Granulocyte Collection by Continuous-flow Filtration Leukapheresis


A safe and simple technique for the collection of large numbers of granulocytes from normal donors, based on the reversible adhesion of granulocytes to nylon wool filters, is described. The procedure is well tolerated by donors, and the granulocytes obtained show nearly normal bactericidal and phagocytic capacity in vitro. However, a surprisingly small median 1-hr posttransfusion increment of 233/cu mm was observed in leukopenic recipients lacking preformed leukocyte antibodies, as compared to the median increment of 850/cu mm reported following transfusion of leukocytes obtained by differential centrifugation. The safety and effectiveness of these granulocytes for the treatment or prevention of bacterial infections in granulocytopenic patients remains to be proven, however our initial experience with such transfusions has been encouraging.

The ability of granulocytes (PMN's) to adhere to a variety of natural and synthetic materials has been well established. Optimum binding to glass beads requires the presence of viable granulocytes at 25–44°C, calcium and magnesium in physiologic concentrations, and fresh serum. Bound PMN's can be released by removing free calcium and magnesium from the suspending medium; this can be achieved by chelation with EDTA or citrate.

Recently, Djerassi et al. have described a method, filtration leukapheresis (FL), for obtaining large numbers of PMN's for transfusion from single donors by reversible absorption to nylon-wool filters (Leuko-Pak, Fenwal Laboratories, Morton Grove, Ill.).

Our initial attempts to use Djerassi's method resulted in variable PMN recovery, and when PMN's collected by FL were transfused, the posttransfusion increments were significantly less than those observed with PMN's collected by differential centrifugation. Because of these difficulties, the present study was initiated in order to define more clearly the factors governing the binding and release of PMN's by nylon-wool columns, and to assess the viability, in vitro and in vivo, of PMN's collected by FL. Based on our findings, a modification of Djerassi's collection technique is presented and our preliminary experience with transfusion of these PMN's is reported.

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Submitted August 2, 1971; revised October 1, 1971; accepted October 5, 1971.

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MATERIALS AND METHODS

Donors
Healthy normal adults were selected as previously described for donors on the Continuous-Flow Blood Cell Separator (CFC). All donors were fully informed as to the risks of the procedure. A complete blood count (CBC) was performed at the beginning and end of each procedure to determine the effect on donor hemogram. In addition, blood chemistries and renal and hepatic function tests were done periodically on all donors.

Recipients
All PMN transfusion recipients were granulocytopenic patients with malignancy having no preformed leukocyte antibody (leukoagglutinin or lymphocytotoxin) and no ABO incompatibility to the donor. However, all recipients had received numerous RBC and platelet transfusions from random blood bank donors for prior episodes of pancytopenia.

Filtration Procedure
The technique of Djerassi et al. was modified to permit continuous-flow filtration of blood using peristaltic pumps and incorporating the safety features available on the CFC. Bilateral antecubital fossa venipunctures were performed with 14-gauge plastic catheter needles (Longdwell, Becton, Dickinson Co., Rutherford, N. J.). Anticoagulated blood was withdrawn from one arm at 30-60 ml/min, passed over one or more nylon-wool columns, and returned to the other arm. After 2-3 hr of continuous-flow processing, the procedure was terminated by flushing each Leuko-Pak (LP) with approximately 50 ml of normal saline to return the red blood cells (RBC's) to the donor. Sterile plastic tubing sets (Cobe Laboratories, Denver, Colo.), designed for CFC, were used with minimal modification in combination with an LP adapter set (Cobe Laboratories). Variable-speed, precision peristaltic pumps were used.

Differential white blood cell (WBC) counts were performed on blood entering and leaving each LP at 30-min intervals throughout the procedure in order to determine accurately the number of adherent cells.

Anticoagulant
At the start of the procedure, 2500 U of heparin were given intravenously. Additional heparin, alone or in combination with sodium citrate (40% solution, NIH Clinical Center Pharmacy) was administered continuously as described for CFC. A maximum of 4 U heparin/ml of blood processed, or 20,000 U/procedure, whichever was less, was given.

Fig. 1. Diagram of system for continuous-flow filtration leukapheresis. To insure equal flow through both LP's, separate peristaltic pumps must be used for each. Asterisk shows sample port.
Lee-White clotting time of donor blood usually remained less than 60 min. The heparin-sodium citrate anticoagulant used by Djerassi et al.\textsuperscript{12} was prepared as a solution containing 5.66 mg/ml sodium citrate and 130 U/ml of heparin, which was administered continuously at a rate of 0.25 ml/10 ml blood flow. Protamine sulfate, 50 mg, was given intravenously at the end of the procedure.

**Elution Procedure**

Upon completion of the filtration procedure, each LP was flushed with an eluting solution to release the adherent PMN’s. Complete elution, as determined by monitoring the WBC count in the filter effluent, required 1000–1500 ml of eluting solution per LP at a flow rate of 35 ml/min. To elute under increased back pressure, as recommended by Djerassi et al.,\textsuperscript{13} the out-flow tubing from the LP was occluded with a pinch clamp until the desired pressure was achieved. The WBC-rich effluent was collected in 600 ml plastic bags (TA-1, Fenwall Laboratories, Morton Grove, Ill.) and centrifuged at 2500 rpm for 5 min in a Sorvall RC3 centrifuge at 20°C. The resulting concentrates were resuspended and combined in the volume of ACD plasma desired for transfusion. All transfusion and in vitro studies of PMN function were performed on this final preparation.

The following eluting solutions were studied:

1. ACD plasma, adjusted to pH 6.5 with additional ACD (NIH Formula A);
2. 20\% ACD plasma-saline, ACD plasma diluted 1:5 with normal saline and adjusted to pH 6.5, as above;
3. Heparinized plasma, 40 U heparin/ml;
4. Acidified heparinized plasma, adjusted to pH 6.5 with hydrochloric acid;
5. 0.2\% EDTA in 20\% heparinized plasma/saline, adjusted to pH 7.4 with sodium hydroxide.

**Collection of LP Plasma**

When desired, donor plasma was obtained by collecting approximately 300 ml of effluent blood from the LP and centrifuging at 4000 rpm for 5 min. The packed RBC’s were returned to the donor and the procedure was repeated until the required volume of plasma had been collected.

**PMN Function Studies**

To serve as a control, whole blood was drawn into plastic bags (TA-3, Fenwal Laboratories) containing the same anticoagulant (heparin or heparin + sodium citrate) used for the filtration collection. The blood was allowed to stand at room temperature for 2.5–3 hr prior to centrifugation at 2500 rpm for 5 min and resuspension in ACD plasma, as described above for PMN collections.

**In Vitro Studies**

All WBC concentrates were freed of contaminating RBC’s by dextran sedimentation followed by hypotonic saline lysis. WBC morphology and viability were examined by phase microscopy after incubation at 37°C. On one occasion specimens were also examined electron microscopically by standard techniques.\textsuperscript{17} Phagocytic capacity was assessed using radiolabeled bacteria as described by Root et al.\textsuperscript{18} In brief, PMN suspensions were incubated with 14C-labeled microorganisms and uptake of the labeled bacteria by the PMN’s was determined at various time intervals. Thus, both the rate of phagocytosis and maximal phagocytic capacity can be evaluated. Hexose monophosphate shunt (HMS) activity, unstimulated and after incubation with polystyrene latex particles, was measured as an additional indicator of phagocytic ability.\textsuperscript{19} Bactericidal capacity against *Staphylococcus aureus* was also determined by the assay of Cohn and Morse.\textsuperscript{20}

**In Vivo Studies**

Posttransfusion Increments: All transfusions were given over a 30–60 min period. Differential WBC counts were performed on all recipients prior to transfusion and at 1 hr and 18 hr posttransfusion to determine PMN increments.
PMN Intravascular Survival: PMN half-life was determined in dogs by DF32P labeling using standard techniques. Distribution of transfused 51Cr-labeled PMN's was followed by external body scanning, as previously described.

Calculations

The number of PMN's adherent to the LP was determined in the following manner: PMN in = PMN/cu mm in input line to LP; PMN out = PMN/cu mm in output line to LP; Δ PMN = PMN in-PMN out; Δ PMN was determined at 30-min intervals throughout each procedure; Δ PMN × total blood flow during 30-min interval = 30-min PMN adherence; Total PMN adherent = Σ 30-min PMN adherence; Total WBC adherent can be calculated in the same fashion.

The total number of WBC's and PMN's collected was determined by measuring the differential WBC count and the volume of the final WBC concentrate.

For the purposes of this study PMN recovery was defined by the following relationship: PMN recovery (%) = (total PMN collected/total PMN adherent to LP) × 100.

Posttransfusion increments were determined as follows: Absolute 1-hr PMN increment = PMN count/cu mm 1 hr posttransfusion - PMN count/cu mm pretransfusion.

To facilitate comparison of PMN increments, a standard PMN increment also was calculated based on a recipient of 1 sq m body surface area (BSA) receiving a transfusion of 1010 PMN's.

Standard 1-hr PMN increment = absolute 1-hr PMN increment (/cu mm) × BSA (sq m) / number of PMN transfused (× 1010)

RESULTS

Effects on Donor

A total of 33 WBC collections was obtained from 17 donors. The procedure was generally well tolerated. Although an occasional donor complained of chilliness, no shaking chills or fevers occurred. The effect of FL on the donor hemogram is shown in Table 1, accompanied by published CFC data for comparison. A modest decrease in hemoglobin (median decrement 0.85 g/100 ml, per procedure) and a substantial drop in platelet count (median decrement 39,000/cu mm, per procedure) were seen.

In six donors two or more daily procedures were done; however, progressive thrombocytopenia prevented more than three consecutive donations from any one individual. No collection was initiated with a donor platelet count below 150,000/cu mm.

The most striking change, not apparent from the tabular data but illustrated in Fig. 2, was a marked but transient granulocytosis observed in 12 of 17 donors (median increase 120% of the initial PMN count). Maximum levels were reached in 60–120 min and sustained for approximately 1 hr. To examine the cause of the leukocytosis, four donors who had previously experienced a granulocytosis were infused with autologous LP plasma, at a time when their WBC counts had returned to normal. A prompt granulocytosis was seen in three of the four donors tested, without accompanying fever or chills. The LP plasma was infused within 48 hr of collection in the three responders, while for the donor who did not respond there had been a 4-mo delay before returning the plasma. In order to exclude the LP itself as a source of the leukocytosis-inducing substance, a single unit of WBC-poor plasma was collected from one of the responders by standard plasmapheresis technique, passed through an unused LP, and retransfused. There was no resulting leukocytosis.
Table 1. Change in Donor Hemogram: Comparison of FL and CFC

<table>
<thead>
<tr>
<th>Type of Procedure</th>
<th>No. of Collections</th>
<th>Volume Processed (liters)</th>
<th>Δ Hb (g/100 ml)</th>
<th>Δ WBC ($\times 10^7$/cu mm)</th>
<th>Δ Platelets ($\times 10^9$/cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL</td>
<td>28</td>
<td>7.1* (5.5 to 11.8)†</td>
<td>−0.85 (−2.8 to +0.3)</td>
<td>+1.4 (−0.6 to +13.5)</td>
<td>−39 (−154 to +18)</td>
</tr>
<tr>
<td>CFC</td>
<td>124</td>
<td>9.6 (3.6 to 12.6)</td>
<td>−0.67 (−2.3 to +1)</td>
<td>0 (−4.2 to +8.3)</td>
<td>−50‡ (−180 to +220)</td>
</tr>
</tbody>
</table>

*Number indicates median.
†Brackets indicate range.
‡Platelet decrements with CFC represent lymphocyte collections or simultaneous PMN and platelet collections.
GRANULOCYTE COLLECTION

There have been no hemorrhagic problems or other donor complications. Serial blood chemistries and hepatic and renal function tests have been followed without significant change after as many as seven donations over a 5-mo period.

Effect of LP Loading and Anticoagulation on PMN Recovery

Early experiments demonstrated a direct relationship between PMN recovery and the extent of LP loading. As can be seen in Fig. 3, there is an approximately linear increase in PMN recovery from the LP with increasing loading, in the range of $2 \times 10^9$ to $15 \times 10^9$ PMN per LP. With additional loading, there is little increase in recovery, the maximum approaching 75% of the adherent PMN’s. Since the total capacity of a single LP is approximately $40 \times 10^9$ PMN’s, a maximum recovery of $30 \times 10^9$ PMN’s per LP can be expected with complete loading. In order to exclude variables among donors and procedures, two or more LP’s were loaded to different degrees during a single procedure in each of three subjects, confirming the relationship established between LP loading and PMN recovery.

In order to examine the effect of anticoagulant on PMN recovery, additional studies were carried out with heparin, or heparin plus sodium citrate, while LP loading remained constant; no significant difference in PMN adherence (90-95% of PMN’s processed) or PMN recovery was found.

Effect of Eluting Solution on PMN Yield

After determining the above factors that influenced PMN recovery, all subsequent studies of the variables governing elution were performed using heparin anticoagulation. For each experiment, two LP’s were loaded simultaneously with a minimum of $10 \times 10^9$ PMN’s from a single donor. Care was taken to insure loading of similar quantities of PMN’s on each LP, under
identical conditions. One LP was flushed with ACD plasma (pH 6.5), as recommended by Djerassi et al.,\textsuperscript{12,13} to serve as a standard while the second LP was flushed with the eluting solution under examination. By this method, both 20% ACD plasma-saline and 0.2% EDTA were found to be as effective as the ACD plasma for elution, while acidified heparinized plasma was relatively ineffective, eluting only 35% of the PMN's released by the ACD plasma (Table 2). The use of cold (4°C) eluting solutions or elution under increased back pressure did not increase PMN recovery significantly.

**PMN Function Studies**

Microscopic examination of Wright's-stained preparations of the WBC concentrates obtained by FL revealed normal morphology. Phase microscopy of wet preparations at 37°C showed good motility, but 15-20% of the PMN's exhibited marked cytoplasmic vacuolization and a ragged appearance to the cell membrane. Electron microscopy confirmed these changes.

Functional capacity was evaluated in four PMN collections with the in vitro tests summarized in Table 3. PMN viability by phase microscopy (confirmed by trypan blue dye exclusion) was 90-96% compared to > 98% viability for the peripheral blood control. Phagocytic and bactericidal capacity were both re-

**Fig. 3. Relationship between LP loading and PMN recovery. Brackets indicate number of determinations per point. Note that loading with 12.5 × 10^9 PMN/LP is required for 50% recovery.**

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**Table 2. Effect of Eluting Solution on PMN Recovery from LP**

<table>
<thead>
<tr>
<th>Eluting Solution</th>
<th>pH</th>
<th>PMN Recovery (%) of ACD Plasma Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACD plasma</td>
<td>6.5</td>
<td>100</td>
</tr>
<tr>
<td>20% ACD plasma-saline</td>
<td>6.5</td>
<td>97*</td>
</tr>
<tr>
<td>0.2% EDTA</td>
<td>7.4</td>
<td>87</td>
</tr>
<tr>
<td>Heparinized plasma</td>
<td>6.5</td>
<td>35</td>
</tr>
</tbody>
</table>

*Mean of four experiments.*
Table 3. In Vitro Functional Capacity of PMN's Collected by FL

<table>
<thead>
<tr>
<th>Assay</th>
<th>WBC Preparation</th>
<th>Reduction of FL From Control (%v)</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>Control (2)*</td>
<td>FL (4)</td>
<td></td>
</tr>
<tr>
<td>Viability†</td>
<td>98.5 ± 0.5‡</td>
<td>92 ± 1.4</td>
<td>6</td>
</tr>
<tr>
<td>Radiolabeled phagocytosis§</td>
<td>21,502 ± 2</td>
<td>19,560 ± 187</td>
<td>9</td>
</tr>
<tr>
<td>HMS activity†§</td>
<td>276 ± 8</td>
<td>217 ± 21</td>
<td>21.4</td>
</tr>
<tr>
<td>Bactericidal capacity†</td>
<td>91.8 ± 2</td>
<td>81.1 ± 1.6</td>
<td>11.7</td>
</tr>
</tbody>
</table>

*Brackets indicate number of determinations.
†Percentage of PMN viable on phase microscopy.
‡Mean ± SE.
§Maximum disintegrations per minute incorporated after incubation with 14C-labeled S. aureus.
| nmoles 14C-1-glucose oxidized, polystyrene latex particle stimulation, 5 × 10⁶ PMN x 60 min incubation. |
| Per cent S. aureus killed, 60 min incubation. |

Reduced approximately 10% from the control (p <0.02). HMS stimulation by polystyrene latex particles, another measure of phagocytic ability, showed a mean reduction of 20% from the control, but this was not statistically significant. Both the use of increased back pressure, and 20% ACD plasma-saline for elution were associated with somewhat poorer in vitro function.

In order to study their in vivo function, PMN's collected by FL from 12 normal donors were transfused into six granulocytopenic patients with leukemia (a total of 13 donor-recipient pairs). Under similar conditions with normal WBC transfusions obtained by CFC given to recipients without pre-formed leukocyte antibody, the median standard 1-hr PMN increment previously has been reported to be 850 PMN/cu mm.¹⁴ However, as is shown in Table 4, the median standard 1-hr increment seen with cells obtained by FL was only 233 PMN/cu mm, or 27% of the CFC increment. Four of the FL transfusions were obtained by elution with 20% ACD plasma-saline. A median standard 1-hr post-transfusion increment of 500/cu mm (range 207–530) was observed, as compared to 172/cu mm (range 28–1320) for undiluted ACD plasma. This difference is not statistically significant (p <0.1). As further confirmation of the shortened intravascular survival of FL PMN's, WBC's were collected both by CFC and FL from a single donor for each of three donor-recipient pairs shown in Table 4. In each case, the PMN increment following cells collected by FL was significantly smaller.

In order to evaluate these poor transfusion increments, the in vivo circulation of FL PMN's was studied in dogs using DF³²P and ⁵¹Cr labeling techniques. Figure 4 shows the DF³²P survival curves for PMN's obtained by FL compared to whole blood from the same donor and to previously published survival curves for cells obtained by CFC.²⁴ The markedly shortened survival of the cells obtained by FL is apparent. Labeling of PMN's with ⁵¹Cr confirmed the short half-life of these cells, and external body scanning revealed rapid accumulation of labeled FL-collected cells in the spleen, and to a lesser extent in the liver.
In 10 of 18 transfusions, the WBC recipients had transfusion reactions characterized by fever and chills similar to those observed with leukoagglutinin-positive WBC transfusions. However, as previously mentioned, no leukoagglutinins were demonstrated in these patients.

Table 4. Comparison of Standard 1-Hr Posttransfusion PMN Increments for FL and CFC

<table>
<thead>
<tr>
<th>Transfusions</th>
<th>CFC</th>
<th>FL</th>
<th>FL/CFC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB/CH</td>
<td>960</td>
<td>97</td>
<td>10</td>
</tr>
<tr>
<td>NC/CH</td>
<td>374</td>
<td>103</td>
<td>28</td>
</tr>
<tr>
<td>NB/PB</td>
<td>486</td>
<td>254</td>
<td>52</td>
</tr>
<tr>
<td>All</td>
<td>850</td>
<td>233</td>
<td>27</td>
</tr>
</tbody>
</table>

1-Hr PMN Increment* × BSA in sq m.

*PMN increment/cu mm × 10^10 PMN transfused
†Median of 60 transfusions; brackets indicate range.
‡Median of 18 transfusions; brackets indicate range.
GRANULOCYTE COLLECTION

Based on the above findings we have standardized the FL technique using heparin anticoagulation, loading each LP with a minimum of $10 \times 10^9$ PMN's and eluting with 20% autologous (donor) ACD plasma-saline, adjusted to pH 6.5 with additional ACD. The usual procedure requires 2.5 hr of donor time and an additional hr for elution of the LP's. Nine WBC concentrates have been collected from four donors, using this technique with yields ranging from $8.1 \times 10^9$ to $37.8 \times 10^9$ PMN's (median $11.8 \times 10^9$). This represents 50–72% (median 63%) of the total PMN's processed. One patient has been transfused solely with PMN's obtained by this standard technique.

CASE HISTORY

N. T., a 5-yr-old boy with acute lymphocytic leukemia in relapse, was admitted to the NIH Clinical Center on May 30, 1971, with fever and rectal pain (Fig. 5). Physical examination revealed a systolic blood pressure of 60 mm Hg and a painful rectal fissure. A diagnosis of septic shock was made (confirmed by blood cultures positive for Escherichia coli), and broad-spectrum antibiotic treatment was begun. Blood pressure was maintained with intravenous isoproterenol. After 36 hr of antibiotic therapy the patient remained febrile to 40°C, and disseminated intravascular coagulation (DIC) developed. Because of severe granulocytopenia, PMN transfusions were begun from the patient’s ABO-compatible father. Four transfusions were given over the next 7 days. The median dose was $21.4 \times 10^9$ PMN's (range $12.5 \times 10^9$ to $30.9 \times 10^9$) producing a median standard 1-hr PMN increment of 350/cu mm. During this period the patient’s fever progressively lessened; isoproterenol was tapered and finally discontinued, and clotting studies returned toward normal permitting the cessation of heparin therapy. Repeated blood cultures remained...
negative and the rectal fissure showed slow improvement with formation of a localized abscess. At no time were performed leukoagglutinin or lymphocytotoxic antibodies against the donor demonstrated.

On June 10, 1971, 3 days after the last WBC transfusion, the patient was afebrile and clinically stable in spite of a WBC count of 50/cu mm. A bone marrow biopsy revealed marked hypocellularity with 60% lymphoblasts. Twelve days later the PMN count reached 1000/cu mm, and antibiotics were discontinued. The patient remained afebrile without clinical evidence of infection.

DISCUSSION

The factors involved in adhesion of granulocytes to glass beads have been described in detail. Similar factors appear to govern the reversible binding of PMN's to nylon wool fibers. When fresh heparinized blood is passed over a nylon wool filter at flow rates up to 35 ml/min, a rate not exceeded in this study, 90–98% of the granulocytes are removed. As saturation of the LP is approached, increasing numbers of PMN's appear in the filter effluent. Once adherent, the addition of chelating agents, which remove calcium and magnesium ions, results in release of PMN's as is obtained with elution by EDTA-plasma solutions, at neutral pH. Acidification of the LP and its adherent PMN's as suggested by others, is not sufficient to cause release as shown by the failure of elution attempts with acidified heparinized plasma which lacks chelating ability. Similarly, Bryant et al. and Garvin have found acidification to have no influence on the adhesion of PMN's to glass surfaces. However, when dealing with ACD solutions, pH reflects citric acid concentration and is thus an indirect measure of chelating capacity. Elution with increased back pressure to insure complete penetration of the LP increased PMN yield by no more than 10% and appears to have resulted in impaired in vitro PMN function perhaps through damage to PMN's as they are forced through the tubing constricted by the pinch clamp.

When collecting PMN's for transfusion, the most convenient solution for washing the LP is ACD plasma; however, it is more economical of plasma to use the 20% ACD plasma-saline mixture described. In this way, 400 ml of plasma suffice for 2 liters of eluting solution, and this quantity of plasma can be obtained easily from the WBC donor during the filtration procedure. When 20% ACD plasma-saline is used for elution, there is no sacrifice in PMN recovery, and the risk of hepatitis in the WBC recipients is greatly reduced by the use of single donor blood products in lieu of 6 U of random donor plasma. Preliminary findings suggest that the in vitro function of PMN's eluted with undiluted ACD plasma may be slightly better, but this may be balanced by the improved post-transfusion PMN increments found with the 20% ACD plasma-saline. Thus, although additional studies of in vitro function and in vivo survival are planned, 20% autologous (donor) ACD plasma-saline appears to be the eluting solution of choice for the present.

In order to insure efficient PMN recovery from LP's, each filter should be loaded with at least $12 \times 10^9$ PMN's which, taking advantage of the linear relationship between LP loading and recovery, will result in a yield of more than 50% of the adherent PMN's. The poor PMN recovery observed with reduced loading might be explained by the loss of the first-loaded PMN's...
through tighter binding, or lysis. This hypothesis can be tested easily by loading studies during which aliquots of labeled PMN’s are added at various time intervals throughout the procedure.

Another factor contributing to larger PMN yields is the marked donor leukocytosis frequently observed. This leukocytosis has been shown to be produced by a substance released into the plasma effluent of a LP during the process of PMN adhesion. It is not pyrogenic and may be related to release of proteases or other substances during the degranulation that accompanies phagocytosis.

It is clear that PMN’s can be obtained with great efficiency (up to 75% recovery of PMN’s processed) and in large quantity (up to $5 \times 10^{10}$ PMN’s per 2.5-hr procedure) from normal donors by the technique of reversible adhesion to nylon wool. Processing the large volumes (4–8 liters) of blood required can be accomplished easily, maintaining donor safety, with the continuous-flow system described. For comparison, it should be recalled that WBC collection by CFC results in average yields of $4 \times 10^9$ PMN per 4-hr collection or 14% of the PMN’s processed. This represents only 20% of the maximal efficiency possible by FL. Recently, McCredie and Freireich have reported improved CFC yields by administration of etiocholanolone and hydroxyethyl starch to normal donors. Under these circumstances, CFC yields may approach the FL recoveries in magnitude, but still not in efficiency.

The procedure is well tolerated by donors and potential donor risk is probably no greater than for CFC. However, because of the significant platelet losses incurred, a single donor should be used for no more than two or three consecutive procedures, while five or more consecutive donations are possible by CFC.

PMN’s collected by FL have nearly normal phagocytic and bactericidal function in vitro. However, on transfusion the PMN’s have a markedly shortened intravascular survival as compared to CFC PMN’s. Chromium labeling studies in conjunction with external body scanning have implicated splenic sequestration as the cause of this shortened survival. Presumably, the process of adhering to the nylon wool alters the PMN surface in a way that leads to splenic uptake, even though in vitro tests indicate continued viability. Work is now in progress in an attempt to modify collection and elution procedures to improve PMN survival.

No definite conclusion can be made as yet regarding the effectiveness of these PMN’s in the treatment of bacterial infections in granulocytopenic patients. Because of the large numbers of cells obtained, absolute 1-hr post-transfusion increments are similar to those observed with the average CFC transfusion, despite the shortened intravascular survival. One disturbing observation has been the frequent occurrence of apparent transfusion reactions characterized by fever and chills, even in the absence of performed leukocyte antibodies or ABO incompatibility. It is possible that these symptoms are the result of rapid destruction of the transfused cells following splenic sequestration. The potential risks of DIC, reticuloendothelial system blockage, or pulmonary congestion following repeated transfusion of FL PMN’s must also be considered and will be the subject of further investigation.
In spite of these reservations, the remarkable recovery of the single patient we have supported entirely with PMN's collected by this technique is encouraging, especially in light of the poor prognosis (30% survival) of such patients when treated with appropriate antibiotics alone. Further controlled clinical trials of FL PMN's are warranted to evaluate recipient toxicity, intravascular survival, and effectiveness in the management of life-threatening infections.

ACKNOWLEDGMENT

The excellent technical assistance of Mrs. Ruth Estabrook, R. N., Mrs. Shirley House, Miss Carole Novick, and Mrs. Regina Dowling, R. N., is greatly appreciated. Electron micrographs were kindly performed by Mr. R. L. Reagan.

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