Granulocyte Bactericidal Capacity and Chemotaxis as Affected by Continuous-Flow Centrifugation and Filtration Leukapheresis, Steroid Administration, and Storage

By Roy T. Steigbigel, John Baum, James L. MacPherson, and Jacob Nusbacher

While granulocyte transfusions have been shown to be effective in the treatment of patients with infections, the effects of the leukapheresis collection procedure on certain granulocyte functions remain in question. We studied the bactericidal function of granulocytes collected by continuous-flow centrifugation (CFC) and filtration leukapheresis (FL) with *S. aureus*, *E. coli*, and *S. typhimurium*. Killing of all organisms was normal with CFC and FL cells if the medium in which the cells were suspended was buffered. Low pH suspension medium had an adverse effect on bactericidal function. Chemotaxis of FL cells was slightly but not significantly reduced. Adrenocorticosteroids are frequently administered to white blood cell donors in order to increase the yield of collected cells. Granulocytes collected by routine venipuncture from subjects who had taken a single dose of prednisone or dexamethasone had normal bactericidal function. Prednisone administration appeared to increase migration of granulocytes in vitro. Evaluation of function of FL granulocytes stored at 4°C showed significant decrease in bactericidal function at 72–96 hr with a more rapid and significant decrease in chemotactic function at 24 hr. These studies provide guidelines for methods of collection and storage of granulocytes that will preserve optimal cell function.

Infection remains as the leading cause of death in patients with hematologic malignancies and solid tumors. The development of neutropenia in these patients appears to be the most important factor contributing to the occurrence of fatal infections. However, sufficiently large numbers of granulocytes can now be collected from normal donors so that white blood transfusions are an effective therapeutic modality controlling infection in neutropenic hosts. Two major methods for collecting granulocytes are continuous-flow centrifugation (CFC) and filtration leukapheresis (FL). Although previous studies have examined the functional capabilities of collected granulocytes both in vitro and in vivo, important questions remain, particularly with regard to the functional integrity of cells collected by FL.

We report here the results of the following studies: (1) an examination of the bactericidal capacity of granulocytes collected by CFC and FL; (2) study of the...
chemotactic ability of granulocytes collected by standard venipuncture (VP) from steroid-treated donors and of granulocytes collected by FL; (3) the effect of steroid treatment of donors upon bactericidal function of granulocytes collected by CFC, FL, and VP; (4) the role of pH in the function of leukapheresis-acquired granulocytes; and (5) the effect of storage for 96 hr at 4°C upon the bactericidal and chemotactic function of FL granulocytes.

MATERIALS AND METHODS

Donors. All donors were normal adult volunteers who fulfilled American Red Cross criteria for plasmapheresis donations. Informed consent was obtained for all studies in accordance with the Helsinki Declaration.

Granulocyte collection. Granulocytes were collected by CFC, FL, or from whole blood obtained by standard VP technique. For study of granulocytes obtained by CFC, cells from five volunteers (4 males) were collected using the Aminco cell separator (American Instrument, Silver Springs, Md). The method of collection by CFC was previously described. Donor flow rates of 30-45 ml whole blood/min were usually achieved; granulocyte collection flow rates were 2-4 ml/min. The centrifuge rate varied from 30 to 70 g, depending upon donor flow rate and degree of red blood cell-plasma separation achieved. The procedure was terminated after approximately 3 hr.

Granulocytes were collected by FL from 22 donors (13 males) in 23 separate collections. The collection was carried out according to methods previously described. Flow rates of up to 80 ml/min were achieved, depending upon the maximum flow that could be achieved from the donors’ veins. The mean time for collection by FL was 138.5 ± 5.6 (SEM) min. Elution of granulocytes from the nylon filters was performed using 1500 ml eluting mixture containing 250 ml acid citrate dextrose (ACD), NIH formula A, 250 ml group AB plasma, and 1 liter 0.9% saline. The mean pH of this solution in the 23 collections was 5.76 ± 0.08. As the eluting solutions rapidly flowed through the filters they were continuously tapped with a blunt instrument. The effluent was collected in transfer pacs that were centrifuged at 2800 g for 10 min at 4°C. Most of the supernatant eluting fluid was removed, giving a final volume of approximately 325-350 ml. In nine collections, a portion of the sedimented cells were immediately resuspended in a solution in which phosphate-buffered saline (PBS) was substituted for the saline. The mean pH of this buffered solution was 6.67 ± 0.13. The granulocytes remained in the buffered or nonbuffered media for approximately 2.5 hr prior to their removal and preparation for the bactericidal and chemotaxis studies, all performed in media with pH of 7.2.

Corticosteroid stimulation. Administration of corticosteroids increases the yield of granulocytes collected by increasing the donor’s granulocyte count. All donors undergoing CFC received 60 mg prednisone orally approximately 10-12 hr prior to the collection procedure. To assess the effect of prednisone administration alone on the bactericidal capacity of the granulocytes, studies were performed on cells collected from these donors by venipuncture just prior to the CFC procedure; blood samples drawn 1 wk later served as controls.

Thirteen of the donors whose cells were collected by FL received corticosteroids prior to the FL procedure. Of these 13, 11 received 6 mg dexamethasone intravenously (i.v.), immediately after collection of the venipuncture blood sample and prior to collection by FL. Two received steroids orally (60 mg prednisone in one instance, 9 mg dexamethasone in the other) approximately 10-12 hr before the FL procedure. The functional capacity of the granulocytes collected by venipuncture before steroid administration was compared with the function of cells collected by FL after steroid administration.

To assess the effect of i.v. administration of dexamethasone alone on VP granulocyte function, six subjects received an i.v. dose of 6 mg dexamethasone. The bactericidal capacity of their granulocytes collected by VP immediately before and 1.5 and 3.0 hr after dexamethasone administration was compared.

Thus the following comparisons of bactericidal capacity were made: CFC granulocytes compared to VP granulocytes from same donor 10-12 hr after 60 mg prednisone; VP granulocytes 10-12 hr after prednisone to VP granulocytes without prednisone; FL granulocytes to VP granulocytes of same donors; FL granulocytes immediately after 6 mg dexamethasone to VP granulocytes of same donor without steroid- and VP granulocytes without steroid to VP granulocytes 1.5 and 3 hr after 6 mg dexamethasone.
PMN FUNCTION WITH FL, STORAGE, STEROIDS 199

Bactericidal assay. Granulocytes were prepared from heparinized venous blood samples as previously described. They were separated by Ficoll-Hypaque density gradient sedimentation. Red cells were removed by dextran sedimentation and ammonium chloride lysis. Granulocytes were washed twice in Earl's balanced salt solution (EBSS). Cells collected by FL consisted almost entirely of granulocytes, so that dextran sedimentation and washing with ammonium chloride was sufficient to achieve pure preparations of granulocytes from this source. Cells were counted and adjusted to a concentration of 1 x 10^7/ml in EBSS with 0.1% gelatin (EBSS-gel). Trypan blue dye exclusion showed that more than 98% of cells from all sources were viable.

The three organisms used to test for bactericidal capacity were Staphylococcus aureus 502A, Escherichia coli, and Salmonella typhimurium (C58). They were prepared as previously described and adjusted to a concentration of 1 x 10^8 organisms/ml in EBSS. Incubation mixtures of 1 ml containing 5 x 10^6 granulocytes, 1 x 10^7 bacteria, and 10% autologous plasma in EBSS-gel were tumbled end over end at 37°C. Control suspensions of bacteria in plasma without granulocytes were studied in all experiments. After 1 and 2 hr incubation, 0.1-ml aliquots were removed and sonicated so as to disrupt the granulocytes and free and disperse intracellular organisms. Appropriate dilutions were plated on trypticase soy agar as previously described. The number of colony-forming units (CFU) was determined at 24 hr.

Chemotaxis assay. Chemotaxis of leukocytes collected by VP and FL from 12 donors who did not receive steroids was studied in triplicate using the modification by Baum and co-workers of the Boyden chamber technique. Chemotaxis assays were performed with dextran settled granulocytes without Ficoll-Hypaque separation. Human serum activated with E. coli endotoxin was used as the source of the complement-mediated chemotactic stimulus. The number of polymorphonuclear leukocytes in ten high-power fields (50 mm²) on the upper and lower surfaces of each filter were counted with a Bausch & Lomb particle counter (Bausch & Lomb, Rochester, N.Y.) and the results expressed as a ratio, the chemotactic index. The effect of pH on chemotaxis was examined in triplicate with eight samples of granulocytes collected by FL and immediately suspended in both nonbuffered and buffered solutions. In addition to the donors' VP samples, a second control consisting of cells obtained by VP from another normal subject was studied on each occasion.

The effect of corticosteroids alone on chemotaxis of granulocytes collected by standard VP and dextran sedimentation was studied in triplicate in nine subjects. Chemotaxis was studied prior to and 10-12 hr after the oral administration of 60 mg prednisone.

Effect of storage. The effect of storage upon bactericidal capacity of cells collected by FL was studied using cells collected from six donors. The bactericidal assay was performed on the day of collection and 24, 48, 72, and 96 hr later. The cells were stored in the buffered and nonbuffered elution solutions in collection packs continuously rocking at 4°C. Aliquots were removed at the stated times for assay of bactericidal capacity, cell counts to determine cell loss, and trypan blue dye exclusion. The comparison of bactericidal capacity was made on the same granulocytes on five successive days. To avoid expression of differences in results reflecting day-to-day variation in initial inoculum and growth of bacteria, the results for stored cells are expressed as a killing index.

\[
\left(1 - \frac{\text{viable bacteria in suspensions of granulocytes and bacteria}}{\text{Viable bacteria in suspensions of bacteria without granulocytes, at same time period}}\right) \times 100.
\]

The effect of storage upon chemotaxis of cells collected by FL was studied using the cells from eight donors. The chemotactic ability of cells collected by FL was studied simultaneously with cells collected by VP.

Statistical analysis. Significance of differences in bactericidal capacity between control and test cells was analyzed by paired t and sign tests and chemotactic studies by paired t test, sign test, and Wilcoxon matched pairs sign rank test.

RESULTS

Effect of corticosteroids on bactericidal capacity of granulocytes. Prednisone 60 mg orally administered 10-12 hr prior to cell collection by VP had no significant effect on bactericidal capacity when compared to control VP cells of...
One or two-hour determination of percent of initial bacterial inoculum killed ± SE. Before dexamethasone.

Fig. 1. Bactericidal capacity of granulocytes collected by venipuncture (veni) and continuous-flow centrifugation (CFC). a, percentage of initial inoculum of S. aureus, E. coli, and S. typhimurium killed at (A) 1 hr and (B) 2 hr by granulocytes collected by venipuncture and CFC from donors with and without prednisone treatment. Horizontal bars, mean for each group. Comparison of killing by venipuncture with CFC granulocytes or prednisone with non-prednisone-treated donors showed no significant differences.

same donors (Fig. 1). Similarly, the effect of intravenous dexamethasone administration on the bactericidal capacity of granulocytes obtained by VP was evaluated (Table 1). There was no significant difference in the killing of E. coli and S. typhimurium by granulocytes collected 1.5 or 3 hr after dexamethasone compared to control granulocytes.

Table 1. Effect of Dexamethasone Administration on Granulocyte Bactericidal Capacity

<table>
<thead>
<tr>
<th>Organism</th>
<th>Aliquot</th>
<th>Control 1.5 hr</th>
<th>Control 3 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>1</td>
<td>90 ± 4</td>
<td>94 ± 3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>80 ± 16</td>
<td>97 ± 1</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>1</td>
<td>72 ± 15</td>
<td>84 ± 4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>55 ± 17</td>
<td>81 ± 7</td>
</tr>
</tbody>
</table>

*One- or two-hour determination of percent of initial bacterial inoculum killed ± SE.
†Before dexamethasone.
Table 2. Bactericidal Capacity of Granulocytes Collected by Filtration Without Steroids

<table>
<thead>
<tr>
<th>Organism</th>
<th>N*</th>
<th>Hour</th>
<th>Venipuncture t</th>
<th>Filtration t</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>10</td>
<td>1</td>
<td>49 ± 14</td>
<td>46 ± 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>62 ± 10</td>
<td>52 ± 12</td>
</tr>
<tr>
<td>E. coli</td>
<td>10</td>
<td>1</td>
<td>95 ± 2</td>
<td>95 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>94 ± 4</td>
<td>95 ± 1</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>10</td>
<td>1</td>
<td>90 ± 5</td>
<td>89 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>91 ± 5</td>
<td>89 ± 7</td>
</tr>
</tbody>
</table>

* N, number of paired experiments; no significant difference in bactericidal capacity for VP versus filtration cells demonstrated.

†Mean ± SE of percentage of initial inoculum of bacteria killed.

Bactericidal capacity of cells collected by CFC. Figure 1 shows the percentage of initial inoculum of $1 \times 10^7$ bacteria killed by the different granulocyte preparations. Granulocytes acquired by CFC after prednisone ingestion showed no difference in bactericidal capacity when compared with VP controls with or without prednisone pretreatment ($p > 0.05$, paired $t$ test).

Bactericidal capacity of cells collected by FL without steroid administration. The effect of FL process itself on the bactericidal capacity of granulocytes collected from donors without steroid administration is shown in Table 2. The killing of S. aureus, E. coli, and S. typhimurium by granulocytes collected by FL without steroid administration was not significantly different from the killing by granulocytes collected by routine VP.

Bactericidal capacity of cells collected by FL after dexamethasone administration. As shown in Table 3, granulocytes collected by FL after dexamethasone administration killed a smaller percentage of the initial inoculum of S. aureus and E. coli than did control VP granulocytes collected prior to steroid administration. At 1 hr these differences were statistically significant ($p < 0.01$ for S. aureus, $p < 0.05$ for E. coli, paired $t$ and sign tests) but were small in magnitude. There were no significant differences in the killing of S. typhimurium.

Effect of pH on bactericidal capacity of cells collected by FL. The bactericidal capacity of granulocytes collected by FL and immediately suspended in buffered and nonbuffered media is compared in Table 4. There was significantly better killing of S. aureus, E. coli, and S. typhimurium by the cells immediately suspended in the buffered as compared with the nonbuffered media. A compari-

Table 3. Bactericidal Capacity of Granulocytes Collected by Filtration With Dexamethasone Stimulation

<table>
<thead>
<tr>
<th>Organism</th>
<th>N*</th>
<th>Hour</th>
<th>Venipuncture/Granulocytes t</th>
<th>Dexamethasone/ Filtration Granulocytes t</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>13</td>
<td>1</td>
<td>75 ± 8</td>
<td>64 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>90 ± 4</td>
<td>72 ± 14</td>
</tr>
<tr>
<td>E. coli</td>
<td>13</td>
<td>1</td>
<td>96 ± 2</td>
<td>92 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>97 ± 2</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>13</td>
<td>1</td>
<td>92 ± 4</td>
<td>90 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>98 ± 2</td>
<td>95 ± 3</td>
</tr>
</tbody>
</table>

*N, number of paired experiments.

†Mean ± SE of percentage of initial inoculum killed.
son of the killing by granulocytes collected by FL and suspended in the buffered media with granulocytes concurrently collected by VP showed no significant difference in the killing of any of the three organisms. This was true for cells collected with and without dexamethasone administration (data not shown).

**Chemotactic capacity of granulocytes collected by FL.** Figure 2 compares the ability of granulocytes collected by FL with granulocytes collected by VP to respond to a complement-mediated chemotactic stimulus. In 11 of the 12 studies performed the chemotactic capacity of FL cells was reduced when compared

![Fig. 2. Comparison of chemotactic capacity of granulocytes collected by routine venipuncture (VP) and filtration leukapheresis (FL). Broken lines connect the chemotactic index for VP and FL granulocytes. Mean chemotactic index for VP and FL granulocytes connected by solid line. A small decrease in chemotactic index was seen with the FL granulocytes from 11 of the 12 individuals.](image-url)
Table 5. Effect of Prednisone Administration Upon In Vitro Granulocyte Migration and Chemotaxis

<table>
<thead>
<tr>
<th>Stimulus*</th>
<th>HBSS</th>
<th>HBSS + Endotoxin</th>
<th>Serum</th>
<th>Serum + Endotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Prednisone</td>
<td>Control</td>
<td>Prednisone</td>
</tr>
<tr>
<td>1</td>
<td>36</td>
<td>56</td>
<td>56</td>
<td>237</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>39</td>
<td>56</td>
<td>191</td>
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<td>3</td>
<td>22</td>
<td>29</td>
<td>54</td>
<td>107</td>
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<tr>
<td>4</td>
<td>72</td>
<td>140</td>
<td>98</td>
<td>111</td>
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<tr>
<td>5</td>
<td>11</td>
<td>152</td>
<td>78</td>
<td>109</td>
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<td>6</td>
<td>35</td>
<td>63</td>
<td>39</td>
<td>103</td>
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<td>7</td>
<td>65</td>
<td>136</td>
<td>196</td>
<td>185</td>
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<tr>
<td>8</td>
<td>225</td>
<td>80</td>
<td>149</td>
<td>196</td>
</tr>
<tr>
<td>9</td>
<td>21</td>
<td>110</td>
<td>23</td>
<td>152</td>
</tr>
</tbody>
</table>

Chemotactic index, mean of triplicate determination.

*Contents of lower chamber.

with VP cells. This consistent decrease in chemotaxis was significant by sign test \((p = 0.001)\). However, the magnitude of the difference in chemotactic capacity between VP and FL cells was relatively small and not significantly different by paired \(t\) test. The chemotactic capacity of FL cells eluted and suspended in buffered medium was not significantly different (sign test, paired \(t\) test) from the chemotactic capacity of granulocytes suspended in the non-buffered medium.

**Effect of corticosteroids on nondirected migration and on chemotactic response of granulocytes.** Granulocytes obtained by VP from the nine normal subjects who took 60 mg prednisone 10 hr before VP were evaluated for their ability to migrate and respond to a chemotactic stimulus. As shown in Table 5 the number of cells migrating to the "attractant" side of the filter was significantly greater when the subjects had taken prednisone \((p < 0.05\), Wilcoxon test). This increase was noted with HBSS and serum as well as with HBSS alone or serum with \(E. coli\) endotoxin in the lower chamber.

**Effect of storage on bactericidal capacity of granulocytes collected by FL.** Studies were performed to determine the effect of storage on maintenance of bactericidal capacity of FL granulocytes. Table 6 shows the killing index for the three organisms studied. A decrease in killing of staphylococcus by FL leukocytes was noted with both buffered and nonbuffered cells at 24 hr storage but was not significant \((p < 0.05\), \(t\) test) until 96 hr storage. The killing of

Table 6. Effect of Storage on Bactericidal Capacity of Granulocytes Collected by Filtration

<table>
<thead>
<tr>
<th>Organism</th>
<th>Medium</th>
<th>Hours of Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>(S. aureus)</td>
<td>Nonbuffered</td>
<td>45.8 ± 19.0</td>
</tr>
<tr>
<td></td>
<td>Buffered</td>
<td>64.3 ± 16.6</td>
</tr>
<tr>
<td>(E. coli)</td>
<td>Nonbuffered</td>
<td>94.6 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>Buffered</td>
<td>94.8 ± 4.6</td>
</tr>
<tr>
<td>(S. typhimurium)</td>
<td>Nonbuffered</td>
<td>96.8 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Buffered</td>
<td>97.1 ± 1.0</td>
</tr>
</tbody>
</table>

Mean killing index ± SEM after 120 min for five subjects; see text for definition of killing index.
E. coli was not significantly decreased until 72 hr storage of both buffered and nonbuffered cells. The killing of salmonella was significantly decreased at the 1-hr determination in both buffered and nonbuffered cells stored for 72 hr and for both 1- and 2-hr determinations after 96 hr storage. Bactericidal capacity appeared to be preserved better in buffered than nonbuffered media, but the difference was not significant.

Effect of storage on cell numbers, viability, chemotactic capacity. The percentage of FL cells remaining and the percentage of these cells that remained viable after storage is shown in Fig. 3. The number of cells decreased by 9.9% at 24 hr of storage, 21.7% at 48 hr, and 32% at 72 hr; no further decrease was noted at 96 hr. Over 99% of these cells were viable, as determined by trypan blue dye exclusion, for the first 24 hr of storage, 98% at 48 hr, 95% at 72 hr, and 80% at 96 hr. The granulocytes were used in the bactericidal assay without regard to the trypan blue dye result. It can be seen from comparison of the percentage viable and killing index curves that some cells capable of exclusion of trypan blue dye had lost bactericidal capacity. Chemotactic capacity diminished before bactericidal capacity. There was a 57% decrease in mean chemotactic index of FL cells by 24 hr and an 88% decrease by 48 hr. The buffered and nonbuffered cells had very similar decline of chemotactic capacity. The chemotactic capacity of cells collected by VP and stored concomitantly with the FL cells decreased by only 25% after 24 hr of storage. By 48 hr, however, these cells also had 88% reduction in chemotactic capacity.

DISCUSSION

This study indicates that granulocytes collected by CFC have normal bactericidal capacity for S. aureus. These data are in agreement with previous
studies that have shown that CFC cells are equivalent to VP cells in their capacity to kill *S. aureus* and *S. epidermidis*, have normal O₂ consumption, and have normal quantitative nitroblue tetrazolium dye reduction.⁶,¹⁰ In addition, we showed that CFC granulocytes maintain normal bactericidal capacity for *E. coli* and *S. typhimurium*.

Collection of granulocytes by FL is less expensive, applicable in a greater number of blood banks, produces greater yields of collected cells, and requires less total donor time than does collection by CFC.⁵,¹⁴ However, the functional capacity of FL cells remains controversial. The results of the present study indicate that the killing of *S. aureus*, *E. coli*, and *S. typhimurium* by granulocytes collected by FL in donors not premedicated with steroids is not significantly different from granulocytes obtained by VP. Granulocytes collected by FL from donors who had received intravenous dexamethasone immediately prior to collection did show slight impairment of killing of *S. aureus* and *E. coli* but not of *S. typhimurium*. These results illustrate the need for testing of bactericidal capacity with more than one organism. Stressing the bactericidal capacity of the granulocytes with a higher ratio of bacteria to phagocytic cells than was used in the present study might reveal defects that we were unable to detect. The clinical significance of such defects are difficult to interpret, however.

Bactericidal capacity was significantly greater with FL cells resuspended in buffered medium than with FL cells resuspended in standard, more acidic medium. Granulocytes collected by FL that were suspended in buffered medium showed killing not significantly different from that seen with VP cells studied concurrently. When the buffered medium was used, normal killing was observed, even with FL cells from donors who had received dexamethasone. The data from this study therefore suggest that resuspension of FL cells in a buffered media will help to preserve normal bactericidal function.

Results from five previous studies that examined bactericidal function of human granulocytes collected by FL are conflicting (Table 7). In the three

<table>
<thead>
<tr>
<th>Authors (Reference)</th>
<th>Bactericidal Capacity</th>
<th>Collection Time (hr)</th>
<th>Steroid Pretreatment of Donors</th>
<th>pH of Elution-Transport Fluids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harris et al.¹¹</td>
<td>Normal killing of <em>S. aureus</em></td>
<td>2</td>
<td>Yes</td>
<td>6.5</td>
</tr>
<tr>
<td>Herzig et al.¹²</td>
<td>Small but significant reduction in killing of <em>S. aureus</em></td>
<td>2-3</td>
<td>None</td>
<td>6.5</td>
</tr>
<tr>
<td>Higby et al.⁷</td>
<td>Normal killing of <em>E. coli</em>, enterobacter, klebsiella; small, nonsignificant decrease in killing of <em>S. aureus</em></td>
<td>2.5</td>
<td>Yes</td>
<td>6.5</td>
</tr>
<tr>
<td>McCullough et al.¹⁰</td>
<td>Significant decrease in killing of <em>S. aureus</em></td>
<td>2.5</td>
<td>None</td>
<td>*</td>
</tr>
<tr>
<td>Wright et al.¹³</td>
<td>Significantly decreased killing of <em>S. aureus</em></td>
<td>3</td>
<td>†</td>
<td>Not stated</td>
</tr>
<tr>
<td></td>
<td>Nonsignificant reduction in killing of <em>S. aureus</em></td>
<td>1-2</td>
<td>None</td>
<td>Not stated</td>
</tr>
</tbody>
</table>

*Not stated; solution used identical to nonbuffered (pH 5.76) solution of present study.
†Killing of *S. aureus* by FL cells collected over 3 hr not significantly different in steroid-treated versus non-treated donors.
studies in which significant reduction of bactericidal capacity was noted, the collection time was in excess of 2.5 hr. Wright et al. showed that the bactericidal function of granulocytes allowed to adhere to the filter for 3 hr was significantly depressed, while cells adhering for 1-2 hr had no significant decrease in bactericidal function. The mean collection time in our study was only 139 min.

The effect of the pH of the elution and suspension medium on bactericidal function of the granulocytes has not previously been examined. As can be seen in Table 7, in the study where the pH of the suspension material may have been comparable to our unbuffered low pH medium a decrease in bactericidal function was noted. The collection time in that study was 2.5 hr. The results of the previous and present studies taken together suggest that granulocytes collected by FL maintain normal bactericidal capacity for S. aureus and the gram-negative bacteria E. coli and S. typhimurium if the collection process (time on filter) is limited to approximately 2 hr and if the solution in which the cells are suspended is buffered to a pH of at least 6.5.

We observed a consistent but small (37%) decrease in the ability of FL cells, as compared with VP cells, to respond to a complement-mediated chemotactic stimulus. Harris et al. found that granulocytes collected by FL during a 2-hr period, as in the present study, responded normally to a complement-mediated chemotactic stimulus. The FL collection in that study differed slightly from the others listed in Table 7. The collection was performed with the leukapherator of Djerassi, in which blood is propelled by air pressure without use of roller pumps; the cells were eluted from the filters with ACD plasma. Wright et al. noted a significant (65%) decrease in chemotactic function of cells collected over 3 hr but not in cells collected for 1.5 hr, and they also noted that dexamethasone pretreatment of donors yielded a chemotactic response that was normal even with a 3-hr collection period. Our chemotactic studies of FL cells were performed on donors who did not receive steroids. McCullough et al. studied chemotactic response to bacterial factors and found normal response of FL cells collected over 2.5 hr when the cells were suspended in fresh-frozen plasma but not when the cells were suspended in Medium 199. Other workers have shown that FL cells are able to migrate to skin windows.

Since leukapheresis donors are frequently pretreated with corticosteroids in order to increase yields of cells collected, we evaluated the effect of steroid pretreatment per se on the function of granulocytes collected by routine VP. We found no alteration in the granulocyte killing of S. aureus, E. coli, or S. typhimurium 10 hr after ingestion of 60 mg prednisone. In addition, the granulocyte killing of E. coli and S. typhimurium was unaffected 1.5 or 3 hr after an i.v. dose of 6 mg dexamethasone. We did not evaluate bactericidal function of granulocytes collected after dexamethasone to S. aureus.

Previous studies have evaluated the effect of steroids on human neutrophil bactericidal capacity by addition of drug to systems in vitro. The results of the effect in vivo of steroids reported here are in agreement with prior studies in vitro that reported no effect upon bactericidal function of human neutrophils at pharmacologic concentrations of drug. While we did not observe any adverse effect of steroid administration upon bactericidal function of VP granulocytes...
cytes, we noted that granulocytes collected by FL from dexamethasone-treated donors did have reduced bactericidal capacity compared to their VP granulocytes taken prior to steroid administration. These results suggest that while either dexamethasone treatment or FL collection alone will not result in a significant reduction in bactericidal function, the combination of both procedures may have an additive adverse effect on granulocyte function.

The effect of oral prednisone upon chemotaxis of granulocytes collected by standard VP and dextran sedimentation, but without Ficoll-Hypaque purification of granulocytes, indicates that prednisone may stimulate nondirected migration in a system in vitro. The migration of granulocytes through the filter in the presence of either unactivated or activated serum was greater when the donors had taken prednisone. In addition, prednisone increased migration with HBSS or HBSS-gel on the "attractant" side, indicating prednisone was enhancing migration. Stevenson\textsuperscript{19} showed similarly that granulocytes from patients taking prednisolone had increased migration. The increased migration was present only when the chemotaxis assay was performed with mixed leukocyte preparations, which contain mononuclear cells, the type employed in the present study. Purified neutrophils showed decreased migration.\textsuperscript{19} Stevenson suggested that in the presence of steroids, mononuclear cells release a factor that stimulates neutrophil migration. Other workers found that prednisone therapy has no significant effect on neutrophil migration.\textsuperscript{20} Steroids added into the test systems were reported to have no effect\textsuperscript{21} or an inhibitory effect\textsuperscript{22} upon migration and chemotaxis. Differences in the preparation of the cells and assay may account for some of these discrepancies.

Since there is an inevitable delay between collection of granulocytes and transfusion, the effect of aging on the function of cells obtained by leukapheresis is of importance. We examined viability and bactericidal and chemotactic function of FL cells over a 96-hr period to determine the feasibility of transport and storage of these cells. The bactericidal capacity of FL cells was not significantly decreased below that of fresh FL cells until after 3 days of storage. Prior studies of the preservation of function of stored granulocytes have been performed on white blood cells separated from whole blood collected by standard VP technique.\textsuperscript{23,28} Skeel et al. noted a 60\% decline in latex particle--stimulated granulocyte hexose monophosphate shunt activity at 72 hr and 70\% decline at 96 hr storage at 4°C.\textsuperscript{23} In the present study a significant decrease in killing of \textit{S. aureus} by FL granulocytes was first seen after 96 hr of storage. This finding is in agreement with that of McCullough and co-workers, who studied \textit{S. aureus} killing by granulocytes from stored whole blood samples.\textsuperscript{24,25} We observed a significant decrease in killing of gram-negative organisms by FL cells by 72 hr storage. Our studies therefore indicate that cells collected by FL can be stored at 4°C for up to 72 hr before any significant loss of bactericidal capacity occurs.

The decline in chemotactic capacity of stored FL cells was more rapid, however. At 24 hr storage of FL cells there was a 57\% decline in chemotactic function, and by 48 hr the cells had minimal chemotactic ability. We observed a slower rate of decline with stored VP cells. McCullough et al. made similar observations for VP cells.\textsuperscript{27} The results of these chemotactic assays in vitro do
not necessarily predict the effect of storage upon the ability of FL granulocytes to migrate to sites of infection. Price and Dale recently showed that the granulocytes in rabbit whole blood stored at 4°C for 24 hr maintained ability to localize into subcutaneous sponges.28

The studies described here lead to the conclusions that CFC cells have normal bactericidal capacity, that FL cells should be suspended in a buffered medium, since a low pH media may damage the cells, that single-dose prednisone and dexamethasone therapy per se will not adversely affect bactericidal or chemotactic function, and that storage of FL cells at 4°C may be practical for at least 24 and perhaps 48 hr.

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