Effect of Low- and Intermediate-Purity Clotting Factor Therapy on Progression of Human Immunodeficiency Virus Infection in Congenital Clotting Disorders

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Low- and intermediate-purity clotting-factor therapies are believed to accelerate human immunodeficiency virus (HIV) progression in hemophiliacs through adverse immune effects of the other plasma proteins in the preparations. To investigate this postulate, we evaluated data from six clinical centers that observed persons with congenital factor deficiencies at 6-month intervals. The present analysis is based on HIV-infected subjects who received intermediate purity factor VIII or factor IX concentrates, or cryoprecipitate. For long-term outcome, we classified 374 subjects by the type and amount of treatment during our first year of observation, and determined the subsequent rate of progression to a CD4 count less than 200 cells/µL. A second analysis of this group used a repeated-measures, random-effect model that allowed for individual differences in CD4 decline. Finally, we compared short-term rates of change in CD4 count in each treatment interval of 525 subjects with the type and amount of factor therapy received in the same interval. There was no overall or dose-related deleterious effect of any form of treatment on CD4 trend. The CD4 decrease was less when cryoprecipitate was administered alone or combined with concentrate, but not significantly so. Our results counter the assertion that low- and intermediate-purity products accelerate the rate of CD4 decrease in HIV-1-infected hemophiliacs.

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Since the mid-1980s it has been generally accepted that plasma proteins in factor therapies of low and intermediate purity have an adverse effect on cellular immunocompetence. In addition, it has been suggested that the larger the amount of concentrate administered, the greater the effect in depressing the CD4 count. However, in a preliminary report from the Transfusion Safety Study we found no effect of the amount of concentrate administered to human immunodeficiency virus type 1 (HIV-1)-infected persons. Furthermore, an extensive analysis from our group failed to find any effect of type or amount of factor therapy on CD4 counts among uninfected hemophiliacs.

Evidence from therapeutic trials suggests that the administration of very high purity factor VIII concentrates slows or even halts the decrease in CD4 counts among HIV-1-infected persons for periods of 2 years or longer. In the Transfusion Safety Study we have also found that HIV-1-infected persons who received a slower rate of CD4 decrease during periods when they were receiving very high purity factor VIII concentrates. If low- and intermediate-purity treatments do not accelerate the CD4 rate of decrease, it cannot be argued that the mechanism by which very high purity concentrates slow CD4 decline is by removal of an adverse effect. The slowing must be attributed to something associated with the use of very-high-purity concentrates themselves.

This report is a detailed set of analyses of cryoprecipitate and of intermediate-purity factor VIII (FVIII) and FIX concentrates in a well-characterized group of HIV-1-infected hemophiliacs. Clinical and immunologic data were related to detailed information about factor therapy for observational periods ranging up to 7.2 years (mean = 3.1 years, median = 2.7 years). The indices of HIV-1 progression we used were the long-term rate of progression to a CD4 count less than 200 cells/µL, and the short-term rate of change in CD4 counts. We also considered long-term progression to an acquired immunodeficiency syndrome (AIDS)-defining condition.

MATERIALS AND METHODS

Patients and clinical evaluation. From August 1985 through mid-February 1989, the Transfusion Safety Study enrolled patients with congenital clotting disorders. Institutional review boards approved all protocols and questionnaires at each site. All subjects gave written consent, including that specifically for HIV testing. The six centers that participated in recruiting and observing patients were located in New York, NY; Miami, FL; Detroit, MI; Seattle, WA; San Francisco, CA; and Los Angeles, CA. Recruitment, evaluation, and data collection are described elsewhere. Anti-HIV status was determined by repeated enzyme-linked immunoassay supplemented by Western immunoblot. Follow-up visits were at 6-month intervals. The diagnosis of AIDS was accepted if it was recorded by the subject’s physician or supervisory clinic with principal responsibility for the patient’s care, and confirmed to the 1987 criteria of the US Centers for Disease Control.

A total of 683 anti-HIV-1 persons with congenital clotting disorders were enrolled in the study. This report analyzes short-term CD4 changes in 525 of these patients who had ≥2 visits, did not have a diagnosis of AIDS antedating entry (visit 1), and did not have a CD4 count less than 200 cells/µL at entry. Of these 525 patients, 438 had FVIII deficiency, 70 had FIX deficiency, and 17 had other forms of congenital clotting disorders. For long-term changes, we required ≥4 visits with no diagnosis of AIDS or CD4 count less...
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than 200 cells/µL by visit 3. There were 374 subjects meeting the latter criteria. The present analysis covers observations through January 1993.

**CD4 counts.** Each clinical center performed complete blood counts, and analyzed the peripheral blood mononuclear cell subsets using a whole-blood staining technique and two-color flow cytometry (Coulter EPICS-C, Hialeah, FL).\(^{15,16}\) All immunology laboratories used the same lots of monoclonal antibodies (Coulter Diagnostics, Hialeah, FL) and used standardized protocols. CD4 cells were measured in three pairings (with anti-CD8, anti-CD29, and anti-CD45RA) from August 1985 through June 1989; thereafter, pairings with anti-CD26 and anti-DR were added. The value used as the best estimate of CD4 was the median of the values available. A quality-control program maintained interlaboratory and intralaboratory comparability both cross-sectionally and longitudinally.

**Factor therapy.** At the time of each follow-up visit, the subjects were questioned concerning the type and amount of interim factor therapy. Self-treatment logs were reviewed, as were clinic and pharmacy records. Patients have been classified according to administration of FVIII concentrates only, FIX concentrates only, cryoprecipitate only, or combinations of these therapies. We included the infrequent use of fresh-frozen plasma (FFP) with that for cryoprecipitate. Each donation-derived bag of cryoprecipitate was assumed to contain 100 clotting factor units of FVIII; each bag of FFP, 200 U of FVIII.

Initially in 1985, all clotting factor concentrates were intermediate in purity with specific activities estimated in the range of 1 to 2 clotting units/mg total heterogeneous proteins. During the period of observation through January 1993, purity was improved in many products and the extent of other plasma protein was variably reduced. Accordingly, from 1988 the manufacturer and trade name of all clotting factor concentrates were added to the database to define specifically the individual products. Classified as "high-purity" products are Profilate SD (6 to 8 U/mg), Profilate OSD (6 to 10 U/mg) (Alpha Therapeutic Corp, Los Angeles, CA); Koate HS (15 U/mg), Koate HP (9 to 22 U/mg) (Miles Inc, West Haven, CT); and MelATE SD (50 to 150 U/mg) (New York Blood Center, New York, NY). Classified as 'very-high-purity' products are those that yield 2,000 to 3,000 U/mg before dilution with albumin for stabilization of clotting activity.\(^{17}\) The range of protein isotopic heterogeneity in very high purity concentrates is undoubtedly greatly reduced.

**Antiretroviral therapy.** At each visit the administration of antiretroviral agents, either as part of a clinical trial or for therapy, was recorded. These were limited to zidovudine and/or didanosine among our subjects.

**Statistical analyses of treatment effects.** Administration of any very-high-purity FVIII concentrates (as defined) during any interval of follow-up resulted in censoring of the analyses at the beginning of, and subsequent to, that interval; no very-high-purity FIX concentrates were administered in our population sample. In the analyses of the effect of intermediate-purity FVIII concentrate alone on the change in CD4 count, data were censored at the beginning of the first interval in which any other type of therapy was administered (ie, FIX concentrate or cryoprecipitate).

For the long-term analyses of HIV-1 progression, we found that amount of therapy for the first year (from visit 1 until visit 3) adequately estimated the amount per unit time administered after visit 3 (r = .81 for comparison of quantities of factor treatment). We used standard Cox analysis methods\(^{8}\) to determine the time to a CD4 count less than 200 cells/µL and (separately) to AIDS for these 405 subjects. Their data were stratified by age and by the average CD4 count at visits 1, 2, and 3. The calculation per unit time used both the total treatment and the amount per unit body weight. For this and the following analysis we excluded the 147 patients who did not have ≥4 visits or whose CD4 count had decreased below 200 cells/µL, or had developed AIDS, by visit 3.

To allow for between-person variations in the rate of HIV progression, we also used a repeated-measures, random-effects procedure.\(^{18}\) This method provides for multivariate estimation both of fixed effects (those acting equally on all subjects) and random effects (those that differ from person to person, but with values constrained to come from a particular distribution). Two measures of the extent of exposure to various therapies were examined. The first was the sum of the number of intervals from visit 3 to the time point under consideration in the analysis, during which any treatment was administered, weighted for the interval lengths. This measure provided an approximate index of the total 'duration' of exposure during the period of observation.

In the random effects model, the second index of the extent of treatment exposure was a weighted sum, across all intervals, of the 'intensity' of treatment during each interval. Treatment intensity was calculated as the average daily dose, logarithmically transformed to correct a pronounced skewness in the data and to reduce the influence of the occasional intervals with extremely high doses. The dependent variable in all analyses was the rate to CD4 less than 200 cells/µL or to AIDS. Data were stratified by age and by the average CD4 count at visits 1, 2, and 3.

For the analysis of short-term effects, we evaluated CD4 changes by the concurrent factor treatment preceding the visit when CD4 was measured. For the total of 525 subjects defined by the criteria for visit 1 (above), each of their total of 3,724 intervals was classified by the treatment type and amount within that interval, and then correlated by standard methods with the percentage change in CD4 count for that same interval. The results of these analyses were then verified by omitting alternate intervals for each particular person (to avoid the inevitable negative correlations between successive CD4 changes), and by allowing for between-person variations in the rate of CD4 change, again using a repeated-measures, random-effects procedure.

All statistical significance levels (P values) are two-sided (2P).

**RESULTS**

The type of factor treatment during visits 1 through 3 for the 374 patients in the long-term analyses are shown in Table 1 by type of clotting disorder. Three hundred nine (82.6%) had FVIII deficiency. The remainder had FIX or other deficiencies. FVIII concentrate was administered alone or in combination to 267 subjects; FIX concentrate alone or with FVIII concentrate to 71; and cryoprecipitate alone or with FVIII concentrate to 22. Thirty-four subjects received no therapy during the first year of observation.

<table>
<thead>
<tr>
<th>Type of Deficiency</th>
<th>None</th>
<th>FVIII Only</th>
<th>FIX Only</th>
<th>FVIII and FIX</th>
<th>FVIII and Cryo</th>
<th>Cryo Only</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII</td>
<td>26</td>
<td>243</td>
<td>12</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td>309</td>
</tr>
<tr>
<td>FIX</td>
<td>4</td>
<td>40</td>
<td>49</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>4</td>
<td>40</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>247</td>
<td>61</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>374</td>
</tr>
</tbody>
</table>

Abbreviation: Cryo, cryoprecipitate and FFP.

* All concentrates were of intermediate purity as defined in the text.
Figure 1 shows the subsequent (after visit 3) progression to a CD4 count less than 200 cells/µL and to a diagnosis of AIDS in these 374 patients divided into three groups. One was the group of 34 with no treatment; the second received less than the standardized median total factor treatment between visits 1 and 3; and the third received the median and larger amounts. Figure 1 shows no overall effect of treatment.

Of the 374 patients, 97 (25.9%) received zidovudine and/or didanosine during 250 (10.2%) of the 2,458 intervals. Stratification of subjects according to whether they received antiretroviral drug during any interval showed the same lack of effect of factor treatment in both groups.

The relative rate of subsequently developing a CD4 count less than 200 cells/µL was evaluated among the 269 patients with FVIII deficiency who received either no treatment, or FVIII concentrate alone (see Table 1). The reference group was the 26 persons with FVIII deficiency who were untreated in that interval. For the remaining 243 subjects (see Table 1), the amount of intermediate-purity FVIII concentrate was examined by quartile. There were 44 CD4 events (ie, CD4 count became less than 200 cell/µL) among the 269 subjects.

No effect of any amount of FVIII concentrate therapy compared with no treatment was seen (Fig 2). Adjustment of the amount of therapy to take into account body weight gave the same result. Progression to AIDS occurred with a much lower frequency (12 diagnoses); there was again no suggestion of a relationship to therapy or its amount.

The repeated-measures, random-effects analysis agreed with the conclusion that there was no deleterious effect of FVIII therapy on HIV-1 progression.

The amount of treatment with FVIII concentrate between each of the visits after entry (visit 1) and their concurrent rates of decrease in CD4 count also showed no pattern (Table 2). The average CD4 decrease per 6 months in periods in which no factor treatment was received was 10.6%. This compares with an overall average decrease of 8.2% in periods in which FVIII concentrate was administered, and there is no evidence of a dose-response effect. This conclusion was confirmed by the analysis using data restricted to alternate time intervals in the repeated-measures, random-effects model. The average CD4 decrease for 6 months in periods in which no factor treatment was received was 12.2%, and in periods in which FVIII was administered was 8.4%, again with no dose-response trend.

Table 3 considers the effects of all types and combinations of factor therapy between visits 1 and 3 on progression to CD4 less than 200 cells/µL. There was no evidence of significant differences among the forms of factor treatment ($\chi^2 = 5.80, 2P = .12$). Administration of FIX concentrates alone or in combination with FVIII concentrate had a relative rate of progression to a CD4 count less than 200 cells/µL of 1.06, but this was not statistically significantly different from those administered no treatment. Administration of any cryoprecipitate either alone or with FVIII concentrate had a relative rate of 0.17, which was not statistically significantly lower than other treatments ($\chi^2 = 2.72, 2P = .10$) for comparison of any cryoprecipitate therapy with all other subjects combined.

FIX concentrate and cryoprecipitate therapies were examined for a dose-response relationship as a short-term (concurrent) effect. Omitting the intervals in which any cryoprecipitate was administered, there was no consistent effect of FIX concentrate by tertile of treatment (Table 4). For cryoprecipi-
Fig 2. Kaplan-Meier analysis of the rate of progression to a CD4 count less than 200 cells/μL among FVIII-deficient subjects given no treatment (—), the first quartile of the amount when FVIII concentrate only was administered (—), the second quartile (—), the third quartile (—), and the fourth quartile (—) (N = 269).

Table 2. Effect of Amount of Intermediate-Purity FVIII Concentrates on Concurrent CD4 Counts in HIV-Infected Subjects With FVIII Deficiency

<table>
<thead>
<tr>
<th>Amount of Treatment per 6 Mos* (FVIII units)</th>
<th>No. of Intervals Observed (no.)</th>
<th>Mean % Change in CD4 Count per 6 Mos</th>
<th>GL Model</th>
<th>LRE Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>No therapy</td>
<td>232</td>
<td>-10.6</td>
<td>-12.2</td>
<td>-12.2</td>
</tr>
<tr>
<td>≤12,000</td>
<td>250</td>
<td>-8.8</td>
<td>-10.0</td>
<td>-10.0</td>
</tr>
<tr>
<td>13,000-30,000</td>
<td>250</td>
<td>-6.9</td>
<td>-7.5</td>
<td>-7.5</td>
</tr>
<tr>
<td>31,000-61,000</td>
<td>250</td>
<td>-10.0</td>
<td>-9.9</td>
<td>-9.9</td>
</tr>
<tr>
<td>&gt;62,000</td>
<td>250</td>
<td>-7.0</td>
<td>-6.0</td>
<td>-6.0</td>
</tr>
</tbody>
</table>

Abbreviations: GL, general linear model; LRE, longitudinal random-effects model.
* Observations were censored at the beginning of any interval in which high-purity or very-high-purity FVIII concentrate, FIX concentrate, or cryoprecipitate were administered.

Table 3. Effect of Treatment Type in First Year of Observation on Progression to a CD4 Count <200 Cells/μL

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Subjects Studied (no.)</th>
<th>Progressed to CD4 &lt;200* (no.)</th>
<th>Relative Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>34</td>
<td>8</td>
<td>1.00</td>
</tr>
<tr>
<td>FVIII only</td>
<td>247</td>
<td>40</td>
<td>0.97 (0.43, 2.14)</td>
</tr>
<tr>
<td>Any FIX</td>
<td>71</td>
<td>27</td>
<td>1.06 (0.46, 2.44)</td>
</tr>
<tr>
<td>Any cryo</td>
<td>22</td>
<td>1</td>
<td>0.17 (0.02, 1.39)</td>
</tr>
</tbody>
</table>

* CD4 count >200 cells/μL at visits 1-3; progressed to count <200 cells/μL after visit 3.
† Adjusted for log (mean CD4 count for visits 1-3) in the following groupings: 200-344, 350-499, 500-699, and ≥700 cells/μL.
mediate-purity FVIII concentrates in vitro, have stated that such data do not necessarily imply that such products induce immune abnormalities in patients. In a separate preliminary report, they also concluded from observations of 111 hemophiliacs that intermediate-purity concentrate usage had no relationship to HIV progression.27

The results of the present analyses as well as those of Pasi et al28,29 fail to indicate any effect of low- and intermediate-purity factor preparations on HIV-1 progression. Therefore, if very-high-purity FVIII preparations do slow the rate of CD4 decrease, it is not because of removal of a deleterious influence. One of our previous analyses using the same cohort and the same random effects model also showed some slowing of the CD4 decrease by very-high-purity concentrates compared with intermediate purity.18 If a slowing of the CD4 decrease is confirmed by more adequate evidence, its relationship to other indices of HIV-1 progression should be thoroughly examined. The most immediate assumption is that very-high-purity concentrates slow the HIV-1 destruction of CD4 cells, but the same result would be seen if they were mobilized into the peripheral blood or otherwise changed their trafficking. If the decrease is slowed by some mechanism, any CD4 effect is of unknown duration and its clinical importance remains to be determined.

ACKNOWLEDGMENT


REFERENCES


Table 4. Effect of Amount of Intermediate Purity FIX Concentrates on Concurrent CD4 Counts in HIV-Infected Subjects

<table>
<thead>
<tr>
<th>Amount of Concentrate per 6 Mos (FIX units)</th>
<th>Intervals Observed (no.)</th>
<th>Mean % Change in CD4 Count per 6 Mos</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>341</td>
<td>-9.7</td>
</tr>
<tr>
<td>Treated, but no FIX or cryo</td>
<td>1,031</td>
<td>-8.2</td>
</tr>
<tr>
<td>&lt;18,000</td>
<td>140</td>
<td>-9.5</td>
</tr>
<tr>
<td>18,000-47,000</td>
<td>141</td>
<td>-12.2</td>
</tr>
<tr>
<td>≥48,000</td>
<td>141</td>
<td>-8.0</td>
</tr>
</tbody>
</table>

Observations were additionally censored at the beginning of any interval in which cryoprecipitate was administered.

Abbreviations: GL, general linear model; LRE, longitudinal random-effects model; cryo, cryoprecipitate.

Table 5. Effect of Amount of Cryoprecipitate on Concurrent CD4 Counts in HIV-Infected Subjects With FVIII Deficiency

<table>
<thead>
<tr>
<th>Amount of Cryoprecipitate per 6 Mos (FFP units)</th>
<th>Intervals Observed (no.)</th>
<th>Mean % Change in CD4 Count per 6 Mos</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>341</td>
<td>-9.7</td>
</tr>
<tr>
<td>Treated, but no FIX or cryo</td>
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</tr>
<tr>
<td>≥48,000</td>
<td>141</td>
<td>-8.0</td>
</tr>
</tbody>
</table>

Observations were additionally censored at the beginning of any interval in which FIX concentrates were administered.

Abbreviations: GL, general linear model; LRE, longitudinal random-effects model; cryo, cryoprecipitate and/or FFP (see text for conversion to FVIII units).

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