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PREVALENCE OF HTLV-I ANTIBODY AMONG TWO DISTINCT ETHNIC GROUPS INHABITING THE AMAZON REGION OF BRAZIL

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SUMMARY

HTLV-I seroprevalences of 3.63% (02/55), 12.19% (10/82) and 13.88% (10/72) were demonstrated among Tiryio, Mekranoiti and Xicrin Amazonian Indians, respectively, by the Western blotting enzyme assay (WBEI). By indirect immuno electron microscopy (IIEM), 2 Tiriyo, 9 Mekranoiti and 6 Xicrin Amerindians were reactive. Of 44 serum samples from Japanese immigrants, none reacted by any of the techniques before mentioned. One, 8 and 6 serum samples from Tiryio, Mekranoiti and Xicrin Indians, respectively, were both WBEI and IIEM positive. Our results strongly suggest that HTLV-I and/or an HTLV-I antigenic variant circulate (s) among populations living in the Amazon region of Brazil.

KEY WORDS: Human T-lymphotropic virus type I (HTLV-I); Amazonian Indians; Amerindians; Japanese Immigrants.

INTRODUCTION

Seroepidemiological surveys have disclosed HTLV-I widespread infection in diverse human ethnic groups[3,14,22,37,40] Geographical clusters of high levels of HTLV-I infection have been detected in the Southern islands of Japan[14,21], Caribbean basin[4], Central Africa[15,37] and Southern Italy[22]. Besides these areas, groups of apparently low endemicity to HTLV-I pinpoint around the world[1,10]. Some investigators have reported high rates of HTLV-I- like seroprevalence in isolated communities in the Southwestern Pacific islands[2,18]. Also Indians from Northern South America have been mentioned as seropositive to HTLV-I[1,25,27] HTLV-I seroprevalence of 20% among Japanese immigrants and their offspring was detected in Hawaii[5]. Also KITAGAWA et al[19] found anti-HTLV-I antibodies among Japanese immigrants in South Brazil.

The human T-cell lymphotropic virus type I is implicated as an etiologic agent of adult T-cell leukemia/lymphoma (ATL) and the neuromyelopathies; HTLV-I associated myelopathies (HAM) in Japan, and tropical spastic paraparesis (TSP) in the tropics[11,12,28,29,32,35,42] . ATL is highly endemic in the Southwestern Japan, in Kunamoto, Miyazaki, Kochi prefectures, Goto islands and the Yaeyama district of Okinawa, yielding its overall HTLV-I seroprevalence, based on seroepidemiological surveys, of 3.6%, 9.9%, 23.6% and 15.3%, respectively[17].

Tropical spastic paraparesis is endemic in Peru, Colombia, Jamaica, Martinique and Seychelles islands, where HTLV-I infection has been shown to be prevalent[9,20,33,34].

Among different groups of population surveyed for antibodies to HTLV-I, Japanese and Blacks were initially found to be the most commonly infected[5,15,21,37]. More recent work has provided both serologic and virological evidences of HTLV-I infection among Cauca-

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sians,[22,39] native South Americans[1,25] and mixed racial populations of Central America and Mexico.[16,31]

In this seroepidemiologic study two distinct human ethnic groups inhabiting the Amazon area of Brazil were surveyed for the presence of antibodies to HTLV-I by western blotting enzyme immunoassays and indirect immunogold electron microscopy.

MATERIALS AND METHODS

Three relatively isolated communities of aborigenes, one located in the Northern Para state, at the borderline of Surinam -- the Tiryio -- and the other two situated in the Southwest of Para state – the Mekranoiti and the Xikrin -- were selected for HTLV-I antibody tests (Fig. 1). Serum specimens were obtained from 55, 82 and 72 normal individuals of the Tiriyo, Mekranoiti and Xikrin tribes, respectively, and kept frozen at –20°C until the time of serologic tests. Sera from the Xikrin tribe were obtained in 1974 and 1978, from Mekranoiti and Tiriyo tribes in 1978 and 1980, respectively. These serum samples had previously been thawed for arbovirus and rotavirus antibody surveys.

Serum specimens were obtained from 44 normal Japanese and their descendents in a Japanese immigrant colony located at the Northeast of Para state, in the city of Tomé-Açú (Fig. 1), and kept frozen at –20°C until proceeded for serologic tests. These sera were collected in 1987. Fifteen of these Japanese migrants were from Southwestern Japan (Fukuoka, Kumamoto, Kagoshima, Miyazaki and Oita) and the rest from various, mostly Northern Japanese prefectures.

The Western Blot Enzyme Immunoassay (WBEI) was performed basically as described elsewhere[7]. Partially purified HTLV-I virus preparation was obtained by differential centrifugation from supernatant of HTLV-I infected cell cultures of Lma-66 cell line[16], banded in sucrose density gradient. The virus containing band was resuspended in sample buffer and heated to 100°C for 2 minutes, cooled, electrophoresed in 12% SDS-polyacry-

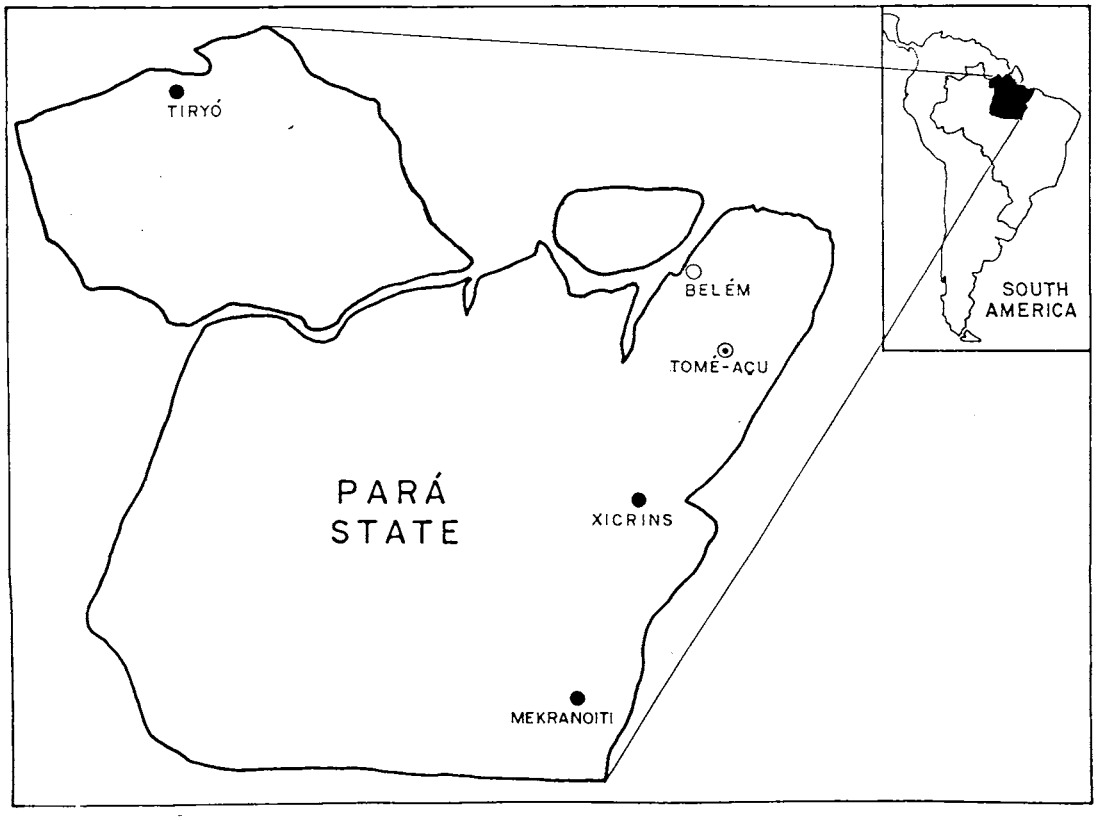


Fig. 1 – Map of Pará state showing Tomé-Açú and Indian villages.

lamide gel and electroblotted to a durapore membrane.

Membrane strips were blocked with 3% skim milk, incubated with subject's serum diluted 1:20, or with mouse monoclonal antibody to HTLV-I gag protein p15, anti-HTLV-I p19 or with anti-HTLV-I p24. The strips were then transferred to rabbit anti-human IgG peroxidase conjugate in tests of human sera, or to goat anti-mouse IgG peroxidase conjugate in testes of mouse monoclonal antibody. After extensive washing, the strips were transferred to the enzyme substrate and incubated for 30 minutes at room temperature, rinsed and dried. Molecular weights of resultant bands were determined based on reference standard protein molecular weight markers that were electrophoresed on the same gel, transferred to the same membrane and stained with Aurodye overnight at room temperature. Sera forming p15, p19 and/or p24 bands of HTLV-I were scored WBEI-positive.

The Indirect Immunogold Electron Microscopy (IIEM) was carried out as described[23]. Lma-66 cells were washed in phosphate buffered saline (PBS) and fixed in suspension in 3% glutaraldehyde in PBS pH 7.4 for 5 minutes at 4°C. Cells were dehydrated through the ethanol series and embedded in Lowicryl K4M (Chemische Werke Lowi Gmbh, Walkraiburg, FRG). Resin polymerization was achieved at –20°C by UV irradiation, using Ultraviolet Polymerizer (Dosaka EM Co. Ltd, Kioto, Japan). Preparation and mounting of ultrathin sections were mounted on the nickelgrid extensively cleaned just before use. In order to prevent nonspecific reactions, the sections were treated with 5% bovine serum albumin (BSA) in PBS pH 7.4 for 1 hour, at room temperature. Thereafter, without washing, the section was exposed to subject's serum, diluted 1:1,000 with PBS containing 0.05% Tween 20 and 0.1% BSA (PTB) for 30 minutes at 37°C in moist chamber and extensively washed with PBS containing 0.05% Tween 20 (PT) and then coated with biotinylated anti-human IgG (Vector Laboratories, Inc., Burlingame, CA) for 30 minutes at 37°C. After thorough washing, streptavidin colloidal gold conjugate (Bethesda Research Laboratories Life Technology, Inc, Gaithersburg, MD) was dropped on the section and allowed to react for 30 minutes at room temperature. Following washing in PT and in distilled water (DT), the section was treated with 0.1% osmium tetroxide for 15 minutes at room temperature, washed vigorously in DT and then treated with uranyl acetate at room temperature for 15 minutes. Thereafter, the section was vigorously washed in DT and exposed to 0.4% lead citrate for 1 minute, washed and observed in a Hitachi H-700H transmission electron microscope. The average gold-grain count was calculated on each virus particle to quantitate the reaction. Sera showing specific gold tagging on virus particles with or without tagging on cells were scored as IIEM-positive.

RESULTS

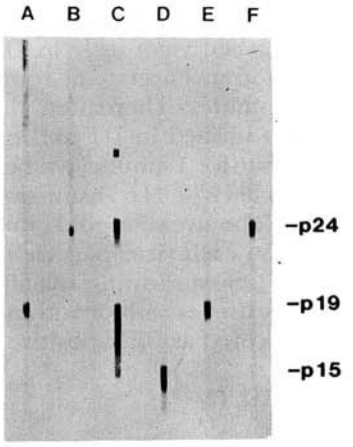
Two Tiriyo out of 55 (3.6%) were positive by WBEI. One of the WBEI-positive sera was also positive by IIEM. Among the Mekranoiti, 10 out of 82 tested (12.2%) were seropositive by the WBEI, of which 8 were also positive by IIEM.

Ten of the 72 individuals (13.9%) of the Xikrin tribe were seropositive by the WBEI and 4 subjects out of 72 (5.5%) were IIEM-positive. All sera positive by IIEM were also positive by WBEI. Some of the WBEI positive sera from Indians are showed in Fig. 2.

Among Japanese immigrants, none (0 out of 44) was positive by either IIEM or WBEI.

DISCUSSION

The prevalence of HTLV-I antibody among the Tiriyo Indians is apparently lower than in the two other Amerindian populations, which leads us to postulate that the former community has been less exposed to viral infection than the latter ones. One possible explanation for this difference is that – at the time of collection of serum samples – contacts with urban man (a likely source of infection) were more frequent among those Indian communities inhabiting the South of Para state, as compared with the Tiryio, a remote, very isolated population living in the North of Para state, at the border of Surinam. In addition to this, malaria was endemic among the Mekranoiti and Xikrin aborigenes, a condition that has been prevailing to date. In this respect it should be pointed out that BIGGAR et al.[4] have previously found that Plasmodium falciparum antibody titres correlate with the prevalence of ELISA HTLV-I reactivity in Ghana. Whether, however, endemic malarial infection would



Western blots of HTLV-I with different sera.

A : Serum (1:20) from a normal Mekranoiti Indian.
B : Serum (1:20) from a normal Xicrin Indian.
C : Serum (1:100) from a Japanese with ATL.
D : Monoclonal antibody (1:20,000) to HTLV-I-p15.
E : Monoclonal antibody (1:500) to HTLV-I-p19.
F : Monoclonal antibody (1:5,000) to HTLV-I-p24.

generate cross-reactive antibodies to HTLV-I in the WBEI and IIEM tests is not known.

In Maranhão, a neighboring state to Para, ANDRADA-SERPA et al.[1] did not detect any HTLV-I seropositive subject among Amerinds of that area, contrary to our results. Probably the techniques employed by these authors to detect HTLV-I antibodies were too restrictive to specific HTLV-I antigens of a single prototype strain commonly found in endemic areas, while methodology employed by us, utilizing partially purified virus preparations, would involve a broader antigenic spectrum,[26] which would possibly allow detection of epitopes shared in common by different HTLV-I strains or any other retrovirus. Nevertheless, we cannot rule out the possibility that this population is a true seronegative one, reflecting a non-endemic situation.

HTLV-I seroprevalence determined by us among Amerinds is similar to the results obtained by ASHER et al.[2] in Amerinds of the Southwestern pacific islands and to those obtained by MERINO et al.[25] in Yanomani Amazonian Indians. These results may indicate a possible antigenic relatedness between strains circulating in the Amazon region and those from U.S.A., Japan or Africa.

The lack of positive results among the Japanese migrants suggests that these people are originally from non-endemic areas in Japan (data on this particular aspect not available). It should, however, be pointed out that a too small sample of the population was tested, leading to an apparent absence of immunity against HTLV-I. Further testing of a large number of samples of this population would give a better comprehension of HTLV-I incidence among Japanese migrants in the Amazon areas of Brazil.

Although South American Indians and Japanese are known to be derived from a single racial root, the Mongoloid,[36] different environmental conditions might influence genetic variability determinant for susceptibility to a pathogen, while racial features predisposing to HTLV-I infection are not known. HTLV-I infection is highly endemic among negroes in Africa and in other continents to that they have migrated; also among Japanese in Southwestern Japan, where a clinical entity, ATL, is highly endemic.

RESUMO

Prevalência do anticorpo HTLV-I em dois grupos étnicos distintos habitando a região da Amazônia Brasileira.

Soroprevalências para HTLV-I de 3,63% (02/55), 12,9% (10/82) e 13,88% (10/72) foram demonstradas entre os Tiryió, Mekranoiti e Xicrin, respectivamente -- indígenas habitantes da Amazônia --, utilizando-se a técnica de "Western Blot" (WBEI). Por outro lado, a imunomicroscopia eletrônica indireta (IIME) revelou como positivos 2 Tiryió, 9 Mekranoiti e 6 Xicrins. Das 44 amostras de soro oriundas de migrantes japoneses, nenhuma resultou positiva pelas duas técnicas antes mencionadas. Foram reativos por ambos os métodos, 1, 8 e 6 amostras dos índios Tiryió, Mekranoiti e Xicrin, respectivamente. Nossos resultados representam uma forte evidência de que o HTV-I e/ou variante(s) antigenicamente similar(es) circula(m) entre populações que habitam a região amazônica do Brasil.

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