Mobilization and Exocytosis of Specific (Secondary) Granules by Human Neutrophils During Adherence to Nylon Wool in Filtration Leukapheresis (FL)


We studied the interaction of human neutrophils with nylon wool in vitro and during filtration leukapheresis (FL) to better understand cellular events detrimental to neutrophils that occur during FL. When neutrophils purified from blood by gradient centrifugation and sedimentation were incubated with nylon wool in vitro, release of lysozyme from the cells occurred rapidly and to a degree that was related to the ratio of nylon wool bulk to the number of cells. Extracellular release of lysozyme was greater than that of β-glucuronidase, and this differential release of granule-associated enzymes reflected a preferential degranulation of the neutrophil-specific (or secondary) granules. Specific and azurophil (primary) granules were separated from neutrophils purified directly from blood and also from cells obtained by FL, and a partial degranulation of specific granules with loss of their associated lysozyme was found with FL neutrophils. Exposure of neutrophils to nylon wool in vitro was found to cause extracellular release of a granule product that activates serum complement and generates the chemotactic C5 fragment, C5a. Plasma from blood leaving nylon wool filters during FL was found to have increased lysozyme activity, reduced hemolytic complement, and increased chemotactic activity attributable in part to C5a production. In addition, neutrophils obtained by FL were found to have changes in surface charge similar to those caused by the exposure of neutrophils to chemotactic factors (such as C5a) or to degranulating stimuli. When plasma and leukocyte-enriched blood were diverted by a cell separator before passage through nylon wool filters during FL, complement activation and consumption were evident only in plasma from blood containing leukocytes, indicating that extracorporeal complement activation during FL is dependent in large part upon the interaction of leukocytes with nylon wool. Detectable quantities of histamine were not released within filters during FL, indicating that extracorporeal basophil degranulation with histamine secretion does not occur in FL to a significant degree as does neutrophil degranulation. These studies support the concept that neutrophil degranulation and secretion of granule contents are of central importance to the functional changes that occur in these cells during FL.

TRANSFUSION OF NEUTROPHILS from normal donors into severely neutropenic patients is now accepted as a useful form of hematologic support. Animal models have shown that neutrophil transfusion augments host defenses against bacterial infections during experimental marrow aplasia, and

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controlled trials of neutrophil transfusion in humans with leukemia have indicated that this procedure can increase the survival of certain patients when chemotherapy is complicated by sepsis. Nonetheless, technical problems continue to restrict the usefulness of neutrophil transfusion. There are important limitations to the numbers of neutrophils that can be collected from donors as well as significant problems in storing these cells prior to transfusion. Moreover, there is an incomplete understanding of functional changes that may occur in neutrophils during collection of these cells from donors.

Filtration leukapheresis (FL) has been an attractive method of collecting neutrophils for transfusion because of its simplicity and efficiency. However, cells obtained by this technique have various functional and morphologic abnormalities that are induced during adherence of cells to nylon wool filters. In addition, both donors during leukapheresis and recipients during transfusion may experience significant undesirable reactions when the FL technique is used.

The following studies were done in order to understand more fully the cellular events that occur during the interaction of neutrophils with nylon wool filters. In these studies we investigated adherence of neutrophils to nylon wool with respect to neutrophil degranulation, extracellular secretion of granule contents, and cell surface changes. These studies show that adherence-induced degranulation during FL affects the specific (secondary) neutrophil granules preferentially and thereby provides a mechanism for complement activation through the release of granule-derived mediators. These studies also indicate that extracorporeal complement activation during FL is in part a leukocyte-dependent phenomenon. Our findings suggest that neutrophil degranulation and secretion during adherence to nylon wool are of central importance in understanding FL donor reactions and the functional abnormalities of neutrophils collected by FL.

MATERIALS AND METHODS

**Neutrophil donors.** Healthy adults of both sexes (ages 25-40 yr), including regular leukapheresis donors for support of patients at NCI, provided neutrophils for study. Informed consent was obtained from all donors prior to leukapheresis.

**Collection of neutrophils.** For studies in vitro, essentially pure preparations of human neutrophils were separated from heparinized venous blood (10 units heparin/ml) by centrifugation through Hypaque-Ficoll gradients followed by dextran sedimentation and hypotonic lysis of residual erythrocytes. Neutrophils were also obtained by FL using nylon wool filters (Leukopak; Fenwal, Morton Grove, Ill.) as reported previously. Neutrophils were eluted from filters after 2.5 hr of FL (blood flow rates through filters 40-50 ml/min) using 300 ml ACD-plasma solution per filter and gentle tapping of the filters, as described previously. Like the Hypaque-Ficoll/dextran sedimented cells, FL neutrophils underwent hypotonic lysis of residual erythrocytes before further study.

**Studies of neutrophil granule secretion in vitro and during FL.** Neutrophils separated directly from blood were suspended in modified Hank's solution with or without 5% autologous plasma and then drawn into small syringes (3-ml disposable syringe, Monoject; Sherwood Medical, Deland, Fla.) that had been packed with varying amounts of nylon wool (Leukopak). After incubation at 37°C, the suspensions were expressed from the syringes, residual cells were removed by centrifugation (150 g for 10 min), and the extracellular media were assayed for the granule-associated enzymes, lysozyme, and β-glucuronidase as well as for the cytoplasmic enzyme lactic dehydrogenase (LDH). Lysozyme activity was determined by measuring the rate of lysis of *M. lysodeikticus* (Worthington Biochemical, Freehold, N.J.) at pH 6.2 according to a
Enzyme activity was expressed as μg/ml egg white lysozyme standard (Worthington). β-Glucuronidase was assayed by measuring the release of phenolphthalein from its β-glucuronate (Sigma Chemical, St. Louis, Mo.) after 4 hr incubation at pH 4.5. Activity was expressed as μg/phenolphthalein/10⁴ neutrophils/4 hr. LDH was assayed by measuring the consumption of DPNH during the conversion of pyruvate to lactate and was expressed in Wroblewski units. Enzymes were determined in whole cell or granule suspensions after disruption with 0.1% Triton X-100 (Rohm and Haas, Philadelphia, Pa.).

For certain studies the incubation media of neutrophils exposed to nylon wool in vitro without plasma was subsequently incubated with 10% pooled AB serum in veronal buffer and then assayed for neutrophil chemotactic activity, as described previously. In addition, at varying times during FL procedures blood was sampled immediately before entering and immediately after leaving the nylon wool filters. The plasma was then separated from these samples by centrifugation (250 g, 10 min) and evaluated for enzyme activities, chemotactic activity, total hemolytic complement, and the presence of free histamine. Complement measurements were kindly performed by Dr. Michael Frank’s laboratory using standard methods. Chemotactic activity of serum that had been exposed to the media of neutrophils incubated with nylon wool in vitro and of plasma samples obtained during FL was measured by a radioassay that employed ⁵¹Cr-labeled granulocytes and double-micropore-filter chemotactic chambers, as described previously. The chemotactic activity of test samples was expressed as corrected counts per minute in the lower filter (cor cm LF), reflecting the numbers of cells that migrated in response to a chemotactic stimulus. Plasma samples obtained during FL were evaluated for the presence of free histamine with the assistance of Dr. Michael Beaven using a radioenzymic procedure described previously.

Separation of neutrophil granules. The relative granule content of neutrophils obtained by FL was compared with that of neutrophils obtained directly from the blood of the same donors by Hypaque-Ficoll/dextran sedimentation techniques. Neutrophils were suspended in 0.34 M sucrose and were lysed in the presence of sodium heparin 1000 U/10⁴ neutrophils/ml by repeatedly aspirating and expressing the cell suspensions through a 20-gauge, 9-cm spinal needle. The extent of lysis was followed by phase microscopy and by cell counts until more than 85% of cells were lysed. Lysates were then passed sequentially through two 13-mm-diameter polycarbonate filters (Nuclepore; Neuroprobe, Bethesda, Md.), the first having a pore size of 5 μm and the second a pore size of 2μm. Granule-rich lysates were layered over continuous sucrose gradients and centrifuged at 95,000 g for 4 hr as has been described permitting the separation of three granule-rich bands. The gradients were then pumped out from the bottom at a constant rate (1 ml/min) and fractionated. Granule-rich fractions were identified by measuring the optical density of the fractions at 450 nm, and enzyme determinations in the fractions were carried out in the presence of 0.1% Triton X-100.

Measurement of neutrophil surface charge. The surface charge of neutrophils separated from the peripheral blood of donors and of neutrophils obtained from the same donors by FL was measured by an electrophoretic mobility assay reported previously. Neutrophils were washed twice in 0.14 M phosphate 5% sorbitol (pH 7.2) and suspended in the phosphate-sorbitol buffer at a concentration of 3.0 × 10⁶ cells/ml. Electrophoretic mobility of the cells was then determined by a cytopherometer (Zeiss, New York, N.Y.). All measurements were made in the frontal plane at 23°C, and for each experimental point 20 determinations were made on ten different cells, with the second measurement of each cell made after reversal of polarity. Neutrophil surface charge was calculated as described previously and expressed as μm/sec/V/cm.

RESULTS

Release of lysozyme from neutrophils exposed to nylon wool. When neutrophils (10⁷/ml) were incubated with 100 mg nylon wool in the presence of 5% autologous plasma, up to 25% of the total cellular lysozyme was released into the media during 60 min incubation (Fig. 1A), while significant release of β-glucuronidase and the cytoplasmic enzyme LDH did not occur under these conditions. Lysozyme release increased with the amount of nylon wool to which the cells were exposed (Fig. 1B). Detectable release of β-glucuronidase, in addi-
Fig. 1. Extracellular release of lysozyme and β-glucuronidase from human neutrophils when incubated with nylon wool. (A) Kinetics of release: neutrophils (10⁷/ml) incubated with 50 mg nylon wool/2 ml cell suspension for 0–60 min. (B) Dose response of enzyme release to varying amounts of nylon wool: neutrophils (10⁷/ml) incubated with 0–200 mg nylon wool/2 ml cell suspension for 30 min. Total lysozyme, 21.3–36.2 μg egg white lysozyme equivalents/10⁷ neutrophils; total β-glucuronidase, 7.8–18.8 μg phenolphthalein released/4 hr/10⁷ neutrophils; total LDH, 931 U/10⁷ neutrophils. Results from representative study (A) and from six replicate studies, means ± SEM (B).

Table 1. Extracellular Release of Lysozyme From Human Neutrophils Incubated With Nylon Wool

<table>
<thead>
<tr>
<th>Incubation Conditions*</th>
<th>Incubation Medium</th>
<th>Lysozyme Release† (Percent of Total Cellular Lysozyme)</th>
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<tbody>
<tr>
<td>No nylon wool</td>
<td>Buffer alone</td>
<td>5.2 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Buffer + plasma</td>
<td>5.7 ± 1.6</td>
</tr>
<tr>
<td>With nylon wool</td>
<td>Buffer alone</td>
<td>25.3 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>Buffer + plasma</td>
<td>22.5 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>Buffer + serum</td>
<td>22.8 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Buffer + human albumin (2 mg/ml)</td>
<td>21.3 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>Buffer + heated plasma (56°C, 30 min)</td>
<td>22.9 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Buffer + serum, activated by E. coli endotoxin§</td>
<td>27.8 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Buffer + C5a⁹</td>
<td>29.5 ± 3.8</td>
</tr>
</tbody>
</table>

*Neutrophils were incubated with or without nylon wool (50 mg/10⁷ neutrophils/ml) for 30 min at 37°C.
†Mean ± SEM, four determinations.
‡Autologous plasma or serum, 5%.
§Serum incubated with 300 μg/ml E. coli lipopolysaccharide at 37°C for 60 min, then at 56°C for 30 min.
⁹References 17 and 32.
with nylon wool in the absence of plasma, serum, or albumin resulted in an even greater release of enzyme (Table 1), some of which could be attributed to increased cell fragility and cell damage, since release of LDH was detected under these conditions. Similarly, release of lysozyme by neutrophils exposed to nylon wool in the presence of plasma or serum did not depend upon an intact complement system, since lysozyme release was similar when plasma or serum heated at 56°C was used in the incubation conditions (Table 1). However, as is also shown in Table 1, lysozyme release was enhanced slightly when neutrophils were exposed to nylon wool in the presence of serum activated by E. coli endotoxin or in the presence of partially purified chemotactic C5 fragment (C5a).17,22

Lysozyme release, observed when neutrophils are exposed to nylon wool in vitro, was also found to occur during FL. When blood samples were drawn from tubing leading to and returning from nylon wool filters during FL, lysozyme activity in the plasma leaving a filter was found to be significantly greater than that in plasma obtained at the same time from blood entering the filter (Fig. 2). Increased plasma lysozyme in blood passing through nylon wool filters was apparent by 15-30 min of FL and was maximal by 90-150 min, by which time filters are usually saturated with cells. Smaller but definite increments in β-glucuronidase were also found in the plasma obtained from blood that had passed through the filters, particularly during the latter portions of FL.

Specific (secondary) granule degranulation of neutrophils collected by FL. The finding of a differential release of granule enzymes, lysozyme, and β-glucuronidase during adherence of neutrophils to nylon wool suggested that the degranulation that occurs under these conditions affects different granule species unequally. It could be shown that nylon wool adherence, like other degranulating stimuli,20 causes a preferential release of neutrophil-specific or “secondary” granules. Azurophil (primary) and specific (secondary) granules were separated from neutrophils obtained by FL and also from neutrophils obtained concurrently from the same donor by Hypaque-Ficoll centrifugation and dextran sedimentation of peripheral blood. Granule separations achieved by continuous sucrose density gradient centrifugation are illustrated in Fig. 3. The granules of lightest density separated by these techniques (band C) have been shown to be small, pleomorphic, and histochemically peroxidase negative and thereby have
B

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Fig. 3. Preferential loss of specific granules from neutrophils obtained by FL (A). Separation of cytoplasmic granules from neutrophil lysates by centrifugation through continuous sucrose gradients (sucrose of specific gravities 1.28–1.32; lysate of 10⁶ neutrophils/gradient). Control cells, obtained directly from donor blood by Hypaque-Ficoll gradient centrifugation and sedimentation, compared with cells obtained from donor by FL. Gradients were suspended against black background and illuminated from above by single-point light source to equalize illumination of each gradient. Shadows cast by apparatus, from which gradients were suspended, darken outer margins of granule bands C and B. (B) Measurement of lysozyme and β-glucuronidase in sucrose gradient fractions obtained by pumping out gradients from bottom. Granule-rich fractions (A, B, C) located by turbidometric measurement of fractions. Enzyme units: lysozyme, 2.2 g egg white lysozyme equivalents/mI/unit; β-glucuronidase, 1.7 μg phenolphthalein released/4 hr/ml/unit.

been identified as specific granules. The denser granules of sedimentation bands A and B, which were larger and peroxidase positive, have been identified as azurophil granules. As is shown, a partial disappearance of the C band granules was apparent in granule preparations from FL neutrophils compared with preparations of control neutrophils from the same donor. When the gradients were pumped out from the bottom and fractionated, the granule bands could be located by measuring the turbidity of the fractions. As shown in Fig. 3B, the loss of specific granules (C) from FL neutrophils was found to be associated with a loss of the lysozyme normally recovered from these granules, without a similar, concomitant change in the recovery of β-glucuronidase from the azurophil granules (A and B).

Evidence for complement activation during FL by neutrophil-derived mediators. Compelling evidence has been presented that extracorporeal complement activation occurs during FL in a manner similar to that observed during hemodialysis, and it has been suggested that complement activation may explain in part the donor reactions that occur during FL. In agreement with these observations, we found that plasma from blood that had passed through nylon wool filters had slightly diminished levels of total hemolytic complement (CH50) compared with plasma obtained concurrently from blood about to enter the filters. In addition to complement consumption, it was also evident that biologically active products of complement activation were being generated in the plasma of blood during its passage through nylon wool filters. As shown in Table 2, the chemotactic activity of plasma from blood leaving a filter during FL
Table 2. Chemotactic Activity of Plasma in Blood Leaving a Nylon Wool Filter During FL

<table>
<thead>
<tr>
<th>Plasma Sample</th>
<th>Chemotactic Activity (Cor cpn IF*)</th>
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<tbody>
<tr>
<td>Control plasma†</td>
<td>579 ± 82</td>
</tr>
<tr>
<td>Plasma from blood leaving the nylon wool filter during leukapheresis at:</td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>804 ± 229</td>
</tr>
<tr>
<td>30 min</td>
<td>837 ± 112</td>
</tr>
<tr>
<td>60 min</td>
<td>879 ± 146</td>
</tr>
<tr>
<td>90 min</td>
<td>951 ± 211</td>
</tr>
<tr>
<td>120 min</td>
<td>860 ± 114</td>
</tr>
<tr>
<td>180 min</td>
<td>832 ± 178</td>
</tr>
</tbody>
</table>

*Corrected counts per minute in the lower filter; † mean ± SEM, four determinations.

Plasma of blood obtained from tubing leading to the filter at the beginning of FL; chemotactic activity of plasma in blood entering the filter at later times during FL was not significantly different.

was increased compared with that of plasma from blood entering the filter, and this increase in chemotactic activity occurred early during FL, as did the increase in plasma lysozyme in blood passing through a filter. It could be shown by Sephadex chromatography of the plasma that the increase in chemotactic activity was related in part to the appearance of a chemotactic molecule in the plasma (15,000-20,000 daltons) that was stable at 56°C for 30 min and was inactivated by antiserum to human C5, characteristics of (C5a).32

It has been proposed that the extracorporeal complement activation that occurs during FL is a result of a direct interaction between plasma and nylon wool.15,16 However, our studies indicated that complement activation during FL was in large part mediated by the neutrophils adherent to nylon wool. During certain FL procedures, blood was circulated through nylon wool filters in tandem with a cell separator (Celltrifuge; Aminco, Silver Spring, Md.) that diverted the blood into plasma, essentially free of leukocytes (Fig. 4, “plasma alone”) and into leukocyte-enriched blood (Fig. 4, “plasma + cells”), each of which was then passed through a nylon wool filter before return to the donor. An increase in plasma chemotactic activity and a concomitant decrease in hemolytic complement (CH50) at 90 min of FL were found only in plasma from blood that had passed through the filter in the “plasma + cells” line (Fig. 4).

![Fig. 4. Chemotactic activity and CH50 of plasma without leukocytes and of plasma from blood enriched with leukocytes after passage through nylon wool filters (leukopak) during FL. (A) Diagram of FL with filters arranged in tandem with cell separator. Sites at which blood or plasma was sampled in extracorporeal circulation indicated by "PRE" and "POST." (B) Chemotactic activity and CH50 of plasma in blood leaving filters or cell separator ("POST") expressed as percentages of those of plasma in blood entering the filters or cell separator ("PRE"). All "PRE" and "POST" blood samples obtained at 90 min of FL.](image-url)
Table 3. Extracellular Release of a Serum Activator From Neutrophils Incubated With Nylon Wool

<table>
<thead>
<tr>
<th>Test Stimulus</th>
<th>Chemotactic Activity (cor cpm LF)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum + buffer alone†</td>
<td>747 ± 54</td>
</tr>
<tr>
<td>Serum + buffer incubated with nylon wool (50 mg/ml) for 30 min at 37°C</td>
<td>753 ± 147</td>
</tr>
<tr>
<td>Serum + buffer incubated with neutrophils (10⁷/ml) for 30 min at 37°C</td>
<td>1019 ± 87</td>
</tr>
<tr>
<td>Serum + buffer incubated with neutrophils and nylon wool (50 mg/10⁷ neutrophils/ml) for 30 min at 37°C</td>
<td>1301 ± 68</td>
</tr>
</tbody>
</table>

*Corrected counts per minute in the lower filter;¹²⁶ mean ± SEM, four determinations.
†Buffer alone, buffer incubated with nylon wool, and buffer incubated with neutrophils with or without nylon wool lacked intrinsic chemotactic activity in the absence of serum.

We previously showed that neutrophils, during phagocytosis of latex particles and during exposure to ionophore A23187 (5 x 10⁻⁷ M), release into the incubation media a product that can activate serum complement in vitro.¹⁷,²⁴ We further showed that this complement-activating mediator is derived from the specific (secondary) granules.¹⁷ The incubation media of neutrophils exposed to nylon wool in vitro was also found to activate serum complement in vitro (Table 3), a finding that was not unexpected because adherence of neutrophils to nylon wool had been found to result in degranulation of the specific granules with extracellular release of their contents.

Absence of histamine release within nylon wool filters during FL. Although the recirculation of activated complement products appears to be the basis at least in part for reactions experienced by donors during FL,¹³,¹⁶,¹⁸ it was thought possible that degranulation of basophils in addition to that of neutrophils might occur in blood passing through filters. In particular, the occasional occurrence of flushing or itching in donors during FL suggested the possibility of extracorporeal histamine release. However, measurements of plasma histamine in blood that had passed through nylon wool filters failed to show extracellular release of histamine during FL. Histamine was undetectable.
(<2 ng/ml) in plasma from blood either before or after passage through filters at any time point during ten separate FL procedures with five different donors.

**Altered surface charge of neutrophils collected by FL.** Neutrophils collected by FL showed a decrease in negative surface charge when compared with neutrophils collected concurrently from the peripheral blood of the same donors by gradient centrifugation and sedimentation techniques (Fig. 5). This change in surface charge correlated with the extent of adherence of the cells to filters, as has been shown to be the case with functional abnormalities of FL neutrophils. Neutrophils that could be eluted from filters without tapping of filters showed smaller changes in surface charge than did the more adherent cells that were eluted with gentle or vigorous tapping. These changes in surface charge were in general greater than those that occur with neutrophils exposed to chemotactic factors such as C5a and resembled surface charge changes found in neutrophils exposed to degranulating stimuli.

**DISCUSSION**

FL is an efficient, simple, and relatively inexpensive technique of harvesting neutrophils from normal donors, and for these reasons FL has been widely used to obtain leukocytes for transfusion. Neutrophils collected by this technique, however, have markedly abnormal posttransfusion kinetics, impaired function in vitro, and altered morphology. Although clinical trials have indicated that transfusion of FL leukocytes benefits certain severely neutropenic leukemia patients who have become septic during ablative chemotherapy, the protective effects of these cells appear to be provided by a limited proportion of all FL cells transfused. It is evident that at most only 20–25% of neutrophils collected by FL circulate in recipients, have relatively normal function in vitro, or show an ability to reach sites of inflammation. Functional and morphologic changes in neutrophils collected by FL have been shown previously to be related to the adherence of these cells to nylon wool filters, and the current studies were undertaken to gain a more complete understanding of the cellular events that occur during the interaction of neutrophils with nylon wool.

Previous studies have shown that the adherence of neutrophils to nylon wool results in a partial degranulation of these cells and, like other degranulating stimuli, causes a burst of oxygen metabolism by the cells via the hexose monophosphate shunt. It has been suggested that the production of toxic oxygen metabolites such as superoxide anion at neutrophil surfaces is damaging to these cells and is the basis for the functional and morphologic abnormalities of FL neutrophils. Other studies have suggested, however, that the principal cause of abnormalities in FL neutrophils may be the activation of complement in plasma exposed to nylon wool and that activated complement products cause neutrophil degranulation and other changes in the cells as secondary events.

Our studies indicate that the degranulation of neutrophils adherent to nylon wool is not dependent on complement, and also that this degranulation affects the specific (secondary) neutrophil granules preferentially. Previous work by others provided evidence that adherence of neutrophils to nylon wool induces
degranulation of both peroxidase-negative (specific) and peroxidase-positive (azurophil) granules.\textsuperscript{9,10} While we also observed some degranulation of neutrophil azurophil granules during FL with extracellular release of their contents, degranulation of neutrophil-specific granules greatly predominated. Moreover, under certain conditions in vitro, adherence of neutrophils to nylon wool appeared to result in a selective degranulation of specific granules, as do certain other degranulating stimuli.\textsuperscript{29} In addition, our studies indicate that extracorporeal complement activation in the plasma of blood passing through nylon wool filters is in part dependent upon the interaction of leukocytes with the filters. When blood was separated into plasma and leukocyte-enriched blood, both of which were then passed simultaneously through nylon wool filters before return to the donor, it was found that only the plasma of the leukocyte-enriched blood showed evidence of complement activation. Others have also found that extracorporeal complement activation during FL appears to depend in part upon the presence of leukocytes in blood exposed to nylon wool,\textsuperscript{14} and it is evident that the secretion of specific granule products by neutrophils adherent to nylon wool may provide a mechanism for leukocyte-dependent complement activation. We showed previously that neutrophils during phagocytosis of latex particles, or during exposure to the degranulating stimulus ionophore A23187, secrete a product stored in the specific granules that activates serum complement in vitro, thereby generating the chemotactic C5 fragment C5a.\textsuperscript{17,24}

Extracorporeal complement activation provides a source of phlogistic mediators that may explain at least some of the adverse side effects of FL upon donors, which include transient neutropenia, hypotension, urticaria, and lower abdominal cramping in females.\textsuperscript{13-16,18} Although some of these transient side effects mimic acute hypersensitivity reactions, our studies indicate that histamine is not released extracellularly in blood as it passes through nylon wool filters.

Ruffling and involution of the plasma membrane of neutrophils collected by FL have been shown electromicroscopically\textsuperscript{9,10} and indicate cell surface changes that we found are also reflected by alterations in the surface charge of these cells. Altered surface charge, like the altered function in vitro of FL neutrophils,\textsuperscript{8} is directly related to how firmly the cells have adhered to nylon wool filters. The changes in neutrophil surface after adherence to nylon wool may reflect in part the interaction of these cells with activated complement products,\textsuperscript{29} or they may simply reflect plasma membrane changes that accompany degranulation and granule exocytosis.\textsuperscript{31}

In agreement with the studies of others,\textsuperscript{10,12} our findings support the conclusion that neutrophil degranulation during adherence to nylon wool is of central importance for an understanding of the changes that occur in neutrophils during FL. Our studies also suggest that extracorporeal complement activation in FL is in part, if not largely, a consequence of neutrophil degranulation and secretion of granule products, rather than the latter resulting from the former. Neutrophil degranulation has been associated with changes in the function of these cells in a variety of experimental systems,\textsuperscript{39-41} and these changes may be caused in part by the metabolic events that accompany degranulation, as has been suggested in the case of nylon wool adherence.\textsuperscript{12} Circulating neutrophils are relatively short-lived cells in vivo with little capacity for protein synthesis de novo. Both the migration of these cells into
tissues and subsequent degranulation in response to phagocytosis or other stimuli appear to be irreversible. We therefore believe that efforts to improve the functional quality of FL neutrophils are best directed toward technical modifications that prevent adherence-related changes to neutrophils rather than those that attempt reconstitution of the cells. Corticosteroids have been shown to inhibit neutrophil degranulation both in vitro and in vivo, and this effect, either directly or indirectly by inhibition of cell adhesiveness, may explain why corticosteroid pretreatment of FL donors, as done in certain FL programs, appears to improve the functional quality of cells collected by FL. Pretreatment of FL donors with other agents that inhibit neutrophil responses to degranulating stimuli, such as colchicine, also deserves evaluation as a means of preventing the functional impairment of FL neutrophils.

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Mobilization and exocytosis of specific (secondary) granules by human neutrophils during adherence to nylon wood in filtration leukapheresis (FL)

DG Wright, JC Kauffmann, GK Terpstra, RG Graw, AB Deisseroth and JI Gallin