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Human T-Cell Leukemia Virus (HTLV-I) Antibodies in Africa

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Abstract. *Antibodies specific for human T-cell leukemia-lymphoma virus type I (HTLV-I) were demonstrated in serum samples from various groups of people in South Africa, Uganda, Ghana, Nigeria, Tunisia, and Egypt. The samples had been collected for other purposes and were presumably selected without bias toward clinical conditions associated with HTLV infections. Regional differences in antibody positivity were observed, indicating widely distributed loci of occurrence of HTLV on the African continent in people of both black and white ancestry. Two patients with high titers of antibody to HTLV-I had some signs of adult T-cell leukemia-lymphoma. In several groups a high frequency of false positive serum reactions was indicated when specific confirmation steps were included in the assay. Further characterization of these sera revealed highly elevated immunoglobulin levels, possibly due to polyclonal activation of immunoglobulin synthesis in these subjects. The possibility that related cross-reactive human retroviruses coexist in the same groups was not eliminated.*

The human T-cell leukemia-lymphoma viruses (HTLV) are a family of related retroviruses originally isolated in the United States from patients with T-cell lymphoma and cutaneous manifestations (*1*). A particular subgroup of the family, HTLV type I, is linked to the cause of these malignancies, which share clinical and epidemiologic features with the disease called adult T-cell leukemia-lymphoma (ATL) that occurs in certain regions of Japan (*2, 3*) and in persons of African ancestry in the Caribbean Basin (*4*) and in the southeastern United States (*5*). An atypical chronic lymphocytic leukemia in Nigeria is also suggestive of an association with HTLV (*6*), as is the high incidence of antibodies cross-reactive with HTLV-I in Old World primates captured in Kenya and Ethiopia and housed in West Germany (*7*) and the United States and Russia (*8*). Although the mechanism of transmission of HTLV is currently unknown, horizontal transmission is clearly implicated by molecular and epidemiologic analyses (*9, 10*). HTLV seropositivity in regions endemic for ATL is elevated overall in the general population and further elevated among close family members of cases and in recipients of blood transfusions (*11, 12*).

The present study, which is mainly descriptive, was undertaken to investigate the occurrence of antibodies to HTLV-I in various groups of people in widely distributed areas of the African continent. We studied serum samples that had been collected for surveys of diseases with no known association with HTLV-I and samples from hospital-based clinic patients. We used a highly sensitive enzyme-linked immunosorbent assay (ELISA) to detect antibodies to HTLV-I (*13*) (see Table 1). Because of the diversity of the test groups, our data cannot be used to make strict epidemiological comparisons, but can be used as a means to compare the distribution of virus antibody positivity with previously reported studies of exposure to the virus (*2, 12, 14*) in similar or analogous groups.

The testing procedure was performed in two steps (legend to Table 1). In step 1, all samples were screened to determine quantitative levels of antibody binding to HTLV-I. In step 2, "candidate" positive sera were selected and tested for specificity in one or more confirmatory steps. The screen-test results are expressed as a ratio (R) to a standard reference normal serum to control daily variations in test results (*13*). The threshold level, $R \geq 2$, was not expected to exclude negative sera. The use of this cutoff for confirmation was based on prior experience with normal U.S. blood donors where samples with a screening ratio of <2 are negative in the confirmation assay. This reference normal serum level and the threshold for detection of sera confirmed as being positive for antibody to HTLV-I were derived from an analysis of 1210 U.S. blood donors (*15*).

Specificity was considered confirmed when sera passed either one of the confirmatory tests described in Table 1. The accuracy and precision of the antibody-blocking procedure was verified by measuring the fractional reduction of antibody binding for mixtures containing a predetermined ratio of HTLV-positive antibodies to the reference normal serum. The results plotted in Fig. 1 show excellent agreement with the predicted results at low levels of positive antibody and deviation within acceptable limits due to incomplete blockade at the higher levels of human antibody. Of those sera failing confirmation by antibody blocking, only three were confirmed by absorption with virus-positive cells.

The values for the numbers of sera from the groups exceeding the screen-test threshold and for the numbers of confirmed positive sera in each of these groups are presented in Table 1. The median values for screen ratio (R) within the groups were in most cases close to the median value of 1.17 found for U.S. donors (*15*). However, median values of R for samples from Tunisia and Ghana were two to three times higher. This reflected the absence of a simple correlation between the prevalence of confirmed positive sera and the proportion of sera exceeding the screen threshold level ($R \geq 2$); for example, the proportion of confirmed positive donors in the Ugandan group (21 percent, which was highest of all groups) was two times higher, while the proportion of samples exceeding $R \geq 2$ was only one-half that of the Ghanaian groups.

We investigated some of the reasons for this apparent high rate of false positivity. Among the Ghanaian samples, 28 out of 67 with high ratios ($R \geq 6$) were nonconfirmed. The mean and median immunoglobulin G (IgG) levels of these 28 samples were, respectively, 130 and 106 mg/ml compared to 9 mg/ml for the standard control serum (measured by ELISA with immunopurified goat antise-

rum to human IgG). Reconstitution experiments showed that these concentrations of IgG could account for elevation in R values in all but three cases (*16*).

While it is possible that cross-reacting antibodies with different HTLV subtype specificities may be a source of apparent nonspecificity in some cases, we view this as unlikely since a cross-reactive antibody should be susceptible to competition by the HTLV-I–specific antiserum used in the confirmation test. A possible improvement might be to use a purified viral protein as test antigen. While the confirmatory tests are able to circumvent nonspecificity in the sense of excluding sporadic false positive reactions, for example, reactivity with contaminants of cellular debris or a nonrelevant elevation of serum IgG level, true positive sera would tend to be underestimated in the presence of generalized nonspecific elevation of the test background because of the stringency of the confirmatory test. Thus far, the ELISA with a purified, unfractionated virus preparation has resulted in greater sensitivity and specificity than purified p24 core antigen. These assays may be improved as more purified HTLV proteins become available for testing and more generally applicable confirmatory procedures are developed.

As summarized in Table 1, 131 (6.9 percent) of 1890 sera tested were positive for HTLV-I antibodies. Since this study was not designed to systematically explore disease relationships, positive results may only be interpreted as establishing exposure to HTLV-I in the groups tested. Furthermore, rates of positivity in some of the groups may vary from rates in the general population.

Two serum collections from Ghana collected over the years 1968 to 1981 by the Burkitt Tumor Project, Accra (*17*) were tested for HTLV-I antibodies. One represented a group of 510 Burkitt's lymphoma patients and the other a group of 236 normal persons from the same families and communities as the patients (*17*). The numbers positive for HTLV-I antibody in these two groups (not corrected for age) were 10 and 8 percent, respectively (Table 1). Controlling for age, there were no statistically significant differences in HTLV-I antibody rate between these groups. Both groups contained high titers of antibodies to HTLV-I (Table 2). Since there is no known association between HTLV-I and Burkitt's lymphoma and since there was only a slight difference between the values for these groups, this prevalence probably reflects the general population prevalence for that age group. These positivity rates are high given the young age of the persons studied (mean, 13

Table 1. Distribution of HTLV-I antibody among African donors. An indirect ELISA microtest to detect serum antibodies was used (*13*). Briefly, HTLV-I was purified by rate-zonal ultracentrifugation, disrupted, and coated into the wells of microtiter plates. Portions (5 μl) of test sera, control positive sera, and control negative human sera were incubated overnight at 4°C in wells containing 100 μl of 20 percent heat-inactivated normal goat serum and were quantitated by measurement of absorbance at 490 nm after reaction with peroxidase-labeled goat antiserum to human IgG. Sera with absorbance values two times greater than the normal control level were verified primarily by confirmatory neutralization, which involved the same procedure as the screening test but included an additional 2-hour incubation period before the test sample was incubated with the antigen-coated wells. During this extra 2 hours of incubation, the wells were exposed to unlabeled sheep antibody to HTLV-I which reacted with and saturated HTLV antigen sites on the well, thus preventing the test serum from attaching to the well in the subsequent step. As a control in the test, adjacent wells were exposed to normal sheep serum during the additional incubation period. Sheep antiserum was used at a dilution of 1:2 and had a titer of 100,000 or more. Sheep antiserum showing reactivity with proteins from phytohemagglutinin (PHA)–stimulated human lymphocyte preparations coated on microtiter plate wells were absorbed with PHA lymphocyte preparations until the reactivity was removed. The sheep antiserum used in these experiments required absorption with one volume of cell equivalents per three volumes of serum to reach the end point. A suppression of the absorbance by >50 percent in the sample exposed to the unlabeled sheep antiserum to HTLV-I, relative to a standard normal human serum, was considered a positive confirmatory result for the presence of antibody to HTLV-I. Sera failing the confirmatory test were absorbed with detergent-released cytosols prepared from PHA-stimulated normal human lymphocytes and with HTLV-I–producing cells and retested for binding to HTLV-I (*13*). Samples were scored positive if the difference between absorption with virus-positive and -negative cell preparations was >50 percent. Titers of positive sera were determined by serial dilution, regression analysis of the titration curves, and solving for the dilution giving results equivalent to a 1:20 dilution of the reference negative control serum tested in wells of the same plate. R is the ratio of the sample to the negative control; all groups followed log-normal distributions of R.

Geographic and racial background of donors	Group characteristics	Number tested	R median	Number with $R \geq >2$	Number positive
Egypt, white*	Infectious disease clinic (no malignancies)	101	0.90	12	2
Tunisia, white†	Malignant lymphoma	22	1.31	7	2
	Mammary carcinoma	256	2.10	136	6
Ghana, black‡	Burkitt's patients	510	3.32	336	52
	Normal comparison population	236	3.30	200	19
Uganda, black‖	Burkitt's patients and normal comparison population	86	1.71	31	18
Nigeria, black§	T-cell lymphoma	9	1.60	2	2
South Africa					
Cape Town, black and white¶	All donors	283	0.90	38	15
	Lymphoid malignancy	22	0.86	2	1
	Myeloid malignancy	104	0.84	16	9
	Solid tumors	59	1.02	11	3
	Nonmalignant disease and healthy blood donors	98	0.80	9	2
Johannesburg, black#	Healthy blood donors	104	0.9	5	0

*Sera from Egypt were collected from infectious disease patients, Navy Medical Research Unit No. 3, Cairo, Egypt. †Sera from Tunisia were provided through the Collaborative Breast Cancer Project between the Institut Salah Azaiz and the National Cancer Institute in Bethesda (*27*). These sera were collected as part of an epidemiologic survey of inflammatory breast carcinoma and incidental cases of non-Hodgkin's lymphoma of Mediterranean origin. The latter were diagnosed as B cell (n = 16), T cell (n = 2), and uncertain (n = 4). ‡These Burkitt's lymphoma patients were being followed by the Burkitt's Tumor Project, Korle Bu Hospital, University of Ghana, and were chosen on the basis of clinical status (divided between untreated patients and patients in remission) and availability of sufficient sera to perform confirmation studies. Comparison groups were family and community study subjects matched to a subset of Burkitt's cases (*17*). ‖Sera from Uganda were from Burkitt's lymphoma patients reported to the Burkitt's lymphoma project in Arua, Uganda, and seen at Kuluva Hospital (*18*). Comparison subjects were matched by age and sex. §Sera from Nigeria were collected prospectively for this study over a period of 2 months at the University College Hospital, Ibadan, Nigeria (*19*) from available patients with lymphoid malignancy. ¶Sera were from unselected patients with leukemias and solid tumors at the University of Cape Town Medical School, Department of Hematology, Clinical Science and Immunology. Racial backgrounds of the patients were: white, 76; black, 9; mixed, 41; and unknown, 157; of the patients with positive sera, white, 9; black, 0; mixed, 2; and unknown, 4. The lymphoid malignancies were diagnosed as acute leukemia (n = 18) and chronic leukemia and lymphoma (n = 4). #These sera were collected at the South African Blood Transfusion Service serving the Baragwanath Hospital, which is the major general hospital serving the black community of Johannesburg.

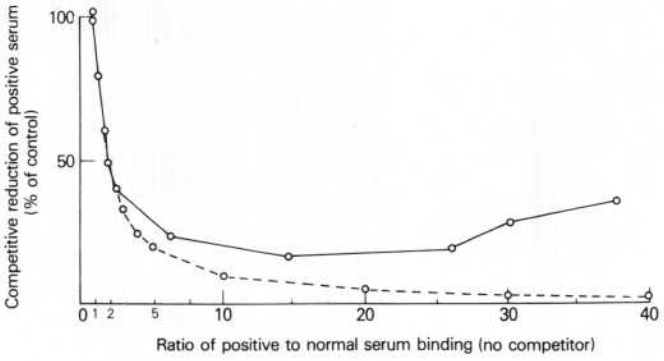

Fig. 1. Correlation between specific antibody binding and its susceptibility to competition by HTLV-specific antiserum. A dilution series containing varying ratios of serum positive for HTLV-I antibodies to serum negative for antibodies was constructed by diluting a positive serum (serum F4608, titer = 3000) with our standard negative serum (serum F4660). Each dilution and the negative control serum were tested for HTLV-binding in wells pretreated with unlabeled sheep antiserum to HTLV-I and normal sheep serum as described in Table 1. The ELISA absorbances at 490 nm were measured and expressed as the ratio to negative human serum treated with normal sheep serum (abscissa); and the relative reduction of each diluted positive human serum after incubation with sheep antiserum to HTLV-I (ordinate). Solid line: a theoretical curve was constructed on the basis of the expected relationship between the fractional content of specific antibodies (abscissa); broken line: the maximum achievable reduction (ordinate).

years) compared to endemic populations in Japan where the antibody positive rate for the equivalent age group is approximately 5 percent (*14*).

The highest rate of antibody positivity, 21 percent, was found in a serum collection from Uganda (Table 1). The sera had been collected from patients with Burkitt's lymphoma and from normal comparison groups in the West Nile region of Uganda over the years 1970 to 1972 as described previously (*18*). Since we were unable to discern any difference in the positivity rates between these groups they are listed together in Tables 1 and 2.

In a survey of patients with T-cell leukemia-lymphoma diagnosed in Nigeria (*19*), two cases fitting the typical characteristics of HTLV-associated disease were identified. The first case, a 19-year-old male student from Lagos, had an aggressive T-cell lymphoma with a high count of white blood cells that included cells of pleomorphic morphology, cutaneous involvement, and hypercalcemia. The second, a 57-year-old woman, had a clinically aggressive leukemia-lymphoma with generalized adenopathy and visceral involvement. She died shortly after diagnosis. Sera from both patients contained a high titer of antibodies to HTLV-I (see Table 2). One additional patient with ATL with high HTLV antibody titer, a native of Zaire, has been observed in Paris (*20*).

In Cape Town, 5 to 10 percent of patients with various malignant diseases had HTLV antibody (Table 1). There were no reported cases of ATL, and the greatest number of HTLV-I antibody-positive cases occurred among the myeloid malignancies (10 percent), although one patient with T-cell leukemia was positive. In areas of southwestern Japan that are endemic for ATL, the frequency of HTLV among patients with myeloid leukemias was 16 percent (*2*). Our present results with regard to the distribution of HTLV-I antibody within disease categories agree very well with the pattern found in the Kanto district, a nonendemic area of Japan (*12*). In that district, two important factors contributed to the high rate of HTLV-I antibody positivity in patients with diseases not linked with this virus. One was that the patient population largely originated from an endemic area and the other was the frequent use of blood transfusions in the management of myeloid leukemias (*12*).

With one exception, serum samples positive for HTLV antibodies were found in groups from all of the African subcontinental regions tested: Tunisia; Ghana; Nigeria; Uganda; Cape Town, South Africa; and Egypt. The absence of HTLV-I antibody in sera from the Johannesburg group may be related only to sample size and probably indicates a lower prevalence than in the other African groups. Race did not appear to be a disposing factor since positive serum samples from Cape Town were mainly of white origin. Although antibody-positive samples were detected in the Tunisian (taken as a whole) and Egyptian groups, the combined factors of frequency and titer found (Tables 1 and 2) were not significantly higher than the baseline for normal donors in the United States (*15, 21*). Both of the positive samples from Tunisian patients with lymphomas had very low titers, that is, 30 and 37, and the lymphomas were of B-cell origin. For reference purposes, among normal blood donors determined by comparable techniques, the HTLV-I antibody positivity rates between nonendemic and endemic regions of Japan range from 2 to 12 percent (*2*) and in the United States, 0.9 to 2.8 percent (*15, 21*).

The typical HTLV-I–associated disease as it occurs endemically in Japan, the Caribbean Basin, and sporadically elsewhere (*22*) is characterized by the occurrence of malignant cells of varying size and pleomorphic morphology with deformed nuclei and mature T-cell surface marker phenotype. It is often characterized by its onset at a relatively young age; by its aggressive clinical course with poor prognosis; and by the enlargement of lymph nodes, spleen, or liver; elevation of white blood cell count; hypercalcemia; and occasional skin involvement. Our data reveal increased levels of HTLV-I–specific antibodies in diverse African groups [compare with (*23*)] and suggest that the antibody levels in regions of South Africa, Ghana, Nigeria, and Uganda equal or exceed those found in previously described areas where HTLV-I and ATL coexist. It

Table 2. Comparison of titers of antibodies to HTLV-I in African groups. Procedures and subject groups are described in Table 1.

Origin and condition of serum donors	Titers (reciprocal serum dilution)				
	20	100	1000	>9999	Range (low-high)
Egypt	1	1			95–960
Tunisia	6	2			30–122
Ghana					
Burkitt's patients	24	23	5		30–4,915
Normal comparison population	5	13		1	46–100,000
Nigeria			2		1,700–4,000
Uganda	3	14		1	40–22,500
Cape Town					
Lymphoid malignancy		1			120
Myeloid malignancy	3	5		1	50–10,000
Nonhematopoietic malignancy		3			400–540
Nonmalignant disease and healthy blood donors	1	1			32–200

would be interesting to conduct systematic surveys of patients with adult non-Hodgkin's lymphoma in various regions of the African continent, with an emphasis on clinical, pathologic, and immunopathologic features of the disease. A study of sera from African patients with known or suspected T-cell malignancies, including the acquired immune deficiency syndrome (AIDS) (*24*), would help to clarify the distribution of the HTLV family and the diseases associated with it, especially in view of the high HTLV-I antibody level in the Ugandan group, the occurrence of AIDS in neighboring Zaire (*25*), and the occurrence of Kaposi's sarcoma along the equatorial region of Africa with its highest prevalence in eastern Zaire and western Uganda (*26*).

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16. We expect that these and other types of indirect assays currently applied without a specific confirmatory step, including immunofluorescence assays that rely on the use of live or fixed HTLV-I–infected lymphocytes, would also be sensitive to such drastic elevations in IgG and could lead to overestimation of seropositivity in similar cases.
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