CONCISE REPORT

An Immunologic Study of Spouses and Siblings of Asymptomatic Hemophiliacs

By Margaret V. Ragni, Franklin A. Bontempo, Jessica H. Lewis, Joel A. Spero, and Bruce S. Rabin

A transmissible agent may be responsible for the recent occurrence of the acquired immune deficiency syndrome (AIDS) in female heterosexual partners and infant contacts of AIDS victims or of persons at increased risk for AIDS. Therefore, we evaluated the clinical and immunologic status of 18 hemophiliac–spouse pairs and 19 hemophiliac–sibling pairs. Using surface marker assays of lymphocyte subpopulations, we found a mean T helper cell/T suppressor cell (H/S) ratio of 1.11 ± 0.15 (11 < 1.00), in 18 hemophiliac adults, 1.11 ± 0.15 of 18 hemophiliac adults of 1.11 ± 0.15 (11 < 1.00), in 18 hemophiliac children of 1.54 ± 0.11 (3 < 1.00), and in their 19 siblings of 1.87 ± 0.11 (none < 1.00). Both hemophiliac adult and hemophiliac children mean H/S ratios differed significantly (p < 0.05) from the control mean ratio of 2.22 ± 0.16 and from the mean of their respective nonhemophiliac spouse or sibling group (p < 0.05). The mean ratio of hemophiliac adults differed significantly from that of hemophiliac children (p < 0.05). These findings suggest that lymphocyte alterations in hemophiliacs are secondary to their therapy, are not influenced by genetic factors, and are not related to an infectious agent that is easily transmissible by close contact.

Clinical Data

All 37 hemophiliacs and their spouses and/or siblings were clinically well, none having experienced fever, night sweats, or weight loss. There was no known history of intravenous drug use or homosexuality. Factor VIII levels were less than 0.01 U/ml in 11 adults and 14 children, and between 0.02 and 0.03 U/ml in 2 adults and 4 children; factor IX levels were less than 0.01 U/ml in 4 adults and 1 child, and 0.07 U/ml in 1 adult. Anti-VIII levels in the 2 adults and 2 children ranged between 0.4 and 40.0 Bethesda U/ml. Fifteen adults and 14 children have persistent anti-HBs, 5 of which were acquired following Hepatavax. One adult and 5 children with no hepatitis B markers are currently receiving Hepatavax-B. Of the 2 remaining adults, 1 is persistently HBsAg positive, and the other demonstrates anti-HBc only. One hemophiliac child, age 1, developed autoimmune thrombocytopenic purpura 1 mo prior to study; this was associated with a platelet count of 60,000/cu mm and a positive platelet-associated IgG of 219 ng/10^11 platelets.

On examination, one hemophiliac adult, previously reported, and one hemophiliac child had diffuse lymphadenopathy involving the axillary, inguinal, supraclavicular, and suboccipital areas. Nine other hemophiliac adults, 2 spouses, 12 hemophiliac children, and 2 siblings had shotty adenopathy, primarily in the cervical area. Hepatosplenomegaly was found in one and hepatosplenomegaly in two hemophiliac adults with coincident chronic liver disease. Two additional hemophiliac adults and two hemophiliac children had minimal splenomegaly (spleen tip palpable only), with no associated hematologic or liver abnormality.

Laboratory Data

The results of laboratory tests, including mean values for the controls, are included in Table 1. A white blood count and differential were performed on all patients. Enumeration of T lymphocytes.

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Table 1. T Helper / T Suppressor Cell Ratios in Spouses and Siblings of Asymptomatic Hemophiliacs

<table>
<thead>
<tr>
<th>No. of Pairs</th>
<th>Mean H/S Ratio*</th>
<th>No. With H/S Ratio &lt; 1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 Spouse</td>
<td>(18.9) Hemophiliac</td>
<td>1.11 ± 0.15†</td>
</tr>
<tr>
<td></td>
<td>(18.9) Spouse</td>
<td>1.88 ± 0.13</td>
</tr>
<tr>
<td>19 Sibling</td>
<td>(19.9) Hemophiliac</td>
<td>1.54 ± 0.10†</td>
</tr>
<tr>
<td></td>
<td>(139, 6) Sibling</td>
<td>1.87 ± 0.11</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM. The mean H/S ratio of 20 controls was 2.22 ± 0.16.
†Statistically different from control, p < 0.05 by Student’s t test, and statistically different from respective spouse or sibling group, p < 0.05, by paired Student’s t test.

including T helper and T suppressor cells, was obtained by flow cytometry using the FACS IV instrument and monoclonal antibodies Leu-4 (total T), Leu-3a + b (T helper), and Leu-2a (T suppressor) (Becton Dickinson, Sunnyvale, CA). Serum immunoglobulins were quantitated by a rate nephelometer (Beckman ICS Analyzer, Fullerton, CA). Cytomegalovirus (CMV) antibody was determined by indirect immunofluorescence with CMV-infected tissue culture cells as the substrate (Electronucleonics, Bethesda, MD). Statistical analysis was performed by an analysis of variance for T helper/T suppressor values based on the logarithms of the ratios. The Student’s t test and paired Student’s t test were used to compare the groups with controls and with each other.

RESULTS

Table 1 summarizes the results of the T lymphocyte studies in the 37 hemophiliacs and their spouses or siblings and in the 20 controls. A T helper cell/T suppressor cell (H/S) ratio of less than 1.00 was found in 11 of 18 hemophiliac adults and in 3 of 19 hemophiliac children, but in none of the 18 spouses or 19 siblings. One of the three hemophiliac children, in addition, has newly diagnosed autoimmune thrombocytopenic purpura. Of the hemophiliacs with an H/S ratio less than 1.00, six adults and two children are treated with factor VIII concentrate, three adults with factor IX concentrate (all three were previously exposed to factor VIII concentrate before developing acquired inhibitors to factor VIII), one adult and one child with cryoprecipitate, and one adult with fresh frozen plasma.

The mean H/S ratio ± SEM for 18 hemophiliac adults was 1.11 ± 0.15 (p < 0.05), for the 18 spouses was 1.88 ± 0.13, for the 19 hemophiliac children was 1.54 ± 0.10 (p < 0.05), and for the 19 siblings was 1.87 ± 0.11, as compared with the mean for controls of 2.22 ± 0.16. Mean values for spouses and siblings were not different from each other or from normal controls (p > 0.05). The mean ratio of hemophiliac adults was also statistically different from that of hemophiliac children (p < 0.05).

Altered H/S ratios were associated with no particular patterns of decreased T helper and/or increased T suppressor cells. Based on the range of T helper cells (551–1,044/cu mm) and T suppressor cells (232–516/cu mm) in the 20 controls, decreased T helper cells were found in 6 hemophiliac adults, 7 spouses, 6 hemophiliac children, and 2 siblings, and increased T suppressor cells were found in 5, 4, 8, and 6 subjects, respectively.

Elevated serum immunoglobulins were present in hemophiliacs and their spouses and/or siblings, although not necessarily in those with H/S ratios less than 1.00. IgG was elevated in 78% of hemophiliac adults versus 6% of their spouses and in 47% of hemophiliac children versus 16% of their siblings, IgA in 17% versus 17% and 5% versus 0%, and IgM in 72% versus 44% and 37% versus 47%, respectively. A total of 17 hemophiliac adults, 15 spouses, 5 hemophiliac children, and 3 siblings were CMV/IgG seropositive, with no clinical evidence of infection.

DISCUSSION

Recent reports of AIDS and AIDS-like immunologic abnormalities in sexual and household contacts of AIDS victims, including homosexuals, Haitians, and drug abusers, have raised the possibility that the disorder may be transmitted heterosexually, vertically, and by close contact. To date, the clinical and immunologic status of contacts of hemophiliacs with AIDS or contacts of asymptomatic hemophiliacs with AIDS-like immunologic abnormalities has not been evaluated. This study demonstrates that none of the spouses or siblings of asymptomatic hemophiliacs have clinical or immunologic abnormalities suggestive of AIDS or its prodrome: T helper cell/T suppressor cell (H/S) ratios of spouses and siblings did not differ from those of controls. These results imply that there is no increased risk of immunologic changes associated with AIDS in spouses and siblings of asymptomatic hemophiliacs.

Mean H/S ratios of hemophiliac adults and children, however, did differ significantly (p < 0.05) from controls, in agreement with other studies of asymptomatic hemophiliacs. However, abnormal H/S ratios (<1.00) occurred not only in those exposed to factor VIII concentrate (8 of 15) and factor IX concentrate (3 of 7), but also in those exposed to cryoprecipitate (2 of 13) and fresh frozen plasma (1 of 2). It thus appears that single-donor products, such as cryoprecipitate and fresh frozen plasma, as well as multiple-donor concen-
trates may be associated with altered lymphocyte subpopulations.

Hemophiliac adult mean H/S ratios differed significantly ($p < 0.05$) from those of hemophiliac children. It is likely that this difference relates to the type, amount, and duration of exposure to blood products. In order to decrease the risk of hepatitis, children in Pittsburgh receive primarily single-donor products; in addition, because of their smaller size and younger age, children have received a smaller total amount of blood products for a shorter time than adults and would theoretically have less exposure than adults to a blood-borne agent responsible for transmission of AIDS.

The fact that mean H/S ratios in siblings of asymptomatic hemophiliacs were not different from controls, and that no sibling had an H/S ratio less than 1.00, implies that close contact is of limited importance and that there is no genetic predisposition for acquiring lymphocyte subpopulation changes. Of interest, two of the hemophiliac children had hemophiliac brothers (not in this study), neither of whom had an H/S ratio less than 1.00. Among the adult hemophiliacs were two sets of brothers, each married and living apart from his respective brother, all of whom had H/S ratios less than 1.00, presumably related to previous concentrate exposure.

Finally, one of the hemophiliac children with an H/S ratio less than 1.00 was found, 1 mo prior to this study, to have autoimmune thrombocytopenic purpura (AITP). Homosexual men, some with abnormal H/S ratios, have also developed AITP, and five patients with hemophilia-A are also known to have AITP. These observations support the possibility that AIDS or its prodrome may involve disordered immune regulation allowing the production of autoantibodies.

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