Sequence Note

High HIV Type 1 Subtype Diversity and Few Drug Resistance Mutations among Seropositive People Detected during the 2005 Second Generation HIV Surveillance in Madagascar

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ABSTRACT

Subtype determination and detection of drug resistant-associated mutations (DRM) were performed on 31 HIV-1 Western blot-positive sera during the 2005 second-generation HIV surveillance in Madagascar. Amplification and sequencing of at least one of the partial reverse transcriptase, protease, and partial envelope genes were successful for all strains. All three gene sequences were obtained for 28 strains. A high degree of subtype or circulating recombinant forms (CRF) was observed for these 28 strains: A-A1 (eight cases), CRF02_AG (six cases), B (five cases), C (three cases), CRF06_cpx (three cases), CRF10_CD, BC_CRF, and unique RF (one case each). According to the ANRS September 2005 DRM list and algorithm, no DRM was detected in the reverse transcriptase and only one strain bore three major DRM in the protease M46I, I84V, and L90M leading to resistance to indinavir, saquinavir, nelfinavir, atazanavir/ritonavir, and possibly lopinavir.

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1), which is a member of the Lentivirus genus, Retroviridae family, is characterized by a high level of genetic variation. The continuous expansion of HIV-1 genetic diversity over time is driven by the high error rate of the viral reverse transcriptase, by the rapid turnover of HIV-1 in infected individuals, by recombination events, by pressure generated by the host immune responses, and by antiviral drugs. The rate of genetic recombination in retroviruses is high, and this greatly contributes to this genetic variation.1 The existence of this extraordinary degree of genetic diversity has major implications in biology, such as infectivity, transmissibility, and vaccine development. It may also influence the diagnosis of HIV infection and the capacity of HIV-1 to develop drug resistance mutations.2 HIV-1 variants have been assigned to three groups: M (major), O (outlier), and N (outlier). The group M has been subdivided further into subtypes A-D, F, G, H, J, and K. On the basis of phylogenetic analysis of the complete genome, 16 recombinant viruses between subtypes have been identified (CRF_01 to CRF_16) (http://hiv-web.lanl.gov/content/hiv-db/HelpDocs/subtypes-more.html). The geographic distribution of the different groups and subtypes, in Africa, is very heterogeneous. HIV-1 group O seems to be endemic in Cameroon and neighboring countries in west-central Africa and group N viruses have been identified in only a limited number of individuals from Cameroon. In group M, although subtypes A, C, and CRF02_AG are the most frequent in Africa, the distribution of HIV variants is very heterogeneous across this continent (http://www.hiv.lanl.gov/content/hiv-db/geography/geography.comp).

HIV prevalence in Madagascar was estimated in 2004 as around 1.8% among the 15–49 year old group and transmission is being largely driven by unprotected heterosexual contact.3 Because HIV-1 variants have not yet been described in the country, we conducted a molecular study to determine the subtype distribution and the prevalence of HIV-1 drug resistance-associated mutations (DRM) based on seropositive people detected during the 2005 second-generation HIV surveillance in Madagascar.

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Forty sera out of the 41 positive detected during this 2005 surveillance were available for our study. The 41 sera were detected positive with the two rapid tests Determine HIV1/2 (Abbott Laboratories, Tokyo, Japan) and ImmunoCom II HIV 1 & 2 BliSpot (Organics, Yavne, Israel) among the 8418 persons sampled during the 2005 second-generation HIV surveillance in Madagascar. The populations studied included female sex workers (SW), patients with sexually transmitted infection (STI), and pregnant women (PW). These 40 sera were tested for confirmation using HIV-1 Western blot (HIV-1 New LAV-Blot, Bio-Rad, Marnes la Coquette, France).

RNA was extracted from the 40 sera using the QIAamp Viral RNA mini kit (Qiagen, Courtaboeuf, France), reverse transcribed to cDNA, and amplified for partial reverse transcriptase (rt), protease (prot), and envelope (env) (C2V3) genes using the “Agence Nationale de Recherche sur le Sida” (ANRS) consensus sets of primers (http://www.hivfrenchresistance.org/2005/ANRS_procedures_03_2005.pdf). The polymerase chain reaction (PCR) amplification products were detected by electrophoresis on a 2% agarose gel containing ethidium bromide. The fragments obtained were sequenced on both strands using Genome Express Company (Meylan, France). For some samples, due to the presence of nonspecific bands, PCR products were purified with the QiaQuick gel extraction kit (Qiagen, Courtaboeuf, France) before sending to the company. Unverified sequences and chromatograms sent by the company were compared and sequences were corrected when needed. Subtype identification was performed using the Blast algorithm from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi). The presence of DRM in the protease and the reverse transcriptase was detected, and drug susceptibility based on subtype B was predicted from the ANRS algorithm updated in September 2005 (http://www.hivfrenchresistance.org/tab2005.html).

Thirty-one of the 40 sera were confirmed HIV-1 positive using HIV-1 Western blot (HIV-1 New LAV-Blot, Bio-Rad, Marnes la Coquette, France). The distribution of the 31 HIV-1 confirmed seropositive patients is indicated in Fig. 1 according to the place of sampling and the population status. Amplification of partial rt, prot, and env (C2V3) genes was positive for 31, 29, and 28 patients, respectively. No amplified product was obtained from the 9 HIV-1 Western blot nonconfirmed seropositive patients. The result of the subtyping based on the sequences of the three amplified fragments was available for the strains of 28 patients. Eight subtypes or circulating recombinant forms (CRF) were detected among the 28 strains (Fig. 1). Subtype A,

![Map of Madagascar showing distribution of HIV-1 Western blot confirmed seropositive patients and serotypes according to the 13 HIV surveillance sites in Madagascar in 2005: (●) sites for pregnant women (PW); (■) sites for patients with sexually transmitted infections (STI); (▲) sites for female sex workers (SW). *One B strain in Mahajanga and one B strain in Toliara subtyped using only the rt sequence and one C strain in Antsiranana subtyped using the rt and prot sequences.](image)
CRF02_AG, and subtype B were the main predominant forms (eight, six, and five strains, respectively) followed by subtype C (n = 3), CRF06_cpx (n = 3), CRF10_CD (n = 1), BC_CRF (n = 1), and unique RF (n = 1). The protease, reverse transcriptase, and env regions of the URF were clearly CRF02_AG, CRF06_AG, and CRF02_AG, respectively. Two additional subtype B were detected when subtyping was based only on the rf gene for two patients (env and prot sequences not available) and one additional subtype C when based on rt and prot genes for one patient (env sequence not available). Although no association could be found between subtype distribution and groups or sampling sites, interestingly, CRF02_AG, the most detected subtype, was found only in Antsiranana sampling sites and among all the sample groups (Fig. 1). No DRM was found in the rt. Minor DRM were detected in prot, linked for some of them to the polymorphism of the subtypes, i.e., mainly I13V, E35D, M36I, R41K, and H69K (data not shown). Only three major DRM, M46I, I84V, and L90M, were detected in the protease and all of them in the BC_CRF strain of a female sex worker sampled in Mahajanga. When using the ANRS algorithm this strain was found resistant to indinavir, saquinavir, nelfinavir, atazanavir/ritonavir, and possibly lopinavir. Five other strains (from subtype A, A1, or CRF02-AG) carrying a combination of four minor mutations among L10I, I13V, K20R, L33F, M36I, or H69K were possibly resistant to tipranavir/ritonavir.

This is the first study providing data about the subtypes circulating in Madagascar. One should notice the high degree of subtype diversity. This diversity and the low prevalence observed in Madagascar in the 2005 HIV serosurveillance favor multiple and recent entries of HIV-1 strains in the country. Furthermore, other subtypes or CRF could be present in Madagascar. Actually, according to the sampling size of the serosurveillance and the prevalence of HIV-1 infection observed in 2005, we could detect a subtype or a CRF if it were present with a power of 20%. Because all CRF02_AG strains have been identified in only one sampling site (Antsiranana) and among the three groups (SW, PW, and STI), it could be hypothesized that an older and/or a more active chain of transmission is occurring in this area. More extensive HIV surveys in this area would confirm or not confirm these data. Subtype A (or CRF02_AG) predominates in West and West-Central Africa; however, there is a decrease in the proportion of subtype A from West to East Africa and La Réunion island, through Uganda, Kenya, Burundi, and Tanzania.2 The detection of 50% of subtype A and CRF02_AG among the 28 samples (with complete subtyping) is in agreement with this observation. Subtype C was detected in only three cases and subtype B in the same proportion (five cases). Subtype C is largely predominant (>85%) and subtype B is quasi-absent in South and South-Eastern countries situated at the same latitudes as Madagascar.2,5 The more regular and frequent exchanges between Madagascar and France (including La Réunion Island close to Madagascar, where subtypes A and B are predominant) and between Madagascar and the francophone countries could explain this distribution. The CRF06_cpx (three cases), CRF10_CD (one case), and BC_CRF (one case) have also been sporadically described in African countries (http://hiv-web.lanl.gov/components/hivdb/new_geography/geography comp?region=world&form=al l). The prevalence of resistance of the strains (1/28) was at the same level as expected (0-5%) from untreated subjects in re-source-limited countries where HIV treatment has been recently available (http://www.who.int/bij5/publications/guidelines/en/ execsumm.pdf). However the 95% CI of prevalence of resistance of the strains is large (0.2-20.2%) because our sample size was limited: HIV prevalence observed during the 2005 serosurveillance in Madagascar was lower than expected according to the previous surveys. Consequently, further evaluation of transmitted HIV drug resistance using specimens from the serosurveillance should be based on a larger sample size. Furthermore, the increase of the sample size could allow the detection of seropositive cases in the highlands (Antananarivo and Fianarantsoa provinces) and consequently the subtyping of strains from this area.

SEQUENCE DATA

GenBank accession numbers for the sequences reported in the study are DQ345035 to DQ345065 for the partial reverse transcriptase gene, DQ345066 to DQ345034 for the protease gene, and DQ344978 to DQ345005 for the C2V3 envelope gene.

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