First seroepidemiological study and phylogenetic characterization of human T-cell lymphotropic virus type I and II infection among Amerindians in French Guiana

Antoine Talarmin, Bruno Vion, Abel Ureta-Vidal, Guénola Du Fou, Christian Marty and Mirdad Kazanjian

1 Laboratoire de Rétrovirologie, Institut Pasteur de la Guyane, BP 6010, 23 Av. Pasteur, 97300 Cayenne, French Guiana
2 Direction de l’Action Sanitaire et Sociale de la Guyane, 97300 Cayenne, French Guiana
3 Unité d’Immunité Cellulaire antivirale, Institut Pasteur, 75015 Paris, France

We investigated the serological, epidemiological and molecular aspects of human T-cell lymphotropic virus type I and II (HTLV-I/II) infection in the Amerindian populations of French Guiana by testing 847 sera. No HTLV-II antibodies were detected, but five individuals (0.59%) were seropositive for HTLV-I. Analysis of the nucleotide sequences of 522 bp of the env gene and the compete LTR showed that all of the strains from French Guiana belonged to the cosmopolitan subtype A. The similarities were greater between Amerindian and Creole strains than between Amerindian and Noir-Marron strains or than between Creole and Noir-Marron strains. Phylogenetic analysis showed two clusters: one of strains from Amerindians and Creoles, which belong to the transcontinental subgroup, and the other of strains from Noirs-Marrons, belonging to the West African subgroup. Our results suggest that the Amerindian HTLV-I strains are of African origin.

The human T-cell lymphotropic viruses, types I (HTLV-I) and II (HTLV-II), are members of a group of mammalian retroviruses with a tropism for T-lymphocytes. HTLV-I is the causative agent of adult T-cell leukaemia/lymphoma (ATLL) (Poiesz et al., 1980) and tropical spastic paraparesis/HTLV-I-associated myelopathy (Gessain et al., 1985). HTLV-I is endemic in some geographical areas such as southern Japan, sub-Saharan Africa, the Caribbean Basin and parts of South America (Gessain et al., 1996). Molecular epidemiological studies have shown few nucleotide changes in strains of HTLV-I that are specific for the geographical origins of patients. Four major geographical genotypes have been described (cosmopolitan, HTLV-I subtype A; Central African, HTLV-I subtype B; Melanesian, HTLV-I subtype C; and HTLV-I subtype D) after sequence analysis or restriction fragment length polymorphism from env and the long terminal repeat (LTR) (Gessain et al., 1992; Miura et al., 1994; Saksena et al., 1992; Ureta-Vidal et al., 1994b). Within subtype A, four subgroups can be distinguished: a transcontinental, a West African, a North African and a Japanese subgroup (Ureta-Vidal et al., 1994a, b).

HTLV-II has been shown to be endemic in various American Indian populations in the Americas (Biggar et al., 1996; Hjelle et al., 1993; Lairmore et al., 1990; Levine et al., 1993; Pardi et al., 1993). In South America, a number of distinct populations in Colombia (Ijichi et al., 1993; Switzer et al., 1995), Argentina (Biglione et al., 1999) and Brazil (Biggar et al., 1996; Black et al., 1994) have been found to be infected with this virus. HTLV-II infection has recently become epidemic among intravenous drug users (IVDUs) in North America (Hall et al., 1992; Murphy et al., 1998) and in Europe, especially in Italy (Salemi et al., 1998) and Spain (Soriano et al., 1993). Two molecular subtypes, HTLV-IIa and HTLV-IIb, have been found both in Amerindians and IVDU populations, but the HTLV-II subtype b, mostly prevalent in the Amerindians groups, is often referred to as the palaeo-Indian subtype (Dube et al., 1993; Eiraku et al., 1996; Lee et al., 1993; Switzer et al., 1996).

French Guiana is an overseas French administrative district in the Amazonian forest complex on the north-east coast of the South American continent, between Brazil and Surinam (Fig. 1). The population is made up of a large variety of ethnic groups, including Creoles (50%), who are mixed European and African descent, Amerindians (4%), Noirs-Marrons (5.4%), immigrants from Haiti (20%), Brazil (43.4%) and various Asian countries (Chinese, Hmongs (2-1%) and whites, mainly from metropolitan France (14.2%).

HTLV-I infection is well documented in French Guiana (Tuppin et al., 1995). A high seroprevalence of HTLV-I (8.0%) and a high incidence of cases of ATLL were found among the Noirs-Marrons, an isolated population descended from slaves.
who escaped from Surinam in the eighteenth century (Gerard et al., 1995; Plancoulaine et al., 1998). HTLV-I infection is less frequent among Creoles (A. Talarmin, unpublished data), and HTLV-I and -II infections have never been detected among the Amerindian populations of French Guiana. Six Amerindian tribes comprising about 4900 individuals live in French Guiana (Fig. 1). The Galibi and Wayana belong to the Karib linguistic family and are related to many tribes located between the Amazon and Orinoco rivers. Palikur and Arawack belong to the Arawack linguistic family, which is scattered throughout the Amazon Basin. The Wayampi and Emerillon, of the Tupi-Guarani linguistic family, are the northern tribes of an ethnic group which came from southern Brazil and Paraguay. The prevalence of HTLV-I/II infections in various Amerindian groups in French Guiana was investigated in a serological study. In order to determine the routes of transmission of these viruses, a familial enquiry, molecular characterization of the HTLV-I strains and a comparison with strains from other ethnic groups in French Guiana were carried out. We present here the first phylogenetic analysis of HTLV-I strains from French Guiana.

Sera were collected from Amerindians, after informed consent, between January 1992 and May 1997. All of the serum samples were screened for antibodies to HTLV-I/II by an enzyme immunoassay (Cobas Core Anti-HTLV-I/II EIA, Roche). Positive or borderline samples were analysed by Western blotting (HTLV blot 2.3, Diagnostic Biotechnology; Cambridge Biotech).

DNA extracted from uncultured peripheral blood mononuclear cells from some of the HTLV-I-seropositive Amerindians and from Noirs-Marrons and Creoles known to be infected with HTLV-I from previous studies (Talarmin et al., 1997) were used for PCR. As previously described by Gessain et al. (1992) and Mahieux et al. (1997) semi-nested PCRs were performed with several specific HTLV-I primers located within the LTR and env regions. PCR products were then purified, cloned and sequenced. Previously published env and LTR HTLV-I sequences and those isolated in French Guiana, were aligned with the clustal W program and then analysed by various programs in the PHYLIP package version 3.52c and the MEGA program version 1.025. Two methods were used to generate phylogenetic trees: the ‘maximum parsimony’ method with the DNAPARS program and the ‘neighbour-joining’ program with the modified approach (Mahieux et al., 1997). The SEQBOOT program was used to generate 100 to 500 data sets that are random resampled versions of the previously aligned sequences. For both methods, a consensus tree was constructed with the CONSENSE program and the ‘majority rule’ criteria.

A total of 847 sera from 545 Amerindian women and 302 Amerindian men (mean age, 24.5 ± 13.2; range, 2–83 years) were tested for antibodies to HTLV, with 35 positive reactions in the EIA test. With Western blotting, no HTLV-II-specific antibodies were detected; five women (0-59%) had antibodies to HTLV-I (one Ararwack aged 52, two Palikurs aged 22 and 53, and two Wayampis aged 16 and 28); 30 individuals had sera of indeterminate positivity. The HTLV-I rates of infection differed between the groups: Arawacks, 1/54 (1.9%); Galibis, 0/136; Palikurs, 2/78 (2.6%); Emerillons, 0/56; Wayanas, 0/385; Wayampis, 2/138 (1.4%). A family survey was conducted in the Haut-Oyapock area (Fig. 1) among the relatives of the two Wayampi women infected with HTLV-I. Neither mother of these women was infected with HTLV-I. The first husband of one of the women had an HTLV-I infection, but the route of transmission could not be determined for the second woman.

Sequence analysis of the 522 bp fragment of the gp21 showed that the four strains from the Amerindians were very closely related to each other (>99.4% nucleotide identity) and to the HTLV-I cosmopolitan prototype ATK (97.7–98.1% identity). The nucleotide identity was greater between the Amerindian and Creole strains (98.9–99.8%) than between the Amerindian and Noir-Marron strains (98.3–99.3%) or between the Creole and Noir-Marron strains (98.1–99.4%).

The four strains from the Amerindians were closely related with regard to the 424 bp fragment of the LTR sequence (>99.1% nucleotide identity) and were also related to the strains from the Creoles (98.1–100% identity). In contrast, the Noir-Marron strains were less similar (3.5–4.7% and 3.1–5.2% divergence with the Amerindian and Creole strains, respectively). The 14 new LTR sequences aligned almost perfectly with the HTLV-I ATK; however, some specific mutations were observed (data not shown).

A comparison of the entire LTR for one strain of each ethnic group demonstrated strong homology between the Amer-
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indian and creole strains (99.1% identity). the two strains were less similar to the noir-marron strain, with 97.1% identity for the amerindian strains and 97.2% for the creole strains. comparison with the prototype strains indicated clearly that all of the strains from french guiana belonged to the cosmopolitan subtype a. the nucleotide identity with atk strain (cosmopolitan subtype) was 97.9% for the amerindian strains, 98.0% for the creole strains and 97.1% for the noir-marron strains, whereas the percentage identity of the strains from french guiana varied from 94.8 to 95.1% with the central african subtype b el strain, from 90.9 to 91.3% with mel5 (melanesian subtype) and from 94.7 to 95.0% with pygi9 (subtype d).

two portions of the htlv-i genome were used for the phylogenetic analysis. the first was a 522 bp fragment of gp21 obtained from four amerindian, five creole and five noir-marron strains and the second portion was the ltr.

phylogenetic analysis of the gp21 env gene was performed with 60 htlv-i sequences (mahieux et al., 1997; yamashita et al., 1998), including the 14 new strains from french guiana and selected htlv-i sequences from each subtype. as seen in the phylogenetic tree (fig. 2), all strains from french guiana belonged to the cosmopolitan subtype (htlv-i subtype a), and two molecular clusters could be distinguished among them. the first corresponded to amerindian and creole strains, which were very similar to the strains in the transcontinental subgroup a, whereas the second cluster was made up of strains from noirs-marrons, which were closely related to the strains from the west african subgroup.

two phylogenetic studies were done for the ltr analysis, with either the complete ltr (fig. 3a) or a 301 bp ltr segment (fig. 3b). comparison of the ltr sequences of strains from french guiana with strains of other geographical origins confirmed the results of the env gene analysis (fig. 3).

although htlv-ii infection is endemic in many indian populations in south america, especially in brazil, this virus was not detected in our study. htlv-ii is therefore either not present or is present at very low prevalence in the amerindian population of french guiana. this situation might be due to the fact that the ancestors of the existing amerindians were not infected with htlv-ii.

htlv-i-infected amerindians were found only in tribes living along the oyapock river and near cayenne (wayamps, palikurs and arawacks). curiously, no htlv-i-infected individuals were detected among the indians living in the haut-maroni area, although they are in contact with the noirs-marrons, who are known to have high seroprevalence rates; however, interethnic mixing is rare between noirs-marrons and amerindians.

the molecular epidemiology of various htlv-i strains from french guiana indicates that amerindian and creole strains are closely related, suggesting a common origin for these strains. it is thought that amerindian populations have been infected through contacts with people from african origin after the slave trade (gessain et al., 1996; van dooren et al., 1998). however, it has recently been suggested that some amerindian populations were infected with htlv-i at the time of their migration to south america, since south american isolates are distinct from african strains (yamashita et al., 1998). these authors report that the difference between their view and the prevailing view is due to differences in the genomic regions used for the phylogenetic analysis. most researchers had previously used the env gene, while yamashita et al. (1998) used the ltr region. in our study, both regions were sequenced, and the phylogenetic analysis of the env gene shows that one amerindian strain from argentina is closer to strains from noirs-marrons than to strains from amerindians in french guiana (fig. 2). the comparison of a
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Fig. 3. Phylogenetic trees constructed by the ‘neighbour-joining’ method with the MEGA program with 37 HTLV-I isolates, including three new strains from French Guiana (CAM, NAR, NM1626), for the complete LTR (755 bp) (a); or with 52 HTLV-I isolates, including 14 new strains from French Guiana and five strains from Amerindians in Argentina (in bold), for a fragment of 301 bp of the LTR (b). For the LTR amplification two semi-nested PCRs were carried out to amplify the LTR region. A first fragment of 433 bp was amplified with 8255 and LTRU5E as the outer primers and 8255 and 420LTR as the inner primers, and a second fragment of 418 bp was amplified with P3LTR and 5PLTR as the outer primers and Tatabox and 5PLTR as the inner primers (Mahieux et al., 1997). Ptm3 (STLV-I isolate) was used to root the tree. The numbers indicated at some nodes (bootstrap values) represent the frequency of occurrence out of 500. The GenBank accession numbers of the nucleotide sequences for the complete HTLV-I LTR are AF063819 to AF063821 and AF076254 to AF076267 for the short fragment (424 bp) of the LTR.

common portion of the LTR (301 bp) of all strains from French Guiana and five Amerindian isolates from Argentina indicates that all these strains are closely related, suggesting a common origin (Fig. 3b). If the hypothesis of Yamashita et al. (1998) is correct, Creoles were infected through sexual contact with Amerindians, and the introduction of the virus into this ethnic group would be more recent than in Amerindians. Therefore, because the risk factors for HTLV-I infection are at least as great among Amerindians and Creoles, the seroprevalence should be higher in Amerindians. A recent study conducted in French Guiana to determine the seroprevalence of HTLV-I in the Creole population showed that HTLV-I seroprevalence is much higher among Creoles than among Amerindians (A. Talarmin, unpublished data). Therefore, the African origin of the Amerindian strains is indubitable and is supported by recent results providing evidence for post-Colombian in-
roduction of HTLV-I into Latin America (Van Dooren et al., 1998). Other factors in our study also lead to the conclusion that HTLV-I was introduced recently among Amerindians in French Guiana. First, the familial enquiry indicated that the two HTLV-I-infected women who agreed to participate were not infected through breast feeding, since their mothers were not infected. Second, if the virus had been introduced a long time ago, it should be more prevalent.

We are grateful to Dr A. Gessain for having initiated the prospective epidemiological study concerning HTLV-I in French Guiana and for critically reading the manuscript. To Dr R. Mahieux for providing us with some HTLV-I sequences and to Dr Joëlle Sankale-Suzanon and Dr André Lacante for their cooperation in this study. We also thank J. F. Pouliquin for excellent technical assistance.

References


Received 5 May 1999; Accepted 18 August 1999