In the North of Argentina, an endemic area for HTLV-1, intrafamilial transmission of this virus has been observed. The HTLV-1 status in 13 family members of a seropositive blood donor from the central region of Argentina (non-endemic area) was investigated. According to serological and molecular assays, four members of this family (the blood donor, the husband, a son, and a daughter-in-law) proved to be HTLV-1 positive. LTR, tax, and env sequences from the provirus infecting the family members were identical. This strongly suggests the intrafamilial transmission of the virus. This study demonstrated intrafamilial transmission of HTLV-1 in a non-endemic area of Argentina.

MATERIALS AND METHODS

Study Group

Thirteen members of the HTLV-1 seropositive blood donor's family were studied. All were born and live in the provinces of Cordoba and Entre Rios (a non-endemic area). After signing an informed consent form, these subjects were interviewed in order to collect demographic and epidemiological data, some information about their potential exposure to retroviral infection and about their previous personal and familial morbidity. This study group also underwent a detailed clinical examination and blood collection for laboratory testing. The Study Protocol was approved by the Institutional Review Board of the School of Medicine, National University of Cordoba, Argentina. The pedigree of the family is shown in Figure 1.

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Serological Assays

The diagnostic algorithm included a screening for antibodies against HTLV-1/2 by particle agglutination assay (Serodia HTLV-I, Fujirebio, Inc., Tokyo, Japan). Reactive samples were tested further with an “in house” indirect immunofluorescence assay (IFA) on MT-2 and Mo-T cell lines, and with Western blot (Bioblot HTLV, Biokit, Barcelona, Spain) according to the manufacturer’s instructions. All samples reactive by screening and positive by IFA and Wb were considered positive for HTLV-1 or HTLV-2 infection.

PCR Assays

High molecular weight DNA was extracted from the whole-blood samples of all the 14 subjects (the seropositive blood donor and the 13 family members). Polymerase chain reaction (PCR) was carried out to amplify 219-bp fragment of the \textit{tax} gene from 500 ng of genomic DNA samples following the protocols described by Vandamme et al. [1997]. All HTLV-1/2 positive samples were typed by specific Nested-PCR for HTLV-1 and HTLV-2 targeting the \textit{tax} region [Vandamme et al., 1997]. The PCR products were separated on 2% agarose gel and visualized under UV light after ethidium bromide staining.

A 657 bp fragment of the \textit{env} gene and the 672 bp fragment of the LTR region corresponding, respectively, to position 5684–6340 and position 124–796 in ATK (prototype Japanese HTLV-1 strain) were amplified by nested-PCR as were reported previously [Liu et al., 1994; Yang et al., 1997].

Sequencing and Molecular Subtyping

PCR products corresponding to HTLV-1 LTR, \textit{env}, and \textit{tax} fragments were purified using QIAamp PCR purification kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The LTR (672 bp), \textit{env} (657 bp), and \textit{tax} (219 bp) fragments were directly sequenced on both strands from the internal primer set by using BigDye Terminator Cycle Sequencing Ready reaction Kit and ABI model 377 automated DNA sequencer (Applied Biosystems). The alignment of the LTR, \textit{env}, and \textit{tax} sequences from family member’s strains was done using Clustal W and they were compared using Pairwise/Blast/NCBI. Genetic distance between the family’s isolates and the HTLV-1 homologous sequences strains from Argentina and South America were determined using MEGA version 7.0. These sequences were obtained from the GenBank using LTR region for which a fragment of 411 bp corresponding to the position 240–650 in the ATK strain were available. Evolutionary distances were estimated using the Kimura two-parameter method and relationships were determined using the neighbor-joining (NJ) method. Phylogenetic stability of the branching was statistically evaluated by performing 1,000 bootstrap replications. \textit{env} sequences with 570 bp were compared using Pairwise/Blast/NCBI. The new nucleotide sequences from the present study have been deposited in GenBank and assigned Accession Nos AY 347748 to AY 347751 and AY 349143 to AY 349146. Other HTLV-1 strains included in the analysis were: Mel 5 L02534 (outgroup); ATK j02029; PNG1 M8507; EL S74562; GB233 D23692;
HS35 D13784; PR52 U12806; BO U 12804; OD U12805; ARGMF AF007754; H5 M37299; BRASP22 AF014661; ATM j02030; MT2 M37747; ARGFF AF007752; KUW1 L42253; KUW2 L42225; KUW3 L42255; CH25 D23690; L195 D38396; SB170 D13784; ARGGAY AF007753; BRASP01 AF014655; ARGDOU AF007751; ARGDOT AF007755; CR1 K02722; AINU D23694; B12 Y16482; RK13 AF042071; WHP AF259264; ITIS Z32527, PYG19 L76310.

RESULTS

Of thirteen members of the HTLV-1 infected blood donor’s family studied, the husband (ARGF 4), a son (ARGF2), and a daughter-in-law (ARGF3, ARGF2’s wife) were found to be positive for specific antibodies against HTLV-1. The seropositive individuals were confirmed positive for HTLV-1 tax gene by PCR. The rest of the family members were negative for HTLV-1 and HTLV-2 by serological and molecular assays (Fig. 1).

All the family members were Spanish descendents. They were born and live in the central region of the country. Although ARGF4 had arthritis, all the other infected individuals were asymptomatic. All had low HTLV-1 antibody titer by IFA (≤1/64).

Index case, ARGF1, is a 60-year-old woman who was a prostitute during her youth. She received blood transfusion three times in her life after the birth of her son (ARGF2). In 1990, she received the last transfusion. She had a sexual relationship with her first husband (ARGF4) for 12 years.

ARGF2, the first son of ARGF1 and ARGF4, is a 42-year-old man who never received blood transfusion but who had sexual intercourse with several prostitutes without a condom. His mother (ARGF1) breast-fed him for more than 1 year.

ARGF3, wife of ARGF2, is a 43-year-old woman who received a blood transfusion in 1988. She had five sexual partners during her life and had never used a condom for sexual relationships. She was breast-fed for 2 years. She has been married to ARGF2 since 1978.

ARGF4 is an 84-year-old man who received blood transfusion in 2000. He has had 43 sexual partners during his life and he usually had sexual intercourse with prostitutes. He was breast-fed during 1 year. Nowadays, he has prostate cancer and arthritis.

The LTR, env, and tax partial sequences (1,500 bp) from the infected members of the family showed 100% identity (0.00% of genetic distance) (data not shown). The LTR sequence variation between Argentinean isolates and other South-American strains was less than 2% (Fig. 2).

Phylogenetic analysis of the 411 bp of LTR region and 570 bp of env gene of these samples confirmed the infection with HTLV-1a Cosmopolitan subtype and demonstrated that the strains clustered closely with other isolates from Argentina (Fig. 2).

DISCUSSION

The familial clustering of HAM/TSP in HTLV-1 endemic areas suggests that mother-to-child transmis-
sion of HTLV-1 within families might be the main route of infection. This assumption is supported by genetic evidence [Liu et al., 1994; Mahe et al., 2004]. On the other hand, it is generally accepted that sexual transmission of HTLV-1, especially male-to-female, is another important route of infection [Tajima et al., 1982; Stuver et al., 1993]. Despite a recent report about the sexual transmission of HTLV-1 between spouses in Japan [Iga et al., 2002], there is not enough genetic evidence of transmission of HTLV-1 between sexual partners.

Intrafamilial transmission of HTLV-1 in endemic areas for HTLV-1 infection has been well documented [Liu et al., 1994]. In the North of Argentina, an endemic area for HTLV-1, the risk factors for the infection and the routes for HTLV-1 transmission within families are under investigation. This study presents evidence of sexual and mother-to-child intrafamilial transmission of the virus in a non-endemic region of Argentina (Fig. 1).

Within this family, ARGF1 and ARGF4 had different risk factors for HTLV-1 infection. ARGF1 was a prostitute during her youth, and she had three blood transfusions during her life. As the transfusions occurred after her son’s birth (ARGF2), it can be inferred that she acquired the HTLV-1 infection by sexual contact and that the virus was transmitted to her son (ARGF2) afterwards, most probably by breast-feeding.

ARGF4 had 43 sexual partners in his life and never used a condom. This suggests that sexual transmission is likely to have been the route of infection. The complete identity of the provirus LTR sequences and a 100% similarity of the sequences corresponding to the structural genes gp46 and gp21 from the spouses, ARGF1 and ARGF4, strongly support the sexual transmission of the virus between them but due to the fact that ARGF1 and ARGF4 had several risk factors for HTLV infection during their lives, it is not possible to determine who transmitted the infection to the other. According to previous studies, the rate of infection by sexual route is higher for women than for men [Kajiya and Kashiwagi, 1989].

ARGF2, the son of ARGF1 and ARGF4, was breast-fed by ARGF1 for more than 1 year. The majority of the cases of HTLV infection in children occur in the postnatal period via breast-feeding with a confirmed transmission rate, which ranges from 18% to 39% [Tsuij et al., 1990; Wiktor et al., 1997]. The risk of transmission increases when breast-feeding is prolonged (>6 months) [Wiktor et al., 1997], and several maternal risk factors seem to be involved in enhancing the transmission rate, such as the high level of in vitro HTLV-1-expressing lymphocytes in breast milk and in peripheral-blood mononuclear cells [Sugiyama et al., 1986; Li et al., 2004]; a high titer of anti-HTLV-1 antibodies [Hino et al., 1987; Ureta-Vidal et al., 1999] and the presence of anti-Tax antibody [Hirata et al., 1992]. The long breast-feeding period ARGF2 received, and the complete identity of LTR and env proviral sequences from mother and son strongly suggest the vertical transmission of the virus.
ARGF3, wife of ARGF2, received a blood transfusion 12 years ago. However, the fact that she had provirus DNA sequences identical to her husband’s indicates sexual transmission of the virus from ARGF2 to ARGF3.

The epidemiological data and the identical LTR and env sequences found in the study of the family members infected with HTLV-1 strongly suggest horizontal transmission between husband and wife (ARGF1–ARGF4 and ARGF2–ARGF3) and the vertical transmission from mother-to-child (ARGF1–ARGF2), presumably via breast-feeding [Hino et al., 1995]. The complete identity of sequences in the family members shows the transmission within the family cluster and the high genetic stability of the HTLV-1 strain over extensive periods of time.

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REFERENCES


