Continuous flow filtration leukopheresis (FL) is a relatively simple, inexpensive, and efficient technique of harvesting blood neutrophils from normal donors for transfusion into neutropenic recipients. There has been concern, however, that neutrophils may be functionally altered during this leukopheresis procedure. Human neutrophils obtained by various FL techniques were studied for in vitro chemotaxis by a $^{51}$Cr-radiolabel method and for in vitro killing and phagocytosis of Staphylococcus aureus. We compared their function with neutrophils obtained by the NCI-IBM cell separator and by dextran sedimentation from whole blood. FL neutrophils eluted from nylon filters after 3-hr collection periods were functionally abnormal by all parameters tested, while neutrophils obtained by cell separator after similar collection times were not significantly different from control cells. However, neutrophils from 3-hr FL collections were found to include both normal and abnormal populations of cells. Loosely adherent cells, eluted easily without tapping the filters, were functionally normal; more adherent cells, eluted after tapping the filters and representing the bulk of cells collected, were progressively more abnormal the less readily they were eluted. Shortened FL collection times (1-2 hr) were found to decrease the functional defects. Also, administration of dexamethasone to donors prior to filtration leukopheresis diminished the functional defects of FL neutrophils perhaps by altering adherence characteristics of the cells. These studies show that neutrophils obtained by filtration leukopheresis are functionally abnormal in relation to the time and extent of adherence to nylon filters.


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and inexpensive for this technique to be accessible to most well-staffed medical center blood banks. However, experience with neutrophils obtained by FL techniques in both humans and in animal models has suggested that adherence to nylon may significantly alter the cells in a way that diminishes their useful function once transfused. FL neutrophils do not circulate normally after transfusion but disappear rapidly from the circulation. Radiolabeling studies have implicated splenic sequestration as the cause of shortened intravascular survival. Peak increments in circulating neutrophils after transfusion of FL cells have been found to occur not immediately but 2-3 hr after transfusion. These findings suggest that transfused FL neutrophils may be released secondarily into the circulation after initial sequestration. In addition, recipients of FL neutrophils commonly experience transfusion reactions that cannot be attributed to ABO or HL-A incompatibility nor to leukoagglutinins. There have been conflicting reports concerning the functional capacity of FL neutrophils as determined by a variety of in vitro tests, some indicating that they are normal and others indicating that they are defective. The present studies demonstrate that collection of neutrophils by filtration leukopheresis clearly alters the in vitro chemotactic, phagocytic, and bactericidal capacities of these cells and that these functional abnormalities are related to the time and extent of adherence by the cells to the nylon wool surfaces used in cell filtration.

MATERIALS AND METHODS

Neutrophil Donors

Healthy adults of both sexes (23-42 yr) who were regular leukopheresis donors for granulocyte support of patients at the National Cancer Institute were the sources of neutrophils studied. Informed consent was obtained from all donors prior to leukopheresis. For certain studies, neutrophils were collected from donors who had taken dexamethasone (4 mg/sq m) by mouth 12 hr before leukopheresis.

Collection of Neutrophils

Dextran sedimentation of venous blood. Fifty to seventy milliliters of heparinized blood (10 U heparin/ml) was obtained from all donors prior to leukopheresis procedures. Leukocytes were separated by sedimentation in 3% dextran (T250, Pharmacia, Uppsala, Sweden) followed by hypotonic lysis of red cells. Leukocytes obtained in this way were 70%-85% neutrophils. The in vitro functions of neutrophils obtained by leukopheresis from each donor were compared in each case with the functions of dextran-sedimented cells prepared concurrently as controls.

Filtration leukopheresis (FL). FL procedures, utilizing nylon wool filters (Leukopak, Fenwall, Morton Grove, Ill.), were essentially as reported previously. Donors received 500 U of heparin intravenously at the start of the procedure. Thereafter, a solution of 500 ml normal saline + 40,000 U heparin was infused, 2 ml/min, into the efferent line leading to the filter or filters during the first hour of the procedure; during the following 2 hr, 1 ml/min of this saline-heparin solution was infused into the efferent line. Filters were perfused with heparinized blood at a flow rate of 40-50 ml/min by peristaltic pump. At the end of 3 hr the filters were flushed with 100-150 ml of normal saline to return residual red cells to the donor. Neutrophils were then eluted from the filters with 300 ml of ACD-plasma solution per filter (ACD: Acid citrate dextrose, NIH formula A), while the filters were gently tapped. The average neutrophil yield from single filters was 1.2 x 10^10 cells. For certain studies neutrophils were collected from two filters simultaneously, one being eluted after 1, 1½, or 2 hr and the other being allowed to collect cells for the full 3 hr. In other studies separate elution fractions were collected in sequence from a single 3-hr FL filter with and without tapping the filter. For certain studies, the concentration of plasma in the ACD-plasma eluting solution was varied from 20% to 100% to match eluting solutions used by
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In some cell collections autologous plasma was used in the eluting solutions instead of pooled ABO-compatible plasma.

NCI-IBM cell separator (CS). CS procedures were as reported previously.5 The donors were injected with 2500 U heparin at the beginning of the procedure. Thereafter, a solution of 500 ml normal saline + 20,000 U heparin and ACD solution (acid citrate dextrose, NIH formula A) were each infused into the effluent line leading to the cell separator (Celltrifuge, Aminco, Silver Spring, Md.) at 1 ml/min. During a routine collection period of 4 hr, 5 x 10⁷ neutrophils were recovered.

For all leukopheresis procedures, cells used in functional assays were taken directly from collection bags used subsequently for transfusion. Like the dextran-sedimented leukocytes, cells from leukopheresis procedures underwent hypotonic lysis of residual red cells before the leukocytes were washed and counted for use in the functional assays.

Assay of Chemotactic Activity

Chemotaxis was measured with a radioassay that employed ¹¹⁹Cr radiolabeling of leukocytes and double microple filter chemotactic chambers.16 For this assay leukocytes obtained by dextran sedimentation or by leukopheresis procedures were labeled with ¹¹⁹Cr (sodium chromate, Amersham-Searle, Arlington Heights, Ill.) and placed in the upper compartment of a modified Boyden chamber, separated from a lower compartment by two 3-μm microple filters (Sartorius, Beckman Instruments, Mountainside, N.J.). The neutrophils were suspended at a concentration of 2.3 x 10⁶ neutrophils per ml in Gey's medium.16 A chemotactic stimulus [fresh serum activated by E. Coli endotoxin (lipopolysaccharide B 0127:B8, Difco Labs., Detroit, Mich.) or sodium caseinate (Difco Labs.)] was added to the lower compartment. The chemotactic chambers were incubated at 37°C for up to 3 hr. It has been shown previously that only neutrophils migrate into the lower filter during incubation and that the number of migrating cells is proportional to the radioactivity incorporated into the lower filter. After adjustment of variable specific activity and uptake of the chromium into the leukocytes, chemotaxis was expressed as corrected counts per minute in the lower filter (COR CPM LF).16 The chemotactic activity in each experimental condition for all experiments was the mean of four replicate determinations.

Assay of Phagocytic Capacity

The phagocytic uptake of ¹⁴C-radiolabeled heat-killed Staphylococcus aureus was measured by a method reported previously.17 Neutrophils were incubated with the bacteria at a ratio of five to ten bacteria: one neutrophil. After 20-min incubation periods, phagocytosis was stopped by the addition of iced buffer, and the leukocytes were centrifuged at low speed (75 g). Supernatants were aspirated to remove non-cell-associated bacteria, the cells resuspended, and the procedure repeated multiple times. It has been shown previously that the cell-associated radioactivity under these conditions closely agrees with other measures of phagocytosis.17 Phagocytosis was expressed as per cent of total bacteria phagocytized: (average cell associated cpm/ total cpm in incubation mixtures) x 100.

Assay of Bactericidal Capacity

Bactericidal assays measured the clearance of viable S. aureus in neutrophil-bacteria incubation mixtures.18 Neutrophils (5 x 10⁶) were incubated with bacteria, prepared from washed overnight cultures, at a ratio of four bacteria: one neutrophil. Aliquots of the incubation mixtures were removed at designated intervals with a calibrated wire loop, diluted with sterile water to lyse the leukocytes, and transferred to pour plates which were counted for bacterial colonies 24 hr later. Killing of bacteria was expressed as per cent of inoculated bacteria surviving at each interval.

Preparation of Neutrophils for Morphology

Small aliquots of neutrophil suspensions prepared by the various procedures described were fixed onto glass slides with a cytocentrifuge (Cytospin, Shandon, Sewickly, Pa.) and then stained with Wright's stain for inspection by light microscopy.
Statistical Analysis of Results
The Student's t test was used throughout to compare the means of different experiments.

RESULTS

In Vitro Function of Neutrophils Obtained by Filtration Leukopheresis

The chemotactic, phagocytic, and bactericidal capacities of neutrophils obtained from 3-hr FL collections were consistently abnormal when compared to control neutrophils prepared concurrently from the same donors by dextran sedimentation of heparinized blood. Migration of FL neutrophils in response to chemotactic stimuli was reduced by up to 65% compared to dextran-sedimented control cells (Fig. 1). In contrast, neutrophils obtained after 3–4 hr collections by cell separator demonstrated chemotactic activity that was not significantly different from control cells (Fig. 1). Neutrophils from 3-hr FL collections were also abnormal with respect to phagocytic (Table I) and bactericidal capacities (Fig. 2). These abnormalities were consistent for all donors and were highly significant. Increasing the percentage of plasma in the eluting solution or use of autologous plasma did not diminish in any way these functional abnormalities of the FL neutrophils.

The functional abnormalities noted in neutrophils from 3-hr FL collections could reflect either abnormalities present in all cells from these collections or...
average functions of different populations of cells, some of which were normal and others impaired. Therefore, studies were undertaken to test whether 3-hr FL collections might contain a subpopulation of normal cells. Rather than eluting neutrophils from the nylon filters in a single bulk collection, different elution fractions were collected in sequence. Cells that were eluted from the filters without tapping were separated from cells that were eluted subsequently with gentle or vigorous tapping (Table 2). In this way the less-adherent cells were separated from those more adherent to the nylon. When neutrophils in separate elution fractions were compared functionally with control dextran-sedimented cells, it was apparent that cells least adherent to the filters (fraction 1) were not significantly different from the controls, while the more adherent

Table 1. Phagocytosis of S. aureus by Neutrophils From Filtration Leukopheresis (FL)

<table>
<thead>
<tr>
<th>Neutrophil Preparation</th>
<th>Per Cent Phagocytosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran-sedimented control cells</td>
<td>45.4 ± 2.5</td>
</tr>
<tr>
<td>FL neutrophils 3-hr collections</td>
<td>30.1 ± 1.5</td>
</tr>
</tbody>
</table>

*Mean ± SEM; nine experiments; per cent of bacteria phagocytized in 20 min; differences are significant, \( p < 0.001 \).

Table 2. Elution Fractions Obtained From Single Filters in 3-hour Filtration Leukopheresis

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Elution Procedure and Volume</th>
<th>Per Cent of Total Cells Collected*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No tapping, 150 ml</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>No tapping, 150 ml</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Gentle tapping, 150 ml</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Gentle tapping, 150 ml</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>Vigorous tapping, 150 ml</td>
<td>5</td>
</tr>
</tbody>
</table>

*Mean of five separate collections.

Fig. 2. Bactericidal capacity of filtration leukopheresis (FL) neutrophils from 3-hr collections. Killing of S. aureus at 1 and 2 hr of incubation with neutrophils. Differences between FL cells and FL donor control cells (12 experiments) are significant: 1 hr, \( p < 0.001 \); 2 hr, \( p < 0.001 \).
Fig. 3. Functional capacities of neutrophils in different elution fractions of 3-hr FL collections (see Table 2). Differences between fraction 1 and fractions 3 and 5 are significant: chemotaxis (four experiments), \( p < 0.001 \); phagocytosis (nine experiments), \( p < 0.001 \); bacterial killing (11 experiments), \( p < 0.001 \).

cells from subsequent elution fractions (representing approximately two-thirds of the total cells collected) were progressively more abnormal by all parameters tested (Fig. 3). Since it was likely that the extent of neutrophil adherence to nylon and the functional defects associated with adherence were related to the time that cells were exposed to the filters, the functional capacities of neutrophils from FL collections of different times (1–3 hr) were compared. In all cases, the shorter the collection times, the more functionally normal were the neutrophils collected (Table 3).

Table 3. Functional Abnormalities of Neutrophils Obtained by Filtration Leukopheresis (FL) in Relation to the Time of Exposure to Nylon Filters

<table>
<thead>
<tr>
<th>In Vitro Function</th>
<th>FL Collection Time (hr)</th>
<th>Number of Experiments</th>
<th>Per Cent of Controls*</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotaxis†</td>
<td>1\ 1/2</td>
<td>7</td>
<td>69 ± 4.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12</td>
<td>32 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>1</td>
<td>9</td>
<td>81 ± 3.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9</td>
<td>75 ± 3.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9</td>
<td>67 ± 3.7</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Bacterial killing‡</td>
<td>1</td>
<td>9</td>
<td>94 ± 1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9</td>
<td>82 ± 2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12</td>
<td>78 ± 3.3</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Mean ± SEM (number of experiments).
†Chemotactic response to E. coli endotoxin-activated serum.
‡Per cent killing of S. aureus after 2 hr incubation.
Fig. 4. Morphology of dextran-sedimented and filtration leukopheresis (FL) neutrophils. Cells prepared concurrently from one donor. Magnification: Dextran sedimented, 3-hr FL, and 3-hr FL fraction 5, × 1200; 1-hr FL and 3-hr FL fraction 1, × 860.
Correlation of Functional and Morphologic Abnormalities

Neutrophil morphology has been shown to be altered by adherence to nylon wool filters. Morphologic changes in neutrophils were found to be related to the observed in vitro functional abnormalities induced by adherence of the cells to nylon filters (Fig. 4). Cells from 1-hr FL collections, found to have relatively normal function, could be shown also to be relatively normal morphologically, whereas cells from 3-hr FL collections that were functionally abnormal appeared by morphology to include two populations of cells: those that were normal in appearance and those with varying degrees of vacuolization and ragged boundaries. When neutrophils from 3-hr collections were separated according to their adherence to the filters, as described, it could be shown that cells from fraction 1, which were functionally normal, were of normal appearance, while cells from fraction 5, which were functionally very abnormal, were extensively vacuolated and of ragged appearance.

Effect of Pretreating Donors With Dexamethasone

Pretreatment of leukopheresis donors with single-dose corticosteroids has been advocated as a means of increasing leukocyte yields by inducing temporary elevations in the donors' circulating white blood cell counts. Also, it has been found that neutrophils from patients receiving corticosteroids show less spontaneous adherence to nylon surfaces than do cells from normal controls. Therefore, we studied the functional capacities of neutrophils from donors both with and without pretreatment with oral dexamethasone (4 mg/sq m) 12 hr before filtration leukopheresis. In each case the donors served as their own controls with and without steroid treatment at 1-wk intervals. With the three donors studied, the chemotactic defect in neutrophils from 3-hr FL collections was significantly reduced or eliminated when the donors were pretreated with the steroid (Fig. 5). Although the phagocytic and bactericidal...
capacities of neutrophils were improved when donors received steroids, these
differences were not significant in the studies performed.

**Exposure of FL Neutrophils to Divalent Cations**

Because it has been reported that incubation of neutrophils obtained from
dogs by filtration leukopheresis with supraphysiologic concentrations of mag-
nessium (1 mM) after FL collections diminished functional defects noted in
these cells, we incubated cells from 3-hr FL collections with calcium and/or
magnesium at this concentration before functional assays. Exposure of hu-
man FL neutrophils to these divalent cations did not alter their in vitro func-
tional defects.

**DISCUSSION**

These studies demonstrate that neutrophils are functionally altered in the
process of filtration leukopheresis and that these alterations are related to the
time and extent of adherence to the nylon filters used in this technique. Plasma
content of the eluting solution did not play a role in the functional alterations
observed. Divalent cations are known to be important in neutrophil adherence,
chemotaxis, and phagocytosis. Our results, however, could not be explained
by intracellular depletion of cations (calcium and magnesium) during elution
with ACD-plasma solution, for all FL neutrophils with both normal and ab-
normal function were exposed to the same eluting solutions. Moreover, addi-
tion of divalent cations did not diminish the observed defects in these human
FL neutrophils as has been noted with canine FL cells that were exposed to
supraphysiologic concentrations of magnesium.

Our data provide an explanation for the normal functions of FL neutrophils
as documented recently by Harris et al. in conflict with prior reports. These
authors suggested that their results might be explained by differences in
methods; in their study cells were collected after no more than 2 hr of exposure
to the filters, and in addition all donors were pretreated with intravenous hy-
drocortisone. Other investigators have also modified their FL procedures
toward more gentle elution techniques and suggest that these modifications
improve the clinical usefulness of the cells collected. Our studies clearly show
that shortened collection times, incomplete elution of filters, and steroid pre-
treatment of donors reduce the functional alterations in cells obtained by filtra-
tion leukopheresis that result from adherence to the nylon filters.

A major advantage of filtration leukopheresis has been the large numbers of
neutrophils that may be collected by this technique compared to numbers of
cells obtainable by cell separator techniques presently available. This advantage
may be more apparent than real, however, for our studies indicate that the FL
techniques that provide greatest cell yield (long collection times and vigorous
elution procedures) result in large percentages of functionally abnormal cells.
This observation is consistent with retrospective clinical evidence that more
neutrophils obtained by FL may be required for transfusion into neutropenic
patients to equal the protective effect of neutrophils obtained by other means.
However, prospective controlled trials comparing the efficacy per
cell transfused of different leukopheresis procedures have yet to be completed.
At present, filtration leukopheresis remains a promising technique by which neutrophil transfusion may become a more generally available procedure in the care of neutropenic patients. Our studies support the observation that neutrophils may be obtained by filtration leukopheresis that are functionally normal by in vitro testing. At the same time, however, our studies indicate that functional alterations in neutrophils are directly related to the time and extent of adherence by the cells to nylon fibers used in this leukopheresis procedure. This finding suggests that even with optimal collection conditions neutrophils may undergo subtle changes during adherence to nylon fibers that are not detected by in vitro functional assays. These studies provide experimental grounds for the refinement of FL techniques: minimum collection times with multiple filters used in series, gentle elution procedures, and pretreatment of donors with corticosteroids, as have been suggested by others for diverse reasons. It remains to be seen whether these refinements will reduce the incidence of transfusion reactions associated with neutrophils obtained by filtration leukopheresis or will improve the post-transfusion circulation and clinical effectiveness of these cells.

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Functional abnormalities of human neutrophils collected by continuous flow filtration leukopheresis

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