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SEROEPIDEMIOLOGY OF HUMAN T CELL LYMPHOTROPIC VIRUS IN THE REPUBLIC OF PANAMA

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Abstract. The human T-lymphotropic virus (HTLV) and associated diseases, adult T cell leukemia and spastic paraparesis, appear to be endemic in southwestern Japan and the Caribbean. This cross-sectional population-based study was conducted to describe the seroepidemiology of HTLV in the Republic of Panama. HTLV antibody was measured by first generation and commercial ELISA tests and confirmed by competitive binding ELISA, a radioimmunoassay for anti-p 24, and Western blot. Of 3,231 subjects ≥15 years of age, 135 (4.2%) had antibody detected in ELISA screening tests, but because only 20% were confirmed positive, HTLV seroprevalence varied from 0.2–2% throughout the Republic. Infection with HTLV clustered in Guaymi Indians living in Bocas del Toro province (9.9% prevalence rate). With the exception of Guaymi Indians, no major geographic, urban/rural, male/female or racial differences in antibody prevalence were observed; specifically, HTLV infection rates were not elevated in black Panamanians. Clustering of infection in an isolated Amerind population must be further investigated. The small proportion of screen-positive sera which confirmed positive illustrates the importance of strict uniform criteria for seropositivity.

The human T cell lymphotropic virus (HTLV) is an exogenous human retrovirus which is etiologically associated with adult T cell leukemia/lymphoma (ATL)[1] and with a chronic progressive degenerative neurologic disorder which presents as spastic paraparesis[2] and is currently referred to as HAM/TSP.[3] Several studies have documented that HTLV, ATL, and HAM/TSP are endemic in the Caribbean basin.[4] Most published studies have been observational and have not sampled well-defined populations; therefore, the actual prevalence of infection and incidence of associated diseases remain unknown. In addition, serologic assays for HTLV antibody have greatly improved and become more standardized since the first studies were done almost 10 years ago.[5] It is extremely important to compare newer laboratory methods with first generation tests in order to better interpret previously published studies.

We have previously reported that the prevalence of HTLV infection in Panama City is similar to other countries in the region.[6] We have also documented that HTLV-associated HAM/TSP is a common cause of neurologic disease in the population of Panama City and, although HTLV-associated ATL also occurs in Panama, it is not as prevalent as in other Caribbean populations.[7,8] In order to understand the background of HTLV infection against which disease manifestations occur, we have initiated cross-sectional studies to describe the seroepidemiology of HTLV in defined, representative populations throughout the Republic of Panama.

MATERIALS AND METHODS

Population base

The Republic of Panama is a developing tropical Latin American country located 7° above the equator. There are ~2 million inhabitants, with >30% of the population living in the Panama City metropolitan area. The country shares a mountainous western border with Costa Rica; the isolated, sparsely populated Darien rain forest forms the eastern border with Colombia. Large parts of the narrow isthmus are devoted to agriculture or remain as tropical rain forest. The majority of the population is mestizo, but there is a substantial black population, comprised of "Colono blacks," descendents of slaves brought to mainland Latin America by the Spanish in the 16th and 17th centuries, and "West Indian

blacks," descendents of immigrants from the English speaking Caribbean who immigrated to Panama in the late 1800s and early 1900s. In addition, there are 3 major Amerind groups: Guaymi, Cuna, and Chocoes.

Serologic surveys

Metropolitan population surveys: Panama City and Colon. Sera from a representative sample of metropolitan Panama were collected in 1981 during studies of ECHO virus 4 aseptic meningitis and enterovirus 70 acute hemorrhagic conjunctivitis in Panama City and Colon. These 2 cities comprise Panama's central metropolitan region and are the termini of the Panama Canal. The sera were gathered in cross-sectional studies which enrolled entire families representative of the city populations.[9,10] We have previously published a descriptive study of HTLV by first generation assays on this collection.[6] The present study tested sera from individuals ≥15 years of age.

National serologic survey. In 1978, the Gorgas Memorial Laboratory and Panamanian Ministry of Health collaborated to conduct a national serologic survey in order to assess immunity to diseases for which routine vaccination programs existed. The survey sampled every Province except San Blas and Darien, which are isolated frontier areas; neither Panama City nor Colon were included in the survey. Standard methods were used to select the population.[11] All accessible districts (counties) in each province were included (isolated, inaccessible districts were not included) and at least one urban and one rural corregimiento (borough) were randomly selected. Within selected corregimientos, census tracts were randomly selected, then households were randomly selected within these census tracts. By including all household members >1 year of age, ~1% of the total population would be sampled. The survey obtained sera from 0.8% of the target population. Compliance varied (36–80%) and was lowest in higher socioeconomic census tracts and adult males. The present study used sera from individuals ≥15 years of age.

Darien Region. Sera from populations in Darien province were collected during several studies conducted between 1983 and 1985 to determine the seroprevalence of leishmaniasis. Darien is a tropical rain forest frontier area which borders Colombia. The major populations are Mestizo, Colono-black, Choco, and Cuna Indian. Villages were visited for several days, during which we attempted to obtain blood specimens from all residents ≥1 year of age. Nine different settlements were visited and 318 sera from residents ≥15 years of age were selected for HTLV antibody testing.

Changuinola environmental impact study. This sample was obtained in 1980 during an environmental impact study in conjunction with a proposed hydroelectric project. The Teribe/Changuinola River basin occupies an isolated low coastal region in Bocas del Toro province. The population is primarily Guaymi Indian, Teribe Indian, mestizo, and West Indian black. We attempted to visit all settlements along the 2 rivers and sample all residents ≥1 year of age. In total, 1,300 subjects were recruited and 539 sera from those >15 years old were tested for HTLV antibody.

Guaymi Indian survey. In 1987, we conducted a special survey to obtain additional serum specimens from Guaymi Indians. In this survey, we attempted to enroll ~200 adult Guaymi as they attended Ministry of Health/Social Security Health Centers. Six Health Centers in Chiriqui Province and the out-patient clinic of the general hospital in Bocas del Toro were visited and serum specimens were solicited from all adult Guaymi in attendance.

Interview data

The populations were sampled during a number of studies designed for different purposes. All studies were reviewed by the Gorgas Memorial Laboratory Human Subjects Committee and all study subjects participated voluntarily. In all instances, surveys attempted to obtain blood from all willing members of selected households, but information was not always available to link family or household members. Standard information included age, sex, race, locality of residence, and locality of birth.

Laboratory

In all studies, vacutainer systems were used to collect venous blood, which was allowed to clot at room temperature and then held on wet ice for 1–12 hr. Serum was separated by centrifugation, aliquoted into small volumes, and stored at ≤−20°C until tested. Sera were tested inde-

TABLE 1
Summary of HTLV seropositivity

	No. sera	Binding ratios			
		Range	Mean	Median	SD
Screen-positive	135*	4–20.9	7.6	6.5	3.8
Positive HTLV	19	4.7–20.3	11.8	12.2	4.1†
Equivocal HTLV	12	4.2–14.6	9.3	10.3	3
No HTLV antibody	80	4–20.9	6.8	5.2	3.7†

* Twenty-four sera were not available for retesting following screen.
† One-way analysis of variance.
Mean binding ratios, HTLV negative vs. HTLV positive sera: $F = 27.11$ $P < 0.001$.

pendently, under code, in 4 HTLV antibody assays: a first generation screening test, a competitive binding confirmation assay, a commercial ELISA, and a solid phase radioimmunoassay (RIA). In addition, Western blot was used as a confirmatory test for discordant sera, if sufficient quantity was available.

First generation screening test. This test has been described in detail.[12,6] Briefly, it was an ELISA using HTLV-I whole virus antigen purified by zonal ultracentrifugation and which was detergent disrupted. Sera with binding ratios >4 were considered "screen-positive" and were retested by other methods.

Competitive binding confirmation assay. This first generation confirmation assay involved retesting screen-positive sera in the presence of heterologous HTLV-I antiserum.[13] Throughout this paper, this will be referred to as the blocking test.

Commercial ELISA. Sera which were screen-positive in the first generation screening-test were tested using the Dupont HTLV ELISA kit (Dupont de Nemours, Wilmington, DE), according to the manufacturer's instructions. Throughout this paper, this will be referred to as the ELISA.

Radioimmunoassay. Sera found positive in the first generation test were also tested by a radioimmunoassay (RIA) to measure antibody against HTLV p24, a major core protein.[14] The viral polypeptide p24 was purified by cation exchange and size exclusion chromatography. Sera were diluted 1:100 and incubated at 4°C overnight with ^{125}I-labeled HTLV-I p24. Formalin-fixed Staphylococcus aureus (STAPH-A) was added to each sample; samples were then incubated for 30 min at 37°C. Mixtures were then pelleted, washed twice, and precipitated ^{125}I-labeled p24 was counted. Sera were scored positive for HTLV p24 antibody if they precipitated at least 10% of the counts from a positive control. Throughout this paper, this will be referred to as the p24 RIA.

Western blot. Sera which gave discordant results in the ELISA and the p24 RIA were further tested by Western blot. HTLV-I was obtained from HUT-102 cells and prepared by standard methods.[14] Sera were scored Western blot positive if both p19 and p24 bands could be seen, regardless of intensity or the presence of other bands. Sera with no bands were classified negative and all other Western blot patterns were considered indeterminate.

RESULTS

As noted, first generation screen-test binding ratios of ≥4 were subjected to confirmatory testing. In order to verify this cut-off, 297 serologic survey sera (13% of total) with screen ratios <4 were tested by ELISA and p24 RIA. Only 1 specimen was ELISA positive and was clearly negative by p24 RIA and Western blot.

Of 3,231 sera collected from population surveys (Changuinola excluded), 135 (4.2%) were first generation screen-positive for HTLV antibody (Table 1). Twenty-four (18% of first generation screen-positives, 0.7% of total) were not available for further testing and have been eliminated from further calculations. Nineteen (0.6% of the total) were considered seropositive; they had a positive blocking test or ELISA and either a positive p24 RIA and/or positive Western blot. Twelve (0.4% of the total) were equivocally positive; they had positive blocking or ELISA reactions, were negative in p24 RIA, and gave equivocal Western blot patterns. Eighty (72% of screen positives) were classified as seronegative; they were either negative in blocking and ELISA or positive in one of these assays and negative by p24 RIA and/or Western blot. The distribution of screen test binding ratios was significantly different for sera defined as positive or equivocal and those classified as negative.

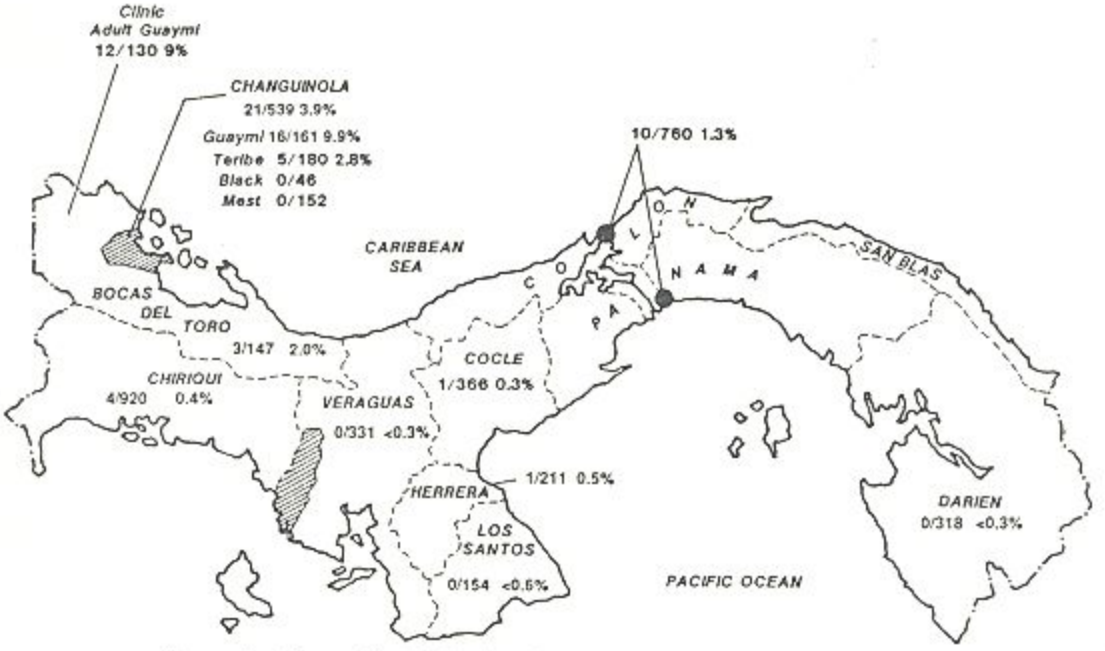

FIGURE 1. Geographic distribution of HTLV antibody prevalence in Panama.

Sera were collected in 7 different studies between 1978 and 1987. With the exception of the Changuinola environmental impact survey population, which included large numbers of Guaymi Indians, results were similar in all provinces (Fig. 1).

Of 760 residents of Panama City and Colon, 42 (5.5%) were first generation screen-positive and 10 had antibody detected by both ELISA and p24 RIA; therefore, the minimum seroprevalence of HTLV in Panama City/Colon was 10/760 (1.3%). Of 318 sera from Darien province populations, 14 (4.4%) were first generation screen-positive, but all were negative in p24 RIA. Thus the minimum seropositivity was <1/318 (0.3%). Of 366 sera from Cocle province, 13 (3.6%) were first generation screen-positive. On retesting, 1 was positive by blocking and p24 RIA, 1 was positive by blocking and equivocal by Western blot, and the others were negative in all tests. Thus, minimum seropositivity was 1/366 (0.3%). Of 211 sera from Herrera province, 6 (2.8%) were first generation screen-positive. On retesting, 1 was positive by ELISA and Western blot, 1 was positive by ELISA and equivocal in Western blot, and the others were negative. Thus the minimum HTLV infection rate was 1/211 (0.5%). Of 154 sera from Los Santos province, 5 (3.2%) were positive in the screening assay. On retesting, 2 were positive by ELISA, negative by p24 RIA, and equivocal in Western blot. Thus, the minimum HTLV infection rate was <1/154 (0.6%). Of 331 sera from Veraguas province, 4 (1.2%) were positive in the screening assay. Three were positive by ELISA, negative by p24 RIA, and equivocal in Western blot, so that the minimum sero-positivity rate was <1/331 (0.3%). Of 934 sera from Chiriqui province, 40 (4.3%) were first generation screen-positive. Four were positive by ELISA and Western blot, 7 were positive by ELISA and equivocal in Western blot, 15 were negative, and 14 first generation screen-positive sera were not available for confirmatory testing. The minimum seropositivity was 4/920 (0.4%). Of 148 sera from Bocas del Toro province, 10 (6.8%) were first generation screen-positive, 3 were confirmed positive by p24 RIA or Western blot, 1 was not available for confirmation, and the remainder were negative; therefore, minimum seroprevalence was 3/147 (2%).

The prevalence of HTLV antibody appeared to be considerably higher in Bocas del Toro than other provinces, but our sample size was small. Therefore, we tested an additional group of sera collected during an environmental impact study in the Changuinola region. Of 539 sera, 41 (7.6%) were first generation screen-positive and 21 were positive by blocking, ELISA, and p24 RIA; thus,

minimum seropositivity was 21/539 (3.9%). Of 161 resident Guaymi Indians (29.9% of total population), 24 (14.3%) were first generation screen-positive and 16 confirmed as positive for an overall Guaymi Indian minimum seropositivity rate 16/161 (9.9%). Of 180 sera (33.4% of total) from Teribe Indians, 10 were first generation screen-positive and 5 confirmed as positive; thus, HTLV prevalence was 5/180 (2.8%). Of 46 sera from West Indian blacks (8.5% of total), 2 (4.4%) were first generation screen-positive but were negative in all other assays. The remaining 152 inhabitants were mestizo and were all seronegative. Guaymi antibody rates were significantly greater than those observed in Teribe ($P = 0.003$, Fisher's exact test), blacks, or Mestizos ($P < 0.001$, Fisher's exact test).

Finally, studies of adult Guaymi attending outpatient clinics in Chiriqui and Bocas de Toro Provinces were conducted in 1987 to ascertain whether seroprevalence was the same as in the 1980 collections. As expected, 12 (9%) of 130 were seropositive in ELISA, p24 RIA, and Western blot assays.

DISCUSSION

The most important finding of this study was that 9.9% of adult Guaymi Indians from Bocas del Toro province had antibody to HTLV or an antigenically similar virus, as compared to only 0.2–2% of the general population. Indeed, seropositivity levels in Teribe Indian, black, and Mestizo subjects residing in the same area as the Guaymi Indians were similar to the remainder of the Republic (5/378, or 1%). It is difficult to account for this by biased sampling since particular attention was taken in study design to obtain population-based samples; furthermore, 3 different collections revealed similarly high rates in the Guaymi. In addition, we have previously documented unusually high rates of chronic hepatitis B virus infection in Guaymi Indians, so at least one other chronic virus behaves differently in this population.[15]

Anthropologically, the Guaymi are unique. Central America was the principal route of colonization to South America; the Guaymi are the descendents of Indians inhabiting this entry corridor.[16] At least 3 other studies have reported high HTLV antibody rates in isolated Amerind groups: 14% (2 of 23) of Yanomami Indians from the State of Amazonas, Venezuela;[17] 4% (13 of 335) of Indians living near the Florida Everglades;[18] and 2–12% (1/42, 5/106, 3/26) of Alaskan Eskimos.[19] These studies enrolled a small number of subjects and did not present details concerning sample selection, so that they cannot be referred to populations. They also used first generation antibody assays, so the results cannot be evaluated based on present laboratory criteria.

The other major finding of this study was that only 17% of sera with positive reactions in the first-generation screening-test could be confirmed as positive by p24 RIA and/or Western blot; an additional 11% had positive blocking results and gave equivocal Western blot patterns; 72% were clearly negative by p24 RIA and/or Western blot (irrespective of blocking test results). At the time of our study, binding ratios ≥ 4 were considered screen-positive, yet sera confirmed as positive had a mean ratio of 11.8, which is similar to data reported in a recent review of 43,445 sera from various geographic localities worldwide.[15] ELISA tests do not apriori detect only antibody against HTLV-specific gene products, and must therefore be confirmed by tests which specifically identify antibody to core and envelope proteins.[5] However, ELISA tests were designed to be highly sensitive, and nonconfirmed positive reactions (particularly at high binding ratios) may well represent antibody against new HTLV types.

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