PATIENTS WITH hemophilia infused with factor VIII concentrates develop alterations of immune function. In those infected with the human immunodeficiency virus (HIV), abnormalities of cellular and humoral immunity are reflected by a progressive and marked decline of CD4 lymphocyte counts, the appearance of anergy to intradermally-injected recall antigens and an increase of serum markers of B- and T-lymphocyte activation, such as β2-microglobulin and neopterin.1-4 HIV seronegative patients have similar but considerably milder alterations of immune function, which may be secondary to their repeated exposure to plasma proteins (especially immunoglobulin aggregates and immune complexes) and alloantigens contained in intermediate-purity factor concentrates.5-8

Factor VIII produced with recombinant DNA technology has been available for clinical trials since 1988. The experience gained to date with more than 300 hemophilic patients treated worldwide with the two currently available products has established safety and efficacy.9,10 Because these products contain only trace amounts of proteins other than recombinant factor VIII and human albumin, they are the epitome of high-purity factor concentrates that should produce little or no immune alterations. To test this hypothesis, we chose to monitor T-lymphocyte subsets and β2-microglobulin prospectively in HIV seropositive and seronegative patients with hemophilia A treated exclusively with recombinant factor VIII.

PATIENTS AND METHODS

Study population. Fifty-four patients with severe hemophilia A (factor VIII <2%) and four patients with moderate hemophilia A (factor VIII 2% to 5%) were enrolled between July 1988 and August 1989 in a phase II/III study designed to evaluate the safety and efficacy of a recombinant factor VIII product (Kogenate, Miles Laboratory, Berkeley, CA). The patients were treated and evaluated in 13 hemophilia centers in the United States and Europe. All patients had been previously treated with plasma-derived factor VIII concentrates, had no measurable factor VIII inhibitor antibody, and were not on treatment with corticosteroids or other immunosuppressive agents. The investigators were allowed to enroll HIV seronegative or HIV seropositive hemophiliacs, provided the latter had no acquired immune deficiency syndrome (AIDS)-defining illness and their baseline CD4 cell counts were 300 cells/mm3 or greater.

Seven patients were excluded from final analysis of immune function (three lacked baseline data, and four were on study less than 2 years). There was one HIV-seropositive patient who had a baseline CD4 count below the inclusion limit (283 cells/mm3), but this violation was not judged to be sufficiently large to warrant exclusion from analysis. Of the 51 evaluable patients, 30 were HIV seronegative, with a median age at entry of 22 years (range, 1 to 72 years); 21 were seropositive but asymptomatic, with a median age of 25 years (range, 8 to 50 years). Even though times of HIV seroconversions were not precisely known in all patients, most of them have seroconverted between 1980 and 1983. No patient was receiving antiretroviral therapy at study entry. During the study, 8 seropositive patients were started on zidovudine according to the policies of their hemophilia centers, usually when their CD4 counts decreased to less than 300 cells/mm3. No patient was hepatitis B surface-antigen positive. Because assays for serum antibody to hepatitis C virus were not available when the study was started, no baseline hepatitis C testing was obtained. Questionnaires sent retrospectively to the participating centers showed that 24 of 26 patients tested were seropositive (92%) for hepatitis C.

Before starting the trial, all patients were on home treatment with a variety of plasma-derived factor VIII concentrates, and none had received previous treatment with monoclonally-purified concentrates. During the trial, the patients used only recombinant factor
VIII, following the same program of treatment for bleeding episodes that they had used when plasma-derived factor VIII was infused. In addition, patients received 50 U/kg of recombinant factor VIII every 3 months for recovery studies. Annual factor usage before study was reported by hemophilia-center investigators based on clinical records and patient reports. Usage on study was recorded on case report forms for each infusion, based on clinic and home diary records.

Follow-up and measurements. Total lymphocyte, CD4, and CD8 cell counting was scheduled at the time of enrollment (baseline) and then at 6-month intervals. At the same time intervals, serum levels of β2-microglobulin were measured. Samples or values were not obtained at all the scheduled time intervals in some instances. However, each patient had at least three lymphocyte subset counts over the follow-up period, which ranged from 2.3 to 3.9 years (median, 3.5 years).

Assays. Lymphocyte subsets on patients treated in the United States were measured within 24 hours of sample collection at a central laboratory (Metpath, Teterboro, NJ). The percentages of CD4 and CD8 cells were determined by fluorescence-activated flow cytometry on whole heparinized blood using mouse monoclonal antibodies (MoAbs) (Cyto-Stat/Coulter Clone, Coulter Corp, Hialeah, FL). Absolute numbers of cells per cubic millimeter of whole blood were calculated from mononuclear cell counts and differentials measured by standard automated methods. The central laboratory had previously shown 48-hour count stability in heparinized samples. In Europe, flow cytometry determination of lymphocyte subset percentages and calculation of absolute T-lymphocyte subset counts were performed within 6 hours from blood sampling at laboratories associated with each hemophilia center. All laboratories were proficient, as judged by the performances obtained in the frame of quality-control programs performed at regular intervals.

β2-Microglobulin was measured on frozen serum samples by microparticle immunoassay (Inx System, Abbott Laboratories, North Chicago, IL).11

Statistical analysis. For description purposes, laboratory measurements were summarized by HIV status with means, standard deviations, and ranges. Between-group comparisons were made with analysis of variance (ANOVA) for repeated measures and either the Student t or Wilcoxon tests, depending on the underlying frequency distribution of the parameter in question. To model changes in CD4 and CD8 cells during the follow-up period, serial absolute counts or percentages were plotted against time for each patient, and the plot of values versus time was fitted by the least squares straight line. For each patient the least square fit, or slope, estimates the yearly absolute change in cell counts (expressed in cells per cubic millimeter per year) or percentage change. The mean slopes were compared with zero for each group and tested (ANOVA) for statistical significance. A slope not significantly different from zero indicates that the cell counts or percentages were stable during the study period in that group. The mean slopes were also compared between groups by the Wilcoxon test. Values of β2-microglobulin were also analyzed in the same way. Spearman’s rank correlation coefficient was calculated between infused amounts of recombinant factor VIII and slopes of CD4 counts to assess the strength of the relation.

RESULTS

Factor VIII usage. During the treatment period, there was a significantly greater annual usage of recombinant factor VIII, compared with the reported usage of plasma-derived factor VIII. HIV positive patients used a median value of 1,528 U/kg/yr (range, 614 to 3,459) versus 987 U/kg/yr (292 to 2,138), HIV negative patients used a median value of 1,886 U/kg/yr (512 to 4,662) versus 1,009 U/kg/yr (31 to 3,795) (P < .001 for both groups).

Changes in CD4 and CD8 cells. During the treatment period, there was not statistically significant difference between mean baseline and end CD4 and CD8 absolute counts for either the HIV positive or HIV negative patient groups. However, CD4 and CD8 absolute counts fluctuated widely over the study period, as reflected by large standard deviations around the means (Fig 1). The same behavior was observed when CD4 and CD8 counts were expressed as percentages (not shown). To account for such variability, the rate of change of cell counts or percentages was obtained for each patient by calculating slopes.
counts during the study period (mean change, -11.1). The larger group of the normal CD4 cell count in young children is higher than in adults, and declines steeply with age until about 6 years of age; among HIV seropositive patients, those identified by closed circles are children under the age of 6 years, among HIV seronegative patients, those identified by closed squares are those on zidovudine treatment during the study period. None of whom were under the age of 6 years, there was no follow-up period, but changes were not statistically different from zero, SD and ranges for slopes (mean change, -0.1 ± 1.3 cells/mm²/yr; P = .70).

HIV seropositive patients. In HIV seropositive patients, none of whom were under the age of 6 years, there was no significant change for CD8 count slopes (mean change, +0.2 ± 9.5 cells/mm²/yr; P = .99). For CD4 cell slopes, there was a significant decrease of 36 ± 44 cells/mm²/yr, which was significantly different from zero, P = .001 (Table 1 and Fig 2). The percentage of CD4 cells for the seropositive patients over 6 years of age tended to increase, but showed no significant difference from zero (mean change, +1.3 ± 2.6 percent/yr; P = .053) (Table 1). Slopes for absolute CD8 counts were not significantly different from zero (mean change, -0.1 ± 1.3 cells/mm²/yr; P = .70).

HIV seropositive patients. In HIV seropositive patients, whose T-lymphocyte subset number must be interpreted separately from those of the older group. The normal CD4 cell count in young children is higher than in adults, and declines steeply with age until about 6 years of age; among HIV seropositive patients, those identified by closed circles are children under the age of 6 years, among HIV seronegative patients, those identified by closed squares are those on zidovudine treatment during the study period. None of whom were under the age of 6 years, there was no follow-up period, but changes were not statistically different from zero, SD and ranges for slopes (mean change, -0.1 ± 1.3 cells/mm²/yr; P = .70).

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In the eight patients receiving zidovudine, the decrease in CD4 counts was similar to that of patients who did not receive the drug (Fig 2). Because these patients were started on zidovudine early in the study, insufficient data are available for the period before zidovudine treatment to allow slope comparison before and after beginning treatment.

No significant correlation between CD4 slopes and annual FVIII usage was present in HIV seropositive and seronegative patients (Spearman’s rank correlation coefficients: -0.26 for HIV seronegative and -0.32 for HIV positive groups; P = .16 and .17, respectively).

Changes in β₂-microglobulin. Mean baseline levels of β₂-microglobulin were significantly higher in HIV seropositive patients than in HIV seronegative patients (2.8 ± 0.7 v 2.3 ± 0.6 mg/dL, P = .02). In either group, there was no significant change of absolute values or their slopes during the study period (data not shown).

Clinical status of patients. None of the 21 HIV seropositive patients has died or developed an AIDS-defining illness. CD4 counts have decreased below 200 cells/mm² in 3 of 21 HIV seropositive patients, giving them AIDS diagnoses according to the 1993 definition.13 Whereas 16 patients have remained currently asymptomatic, five patients have reported symptoms related to HIV infection, including oral candidiasis, herpes zoster, and weight loss. Three patients, including two of those with symptoms, have intermittent mild thrombocytopenia (platelets, 75,000 to 100,000 cells/mm³). Eight of the HIV seropositive patients were started on zidovudine treatment (see above). After 30 months of zidovudine treatment, 1 patient was changed to dideoxynosine because of continuing decrease in CD4 count (325 cells/mm³ baseline to 105 cells/mm³ at month 43), however, he remains asymptomatic. Ten of the HIV seropositive patients and 7 of the HIV seropositive patients have had intermittent alanine aminotransferase elevations before and during the study, attributed to chronic hepatitis C from previous exposure to plasma products. None of the patients have developed an inhibitor antibody.

DISCUSSION

Fifty-one patients with hemophilia treated exclusively with recombinant factor VIII were monitored for an average period of 3.5 years to assess changes of immune function. The study’s duration of 3.5 years represents the longest period over which immune function has been monitored in a cohort of hemophilic patients treated with a single high-

![Fig 2. CD4 cell-regression slopes by HIV status. Among HIV seronegative patients (n = 30) included a significant number of young children, whose T-lymphocyte subset number must be interpreted separately from those of the older group. The normal CD4 cell count in young children is higher than in adults, and declines steeply with age until about 6 years. When analyzed as a group, the 9 HIV seronegative children under age 6 showed the expected downward trend of CD4 count slopes with increasing age during their years on study (Fig 2). In contrast, the larger group of the HIV seronegative patients over age 6 (n = 21) showed no significant change in absolute CD4 counts during the study period (mean change, -11 ± 83 cells/mm²/yr; P = .53) (Table 1 and Fig 2). The change in percentage CD4 cells for the seronegative patients over 6 years of age tended to increase, but showed no significant difference from zero (mean change, +1.3 ± 2.6 percent/yr; P = .053) (Table 1). Slopes for absolute CD8 counts were not significantly different from zero (mean change, -0.1 ± 1.3 cells/mm²/yr; P = .70).

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In the eight patients receiving zidovudine, the decrease in CD4 counts was similar to that of patients who did not receive the drug (Fig 2). Because these patients were started on zidovudine early in the study, insufficient data are available for the period before zidovudine treatment to allow slope comparison before and after beginning treatment.

Table 1. CD4 and CD8 Cells in HIV Seropositive and Seronegative Hemophilia Patients Treated with Recombinant Factor VIII

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>HIV Seropositive</th>
<th>HIV Seronegative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD and ranges for absolute baseline CD4 cell counts/mm² (patients of all ages)</td>
<td>553 ± 178 (282 to 881)</td>
<td>1,104 ± 651 (331 to 3,864)</td>
</tr>
<tr>
<td>Mean ± SD and ranges for absolute baseline CD8 cell counts/mm² (patients of all ages)</td>
<td>834 ± 473 (153 to 2,333)</td>
<td>573 ± 208 (317 to 1,310)</td>
</tr>
<tr>
<td>Mean ± SD and ranges for slopes of absolute CD4 cell counts/mm²/yr (patients &gt; 6 yrs old)</td>
<td>-36 ± 44* (102 to 67)</td>
<td>-11 ± 83 (145 to 156)</td>
</tr>
<tr>
<td>Mean ± SD and ranges for slopes of percentage CD4 cells (%/yr) (patients &gt; 6 yrs old)</td>
<td>-0.8 ± 1.9 (-3.8 to 3.2)</td>
<td>+1.3 ± 2.6 (-3.2 to 8.9)</td>
</tr>
</tbody>
</table>

* Mean slope significantly different from zero, P = .001.
purity factor VIII product. The yearly usage of factor VIII was significantly greater during the study period with the recombinant product than during the historical period, when patients were treated with plasma-derived products. Increased factor usage may have been a study artifact, as prestudy usage was estimated retrospectively by patients and investigators, whereas recombinant factor usage on study was recorded prospectively. Alternatively, an actual increase in factor usage may have resulted from the novelty of treatment and the freedom from reimbursement constraints. High on-study usage provides assurance that patients received large enough factor exposure to effect any changes in immune function.

The HIV seronegative group of patients over 6 years of age showed no significant change in absolute or percentage CD4 cells, whereas seronegative children under 6 years had a normal CD4 cell decline. In seronegative hemophiliacs there was also no change in CD8 cell counts and β2-microglobulin levels. It is important that recombinant factor VIII did not change these immunological parameters in HIV-uninfected hemophiliacs, because even though recombinant factor VIII is very pure, it is not biochemically identical to plasma-derived factor VIII and this might have affected the immune status of these patients.

In the HIV seropositive patients, over the study period, there was no statistically significant difference between baseline and end CD4 cell counts and β2-microglobulin serum levels, which are important predictors of the development of AIDS. However, using a model that takes into consideration the rate of change over time for each patient, there was a small but statistically significant decrease in the absolute count, but not in the percentage of CD4 cells. It has been reported that in HIV seropositive patients treated with high-purity plasma-derived factor VIII concentrates obtained with MoAb techniques, CD4 cell counts decrease less than in patients treated with intermediate-purity concentrates. To make some comparisons between the data from these studies and our own data, we calculated the annual decrease in CD4 cells using the mean-baseline, mean-end CD4 cell count and on-study time for several different studies. In patients receiving intermediate purity products, mean CD4 cell counts decreased between 5 and 77 cells/mm³/year. In patients receiving monoclonally purified factor VIII, mean CD4 cell counts decreased between 63 and 141 cells/mm³/year. In our study, patients treated with recombinant factor VIII showed a mean CD4 cell decrease of 36 cells/mm³/year. Although differences between studies in terms of duration of follow-up and treatment with antiretroviral agents limit the significance of comparisons, this decrease is in the same range reported for patients treated with monoclonally purified factor VIII derived from plasma.

The varied effects on CD4 cell counts of factor VIII concentrates of different purity may have clinical implications because the CD4 cell count is an important independent predictor of the development of AIDS. However, none of the studies evaluating changes of CD4 counts in HIV seropositive hemophiliacs treated with products of different purity were long enough to evaluate whether the smaller decline in CD4 cell counts seen in patients treated with high-purity concentrates reflected less progression to symptomatic disease and improved survival. Only a large prospective trial based on clinical end points could provide direct comparison data allowing the physician to choose between products of different purity.

APPENDIX

Members of the Kogenate Study Group with patients included in this study:

Department of Pediatrics, University of California at Davis: C.F. Abildgaard, K. Jolly, and J. Harrison; Children's Hospital, Oakland, CA: J.E. Addiego Jr, and R. Jereb; Mount Sinai School of Medicine, New York, NY: S. Arkin, L.M. Aledort, A. Foster, and S. Seremetis; Department of Hematology, University Hospital of Wales, Cardiff, UK: A. Bloom, H. Dasani, and J. Harrison; Cornell University Medical Center, New York, NY: M.W. Hilgarter and N. Boards; St. Joseph's Health Centre, London, Ontario: M.J. Inwood and E. Clegg; Orthopaedic Hospital, Los Angeles, CA: C. Kasper and P. Mockry; Children's Hospital, Akron, Ohio: C. Krill, A. Dery, and E. Miller; Medical Center of Central Massachusetts Memorial, Worcester, MA: D. Brettler, P. Levine, and A. Forsberg; Children's Medical Center, Dayton, Ohio: L. Valdez and S. Jacques; Children's Hospital of Michigan, Detroit: J. Lusher, L. Pfaffman, and I. Warrior; University of Bonn, Germany: H.H. Brackmann; A. Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy: P.M. Mannucci, A. Gringeri, and E. Santagostino; Goethe University, Frankfurt, Germany: I. Scharrer; Haemophilia Centre and Hemostasis Unit, The Royal Free Hospital, London, UK: C. Lee.


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Immune status of human immunodeficiency virus seropositive and seronegative hemophiliacs infused for 3.5 years with recombinant factor VIII. The Kogenate Study Group

PM Mannucci, DB Brettler, LM Aledort, JM Lusher, CF Abildgaard, RS Schwartz and D Hurst

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