Blood Kinetics and In Vivo Chemotaxis of Transfused Neutrophils: Effect of Collection Method, Donor Corticosteroid Treatment, and Short-Term Storage

By Thomas H. Price and David C. Dale

To evaluate effect of collection technique and short-term storage on in vivo cell function, neutrophils were collected from 53 normal subjects by phlebotomy (PB), intermittent flow centrifugation (IFC), or filtration leukopheresis (FL), stored 0 or 1 day, labeled with 32P-diisopropylfluorophosphate, rein infused into the donor, and blood kinetics and/or skin chamber accumulation of labeled cells measured. The blood kinetics of unstored PB and IFC cells were similar; the kinetics of unstored FL cells were markedly abnormal. The percent of infused neutrophils localizing to the skin chamber was 0.1, 0.06, and 0.006 for unstored PB, IFC, and FL cells, respectively. One-day storage substantially decreased chamber accumulation of infused neutrophils. Donor steroid pretreatment had no effect on chamber results. Thus, in vivo chemotactic ability of IFC neutrophils is slightly impaired, whereas that of FL cells is severely impaired. One-day storage of either cell concentrate causes further cell damage.

Neutrophil transfusions are being used with increasing frequency in the support of neutropenic patients. The efficacy of this form of therapy depends on the ability of the transfused cells to function normally in vivo. Factors that have been considered important in influencing the functional capabilities of the transfused cells include the method of collection of the cells from the donor, the time and condition of in vitro cell storage prior to transfusion, and premedication of the donor with corticosteroids.

Neutrophils are generally collected from donors by continuous flow centrifugation, intermittent flow centrifugation, or filtration leukopheresis. Although no differences have been detected in the clinical efficacy of these cell preparations, cells collected by filtration leukopheresis have been shown to be abnormal by several investigators on the basis of in vitro function, blood kinetics, and in vivo chemotaxis in animals. Studies by Wright et al. have suggested that these abnormalities in filtered cells may be minimized or perhaps prevented by premedication of the donor with corticosteroids.

Corticosteroids are frequently given to leukopheresis donors to increase the number of neutrophils collected. Although cells collected after such donor stimulation have been shown to function normally in vitro, their ability to function in vivo has not been assessed.

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The effect of short-term storage on neutrophil viability has been the subject of numerous studies, most of which have suggested that it may be possible to store neutrophils for 1–2 days with only moderate loss of functional capabilities.

To examine these issues we have measured the effect of collection method, donor corticosteroid stimulation, and 24-hr storage on the ability of autologous neutrophils to circulate and to accumulate at an inflammatory site in the recipient.

MATERIALS AND METHODS

Neutrophils were collected from normal subjects by: (1) phlebotomy, (2) filtration leukopheresis, or (3) intermittent flow centrifugation. The cells were stored for 0 or 1 day at 4°C, labeled with 32P-diisopropylfluorophosphate (DF32P), and reinfused into the donor. Blood kinetics and skin chamber accumulation of the transfused labeled cells were determined over the subsequent 24 hr.

Subjects

Thirty-three male and 20 female normal volunteers were studied after giving informed consent. The study was approved by the University of Washington Human Subjects Review Committee. All subjects had normal neutrophils (greater than 1800/cu mm) and hematocrit greater than 35 prior to study and had no history of hematologic disease or recent infection. Each volunteer was studied only once.

Neutrophil Collection

Phlebotomy (PB). Five-hundred milliliters of whole blood was drawn into plastic blood bags (Fenwal PL 130, Travenol, Deerfield, Ill.) containing ACD-A.

Intermittent flow centrifugation (IFC). Collections were performed with the Haemonetics Model 30 Cell Separator (Haemonetics Corp., Natick, Mass.) using 6% hydroxyethyl starch as the red cell sedimenting agent, as previously described.6

Filtration leukopheresis (FL). Neutrophils were collected over a 3-hr period using the standard Fenwal leukopheresis apparatus, as previously described.6 The filters were eluted with 1000 ml of a mixture of 20% autologous plasma, 15% ACD-A, and saline while tapping the filters. Cells were concentrated to a final volume of approximately 300 ml by centrifugation at 400 g for 15 min.

To determine the effect of donor corticosteroid stimulation on the viability of the collected neutrophils, one group of IFC and FL donors was given 60 mg prednisone orally 4 hr prior to the beginning of the collection procedure. Since these studies were autologous, the measurements of in vivo cell function were made in recipients who had been given corticosteroids 7–8 hr previously. As controls for these studies, the blood kinetics and skin chamber accumulation of cells collected by phlebotomy were determined in 5 subjects who had been given 60 mg prednisone 4–7 hr before the phlebotomy, that is, 5–8 hr prior to the infusion of the labeled cells.

Storage

In some experiments the cell preparations were stored, as obtained, in plastic bags at 4°C, without agitation, for 1 day.

Labeling

Neutrophils were labeled in vitro with 50–100 μCi of DF32P.18 The isotope was obtained from the manufacturer (Amersham, Arlington Heights, Ill.) as a radiochemical and was placed in propylene glycol and sterilized by the Nuclear Pharmacy at the University of Washington. After removing an aliquot for determination of neutrophil concentration and specific activity, the cells were reinfused into the donor. Reinfusion time was 5–10 min for cells collected by phlebotomy or IFC. FL cells were administered over approximately 30 min because of the previously demonstrated high incidence of transfusion reactions with these cells.6

Blood Kinetic Measurements

Twenty-milliliter blood samples were obtained at 10 min and at 1, 2, 3, 4, 6, 8, 11, and 24 hr after infusion of the labeled cells. Neutrophils were isolated from each sample by Hypaque-Ficoll sedimentation, and the contaminating red cells were removed by dextran sedimentation and NH4Cl lysis, as
previously described. Neutrophil specific activity was determined by measuring the radioactivity present in a known number of neutrophils.

**Measurement of In Vivo Chemotaxis**

The accumulation of neutrophils at an inflammatory site was measured by the method of Perillie and Finch. Immediately prior to the infusion of the labeled cells, a 1.5 x 1.5 cm abrasion was created on the volar forearm of the subject by scraping off the top layer of the skin with a knife blade. A sterile glass chamber (approximate volume, 9 ml) was placed over the abrasion and fastened in place with tape and rubber bands. The chamber was filled with a mixture of 20% autologous serum in saline to which streptokinase-streptodornase (Varidase, Lederle Laboratories, Pearl River, N.Y.), 100 U/ml, had been added. Six hours after the infusion of the labeled neutrophils, the chamber was emptied, flushed twice with sterile normal saline, and refilled with fresh serum-saline-Varidase mixture. Twenty-four hours after infusion, the chamber was again emptied, flushed twice with saline, and removed. White blood cell and differential counts were performed on all samples obtained from emptying and flushing the chamber. Samples for each time point were combined, the cells washed 3–4 times with 10 ml NH₄Cl, and neutrophil specific activity determined as above.

To evaluate the effect of the timing of the abrasion on the chamber neutrophil accumulation of FL cells, 3 subjects were studied in whom the skin abrasions were created 5–6 hr prior to infusion of unstored labeled cells. In addition, the blood kinetics of unstored neutrophils collected by phlebotomy were studied in 5 subjects without skin abrasions.

**Calculations**

For blood kinetic measurements, neutrophil radioactivity per milliliter blood was calculated for each time point. Neutrophil recovery (percent of injected neutrophils circulating at a given time) was plotted on semilogarithmic paper. The resulting curves were generally linear or near linear over the first 6–10 hr but tended to plateau thereafter. Blood neutrophil half-time was determined from the slope of the line, drawn by the method of least squares, through the most linear portion of the curve. Extrapolated recovery was defined as the recovery obtained on extrapolation of this line to zero time. Initial recovery was defined as the recovery at the 10-min sample. As was previously shown, there often appears to be a temporary sequestration of labeled cells immediately postinfusion with cells collected by filtration leukopheresis, as reflected by a low initial recovery. When this occurred, the initial recovery point was not used to calculate the disappearance curve.

Accumulation of transfused neutrophils in the skin chamber was determined by multiplying the chamber neutrophil specific activity by the number of neutrophils present. Total cell accumulation was the sum of the cells present at 6 and 24 hr. The range of values for total cell accumulation was quite wide, undoubtedly reflecting differences in the extent of the abrasion and the degree of the inflammatory response. To eliminate these variables from consideration, the accumulation of labeled cells was expressed as the number of labeled cells per 10⁶ total chamber neutrophils. The number of labeled neutrophils in the chamber also was expressed as a percent of the injected labeled neutrophils.

Statistical comparisons of the means for groups of subjects were made using the Student’s t test.

**RESULTS**

Mean neutrophil yields were 1.2 x 10⁶, 6.9 x 10⁶, and 17.6 x 10⁶ for phlebotomy, intermittent flow centrifugation, and filtration leukopheresis, respectively; these were similar to values previously reported.

**Table 1. Effect of Skin Chamber on Blood Kinetics of Transfused Unstored PMNs**

<table>
<thead>
<tr>
<th>Method</th>
<th>No Chamber</th>
<th>Chamber</th>
<th>t₁/₂ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phlebotomy</td>
<td>5</td>
<td>6</td>
<td>7.2 ± 0.6</td>
</tr>
<tr>
<td>FL</td>
<td>4</td>
<td>6</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td>IFC</td>
<td>4</td>
<td>6</td>
<td>7.3 ± 0.5</td>
</tr>
</tbody>
</table>

*Previously published data.

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Table 2. Effect of Donor Corticosteroids and of Storage on Blood Kinetics of Transfused PMNs (Mean ± SEM)

<table>
<thead>
<tr>
<th>Cortico-Steroids</th>
<th>No Storage</th>
<th>1-Day Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Recovery (%)</td>
<td>Extrapolated Recovery (%)</td>
</tr>
<tr>
<td>Phlebotomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>6</td>
<td>45.1 ± 2.5</td>
</tr>
<tr>
<td>+</td>
<td>5</td>
<td>60.4 ± 10.8</td>
</tr>
<tr>
<td>FL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>5</td>
<td>5.1 ± 1.2</td>
</tr>
<tr>
<td>+</td>
<td>3</td>
<td>4.6 ± 1.0</td>
</tr>
<tr>
<td>IFC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>36.3 ± 3.1</td>
</tr>
<tr>
<td>+</td>
<td>4</td>
<td>39.2 ± 12.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>24.7 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12.9 ± 2.6</td>
</tr>
</tbody>
</table>
The blood kinetics of unstored neutrophils in subjects with skin abrasions was compared to that in subjects without skin abrasions. Neutrophil recovery and blood half-time were not appreciably affected by the presence of the skin chamber (Table 1).

Blood kinetics of FL or IFC neutrophils stored for 1 day showed decreased recovery and shortened blood half-times (Table 2). The blood kinetics of cells obtained from donors who had received prednisone were similar to those of donors not given prednisone (Table 2); the only significant changes ($p < 0.05$) were a lengthening of the blood half-time for cells collected by phlebotomy and a decrease in recovery with IFC cells stored for 1 day.

The total number of white blood cells in the skin chamber by 24 hr was $101.8 \pm 9.6 \times 10^6$ for subjects not given prednisone and $110.8 \pm 26.8 \times 10^6$ for subjects given prednisone 5–8 hr prior to the skin abrasion; values were not significantly different ($p > 0.5$). These cells were $96\% \pm 2\%$ neutrophils.

The skin chamber accumulation of labeled cells for subjects not given prednisone is shown in Fig. 1 (open bars). For unstored cells collected by phlebotomy, $0.10\% \times 0.01\%$ of the infused cells were present in the chamber at 24 hr. Unstored IFC cells showed significant impairment ($p < 0.05$) in their ability to migrate into the chamber (mean $0.058\% \pm 0.013\%$), but the difference was only moderate with values overlapping those of cells collected by phlebotomy. In contrast, FL cells were markedly abnormal. Only $0.006\% \pm 0.002\%$ of the infused cells localized in the chamber by 24 hr, a value significantly different from that of both phlebotomy and...
IFC cells ($p < 0.05$). In 3 subjects in whom the skin abrasion was created 5–6 hr before infusion of labeled FL cells, a similar result was obtained (0.002% ± 0.001%). Premedication of the donors with corticosteroids resulted in slightly lower mean chamber accumulation of labeled cells in all groups, but none of these differences was significant (Fig. 1).

Storage of either IFC or FL cell concentrates for 1 day resulted in substantial cell damage as reflected by reduced chamber accumulation (Fig. 1). With IFC cells, the difference was significant ($p < 0.05$), with approximately one-fourth as many cells accumulating at the inflammatory site. In vivo localization of unstored FL cells decreased to approximately one-third that of the unstored cells, although the difference was not quite significant ($p < 0.2$). The effects of storage were not altered in cells collected from subjects given prednisone.

**DISCUSSION**

Neutrophils for transfusion are generally harvested from normal donors by methods involving either centrifugation or the reversible adherence of neutrophils to nylon fibers. The effect of these various collection techniques on the integrity of the neutrophils obtained has been the subject of numerous studies. The function in vitro of cells obtained by centrifugal techniques has been found to be essentially normal by several investigators. In contrast, FL cells have generally been shown to exhibit a modest impairment in function in vitro, an impairment that may be minimized, or perhaps prevented, by premedication of the donor with corticosteroids or by decreasing the time of filter-cell contact.

The ability of the neutrophils in these preparations to circulate in the recipient has been most reliably determined by kinetic studies of autologous neutrophils labeled with DF$^{32}$P in normal subjects. These studies have shown that blood kinetics of cells collected by either continuous or intermittent flow centrifugation are normal or near normal. In contrast, FL cells are severely impaired, with markedly decreased blood recovery and shortened blood disappearance time. These studies confirmed impressions on the effect of the collection method that have been obtained from animal studies and from posttransfusion increments and occasional isotopic studies in neutropenic, usually infected, patients.

Some previous work indicates that both CFC and FL neutrophils are capable of accumulating to some degree at inflammatory sites in patients receiving neutrophil transfusions. Most of these studies, however, are not quantitative. Appelbaum et al., using quantitative methods similar to those reported here, found that the skin chamber accumulation of transfused FL neutrophils was several-fold less than that of transfused CFC cells in neutropenic dogs. These same investigators have shown that 2.5–3 times as many FL cells as CFC cells are required to affect the survival of neutropenic septicemic dogs.

Neutrophil donors are often given corticosteroids prior to leukopheresis in order to shift neutrophils from the marrow storage pool into the circulation and thereby increase the number of cells collected. There are several possible effects of this practice on the functional capabilities of the cells obtained. One of the known effects of corticosteroids on neutrophil blood kinetics is to slow the egress of cells from the circulation to the tissues, and several studies have demonstrated decreased migration of neutrophils to inflammatory sites in humans or animals.
given corticosteroids.\textsuperscript{30-32} The in vitro function of neutrophils collected by centrifugation from steroid-treated donors has been shown to be normal,\textsuperscript{8,9} but there is no information on whether such cells function normally in vivo. In addition, it has been suggested by Wright et al.,\textsuperscript{5} on the basis of a variety of in vitro function tests, that the abnormalities in cells collected by FL may be prevented by pretreatment of the donor with corticosteroids. However, skin chamber studies in dogs have failed to show any effect of donor steroid treatment on the ability of FL cells to accumulate at an inflammatory site.\textsuperscript{7} Finally, it is conceivable that neutrophils collected after donor stimulation are more resistant to storage-induced damage.

Although neutrophils are usually administered to recipients as soon as possible after collection, the ability to store cells, at least short-term, without inducing irreversible cell damage would be of practical importance. Although for years it was presumed that neutrophils were fragile cells that rapidly deteriorated in vitro, recent evidence has suggested that short-term storage may be possible in some circumstances. McCullough et al. studied the function in vitro of neutrophils stored in whole blood and found the cells to be near normal after 24-hr storage.\textsuperscript{11,12} Other studies have suggested that FL or IFC cells may be stored at 4\textdegree C for 24 hr without substantial damage as judged by in vitro function tests.\textsuperscript{10,11} In vivo studies in the rabbit have documented that neutrophils may be stored in whole blood for up to 3 days without seriously impairing their ability to circulate or to accumulate at an inflammatory site.\textsuperscript{14} Autologous studies in humans have shown that the blood kinetics of IFC or FL neutrophils are at most moderately abnormal after 2-day storage at 4\textdegree C.\textsuperscript{6}

The blood kinetic data in this report confirm the results of previous studies,\textsuperscript{6} namely that the kinetics of unstored IFC cells is near normal whereas that of unstored FL cells is markedly abnormal. Storage at 4\textdegree C for 1 day results in moderate blood kinetic abnormalities for both IFC and FL cells. The skin chamber results confirmed that unstored IFC cells were, at most, moderately abnormal. In contrast, the chamber accumulation of unstored FL cells was markedly decreased.

It seemed possible that the decreased neutrophil accumulation seen with FL cells might be due to the timing of the abrasion. If several hours were required for inflammation to become established and for cells to begin localizing in the chamber, a phenomenon seen in our earlier animal studies,\textsuperscript{14} the decreased accumulation might merely reflect the fact that few labeled cells were circulating at the time of most rapid cell migration into the chamber. To investigate this possibility, 3 subjects were studied in whom the skin abrasion was created 5–6 hr prior to infusion of the labeled FL cells. The 5–6 hr interval was considered adequate for these purposes, based on previous animal studies.\textsuperscript{14} The low values obtained in these subjects indicate that the failure of FL cells to accumulate in the chamber is probably due to an abnormality of the cells rather than to the kinetic characteristics of the inflammatory site.

Although these data clearly show that on a per-cell basis the chemotactic capabilities of FL cells in vivo are severely impaired, it is conceivable in the clinical transfusion setting that this deficit would be overcome by the larger number of cells routinely obtained and transfused with this technique. To evaluate this possibility, the results were alternatively expressed as the total number of infused labeled neutrophils present in the chamber (Fig. 2). With unstored phlebotomy cells, mean
chamber accumulation was $1.2 \times 10^6$ labeled neutrophils. When 5–6 times this number of IFC cells was transfused, 3.5 times as many labeled cells were found in the chamber. With FL cells, although 15 times as many neutrophils were infused, mean chamber accumulation was not different from that with unstored phlebotomy cells. Based on these data, it can be calculated that an average FL transfusion would be expected to be about as effective as giving a unit of fresh blood and about one-third as effective as an average IFC transfusion. To obtain equivalent numbers of neutrophils at an inflammatory site, it would appear that approximately ten times as many FL cells must be transfused as IFC cells, a ratio not normally approached clinically.

In light of these results, it is of interest to note that clinical studies have failed to detect any difference in the efficacy of FL and CFC preparations, suggesting that the clinical criteria are not sufficiently sensitive or, alternatively, that factors other than local neutrophil accumulation may be of clinical importance.

Treatment of the subjects with prednisone did not significantly affect the skin chamber results in any group studied. The lack of effect on the phlebotomy group serves as a control and indicates that the skin chamber kinetics are not appreciably altered in recipients who have received steroids 5–8 hr previously. Donor stimulation does not appear to adversely affect the in vivo chemotactic ability of either IFC or FL cells. Of equal importance, there is no evidence from these data that the functional capabilities of FL cells are improved by premedication of the donor with steroids. Finally, stimulation of the donor with prednisone did not influence the degree of cell damage induced by short-term storage.

In contrast to the blood kinetic data, which suggest that storage of IFC or FL neutrophils for 1–2 days results in at most moderate cellular damage, the skin chamber results suggest that storage causes substantial damage. The difference is not significant with the FL cells, probably because the values for the unstored cells
are so low. It is of interest that measurement of in vivo chemotaxis appears to be a more sensitive test of cellular damage than measurement of the blood kinetics, a phenomenon previously observed in animal studies.14

In these studies we observed that the presence of the skin chamber does not affect either the blood neutrophil recovery or half-time. This is in contrast to a group of subjects studied by Boggs et al.15 in which similar inflammatory sites were reported to have resulted in enlarged blood marginal neutrophil pools. Other studies have demonstrated that neutrophil kinetics are altered in inflammation,35,36 and failure to detect changes in these studies is probably related to the magnitude of the inflammatory stimulus we created.

All of these studies have been performed on neutrophil preparations as conventionally obtained and stored without modification. Whether the cellular defects induced by filtration leukopheresis or by storage can be minimized by manipulation of factors such as pH or cell or glucose concentration requires further investigation.

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Blood kinetics and in vivo chemotaxis of transfused neutrophils: effect of collection method, donor corticosteroid treatment, and short-term storage

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