Immunological studies in haemophiliac children

N. A. Nicholson, C. D. Karabus, D. W. Beatty, W. B. Becker

Summary
A majority of haemophiliacs who have received large-pool plasma products within the past 5 years have been exposed to the putative agent of the acquired immunodeficiency syndrome (AIDS) — HIV. It is not known what the risk of infection is among patients in South Africa. A study was made of 39 children with congenital coagulation disorders attending the Red Cross War Memorial Children’s Hospital Haemophilia Clinic. All but 3 had been treated exclusively with small-pool lyophilised cryoprecipitate or a factor IX concentrate prepared by local blood transfusion services. Three patients had also received imported non-heat-treated commercial products FEIBA (Immuno), Autoplex, Proplex (Hyland) or Factorate (Armour). Absolute lymphocyte counts were normal in all patients but the OKT4/OKT8 ratio was reduced below 1.0 in 9 children including 2 of the 3 who had received commercial plasma concentrates. A high titre of HIV antibody was present in 2 of the 38 patients tested. Both of these children had received imported plasma concentrates and 1 shows some features of the AIDS-related complex. These results suggest that haemophiliacs who receive non-heat-treated commercial concentrates may be at greater risk of HIV infection than patients treated with locally produced plasma products.

Haemophilia and Immunology Services, Department of Paediatrics and Child Health, University of Cape Town and Red Cross War Memorial Children’s Hospital, Cape Town

N. A. Nicholson, M.B. Ch.B.
D. W. Beatty, M.B. Ch.B., F.C.P. (S.A.)
Department of Medical Virology, University of Stellenbosch and Tygerberg Hospital, Parowvallei, CP
W. B. Becker, M.D., M.Med. (Path.)

Subjects and methods
A group of 39 children between 2 and 18 years old with congenital coagulation disorders attending the Haemophilia Clinic was studied between November 1983 and December 1984. Of the group, 30 patients had haemophilia A, 7 had haemophilia B and 1 each had von Willebrand’s disease and afibrinogenaemia. Twenty-two children were severe bleeders; 6 had moderate and 11 mild disease. All except 3 had been treated exclusively with locally manufactured products — a small-pool (4-donor) lyophilised cryoprecipitate for the haemophilia A patients and a factor IX concentrate prepared by the National Blood Fractionation Centre for the haemophilia B children. Three patients had also received imported non-heat-treated material — Autoplex (Hyland) and FEIBA (Immuno) (1 patient), Proplex (Hyland) (1) and Factorate (Armour) (1) because of factor VIII inhibitors in the first 2 patients and allergic reactions to cryoprecipitate in 1.

Duration of treatment. This ranged from less than 1 year to over 15 years; 18 children had been treated for less than 5 years, 19 for 5-15 years and only 2 for more than 15 years.

Factor use. Factor VIII or IX use in the previous year was calculated for each patient. Five children had not received any cryoprecipitate during the previous year, 24 had received less than 1000 U/kg/yr of either cryoprecipitate or factor IX concentrate, 6 between 1000 and 2000 U/kg/yr, 2 between 2000 and 3000 U/kg/yr and 2 between 3000 and 4000 U/kg/yr. The patient receiving FEIBA had received over 7000 U/kg of this product during the previous year because of recurrent intracerebral bleeding.

All the patients were healthy apart from 1 who had marked generalised lymphadenopathy of long standing.

A control group was used for comparison of the immunology results. This consisted of 30 healthy individuals, either children where blood was routinely drawn before surgery for conditions unlikely to affect immune status or laboratory personnel. Ages ranged from 2 to 40 years. Other results were compared with the normal range for age as determined by the laboratories in which the tests were performed.

Antibodies to the HIV virus were measured by an immunofluorescence technique as previously described. Hepatitis B virus (HBV) surface antigen and antibody were detected by standard radio-immunoassay.

Routine chemistry tests included measurement of serum alanine aminotransferase and serum immunoglobulin levels.

Direct Coombs testing was performed on each patient in addition to a full blood count including platelet count, total lymphocyte count and total monocyte count.

Immunology
Peripheral blood lymphocytes (PBL) were isolated on Ficoll-Hypaque gradients as previously described. T cells and T-cell subsets were counted by indirect immunofluorescence using monoclonal antibodies OKT3 (total T cells), OKT4 (helper T
cells) and OKT8 (suppressor-cytotoxic T cells) (Ortho Diagnostics, New Jersey, USA) as follows: 1 x 10^9 mononuclear cells suspended in 200 μl RPMI culture medium with 5% fetal calf serum was incubated for 30 minutes at 4°C with 10 μl (0.05 μg) of monoclonal antibodies. The cells were then washed twice at 4°C with phosphate-buffered saline (PBS) pH 7.4 and 20 μl of 1/40 fluorescently labelled anti-mouse IgG (Miles-Yeda, Rehovot, Israel) was added. This was incubated for 30 minutes at 4°C, washed, resuspended in 30% glycerol in PBS pH 7.4 and counted using fluorescent microscopy. Results were expressed as a percentage and in absolute numbers of mononuclear cells. Lymphocyte proliferative responses to phytohaemagglutinin (PHA), (Wellcome Diagnostics) in autologous and group AB serum was performed in microculture as previously described. The PHA response was defined as the computed mean of three samples. PBL from at least 1 normal adult control were tested with each run.

The BMDP® statistical package was used to compute t-tests with the Bonferroni procedure for multiple comparisons and to perform linear regression.

Results

A high titre (>1:280) of HIV antibody was present in 2 of the 38 patients tested. Both of these children had received imported plasma concentrates and 1 showed some features of the AIDS-related complex (ARC). Three patients were HBV surface antigen-positive and 25 of 34 tested were antibody-positive.

Serum alanine aminotransferase levels (ALT) were raised above the normal upper limit of 30 μl/l in 13 children at least once during the preceding year. In 11 the ALT level exceeded 50 μl. Five of these patients had T4/T8 ratios of less than 1. However, the mean T4/T8 ratio for these 13 patients was not significantly different from the mean of the total group of haemophiliacs or the controls. Simple linear regression tests revealed no statistically significant correlation between serum ALT and serum IgG, or amount of blood product received.

Serum immunoglobulin levels were above normal for age in 18 patients. In 9 this involved a single fraction (usually IgG), in 8 two fractions (usually IgG and IgA) and in 1 all three immunoglobulin fractions. In 3 patients the values were at least twice normal for age. All patients had normal platelet counts and negative direct Coombs tests.

Absolute lymphocyte counts were normal in all patients. The OKT4/OKT8 ratios (Fig. 1) were less than 1 in 9 of the bleeders compared with 1 person in the control group. The low ratios in the haemophilia group were associated with a low percentage of OKT4 cells in 5 patients (of which 3 showed a decrease in absolute number of OKT4 cells) and an increased percentage of OKT8 cells in 3 children.

Mean values of the T-cell subset numbers and ratios were not significantly different from the controls. No statistically significant associations were found between T-cell subset percentages or the OKT4/OKT8 ratio and age, severity of disease, duration of treatment or amount of factor used.

Lymphoproliferative responses to PHA (Fig. 2) were significantly higher in the haemophilia group than in the control group. The patient who had the lowest response in the haemophilia group had an OKT4/OKT8 ratio of 0.8 and a decrease in both absolute number and percentage of OKT4 cells.

Characteristics of patients with T4/T8 ratio below 1.0 (Table I)

The 9 patients with inverted OKT4/OKT8 ratios were between 4 and 17 years of age. Four had received less than 5 years of treatment, while 1 had been treated for more than 15 years. Two patients with mild haemophilia B had received small amounts of factor IX in the preceding year. The remaining 7 in this group had haemophilia A, of whom 5 had severe and 2 moderate disease. Two of these patients had not received any cryoprecipitate in the preceding year, 2 less than 500 U/kg, 2 between 1 000 and 2 000 U/kg and 1 more than 2 500 U/kg. One patient had received over 7 000 U/kg of FEIBA in addition to a small amount of cryoprecipitate in the previous year. Two of the patients have factor VIII inhibitors. Six of the 9 had raised immunoglobulin values, 3 had ALT levels above 50, and 6 were HBV surface antibody-positive.

Characteristics of HIV antibody-positive children

The 2 HIV-positive patients each had an OKT4/OKT8 ratio of 0.7. One patient is a 4-year-old with severe haemophilia A who has been receiving treatment for 3 years. He has factor VIII inhibitors and received more than 7 000 U/kg of FEIBA in addition to 400 U/kg cryoprecipitate during the past year for recurrent intracerebral haemorrhages. He has longstanding generalised lymphadenopathy but no other features of ARC. His IgG level is raised to twice normal for his age. His response to PHA stimulation was normal and a skin test for delayed hypersensitivity to Candida antigen was positive. The low OKT4/OKT8 ratio in his case was due to a decreased percentage of OKT4 cells together with a mild increase in OKT8 percentage.

The second patient is a 16-year-old boy with severe haemophilia A who received approximately 400 U/kg of Factorate owing to allergic reactions to cryoprecipitate. He is clinically healthy but has raised IgG levels and persistently raised ALT and antibody to HBV surface antigen was present. All other investigations were normal. An increased percentage of OKT8 cells accounted for his low OKT4/OKT8 ratio.
### TABLE I. CHARACTERISTICS OF HAEMOPHILIC CHILDREN WITH T4/T8 RATIOS BELOW 1

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yrs)</th>
<th>Factor deficiency</th>
<th>Factor usage (U/kg/yr)</th>
<th>Inhibitors (Bethesda units)</th>
<th>HIV abs</th>
<th>Hepatitis studies</th>
<th>Immunology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HBAg</td>
<td>HbAB ALT</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>VIII</td>
<td>0</td>
<td></td>
<td>Neg.</td>
<td>Neg.</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>VIII</td>
<td>420</td>
<td></td>
<td>0</td>
<td>Pos.</td>
<td>107</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td>VIII</td>
<td>1800</td>
<td></td>
<td>Neg.</td>
<td>Neg.</td>
<td>25</td>
</tr>
<tr>
<td>13</td>
<td>17</td>
<td>VIII</td>
<td>0</td>
<td></td>
<td>6</td>
<td>Neg.</td>
<td>42</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>VIII</td>
<td>471</td>
<td>2</td>
<td>Pos.</td>
<td>Neg.</td>
<td>10</td>
</tr>
<tr>
<td>19</td>
<td>5</td>
<td>VIII</td>
<td>2567</td>
<td></td>
<td>0</td>
<td>Neg.</td>
<td>13</td>
</tr>
<tr>
<td>24</td>
<td>12</td>
<td>IX</td>
<td>26</td>
<td></td>
<td>0</td>
<td>Neg.</td>
<td>31</td>
</tr>
<tr>
<td>30</td>
<td>15</td>
<td>IX</td>
<td>13</td>
<td></td>
<td>0</td>
<td>Neg.</td>
<td>100</td>
</tr>
<tr>
<td>34</td>
<td>15</td>
<td>VIII</td>
<td>1094</td>
<td></td>
<td>Neg.</td>
<td>Neg.</td>
<td>70</td>
</tr>
</tbody>
</table>

Normal values

*Factorasle (Armour).
*FEIBA (Immuno).

**Discussion**

In Europe and the USA, studies have shown that a majority of healthy haemophiliacs (54 - 74%) who have received large-pool plasma products such as factor concentrates within the past 5 years have antibodies to HIV. Our study shows a very low overall HIV seropositivity rate (5,4%) in this group of haemophiliac children who have predominantly been treated with small-pool locally prepared plasma concentrates. No patients treated exclusively with local products were HIV positive. The 2 children with HIV antibody had received non-heat-treated imported commercial plasma concentrates. Both had inverted OKT4/OKT8 ratios, and in addition, 1 shows some features of ARC. The incidence of HIV seropositivity in children receiving large-pool imported products was 66% (2 of 3), which is similar to the figures reported from other countries.

A significant number of healthy haemophiliacs have an abnormal T4/T8 ratio. Decreased percentages of helper T lymphocytes, increased percentages of suppressor T lymphocytes, increased levels of serum immunoglobulins and diminished mononuclear responses to PHA and pokeweed mitogen have all been reported. Our study confirms that these immunological abnormalities are also seen in South African haemophiliac children but are unrelated to the amount of plasma products used or to the age of the patient, as has previously been suggested. Although only 19% of patients receiving locally prepared materials had immunological abnormalities when compared with 2 out of 3 patients who received imported products, this study indicates that immune deficiencies do occur in patients who have exclusively used local small-pool plasma concentrates and who show no evidence of exposure to HIV. These findings agree with those reported in Australian and Scottish haemophiliacs who were found to have immunological abnormalities despite not having been exposed to commercially prepared factor VIII concentrates.

Our results suggest that patients receiving non-heat-treated commercial concentrates seem to be at greater risk of HIV infection and possibly of immunological abnormalities. This may indicate a relative absence of the virus among the local blood donor population as well as the greater safety of using small-pool plasma products. It will be interesting to follow up this group of children over a longer period to see whether the prevalence of HIV infection increases with prolonged exposure to local plasma products.

We wish to thank Mrs A. Buchan, Mrs M. Cooper and Miss E. J. Hughes for technical assistance, Mrs L. Heuer for typing and Dr M. Power and Dr J. du Plessis for help with the statistics. We also wish to thank Dr J. G. L. Strauss, former Medical Superintendent of the Red Cross War Memorial Children’s Hospital, for permission to publish.

**REFERENCES**