Hemodialysis Coil-induced Transient Neutropenia and Overshoot Neutrophilia in Normal Man


Profound transient neutropenia, followed by an overshoot of the neutrophil (N) count to an average of 2.5 ± 1.0 (mean ± 1 SD) times higher than average control N count, has been induced in seven normal subjects by reinfusion of heparinized blood that had been stagnated in a hemodialysis coil for 15 min. This was similar to the neutropenia-neutrophilia cycle occurring shortly after the initiation of hemodialysis in uremic patients. At the time of neutropenia, profound monocytopenia was also observed, but only a slight drop in lymphocyte count occurred. Neither monocytes nor lymphocytes subsequently recovered to higher than control values. A DF32P standard N survival procedure, performed in one subject with Hodgkin's disease in complete unmaintained remission, showed that a large number of unlabeled N appeared during the overshoot, suggesting their marrow origin. Although in two of three attempts neutropenia was produced by reinfusion of blood through the coil without stagnation, no significant overshoot of N count following neutropenia occurred without the stagnation step. Simple phlebotomy and reinfusion of blood without the coil had no effect on the N count. This model may prove useful in studying the possible immediate regulation of the N count by marrow release of N and marrow N reserves in various patients.

A NEUTROPENIA-NEUTROPHILIA CYCLE beginning promptly with initiation of hemodialysis in uremic patients has been described in our earlier report. Evidence was presented that the rebound neutrophilia or overshoot, which peaks from 1½ to 3½ hr after starting hemodialysis, is caused both by the return to circulation of the neutrophils (N) temporarily sequestered (presumably in the lungs) during profound neutropenia and by the addition of new N from the bone marrow. The present studies demonstrate that the same neutropenia-neutrophilia cycle can be induced in normal human subjects.

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MATERIALS AND METHODS

Six normal male subjects, ages 22–37 yr, and one 22-yr-old male patient with Hodgkin’s disease in complete remission were studied by the following procedure:

Blood, 500 ml, was drawn into a bag containing 2250 U of heparin (Fenwal, JH-1, Division of Travenol Laboratories, Inc., Morton Grove, Ill.). The blood was infused promptly, via a Sarns roller-type blood pump, into a saline-primed UF-145 cellophane dialysis coil (Travenol Laboratories, Morton Grove, Ill.) immersed in standard circulating dialysate (K⁺ raised to 4 meq/liter) kept at 37°C. When the first blood appeared in the return tubing, the blood pump was turned off, and the outlet tubing was clamped. The blood was stagnated in the coil for 15 min. After this, the blood was reinfused through the phlebotomy needle at the rate of 50 ml/min until it had been totally returned to the subject. Samples of blood were drawn from a vein in the opposite forearm at frequent intervals before, during, and for 3–4 hr after blood reinfusion. A total white count on a Coulter Counter (Coulter Electronics, Hialeah, Fla.), a 300 cell differential count, and a hematocrit were determined on each sample. Because saline was used both to wash the blood from the coil and to keep open the intravenous needle from which the small blood samples were periodically drawn for white cell counts, there was some dilution of blood by this saline after the first blood sample. To correct for this, all white cell counts were multiplied by the ratio: first sample hematocrit/diluted sample hematocrit. In most studies, the diluted sample hematocrits were 2–4 hematocrit units lower than the first sample, but the apparent dilution was rarely greater than this (in one study the sample hematocrits varied from 43.5 to 33). In the differential counts, N were classified in the following manner: segmented N, seg., those with a filament connecting lobes; nonsegmented N, nonseg.; and bands, those with parallel nuclear sides. In case of doubt, the N was considered to be segmented.

Three uremic patients were also studied by withdrawing 500 ml blood into the JH-1 bag containing heparin, then reinfusing the blood through the coil with the 15-mm stagnation. For comparison, patients were studied during a period of regular hemodialysis on the same type of coil. At the initiation of hemodialysis, the patients were given 5000 U of heparin intravenously plus 5000 U into the arterial (inlet) tubing of the coil.

The study in the patient with Hodgkin’s disease (subject No. 5) was done during a white cell intravascular survival procedure using 100 μCi of DF32P (diisopropylfluorophosphate-32P, PB-117P, Amersham/Searle Corp., Arlington Heights, Ill.) by a procedure similar to that previously described.1

As controls, three studies were done in the same subjects by reinfusing 500 ml heparinized blood through the UF-145 coil at 50 ml/min without stagnation. In four additional studies, 500 ml heparinized blood was drawn into the bag, allowed to remain in the bag 25 min, and was reinfused directly, without going through a coil. It usually took 25 min to infuse the blood into the coil and to allow the 15-min stagnation time, so the latter controls were done to determine the effect of temporary phlebotomy only on the subject’s N count.

During all of these studies, the subjects were reclining in a comfortable phlebotomy chair or in bed and frequently dozed between samples. Lidocaine 1% was used to eliminate pain from venipuncture. These studies were approved by the Committee for Research Involving Human Subjects of the University of Missouri Medical Center. Informed consent was obtained in each case. No untoward effects were observed during any of these studies, but one subject had a mild facial flushing during the neutropenia as described later.

RESULTS

Total seg., nonseg., and band N counts observed during control and postreinfusion periods of the studies with the 15-min stagnation (stasis) are shown in the top section (1) of Table 1. Profound neutropenia occurred in each study; the lowest point was reached from 6 to 11 min following the start of reinfusion. In all studies, an overshoot of all three types of N to
Table 1. Summary of Neutrophil Counts*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control (Average)</th>
<th>Neutropenia (Lowest)</th>
<th>Rebound Neutrophilia (Highest)</th>
<th>Ratio: Highest Count/Average Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Nonseg</td>
<td>Band</td>
<td>Total</td>
</tr>
<tr>
<td>1. Normals, infusion through coil with 15-min stagnation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4151</td>
<td>449</td>
<td>49</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>2383</td>
<td>138</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>7987</td>
<td>429</td>
<td>58</td>
<td>757</td>
</tr>
<tr>
<td>4a</td>
<td>3002</td>
<td>195</td>
<td>21</td>
<td>39</td>
</tr>
<tr>
<td>4b</td>
<td>4066</td>
<td>340</td>
<td>69</td>
<td>472</td>
</tr>
<tr>
<td>5</td>
<td>2517</td>
<td>74</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>2737</td>
<td>121</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>2844</td>
<td>83</td>
<td>12</td>
<td>59</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3713 ± 1652</td>
<td>229 ± 155</td>
<td>28 ± 27</td>
<td>180 ± 279</td>
</tr>
<tr>
<td>2. Uremic patients, infusion through coil with 15-min stagnation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5390</td>
<td>512</td>
<td>0</td>
<td>172</td>
</tr>
<tr>
<td>B</td>
<td>3813</td>
<td>400</td>
<td>0</td>
<td>86</td>
</tr>
<tr>
<td>C</td>
<td>3035</td>
<td>668</td>
<td>30</td>
<td>193</td>
</tr>
<tr>
<td>3. Normals, infusion through coil without stagnation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2804</td>
<td>117</td>
<td>11</td>
<td>2710</td>
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<tr>
<td>2</td>
<td>2843</td>
<td>75</td>
<td>3</td>
<td>87</td>
</tr>
<tr>
<td>7</td>
<td>2954</td>
<td>78</td>
<td>6</td>
<td>668</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3705 ± 591</td>
<td>156 ± 66</td>
<td>19 ± 13</td>
<td>2526 ± 529</td>
</tr>
</tbody>
</table>

*Counts given as neutrophils/μm.
†Averages during period 0.25–2.0 h (no rebound peak observed).

In section 1, differences between average control and highest rebound total neutrophil counts are significant (p < 0.01) by paired t analysis. This is also true if results in studies 3, 4a, or both are not included in the analysis. Similarly, differences between average control and highest rebound nonseg plus band neutrophil counts were significant (p < 0.02) by paired t analysis, except when 4a was excluded from analysis and then p < 0.05.
levels significantly higher than the corresponding control values was observed. The peak of the total N count, 1.81–4.98 times the average control values, occurred from 10 to 91 min following the lowest point. The overshoot/average control ratio for nonseg. and band N was consistently much higher than the same ratio for total N.

In the study in subject 3 we attempted to remove the N from the bag by filtering the blood through a Fenwal Leuko-Pak prior to the stagnation in the coil. However, only a few cells were removed. Therefore, we included this study in the list. The first study in subject 4 was the first time that this subject had ever undergone phlebotomy. At the end of phlebotomy he became sweaty and pale (while denying symptoms). The donor chair was tilted back, and he recovered fully within a few minutes. Since results in the other six subjects were different from his, he willingly underwent a repeat study that was entirely uneventful. The results of this repeat study were different from the first and were more similar to studies in the other six subjects. We believe that the donor reaction during the first study in this subject may have contributed to an unusually large overshoot.

Figure 1 illustrates the relative changes in N, monocytes, and lymphocytes with time in all seven subjects. The repeat study in subject 4 is not included in this figure. N deceased transiently to nearly zero but then recovered to levels markedly greater than the control counts. Monocytes also decreased transiently to nearly zero but thereafter showed no consistent recovery pattern. Lymphocytes showed a minor but consistent decrease at the time of profound neutropenia and thereafter had no consistent pattern.

Section 2 of Table 1 shows results in the three uremic patients. These patients were studied in exactly the same manner as the normal subjects listed in section 1.

Two of these patients were also studied during regular hemodialysis using the same type of coil. A comparison of the two types of studies in uremic patients is shown in Fig. 2. Following neutropenia, the N count of one of these patients (C) recovered to only the control level. The number of nonseg. and band N present during the recovery phase was essentially the same as during the control period. This implies that patient C did not release neutrophils from the bone marrow following neutropenia. The N count of patient A did show an overshoot of total, nonseg., and band N during the period following neutropenia. It is evident from Fig. 2 that reinfusion of blood from the bag through the coil (with stagnation) produces effects on the N count that are similar to the effects produced during initiation of hemodialysis. The major differences seem to be a longer duration of neutropenia and a longer time to the peak of the neutrophilia during the dialysis. However, both the depth of neutropenia and the height of the neutrophilia seem similar as judged by these two studies.

Figure 3 shows a simultaneous plot of total N count and the specific radioactivity of mixed cells during the DF32P study in the patient with Hodgkin's disease (subject 5). Shortly after the start of reinfusion the N count dropped nearly to zero. Thereafter, the specific activity fell sharply and remained at levels lower than expected if the specific activity of the control period were
Fig 1. Manipulation of neutrophil, monocyte, and lymphocyte counts in seven normal volunteers by autotransfusion of heparinized blood through hemodialysis coil. In each study, blood was stagnated for 15 min in coil prior to reinfusion. Curves were normalized as indicated on vertical axis. Monocytes and lymphocytes disappear from circulation simultaneously with neutrophil disappearance but do not rebound higher than control values. Lymphocyte counts show only a slight, but consistent, decline when neutrophils disappear.
Fig. 2. Comparison of neutropenia-recovery cycle in two uremic patients caused by blood reinfusion through hemodialysis coil, allowing 15-min stagnation in coil with a similar cycle caused by initiating regular hemodialysis in the same patient. Maximum depth of neutropenia and peak of recovery phase were almost the same by either procedure.

Fig. 3. Comparison of specific activity of $^{32}$P-labeled white cells with absolute circulating N count in a patient with Hodgkin's disease in complete remission on no therapy who was phlebotomized 500 ml (heparin anticoagulant). Blood was infused into hemodialysis coil, allowed to stagnate 15 min, then reinfused during time interval indicated at the top. Changes in N count and specific activity are similar to that reported in uremic patients on initiation of hemodialysis. Dilution of specific activity during neutrophilic phase, postreinfusion, represents addition of unlabeled N presumably from bone marrow.

extrapolated. This occurred at the same time as the overshoot neutrophilia, indicating that the circulating N pool had been diluted with unlabeled N. In this study there was no indication of N damage; all of the expected number of labeled N returned to circulation during the overshoot period, as determined by the previously outlined method using a semilog plot of total labeled cells vs. time (not shown here).\(^1\)

The three studies shown in section 3 of Table 1 were performed by reinfusing the blood of normals through the coil without stasis. The study in subject 1 did not show neutropenia. However, the same studies in subjects 2 and 7 did have a neutropenic phase, although it was unusually brief. It is possible that we missed a brief neutropenic period in subject 1. The overshoot neutrophilia, consistently seen when we stagnated blood in the coil (sections 1 and 2), was either only questionably present or absent.

In section 4, the same subjects (with one additional) were studied without reinfusion through the coil. The purpose of these studies was to deal with
NEUTROPENIA AND NEUTROPHILIA

Fig. 4. N counts in two normal subjects in which three variations of re-infusion of heparinized blood were performed. Reinfusion through coil with 15-min stasis produced a complete neutropenia-neutrophilia cycle. Reinfusion without stasis produced a shorter duration of neutropenia and little or no overshoot, thereafter. Reinfusion directly from the bag had no apparent effect on N counts.

The question of whether the overshoot neutrophilia represented a response to hemorrhage. These studies showed that simple phlebotomy and reinfusion of heparinized blood (with an interval of 25 min between them) produced no detectable effects on the total, nonseg., or band N counts.

Figure 4 displays the three different types of studies performed in subjects 2 and 7. Its purpose is to show the different duration and depth of neutropenia with and without stasis in the coil. The maximum depth of neutropenia in the study without stasis in subject 2 is similar to that in the study with stasis. However, the duration of neutropenia is greater with stasis than without stasis. In subject 7, both the duration and depth of neutropenia are greater with stasis than without stasis. By examining the studies in which the coil was eliminated and blood was reinfused directly from the bag, one may appreciate the variations in serial N counts determined under these conditions.

DISCUSSION

Other investigators have found that initiation of hemodialysis, using the coil and hollow-fiber dialyzers, in uremic patients causes profound, transient neutropenia in all patients studied. In addition, the Kiil dialyzer has been found to induce a similar transient neutropenia, although one group found no neutropenia with this dialyzer. (However, they sampled at 0, 20, 40, and 60 min and, thus, could have missed it.) Two groups of investigators noted a large increase in band N appearing in the blood following neutropenia. Very few of the reported patients, however, showed any overshoot. In contrast, our previous report showed that all of our patients studied up to that time had an overshoot of the total N count during the rebound phase. We have subsequently found several patients who did not show this overshoot, as illustrated by patient C.
We undertook the present study to see if we could induce the same profound, transient neutropenia in normal subjects. At first, we simply tried drawing a pint of blood in heparin anticoagulant and reinfusing it immediately through the coil (Table 1, section 3, subject 1). When this was unsuccessful, we started allowing the 15-min stagnation. This tactic has been uniformly successful to date in causing a full neutropenia-neutrophilia cycle in normal subjects. We later found that reinfusion through the coil without stagnation can cause neutropenia, although seemingly less profound and of shorter duration. However, whether a marrow release of neutrophils can be provoked by this variation of the technique is uncertain. At least, it was not as consistent in its effects as the stagnation technique. Simple phlebotomy and reinfusion of blood without the coil caused no effects whatever.

To date, all of our studies in normal patients (with stagnation) have shown a marked overshoot of the N count following neutropenia equal to or greater than that seen during hemodialysis in any uremic patient other than patient A. Moreover, in studies in normal subjects (with stagnation), there has been a significant overshoot in the nonseg. and band N during this period, which probably reflected bone marrow release of new N into the circulation. In our previous report, we demonstrated more direct evidence for marrow release of N during the overshoot using the DF³²P-labeling technique. There are two general causes for a prompt increase in the N count: One is marrow release, and the other is a shift from the margined to the circulating pool. The in vitro DF³²P-labeling technique assures that bone marrow N are not labeled. In contrast, both freely circulating N and those in the margined pool have been shown to be labeled equally because of an equilibrium between these two intravascular pools in normal subjects. Consequently, a shift of margined N into the circulating pool to cause a neutrophilia would not change the N specific activity (e.g., Fig. 1 of reference 10). We showed that the same equilibrium existed in one uremic patient by giving her an intravenous epinephrine infusion (0.5 ml of 1/1000 solution during a 1-min period) during a DF³²P survival study. This caused a 50% increase in the total N count with no change in the N specific activity. Furthermore, there was no significant increase in nonseg. and band cells at this time. Thus, when we found a consistent decrease that was more than expected in the N specific activity during the overshoot following neutropenia in five studies in four uremic patients during regular hemodialysis, we concluded that the source of the extra cells was the bone marrow. Figure 3 shows that the same greater-than-expected decrease in specific activity was found in the overshoot phase of the bag-reinfusion-stagnation induced neutropenia-neutrophilia cycle in the hematologically normal patient with Hodgkin’s disease (subject 5). This correlated with an increase at the same time of nonseg. plus band N of approximately eight times the average control value.

The identity of the factor that initiates the pulmonary sequestration of N and causes neutropenia is unknown. Many substances are known to produce prompt, transient, and profound neutropenia, including endotoxin, histamine, nicotonic acid, dextran, and transfusion of leukocytes (both intact
and disintegrated). In the case of histamine, pulmonary sequestration of N was demonstrated. What is common to all of these substances, except dextran (the experiments with dextran were done in rabbits), is that their injection intravenously causes marked, well-known signs and symptoms. It is well known that the induction of routine hemodialysis is not accompanied by chills, fever, flushing, or headache, and we have never observed these. Only one of our subjects has ever detected any sensations around the time of neutropenia (the subjects include all authors except one). A recent repeat study, complete with 15-min stagnation, was done in subject 5 (the patient with Hodgkin’s disease) approximately 7 wk following a course of combination chemotherapy given as remission maintenance treatment. During the period of neutropenia, he reported a mild sensation of facial flushing and very mild dizziness that lasted 2–3 min and then disappeared completely. It was not distressing to him. He had not reported this during the study reported herein. During this second study, his total N count rebounded to 1.5 times average control count, and his nonseg. plus band N count rebounded to 5.23 times the average control count. Both of these numbers are less than the respective numbers given in Table 1. Although this one occurrence might be a subtle clue to the nature of neutropenia-inducing factor, we are more impressed by the lack of such symptoms in all of our other studies.

We and others have observed that repeated stagnation of the blood in the coil can lead to repeated generation of neutropenia-inducing factor. This substance appears to be labile. During one hemodialysis study in a uremic patient, we isolated the first 500 ml of blood to come through the coil; this is more than is needed to produce neutropenia when transfused immediately. This blood was reinfused through the venous line over a few minutes, 5 hr later, without stopping hemodialysis. No change in the N count was observed as a result of this. We have recently performed one study in a normal subject in which we first obtained fresh, cell-free, heparinized plasma, stagnated this in the coil for 30 min, and then reinfused it. The same profound, transient neutropenia (but, curiously, no significant neutrophilia) was produced, showing that neutropenia-inducing factor can be produced in the absence of cells. Saline and albumin infused under similar circumstances had no effect. This is similar to the experience of Nidus and Pineda.

We have previously shown that the process of temporary sequestration in the lungs does not cause permanent damage to the N, since nearly all return to circulation and thereafter have a normal survival. However, this process is shortly followed by a marrow release of N. Two of the possible explanations for this are: (1) that the temporary absence of N, if it is of sufficient duration, can lead to elaboration by the body of a substance causing an accelerated marrow release of N; or (2) that some substance capable of causing an accelerated marrow release of N is produced by the process of stagnating blood in the coil. In regard to the first possibility, Marsh and Levitt recently presented evidence and reviewed the works of others about a plasma factor appearing during neutropenia that caused marrow N release. In regard to the second possibility, marrow N release might be related somehow to N destruc-
tion. Perhaps destruction of a few N in the coil could lead to production of a marrow-N-releasing substance. We are presently conducting experiments to explore both of these possibilities.

A practical use of the induced neutropenia-neutrophilia cycle may be as a test for the ability of the marrow to release N in various malignant and nonmalignant disease in which the marrow may be damaged or for which myelosuppressive drugs need to be given. Such a test of marrow N reserves may be not only more physiologic, but safer, less toxic, and more convenient than the etiocholanolone or endotoxin stimulation tests more commonly used.

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REFERENCES

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