CONCISE REPORT

Antibodies to Human T-Cell Leukemia Virus Type III in Hemophiliacs From Spain

By L. Kitchen, M. Leal, I. Wichmann, E. Lissen, M. Ollero, J.S. Allan, M.F. McLane, and M. Essex

We tested serum samples from 50 hemophiliacs from Sevilla, Spain, for antibody to HTLV-III by indirect membrane immunofluorescence (IMI) and radioimmunoprecipitation with SDS polyacrylamide gel electrophoresis (RIP-SDS/PAGE). All had received commercial clotting factors from the United States with the exception of one hemophiliac who had never been transfused. Thirty-four (68%) reacted with HTLV-III-infected cells (H9/HTLV-III) by both methods, but not with the uninfected line (H9). Of 41 hemophilia-A patients tested, 28 (68%) were positive, and of nine hemophilia-B patients, six (66%) were positive. The nontransfused hemophilia-B patient was negative for antibody to HTLV-III by both methods. One patient with clinical AIDS tested positive as did six of seven with chronic unexplained lymphadenopathy. The eight individuals with AIDS or lymphadenopathy all had hemophilia A. We conclude that exposure to HTLV-III is widespread among asymptomatic hemophiliacs in Spain.

PREVIOUS STUDIES have revealed that seropositivity to HTLV-III is associated with AIDS-related disease in hemophiliacs and is increasingly prevalent among asymptomatic hemophiliacs. Using indirect membrane immunofluorescence (IMI) and radioimmunoprecipitation with sodium dodecyl sulfate/polyacrylamide gel electrophoresis (RIP-SDS/PAGE), we detected HTLV-III antibodies in 30 of 47 (64%) of asymptomatic American hemophiliacs and in three of three (100%) hemophiliac AIDS cases. Hemophilic AIDS cases have been noted in several other countries outside of the United States.

The first published report of AIDS cases among hemophiliacs in Europe to appear in the international medical/scientific literature was from Sevilla, Spain, where three cases were described in early 1983, including a case of fatal AIDS in a 9-year-old hemophiliac. Two of the three cases had become symptomatic by I 982. In I 984 a fourth case of hemophiliac AIDS was reported in a 5-year-old boy from Sevilla. An additional three hemophilic AIDS cases (seven total) and 12 cases of chronic unexplained lymphadenopathy have subsequently been diagnosed in Sevilla; five of the AIDS cases have died (M. Leal, I. Wichmann, unpublished results). Analysis of the HTLV-III antibody response in Spanish hemophiliacs would therefore seem particularly pertinent given (1) the rapidity with which the disease first appeared in Spain, (2) the increasing number of AIDS cases among hemophiliacs in Sevilla, and (3) the exclusive use, in Spain, of clotting factor concentrates imported from the United States.

MATERIALS AND METHODS

We tested serum samples from 50 hemophiliacs from the Hospital General Ciudad Sanitaria Virgen del Rocio, Sevilla, Spain. The average age was 18.8 years with a standard deviation of 12.9 years. Forty-one patients had hemophilia A and nine had hemophilia B. All samples were collected during the year 1983. Forty-two patients were asymptomatic at the time of serum collection; one patient had clinical AIDS, and seven had chronic unexplained lymphadenopathy. All except one patient with hemophilia B had received infusions of commercial clotting factor concentrates imported from the United States; none had received cryoprecipitate therapy. Serum samples from 20 healthy volunteer Spanish blood bank donors were also tested.

All serum samples were initially examined at a 1:4 dilution by IMI on the HTLV-III infected line, designated H9/HTLV-III, and the uninfected line, designated H9. All 50 samples were subsequently tested using RIP-SDS/PAGE. Both uninfected H9 and HTLV-III-infected cells were metabolically labeled with 35S-cysteine according to details described elsewhere.

RESULTS

Of 50 samples tested, 34 (68%) reacted with the HTLV-III-infected cells by IMI, but not with the uninfected cells (Table I). Serum samples from the 20 (healthy Spanish) blood bank donors were all negative when tested by both membrane immunofluorescence and immunoprecipitation. All 34 serum samples positive by IMI precipitated the HTLV-III-encoded glycoproteins 120 and 160 by RIP-SDS/PAGE. These two glycoproteins are the antigens most consistently recognized by antibodies to HTLV-III in AIDS patients, patients with the AIDS-related complex (ARC), and asymptomatic hemophiliacs and homosexual males. In addition, 25 of 34 (74%) samples positive by IMI and containing antibodies recognizing gp120 and gp160 also precipitated the HTLV-III gag-related antigens p24 and p55 (Table I, Fig 1). The seropositive hemophilic serum samples precipitated several other bands in varying proportions, including proteins of about 41 kd (gp41), 27 kd (p27), and 17 kd (p17). P27 is a gene product of the 3' open reading frame of HTLV-III. Of the 34 serum samples positive by IMI and precipitating gp120 and gp160 by RIP-SDS/PAGE, 16 (47%) also precipitated p27.

The 50 hemophiliacs tested included one patient who had clinical AIDS and seven who had lymphadenopathy. All tested positive by both procedures, except for one with lymphadenopathy, and all eight were hemophilia-A patients.
Table 1. Presence of Antibodies to HTLV-III Hemophiliacs and Asymptomatic Blood Bank Donors From Sevilla, Spain

<table>
<thead>
<tr>
<th>Category</th>
<th>No. Tested</th>
<th>HTLV-MA</th>
<th>gp120/gp160</th>
<th>p24/p55</th>
<th>p27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A</td>
<td>41</td>
<td>28 (68)</td>
<td>28 (68)</td>
<td>21 (51)</td>
<td>14 (34)</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>9</td>
<td>6 (66)</td>
<td>6 (66)</td>
<td>4 (44)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>42</td>
<td>27 (64)</td>
<td>27 (64)</td>
<td>22 (52)</td>
<td>13 (31)</td>
</tr>
<tr>
<td>Chronic lymphadenopathy</td>
<td>7</td>
<td>6 (86)</td>
<td>6 (86)</td>
<td>3 (43)</td>
<td>3 (43)</td>
</tr>
<tr>
<td>AIDS</td>
<td>1</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total hemophiliacs</td>
<td>50</td>
<td>34 (68)</td>
<td>34 (68)</td>
<td>25 (50)</td>
<td>16 (32)</td>
</tr>
<tr>
<td>Healthy blood donors from Spain</td>
<td>20</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Antibodies to HTLV-MA were determined by IMI; those to gp120/gp160, p24/p55, and p27 were determined by RIP-SDS/PAGE.

Of the 41 hemophilia-A patients tested, 28 (68%) were positive for antibodies, and of the nine hemophilia-B patients, six (66%) were positive. The one hemophilia-B patient who had never been transfused was negative for antibody to HTLV-III by both methods.

**DISCUSSION**

The present results suggest that exposure to HTLV-III is widespread among asymptomatic hemophiliacs in Spain as well as among those suffering AIDS-related disease. That the seropositivity rate among Spanish hemophiliacs is similar to that seen in American hemophiliacs may be due to the use, in Spain, of commercial clotting factor concentrates imported from the United States. Since the rate of AIDS-related disease and lymphocyte dysfunction in asymptomatic hemophiliacs increases with duration of seropositivity to HTLV-III, and since there is evidence of heterosexual transmission of HTLV-III from seropositive hemophiliacs to their wives, the clinical implications of the high seropositivity rate among hemophiliacs in Spain, as in the United States, are ominous.

**REFERENCES**


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