The Impact of a Very High Purity Factor VIII Concentrate on the Immune System of Human Immunodeficiency Virus-Infected Hemophiliacs: A Randomized, Prospective, Two-Year Comparison With an Intermediate Purity Concentrate

By R. de Biasi, A. Rocino, E. Miraglia, L. Mastrullo, and A.A. Quirino

Pathophysiologic considerations as well as noncomparative clinical results suggest that very high purity concentrates may slow immunologic deterioration in human immunodeficiency virus (HIV)-infected hemophiliacs. In an attempt to evaluate this hypothesis, we prospectively compared CD4 cell counts, skin testing responses, and changes of the clinical status in 20 asymptomatic HIV-positive hemophiliacs, randomly assigned to continue the treatment with an intermediate purity concentrate or to receive a very high purity product, purified by immunoaffinity chromatography with monoclonal antibodies. In the group switched to the very high purity concentrate there was no significant change of the CD4 cell counts over the 96-week follow-up period, whereas in the group continued on the intermediate purity concentrate, a highly significant decline was detected ($P < .013$). Furthermore, in the very high purity group, four of six anergic patients at entry acquired reactivity to skin testing. The results of this study clearly support the use of very high purity concentrates for the replacement therapy of HIV-infected hemophiliacs.

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MATERIALS, PATIENTS, AND METHODS

FVIII concentrates. Kryobulin TIM 3 (Immuno, Vienna, Austria) is an intermediate purity concentrate that shows a specific activity of 1 to 2 IU/mg. Major protein contaminants are fibrinogen, fibronectin, IgA, IgG, IgM, and von Willebrand factor. The concentrate is virally inactivated by steam treatment at 60°C for 10 hours in an oxygen-free inert gas atmosphere at 1,190 mbar plus treatment for 1 hour at 80°C at 1,120 mbar.

Hemofil M (Baxter Hyland Division, Glendale, CA) is a very high purity product with a specific activity of 2,000 to 3,000 IU/mg, before the addition of albumin as a stabilizer. It is purified by immunoaffinity chromatography with a murine monoclonal antibody (MoAb) that specifically binds the FVIII coagulant moiety. Viral inactivation is provided by addition to the starting material of an organic solvent/detergent mixture. Both the concentrates have been previously established to be efficacious and safe in the replacement therapy of hemophilia A patients.

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0006-4971/91/7808-0036$3.00

Table 1. Features of Hemophilic Patients Continued on Intermediate Purity or Switched to Very High Purity FVIII

<table>
<thead>
<tr>
<th>Patient Features</th>
<th>Very High Purity</th>
<th>Intermediate Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. enrolled</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age (yr) (mean and range)</td>
<td>22.1 (15-35)</td>
<td>22.6 (12-37)</td>
</tr>
<tr>
<td>Yearly FVIII usage before the study (IU) (mean and range)</td>
<td>54,000 (28,000-122,000)</td>
<td>46,000</td>
</tr>
<tr>
<td>Yearly FVIII usage during the study (IU) (mean and range)</td>
<td>47,000 (18,000-64,000)</td>
<td>38,000</td>
</tr>
</tbody>
</table>

**Patient population.** Twenty patients (mean age, 22.3 years) with severe hemophilia A (FVIII less than 0.01 IU/mL) and asymptomatic HIV-infection (CDC stages II or III) were enrolled. Selection criteria required that patients have CD4 cell counts of 300 to 600/μL, be negative for HBsAg and HIVp24Ag, have not received any immunomodulating or antiviral therapy, and have previously received definitive treatment with FVIII of more than 500 IU/Kg of body weight per year (mean of the preceding 3 years). Before enrollment, the patients were treated with the intermediate purity concentrate and were willing to remain on the same concentrate for at least 24 months. They were randomly assigned: 10 patients to continue the intermediate purity concentrate and 10 patients to receive the very high purity concentrate. There were no significant differences in age, yearly FVIII usage, or CD4 cell counts between the two groups (Table 1).

**Follow-up investigations.** Patients were administered replacement therapy according to clinical needs, at dosages required for the control of each bleeding episode. No surgical operations were performed during the study period.

Total lymphocyte counts and T-lymphocyte subpopulations were measured 12 weeks before starting the study, at the time of randomization (baseline) and then at 12-week intervals for 96 weeks. Delayed cutaneous hypersensitivity reactions were repeated at 24-week intervals. Physical examinations were performed every 12 weeks. Records were taken of current drug treatment.

**Assays.** T-lymphocyte subsets were studied by direct immunofluorescence with murine MoAbs OKT4 and OKT8 (Ortho Diagnostic System, Milan, Italy) and a flow cytometry system (Spectrum III-Ortho; Raritan, NJ). The skin testing panel used to evaluate delayed hypersensitivity reactions included seven antigens (tetanus toxin, candida, proteus, tubercolin, diphteria toxoid, streptococcus, tricophyton, and a glycerol control; Multitest CMI, Istituto Merieux, Roma, Italy). The results were read by one experienced observer and the response was considered positive when induration measured 2 mm or more. A patient was considered anergic if he did not react to any antigen and not anergic if he reacted to at least one antigen.

**Statistical analysis.** A repeated measures profile analysis was performed to statistically compare the two groups of patients at 12-week intervals over the 96-week observation period. At any given interval, individual comparisons of between-group differences were performed using a two-sample t-test, while the Student's t-test for paired samples was used to compare within-group differences from baseline values. Standard nonparametric tests were used to evaluate differences between the two groups for age, concentrate consumption, and skin testing responses.

**RESULTS**

All 20 patients completed the 96-week study period. During the follow-up period, there were no significant differences in yearly concentrate usage between patients in the two groups of treatment (Table 1). No patient developed clinical and laboratory evidence of inhibitory antibody. The detailed results of lymphocyte subset analysis of the two groups over time is shown in Table 2 and the specific CD4 cell numbers for individual study subjects in Table 3. A significant interaction between the two groups and time of follow-up was detected in terms of CD4 cell counts (P = .0124) and of CD4/CD8 ratios (P = .0080). The two groups showed mean CD4 of borderline statistical significance at 24 weeks (P = .0529) and statistically significant differences first at 36 weeks (P < .03) and then at 48, 60, 72, 84, and 96 weeks (P < .012) (Fig 1). Within the very high purity group there was no significant change of the mean CD4 count over time, whereas the intermediate purity group showed statistically significant decreases of CD4 cell counts from baseline values, first at 24 weeks and then at all the other time intervals (P < .0013). There were no significant differences in CD8 lymphocytes either over time or between the two groups (Table 2). Finally, in terms of CD4/CD8 ratios, the results parallel those of CD4 cell counts with the two groups having significantly different ratios at those time points beyond the 48 weeks of observation (P < .01). There were no differences in the ratios over time for the group on the very high purity product, but the group on the intermediate purity concentrate was found to...
have significantly different CD4/CD8 ratios from baseline values at weeks 24, 36, 48, 60, 72, 84, and 96.

At the baseline, six patients were anergic in the very high purity group and seven in the intermediate purity group. Four patients in the first group acquired reactivity to at least one skin test antigen during the 96 weeks of follow-up. In the intermediate purity group, two patients, who were not anergic at baseline, became anergic during the study period (Table 4). No patient in the very high purity group progressed to symptomatic HIV infection, while two patients in the group of treatment with the intermediate purity concentrate developed oral hairy leukoplakia, oral candidiasis, and deterioration of their clinical status (Table 4).

Fig 1. CD4 cell counts in hemophilic patients continued on (O) intermediate purity concentrate or switched to (●) very high purity FVIII concentrate (means and SD).

**DISCUSSION**

Currently, there is interest in clinically evaluating the impact of extraneous protein in clotting factor concentrates on the immune function of HIV-infected hemophiliacs. The key question is whether protein contaminants may function as a cofactor in the rate of progression to endstage HIV disease. The single randomized controlled trial reported to date showed that the treatment with a high purity concentrate was not clearly superior to treatment with the same intermediate purity concentrate used in the present study. To evaluate the effect of an MoAb immunoaffinity purified very high purity concentrate as compared with an intermediate purity product we have conducted this randomized, controlled study. Our results show striking immunologic differences between the patients in the two arms of the study, with the 10 patients receiving the very high purity concentrate maintaining stable CD4 cell counts, while the 10 patients continuing to receive the intermediate purity concentrate showed a significant decrease. We studied, however, a small number of patients, so, ideally, our results should be confirmed by a larger number of patients. However, in the present study, the careful matching of patients and their randomization to one of either product provides an important indication of outcomes to this clinical issue, especially as it is increasingly difficult to conduct such larger trials for a variety of reasons: intermediate purity concentrates are becoming less available in many countries, because they are being withdrawn from the market and replaced by purer concentrates; many patients voluntarily leave conventional replacement therapy and choose more purified products; additionally, with each year of HIV infection, more patients are becoming symptomatic and require antiviral therapy, which affects the immune status per se and makes changes in the immune function difficult to interpret. Finally, with recent recommendations to initiate zidovudine therapy in early stages of HIV infection in patients with fewer than 500 CD4 cells, many HIV-infected hemophiliacs fall in this group, making their enrollment in other prospective trials impossible.

**REFERENCES**

3. Morfini M, Rafanelli D, Filimberti E, Cinotti S, Piazza E, Longo G, Rossi Ferrini P: Protein content and factor VIII complex


The impact of a very high purity factor VIII concentrate on the immune system of human immunodeficiency virus-infected hemophiliacs: a randomized, prospective, two-year comparison with an intermediate purity concentrate [see comments]

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