

## **Evidence for Exposure to HTLV-III in Uganda Before 1973**

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**Abstract.** Fifty of 75 serum samples collected in the West Nile district of Uganda between August 1972 and July 1973 contained antibodies reactive with human T-cell leukemia (lymphotropic) virus type 3 (HTLV-III; mean titer, 601), while 12 of 75 samples were positive in a similar test for HTLV type 1 (HTLV-I) antibodies (mean titer, 236). The samples were screened by enzyme-linked immunosorbent assay and positive results were confirmed by a newly developed unlabeled antibody-peroxidase procedure with enhanced sensitivity for detection of antibody binding to immunoblots of HTLV-III antigen, demonstrating antibodies to proteins with molecular weights of 24,000, 41,000, and 76,000 in nearly all positive samples. Analysis of titration data indicated enhanced titers of antibody against HTLV-III and HTLV-I when coinfection occurred. The high prevalence and relatively low titers [compared to serum from patients with acquired immune deficiency syndrome (AIDS)] of antibodies recognizing HTLV-III proteins in sera from this population at a time that may predate or coincide with the appearance or spread of the AIDS agent (HTLV-III) suggest that the virus detected may have been a predecessor of HTLV-III or is HTLV-III itself but existing in a population acclimated to its presence. It further suggests an African origin of HTLV-III.

The acquired immune deficiency syndrome (AIDS) is known to occur among homosexual or bisexual men, intravenous drug abusers and their infants, female sexual partners of men with the syndrome, Haitians, and patients with hemophilia (1, 2). Recently, a retrovirus of the human T-cell leukemia (lymphotropic) virus "family" called HTLV-III was isolated with high frequency in serum samples from patients with AIDS and pre-AIDS (3), and antibodies to the virus were found in 88 to 100 percent of AIDS patients and individuals at high risk for AIDS (4). Many data indicate that this virus is the cause of AIDS (5). Similar or identical retroviruses have also been identified (6) and subsequently isolated (7). Some of these are now being directly compared to several isolates of HTLV-III.

AIDS, first recognized as a separate

disease entity in 1981 (1), is diagnosed as a severe, unexplained immune deficiency that usually involves a reduction in the number of helper T lymphocytes and is accompanied by multiple opportunistic infections or malignancies. Where and how the disease arose are open questions, but several observations are consistent with an African origin.

Although the occurrence of AIDS in black central Africans was only recently reported (8), Kaposi's sarcoma is highly prevalent there (9), and cases in children and young adults with a presentation and course similar to those associated with Kaposi's sarcoma in homosexual men with AIDS were documented in 1971 (10). Earlier studies in our laboratory and others indicated that HTLV type 1 (HTLV-I) occurs with high frequency in regions of central Africa (11-14), and closely related retroviruses are present

in many Old World monkeys (13, 15). These and other results led us to suggest an African origin of the HTLV family (16). Furthermore, we recently found a high prevalence of serum antibodies recognizing HTLV-I in Ugandan children with Burkitt's lymphoma and in normal family and community members sampled in 1972 and 1973 (11). We have now extended our observations to the prevalence of antibodies related to HTLV-III in the West Nile district population of Uganda and have found evidence suggesting the existence of a virus related to HTLV-III present at a time predating or coinciding with the earliest report of cases resembling AIDS.

The Ugandan serum tested was primarily from clinically healthy donors randomly selected as controls for Burkitt's lymphoma patients on the basis of age, sex, and community. All samples were collected between August 1972 and July 1973 (17). The mean age of the patient population was 6.4 years (17). Samples from this collection were tested for antibodies recognizing HTLV-III in two stages. First, all the samples were tested by indirect enzyme-linked immunosorbent assay (ELISA) for quantitative levels of immunoglobulin G binding to disrupted HTLV-III virions coated onto the wells of microtiter plates (18). HTLV-III virions were produced in high quantity by specific clones from a permissive human neoplastic T-cell line (19). Test results were normalized to values for a standardized normal human control serum, and all samples with normalized values exceeding a cutoff of the mean + 2 standard deviations (determined from 82 normal donors) (20) were confirmed by the demonstration of antibody binding to virus-specific proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to nitrocellulose strips (21).

Of the 75 samples, 50 of 55 that exceeded the cutoff of 2 standard deviations recognized specific viral bands with an overall positive rate of 66 percent. The most prominent reactions were with antigens having molecular weights of 76K, 55K, 41K, and 24K. Less frequently recognized antigens had molecular weights of 64K, 59K, 32K, and 18K. These values coincide with the previously described molecular weights of HTLV-III antigens recognized by serum from AIDS patients or individuals at risk for AIDS (4, 22). Representative samples from two consecutive experiments are shown in Fig. 1, aligned in order of ascending screening ratios from left to right. Under the same experimental con-

Table 1. Antibodies to HTLV-III and HTLV-I in serum collected in 1972 and 1973 from 75 Ugandan children. All samples that were positive by the Western blotting procedure were included in titration analyses. Titration data were obtained from serial dilutions of a test sample in the standard ELISA protocol. The data were analyzed by using the equation in reciprocal form for molecular binding (28). The slope for the regression line where  $x$  is serum dilution and  $y$  inverse optical density (values of inverse optical density greater than 25 were excluded) was determined and the general equation for a straight line was used to solve for  $x =$  titer. The titration end point was the value of  $y$  for a 1/20 dilution of standard normal serum. NA, not applicable.

| Serum positive for  | HTLV III antibodies |            |   | HTLV I antibodies |            |   |
|---------------------|---------------------|------------|---|-------------------|------------|---|
|                     | Number of samples   | Mean titer | Geometric mean titer (coefficient of variation;* range) | Number of samples | Mean titer | Geometric mean titer (coefficient of variation;* range) |
| HTLV-III            | 40                  | 421        | 263 (46; 43 to 1750)                                    | NA                | NA         | NA  |
| HTLV-III and HTLV-I | 10                  | 1328       | 447 (73; 84 to 7400)                                    | 10                | 264        | 195 (34; 80 to 840)                                     |
| HTLV-I              | NA                  | NA         | NA  | 2                 | 83         | 71 (95; 40 to 125)                                      |
| Total               | 50                  | 601        | 295 (52)  | 12                | 236        | 166 (37)  |

\*Analysis performed on log units (titers).

ditions all 4 of 82 normal donors exceeding the ELISA cutoff were negative in the blotting procedure.

Titers of positive samples were determined and compared with titers determined previously (11) for HTLV-I-positive serum from this group (Table 1). The extent of cross-reactivity between HTLV-I antibody-positive serum samples from individuals in regions in which adult T-cell leukemia is endemic and HTLV-III antibody-positive serum from AIDS patients and high-risk groups is shown in Table 2. The data show little or no cross-reactivity between natural antibodies to HTLV-I and HTLV-III under the conditions of our test. Ten of 50 samples (20 percent) showing a positive antibody response to HTLV-III antigens were also positive for HTLV-I, while 10 of 12 samples (83 percent) positive for HTLV-I were positive for HTLV-III. These percentages are in good agreement with the overall rates of 16 percent for HTLV-I and 66 percent for HTLV-III. The agreement might have been closer, but 11 samples of the original group of 86 had been depleted. Of the 11 samples that were removed 5 were positive for HTLV-I antibody. The mean titer of samples positive in both the HTLV-I and HTLV-III tests was two to three times higher than that of samples that were not. Only one sample (out of 10) had a higher titer with respect to HTLV-I (840 versus 80).

The data show that antibodies recog-

Table 2. Specificity of ELISA tests for HTLV-III and HTLV-I antibodies. Titers were determined as described in Table 1; representative sera are listed individually; ATL, adult T-cell leukemia.

| Serum                               | Titer of antibodies |                  |
|-------------------------------------|---------------------|------------------|
|                                     | Against HTLV-I      | Against HTLV-III |
| <i>Caribbean black ATL patients</i> |                     |                  |
| S0082                               | 76,000              | <20              |
| S0180                               | 44,000              | 120              |
| S0209                               | 100,000             | <20              |
| S0310                               | 310,000             | 40               |
| S0646                               | 81,000              | 40               |
| S0668                               | 520                 | <20              |
| S0669                               | 19,000              | <20              |
| S1050                               | 19,000              | <20              |
| S1089                               | 49,000              | 40               |
| <i>AIDS or at-risk patients*</i>    |                     |                  |
| J8757                               | <20                 | 29,000           |
| J8759                               | 24                  | 3,400            |
| J8760                               | <20                 | 100,000          |
| J8783                               | 23                  | 110,000          |
| J8786                               | <20                 | 29,000           |
| J8800                               | <20                 | 105,000          |

\*Danish white homosexual males with AIDS (J8757) or "asymptomatic" when presenting with venereal disease (29, 30).

nizing a virus related to or identical to HTLV-III were present in most of the Ugandan subjects tested. Since the subjects were chosen to be representative of Burkitt's lymphoma patients by age, sex, and community and since the mean age was low, it is likely that residents of the West Nile region of Uganda have been and continue to be exposed to the virus at a very early age. The immunologic

specificity of the tests argues against cross-reactivity resulting from antibodies against HTLV-I (or vice versa) in these cases (Table 2) (11); and, since antibodies against HTLV type 2 (HTLV-II) (23) would be expected to react more efficiently with HTLV-I than HTLV-III (4, 22), it is unlikely that HTLV-II is responsible for the high prevalence of HTLV-III antibodies seen. As expected, titers of HTLV-III antibody-positive serum against HTLV-II antigens were not significant. Therefore we favor the interpretation that this population subgroup has been exposed to both HTLV-III and, to a lesser extent, HTLV-I (24). Subjects with antibodies reactive with both HTLV-I and HTLV-III had higher titers than those with a single specificity. We believe that certain host or environmental factors may facilitate or enhance exposure, susceptibility, or immune responsiveness to both or even other viruses. Alternatively, it is conceivable that the observed antibody reactivities against HTLV-I and HTLV-III result from unusual cross-reactivity of antibodies against an undescribed variant of HTLV, that is, a type 4. Finally, infection by one of the viruses could increase susceptibility to the other, but studies of areas where HTLV-I is endemic, such as southern Japan and Jamaica, do not support this idea.

If, as we suspect, the antibody reactivities found represent widespread exposure or infection by HTLV-III, then it

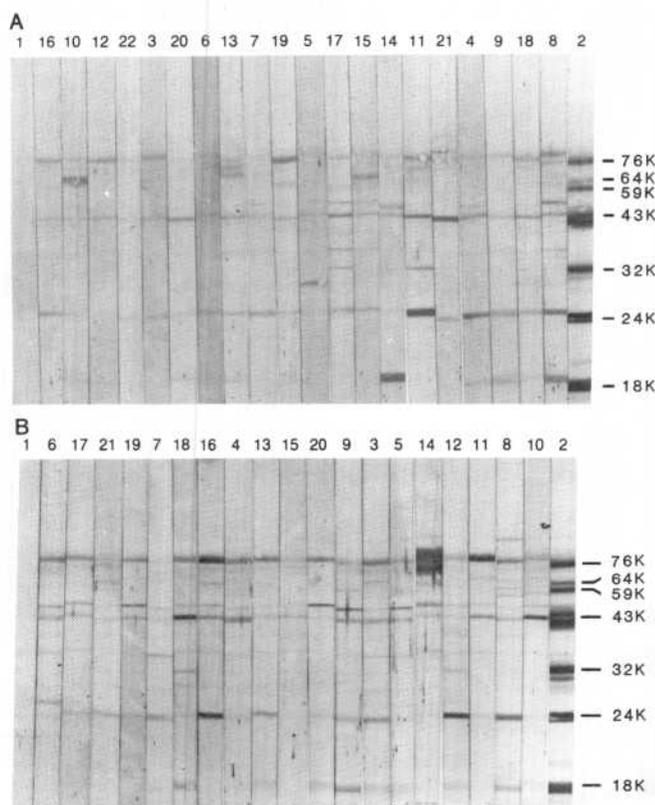


Fig. 1. Identification of HTLV-III antigens recognized by Ugandan serum collected in 1972 and 1973. Absorbance values of samples tested for HTLV-III antibody were expressed in ratio to a standard normal control serum and samples with ratio values greater than the mean + 2 standard deviations = 2.0 for normal donors were subjected to a confirmatory test requiring positive reactivity with the major HTLV-III viral bands separated by SDS-PAGE (200 µg of virus per 12 percent acrylamide slab) and transferred to nitrocellulose sheets by electroblotting (21) overnight at 15°C and 30 volts. The sheets were cut into strips and incubated with human test serum diluted 1:400 in 5 percent nonfat dried milk [Blotto (26)] containing 0.02 percent methionine for 16 hours at 4°C. The strips were washed with wash buffer (18), phosphate-buffered saline (PBS) containing 0.5 percent deoxycholate, 0.5 percent Triton X-100, and 1 mM phenylmethylsulfonyl fluoride (PMSF). Next, the strips were incubated at room temperature for 1 hour with goat antiserum to human immunoglobulin G Fc fragment (Cappel) diluted 1:400 in buffer 1 (18), 20 mM tris-HCl (pH 7.5) containing 1 mM EDTA, 0.2M NaCl, 0.3 percent Triton X-100, bovine serum albumin (2 mg/ml), and 4 percent normal goat serum. The strips were washed as described above and incubated for 1 hour at room temperature with human immunoglobulin G conjugated with horseradish peroxidase (2 µg/ml) (27) in PBS containing 0.5 percent Tween 20 and 5 percent normal goat serum. After washing the peroxidase color reaction was developed by incubation in 25 mM tris (pH 7.5) containing 0.02 percent hydrogen peroxide and 0.025 percent *o*-dianisidine. (A) and (B) show 39 different sera tested in consecutive experiments. Strips are arranged in order of ascending screening ratios from left to right; lane numbers refer to the original position of the strip within the nitrocellulose blot. Lanes 1 in (A) and (B) contain normal control human serum; lanes 2 in (A) and (B) contain positive control human serum from an AIDS patient. Normal (control) human serum at a tenfold higher concentration (1/40) also showed no visible staining.

must be asked why the incidence of AIDS in the Ugandan population (and neighboring Zaire) has gone unnoticed for so long. It is possible that AIDS existed in African populations without being recognized as a separate disease entity. The virus may have originated in Africa in the past and exposure to the virus may be much more common than AIDS itself in some populations. As with many other infectious diseases, host responsiveness may vary between severe and subclinical. If recent reports of AIDS in central Africa suggesting that the disease is newly evolved and spreading are correct (8), then it is essential to determine which of the various host- and virus-related factors are responsible. For example, it is important to know whether the current spread of AIDS is due to a spread of HTLV-III from nonsusceptible to susceptible populations or to a molecular change in the virus. In this regard, our samples were taken from a sparsely populated subsistence-farming environment (17) where AIDS is not known to occur, while the recent spread in African AIDS appears to be in more densely populated urban environments and heterosexual populations (25). Clearly, epidemiologic and virologic studies are needed to examine changes in the occurrence of AIDS and AIDS-related diseases in healthy individuals (particularly children) in central Africa.

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- by Henning Sørensen at the Rigshospitalet, University Hospital of Copenhagen Blood Bank, serving the Copenhagen area. A detailed description by age and sex is given by W. C. Saxinger, G. Lange-Wantzin, K. Thomsen, M. Hoh, and R. C. Gallo (in preparation).
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