CONCISE REPORT

Antibodies Reactive With Human T Cell Leukemia Viruses in the Serum of Hemophiliacs Receiving Factor VIII Concentrate

By James J. Goedert, M.G. Sarngadharan, M. Elaine Eyster, Stanley H. Weiss, Anne J. Bodner, Robert C. Gallo, and William A. Blattner

The third member of the family of T cell leukemia viruses (HTLV III) has been proposed as the primary etiologic agent of the acquired immunodeficiency syndrome (AIDS). A high risk of AIDS has been reported among patients with hemophilia, particularly those with factor VIII deficiency who receive commercial clotting factor concentrates. In a prevalence survey conducted between September 1982 and April 1984, initial serum samples from 74% of hemophiliacs who had ever been treated with commercial factor VIII concentrate, 90% of those frequently treated with factor VIII concentrate, and 50% of those treated with both factor VIII and factor IX concentrates had antibodies reactive against antigens of HTLV III, compared with none of the hemophiliacs treated only with factor IX concentrate or volunteer donor plasma or cryoprecipitate. Two of the seropositive patients have developed AIDS-related illnesses, and a third patient died of bacterial pneumonia. One initially seronegative patient developed antibodies against HTLV III during the study and is currently well. The predominant antibody specificities appear directed against p24 and p41, the presumed core and envelope antigens of HTLV III, suggesting that factor VIII concentrate may transmit the p24 and p41 antigens of HTLV III. However, the presence of infectious retroviruses in clotting factor concentrates and the effectiveness of screening and viral neutralization procedures remain to be determined.

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THE NEWEST member of the family of human T cell leukemia viruses (HTLV III) can be isolated frequently from patients with the acquired immunodeficiency syndrome (AIDS). The continuous production of HTLV III from clones of human T cells led to the development of an enzyme-linked immunosorbent assay (ELISA) for HTLV III antibodies. Such antibodies were detected in the sera of 43 (88%) of 49 patients with AIDS, as well as in 11 of 14 patients with AIDS-related conditions, six of 17 unaffected homozygous male patients with hemophilia, and three of five unaffected intravenous drug users, while only one (0.6%) of 164 normal control subjects. Similar results have been obtained using an ELISA for antibodies to the lymphadenopathy-associated virus (LAV) that was isolated by French investigators. Subsequently, a prospectively monitored cohort of homosexual men has provided compelling statistical evidence that HTLV III seropositivity is associated both with AIDS and with several related conditions.

Patients with hemophilia are at increased risk of developing AIDS. Vilmer et al isolated LAV from a hemophilic AIDS, and from his hemophilic brother, and Essex et al reported that 5% to 19% of hemophiliacs had antibodies reactive with HTLV-infected cell lines. These antibodies were correlated with low helper T cell counts, a subclinical AIDS-type immune abnormality, but not with suppressor/cytotoxic T cell counts. Recently, antibodies against LAV were noted in 14 (64%) of 22 hemophiliacs in Denmark and in 18 (72%) of 25 hemophiliacs in Georgia. In both of these small studies, the relationship between LAV seropositivity and blood products was unclear.

MATERIALS AND METHODS

Sera were separated from the clotted blood of 109 patients attending the hemophilia clinic of the Milton S. Hershey Medical Center between September 1982 and April 1984. The coded samples were stored at or below –20°C until testing in a blinded fashion with larger batches of sera from other studies, using previously reported methods. Lysates of sucrose density-banded HTLV III were coated on 96-well microtiter plates. The test sera were diluted with normal goat serum, added to the wells, and allowed to react overnight at room temperature. The primary immune complex formed between the HTLV III antigens and antibodies in the human sera was detected by adding peroxidase-labeled goat antiserum to human immunoglobulins and assaying for a colored peroxidase reaction product. A ratio of test samples over background was calculated by dividing the mean of duplicate measures of absorbance of each serum specimen by the mean of octuplicates of a standard control serum specimen. A ratio of 3.0 or greater was previously correlated with seropositivity. For this analysis, we used a more stringent criterion (ratio of sample to control, ≥5.0), in order to minimize false-positive test results. The predominant HTLV III
antibody specificities were evaluated by the Western blot technique as previously described.\textsuperscript{14}

### RESULTS

#### Prevalence of HTLV III Antibodies

Fifty-one (74\%) of 69 hemophilia A patients who had ever received factor VIII but not factor IX concentrate had antibodies against HTLV III (Table 1). However, in the subset of 48 patients receiving factor VIII concentrate at least twice a month, 43 (90\%) had HTLV III antibodies (Table 1). In addition, three (50\%) of six hemophilia A patients treated with both factor VIII and IX concentrates (because of a circulating inhibitor of factor VIII) were positive for HTLV III antibodies (Table 1). Two of these three seropositive patients had been heavily treated with factor VIII concentrate during the 1980s. In contrast, HTLV III antibodies were detected initially in none of 12 hemophilia B patients (including one homosexual man) treated with factor IX concentrate and in none of 22 hemophiliacs treated only with fresh frozen plasma or cryoprecipitate (Table 1).

#### Relationship of HTLV III Antibodies to Clinical Conditions

All of the seronegative patients are in good health. Two of the seropositive patients have died. One man had cirrhosis and died of bacterial pneumonia six months after his blood sample. The second, who had had herpes zoster five months before examination, later developed AIDS-type illnesses that included recurrent herpes zoster, extreme weight loss, severe lymphocytopenia, oral candidiasis, and fatal pneumonia 12 months after examination. In addition, a 13-year-old seropositive boy had idiopathic thrombocytopenia when his serum was drawn and developed herpes zoster eight months later.

### Persistence and Confirmation of HTLV III Antibodies

Sera drawn two to 19 months (median, 12 months) after the initial samples from 37 of the same patients were retested by ELISA. Twenty-three factor VIII concentrate recipients remained seropositive. Of the 14 initially seronegative patients who were retested, 11 remained seronegative for 12 to 16 months, including three recipients of cryoprecipitate, seven of factor IX concentrate, and one of factor VIII concentrate. Of the three patients whose ELISA ratios converted to positive, one was confirmed by the Western blot technique as having antibodies against both the 24,000- (p24) and 41,000- (p41) molecular weight proteins that probably represent components of the HTLV III core and envelope, respectively.\textsuperscript{3,4} The other two patients, neither of whom was a factor VIII concentrate recipient, had ELISA ratios that converted from borderline (3.9 to 4.2) to positive (6.9 to 9.0), but these were not confirmed by Western blot. No other false-positive ELISA ratios were found. Four of the other factor VIII recipients with positive ELISA ratios and a fifth with a borderline ratio (4.4) had demonstrable antibodies to both p24 and p41. Five hemophiliacs with borderline ratios (3.7 to 4.3) and six with negative ratios (\leq2.5) were all negative on Western blot.

### DISCUSSION

These data demonstrate that HTLV III antibodies are detectable in the majority of hemophilia A patients, particularly those heavily treated with factor VIII concentrate. As of Oct 15, 1984, 49 hemophiliacs who did not have other risk factors have been accepted as AIDS cases by the Centers for Disease Control, including 45 patients with hemophilia A (classical hemophilia, factor VIII deficiency) and two with hemophilia B (factor IX deficiency).\textsuperscript{14} Estimating the hemophilia population from a 1980 Department of Health, Education, and Welfare survey,\textsuperscript{15} the cumula-

### Table 1. Prevalence of HTLV III Antibodies in 109 Patients With Hemophilia

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Therapy</th>
<th>No. Positive for Antibodies to HTLV III*</th>
<th>No. Tested</th>
<th>Percentage Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A</td>
<td>Factor VIII concentrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ever used</td>
<td>51</td>
<td>69†</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Frequently used†</td>
<td>43</td>
<td>48†</td>
<td>90</td>
</tr>
<tr>
<td>Hemophilia A with cir-</td>
<td>Factor VIII and IX concent-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>culating inhibitor</td>
<td>rates</td>
<td>3</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>Factor IX concentrate</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Hemophilia A or B</td>
<td>Only plasma and/or cryo-</td>
<td>0</td>
<td>22</td>
<td>0</td>
</tr>
</tbody>
</table>

*Defined as an HTLV III ELISA ratio of \geq5.0. These data include only the initial seroprevalence results.
†Factor VIII concentrate was used an average of two or more times per month.
‡One initially seronegative patient developed HTLV III antibodies at a later date.
tive attack rate of AIDS is six times greater for patients with hemophilia A (3.6 per 1,000 hemophiliacs) than those with hemophilia B (0.6 per 1,000 hemophiliacs). All 49 of these hemophiliacs with AIDS had been treated with lyophilized clotting factor concentrates (factor VIII concentrate or factor IX concentrate), plasma derivatives that have revolutionized the treatment and dramatically improved the lives of many patients with hemophilia. These products are commercially prepared from the pooled plasma of thousands of donors and distributed nationally and internationally. Although infectious retroviruses apparently can be transmitted in clotting factor concentrates, as evidenced by clinical AIDS in hemophiliacs and by isolations of HTLV III and LAV from hemophiliacs, it is possible that some of the reactivity detected in this survey represents an antibody to incomplete, noninfectious HTLV III antigens. It is critical to determine how frequently intact, infectious viruses can be isolated from patients with hemophilia and from commercial clotting factor concentrate. Such studies are in progress.

Although both factor VIII and factor IX concentrates frequently transmit hepatitis B and non-A, non-B viruses there are major differences in the derivation and purification of these products from frozen plasma, which could affect the ultimate risk of acquiring virus-transmitted illnesses. Factor IX concentrate, also called prothrombin complex concentrate, is affinity-purified from the supernatant of frozen-thawed plasma by absorption on a diethylaminoethyl (DEAE) ion exchange column. In contrast, factor VIII concentrate, also called antihemophilic factor (AHF) concentrate, is prepared from the precipitate of frozen-thawed plasma from which the vitamin K-dependent factors and fibrinogen have been partially removed by cold ethanol fractionation and precipitation. With electron microscopy, a considerable amount of cellular and proteinaceous debris is apparent in cryoprecipitate and reconstituted factor VIII concentrate. It may be that antigens of HTLV III sediment preferentially with the cellular debris. This hypothesis is supported by the observations that cell surface antigens (p61 and p65) encoded by HTLV I are recognized, at low titers, by antibodies in the serum of hemophiliacs. Moreover, the specific HTLV III antibodies detected in the ELISA system are directed predominantly against the presumed envelope protein (p41), acquired by the virus during budding from the infected cell surface. The alternatives to factor VIII concentrate therapy for patients with hemophilia A, fresh-frozen plasma or cryoprecipitate, were associated with an HTLV III seropositivity rate of less than 3% in this study. However, most of these patients had mild hemophilia, and the seropositivity rate might well increase with the intensive therapy that would be needed for patients with severe hemophilia. In addition, fresh-frozen plasma and cryoprecipitate are inconvenient for home-based therapy and are difficult to use during surgery or serious hemorrhage, when high levels of clotting factors must be achieved and maintained. A possible alternative to this dilemma, heat-treated factor VIII concentrate, recently has been licensed in the United States in hopes of reducing the incidence of hepatitis B virus infection in hemophiliacs. Until the capacity of heat treatment to inactivate retroviruses without destroying factor VIII activity is demonstrated, however, greatest safety might be achieved by a combination of screening donors for HTLV III antibodies, using cryoprecipitate for patients with newly diagnosed or mild hemophilia, and implementing procedures such as heat treatment to neutralize infectious agents. The need for such measures may be temporary, as treatment with factor VIII that has been cloned by recombinant DNA technology appears to be on the horizon.

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