Prevalence of Antibodies to HTLV-III in AIDS Risk Groups in West Germany

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Abstract

The prevalence of antibodies to human T-lymphotropic virus III was determined in acquired immunodeficiency syndrome (AIDS) risk groups by an enzyme linked immunosorbent assay and confirmatory tests in four different areas in West Germany. Twenty-four of 28 homosexual AIDS patients (86%), 24 of 33 (AIDS) risk groups by an enzyme linked immunosorbent assay and confirmatory tests in four different areas in West Germany. Whereas 36 selected blood donors, and 32 healthy laboratory personnel were all negative. Also no human T-lymphotropic virus III antibodies were detected in sera of 187 homosexuals with lymphadenopathy syndrome or AIDS and hemophiliacs (4). Seropositive studies in several countries indicate that these risk groups are the same groups which are predominantly infected with HTLV-III (5–14). Here we report on the prevalence and distribution of HTLV-III in several AIDS risk groups in West Germany.

Introduction

There is convincing evidence for a major etiological role of HTLV-III and related viruses in AIDS (1–3). The major risk groups for AIDS are promiscuous homosexual men, i.e. drug abusers, and hemophiliacs (4). Seropositive studies in several countries indicate that these risk groups are the same groups which are predominantly infected with HTLV-III (5–14). Here we report on the prevalence and distribution of HTLV-III in several AIDS risk groups in West Germany.

Materials and Methods

Cells and Virus. The HTLV-III producing cell line H 9/HTLV-III was a gift from Dr. R. C. Gallo, Bethesda, MD. Virus was pelleted from the culture supernatant (rotor 19; 18, 000 rpm; 5 h at 4°C). The pelleted virus was purified by two cycles of centrifugation in linear 15–60% sucrose gradients. The banded virus was freed from sucrose and treated with Triton X-100 (final concentration, 0.5%).

ELISA. The ELISA technique was performed as described earlier (15) with some modifications for the detection of antibodies (16). Microwell plates (Immunolon plates; Dynatech, Nürenberg) were coated with 100 μl of virus suspension (10 μg/ml; final concentration of Triton X-100, 0.0015%) in 50 mM carbonate buffer (pH 9.6) for 20 h at 4°C. After washing the plates the wells were coated with 150 μl 5% bovine serum albumin in carbonate buffer at pH 9.6 for an additional 20 min. The diluted test sera were added for 2 h at 37°C followed by horseradish peroxidase coupled rabbit anti-human IgG in PBS plus 0.1% Tween 60 and 1% bovine serum albumin and incubated for another 2 h at 37°C. After washing orthophenylenediamine was added as a substrate. The color reaction was determined in a SLT 210.1 reader (Salzburger Labor Technik, Salzburg, Austria).

Immunoperoxidase Staining. Immunoperoxidase staining with the ELISA positive sera on H 9 and H 9/HTLV-III cells was performed according to standard procedures. Cells (2 x 10⁶) were added into each well of a Terasaki-plate, incubated at 37°C for 30 min, and centrifuged at 1000 x g for 10 min. The cells were fixed with ice-cold methanol/acetone (1:1) for 5 min and air dried. The human sera (10 μl) were added in dilutions of 1:100, 1:300 and 1:1000 in PBS and incubated at 37°C for 60 min. After washing, 10 μl of rabbit anti-human IgG horse liver peroxidase (Dako) diluted 1:100 in PBS was added per well and incubated at 37°C for 60 min. The wells were washed again and substrate was added (20 mg 3-amino-9-ethyl-carbazol dissolved in 5 ml N,N-dimethylformamide and 95 ml 50 mM acetate, pH 5.0, filtered and mixed with 50 μl 30% H₂O₂). After washing under running water the plates were covered with 50% glycerol and evaluated under a light microscope.

Sera. Serum samples were collected in the respective hospitals, shipped by express mail, and kept frozen at −20°C or −70°C. Sera dilutions of 1:50 and 1:200 were used in the ELISA tests.

Results

The results obtained with sera from a total of 677 persons with AIDS or at risk of AIDS and controls are summarized in Table 1.

AIDS and LAS-ARC Patients. Twenty-eight sera were obtained from male homosexuals with AIDS and 33 from male homosexuals with signs of LAS-ARC. Twenty-four of the AIDS patients (85.7%) and 24 of the LAS-ARC patients (72.7%) were positive for HTLV-III antibodies (Table 1). The majority of sera from the AIDS and LAS-ARC cases had titers between 1:1,000 and 1:50,000 (Chart 1).

Homosexuals at Risk of AIDS. Sera were collected from 113 homosexuals at risk of AIDS in Munich between August and October 1984. Of these sera, 44 (38.9%) were positive for HTLV-III antibodies. Some of the sera showed high titers of more than 1:100,000 (Chart 1).

Prostitutes. In contrast all the 187 sera tested from prostitutes in Munich were HTLV-III antibody negative (Table 1).

Hemophiliacs. Three groups of sera from a total of 123 hemophiliacs from three different West German areas were tested. The first group consisted of sera collected in January 1983 in Ulm (35 patients). The second group comprised sera from 65 patients from Cologne obtained between January and June 1984. The third group were sera taken from 23 hemophiliacs in Homburg-Saar during September and October 1984. Seven of the 35 sera from Ulm, 25 of the 65 sera from Cologne, and 19 of the 23 sera from Homburg-Saar were positive (20.0, 38.5, and 82.6%, respectively). The results are summarized in

1 Presented at the HTLV Symposium, December 6 and 7, 1984, Bethesda, MD. This study was supported in part by the Bundesminister für Forschung und Technologie (Project NT/A-MT 0569 01 ZO 0565).
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: HTLV-III, human T-lymphotropic virus III; AIDS, acquired immunodeficiency syndrome; ELISA, enzyme-linked immunosorbent assay; LAS, lymphenopathy syndrome; ARC, acquired immunodeficiency syndrome-related complex; PBS, phosphate-buffered saline.
Patients with Multiple Transfusions. Thirty-six patients polytransfused with blood products from local donors were tested for HTLV-III antibodies. Two of them were positive. But 36 blood donors selected from the donor pool of the polytransfused patients were negative.

Laboratory and Clinical Personnel. None of the hospital workers having contact with AIDS and LAS-ARC patients was antibody positive. Also all laboratory personnel involved in AIDS and/or in HTLV-III diagnostic work were negative.

Discussion

The seroepidemiological study reported here indicates a distribution of HTLV-III virus in AIDS risk groups in West Germany comparable to that observed in other countries which have been surveyed (5–12). Homosexual AIDS patients as well as homosexual patients with LAS-ARC are to large extent antibody positive. According to virus isolation studies these individuals have to be regarded as infectious virus carriers (1, 2, 16–19) but also antibody negative AIDS patients may harbor infectious HTLV-III virus as reported by Groopman (20) elsewhere in this supplement. Antibody negativity for example might be due to AIDS-related failure to produce antibodies or existing antibodies might escape detection if certain antigenic determinants which are recognized by these antibodies are not exposed on the Triton X-100 treated HTLV-III used in the assays. Indeed some of our antibody negative AIDS patients were antibody positive when urea treated HTLV-III virus was used as antigen instead of Triton X-100 disrupted virus. This indicates the presence of antibodies in some AIDS patients which are specific for one or a few epitopes that can only be detected with appropriate antigen preparations.

Asymptomatic homosexuals at risk of AIDS from the Munich area who consulted medical doctors because of their contacts with other homosexuals at risk or with persons who developed AIDS later also show a remarkable proportion of antibody carriers. Many of the positive reactors had contact with persons from New York or were in New York themselves in the past years. Prostitutes, especially those who are i.v. drug abusers, have also been reported to be HTLV-III antibody positive [Ginzburg (21), this supplement]. In our study none of 187 prostitutes in Munich who are under permanent medical control was positive. This indicates that registered prostitutes under constant medical control are not at present a reservoir for HTLV-III in Munich. We have no data yet, however, on unregistered prostitutes.

An increasing number of HTLV-III antibody positive persons was found among otherwise healthy hemophiliacs. The percentage increase from January 1983 to October 1984 probably reflects a general trend observed also by others (12, 22) as well as distinct local situations. All HTLV-III antibody positive patients from Ulm in 1983 and all HTLV-III positive hemophiliacs from Homburg in 1984 had received blood products imported from the USA (23). Also, two patients who had received multiple blood transfusions from local donors showed HTLV-III antibodies.

In summary our data show that HTLV-III is present in the well-known risk groups in West Germany and that HTLV-III has spread already to other population groups. An extended study of blood donors in West Germany also yielded a small percentage of HTLV-III antibody positive individuals (24). Large seroepidemiological studies will have to be performed in order to detect all possible routes of transmission, particularly by HTLV-III positive blood donors. This is a prerequisite for the successful control of a further distribution of the virus.

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