Filtration Leukapheresis: Effects of Donor Stimulation With Dexamethasone

By D. J. Higby, E. S. Henderson, D. Burnett, and E. Cohen

Dexamethasone was administered to 51 donors prior to filtration leukapheresis. The results of this maneuver, including the consequences of transfusion, were contrasted with results in 52 donors who did not receive the steroid. Yields of polymorphonuclear leukocytes, the posttransfusion increments in recipients, the morphologic polymorphonuclear leukocytes obtained, and the incidence of donor and recipient reactions were all beneficially influenced by this manipulation. Possible mechanisms responsible for these observations are discussed. It is recommended that dexamethasone stimulation be used in filtration leukapheresis when circumstances do not otherwise contraindicate such a maneuver.

CONTINUOUS-FLOW FILTRATION LEUKAPHERESIS (CFFL) is a useful technique for obtaining polymorphonuclear leukocytes (PMN) for transfusion into neutropenic patients. Variations in the technique of CFFL, however, can result in significant alterations in both the quality and quantity of PMN. Yields reported by various investigators range from 1.0 to 5.0 x 10^11 PMN per procedure; preparation of donors may or may not involve pretreatment with steroids. The elution of PMN from the filters has been accomplished with solutions varying from EDTA-containing crystalloids to undiluted acid-citrate-dextrose (ACD) plasma. Wright et al. have demonstrated that cells obtained during different phases of the elution procedure exhibit differences in functional characteristics, and that the administration of dexamethasone to the donor increases the yield of normal-appearing and functioning cells. The degree to which the transfused PMN function in vivo cannot be easily determined. Nevertheless, migration of PMN into skin chambers and mouth ulcers suggests that PMN from steroid-stimulated and unstimulated donors behave similarly, and, most importantly, control of infection can be achieved with cells obtained with and without steroid stimulation of the donor.

This paper describes the results of a study comparing yields, posttransfusion increments, morphologic integrity of PMN, and donor and recipient reactions when granulocytes are obtained from donors who either are or are not stimulated with dexamethasone.

MATERIALS AND METHODS

The CFFL procedure used in our laboratory has been previously described. Basically, blood was propelled by means of a roller pump from a large vein in the antecubital fossa through two filters arranged in parallel and into a holding bag, where it returned to the opposite arm by gravity. Donors were given 17,500 units of beef lung heparin at the beginning of the procedure.

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Table 1. Morphology of PMN With and Without Steroid Prestimulation

<table>
<thead>
<tr>
<th></th>
<th>Normal (%)</th>
<th>Slightly Altered</th>
<th>Markedly Altered</th>
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<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>66 (56-70)</td>
<td>25 (14-33)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>20</td>
<td>83 (72-90)</td>
<td>14 (10-20)</td>
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*Intact cells with two or fewer vacuoles or aggregates of granules.
†Cells with more than two vacuoles or aggregates of granules, or with membrane disruption.

The procedure varied in length, but in all cases, 10 liters of blood were processed through two filters. After the procedure, PMN adherent to the two filters were eluted with a mixture of 2 units of ACD plasma in lactated Ringer's solution to a total volume of 1000 ml and then were buffered to a pH of 7.0 with a concentrated solution of sodium citrate. During elution, the filters were tapped gently, and the final product was concentrated by centrifugation to a volume of 100-150 ml. Donors were not given protamine after the procedure, but were observed until clotting had taken place at the venipuncture sites (about 1-2 hr postprocedure). Recipients received the product at a rate of 10^10 cells/30-75 min.

Neutropenic infected adult patients with acute leukemia were assigned to arm A or arm B of the study. In arm A, all donors of PMN received 4 mg/sq m (no more than 6 mg total) of dexamethasone intravenously immediately before the procedure. In arm B, no dexamethasone stimulation was given to donors. Yields, morphology, donor and recipient reactions, and posttransfusion increments in recipients were analyzed. Subjects involved in these studies gave appropriate informed consent.

RESULTS

Fifty-one donors who received dexamethasone, and 52 who did not were analyzed; 82 transfusions of dexamethasone-obtained cells and 84 of the cells obtained without dexamethasone were compared. Significance of data was determined by Student's t test, and the \( \chi^2 \) test.

Morphology

Table 1 summarizes morphologic observations on PMN collected with and without dexamethasone: 100 cells on each Wright-Giemsa stained slide were examined, and classified as (1) normal, (2) slightly altered (if the cell was intact, but showed 1-2 vacuoles or aggregates of granules), or (3) markedly altered (if the cells had more than 2 vacuoles or aggregates, or showed nuclear or plasma membrane disruption). In the control group, a median of 66% of cells were normal, while in the dexamethasone stimulated group, 83% were normal. The percentage of both slightly and markedly altered cells was reduced in the latter group.

Yields, Donor Increments, and Filter Clearance

Table 2 summarizes PMN yields and the degree of PMN leukocytosis noted in donors at the end of the procedure. In the steroid-stimulated group, the mean
yield was $3.2 \times 10^{10}$ PMN/10 liters of blood processed, while in the control group, the mean yield was $2.8 \times 10^{10}$ PMN. These differences were significant (Student's $t$ test, $p < 0.05$). In the steroid-stimulated group, the mean increment in PMN at the end of the procedure was 2700/cu mm, and in the control group, 200/cu mm ($p < 0.025$). It was noteworthy that in the steroid-stimulated group the range of observations was very narrow, compared to that in the control group.

The ability of the filters to clear blood of PMN can be defined as “filter efficiency” (FE), which may be expressed at a given point in time as a percentage:

$$\text{Filter Efficiency} = \frac{\text{PMN/cu mm in afferent blood} - \text{PMN/cu mm in efferent blood}}{\text{PMN/cu mm in afferent blood}}.$$

In ten procedures in each study group, FE was calculated at the beginning, midpoint, and end of the procedure. Figure 1 demonstrates that in the steroid-stimulated group the FE dropped rapidly to about 22%, and then more slowly to a final figure of near 10%. In the control group, the drop was more gradual, with a final figure of about 30% of the initial value. Although FE was lower in the steroid-stimulated group, a much higher leukocytosis accounts for the slightly higher yields.

**Recipient Increments**

In patients receiving PMN transfusions for infection, the posttransfusion increments (PMN/cu mm/$10^6$ PMN transfused/sq m body surface area) may be.
affected by the type and severity of the patient's infection, the rate of PMN
transfusion, the presence in the recipient of antileukocyte or antiplatelet anti-
bodies, the height of the fever, and probably several other factors. The large
number of observations in this study reduced the relative importance of such
factors. The increments produced by cells obtained from stimulated donors
tended to be higher than those produced by cells from unstimulated donors at
1 hr (210 versus 90/cu mm) and 24 hr (165 versus 59/cu mm), although sig-
nificance was not reached at 24 hr. In addition, the percentage of transfusions
showing measurable increments was greater in the group receiving PMN from
stimulated donors at 1 hr (79% versus 38%), although at 24 hr no difference was
noted (58% versus 56%). (See Table 3.)

Donor Reactions

Table 4 describes the frequency of donor reactions in the two groups (dexa-
methasone treated and control). True syncope was not noted in this study, al-
though it occasionally has been seen. Faintness, as defined by the presence of a
sensation of weakness, apprehension, and a sense of clouding of consciousness,
accompanied by diaphoresis and often a marked slowing of pulse, occurred in
23.5% of nonstimulated donors and only 7.7% of stimulated donors. Transient
drops in blood pressure were noted in 40% of patients experiencing faintness
(range 85–100 systolic), but rapidly returned to normal with lowering of the
head and elevation of the feet. Although chills (rigor) were rarely seen in either
group, they occurred less often in the steroid-stimulated donors (2% versus
5.8%).

Conjunctival hyperemia, a common but usually asymptomatic condition,
also occurred less frequently in the steroid-stimulated donors (23% versus 47%).
Finally, we have noted a postapheresis syndrome, usually occurring 12–24 hr
following the procedure and lasting no more than 12 hr, which was manifested
by myalgia and/or general cutaneous dysesthesia, and occasionally a sensation
of chills; in short, a flulike syndrome. As recognition of this event is entirely
subjective, the figures in the table are probably underestimates. The syndrome

Table 3. Influence of Donor Steroid Prestimulation on Adjusted 1-hr
Posttransfusion Increments in Recipients

<table>
<thead>
<tr>
<th></th>
<th>Increment* (Median and Range)</th>
<th>Percent Showing an Increment at</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>1 hr</td>
</tr>
<tr>
<td>Control</td>
<td>84</td>
<td>90 (5–255)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>82</td>
<td>210 (30–760)</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>0.05†</td>
</tr>
</tbody>
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*Adjusted increment: increase in circulating PMN/ cu mm/10^10 PMN transfused/sq m body surface area.
†Mann-Whitney U test.

Table 4. Influence of Steroid Prestimulation on Donor Reactions

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Faintness (%)</th>
<th>Chills (%)</th>
<th>Conjunctival Hyperemia (%)</th>
<th>Postapheresis Syndrome (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>52</td>
<td>23.5</td>
<td>5.8</td>
<td>47</td>
<td>5.7</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>51</td>
<td>7.7</td>
<td>2</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.1</td>
<td>&lt;0.025</td>
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Table 5. Influence of Donor Steroid Prestimulation on Recipient Reactions

<table>
<thead>
<tr>
<th></th>
<th>Temperature Elevation 2°C (%)</th>
<th>Chills (%)</th>
<th>Respiratory Syndrome (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>20</td>
<td>6</td>
<td>1.3</td>
</tr>
<tr>
<td>( p )</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

was reported in 2% of dexamethasone-stimulated donors versus 5.7% of control donors.

Recipient Reactions

Transfusions of CFFL-procured PMN frequently are associated with fever and chills, which do not appear to be related to the presence of preformed leukocyte antibodies. Since patients are infected at the time of transfusion, it is often difficult to determine whether the reaction is a consequence of the transfusion or if it is related to the natural history of the infection. Nevertheless, reactions during transfusions occurred less often with PMN obtained from steroid-stimulated donors (Table 5). Temperature elevations occurred with 20% of the transfusions of PMN from steroid-stimulated donors, compared to 34% of the control transfusions, and chills occurred in 6% of transfusions obtained from steroid-stimulated donors, versus 22% of transfusions from the control group. In addition, recipients occasionally manifested slight to moderate tachypnea with CFFL transfusions. Although this syndrome is invariably associated with increased temperature, we have not observed radiologic evidence of pulmonary infiltration. Tachypnea disappeared when the transfusion was slowed, and never persisted after it was completed. The incidence of this reaction with transfusions obtained from steroid-stimulated donors was 1.3%, in contrast to 13% with transfusions obtained from control donors.

DISCUSSION

Stimulation of CFFL donors with dexamethasone immediately prior to donation resulted in increases in yields, preservation of cellular integrity, increases in posttransfusion increments in recipients, and reduction in adverse reactions in donors and recipients. It has not been determined whether PMN from stimulated donors are functionally equivalent to those from unstimulated donors on a cell-for-cell basis, although in our experience transfusions obtained from either stimulated or unstimulated donors appear to be efficacious.

Dexamethasone probably improved yields of PMN by increasing the level of circulating cells in the donor. Leukocytosis due to dexamethasone alone usually reaches a peak 2-6 hr after injection. In this study, there was a marked increase in donor leukocytosis when steroid stimulation was used, despite the fact that the average 10-liter leukapheresis was accomplished in less than 90 min. Leukocytosis as a result of the CFFL procedure has been noted by others and this study suggests that there may be some potentiation of the effects of CFFL by dexamethasone.

The yields achieved with steroid stimulation were not as great as would have been expected by the leukocytosis alone. We noted a decrease in the percentage
of PMN adhering to the nylon fibers. This observation has also been made by McPherson et al. Fehr et al. have postulated that activation of complement plays a role in granulocyte adherence to nylon fibers. Thus the decrease in granulocyte adherence noted in this study may have been a result of steroid inhibition of complement activation.

Wright et al. have demonstrated that there is selective degranulation of "specific" granules in PMN exposed to nylon fibers. Improvement in morphologic integrity of PMN in steroid-stimulated donors is probably related to stabilization of lysosomal membranes. Reduction of egress of lysosomal contents into the supernatant of the PMN preparations may also account for the decrease in incidence of adverse reactions in donors and recipients.

The trend toward an increase in posttransfusion increments in recipients, and the increased number of recipients having such increments when steroid-stimulated donors were used to obtain PMN, may be secondary to a decreased rate of egress of transfused PMN from the circulation of the recipient. It has been postulated that the usually low posttransfusion increments following transfusion of CFFL-procured PMN are the result of rapid sequestration of cells because of increased membrane adheriveness. Stabilization of membranes by steroids may also account for our improved increments, and also for the better increments noted with transfusions obtained by continuous-flow centrifugation leukapheresis, in which steroids are commonly used to improve yields.

The flulike syndrome noted in a few donors may or may not be related to CFFL. The symptoms noted with a viremia as well as the syndrome noted here may be mediated by an endogenous pyrogen reaction related to alterations in leukocyte function.

The slight to moderate tachypnea noted in this study in some recipients may be a variant of the more acute respiratory syndrome which has been noted with rapid infusion of CFFL PMN. While such reactions are apparently not related to preformed antibodies in the recipients, improved ability to detect specific antigranulocyte antibodies may result in better understanding of this phenomenon. In any event, adverse recipient reactions to CFFL PMN transfusions are abrogated by slowing the transfusion; usually this measure permits the entire transfusion to be given.

While such single doses of steroids are of minimal risk, caution must be exercised when a donor is used repeatedly. Our policy is to use dexamethasone stimulation no more than a total of four times, and no more than twice weekly when a donor is repeatedly leukapheresed.

In view of the results with dexamethasone, we currently use such stimulation in all donors, and believe that the evidence is sufficient to advocate this approach routinely.

ACKNOWLEDGMENT

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