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First Description of Endemic HTLV-II Infection Among Venezuelan Amerindians

Gloria Echeverria de Perez, Matilde Leon-Ponte, *Oscar Noya, *Carlos Botto, †Dana Gallo, and Nicolas Bianco

*Institutes of Immunology and *Tropical Medicine, Faculty of Medicine, Central University of Venezuela, Caracas, Venezuela; and †Viral and Rickettsial Disease Laboratory, Division of Laboratories, California Department of Health Services, Berkeley, California, U.S.A.*

Summary: We describe for the first time the presence of human T lymphotropic virus type II (HTLV-II) infection in Venezuela, among the Pume Amerindians living in the southern plains of the country. Antibodies to HTLV-II antigens were assessed by enzyme immunoassays (Elisa), Western blot, radioimmunoprecipitation, and immunofluorescence; titration studies against HTLV-I– and HTLV-II–infected cell lines were very useful in the differentiation of HTLV-I and HTLV-II antibodies. The HTLV-II general prevalence was 5%; however, there is a striking difference in prevalence between the truly isolated villages (0%) when compared to those living along the riverside and thus in contact with outsiders (9%). Preliminary evidence suggests sexual contact as the main source of transmission. These findings might suggest that HTLV-II in Venezuela originated through contact with outsiders rather than ancient infection related to the origins of the Pume. **Key Words:** HTLV-II—Amerindians—Pume—Yaruro.

The human T cell lymphotropic virus type I (HTLV-I) reported in 1980 (1) was the first pathogenic human retrovirus to be discovered. HTLV-I has been etiologically associated with adult T cell leukemia/lymphoma and to a neurologic syndrome referred to as HTLV-I–associated myelopathy (HAM) in Japan or tropical spastic paraparesis (TSP) in the Caribbean.

A second subtype, HTLV-II, was first isolated from a patient with a T-cell variant of hairy cell leukemia (2), but its role in human disease remains unknown.

Infection by HTLV-I appears to occur worldwide, with high prevalence in Japan (3,4), the Caribbean (5,6), West Africa (7), and the Pacific Coast of South America (8). Lee et al. (9) found that 91% of confirmed HTLV-infected intravenous drug abusers in New Orleans were infected with HTLV-II. Native Americans in New Mexico (10), Florida (11), Panama (12), and Brazil (13) have been found to be endemically infected, suggesting Amerindians may be a primary focus of infection; furthermore, it has been suggested that HTLV-II might be a "new world retrovirus" as opposed to HTLV-I as an "old world virus" (13). However, this theory has been recently challenged by the demonstration of HTLV-II antibodies among Pygmies of Central Africa (14).

In a previous publication, we reported the absence of HTLV-I/II infection among 305 Amerindians from five different ethnic groups (15); these data have been further confirmed in at least two of these Venezuelan tribes (13).

More recently, we have been involved in the study of another Amerindian group, called Pume or Yaruro, ethnically distinct from previously studied

Address correspondence and reprint requests to Dr. G. Echeverria de Perez at Retroviral Infection Program, Institute of Immunology, Apartado 50109, Caracas 1050A, Venezuela.

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groups, and also geographically different since they inhabit the southern plains of Venezuela, close to the border with Colombia. Serum samples were analyzed for the presence of antibodies to HTLV and its subtypes by different methods, including enzyme immunoassays (EIA), Western blot (WB), radioimmunoprecipitation (RIPA), and indirect immunofluorescence (IF).

MATERIAL AND METHODS

Population

The Pume ethnic group includes a little over 6,000 individuals, living in small villages scattered among the plains of the Apure state. The Pume Amerindians are partly nomad and remain largely isolated, thus preserving their language and culture.

We have studied two different settings: (a) two villages located along the rivers, with a higher contact with outsiders and thus some acculturation, and (b) four villages located in the plains situated between the rivers, and remaining isolated. Among the six villages visited, a total of 440 individuals were enumerated; 210 were bled for the study, representing 36.3% and 47.3% of the total population of river-side (a) and isolated-plains (b) groups, respectively.

Data and Specimen Collection

Anthropological, cultural, and medical information was collected on each field trip by different participants on this collaborative study. Three to 10 ml of peripheral blood were collected on 210 individuals that volunteered for the study, after being thoroughly informed through an interpreter. Serum specimens were separated, aliquoted, and stored at −20°C the same day they were collected.

Enzyme Immunoassay

The 210 sera were screened for HTLV-I/II antibodies by at least two different commercially available EIA reagents (Abbott Laboratories, North Chicago, IL; Organon Teknika Corp., Belgium and Cellular Products Inc, CPI, Buffalo, NY).

Western Blot

Samples that were repeatedly reactive by at least one EIA test were tested by an HTLV-I/II WB made of viral lysate spiked with an env recombinant protein, gp21e (Cambridge Biotech, Worcester, MA).

Radioimmunoprecipitation Assay

HTLV-I and HTLV-II RIPA were performed by using radiolabeled extracts from MT2 and clone 19 cell lines as previously described (16).

Immunofluorescence Assays

Twenty-five samples, including 11 of the 12 confirmed as positive by WB and 14 negatives, were tested by IF against HTLV-I and HTLV-II transformed cell lines, according to methods previously published (17). Briefly, specimens were tested at 1:10 dilution in PBS, fluorescence was read in a Zeiss microscope, and reactions were compared with the positive control and subjectively scored from 1+ to 4+. In order to identify antibody reactivity to either HTLV-I or HTLV-II, IF endpoint determinations were assessed by titration of samples in fourfold dilutions, and the endpoint was the highest dilution exhibiting 1+ fluorescence. Specimens displaying similar endpoints with both cell lines were retested in twofold dilutions; those displaying nonspecific fluorescence were absorbed with uninfected cell pellets and retested.

RESULTS

Two hundred and ten individuals aged 1 to 80 years were studied; 107 were females and 103 males with similar median age (28 ± 17 and 28 ± 21 years, respectively).

When the 210 samples were tested by EIA with two commercially available reagents, 12 sera (5.7%) were repeatedly reactive with both reagents, and two (0.9%) were reactive with only one of the EIA.

Twelve out of 14 samples repeatedly reactive by EIA were confirmed as positive by WB according to the ASTPHLD criteria (18); samples 20 and 26 were negative (Fig. 1). Core band p24 was identified in all positive samples, whereas p19 was present in nine of 12. Although both core bands were easily identified, p24 was stronger in all samples. The recombinant envelope protein gp21e was strongly reactive in every positive serum.

HTLV-II env bands gp21 and gp67 were demonstrated by RIPA in the 11 positive sera that were tested (sample 31 not tested), and gp68 band in the RIPA HTLV-I antigen lysate was identified in 10 of 11 samples, negative in 19 (Fig. 2). Sample 26 was considered negative, since envelope bands gp21, gp67, and gp68 were not identified; this serum was also negative by WB and IF.

Eleven specimens out of the 25 tested by IF were positive with both MT2 and clone 19 antigens; these were the same specimens that were positive by WB and RIPA. As shown in Table 1, differences in fluorescence intensity between the two antigens were found at the screening dilution, and the brightest staining was consistently observed with the HTLV-II antigen. Nonspecific fluorescence was seen with 6 of the 25 specimens, but this was removed by absorption with H9 cell pellets.

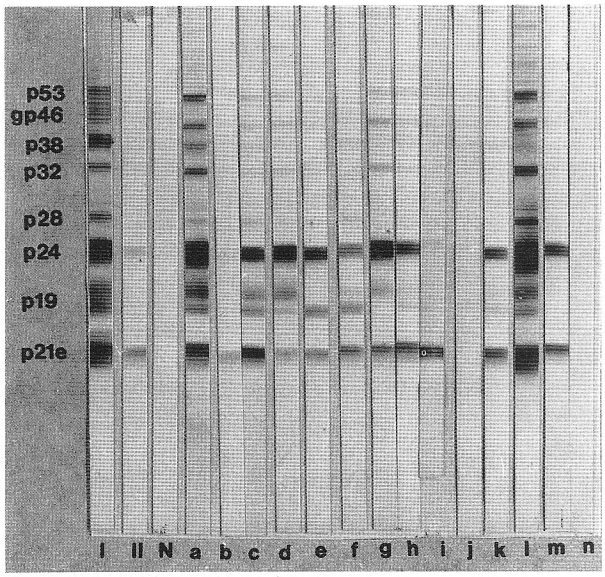

FIG. 1. HTLV-I/II Western blot patterns of Pume Amerindians. Lanes I and II are HTLV-I, HTLV-II positive and N negative controls. Lanes a–n are individual sera; j and n are negative (samples 20 and 26); lanes b and i correspond to low-titer seropositives, 02 and 19, respectively.

When IF endpoints were determined by fourfold serial dilution, all samples tested exhibited at least a fourfold higher titer against HTLV-II. Nine of 11 (81.8%) had titers of ≥1:4,096 with the HTLV-II antigen, and only two individuals, 02 and 19, showed low titers, with end points of 1:64. (Table 1). These two sera, although reactive, gave weaker bands by WB (Fig. 1, lanes b and i).

Eight of 11 (73%) HTLV-II infected individuals are females, and interestingly enough, the median age is significantly higher when compared to negative women (47 ± 20 vs. 27 ± 17 years, $p < 0.01$).

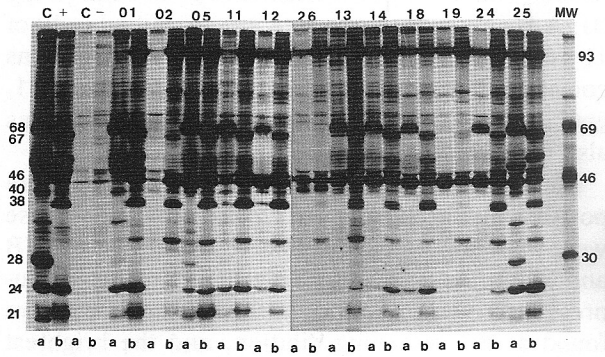

FIG. 2. RIPA bands in Pume Amerindians. Lane a corresponds to MT2-HTLV-I and b to clone 19-HTLV-II antigen. C+ and C− are positive and negative controls. Samples 01–25 are positive, and 26 is negative. MW, molecular weight marker.

TABLE 1. *Antibody determinations by EIA and IF*

		IF results			
		Screening[b]		Titer[c]	
Serum	EIA	HTLV-I	HTLV-II	HTLV-I	HTLV-II
01	R/R	3+	4+	1,024	>4,096
02[a]	R/N	1+	2+	16	64
05	R/R	3+	4+	256	4,096
11	R/R	2+	3+	256	4,096
12	R/R	1+	4+	256	4,096
13	R/R	2+	4+	256	>4,096
14	R/R	3+	4+	1,024	>4,096
18	R/R	2+	3+	256	>4,096
19[a]	R/N	1+	2+	8	64
24	R/R	2+	3+	64	4,096
25	R/R	4+	4+	4,196	32,768

[a] Reactive with Organon-Teknika and negative by Cellular Products/Abbott.
[b] Fluorescence graded from 1+ to 4+ at 1:10 serum dilution.
[c] Reciprocal of endpoint dilution.

Regarding the villages, all positive individuals belong to the riverside setting with a prevalence of 8.7% as compared to 0% among the more isolated group ($p < 0.05$).

DISCUSSION

The Pume or Yaruro group remains an isolated tribe inhabiting the plains of Apure State. Some Pume villages are located along the rivers, thus allowing some contact with non-Pume individuals, whereas another group is mainly isolated due to the location deep in the plains.

We found 14 of 210 samples HTLV reactive by EIA, 85.7% confirmed by WB and RIPA as HTLV-I/II. It has been previously reported that HTLV-I and HTLV-II antibodies can be differentiated by the reactions to p19 and p24 in the WB (19). In this study, WB results on the positive samples strongly suggested that the antibodies were due to HTLV-II infection, because the reactions were invariably more intense with the p24 core band than with p19; however, two samples (Fig. 1, lanes a and l) were indistinguishable from the HTLV-I positive control. IF endpoint determinations, another method shown to be reliable for differentiation of HTLV antibody (16), resulted in at least fourfold higher titers with the HTLV-II antigen.

RIPA results confirmed the presence of HTLV-II env bands, gp21 and gp67, in every sample already identified by WB and IF, but failed to identify env bands in the WB-IF negative samples.

Regarding epidemiological studies of Third World

populations, we found IF a very useful test to identify HTLV-I and II subtypes. Although training and reading skills are required, it is a very economic method and slides can be kept frozen for years. The combination of EIA with WB, spiked with a recombinant env protein, and IF using HTLV-1– and HTLV-II–infected cell lines appears to be sufficient for correct identification of antibodies reacting to the two different subtypes of HTLV.

From our results, we conclude that HTLV-II infection is present in the Pume group but not in previously assessed Amerindians residing in Venezuela (15). The reason for this situation remains unknown and perhaps is related to the origin of the Pume population, which might have migrated from Central America through Colombia many years ago. Although we have no definite data to support such an assumption, linguistic studies have suggested that Pume belongs to the Macro-Chibcha filum, along with Indian groups residing in Central America and the Pacific Coast of Colombia and Ecuador (20). As far as we know, the Pume have been located in the Venezuelan plains since 1644, and their inclusion among the Chibchan linguistic group has been debated (20). Besides, the finding of infected individuals restricted to the groups with some contact with outsiders tends to suggest a foreign origin of the virus; it might be interesting to state that the Pume share some territory with another ethnic group, the Cuiva, a subtribe of the Guahibo (21). Ongoing studies in our laboratory have found a high HTLV-II prevalence (25%) among a Guahibo group located in the Bolivar state, just aside Apure, where the Pume live.

Finally, since mainly sexually active females are infected, with a significantly higher median age as compared to uninfected, ongoing family studies including PCR in seronegatives will hopefully provide definitive evidence on our assumption of sexual contact as the main mode of transmission of HTLV-II in the Pume.

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