Mechanisms of Recovery From Neutropenia Induced by Hemodialysis

By Leonard H. Brubaker and Karl D. Nolph

Profound, transient neutropenia is observed early in hemodialysis. The present studies were performed during 11 twin-coil dialyses in eight patients with chronic renal failure to determine if the rapid recovery reflects remobilization of sequestered neutrophils and/or if marrow reserves are utilized. In five studies, in vitro cell tagging with diisopropylfluorophosphate-\(^{32}\)P (DF \(^{32}\)P) and reinfusion were performed 4–6 hr before routine hemodialysis. In all 11 studies a marked decrease of the absolute neutrophil count occurred within 15 min of starting blood return from the coil. In nine studies carried on over a sufficient period of time, a rebound of the neutrophil count to levels significantly higher than the highest control counts occurred by 1½–3½ hr after starting dialysis. In the radioactive studies during the rebound phase, most or all of the expected number of labeled neutrophils returned to the circulation, but simultaneously in all cases a large number of extra unlabeled neutrophils appeared in circulation. Since there are two sources for additional circulating neutrophils—the marginated pool and the marrow reserves—and since the marginated pool under the study conditions would be expected to have the same specific radioactivity as the circulating pool, the data are consistent with the hypothesis that there was an immediate marrow response to neutropenia.

Profound transient neutropenia was noted by Kaplow and Goffinet\(^1\) during the initial hour of hemodialysis using a twin-coil artificial kidney. Gral et al.\(^2\) have observed the same phenomenon with two other types of hemodialyzers. Cell trapping in the coil has been excluded as a quantitatively adequate explanation for the almost complete disappearance of circulating neutrophils.\(^3\) These reports suggest that the neutrophil count returns to normal by 1 hr after starting dialysis and remains normal for the remainder of the dialysis. They have primarily focused on the neutropenic phase, however, with relatively few studies being done after 1 hr. The presence of increased numbers of band neutrophils during the recovery phase has been noted,\(^1,2\) however, suggesting that a bone marrow response plays a role in the recovery phase. The present studies were undertaken to determine whether the missing neutrophils are destroyed and permanently removed from the circulation, and

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the possible mechanisms of recovery from neutropenia. We have found that acute, transient neutropenia occurs, that most of the cells leaving the circulating pool return to it by 1-3 hr after starting dialysis, and that the period of neutropenia is followed by an influx of neutrophils that apparently come from the bone marrow.

MATERIALS AND METHODS

Eight patients with chronic renal failure undergoing twice weekly maintenance hemodialysis and having either an external shunt (two patients) or an internal arterio-venous fistula (six patients) were studied during 11 twin-coil dialyses. Their ages, sexes, and diagnoses are listed in Table 1. Informed consent was obtained prior to each study. A cellophane dialysis coil (UF-145, Travenol Laboratories, Morton Grove, Ill.) was used in all studies except one (patient 8) in which a cuprophane (UF-100, Travenol Laboratories, Morton Grove, Ill.) coil was used.

A white cell intravascular survival procedure was done during five studies in four patients by a modification of the method of Mauer et al. At approximately 7 a.m., 500 ml of blood was withdrawn, labeled for 45 min with 100 pCi of DF32P (diisopropylfluorophosphate, PB-117P, Amersham/Searle Corp., Arlington Heights, Ill.) and with 50 pCi of sodium chromate-51Cr (Cat. No. 0590, E. R. Squibb, Radiopharmaceutical Dept., New Brunswick, N.J. for purpose of determining the blood volume to compare with the volume computed from body surface area), and reinfused. Blood samples were drawn during a 4-6 hr control period prior to initiation of hemodialysis for determination of specific activity of mixed white cells (expressed as cpm p/1017 white cells), total white count done in duplicate on a Coulter Counter (Coulter Electronics, Hialeah, Fla.), and a differential count on 300 white cells. These blood samples were drawn from a vein on the forearm with the A-V shunt or internal A-V fistula, which was the opposite side from where labeled cells were reinfused. No attempt was made to separate band, nonsegmented, and segmented neutrophils in the differential count. The isolation of mixed white cells for determination of radioactivity involved dextran sedimentation and hypotonic lysis of the contaminating red cells and platelets.

Routine hemodialysis was then initiated and continued for 6 hr. The dialyzer and tubing were primed with 0.9% saline. Five thousand units of heparin were injected into both the arterial and venous tubing at the start of dialysis. Blood flow was initially maintained at 50 ml per min and increased gradually over the first 15 min to 100-200 ml per min. Blood traversed the coil and returned to the patient usually within 8 min after starting. Blood samples were taken from the arterial tubing subsequent to starting dialysis; these counts were found to be equal to venous blood counts. Blood volume was computed in the following two ways: from 51Cr red cell volume and from body surface area (B.S.A.) in sq m x 2.68. The two methods agreed in all except one case (patient 1) in which 51Cr volume was greater than B.S.A. volume, and in this case the 51Cr volume was used. Specific activity of mixed white cells was computed as outlined previously. Total circulating labeled neutrophils (N*) at any given time were calculated by the following formula:

\[
N^* = \frac{\text{Total white count} \times \text{fraction neutrophils} \times \text{specific activity}}{\text{Specific activity of earliest sample}}
\]

Aliquots of the patients' plasma and dialysate were assayed periodically to determine the rate of removal of noncell bound DF32P and 51Cr by dialysance. Removal rate was comparable with normal rate of isotope removal by the kidneys, so patients did not undergo unusually high radiation exposure.

RESULTS

Total circulating neutrophil counts observed during control and hemodialysis periods are shown in Table 1. Profound neutropenia occurred in each study, the nadir being reached from 12 to 21 min following start of dialysis
<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Age, Sex</th>
<th>Months on Dial.</th>
<th>Diagnosis</th>
<th>Initial</th>
<th>Neutrophils/cu mm</th>
<th>Ratio: Rebound/Highest Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>White Counts Obtained During DF³°P Labeling Studies</td>
<td></td>
<td>Highest Control</td>
<td>Lowest</td>
</tr>
<tr>
<td>1</td>
<td>65F</td>
<td>24</td>
<td>Polycystic kidneys</td>
<td>5,780</td>
<td>5,780</td>
<td>130</td>
</tr>
<tr>
<td>2</td>
<td>52F</td>
<td>1</td>
<td>Amyloid kidney</td>
<td>5,880</td>
<td>5,880</td>
<td>60</td>
</tr>
<tr>
<td>3a</td>
<td>27M</td>
<td>%</td>
<td>CGN</td>
<td>2,680</td>
<td>2,680</td>
<td>7</td>
</tr>
<tr>
<td>3b</td>
<td>2</td>
<td>(second study)</td>
<td></td>
<td>2,390</td>
<td>2,710</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>38F</td>
<td>1</td>
<td>RPGN</td>
<td>4,020</td>
<td>4,380</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No Radioactive Studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5a</td>
<td>12F</td>
<td>%</td>
<td>Hemolytic-uremic cortical necrosis with shunt infection</td>
<td>11,400</td>
<td>11,400</td>
<td>2,800</td>
</tr>
<tr>
<td>5b</td>
<td></td>
<td>(second study)</td>
<td></td>
<td>12,000</td>
<td>12,000</td>
<td>1,160</td>
</tr>
<tr>
<td>6a</td>
<td>46M</td>
<td>1</td>
<td>Chronic pyelonephritis</td>
<td>3,150</td>
<td>3,580</td>
<td>220</td>
</tr>
<tr>
<td>6b</td>
<td></td>
<td>(second study)</td>
<td></td>
<td>3,060</td>
<td>3,360</td>
<td>160</td>
</tr>
<tr>
<td>7</td>
<td>28M</td>
<td>3</td>
<td>Amyloid kidney</td>
<td>2,700</td>
<td>2,700</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>16F</td>
<td>3</td>
<td>Hereditary nephritis</td>
<td>2,900</td>
<td>2,900</td>
<td>100</td>
</tr>
</tbody>
</table>

As tested by paired t analysis, lowest counts observed are different from initial counts ($p < 0.001$). Rebound counts are different from highest control counts ($p < 0.02$). If studies on patient 5 are excluded because of unusually high control counts, remaining rebound counts are more significantly different from highest control counts ($p < 0.01$). RPGN, rapidly progressive glomerulonephritis; CGN, chronic glomerulonephritis.
and about 4–13 min following the first return of cells to the body. A constant finding was a rebound of the total neutrophil count to significantly higher levels than the highest control values. The rebound peak occurred 1½–3½ hr following starting hemodialysis. This peak was 1.16–1.7 times the respective maximum control counts. This ratio of peak/control neutrophil counts did not correlate with the respective control counts, although control counts ranged in different individuals from 2,390 to 12,000 cells/cu mm.

The patient with the highest neutrophil count (patient 5) had an infection around her external shunt that may have been the cause of her base-line neutrophilia. Because of her youth, a radioactive study was not done. However, two studies of the changes in her circulating counts with hemodialysis were done. Both were almost identical. The results of one of these is shown in Fig. 1. Except for starting at a higher level, the pattern was typical of the response to hemodialysis in all patients studied.

A pronounced fall of the monocyte count to nearly zero was observed during all the studies. There was no consistent increase of monocyte count over base line during the rebound phase, in contrast to neutrophils. Lymphocyte counts showed no significant change during hemodialysis, but the variability of these counts was relatively greater than neutrophils or monocytes.

There were two successive coil ruptures during one study in patient 6, so that observations were performed during initiation of hemodialysis with three coils in quick succession. The results are shown in Fig. 2. The extreme fall in
neutrophil count occurred repeatedly with use of each new coil.

A second study in patient 6 was done with a pause of extracorporeal circulation for 45 min at the point following infusion of most of the saline prime, but just before blood began to return to the body. No disappearance of neutrophils occurred until the clamps were finally released and blood returned to the patient, when there was a prompt fall in the neutrophil count in the usual manner.

Figure 3 shows a plot of total neutrophil count with specific radioactivity of mixed white cells superimposed for patients 1-4. Patient 3 was studied twice with similar results. All studies had the following features: Shortly after the onset of dialysis the total neutrophil count fell to nearly zero; at this time white cell specific activity also fell markedly (the majority of circulating white cells temporarily were lymphocytes, which take up the DF22P tag very poorly6); the neutrophil count then rose rapidly to a value higher than the control values; accompanying the marked rise in total neutrophil count was a modest rise in the white cell specific activity; if the specific activity is projected from the control period through the period of dialysis, as represented by the straight dotted line, it can be seen that there was a persistent reduction of the specific activity accompanying the rebound neutrophilia. This suggests that the circulating neutrophil pool had been diluted with unlabeled neutrophils.

When the number of circulating labeled neutrophils were computed by the formula shown under Methods and these numbers were plotted on a semilogarithmic scale against time, it could be seen that most or all of the original circulating neutrophils returned to the circulation during the rebound period. The values for two studies are shown in Fig. 4. In case 3b, on the left, all but about one-fourth of the expected number of labeled neutrophils returned. On the right is an earlier study in the same patient in which most or all of the expected number of labeled neutrophils returned. The expected number of labeled neutrophils returned in two of the three other radioactive studies.
Fig. 3.—Comparison of specific activity of DF³²P-labeled white cells with total circulating neutrophil count during hemodialysis in four patients studied. Fall in white cell specific activity simultaneous with appearance of neutropenia was due to fact that lymphocytes, which temporarily were almost only cells in circulation, do not label well with DFP. Shortly afterward, during recovery period, continued low level of white cell specific activity was due to presence of increased number of (Legend continued on facing page.)
RECOVERY FROM NEUTROPENIA

Fig. 4.—Circulating labeled and unlabeled white cells during two dialyses on patient 3 as computed by formula in Methods. Slope of dashed line representing projection of number of labeled cells was derived from control period points. On semilogarithmic scale number of labeled cells should decline in linear fashion just as specific activity does. In study on left, after neutropenic phase, number of returning labeled cells reached about three-fourths of expected number, while on right they all returned in expected numbers.

(patients 2 and 4), while in the remaining one (patient 1), again about one-fourth of the labeled neutrophils was missing. The increase of total circulating neutrophils from control period to the rebound peak was an underestimate of the number of unlabeled cells appearing during the rebound in the two studies in which the expected number of labeled neutrophils did not return.

DISCUSSION

Toren et al.8 postulated that some soluble substance was removed from the coil and, once in the blood, initiated neutrophil sequestration, perhaps in the lungs. Figure 2 illustrates that, if such a soluble factor exists, it is not delivered to the circulation in an active form by the saline prime. In all of our studies the neutropenia developed only after some blood had returned to the patient. This has also been shown by Gral et al.2 A second possible mechanism is that the first few neutrophils to pass through the coil are damaged enough so that they are sequestered in the lungs. These may release attractive substances causing other neutrophils to be sequestered and thus multiplying the effect in a manner similar to reversible agglutination of platelets. Compatible with both the above mechanisms is the fact that the disappearance of almost all neutrophils from the circulation occurred when no more than 5%–10% of the total blood volume could have traversed the coil. Thus, the great majority of the neutrophils that disappeared from the circulating blood never entered the extracorporeal circuit.

unlabeled neutrophils. Slope of line projecting specific activity during dialysis was derived from control points except in study of patient 2 in which slope of this line was set equal to slope of points 1½–6 hr after starting dialysis. This was necessary because of initial rise in specific activity, a variation also seen in normal patients.7
The data presented in our studies have two major implications. First, the circulating neutrophils were for the most part only temporarily sequestered from the circulating pool during early hemodialysis. Any damage to these cells was evidently minimal. Second, the period of neutropenia was followed by an influx of unlabeled cells into the circulating pool, as well as a return of previously circulating cells. These new cells, plus the old cells returning, led to an increase of the total neutrophil count above control values.

The source of these new cells was most likely from bone marrow reserves. The only other major source of new cells would have been the marginated intravascular pool that has been shown in normal individuals to be in equilibrium with the circulating intravascular pool. We have similarly demonstrated this equilibrium in a uremic patient. Therefore, cells from this source should have been labeled also. Any shift of cells from the marginated to the circulating pool, therefore, would not have changed the specific activity. The in vitro DFP labeling technique is designed so that by the time blood returns to the patient all excess DFP is hydrolyzed, which assures that bone marrow cells remain unlabeled. The fact that recovery from neutropenia was associated with dilution of white cell specific activity thus strongly suggests that movement of marrow cells into the circulating pool occurred.

Other investigators have shown that neutropenia can cause increased release of mature neutrophils from the bone marrow. Studies in neutropenic rats and dogs have produced evidence that a humoral factor is formed during neutropenia induced by leukophoresis and vinblastine that can induce marrow neutrophil release in normal animals. Morley and Stohlman demonstrated that irradiation-induced neutropenia in shielded-limb mice was associated with an increase in the shielded-marrow release of mature neutrophils at a time when the production of mature marrow neutrophils had not been altered. They observed that the rate of neutrophil release in different mice varied similarly to the degree of neutropenia. The present study is the first demonstration of neutropenia-associated increased marrow release of neutrophils in humans.

Boggs et al. have pointed out the necessity of separating the effect of a neutrophilia-inducing factor from the effect of endotoxin. Endotoxin is a very potent stimulus for neutropenia followed by neutrophilia. Certain pronounced differences between the effects of endotoxin and hemodialysis may be noted. First, endotoxin is associated with fever, which is not a usual feature of hemodialysis. Second, endotoxin produces a pronounced fall in lymphocytes, whereas there is no discernable change in the lymphocyte count during hemodialysis. Third, administration of endotoxin causes a modest neutropenia followed by a pronounced neutrophilia, while the opposite is true during hemodialysis. Fourth, there is no hint of any development of tolerance with repeated hemodialyses. In our study, patient 1 had been dialyzed twice weekly for 2 yr.

Neutrophils and monocytes both have surface adherent properties while lymphocytes do not. This may have some bearing on the absence of any fall in lymphocyte count during hemodialysis. In contrast with the increased numbers of neutrophils found during the rebound phase, no consistent increase was found with monocytes (usually no increase was found). This suggests that
the monocyte count may not be under the same type of control as the neutrophil count.

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REFERENCES


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