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Sequence Note

Human Retroviruses (HIV and HTLV) in Brazilian Indians: Seroepidemiological Study and Molecular Epidemiology of HTLV Type 2 Isolates

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ABSTRACT

To investigate serological, epidemiological, and molecular aspects of HTLV-1, HTLV-2, and HIV-1 infections in Amerindian populations in Brazil, we tested 683 and 321 sera from Tiriyo and Waiampi Indians, respectively. Both HIV-1 and HTLV-2 infections were detected at low prevalence among the Tiriyos whereas only HTLV-1 was present among the Waiampis, also at low prevalence. Analysis of the nucleotide sequence of the 631 bp of the *env* gene obtained from the three HTLV-2 isolates detected among the Tiriyos demonstrated by restriction fragment length polymorphism that these viruses belong to subtype IIa. Phylogenetic analysis of this same fragment showed that these sequences cluster closer to HTLV-2 isolates from intravenous drug users living in urban areas of southern Brazil than to the same gene sequence studied in another Brazilian tribe, the Kayapos. Our results confirm the distribution of Brazilian HTLV-2 sequences in a unique cluster I and cluster IIa and suggest that there is a considerable degree of diversity within this cluster. We also report for the first time HIV-1 infection among Brazilian Amerindians.

INTRODUCTION

HUMAN T CELL LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) and type 2 (HTLV-2) are retroviruses widely spread throughout the world.[1,2] HTLV-1 is related to adult T cell leukemia (ATL)[3] and to a neurologic syndrome called HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).[4,5] Until now there have been no reported oncogenic effects of HTLV-2; however, cases of HTLV-2-associated diseases have been described.[6]

HTLV-2 infections were first detected in intravenous drug users (IDUs) coinfected with HIV-1 in North America, Europe, and Southeast Asia.[7–11] Several seroprevalence studies have demonstrated endemic foci of HTLV-2 in Amerindians.[12–27] In Brazil HTLV-2 is reported as endemic in several Indian populations with high prevalence: Kayapo (33.3–57.9%),[18,24,28,29] Kraho (12.2%),[24] Mundukuru (8.1%), Tiriyo (15.4%), and Arara do Laranjal (11.4%).[29]

In a study of HTLV-2 isolates from IDUs living in the United States two subtypes were suggested (2a and 2b), on the basis of two distinct restriction fragment length polymorphism (RFLP) patterns.[9] Later phylogenetic studies of HTLV-2 have led to the establishment of three main subtypes: HTLV-2a, HTLV-2b,[9,29–32] and HTLV-2d.[33] Although HTLV-2a and -2b

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have been reported in various Amerindians with a predominance of subtype 2b.[12,25,27,34] Only HTLV-2a has been detected in Brazilian Indians[12,19,26,28,29] and in Brazilian IDUs.[15,35] It has been suggested that the Brazilian HTLV-2a isolates might be a distinct molecular variant of the prototype 2a.[29] Furthermore, the study of one Brazilian IDU HTLV-2a isolate showed that this virus does not have the characteristic *tax* stop codon seen in the subtype 2a *tax* gene, thus sharing some similarity with the extended HTLV-2b Tax protein.[15,35]

To date, a high prevalence of HTLV-2 infections and no human immunodeficiency virus type 1 (HIV-1) infections have been demonstrated in Brazilian Indians. In the present article, we report a low prevalence of HTLV-2 and an introduction of HIV-1 subtype B infection in these populations. Furthermore, we report the first *env* phylogenetic analysis of HTLV-2 from Tiriyo Indians and confirm that the HTLV-2 isolates belong to a molecularly distinct variant of the 2a cluster.

MATERIALS AND METHODS

This work was performed in the framework of the Brazilian Ministry of Health National Program for STD/AIDS control. Indeed, because of a suspected AIDS case in a Tiriyo Indian, actions were taken to evaluate the magnitude of a potential HIV outbreak in this population. Thus, a prevalence survey was carried out in almost 100% of the Tiriyo and Waiampi tribes. The Waiampi tribe was included because it is well known that both tribes have relationships with each other.

Study population, serology, and HIV-1 subtyping

The Tiriyo Indian population speaks the Caribe language and lives at the border between Suriname and Brazil. From a total of about 1700 individuals, 750 live in Brazil spread throughout 15 villages. The Waiampi Indian population speaks the Tupi-Guarani language and lives at the border between French Guyana and Brazil. From a total of 1200 individuals, 450 live in Brazil in the State of Amapa. Briefly, the sampling from the two tribes was represented approximately equally between the sexes: 50% male and 50% female. The majority of the individuals (83% among the Tiriyos and 89% among the Waiampis) were under 40 years of age. In this category, 37 and 22% are represented by children between 0 and 10 years old whereas 46 and 67% are represented by individuals of reproductive age (between 11 and 39 years old) in the Tiriyo and Waiampi tribes, respectively.

Samples (5 ml of intravenous blood) were collected from the Tiriyo and Waiampi tribes between April 1997 and May 1997 into tubes containing EDTA and without anticoagulant, respectively. These samples were left at room temperature to allow spontaneous sedimentation and coagulation. Plasma, sera, and buffy coat were collected, frozen in dry ice, and shipped by plane to the Advanced Public Health Laboratory (LASP/CPqGM/Fiocruz) in Salvador, where they were kept at −70°C until use.

According to the algorithm recommended by the Brazilian Ministry of Health, with small modifications, plasma and sera were screened for HIV-1 and -2 and HTLV antibodies. The samples were screened for HIV-1/2 by two enzyme-linked immunosorbent assay (ELISA) methods simultaneously: Enzygnost Anti-HIV-1/2 Plus (Behring, Marburg, Germany) (gp32) and Vironostika R/HIV-UNI-FORMII Plus O (Organon Teknika, Boxtel, The Netherlands). Repeatedly reactive samples were subjected to indirect immunofluorescence assay (IFA) (Biomanguinhos, Fiocruz, Rio de Janeiro, Brazil) and Western blot (WB) (HIV Blot 2.2; Genelab Diagnostics, Singapore). WB patterns were interpreted according to the manufacturer instructions. Specimens were considered HIV-1 positive (two *env* bands [gp160/gp41 and gp120] and *gag* bands [p17, p24, and p55] or *pol* bands [p31, p51, and p66]), negative (absence of virus-specific bands or p17 only), or indeterminate (virus-specific bands present, but pattern does not meet the criteria for positive).

HTLV-1/2 samples were screened by ELISA (rp21e-enhanced EIA; Cambridge Biotech, Worcester, MA). Repeatedly reactive samples were confirmed as HTLV-1 or HTLV-2 by WB (HTLV Blot 2.4; Genelab Diagnostics). Results were interpreted according to the manufacturer instructions, namely (1) HTLV-1 positive: *gag* p19 with or without p24 and two *env* (gp21 and gp41-I; (2) HTLV-2 positive: *gag* p24 with or without p19 and two *env* gp21 and rgd46-II; (3) HTLV positive: *gag* p19 and *env* gp21; (4) indeterminate: specific bands detected but did not meet the criteria for positive HTLV-1, HTLV-2, or HTLV; or (5) negative: absence of specific bands. The HIV-1 subtype was identified by heteroduplex mobility assay (HMA) as described elsewhere.[36]

Polymerase chain reaction

Genomic DNA was extracted from buffy coat from Tiriyo samples with positive and indeterminate serology, using the GFX genomic blood DNA purification kit (Amersham Pharmacia Biotech, Piscataway, NJ). First-round polymerase chain reaction (PCR) was performed on the extracted DNA from the indeterminate serology samples to amplify a fragment of the *env* gene, using the primers 12E3 (5′-GACCACCAACACCATGGG-3′) and 12E4 (5′-GCTAGGGCGGAG ACAAGCC-3′). These primers amplify a fragment of the 987 and 975 bp for the HTLV-1- and HTLV-2-positive samples, respectively, corresponding to nucleotides (nt) 5169–5186 and 6123–6141 of the HTLV-2 Mo isolate. A second round was carried out with nested primers 1E8 (5′-TCAAGAAGTTTCACACCTCAAT-3′) and 1E5 (5′-GATTTCCATGGTATAGAGGGCT C-3′), which amplify a 198-bp fragment of HTLV-1, corresponding to nt 5607–5628 and 5782–5804 of the HTLV-2 Mo isolate. An alternative second round was carried out with the nested primers 8 (5′-CAGCCAAGTGTCCCTTCGA-3′) and 9 (5′-ATTTGAGTATTTTGGTCG-3′), which amplify a fragment of 203 bp for HTLV-2 corresponding to nt 5602–5620 and 5787–5804 of the HTLV-2 Mo isolate. Reactions were performed in 50-μl reaction mixtures containing 1.5 μg of DNA, 2.5 U of *Taq* DNA polymerase, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, deoxynucleotide triphosphates, (0.2 mM each), and 10 pmol of each primer. PCR cycling conditions consisted of 1 cycle at 94°C for 5 min and 35 cycles at 94°C for 40 sec, 55°C for 40 sec, and 72°C for 30 sec. Five microliters of the initially amplified product was used in the nested reaction. First and second rounds of amplifications were cycled as described above. The amplified products were ana-

lyzed by 2% agarose gel electrophoresis followed by ethidium bromide staining.

For restriction fragment length polymorphism and nucleotide sequence analysis, a fragment corresponding to 631 nucleotides from transmembrane gp21 was amplified from samples that were HTLV-2 PCR positive. The first set of primers included BSEF4 (5′-CAGGCAATAACGACAGATAA-3′) and FLENV.R1 (5′-AAGCTTAAGCTTACTGTGGATGGGTCA-ATGGTAGGGG-3′), corresponding to nt 6011–6031 and 6669–6705 of the HTLV-2 Mo isolate, respectively, followed by second-round PCR using primers GP21F1 (5′-CTG-CAACA-ACTCCATTATCCT-3′) and GP21R1 (5′-CTGCAG-AAGCTAGCAGGTCTA-3′), corresponding to nt 6031–6051 and 6641–6661 of the HTLV-2 Mo isolate. Reactions were performed in 100-μl reaction mixtures containing 2 μg of DNA, 2.5 U of *Taq* DNA polymerase, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, deoxynucleotide triphosphates (0.25 mM each), and 100 pmol of each primer. PCR cycling conditions consisted of 1 cycle at 94°C for 5 min and 30 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min. Five microliters of the initial amplified product was used in the nested reaction. First- and second-round amplifications were cycled as described above. The amplified products were analyzed by 2% agarose gel electrophoresis followed by ethidium bromide staining.

Restriction fragment length polymorphism analysis

Restriction fragment length polymorphism (RFLP) analysis was done on the amplified 631-bp *env* HTLV-2 fragments to identify the HTLV-2 subtypes. The amplified products were purified with the Promega (Madison, WI) Wizard PCR Prep system and subsequently digested with *Xho*I restriction endonuclease (GIBCO-BRL, Gaithersburg, MD) as previously described.[29] The same fragment was digested with *Sac*II (New England BioLabs, Beverly, MA), according to the manufacturer instructions. Digested and undigested samples were directly electrophoresed on 3% agarose gels followed by ethidium bromide staining.

Nucleotide sequencing and phylogenetic analysis

PCR products amplified for RFLP analysis were purified as described above and sequenced in a Perkin-Elmer/ABI (Foster City, CA) Prism 377 DNA Stretch sequencer using *Taq* FS dye terminator cycle sequencing. The same PCR primers were used in the sequencing reactions. The multiple sequence alignment of the Tiriyo Indian samples together with related HTLV-2 sequences from the GenBank/EMBL database was done with the Dambe program using the CLUSTAL algorithm, and further edited with the GeneDoc program. The empirical transition:transversion was determined by the Puzzle 4.0.2 program. Aligned sequences were used with PHYLIP version 3.572[37] to construct neighbor-joining (NJ) and maximum likelihood (ML) trees, using the F84 substitution model. The reliability of the NJ trees was evaluated by analyzing 1000 bootstrap replicates. The trees were drawn with the TreeView 1.4 program.

RESULTS

Prevalence of human retroviruses in Brazilian indian populations

Serum and plasma samples from a total of 1004 Indians belonging to the Tiriyo and Waiampi tribes were initially screened by ELISA for HIV-1/2 antibodies. Fifty of 683 Indians from the Tiriyo tribe were repeatedly reactive in Vironostika R and only 1 (sample 469) was reactive in the Enzygomost ELISA when screened for HIV-1/2. The analysis of these samples by WB showed the following results: 1 positive (sample 469), 29

TABLE 1. DEMOGRAPHIC AND LABORATORY DATA OF HTLV-INFECTED BRAZILIAN INDIANS[a]

Sample identification	*Village*	*Sex, age*	*ELISA OD CO*		*WB bands*			*PCR*	
			HIV-1	*HIV-2*	*HTLV-1*	*HTLV-2*	*Ind.*	*HTLV-1*	*HTLV-2*
Tiriyo									
22	Missão Tiriyo	F, 42	2.91	4.18	—	21, 24, 38, 53, 46II		—	+
26	Missão Tiriyo	M, 11	4.0	5.31	—	21, 19, 24, 38, 53, 46II		—	+
525	Pönötö	F, 18	1.18	1.52			19, 26, 28, 32	—	+
611	Munemi	M, 51	1.51	1.78			19, 26, 28	—	—
Waiampi									
10	Manilha	M, 43	1.04	1.12			46I	ND	ND
35	Camopi	F, 26	4.89	6.40	21, 19, 24, 26, 28, 32, 38, 46, 53, 46I			ND	ND
75	Mariry	M, 8	1.20	1.38			32	ND	ND
291	Ywyrareta	M, 9	5.60	5.60	21, 19, 24, 26, 28, 32, 38, 46, 53, 46I			ND	ND

Abbreviations: F, Female; M, male.

[a]Samples initially seroreactive on ELISA were further analyzed by WB and PCR/RFLP analysis to differentiate HTLV-1 and HTLV-2 infections (*env*) and HTLV-2a subtypes (*env*); +, positive; —, negative; OD, optical density; CO, cutoff; ND, not done; Ind, indeterminate.

TABLE 2. PREVALENCE OF HUMAN RETROVIRUSES IN BRAZILIAN INDIANS

		HTLV			*HIV*			
							IND	
Indian tribe	n	*1* [n *(%)*]	*2* [n *(%)*]	*IND* [n *(%)*]	*1* [n *(%)*]	*2* [n *(%)*]	*p24* [n *(%)*]	*p17* [n *(%)*]
Waiampi	321	2 (0.62)	0 (0)	2 (0.62)	0 (0)	0 (0)	9 (2.80)	2 (0.62)
Tiriyo	683	0 (0)	3 (0.44)	1 (0.14)	1 (0.14)	0 (0)	18 (2.60)	2 (0.29)
Total:	1004	2 (0.19)	3 (0.29)	3 (0.29)	1 (0.10)	0 (0)	27 (2.70)	4 (0.40)

Abbreviation: IND, indeterminate samples.

negative (27 samples with no bands and 2 samples with p17 only); and 19 indeterminate (18 with p24 only and 1 with p24 and p17). Only 1 specimen (sample 469) was reactive by IFA, 48 were nonreactive, and 1 showed an unspecific pattern of fluorescence. Twenty-nine of 321 Indians from the Waiampi tribe were repeatedly reactive in the Organon Teknika ELISA and none was reactive by the Behring ELISA when screened for HIV-1/2. Twenty samples were negative by WB (18 samples with no bands and 2 with p17 only), 9 were indeterminate (p24), and none were positive. All but two samples were nonreactive by IFA. These two samples yielded a nonspecific pattern of immunofluorescence, but were negative by WB. The only HIV-1-positive sample detected among the Tiriyo Indians was subtyped as B, using HMA.[36] Regarding HTLV antibody evaluation (Table 1), 23 samples from the Tiriyo tribe were repeatedly reactive by ELISA. Of these, 2 were HTLV-2 positive, 2 were indeterminate, and 19 were negative by WB. PCR analyses was performed on these positive and indeterminate samples. PCR amplification of the HTLV-2 *env* region confirmed HTLV-2 infection of the two seropositive samples and of one of the indeterminate samples. Regarding the Waiampi tribe, seven were repeatedly positive by HTLV ELISA. Of these seven samples two were positive, three were indeterminate, and two were negative by WB. Because DNA was not available for these samples PCR was not done.

On the basis of these results, we can assume that the overall prevalences of HIV, HTLV-1, and HTLV-2 were 0.10, 0.19, and 0.29%, respectively, as seen in Table 2. In the Tiriyo tribe HIV-1 and HTLV-2 were detected, whereas only HTLV-1 was detected in the Waiampi tribe. The indeterminate serology results for retroviruses HTLV and HIV could be due to cross-reactivity to infection such as malaria, which induces polyclonal B cell activation, as has been previously suggested.[38]

Molecular characterization

RFLP analysis of the 631-bp fragments amplified from the envelope region encoding the transmembrane protein gp21 was used to identify sample subtype. Thus *Xho*I digestion of these products resulted in two DNA fragments of 180 and 450 bp, indicating that all three samples were infected with the HTLV-2a subtype (Fig. 1). To determine whether there were significant differences in nucleotide sequence between the three Tiriyo Indian HTLV-2 *env* sequences, the amplified products representing the transmembrane gp21 protein gene regions were sequenced and analyzed. The alignment of these sequences with HTLV-2 Mo[39] and HTLV-2 KAY1[29] demonstrated the presence of two mutations (at nt 6580 and 6582) creating a *Sac*II restriction endonuclease site that when cleaved generates two fragments of 530 and 100 bp (Fig. 2). The comparison of the amino acid sequence of the transmembrane gp21 protein between HTLV-2 Mo,[39] HTLV-2a KAY1,[29] HTLV-2b G12,[34] and the three HTLV-2 Tiriyo sequences, showed two amino acid changes: Gln-468 and Arg-469 were replaced by Arg-468 and Gln-469.

Phylogenetic analysis

We restricted our phylogenetic analysis to the transmembrane portion of the *env* gene, considering the greater number

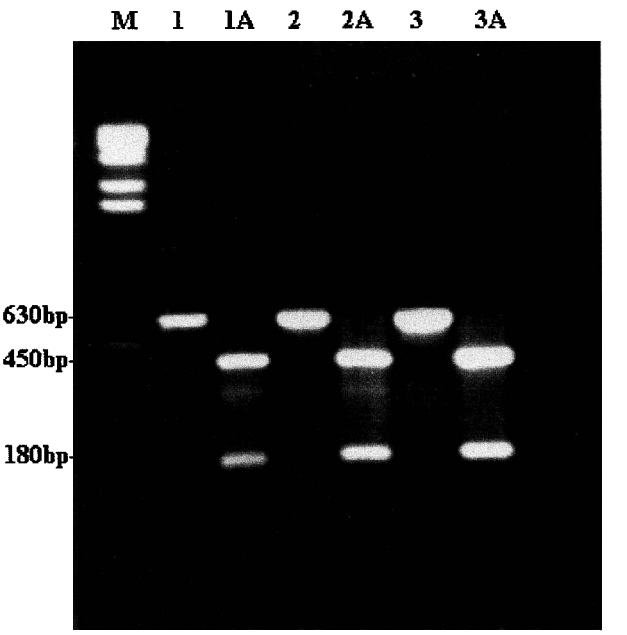

FIG. 1. Restriction fragment length polymorphism (RFLP) analysis of the *env* region encoding the transmembrane protein gp21. The amplified products were digested with *Xho*I and analyzed by 2% agarose gel electrophoresis. Lanes 1, 2, and 3 correspond to DNA from PBMCs of Tiriyo Indians 22, 26, and 525, respectively, before *Xho*I digestion. Lanes 1A, 2A, and 3A represent the *Xho*I-digested products from Tiriyo Indians 22, 26, and 525, respectively. Lane M, molecular weight markers.

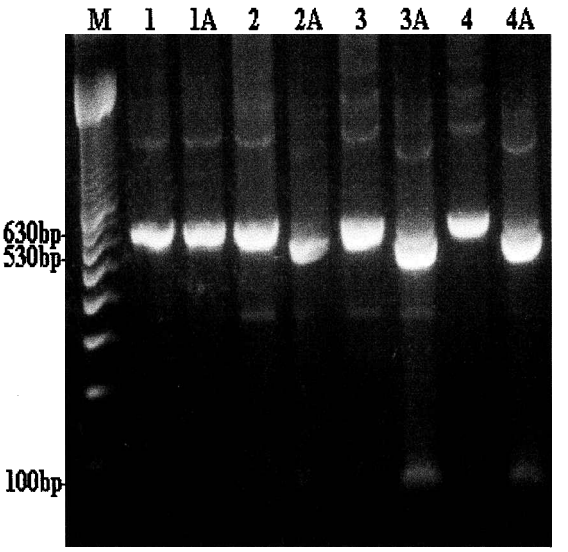

FIG. 2. Gel electrophoretic analysis after *Sac*II digestion of the PCR products from Tiriyo samples to confirm the cleavage site observed in the sequences. DNA from fresh PBMCs was amplified with primers flanking the gene region encoding the transmembrane gp21 protein. Lanes 1 and 1A show nondigested and digested controls: the same *env* PCR fragment was amplified from an HTLV-2a-seropositive IDU. Lanes 2, 3, and 4 represent the undigested products from the Tiriyo Indians PCR fragments 22, 26, and 525, respectively. Lane 2A, 3A, and 4A represent the digested products from the Tiriyo Indians PCR fragments 22, 26, and 525, respectively. Lane M, molecular weight markers.

of gp21 sequences relative to the lesser number of complete *env* sequences deposited in GenBank. Phylogenetic trees were constructed and evaluated by two different methods: NJ and ML. The reliability of the NJ topology was statistically evaluated by using 1000 molecular bootstrap replicates. The three Tiriyo Indian sequences were all identical and clearly clustered in the HTLV-2a group of sequences with a bootstrap support of 98% for NJ (Fig. 3). Within HTLV-2a, they do not cluster with the Brazilian Indian Kayapo sequences. The HTLV-2b clade was supported by 96% of the bootstrap replicates. In addition, when the ML method was used, the branch leading to the HTLV-2a and HTLV-2b subtypes displayed p values < 0.01.

DISCUSSION

Until now, a high prevalence of HTLV-2 infection, few cases of HTLV-1, and no cases of HIV infection have been reported in Brazilian Indian populations.[24,28,29,38,40,41] In the present study, we identified for the first time the introduction of HIV among Brazilian Indians. Also, we demonstrated low prevalences of HTLV-2 and HTLV-1. Indeed, we detected an HTLV-2 prevalence of 0.44% in the Tiriyo tribe, which is much lower than that (15.4%) reported previously.[29] These discordant results could be due to the small sampling size in the previous study. On the other hand, only HTLV-1 infection (0.56%) was observed in the Waiampi tribe, in agreement with previous reports.[42]

In different studies prevalences as high as 57.9% have been reported for HTLV-2 infections in Kayapo Indians.[18,24,28,29] These differences in prevalence rates among the Kayapo and the tribes studied here might be explained by the difference in ancestry of these indigenous populations in addition to the distances between their locations. As a matter of fact, they represent different linguistic groups (Kayapo [Gé], Tiriyo [Caribe], and Waiampi [Tupi-Guarani]), which could also suggest different origins and probably a lack of communication between these tribes.[43] Tiriyo and Waiampi are located 216 miles apart whereas the distance between Kayapo and Tiriyo/Waiampi is 586 miles.

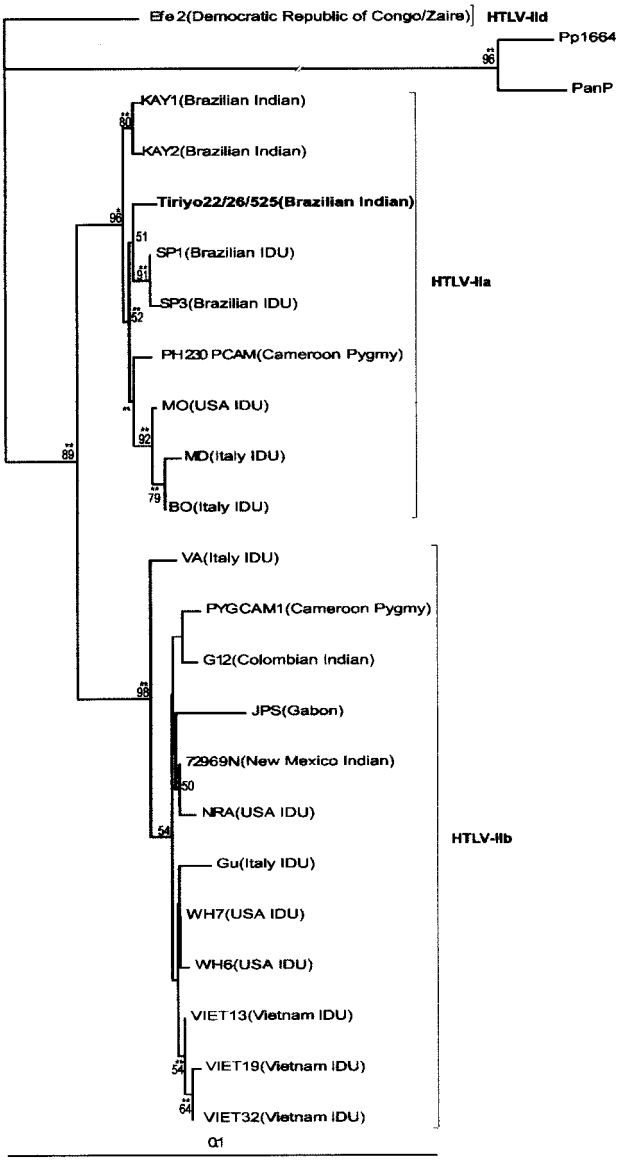

FIG. 3. Rooted NJ tree of three HTLV-2 strains based on a 537-bp fragment of the *env* region. The bootstrap values (above 50% and using 1000 bootstrap samples) on the branches represent the percentage of trees for which the sequences at one end of the branch form a monophyletic group. Efe2, PP1664, and PanP strains are used as outgroups. Geographical origin and ethnic origin are given in parentheses. Newly sequenced *env* included in this analysis are from the following three Tiriyo Indians: 22, 26, and 525 (in boldface). Statistical evaluation of branch lengths results in some clades being better supported than others ($^{**}p < 0.01$; $^{*}p < 0.05$).

We could demonstrate by RFLP and nucleotide sequence analysis that all three infected Indians in the Tiriyo tribe harbored the HTLV-2a subtype, similar to the virus previously reported among the Kayapo.[29] Subtype 2a has been identified among Brazilian Indians,[12,19,26,29] IDUs, and blood donors in Brazil. However, we report here the first *env* gene phylogenetic characterization of HTLV-2 among the Tiriyo Indians. Phylogenetic analysis of long terminal repeat (LTR) and *env* sequences from Brazilian HTLV-2a isolates shows that these viruses formed a separate group inside the 2a cluster.[12,27,29,35] Although we have analyzed *env* gene sequences only, our results clearly demonstrate that the three Tiriyo sequences are identical and cluster within subtype 2a, both in NJ and ML trees with high bootstrap support, and p values < 0.01, respectively. These sequences clustered closely with HTLV-2a isolates present in IDUs from urban areas of southern Brazil, but separately from Kayapo Indians, which is in agreement with data previously reported when the LTR sequence from a Tiriyo Indian was used.[35]

Our results show that there is considerable diversity within the Brazilian HTLV-2a subtype sequences. Further sequencing studies of LTR and *tax* regions of the provirus should determine whether the HTLV-2 in the Brazilian population has unique characteristics.

Different ancestry could explain the difference in prevalence among Brazilian Indian tribes. Besides, infections with HTLV-1 and HTLV-2 among the Waiampi and Tiriyo populations, respectively, could be due to a recent introduction of these viruses through contact with Brazilian non-Indians. In fact, when some of the Tiriyo Indians need medical care they travel to the cities of Macapa and Belem, where they stay for periods up to 1 month to be treated. Furthermore, it has been reported that some of these Indians travel to other cities (Obidos, Oriximina, and Paramaribo) to trade goods.[44] The latest hypothesis is supported by the low overall HTLV prevalence and by the phylogenetic closeness between the Tiriyo and IDU sequences observed in this study. Furthermore, the recent introduction of HIV infection into the Tiriyo population demonstrated in the present study also corroborates contacts with non-Indians.

Finally, considering the susceptibility of Indian populations to acquire infections from non-Indian populations, urgent health measures to prevent serious outbreaks in the Brazilian Indian populations are being implemented.

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SEQUENCE DATA

The GenBank accession numbers of the HTLV-2 *env* fragments included in the phylogenetic study are as follows: U19110, KAY1; U19109, KAY2; U32899, SP1; U32897, SP3; Z46837, PH230PCAM; X80244, MD; X80243, BO; X80242, VA; Z468889, PYGCAM1; S67034, G12; Z47788, JPS; U32904, 72969N; X80241, GU; U32900, WH7; M85226, WH6; U72524, VIET13; U72525, VIET19, U72527, VIET32; M10060, MO[39]; L20734, NRA[31]; and Y14365, Efe2.[33] The GenBank accession numbers of the HTLV-2 *env* fragments sequenced in our laboratory and included in the phylogenetic analysis are as follows: AF197276, Tiriyo22; AF197277, Tiriyo26; and AF197278, Tiriyo525. The GenBank accession numbers of the two STLV-2 *env* fragments included as outgroup in the analysis are Y14570, Pp1664[45]; and U90557, PanP.[46]

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