HTLV-III Infection in Homosexuals and Hemophiliacs in Sweden


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Abstract

Two hundred and three homosexual (HS) men and 114 hemophiliacs in Sweden were examined for serum antibodies to human T-lymphotropic virus type III (HTLV-III) and for alterations of T-lymphocyte subsets. Sera were screened for HTLV-III antibodies by an enzyme-linked immunosorbent assay and/or a dot immunobinding assay, and positive reactions were confirmed by Western blotting. HTLV-III antibodies were demonstrated in 13 of 13 (100%) HS men with acquired immune deficiency syndrome, in 63 of 67 (94%) HS men with persistent generalized lymphadenopathy, in 17 of 45 (38%) asymptomatic HS men, and in 6 of 78 (8%) asymptomatic non-HS men but in none of 108 male blood donors. Seropositive HS men had significantly lower T4/T8 (helper/suppressor) cell ratios and T4 cell numbers than had seronegative HS men. Seronegative HS men had decreased T-cell ratios compared to controls but not decreased T4 cell numbers. Among hemophilia A patients, HTLV-III antibodies were demonstrated in 40 of 48 (83%) cases treated with American factor VIII concentrate and in 17 of 29 (59%) cases treated with both American and Swedish concentrates but in none of 13 cases treated exclusively with Swedish factor VIII. Twenty-one hemophilia B patients treated with Swedish factor IX concentrates were all seronegative, whereas one of 3 hemophilia B cases treated with imported factor IX was seropositive. T4/T8 cell ratios were significantly lower in seropositive as compared to seronegative hemophilia A patients.

Introduction

There is evidence indicating that the newly identified retrovirus called HTLV-III or lymphadenopathy virus is etiologically associated with AIDS and with AIDS-related manifestations such as, for example, PGL (1-3). Recent studies in the USA, France, and England have shown that serum antibodies to this virus are common in patients with AIDS and in persons at risk for AIDS, including homosexual men, hemophiliacs, and intravenous drug abusers (4-9).

HTLV-III selectively infects T-cells of helper-phenotype (T4-cells) (1, 3, 10). Virtually all patients with AIDS and a proportion of persons at risk for AIDS have decreased numbers of T4-cells and decreased ratios between T4 cells and T-cells of suppressor/cytotoxic phenotype (T8-cells) (11).

We report here studies of the prevalence of HTLV-III antibodies and of the relation between seropositivity and alterations of T-cell subsets in Swedish subjects at risk for AIDS, including homosexual men with symptoms or without symptoms and hemophiliacs treated with factor VIII or IX concentrates of American or Swedish origin.

Materials and Methods

Subjects. The following groups of people, all of whom were living in Sweden, were studied: (a) 13 HS men with AIDS according to the Centers for Disease Control definition (12) with opportunistic infections and one with Kaposi's sarcoma; (b) 67 HS men with PGL, defined as lymph node enlargement in two or more extraginal sites lasting for more than 3 months; (c) 45 symptomatic HS men, including 43 men with a transient or minor degree of lymphadenopathy not fulfilling the definition of PGL and 2 men with only B symptoms (prolonged fever and fatigue); (d) 78 asymptomatic HS men; (e) 90 patients with hemophilia A (61 adults and 29 children ≤15 years of age); (f) 24 patients with hemophilia B (20 adults and 4 children); and (g) 108 male blood donors. Blood specimens were collected from these subjects in 1983 and 1984.

Enzyme-linked Immunosorbent Assay. Determination of antibodies to HTLV-III by ELISA was done as described previously (12, 13) using virus produced by clone H9/HTLV-III b (10). Sera with ELISA absorbance values at least 3 times over the value of a standard negative control serum (antibody ratio, ≥3.0) were also tested for HTLV-III antibodies by Western blotting.

Dot Immunobinding Assay. Determination of antibodies to HTLV-III by DIB was done as described previously in detail (13). The antigen used was a lysate of HTLV-III-infected T-cells of line H9 (10). All sera positive by DIB (titer ≥100) were also tested by Western blotting.

Western Blotting. Testing for HTLV-III antibodies by Western blotting was done essentially as described previously (12, 14) using an immunoperoxidase technique.

Determination of T-Lymphocyte Subsets. T-cell subsets in blood were determined by direct or indirect immunofluorescence. Fluorescein isothiocyanate-conjugated monoclonal antibodies to T4 (helper subset), T8 (T-suppressor/cytotoxic subset), and T3 (total T-cells) (Ortho Diagnostics, Raritan, NJ) were used in most instances. In some instances the equivalent unconjugated Leu monoclonals Leu 3, Leu 2, and Leu 4 (Becton & Dickinson, Sunnyvale, CA) were used followed by a fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin. The samples were analyzed in an Ortho Spectrum III flow cytometer.

Statistics. For statistical analysis Student's t-test was used or the Mann-Whitney test when the number of cases was small.

Results

HTLV-III Antibodies and T-Cell Subsets in Homosexual Men. The results of determination of HTLV-III antibodies by ELISA and Western blotting in HS men and in blood donor controls are shown in Table 1. All sera from AIDS cases, PGL cases, and symptomatic HS men which had ELISA antibody ratios above 3.0 were positive by Western blotting. However, in the group of asymptomatic HS men, four sera with ELISA...
antibody ratios between 3 and 7 were negative by Western blotting. Among the blood donor sera with ELISA ratios between 3 and 5, there were two sera which showed faint reactivity by Western blotting and which were considered doubtful.

All of 91 sera from HS men with ELISA antibody ratios ≥3 which were positive by Western blotting were also positive by DIB. In addition to 6 AIDS cases seropositive by ELISA and Western blotting (see Table 1), 7 AIDS cases not tested by ELISA were seropositive by DIB and Western blotting.

The combined results of the serological tests in the four groups of HS men (Table 1) showed that HTLV-III antibodies were present in all of 13 AIDS cases (7 of them not tested by ELISA), in 94% of PGL cases, in 38% of symptomatic HS men, and in 8% of asymptomatic HS men.

Results of T-cell subset determination in relation to the presence or absence of HTLV-III antibodies in the various groups of HS men are shown in Table 2. Seropositive men had significantly lower T4/T8 cell ratios and absolute numbers of T4 cells than had seronegative HS men. Symptomatic as well as asymptomatic seronegative HS men also had decreased T-cell ratios as compared to controls, but their T4 cell numbers were not decreased. The number of T8 cells was increased in seronegative as well as in seropositive HS men, with the exception of AIDS cases (Table 2). The proportions and numbers of T3- (total T-) cells were not significantly different in seropositive HS men as compared to seronegative HS men or controls, except for AIDS patients who had decreased relative and absolute numbers of T3-cells (data not shown).

The finding of a low T4/T8 cell ratio (below 0.8) was associated with HTLV-III seropositivity in 46 of 47 (98%) PGL cases and in 11 of 16 (69%) symptomatic HS men but in only 2 of 11 (18%) asymptomatic HS men.

HTLV-III Antibodies and T-Cell Ratios in Hemophiliacs. The prevalence of HTLV-III antibodies demonstrated by DIB and Western blotting in Swedish hemophiliacs treated with factor VIII or IX concentrates of American or Swedish origin is shown in Table 3. HTLV-III antibodies were found in 85% of adults and in 80% of children with hemophilia A treated with American factor VIII, whereas none of 13 hemophilia A patients treated exclusively with Swedish factor VIII was seropositive. Fifty-nine percent of cases treated with both American and Swedish factor VIII had HTLV-III antibodies. Among patients with hemophilia B, all 21 cases treated with Swedish factor IX were seronegative, while 1 of 3 cases treated with imported factor IX was seropositive.

Results of determination of T4/T8 cell ratios in hemophiliacs in relation to HTLV-III antibody status are shown in Table 4. T4/ T8 ratios were significantly lower in seropositive as compared to seronegative hemophilia A cases.

**Discussion**

The finding of HTLV-III antibodies in all cases with AIDS and in more than 90% of cases with PGL in this study of HS men in Sweden is in agreement with results of similar studies in the USA, France, and England (4–8). In the group of Swedish HS men with a transient or minor degree of lymphadenopathy (symptomatic group), only 38% were seropositive, indicating that lymph node enlargement in this group of men often was due to other causes than HTLV-III infection. The 8% HTLV-III seropositivity found in the Swedish asymptomatic HS men is lower than the corresponding figures in similar studies from the USA, France, and England (4, 5, 7, 8).

Most HS men in the present study participated in a health control study of HS men in Stockholm, including 1000 HS men studied clinically during 1983. Of these men, 5.6% had PGL, and another 17% had enlarged lymph nodes not fulfilling Centers for Disease Control criteria for PGL.
cases, only two cases had PGL, while the other cases were essentially healthy except for their bleeding disorder. It is conceivable that a proportion of the hemophiliacs may have developed HTLV-III antibodies in response to repeated antigen stimulation by non-viable virus in the concentrates rather than in response to infectious virus.

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