Presence of multiple non-B subtypes and divergent subtype B strains of HIV-1 in individuals infected after overseas deployment

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Mariama Lasky, Jean-Luc Perret*[†], Martine Peeters, Fréderic Bibollet-Ruche, Florian Liegeois, Delphine Patrel, Sylvain Molinier*, Claude Gras*[†] and Eric Delaporte

Objective: To identify the genetic subtypes and characteristics of HIV-1 strains from individuals infected after overseas deployment.

Patients and methods: Sixty-one HIV-1-positive individuals detected between 1986 and 1995 in the French army were included in the study. For each patient, the year and country of HIV infection are known. Genetic subtypes of HIV-1 were determined using the heteroduplex mobility assay (HMA) using ED5/ED12 as outer and ES7/ES8 as inner primers. Strains were further characterized by sequencing and phylogenetic analysis of the C2–V3 region. The amino-acid sequences corresponding to the V3 region were aligned on the basis of the subtyping results and were then compared to the consensus V3 sequences of the corresponding subtypes.

Results: Among the 61 patients studied, nine became infected in France, and 52 were HIV-negative before overseas deployment but HIV-positive at their return. The majority (n = 43) deployed in Africa and a limited number of patients deployed in Asia (Cambodia, n = 5) or South America (Guyana, n = 4). The nine individuals who were not deployed overseas were all infected with subtype B strains. The majority of the other patients were infected with non-B strains; eight subtype A, 20 subtype B, 16 subtype C, one subtype D, six subtype E and one subtype F. Five of the six subtype E strains were contracted in Cambodia and one in Djibouti, and all subtype C strains were from Djibouti. Phylogenetic analysis revealed a large diversity among the different strains introduced into France. Analysis of the amino-acid sequences of the V3 loop revealed the introduction of uncommon V3-loop patterns.

Conclusion: In the group of HIV-1-infected individuals that we studied and who were deployed overseas, 63.4% were infected with non-B strains. In addition, the subtype A, B and C viruses in this population were very heterogeneous. Due to the routine occurrence of international travel and deployment, the predominance of subtype B HIV-1 viruses may change in European countries. However, the possible implications on the dynamics of the HIV-1 epidemic needs further follow-up.

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From the Retrovirus Laboratory, ORSTOM, Montpellier, the *Hôpital d'Instructions des Armées, Laveran and the [†]Institut de Médecine Tropicale des Services de Santé des Armées, Marseille, France.

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Requests for reprints to: Martine Peeters, Laboratoire Retrovirus, ORSTOM, 911 Avenue Agropolis, BP5045, 34032 Montpellier Cedex 1, France.

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Introduction

Since the discovery of HIV-1 and HIV-2 as causative agents of AIDS, much has been learned about their molecular characteristics, mechanisms of gene expression, and genomic complexity. Multiple isolates of both viruses have been characterized, their genomes cloned and their nucleotide sequences determined [1]. From these studies we know that genetic variation is a hallmark of this class of viruses, no two viruses are alike, and even within a single individual HIV is present as a quasispecies [2]. With the recent characterization of many isolates of HIV from Africa and from other regions of the world, a phylogenetic structure has emerged that identified numerous subtypes of the virus. This 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 [1,3]. HIV-1 group O viruses are highly divergent HIV-1 viruses and are mainly found in Cameroon or among Cameroonians [4–6]. The geographical distribution of the different HIV-1 genetic subtypes is not well known, since relatively few systematic large-scale attempts to characterize HIV isolates from different parts of the world have been made. Preliminary data indicate a very heterogeneous distribution and dominance of different genetic subtypes depending on the country analysed. Subtype B, which includes the prototype strain (HIV-1_{LAI}) is dominant in Europe and North America [1]. In Africa, all the known HIV-1 genetic subtypes, including group O, are present, and in Southeast Asia, subtype E is predominant [7–13].

The relevance of this subtype classification versus pathogenesis, disease progression and virus transmission is not yet fully understood. HIV-1 subtypes may have effects on the sensitivity of diagnostic assays and natural resistance against anti-HIV drugs [14]. Some data suggest that the genetic heterogeneity of HIV-1 has immune relevance [15]; there is therefore concern that vaccine products based on subtype B may not generate broad enough immunity to protect against the other subtypes that occur around the world.

Given the wide international dispersal of HIV-1 subtypes, and the occurrence of international travel, it is inevitable that strains other than subtype B will spread within Europe and the United States. Recently, the introduction of A, D and E genotypes into the United States by five individuals of the US army personnel after overseas deployment was reported [16]. In addition, in Germany, out of nine new HIV-infected patients three were infected with subtype E, and one with subtype C [17]. It is important to monitor the different genetic subtypes that are introduced in the population in order to study whether the introduction of non-B genetic subtypes could influence the actual epidemic.

In order to contribute to an evaluation on the importance of HIV-1 infections with non-B subtypes in France we analysed the HIV-1 genetic subtypes and risk factors associated with HIV infection in military personnel selected for overseas duties. We focused on this population group because they undergo regular medical visits, and the country and period of overseas deployment are well documented, allowing a better analysis of factors associated with presence of certain HIV-1 genetic subtypes.

Materials and methods

Patients

We analysed the risk factors and possible contamination sites in 61 HIV-1-positive individuals from the French army. Since 1987, the army personnel that are selected for overseas deployment are screened for HIV infection in the year that they are supposed to leave (between 0 and 6 months before their actual departure). Subjects are retested for anti-HIV antibodies within a period of 3 months after their return to France. By this method for each patient the year and the country of infection are known with a high probability. When the HIV test is positive, patients are informed and they undergo medical visits at regular time intervals.

Virus isolation

Peripheral blood mononuclear cells (PBMC) from HIV-positive patients were cocultivated with phytohaemagglutinin (PHA)-stimulated lymphocytes from a healthy (HIV-negative) human donor in RPMI-1640 medium supplemented with 15% heat inactivated fetal calf serum, 0.03% L-glutamine, 2 μg/ml polybreen, antibiotics and 20 U/ml recombinant interleukin-2 (Boehringer, Mannheim, Germany). The culture flasks were incubated at 37°C in a 5% CO_2 atmosphere. The medium was replaced every 3–4 days, and fresh PHA-stimulated donor lymphocytes were added once weekly. The cultures were kept for 6 weeks. The release of viral particles in the culture supernatant was examined by an HIV p24 antigen capturing test (Innogenetics, Antwerp, Belgium).

DNA preparation and polymerase chain reaction

DNA was extracted using the IsoQuick isolation kit (Microprobe Corp, Garden Cove, California,USA) from cultured PBMC or primary lymphocytes when viral isolations were negative. A nested polymerase chain reaction (PCR) technique was used to amplify viral genomic regions for heteroduplex mobility assay (HMA) and sequencing. For HMA, we used ED5/ED12 as outer primers, and ES7/ES8 as inner primers, which have been described elsewhere [18]. From 0.5 to 1.0 μg 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. Three microlitres from this amplification were used for the second round with the inner primers using the same cycling conditions for 40 cycles. The reaction mixture consists of 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 9), 0.1% Triton X-100, 1.4 mmol/l $MgCl_2$, 20 pmol of each primer, 0.2 mmol/l of each dNTP, and 2.5 U *Taq* polymerase.

For sequencing, amplifications were performed on the same starting material with previously described primers, ED31/ED33 for outer primers [19] and BH1E101 (biotinylated, 5′-CTGTTTAATGGCAGTCTAGCA-GA-3′) and V4 (5′-CAGTAGAAAAATTCCCC-3′) for the inner primers. Cycling conditions were as follows: for the first round, three cycles of 94°C for 1 min, 37°C for 1 min and 72°C for 1 min were 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 were performed, with a final extension of 7 min at 72°C. The reaction mixture consisted of 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 9), 0.1% Triton X-100, 1.4 mmol/l $MgCl_2$, 20 pmol of each primer, 0.2 mmol/l of each dNTP, and 2.5 U *Taq* polymerase. The PCR amplification products were detected by electrophoresis on a 1% agarose gel and visualised by ethidium bromide staining.

Heteroduplex formation

Heteroduplex molecules were obtained by mixing two divergent PCR-amplified DNA fragments (the unknown patient strain with a plasmid from typed reference strains) denaturated at 96°C for 3 min and renaturated by rapid cooling on wet ice. The reference strains used in this study were as follows: 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, Zaïre), H3 (VI997, Belgium). The reaction was performed in 100 mmol/l NaCl, 10 mmol/l Tris-HCl (pH 7.8) and 2 mmol/l EDTA in a final volume of 8 µl. The heteroduplex formation was resolved by electrophoresis analysis at 250 V for 3 h on a non-denaturing 5% polyacrylamide gel in Tris–boric acid–EDTA buffer (88 mmol/l Tris borate, 89 mmol/l boric acid, 2 mmol/l 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

Amplified products were purified by immobilization on streptavidin-coated magnetic beads (Dynabeads M280-streptavidin, Dynal AS, Compiègne, France). Both strands were separated by denaturation with NaOH and were purified separately. Sequencing primers were H1E101 (5′-AATGGCAGTCTAGCAGAA-3′, forward) and H1E203 (5′-GTAATTTCTAAATCCC-CTCCTGAG-3′, reverse). Cycle sequencing was performed using fluorescent dye terminator technology (dye terminator cycle sequencing with AmpliTaq DNA polymerase FS, Perkin Elmer, Roissy, France) according to the instructions of the manufacturer. Electrophoresis and data collection was performed on an Applied Biosystems 374 automatic DNA sequencer.

Phylogenetic analysis

Nucleotide sequences were aligned by using CLUSTAL V [20] program with minor manual adjustments considering their predict protein sequence. Regions that could not be aligned unambiguously, due to length or sequence variability, were omitted from the analysis. The newly determined C2–V3 nucleotide sequences were aligned with known HIV-1 sequences representing different genetic subtypes and the sequence of $SIV_{cpz-gab}$. The previously reported HIV-1 sequences are as follows: CA5, LAI, and MN for subtype B; NDK and ELI for subtype D; U455 and KE 89 for subtype A; BZ-126AC for subtype F; ZAM20, NOF and DJ2591 for subtype C; CAR4017.2 and TN2432 for subtype E. Evolutionary distances were computed using Kimura's empirical two-parameter period [21]. A distance matrix was then used to estimate phylogenetic relationships by the neighbour-joining method [22]. Reliability of the branching orders was confirmed by the bootstrap approach [23]. All analyses were performed using CLUSTAL V [24].

Results

Population studied

A total of 61 HIV-positive individuals were studied. All these persons were selected for overseas deployment and were tested for HIV antibodies in the year that they are due to leave for overseas. Among the 61 individuals, nine tested positive for HIV antibodies (screening by enzyme-linked immunosorbent assay and confirmation by Western blot) at this initial visit and were therefore not allowed to deploy overseas. Since their test was positive at the screening visit and based on their epidemiological history, they most probably became infected with HIV in France. None of them

had a history of injecting drug use or transfusion with blood or blood-derived products. The other 52 individuals were HIV-negative at the initial visit but were positive when they came back to France, and therefore most probably became infected overseas. None of these 52 patients had a history of injecting drug use or blood transfusion. Table 1 summarizes the year of infection or diagnosis, age, risk of HIV exposure, the country of overseas duty and the genetic subtype as determined by HMA or sequencing of the C2–V3 region for all the patients in our study. For the individuals who tested positive after overseas deployment, the year of HIV diagnosis corresponds to the year of HIV infection. The mean age for our patients was 33.5 years (range, 26–54 years). The majority of the HIV-infected individuals were deployed to Djibouti (East Africa; n = 33). The remaining 19 subjects performed duties in Cambodia (n = 5), Cameroon (n = 1), Central African Republic (n = 4), Gabon (n = 1) and Chad (n = 2), Côte d'Ivoire (n = 1), Mayotte (located in the Indian Ocean near Madagascar; n = 1) and French Guiana (n = 4). Only one women was included in the study, she was positive at the initial screening visit (patient MP 039).

Genetic subtyping of HIV-1 strains

In order to determine the genetic subtype of the HIV-1 viruses, HMA was performed on heteroduplexes obtained by reannealing the 0.7 kb *env* PCR product, corresponding to the V3–V5 region, of each sample with the corresponding PCR products of the HIV-1 reference strains. All subtype assignments were based on reactions with the reference sequences. In this group of patients, six different genetic subtypes (A, B, C, D, E and F) were identified by HMA and sequencing of C2–V3 sequences as shown in Table 1. Significantly more B subtypes were observed in the patients who acquired HIV-1 infection in France, nine out of nine (100%) versus 20 out of 52 (38.5%) in patients who went on overseas duty. Among the 52 patients who acquired HIV infection after overseas deployment, eight (15.3%) were infected with subtype A, 20 (38.5%) with subtype B, 16 (30.7%) with subtype C, one (1.9%) with subtype D, six (11.5%) with subtype E, and one (1.9%) with subtype F.

All the 16 HIV-1 subtype C-infected individuals were deployed to Djibouti. Five of the six subtype E-infected individuals were deployed to Cambodia and one to Djibouti. Subtype A infections were acquired after deployment to French Guiana (n = 1), Djibouti (n = 5) and Central African Republic (n = 2). Subtype B infections were acquired in Djibouti (n = 11), Cameroon (n = 1), French Guiana (n = 3), Chad (n = 2), Gabon (n = 1), Mayotte (Comores Island; n = 1) and Côte d'Ivoire (n = 1). Subtype D and F were acquired in the Central African Republic.

Since 1987 non-B HIV-1 strains have been introduced into France by this population group, and since then the number and the variety of non-B strains has increased.

Nucleotide sequence and phylogenetic analysis of the C2–V3 region

In order to complete the qualitative analysis started by HMA analysis, approximately 260 base-pairs (the C2–V3 region of the *env* gene) were directly sequenced from the PCR products. The nucleotide sequences were compared pairwise and the homology rates were obtained by alignment analysis. The nucleotide homologies were calculated and the average divergence within the different genetic subtypes is as follows: 16.4% (range, 6.3–21.0%) for subtype A strains, 15.6% (range, 7.4–22.0%) for subtype B strains, 14.8% (range, 6.3–23.9%) for subtype C, and an average of 5.5% (range, 3.1–10.8%) for type E. The highest homology was observed between the type E strains.

In order to perform phylogenetic studies, the nucleotide sequences from our study were aligned among themselves and with databank sequences, in particular with HIV-1 sequences from the different clades (A–H), and with the chimpanzee $SIV_{cpz-gab}$ strain as an outlier, a phylogenetic tree was constructed (Fig. 1). The genetic subtypes identified by HMA analysis were confirmed in the phylogenetic analysis. Seven HIV-1 strains were not typed by HMA, because the amount of amplified product was too low or PCR with ES7/ES8 primers remained negative, probably due to too many mismatches between primers and target DNA. These strains were identified by the phylogenetic analysis of the C2–V3 sequences as subgroup A (n = 1), subgroup B (n = 4), subgroup C (n = 1) and subgroup E (n = 1). The phylogenetic analysis of group A and C suggests two clusters, although supported by relative low bootstrap values. The possible geographic origin of the subtype A strains is very diverse. This group includes isolates from Djibouti, Côte d'Ivoire, Central African Republic and French Guiana, and the infections occurred between 1987 and 1995. The group C strains all originated from Djibouti, although the infections were acquired at different periods between 1990 and 1995.

The group E strains formed a small cluster and there was less variability in this subtype, five of the six subtype E strains were from Cambodia and these individuals were infected between 1992 and 1993. The only subtype F strain obtained from a patient after deployment in the Central African Republic clustered with the reference strains from genetic subtype F. The B strains were obtained from different geographic regions and form a heterogeneous group.

Table 1. Epidemiological summary from the HIV-1-infected individuals included in the study.

Patient	Age (years)	Year of diagnosis	Epidemiological exposure	Risk country of overseas duty	HIV genetic subtype	
					HMA	C2–V3 sequence
MP 023	38	1987	Heterosexual	Djibouti	A	A
MP 058	39	1989	Heterosexual	CAR	–	A
MP 117	34	1990	Heterosexual	Guyana	A	A
MP 020	34	1991	Heterosexual	Djibouti	A	A
MP 031	42	1992	Heterosexual	Djibouti	A	A
MP 033	36	1992	Heterosexual	Djibouti	A	A
MP 026	27	1993	Heterosexual	Djibouti	A	A
MP 246	38	1995	Heterosexual	CAR	A	A
MP 013	40	1986	Heterosexual	–*	B	NT
MP 027	32	1987	Heterosexual?	Chad	B	B
MP 032	33	1987	Heterosexual	Chad	B	B
MP 122	50	1987	Heterosexual	Cameroon	B	B
MP 053	43	1988	Heterosexual	Djibouti	B	B
MP 006	41	1989	Heterosexual	Mayotte	B	B
MP 007	29	1990	Heterosexual	Djibouti	B	B
MP 029	34	1990	Heterosexual	Djibouti	B	B
MP 024	31	1990	Heterosexual	–*	B	NT
MP 078	38	1991	Heterosexual	Djibouti	B	B
MP 018	31	1991	Heterosexual	–*	NT	B
MP 093	32	1991	Heterosexual	Djibouti	B	B
MP 054	39	1992	Heterosexual	Côte d'Ivoire	–	B
MP 045	39	1992	Heterosexual	Djibouti	B	B
MP 052	36	1992	Heterosexual	Djibouti	B	B
MP 016	27	1992	Heterosexual	–*	B	NT
MP 051	29	1993	Heterosexual?	Gabon	B	B
MP 159	23	1993	Heterosexual	Djibouti	B	B
MP 025	34	1993	Heterosexual	Guyana	B	B
MP 036	54	1993	Heterosexual	Djibouti	B	B
MP 047	33	1993	Heterosexual	Guyana	–	B
MP 039	29	1994	Heterosexual	–*	B	B
MP 077	42	1994	Heterosexual?	–*	B	B
MP 030	28	1994	Heterosexual	–*	NT	B
MP 034	29	1994	Heterosexual	–*	NT	B
MP 061	26	1995	Heterosexual	Djibouti	–	B
MP 110	41	1995	Heterosexual	–*	B	B
MP 291	24	1995	Heterosexual	Guyana	–	B
MP 329	30	1995	Heterosexual	Djibouti	B	B
MP 019	40	1990	Heterosexual	Djibouti	C	C
MP 003	40	1991	Heterosexual	Djibouti	C	C
MP 040	35	1991	Heterosexual	Djibouti	C	C
MP 197	32	1991	Heterosexual	Djibouti	C	C
MP 041	31	1992	Heterosexual	Djibouti	C	C
MP 043	25	1992	Heterosexual	Djibouti	C	C
MP 130	30	1992	Heterosexual	Djibouti	C	C
MP 148	38	1992	Heterosexual	Djibouti	C	C
MP 037	28	1993	Heterosexual	Djibouti	C	C
MP 129	44	1993	Heterosexual	Djibouti	C	C
MP 169	27	1993	Heterosexual	Djibouti	C	C
MP 300	31	1993	Heterosexual	Djibouti	–	C
MP 098	32	1994	Heterosexual	Djibouti	C	C
MP 102	27	1994	Heterosexual	Djibouti	C	C
MP 292	24	1994	Heterosexual	Djibouti	C	NT
MP 083	32	1995	Heterosexual	Djibouti	C	C
MP 153	32	1995	Heterosexual	CAR	D	D
MP 059	31	1992	Heterosexual	Cambodia	–	E
MP 038	39	1993	Heterosexual	Cambodia	E	E
MP 044	27	1993	Heterosexual	Cambodia	E	E
MP 048	26	1993	Heterosexual	Cambodia	E	E
MP 126	26	1993	Heterosexual	Cambodia	E	E
MP 323		1995	Heterosexual	Djibouti	NT	E
MP 084	39	1992	Heterosexual	CAR	F	F

*Individuals who were not deployed overseas because of a positive HIV test at the initial medical visit. HMA, heteroduplex mobility assay; CAR, Central African Republic; NT, not tested.

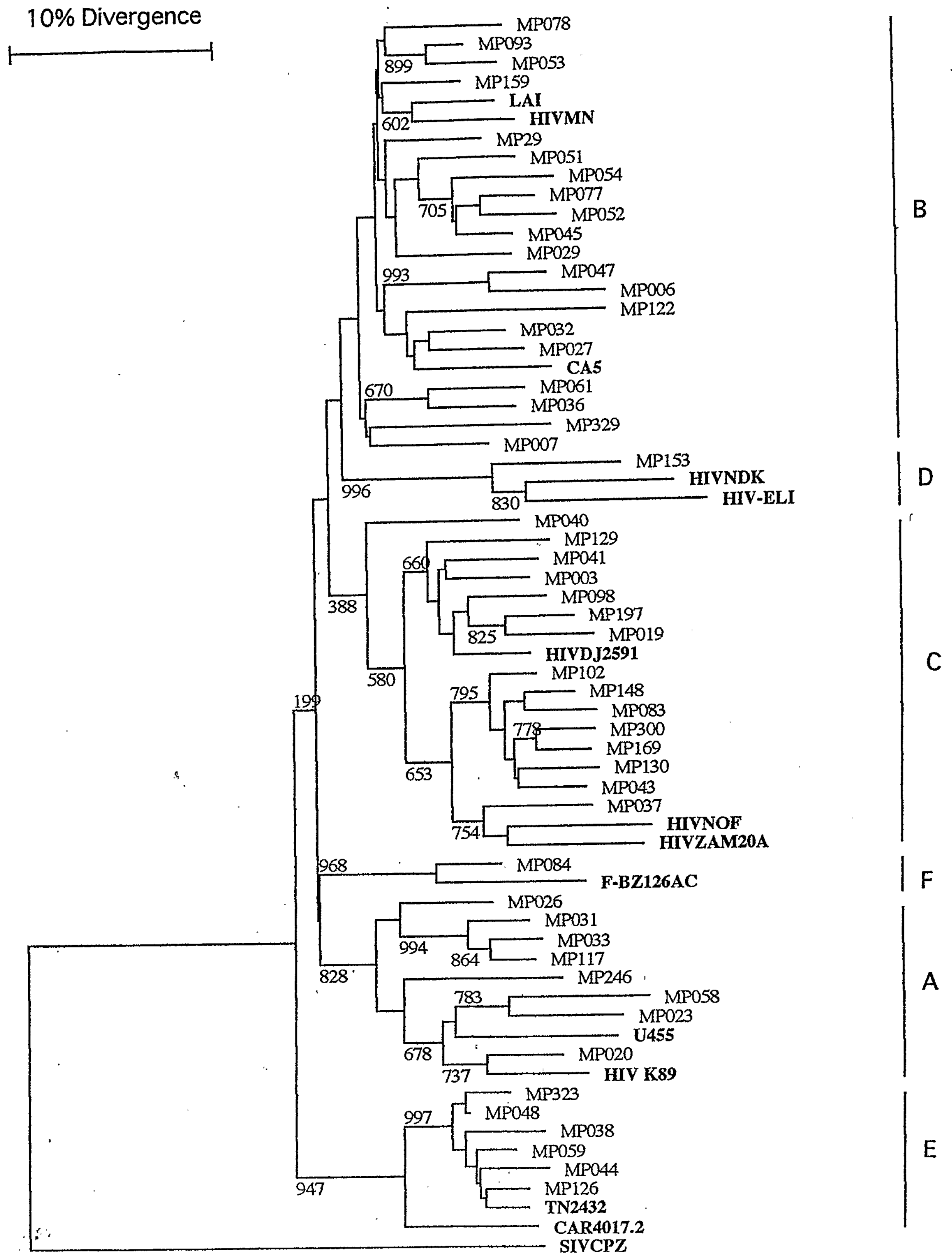

Fig. 1. Phylogenetic tree based on 260 unambiguously aligned positions of the HIV-1 *env* gene C2–V3 region from 51 new HIV-1 isolates and 13 HIV-1 reference strains representing different genetic subtypes. The tree was rooted with a corresponding region of the chimpanzee $SIV_{cpz\text{-}gab}$ isolate being used as an outgroup. The analysis was performed as described in Materials and methods.

Analysis of the V3 loop

The amino-acid sequences corresponding to the V3 region of the HIV-1 strains from our study were aligned on the basis of the subtyping results and were compared to the consensus V3 sequences of the corresponding subtypes (data not shown). The different genetic subtypes of HIV-1 identified in our study present distinct V3-loop patterns. Only in the B subtype GPGR is the most common tip of the V3 loop, and globally for the other genetic subtypes GPGQ is more prevalent. All the subtype A and E strains had the GPGQ tetramer. Among the 15 subtype C strains, 14 were GPGQ and one was GPGR. Twelve of the 16 subtype B strains were GPGR, from individuals infected in France and Djibouti. The GWGR motif was observed in two individuals infected in Djibouti, and the GLGR motif in a strain most probably from Cameroon. In six of the 15 subtype C strains from our study the highly conserved amino-terminal-linked glycolysation site proximal to the first cysteine in the loop was present. In the phylogenetic tree these isolates also form a separate group (MP 003, MP 019, MP 041, MP 129, MP 197 and MP 098).

Discussion

Early studies in Europe noted that the first heterosexual AIDS cases were among travellers, expatriates and workers returning from abroad, and the role of cross-border travel in the transmission of HIV was recognized soon after AIDS was first described [25]. Given the wide dispersal of HIV-1 subtypes internationally and the routine occurrence of international travel, it is inevitable that HIV spreads worldwide, but also that strains other than subtype B will eventually spread within Europe and the United States.

The genetic subtypes of HIV-1 in our study were determined by HMA and phylogenetic analysis of the C2–V3 sequences from the *env* region. The genetic subtypes identified by HMA were identical to those obtained by the phylogenetic analysis based on the corresponding V3 sequences. This confirms the accuracy of HMA for genetic subtyping.

This study demonstrates that strains other than subtype B are entering France. In the population we studied, approximately 53% of the newly detected HIV-1 isolates were non-B subtypes. In addition, in the group of HIV-infected individuals who were deployed overseas, 32 (61.5%) out of 52 patients were infected with non-B HIV-1 strains, and the HIV-1 subtype B viruses observed in this population were very heterogeneous. Two subtype B strains from individuals infected in Djibouti showed the GWGR pattern at the tip of the V3 loop. This pattern was previously only observed in subtype B strains from Brazil and Paraguay [1]. One subtype B strain had the GPGK tetramer motif, previously described in a limited number of B strains from Europe and the United States and in two subtype A strains from Uganda and the Central African Republic. One strain from a patient infected in Cameroon showed the GLGR tetramer motif; among the 967 V3 worldwide sequences described in the Los Alamos database [1], this motif has only been described in six B strains, one E strain from Thailand and one F strain from Cameroon. Among the subtype C strains, one had the GPGR tetramer motif typical for B strains; this is the first C strain described with this sequence at the tip of the V3 loop. For the majority (48 out of 55) of the subtype C strains described in the Los Alamos database, the highly conserved amino-terminal-linked glycosylation site proximal to the first cysteine in the loop is absent, but in our study in 40% (six out of 15) of the C strains this glycosylation site was present. In addition, in the phylogenetic tree, these strains seem to cluster in one group. However, the two clusters of subtype C strains are only supported by 65% bootstrap values and sequences of longer envelope fragments are necessary to confirm this. Divergent forms of the V3 loop may have very different biological and immunological characteristics from viruses which are similar to the consensus. In the population group that we studied the introduction of non-B strains and subtype B strains with an uncommon V3 loop motif had already started by 1987.

A surprisingly high number of individuals became infected with subtype B strains. One of the possibilities is that they became infected with HIV in France during the period between the HIV test and their actual departure, but it seems rather unlikely that 38% of individuals became HIV-positive during this relatively short period, which varies between 0 and 6 months. French Guiana is located in South America were the majority of HIV-1 infections are subtype B. Many of the subtype B infections were contracted in Djibouti which is located at the tip of East Africa. Several Western nations have important military bases in this small country, which means that there is a significant population of expatriates in this country. One of the possibilities is that subtype B strains have been introduced in this community by the expatriates and that this strain circulates among certain networks of prostitutes.

The possibility exists that biological or immunological differences among HIV-1 subtypes can have implications on transmission and efficiency of vaccines. To date, studies of HIV-1 and HIV-2 have provided clear evidence of differences in transmissibility of viral strains. Prospective cohort studies have shown lower mother-to-child transmission rates for HIV-2 compared with HIV-1 as well as less efficient rates of sexual trans-

mission [26–28]. In Thailand, the seroconcordance in couples is significantly higher in patients infected with subtype E strains than with subtype B [29], suggesting a higher risk of heterosexual transmission for subtype E HIV-1 viruses. *In vitro* findings suggest that subtype E viruses grow more efficiently in Langerhans' cells from genital tract mucosa than subtype B viruses [30], raising the possibility that the enhanced transmissibility of subtype E viruses is related to an intrinsic property of subtype E. However, further epidemiological and *in vitro* studies are necessary to confirm the eventual higher sexual transmissibility of subtype E viruses. Because HIV infection in France has been decreasing during the last 3 years in the homosexual population but continues to increase in the heterosexual population and in injecting drug users (IDU) [31], the introduction of non-B subtypes could modify the HIV epidemic in the future. This has been the case in Thailand, and between 1988 and 1993 the proportion of subtype E infections among IDU in Bangkok increased from 2.6 to 43.8% [19,32]. However, the impact of non-B strains on the epidemic in France has to be analysed more in detail and in a prospective manner. The returning army personnel represents only a small proportion of the HIV-infected persons in France, and their influence on changing the predominance of subtype B strains in France will be limited. A study in Belgium showed that non-B HIV-1 subtypes have been present in Belgium for some time without any evidence of an epidemic among heterosexuals [33].

As with diagnostic tests for HIV-1, most vaccine candidates have been based on subtype B and not on subtypes prevalent in some geographic regions. It is therefore important to confirm whether neutralization efficiency is strongly influenced by the degree of genetic heterogeneity predicted by these subtypes. Kostrikis *et al.* [34] showed that neutralization serotypes do not directly correlate with HIV-1 genetic subtypes, although a more extended study is necessary to identify the exact number and nature of all neutralization serotypes of HIV-1. A vaccine developed with a subtype B strain will be less efficient in developing countries or in certain population groups in Europe that are preferentially infected with non-B strains.

As our understanding of the significance of different genotypes and phenotypes increases, knowledge of their frequency and distribution will play an important role in a timely and effective response to the HIV pandemic. The concerns related to the genetic variability discussed above emphasize the importance of monitoring the molecular epidemiology of HIV by systematically collecting isolates from different populations with various transmission risk factors to help understand the degree of genetic diversity within subtypes and to determine which subtypes predominate in these populations. Our study, together with other reports, clearly documents that strains other than subtype B have entered the Western hemisphere from Africa and Asia. Even within subtype B, variants have been introduced with V3-loop sequences which have previously been observed in Brazilian HIV-1 subtype B strains (GWGR motif of the tip of the V3 loop). In the near future, subtypes of new HIV infections should be taken into account in order to follow the natural history of the disease and the effect of antiviral therapy on the different genetic subtypes. For the population that we studied, the year of HIV infection was known, and since that time the individuals have undergone regular and intensive clinical and immunological follow-up. This group of individuals forms a good basis to initiate a prospective study on the eventual impact of the HIV-1 genetic subtype on the disease progression.

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