Nylon-Fiber-Induced Neutrophil Fragmentation

By John C. Klock, Janet Boyles, Dorothy F. Bainton, and Thomas P. Stossel

We have investigated the effects of mechanical elution of neutrophils from nylon-wool fiber (NWF) using the scanning electron microscope and biochemical analysis of elution fractions. We have determined that mechanical removal of neutrophils from nylon-wool fiber disrupts neutrophils adherent to nylon-wool fiber and augments release of granules, release of peripheral cytoplasmic fragments, and release of lactic dehydrogenase, a soluble cytoplasmic enzyme. Mechanical shearing of the adherent cell, and not adherence per se, causes the fragmentation. The extent of fragmentation is proportional to the NWF surface area available to neutrophils and is maximal at the temperature for optimal adherence and spreading. Agents that decrease cell spreading (n-ethylmaleimide and cold) diminish fragmentation. Cytochalasin B, an agent that destabilizes the neutrophil cortex, increases fragmentation. Fragmentation may be an important contributing cause of the abnormal morphology, function, and in vivo survival of nylon-wool-fiber procured human neutrophils. The prevention of fragmentation would appear to be necessary to insure the procurement of optimally functioning cells. Elution of NWF-adherent neutrophils in the cold might be a practical way to diminish neutrophil damage during clinical filtration leukapheresis.

NYLON-FIBER FILTRATION is a popular method for obtaining human neutrophils for clinical transfusion.1,4 This method is efficient for collecting large numbers of neutrophils because these blood cells selectively adhere to nylon fiber. Knowledge of the interaction of neutrophils with nylon-wool fiber (NWF) is important both for understanding the filtration leukapheresis system and for analyzing the functions that the neutrophil performs: cellular adhesion, spreading, phagocytosis, and degranulation.

Adherence of neutrophils to NWF is associated with cell spreading and changes in cellular metabolism. In previous studies we have established that the interaction of phagocytes with NWF results in (1) exocytosis of granules,5 (2) a defect in bacterial function,6 (3) activation of oxygen metabolism in the neutrophil that damages its membrane and makes it subject to ingestion by other phagocytes,6 and (4) rearrangement of cytoplasmic contractile proteins.7,8 The focus of the present studies, in which we continue to explore the interaction of phagocytes with NWF, is on neutrophil fragmentation during removal from NWF.

Adhesion of human neutrophils to NWF is so avid that mechanical removal is usually necessary to elute the cells. Since only a small amount of shear force is required to rupture the human neutrophil,9 one would predict that the mechanical removal of adherent neutrophils from NWF would cause many of them to fragment. Ts'ao and Ruder10 have shown that mechanical injury during elution is
probably the cause of the abnormal morphology of neutrophils obtained by filtration leukapheresis. These studies and our previous work with phagocytes and NWF prompted our investigation of the effects of this mechanical elution of human neutrophils from NWF. We first examined NWF after it had been used in clinical filtration leukapheresis; we also looked for cellular debris from neutrophils in the filtration leukapheresis eluate. We then established an in vitro model of the clinical filtration system, used it to reproduce the phenomena seen in filtration leukapheresis, and examined the variables that influence neutrophils to fragment when they are removed from NWF. These studies document that mechanical removal of neutrophils from NWF causes (1) further degranulation, (2) release of peripheral cytoplasmic fragments, and (3) release of soluble cytoplasmic contents. The studies also provide a basis for effective methods to minimize such effects on neutrophils in vitro and during clinical filtration leukapheresis.

MATERIALS AND METHODS

Clinical Filtration Leukapheresis

The Clinical Transfusion Service of the Moffitt Hospital Blood Bank was the source of elution supernatants and NWF packs used in studies of clinical filtration leukapheresis. Donors were family members of patients receiving therapeutic neutrophil transfusions in the hospital. Cells were obtained using the Buchholz modification of the Djerassi technique as previously described. In this procedure, the donor anticoagulant was heparin (6000 U/hr), and no corticosteroids were given to donors. Leukapheresis was performed for 2 hr at 40 ml/min (vein-to-vein) using Fenwal Leukopak filtration leukopheresis sets (Fenwal Laboratories, Morton Grove, Ill.). Cells were eluted from the fibers at 100 ml/min at room temperature with a solution consisting of: 1500 ml physiologic saline, 250 ml ACD-A, and 250 ml normal plasma (pH 6.5). The fiber packs were vigorously tapped against each other during elution. Neutrophils were concentrated by 1000 g × min centrifugation and were removed for transfusion. Cellular debris for transmission electron microscopy was pelleted by a 1000 g/min centrifugation of the remaining elution supernatant. The fiber packs were fixed for scanning electron microscopy by immediately perfusing them with 500 ml of 1.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) containing 1% sucrose.

Isolation of Neutrophils for In Vitro Studies

Whole blood was removed from normal volunteers by venipuncture and processed as previously described. The final neutrophil suspension (over 95% neutrophils) was diluted to 10⁶ cells/ml with 0.15 M NaCl solution containing 1% twice crystallized human serum albumin (Sigma Chemical Co. St. Louis, Mo.). The suspension was kept at 4°C until it was used in studies. Experiments were performed within 2 hr of isolation of cells.

Preparation of NWF Neutrophils

NWF-adherent neutrophils were prepared by incubating the final neutrophil suspension in sterile 10-ml plastic syringe barrels containing loosely packed NWF (3 denier, scrubbed NWF, Fenwal Laboratories, Deerfield, Ill.). A quantity of 3 × 10⁶ cells (3-ml cell suspension) were allowed to settle on 300 mg of NWF for 0, 5, 15, 30, and 60 min. In order to study variables influencing neutrophil fragmentation, we used n-ethylmaleimide (1 mM), colchicine (0.05 mM), tetracaine (1 mM), and cytochalasin B (10 ng/ml)—drugs with different effects on neutrophil function. These drugs were added either at the start of incubations with NWF or during the last 15 min. To remove neutrophils from the NWF, the syringe was perfused with 20 vol suspending solution after gently tapping the syringe barrel against the palm of the hand. In some experiments, shear forces were applied to neutrophils in a different fashion by placing 10 ml of neutrophil suspension in a 30 × 100 mm polypropylene tube and inserting the tube into a vortex stirring apparatus for 60 sec. In some experiments, NWF-adherent neutrophils were brought to different temperatures during incubation with NWF or at the end of the incubation. This was done by placing the syringe containing NWF in a water bath maintained at 4°C, 10°C,
Centrifugation at $10^4 \text{ g} \times \text{min}$ sedimented whole cells. The supernatants were removed and centrifuged at $10^5 \text{ g} / \text{min}$ to sediment granules and membrane fragments. The final supernatants of this second centrifugation, containing nonsedimentable soluble protein, were dialyzed against distilled water overnight and lyophilized. Each of these three fractions was analyzed for protein content, enzyme activity, and morphology.

**Biochemical Studies**

Protein determination was done by the method of Lowry et al., lactic dehydrogenase activity was determined by the technique of Kornberg, and $\beta$-glucuronidase activity was determined according to the method of Gianetto and deDuve. Sigma Chemical Co. (St. Louis, Mo.) was the source of $n$-ethylmaleimide, colchicine, and tetracaine; these agents were dissolved in suspending medium for use in experiments. Cytochalasin B, obtained from Aldrich Chemical Co., Milwaukee, Wisc., was dissolved in dimethylsulfoxide and diluted with suspending medium to a final concentration of 0.5%. All enzyme assays were done with and without Triton X-100 at 0.1%.
Transmission Electron Microscopy

Specimens for transmission electron microscopy were prepared as described previously. The pellets from the clinical filtration leukapheresis supernatant and from in vitro studies were fixed for 2 hr in nitrocellulose tubes by adding 1.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) containing 1% sucrose and postfixed for 1 hr in 1% OsO₄ in 0.1 M phosphate buffer (pH 7.4) containing 4% sucrose. Samples were en bloc stained with 0.5% uranyl acetate in Michaelis buffer (pH 6.0) containing 3% sucrose and dehydrated. The nitrocellulose tube was dissolved in propylene oxide, and the pellet was embedded in Epon for sectioning.

Scanning Electron Microscopic Studies

Specimens for scanning electron microscopic studies were prepared as previously described. NWF from clinical filtration leukapheresis and in vitro studies were fixed by perfusing the systems with the 1.5% glutaraldehyde fixative as described above. The NWF was then cut into smaller pieces and processed as above through 100% ethanol, critical-point-dried, and coated immediately with 100-150 Å of sputtered gold or gold-palladium. Micrographs were taken at intermediate magnifications to avoid collapse of samples under high current fluxes on a Coates and Welter Quick Scan Model 50 special.

RESULTS

Elution During Clinical Filtration Leukapheresis Caused Neutrophil Fragmentation

Figure 1 shows low power transmission electron micrographs of material recovered from the cell-free supernatant of a clinical filtration leukapheresis eluting solution. The material consists of large and small membrane fragments and a few neutrophil granules. Approximately 1 g of this material was recovered from the cell-free supernatant of a 2-hr clinical filtration leukapheresis in which \(2 \times 10^{10}\) cells were collected. Examination of the NWF by scanning electron microscopy after cells were eluted (Fig. 2) showed that material consisting primarily of damaged neutrophils (Fig. 2A) and large fragments of adherent membrane material (Fig. 2B) remained on the NWF. In Fig. 2C, a neutrophil is shown in the process of being torn away from the NWF. The upper portion of the cell membrane is broken, and membrane fragments and granules are released; the basal portion of the cell's plasma membrane remains on the NWF. This collection of material on the NWF represents approximately 5% of the total neutrophil protein placed on the NWF (data not shown).

Neutrophil Fragmentation Can be Examined In Vitro

To examine the comparability of the clinical procedure and the in vitro system, the \(10^4\) g \times min pellet and the \(10^5\) g \times min pellet in the in vitro system were examined by transmission electron microscopy and compared to the \(10^4\) and \(10^5\) g \times min pellets from the clinical filtration procedure. Results showed that these fractions contained whole neutrophils and membranes and granules, respectively; thus, the in vitro system was felt to be a reasonable reproduction of the clinical NWF procurement procedure (data not shown).

Table 1 shows that after a 1-hr incubation and elution, 4%-5% of the neutrophil protein and 2%-3% of the total neutrophil \(\beta\)-glucuronidase activity was recovered in the membrane-fragment–granule fraction. Twenty percent of the neutrophil protein and 16% of the total neutrophil lactate dehydrogenase (LDH) activity was recovered in the high-speed supernatant. The large amount of \(\beta\)-glucuronidase activity (31%) in the soluble protein fraction represents active degranulation during
Fig. 2. Scanning electron micrograph of nylon-wool fiber from a clinical filtration leukapheresis after elution has been performed. (A) One normal neutrophil (N) stuck to the NWF and a group of damaged neutrophils (D). These cells are considered to be damaged because their shape is abnormal, their surface projections are missing, and in many instances their plasma membranes are broken. Note also the membrane fragment (arrow) from a phagocytic cell that has remained on the NWF after the cell was eluted. (B) In more detail, many such membrane fragments (arrows), and (C) a partially adherent ruptured neutrophil. Note the adherent membrane (m) and the many free granules (g). (A, ×4200; B, ×5000; C, ×3600)
adherence to the NWF. Therefore, it can be concluded that the high-speed supernatant protein represented mostly “cytosol” protein and that the granules seen in the granule–membrane fraction were released largely intact during elution. These results indicate that when the neutrophil was tapped to release it from the NWF, membrane material, cytosol, and intact granules were simultaneously released during adherence, elution, or both.

**Increased Cell Spreading Increased Fragmentation**

Figure 3 shows that an increase in the amount of NWF available to the same number of neutrophils was associated with an increase in fragmentation. As the ratio of NWF to cells increased, the amount of protein recoverable in the granule–membrane fraction increased markedly, suggesting that as more cells were able to spread on NWF, fragmentation increased. Note that some fragmentation occurred during tapping at 37°C in the absence of NWF. The time of incubation of cells with the NWF directly influenced fragmentation, as shown by the appearance of protein and granule enzyme in the membrane–granule fraction and by the appearance of cytosolic protein and LDH in the 10^5 g/min supernatant (Fig. 4). The effect was not pronounced until 15 min, when most of the cells had adhered to the NWF.

**Shear Forces Were the Predominant Cause of Neutrophil Fragmentation**

To assess the effect of mechanical shear on neutrophil fragmentation, we attempted to remove neutrophils from NWF without tapping and to reproduce the

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**Table 1. Percent Total Cell Protein, Granule Enzyme, and Cytosolic Enzyme in Different Eluate Fractions After Removing Neutrophils From NWF**

<table>
<thead>
<tr>
<th>Eluate Fraction</th>
<th>Percent Total Protein</th>
<th>β-glucuronidase (%)</th>
<th>LDH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole neutrophils (After 1 hr on NWF)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10^4 g x min pellet (eluted cells)</td>
<td>79 ± 6†</td>
<td>61 ± 8</td>
<td>81 ± 8</td>
</tr>
<tr>
<td>10^5 g x min pellet (membrane fragments and granules)</td>
<td>4.5 ± 1</td>
<td>2.5 ± .5</td>
<td>2 ± .5</td>
</tr>
<tr>
<td>10^5 g x min supernatant (soluble protein)</td>
<td>21 ± 4</td>
<td>31 ± 3</td>
<td>16 ± 3</td>
</tr>
</tbody>
</table>

*Following NWF after 1 hr.*
†Mean ± standard deviation from five experiments.

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**Fig. 3. Effect of NWF/neutrophil ratio on fragmentation of neutrophils that have been allowed to stick to NWF for 1 hr. The amount of protein in the 10^5 g x min pellet containing membranes and granules increases as more nylon-fiber surface is made available for neutrophil adhesion. Note that some fragmentation occurs when neutrophils are tapped with no NWF.
shear on neutrophils without using nylon fiber. The results of these experiments are shown in Table 2. By simply washing the nylon fiber with eluting solution we were unable to remove more than 6% of the cells; we observed no neutrophil fragmentation as evidenced by negligible protein in the $10^9$ g x min pellets and supernatants. Figure 5 shows the appearance of normal human neutrophils after adherence for 15 min on NWF. The cells are attached to the NWF, and the unadherent surface membrane shows characteristic redundant membrane pleats (ruffles). After a single vigorous tap most cells showed loss of ruffles, some showed considerable peripheral cytoplasmic membrane blebbing, and the NWF showed traces of membrane fragments and cytoplasmic granules (Fig. 6). When we subjected the neutrophils to vortex shear in the absence of NWF at 37°C (Table 2), the extent of neutrophil fragmentation we observed was almost equivalent to that occurring after NWF incubation and tapping.

Table 2. Effect of Shear Force on Appearance of Enzymes and Protein in Different Eluate Fractions After Neutrophil Removal From NWF

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>$10^9$ g x min Pellet (Eluted Cells)</th>
<th>$10^9$ g x min Pellet (Membranes and Granules)</th>
<th>$10^9$ g x min Supernatant (Soluble Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr 37°C elution with tapping</td>
<td>79 ± 6*</td>
<td>4.5 ± 1</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>1 hr 37°C elution without tapping</td>
<td>6.4 ± 2</td>
<td>1.1 ± 0.3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Vortex 1 min 37°C</td>
<td>88 ± 7</td>
<td>3.7 ± 2</td>
<td>17 ± 3</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation from five experiments.
Fig. 5. Scanning electron micrograph of normal human neutrophils after 15 min of incubation with NWF at 37°C. The cells are attached to the NWF and adhere to each other without extensive spreading, maintaining their characteristic surface membrane "ruffles" (arrows). (×2700)

Fig. 6. The same preparation as Fig. 5 except that this specimen has been given a single forceful "tap" before fixation. Cells have been removed from the NWF leaving membrane behind (arrow) as well as free granules (g). The small cell is a platelet. Other cells have withdrawn leaving some membrane on the NWF (m) (×3200). Inset: The neutrophils have lost much of their surface ruffles, possibly as a result of blebbing (B), as seen in this cell (×3800).
Table 3. Effect of Temperature and Pharmacologic Agents on the Appearance of Enzymes and Protein in Different Eluate Fractions After Neutrophil Removal From NWF

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Percent Total Protein $10^8,g\times mm$</th>
<th>Pellet (Eluted Cells)</th>
<th>Pellet (Membranes and Granules)</th>
<th>Supernatant (Soluble Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells on NWF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hr 37°C</td>
<td>79 ± 6†</td>
<td>4.5 ± 1</td>
<td>21 ± 4</td>
<td></td>
</tr>
<tr>
<td>Cells on NWF</td>
<td>95 ± 2</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td></td>
</tr>
<tr>
<td>1 hr 37°C (NEM†)</td>
<td>90 ± 4</td>
<td>1.8 ± 3</td>
<td>4 ± 1</td>
<td></td>
</tr>
<tr>
<td>Cells on NWF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hr 37°C (NEM last 15 min)</td>
<td>82 ± 4</td>
<td>4 ± 1</td>
<td>18 ± 3</td>
<td></td>
</tr>
<tr>
<td>Cells on NWF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hr 37°C (colchicine last 15 min)</td>
<td>80 ± 6</td>
<td>4.7 ± 2</td>
<td>22 ± 3</td>
<td></td>
</tr>
<tr>
<td>Cells on NWF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hr 37°C (cytochalasin B last 15 min)</td>
<td>63 ± 6</td>
<td>6.5 ± 3</td>
<td>25 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

*All cells eluted at 37°C with tapping.
†Mean ± standard deviation for five experiments.
†n-Ethylmaleimide.

Effects of Various Drugs and Temperatures on Neutrophil Fragmentation

Table 3 shows that incubation of neutrophils in the cold or in the presence of n-ethylmaleimide (which decreases adherence and prevents spreading of neutrophils) prevented cell fragmentation during elution. The addition of other drugs that prevent neutrophil adherence, such as 2-deoxyglucose and colchicine, also prevented fragmentation (data not shown). Addition of colchicine after the cells were adherent caused the cells to assume a round appearance but did not prevent

Fig. 7. Scanning electron micrograph of human neutrophils after 60 min on NWF. Cytochalasin B was added during the last 15 min. The cells have rounded up and lost their surface ruffles but show many large and small surface blebs (B) induced by the drug. These cells have not been tapped prior to fixation (×3500).
fragmentation. Similarly, the addition of tetracaine and n-ethylmaleimide to adherent cells caused some of the cells to become round but also did not prevent fragmentation. When adherent neutrophils were treated with these drugs, they showed spontaneous blebbing (data not shown), which probably offset any protection the rounding up of the cell might have caused. Cytochalasin B decreased the recovery of intact cells from NWF and augmented fragmentation, and examination of these cells demonstrated that this treatment of the adherent neutrophil was associated with extensive blebbing of spread cells (Fig. 7). The difference between recovery of cells with and without cytochalasin B was significant ($p < 0.005$); the difference between the amount of protein in the membrane-granule fraction with and without cytochalasin B was also significant ($p < 0.05$), but the difference in the amount of protein in the soluble supernatant with and without cytochalasin B was not significant. This was interpreted as showing that augmentation was mostly due to increased blebbing and the release of intact blebs rather than an increase in the degree of cell rupture. Experiments done using the solvent for cytochalasin B (0.5% DMSO in neutrophil suspending medium) were no different from controls without DMSO.

Because cooling of neutrophils is associated with rounding of the cells, we investigated the effects of cooling on already spread neutrophils. Figure 8 shows the results of experiments in which neutrophils were stuck to NWF for 30 min and then cooled to various temperatures before elution. Temperatures between 4°C and 10°C protected the neutrophils from fragmentation by mechanical elution. The cold eluting temperature was not associated with a decrease in cell yield or in the amount of protein in the 10$^4$ g x min cell pellet (data not shown); thus, by simply reducing the eluting temperature, we were able to obtain a four-fold reduction in fragmentation. Cooling of neutrophils after adhesion to NWF (Fig. 9) resulted in the disappearance of “ruffles” and a substantial decrease in the amount of cell spreading and retraction (cf., Fig. 5). Furthermore, the 4°C temperature also completely protected neutrophils from fragmentation during the higher shear forces used in the vortexing procedure (data not shown).

**DISCUSSION**

These studies have shown that mechanical stress used to elute neutrophils adherent to NWF causes neutrophil fragmentation defined by the loss of a substantial quantity of cell membrane and granules in the eluate and on the NWF.
and by the loss of up to 20% of the cell cytoplasmic protein. Mechanical shearing causes disruption of adherent, spread cells. We do not know if the 20% protein loss into the cell-free supernatant indicates partial disruption of all cells or whether it represents more complete disruption of a minority of cells on the fiber. It probably represents both. Since many of the neutrophils in the $10^4 g \times$ min cell pellet appear relatively normal, and since those cells in closest apposition to the NWF would be expected to be more susceptible to shear, we propose that some of the neutrophils closely adherent to the NWF undergo complete disruption and are never recovered. However, most of the membrane and granule material is derived from other cells disrupted less violently.

In our experiments, factors that optimize adherence and spreading optimized neutrophil fragmentation. These factors were high NWF-to-cell ratio and physiologic temperatures. Drugs that inhibit adherence and spreading of neutrophils (colchicine, tetracaine, and n-ethylmaleimide) reduced fragmentation when added to cells during adherence and spreading on NWF. Physical factors that inhibit spreading also decreased neutrophil fragmentation; cooling reduced the fragmentation of adherent neutrophils and micrographs showed that the spreading of these cells was decreased. Thus, the ability of cells to attach to and spread on NWF were
important prerequisites to neutrophil fragmentation. The ability of the vortexing procedure to rupture nonadherent neutrophils might be interpreted as evidence against this hypothesis, however, the shear forces generated by this procedure are much higher than those during tapping of NWF, and the situations are not comparable.

The extent of spreading could promote fragmentation in several ways. A large area of adherent membrane could act as a resistance to elution of the whole cell, rendering rupture the only way the cell could respond to shear. Additionally, extensive spreading could attenuate peripheral cytoplasm, decreasing the strength of the cell cortex at the shear site. Since cortical cytoplasm moves from the cell body to the pseudopods during cell spreading, the cortical support of the central cell body would also be weakened. The results of the experiments with cytochalasin B support the idea that weakening of cortical cytoplasm promotes fragmentation, as cytochalasin B dissolves the cortical gel structure composed of actin filaments crosslinked by actin-binding protein.

To cause the cell to become round and retract pseudopodia would seem to be the best way to reduce the degree of fragmentation; however, this must not be done at the expense of inducing cytoskeletal, membrane, or cytoplasmic disruption. None of the pharmacologic agents that we used could do this. Colchicine does appear to improve in vitro function of NWF-adherent neutrophils; however, it probably does so without having an effect on neutrophil fragmentation. Tetracaine, if used in higher concentrations, can cause the release of neutrophils from NWF; however, the necessary high concentrations may have toxic effects on the neutrophils. Cold, on the other hand, will reduce the degree of neutrophil spreading and cause the cells to become round without any of the consequences of treatment with pharmacologic agents. Human neutrophils are known to undergo shape changes in the cold, and evidence exists that cold temperatures can affect human granulocyte cytoplasmic viscosity by its action on contractile proteins. Cooling of NWF-adherent neutrophils was associated with diminished fragmentation, peripheral pseudopod retraction, and rounding of cells (Fig. 9). These effects were accomplished without attendant detachment of neutrophils. Since human neutrophil shape, rheology, movement, and spreading are dependent on the state of the contractile protein apparatus (which is temperature dependent), it is likely that a change in the state of these proteins induced by cold is an important factor in the prevention of neutrophil fragmentation.

Clinical Implications

Neutrophil fragmentation occurs during elution of cells from NWF during clinical filtration leukapheresis. Most cell fragments are removed by the initial centrifugation to concentrate neutrophils for clinical transfusion; therefore, most of the recovered cells are relatively normal in appearance and function; however, many of these cells are vacuolated and have lost membrane and cytoplasm as a result of shear. Since no attempts have been made to standardize the tapping procedure during clinical filtration leukapheresis, the differences in tapping techniques and elution solution flow rates might be one cause for the disparate results seen in the function of cells eluted from NWF. Possible adverse effects of this procurement procedure are impairment of neutrophil function and cell damage,
resulting in clearance of cells from the recipient’s circulation by the macrophage system. The role of fragmentation in contributing to these consequences is unclear. Mechanical shear alone does not cause neutrophils to be recognized by other phagocytes, and agents that affect immunologic recognition of NWF-adherent neutrophils do not prevent neutrophil fragmentation. Nevertheless, it seems reasonable to assume that infusion of neutrophil debris and cytosol protein into recipients should be avoided.

We continue to believe that the major injury to neutrophils adherent to NWF results from the activation of their oxygen radical generating system and from degranulation. Most of the events (adherence, oxygen radical generation, and degranulation) that predispose the neutrophil to damage during filtration leukapheresis occur during the first few minutes of contact with NWF. The cells, then, are damaged very early in the procurement process, and thus, shortening the time of contact and using cold eluting temperatures will not likely decrease the damage resulting from these events. The events described here and in previous publications are related to the abnormal morphology, function, and in vivo survival of NWF-procured granulocytes. Attempts to change the procurement protocol by using agents that affect degranulation and oxidative metabolism, or by using oxygen radical scavengers such as mannitol, or by using elution methods that are less harmful to neutrophils (e.g., cationic anesthetics or cold) would appear to be necessary to insure the procurement of optimally functioning granulocytes.

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