-2 subtype A viruses grow easily in culture, whereas attempts to isolate representatives of subtypes C, D, and E, as well as the majority of subtype B viruses, remained unsuccessfull. Therefore it was suggested that subtype-specific differences in HIV-2 biology exist. Subtype A viruses were observed among asymptomatic and AIDS patients whereas subtypes C, D, and E have been described only among asymptomatic individuals. The clinical significance of differential virulence between these subtypes has been hypothesized as a reason for the apparently divergent characteristics of the HIV-2 epidemic in different regions of West Africa. However, no evidence exists to support this hypothesis and long-term prospective studies are necessary to study the natural history of HIV-2 infection in different parts of West Africa and to determine whether there are regional or subtype-specific differences in HIV-2 pathogenicity. Because we were able to amplify only a limited number of HIV-2 samples from our study, the HIV-2 genetic subtypes observed are probably not representative of the HIV-2 viruses actually circulating among CSWs in Bamako. All the HIV-2-positive CSWs from our study were asymptomatic and low viral load can possibly explain the low efficiency rates of the PCRs. Modifications of the PCR conditions as described by Gao *et al.*[16] did not improve the efficiency.

To determine the prevalence of HIV strains in a population group that is at high risk for acquiring and transmitting HIV infection, we have analyzed 66 HIV-1 strains obtained from 56 HIV-1- and 10 HIV-1 + 2-infected CSWs from Bamako. We have found four different genetic subtypes of HIV-1; subtype A is predominant and accounts for 80% of the cases. However, 15% of the HIV-1 infections were subtype G, 2 (3.1%) were subtype C, and 1 (1.5%) was subtype D. Sporadic cases of subtype G infections have been described in Central (Gabon), eastern (Uganda), and western (Benin, Nigeria, and Ghana) African countries.[27–31] Our study shows that 15% of the HIV-1-infected CSWs are infected with subtype G and that the majority of these strains were detected in women who recently started in prostitution (with an

activity of 1 year or less), thus there is a high probability that there infections occurred recently. All the subtype G strains were from epidemiologically unlinked individuals. Further follow-up studies are needed to confirm if the subtype G strains were acquired recently and whether their prevalence increases.

Up to now, subtype C strains have been reported mainly from southern and eastern African countries, and from West Africa; only a sporadic case from Senegal has been described.[32–34] Our data show that the presence of this subtype is rather limited in this population group in western Africa. Subtype D strains were mainly observed in East and Central Africa; however, sporadic cases have been described in Côte d'Ivoire and Ghana.[31,35–37] Our data confirm the low presence of subtype D in West Africa. The number of HIV-1 sequences obtained from strains isolated in Africa has grown rapidly. However, many of these sequences were not generated from any systematic broad-based sampling of different infected populations. Therefore, these viruses may not be representative of the full spectrum of HIV variants in these regions. In addition, the HIV-1 subtype profile may change with the movement of infected individuals between different regions. It has been suggested that in Thailand HIV-1 subtype E may be associated with a higher risk of heterosexual transmission than subtype B, and so the predominance of subtype E in Thailand may have contributed to the particularly rapid spread of HIV in this country.[38]

It is important to continue the surveillance of genetic subtypes on a systematic basis in Mali in the same population group of individuals at high risk for HIV infection and also to extend it to other population groups, in order to see to what extent the proportions of the different genetic subtypes will change over time. As our understanding of the significance of different HIV genetic subtypes and phenotypes increases, knowledge of their frequency and distribution will allow a timely and effective response to the HIV pandemic.

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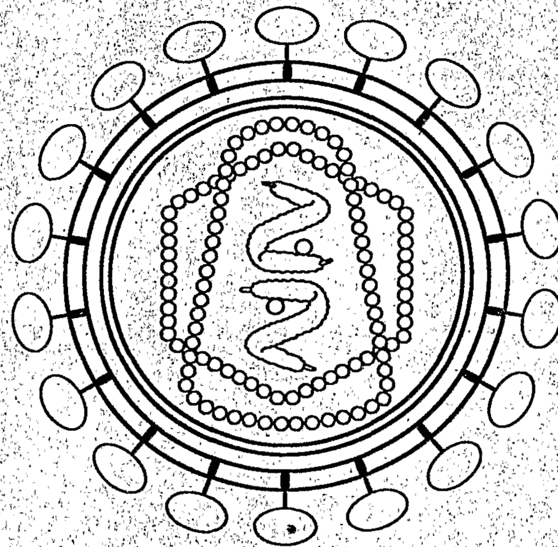

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