Immunologic and Virologic Status of Multitransfused Patients: Role of Type and Origin of Blood Products

By the AIDS-Hemophilia French Study Group

An immunologic and virologic work-up was undertaken in 425 symptom-free multitransfused patients with hemophilias or hemoglobinopathies living in France. Patients were entered into five groups according to the type of blood product they received: local factor VIII, a mixture of local and imported factor VIII, imported factor IX, local factor IX, washed red blood cells. Lymphadenopathy, decreased skin hypersensitivity reactions, relative lymphopenia, and altered ratio of T lymphocyte subsets occurred at significantly higher rates in patients positive for LAV antibody, although such abnormalities were also encountered in LAV serologically negative patients. A correlation between treatment intensity and immunologic disturbances was found in patients infused with factor VIII preparations, irrespective of their positive or negative LAV antibody status. This study has shown the prominent role of LAV in the occurrence of immunologic disturbances in multitransfused patients. However, allogenic or altered proteins present in factor VIII but not in factor IX concentrates seem to play a role of immunocompromising agents. The interplay between LAV and additional factors possibly leading to acquired immunodeficiency syndrome remains to be analyzed.

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) and AIDS-related complex (ARC) are observed with an increasing frequency in hemophiliacs1,2; a similar trend is reported for posttransfusion AIDS in otherwise healthy individuals.3 Before the isolation of the lymphadenopathy-associated virus (LAV) from two hemophilia B patients,4 one of them with AIDS, and before the finding of a high incidence of IgG antibodies to the lymphadenopathy-associated virus (LAV) was 45%. The highest rate was observed in hemophiliacs who received factor VIII concentrates prepared from plasma collected mainly on the American continent; intermediary values were found for hemophilic patients treated with local factor VIII or factor IX concentrates; and the lowest values were found for those who were treated with washed red blood cells. Lymphadenopathy, decreased skin hypersensitivity reactions, relative lymphopenia, and therapeutic regimen.18,19 Because other markers of blood-transmitted infections, such as hepatitis B virus (HBV), Epstein-Barr virus (EBV), and cytomegalovirus (CMV), were unrelated to the observed clinical and biological manifestations, it was suggested that the latter resulted not only from a latent infectious agent, but were also induced by multiple exposures to plasma proteins.

In France, hemophiliacs are treated with concentrates either prepared locally from plasma of volunteer blood donors or imported, mainly from the United States. It appeared, therefore, of interest to determine whether the clinical, immunologic, and virologic statuses differed among patients receiving concentrates of various origins.

MATERIALS AND METHODS

Patients and blood products. The study comprised 425 patients with severe or moderate hemophilia A or B or congenital anemias (thalassemia, sickle cell disease). The two French hemophilia B patients known to present AIDS were excluded from this study. The population of multitransfused patients was divided into five groups according to the diagnosis and the type of blood derivatives used for treatment during the one-year period preceding the entry in the study (Table 1). Hemophiliacs were investigated in seven centers, each contributing 19 to 130 patients. Nonhemophilic patients came from one center. Screening was performed between September 1983 and March 1984 at the time of regular checkups, and all patients or their parents gave informed consent. One physician per center was responsible for clinical examination; no special emphasis was placed on drug exposure or sexual behavior. Clinical findings were recorded on standardized forms.

Group I comprised 80 hemophilia A patients (60 severe and 20 mild or moderate), ranging in age from 1 to 67 years old (median, 15.4). These patients were treated exclusively with factor VIII preparations (frozen or lyophilized cryoprecipitate or intermediate purity concentrate) made from plasma collected in France. Most patients had never received factor VIII of foreign origin. Eleven were treated exclusively with individual or small pool frozen cryoprecipitate. The average amount of factor VIII received by this last subgroup was 18,771 IU/yr (319 IU/kg/yr). The average yearly consumption of the remaining 58 patients treated before the entry was 817 IU/kg/yr.

Group II comprised 219 patients with hemophilia A (191 severe,
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Table 1. Populations Under Study

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Patients</th>
<th>Disease</th>
<th>Product Used</th>
<th>Average Number of Units Received 1 Yr Prior Entry*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>80</td>
<td>Hemophilia A</td>
<td>Local factor VIII</td>
<td>33,181</td>
</tr>
<tr>
<td>II</td>
<td>219</td>
<td>Hemophilia A</td>
<td>Imported and local factor VIII</td>
<td>68,300</td>
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<tr>
<td>III</td>
<td>48</td>
<td>Hemophilia A with anti-VIII:C</td>
<td>Imported factor IX</td>
<td>45,419</td>
</tr>
<tr>
<td>IV</td>
<td>58</td>
<td>Hemophilia B</td>
<td>Local factor IX</td>
<td>34,400</td>
</tr>
<tr>
<td>V</td>
<td>20</td>
<td>Thalassemia, sickle cell disease</td>
<td>Filtered or frozen red blood cells</td>
<td>17†</td>
</tr>
</tbody>
</table>

*This figure does not take into account patients who were not treated during the year preceding entry (11 in group I, two in group II, five in group III, and eight in group IV).

†Donor units of red cells.

28 moderate or mild) (aged 1 to 63 years; median, 15.9) treated with a mixture of imported factor VIII mostly manufactured from American plasma and local intermediate- or high-purity concentrates. The average factor VIII consumption was 1,603 IU/kg/yr. From this total, 42% was of local origin.

Group III included 48 patients with severe hemophilia A who developed antibodies to factor VIII:C (aged 3 to 55 years; median, 16). Although all patients had been treated with factor VIII in the past, activated factor IX concentrates (Autoplex, TravenoLab, Glendale, Calif, or FEIBA, Immuno AG, Vienna) were mostly used for treatment since 1981 or since antibody discovery if at a later date. The ratio of utilization between the two products was approximately 1:1. Twelve of these patients also received nonactivated French factor IX concentrate (P.P.S.B.) (average 13,000 IU of factor IX).

In addition, 13 patients received some factor VIII concentrate, either in small amounts before antibody discovery (five patients treated with less than 10,000 IU) or in large amounts for treatment of severe hemorrhages (23,000 to 570,700 IU).

Group IV comprised 58 hemophilia B patients (40 severe, 18 moderate or mild) (aged 2 to 66 years; median, 20.1). All patients, including the two with anti-factor IX antibodies, had been treated only with the French factor IX concentrate P.P.S.B. The average yearly consumption was 683 IU/kg/yr.

Group V comprised 20 patients with hemoglobinopathies (15 thalassemia major, five sickle disease). This group was included in the study in order to compare the four hemophilic groups with a population of multitransfused patients who did not receive plasma derivatives. The range of age was from 5 to 25 years (median, 13).

Methods. Full blood counts were performed with a Coulter S (Coultronics, Hialeah, Fla), and total lymphocyte counts were calculated from a visual 200 differential. Murine monoclonal anti-human antibodies (Ortho Pharmaceutical, Raritan, Nj) were used to determine the total number of circulating T lymphocytes and to identify and quantify subpopulations of the T lymphocytes. The antibodies included OKT3 for total T cells, OKT4 for helper/inducer (T4), and OKT8 (T8) for suppressor/cytotoxic subsets. Fluorescein-labeled goat IgG antimurine Ig was used as a second antibody. The lymphocyte preparations were scored by eye microscopy with incident fluorescent illumination (Leitz Ortholux, Vetzlar, FRG).

Serum concentrations of IgG were measured by single radial diffusion or by nephelometry. Serum B, microglobulin (Bm) levels were determined by radioimmunoassay (RIA) as previously described.26

Antibodies to viruses were detected by enzyme-linked immunosorbents (ELISA) for LAV27 and CMV. Indirect immunofluorescence was used for detecting antibodies to EBV capsid antigen, RIAs for antibodies to hepatitis A virus (HAV), HBV markers (Abbott Laboratories, Chicago), and for antibodies to HTLV-I protein p 24.28

Delayed cutaneous hypersensitivity (DH) to seven antigens was tested with the Merieux-Multitest (Lyon, France).21 For each antigen, the DH reaction was regarded as positive when the diameter of the induration at 48 hours exceeded 2 mm. Because nearly all hemophiliacs had been immunized with bacille Calmette Guérin, and diphtheria and tetanus antitoxins, DH was considered decreased when positive results were recorded for two or less recall antigens.

Statistical methods. Intergroup and intragroup comparisons were made with the Mann-Whitney test. Association between pairs of variables was assessed with the Spearman's rank correlation coefficient.24 The chi-square test or Fisher's exact test was used for comparing proportions. Averages are expressed as means ± SEM.

RESULTS

Within the four groups of patients with hemophilia, the input of each center, the age of patients, and the decreased skin DH were randomly distributed. In contrast, patients in group V were significantly younger. No patient with clinical signs, such as long-term pyrexia, diarrhea, weight loss, night sweat, or oral candidiasis, was recorded. The prevalence of lymphadenopathy was increased in groups II and IV (P < .01), the T4:T8 ratio, four times with decreased skin reactions to recall antigens, lymphadenopathy, T4 cell counts below 300 × 10^6/L or inverted T4/T8 ratio, five times with serum IgG levels above 16 g/L. A moderate thrombocytopenia (95 × 10^9/L) was present in two further hemophilic patients who had, in one case, skin anergy and a reversal of T4:T8 ratio and, in the other, serum levels of IgG at 37.5 g/L and of B2m at 5.5 mg/L.

Lymphocyte indices. As expected from a population including children, the distribution of the absolute counts of total lymphocytes, T4 and T8 cells did not follow a normal distribution, the shape of the curves spreading toward the high values (Table 2). In group V the counts of total lymphocytes, T4 and T8 subsets were significantly higher than in age-matched controls (P < .01), the T4:T8 ratios remaining within normal range except for one patient. In the hemophilic patients, the intergroup variations in the counts of total and T4 lymphocytes did not reach significance. In
group II the T8 values were increased (P < .02 v group I, P < .001 v group IV, and, considering only patients 10 years or more, P < .05 v adult controls) and resulted in significantly decreased T4:T8 ratios (P < .005 v groups I and III, P < .001 v group IV and controls). The T4:T8 ratios were inversely correlated to the intensity of treatment (group I, r = -.448, P < .01; group II, r = -.210, P < .01). When comparing T8 counts and T4:T8 ratios between group I and II at similar treatment intensity (20 to 2,000 IU/kg/yr) the differences observed in the entire groups remained significant. In group I only, and after exclusion of the 11 patients untreated the year before study, correlations were found between the amount of factor VIII infused and the counts of total, T4, and T8 lymphocytes (P < .005, < .001 and < .025, respectively). In the subgroup of untreated patients (median age, 9 years) one had a T4:T8 ratio below 1.0 with, however, a normal T4 count. Among the 11 patients infused exclusively with local cryoprecipitates, two had an absolute T4 count below 2 SD and two others, an inverted T4:T8 ratio.

Delayed cutaneous hypersensitivity. Two hundred sixteen hemophiliacs underwent skin testing. Positive reactions to three or more antigens were recorded in 147, reactions limited to one or two recall antigens in 60, and anergy in nine. In these nine patients (median age, 11 years; range, 2 to 48), lymphadenopathies coexisted in four, inverted T4:T8 ratios in five once associated with a lymphopenia of 740 × 10⁶/L, serum IgG levels above 16 g/L in seven, and serum B₂m concentrations above 2.6 mg/L in eight. Among the 60 and in group V lgG levels above 16 g/L in seven, and serum B₂m levels were increased over that of controls (P < .001); the highest percentages of levels above 2 SD were found in group II and V. A modest correlation between treatment intensity and serum B₂m concentration was present in group I (r = -.271, P < .05). The prevalence of alanine aminotransferase activities above 30 IU/mL ranged among groups between 55% and 77% without significant differences; levels thrice normal were random among the five groups.

Viral markers. As shown in Table 3, markers for HBV, CMV, EBV were randomly distributed among the four groups of hemophiliacs. Some of them had been immunized with HBV vaccine. Group V had a lower incidence of anti-HBc and anti-HBs markers than the combined groups I through IV (P < .001). Antibody to HTLV-I was detected in one adult of group II.

Antibody to LAV was found in 47% of the hemophilic cohort as compared with 10% in group V (P < .001). The occurrence of LAV Ab was not age-dependent because the mean age of the LAV Ab-positive and LAV Ab-negative patients was 18.3 and 18.5 years, respectively. The youngest hemophiliac with LAV Ab was 15 months old. The distribution of LAV Ab varied between centers and groups, its prevalence being significantly increased in group II

<table>
<thead>
<tr>
<th>Markers</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSAg</td>
<td>2.5</td>
<td>5.0</td>
<td>6.3</td>
<td>5.2</td>
<td>5</td>
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<tr>
<td>HBSAb</td>
<td>89.0</td>
<td>82.0</td>
<td>85.0</td>
<td>88.0</td>
<td>30</td>
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<tr>
<td>HBCAb</td>
<td>59.0</td>
<td>73.0</td>
<td>69.0</td>
<td>67.0</td>
<td>15</td>
</tr>
<tr>
<td>HAV Ab IgG</td>
<td>47.0</td>
<td>56.5</td>
<td>39.0</td>
<td>41.0</td>
<td>35</td>
</tr>
<tr>
<td>CMV Ab</td>
<td>56.0</td>
<td>61.0</td>
<td>40.0</td>
<td>41.0</td>
<td>75</td>
</tr>
<tr>
<td>EBV Ab</td>
<td>87.0</td>
<td>93.2</td>
<td>87.0</td>
<td>85.7</td>
<td>95</td>
</tr>
<tr>
<td>HTLV-I Ab</td>
<td>0.0</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>LAV Ab</td>
<td>33.8</td>
<td>59.6</td>
<td>16.3</td>
<td>43.1</td>
<td>10</td>
</tr>
</tbody>
</table>

Number of patients tested was as follows: group I, 69–80; group II, 203–219; group III, 39–48; group IV, 49–58; group V, 20.
(P < .001 v group I and III, P < .05 v group IV). When matching patients of groups I and II for treatment intensity (20 to 2,000 IU/kg/yr) the increased prevalence of LAV Ab remained significant (P < .05). In group I the proportion of serum positive for LAV Ab did not differ significantly between the patients who, during the preceding year, had received local concentrate, frozen cryoprecipitate, or no treatment. Patients treated with imported factor IX concentrates (group III) had a lower prevalence of LAV Ab than those treated with local nonactivated factor IX concentrates (P < .005).

Impact of antibodies to LAV. The distribution of immunologic abnormalities in each group of patients according to the LAV Ab result is given in Table 4. The presence of 80 children below age 10 in the various groups makes the interpretation of the data difficult. It is worth noticing, however, that among the 34 LAV Ab-positive children, only one had a T4 count below 2 SD from normal adult values and four had a T4:T8 ratio below 1. In addition, using the Mann-Whitney ranking test, a significant association between LAV Ab and relative lymphopenia (total lymphocyte count below 1,200 x 10^6/L) was found (P < .01), and all patients with lymphocyte counts below 1,000 x 10^6/L who had a virologic workup were LAV Ab positive. In group I, the presence of LAV Ab was associated with decreased absolute T4 counts (P < .01 and, for patients above 10 years, P < .001), a trend remaining in the three other groups below statistical significance. Increases in T8 counts in LAV Ab-positive patients reached significance levels in group II only (P < .005). In this group, LAV Ab-positive patients had received more factor VIII (IU/kg/yr) than the LAV Ab-negative ones (P < .02). As shown in Table 5, when patients in groups I, II, and IV were selected on the basis of treatment intensity (20 to 2,000 IU/kg/yr), the presence of LAV Ab was associated with immunologic abnormalities in groups of hemophiliacs receiving factor VIII preparations (groups Ia and Ila), but not in groups of patients receiving factor IX concentrates. In patients with lymphadenopathy, the prevalence of LAV Ab was raised (P < .01), and all nine cases with cutaneous anergy were LAV Ab positive. The proportion of patients with normal findings for DH, lymphocyte counts, T cell subsets, and serum IgG level was significantly decreased in LAV Ab-positive patients (P < .001).

**DISCUSSION**

This prospective study of immunologic and virologic status in multitransfused patients with hemophiliacs or hemoglobinopathies was designed in order to assess the role of type and origin of blood products. We report here the results of the initial workup of a large urban and rural French population divided in five groups defined by the blood products received. Twenty-three percent of hemophiliacs had decreased skin reactivity to recall antigens, 29% had reversal of T4:T8 lymphocyte ratios, and 10% had both decreased skin reactivity and reversal of T4:T8 ratios. The inclusion in this survey of 84 hemophiliacs under age 10 may account for the relatively lower incidence of T lymphocyte disturbances than observed in previous reports. 

Iterative treatment with red blood cells hardly impaired cell-mediated mediated immunity. Details on the two LAV Ab-positive patients in group V have been reported. Significant increases in serum IgG and B2m levels were common to all groups, but these findings may merely reflect the high prevalence of chronic hepatitis in hemophilia and congenital anemias, as evidenced in this study by a 20% incidence of thrice normal alanine aminotransferase activities.

As reported from England and Italy, we found a significant correlation between the amount of factor VIII concentrate administered during the preceding year and the T4:T8 ratios (Table 2). The highest prevalence of immunologic abnormalities (29% of reversed T4:T8 ratios, 24% of skin reactivity to two or less recall antigens, 13% for both)
was seen in the group having received a mixture of imported and local factor VIII concentrates (group II). This finding persisted when matching groups I and II in terms of treatment intensity. It appears thus that factor VIII concentrates prepared mainly from American plasma contain additional factor(s) interfering with immunoregulatory mechanisms. Such an hypothesis has been forwarded previously. Results obtained in group III were surprising and, to a large extent, contradicted those observed in group II. Although both groups were largely treated with products prepared from American plasma, the incidence of immunologic abnormalities in patients receiving Autoplex or FEIBA or both was the lowest of all hemophilic groups. In contrast, treatment with French factor IX concentrates was associated with 25% of abnormal DH scores and 11% of T4:T8 ratio below 1, figures similar to those observed in patients treated with French factor VIII preparations. Furthermore, it is interesting to note that the three French hemophiliacs with full-blown AIDS are factor IX deficient.

The prevalence of LAV Ab in this multitransfused population and its variations among the five groups (10% to 59%) confirms that factor VIII and factor IX concentrates, as well as washed or frozen erythrocytes, may carry the LAV agent. It also illustrates that, in 1982–1983, the likelihood of encountering the LAV, as assessed by the development of LAV Ab, was higher for patients treated with imported factor VIII concentrates than for those infused with local factor VIII preparations (Table 3). The prevalence of antibodies to LAV or HTLV-III of our group of French hemophiliacs treated with factor VIII concentrates of American provenance and of Danish or American patients having received similar products, tested approximately at the same time (early 1984) was similar but lower than reported in more recent studies. However, the incidence of LAV Ab in the two groups of patients treated only with local products (I and IV) was significantly higher than in a group of Scottish hemophiliacs. Patients in group III who were treated with imported activated factor IX concentrates had a lower prevalence of LAV Ab than those of groups II and IV. Two hypotheses may explain this unexpected discrepancy between groups receiving derivatives from American plasma. Most patients had received factor VIII concentrates before 1980, when LAV was unlikely to be transmitted. In 1981 and 1982, these patients were essentially treated with Autoplex, whose preparation from Cohn fraction IV 1 includes precipitations with 20% of ethanol at −8 °C. This concentration was shown to inactivate LAV at room temperature.

Repeated contacts with various viruses (CMV, EBV, non-A, non-B hepatitis viruses) and iterative infusions of allogenic plasma proteins or cellular elements do per se interfere with the mechanisms of immunologic regulation. The presence of LAV Ab heightened the extent and severity of the immunologic abnormalities related to replacement therapy. Strikingly, all nine patients with skin anergy to recall antigens, eight of the ten who had lymphopenia, and two of three of those with lymphadenopathy were LAV Ab positive. However, the close association between level of serum IgG, counts of lymphocytes or their subsets, and LAV Ab observed in this study (Table 5) in patients receiving factor VIII preparations irrespective of their origin suggests that proteins present in factor VIII but not in factor IX concentrates act as immunocompromising agents. The prospective studies we are currently undertaking aim at answering the following questions: (1) Do repeated administrations of procoagulant concentrates modify the “host-virus” relationship in favor of the virus? (2) What is the clinical relevance of LAV antibodies and the protective potential of antibodies reacting with different structural components of the LAV? (3) Will the use of heat-treated coagulation factor concentrates that do no longer carry LAV infectivity influence the natural course of LAV infected patients?

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REFERENCES

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