Neutrophilia-inducing Activity in Plasma of Neutropenic Human Beings

By John C. Marsh and Martin Levitt

Humoral factors have been implicated in the bone marrow release of neutrophils in experimental animals in response to neutropenia. No comparable studies have yet been reported in man, although a humoral mechanism has been described in the neutrophilic response to endotoxin. Three patients with reversible drug-induced neutropenia were studied for the presence of neutrophilia-inducing activity by obtaining plasma during neutropenia and after recovery. When the blood neutrophil concentration and marrow granulocyte reserves were normal, the paired plasmas were returned to the donors on different days, and the blood neutrophil concentrations sequentially measured. Reinfusion of the plasmas obtained from the two patients with marked neutropenia was associated with a 2.5–3.5-fold increase in the total neutrophil concentration and an 8.5-fold increase in the nonsegmented neutrophils, with the peak 30–60 min after infusion. In contrast, plasma obtained when the blood neutrophil concentration was normal did not have this effect. Plasma from the patient with only slight neutropenia was not associated with neutrophilia-inducing activity. Neutrophilia-inducing activity is present in increased concentration in the plasma of severely neutropenic human beings and we suggest that it plays a role in regulating the blood neutrophil concentration by causing release of neutrophils from the bone marrow.

A HUMORAL MECHANISM regulating the release of neutrophils from the bone marrow has been postulated to be one of two negative feedback loops controlling the blood neutrophil concentration; the other loop is thought to mediate neutrophil production, possibly by influencing the rate of stem cell differentiation. Experimental evidence for a humoral mechanism regulating neutrophil release includes the accelerated release of granulocytes from isolated rat hind limbs or femurs following perfusion with plasma from neutropenic animals, and the rapid production of neutrophilia in normal dogs after infusion of plasma from dogs made neutropenic by vinblastine or nitrogen mustard. We have studied the effect on the blood leukocyte concentration of the reinfusion of plasma obtained during reversible neutropenia following antineoplasic chemotherapy in three patients. Plasma obtained following recovery was reinfused as a control.

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MATERIALS AND METHODS

Informed consent was obtained from the three patients studied. Plasmapheresis of 1 or 2 units of blood was carried out using the Fenwal Plasmapheresis Double Blood Pack (PA220), each whole blood bag containing 75 ml of acid citrate dextrose (ACD), U.S.P. Formula A. The blood was centrifuged in a Sorvall RC-2 at 4000 rpm (4470 g) for 10 min. Plasma was then expressed into the transfer packs, frozen at -18°C, and the red cells returned to the donor.

Plasmapheresis was performed in each patient during neutropenia following chemotherapy and when the blood neutrophil concentration had returned to normal. Recovery of bone marrow granulocyte reserve was assessed (in two patients) by the blood neutrophil increment following the injection of endotoxin (Pyrexal). When recovery had occurred, the effects of neutropenic-phase and control plasma were tested in each donor on separate days. Plasma (314–446 ml) was thawed at room temperature and infused over a 30-min period. Capillary blood for total leukocyte counts and differentials was obtained at the beginning and at the end of the infusions and at 30, 60, 120, 180, 240, and 300 min afterward. Total leukocyte counts were done on an electronic counter (Coulter Counter, Model A, Coulter Electronics, Hialeah, Fla.). Two hundred cell differential counts were done on Wright's stained blood smears. Neutrophils were classified as segmented forms only when a clear filament between lobes was discernible. The ranges of normal for the different leukocyte types were those of Orfanakis et al. Serum 11-hydroxycorticosteroids were determined by a fluorometric method.

CASE REPORTS

Case 1

A.A. is a 66-yr-old man who underwent total laryngectomy and radical neck dissection for carcinoma of the left pyriform sinus in 1962. A recurrent lesion at the base of the tongue and a cervical metastasis were found in March 1969. Prior to radiation therapy, he was treated with 30-hr infusions of methotrexate at 2-wk intervals, at doses ranging from 120–180 mg/sq m. The fourth dose, 150 mg/sq m, was given on June 4, 1969. Plasmapheresis was carried out on June 11 (total leukocytes 1600 cu mm and total neutrophils 128/cu mm) and again on June 18 (total leukocytes 5100/cu mm and total neutrophils 3370/cu mm). The response to endotoxin was subnormal (peak neutrophil increment 1800/cu mm at 4 hr) on June 20 but normal on July 1 (peak neutrophil increment 11,360/cu mm at 33½ hr). The control plasma was reinfused on July 2 and that from the neutropenic phase on July 3.

Case 2

R.S. is a 40-yr-old man with Hodgkin's disease since 1965, currently Stage IV-B. Previous therapy included radiation, nitrogen mustard, chlorambucil, vinblastine, and vincristine. He had been well controlled with oral vinblastine (supplied by Eli Lilly & Co. as an investigational drug), 7.5 mg daily, for 18 months, but because of relapse, on February 24, 1970 the daily dose was increased to 10 mg and neutropenia developed. Vinblastine was stopped and on March 5, he was given 0.01 mg/kg of vincristine. Plasmapheresis was carried out on March 12 (total leukocytes 2690/cu mm, total neutrophils 215/cu mm) and on April 3 (total leukocytes 10,050/cu mm, total neutrophils 6340/cu mm). An endotoxin test was not done. The neutropenic-phase plasma was reinfused on April 16 and the control plasma on April 18.

Case 3

W.L. is a 30-yr-old man with Stage IV-B Hodgkin's disease since January 1970. He received cyclic combination chemotherapy delivered in 6-wk periods, with 2-wk intervals of no therapy. The third cycle was begun on March 12, 1970 with 0.4 mg/kg of nitrogen mustard. Vincristine 1.4 mg/ sq m was given intravenously (i.v.) March 13, March 19,
and March 26. Prednisone was given orally in a daily dose of 80 mg from March 13 to April 2, 40 mg from April 2 to April 9, 20 mg from April 9 to April 12 and then further tapered and stopped by April 16. Vinblastine 6 mg/sq m i.v. was given on April 2 and April 9. Procabazine 100 mg/day orally was given from April 2 to April 16, then withheld because of neutropenia. Plasmapheresis was carried out on April 17 (total leukocytes 1980/cu mm, total neutrophils 1130/cu mm) and on May 8 (total leukocytes 4890/cu mm, total neutrophils 3860/cu mm). The endotoxin response on May 11 was normal (peak neutrophil increment at 4 hr of 4300/cu mm). The neutropenic-phase plasma was reinfused on May 15 and the control plasma on May 19.

RESULTS

Neutrophils (Table 1, Figs. 1 and 2)

Reinfusion of the neutropenic-phase plasma from two markedly neutropenic patients (A.A. and R.S.) was associated with a prompt increase in blood neutrophil concentration, evident at the end of the infusion and reaching a maximum level 2.5–3.5 times the baseline value 30–60 min postinfusion. The increase in the concentration of non-segmented cells was particularly marked, increasing from about 600/cu mm to over 5000/cu mm in both patients. Reinfusion of the control plasmas did not produce an early neutrophilia, although R.S. developed a slow rise in both segmented and nonsegmented forms, with maximum levels at 4 and 3 hr, respectively. The maximum increment for the latter was 550/cu mm and the ratio of nonsegmented to segmented forms actually decreased.

The reinfusion of plasma in W.L., whose neutropenia was much less pronounced when plasma was obtained, was not associated with any rise in total blood neutrophil concentration. A slight decrease following both neutropenic-phase and control plasma was, in fact, observed. The nonsegmented granulocyte concentration rose by 200 and 770/cu mm, respectively, but the segmented neutrophil concentration decreased.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma Volume (ml)</th>
<th>Total Leukocyte Concentration (cu mm)</th>
<th>Total Neutrophil Concentration (cu mm)</th>
<th>Time of Neutrophil Peak (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>Peak</td>
<td></td>
</tr>
<tr>
<td>A.A.</td>
<td>56 kg</td>
<td>446</td>
<td>8,870</td>
<td>28,000</td>
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<tr>
<td>NP</td>
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<td>314</td>
<td>7,390</td>
<td>9,940</td>
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<tr>
<td>R.S.</td>
<td>64 kg</td>
<td>366</td>
<td>8,500</td>
<td>18,950</td>
</tr>
<tr>
<td>NP</td>
<td></td>
<td>421</td>
<td>8,130</td>
<td>12,150</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W.L.</td>
<td>75 kg</td>
<td>315</td>
<td>5,180</td>
<td>4,930</td>
</tr>
<tr>
<td>NP</td>
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<td>313</td>
<td>10,380</td>
<td>8,630</td>
</tr>
</tbody>
</table>
Fig. 1.—Segmented neutrophil response to autologous plasma. Numbers in parentheses refer to the total neutrophil concentration when the plasma was obtained. Horizontal lines indicate the normal range of blood segmented neutrophil concentration; solid squares, neutropenic-phase plasma; open squares, control plasma.

Lymphocytes (Fig. 3)

Both plasmas from A.A. were obtained when he was lymphopenic. Reinfusion of each was associated with a rise in blood lymphocyte concentration: one from lymphopenic to normal levels and the other from normal to supranormal levels.

Fig. 2.—Nonsegmented neutrophil response to autologous plasma. Numbers in parentheses refer to the total neutrophil concentration when the plasma was obtained. Horizontal lines indicate the normal range of blood nonsegmented neutrophil concentration; solid squares, neutropenic-phase plasma; open squares, control plasma.
The lymphocyte concentration in R.S. when he was plasmapheresed was in the lower range of normal on both occasions. Upon reinfusion of neutropenic-phase plasma when he was mildly lymphopenic, the lymphocyte concentration rose sharply, and then fell. Infusion of control plasma at a time when the lymphocyte concentration was normal caused little change.

W.L. was markedly lymphopenic when both plasmas were obtained and even more so when they were reinfused. No significant changes were noted.

**Monocytes (Fig. 4)**

Reinfusion of neutropenic-phase plasma in A.A. and R.S. was associated with transient monocytosis as well as neutrophilia. Little change was seen in W.L. No exact correlation with monocyte blood concentration at the time of collection of plasma was evident since infusion of control plasma also was associated with monocytosis in one patient (A.A.).

**Eosinophils (Fig. 5)**

Reinfusion of neutropenic-phase plasma in A.A. and R.S. was associated with a rise in eosinophils, which was also found after reinfusion of control plasma in R.S. No increase was found in W.L. No adverse symptoms were noted during or after any of the infusions. Cortisol concentrations in serum...
Fig. 4.—Monocyte response to autologous plasma. Numbers in parentheses refer to the monocyte concentration when the plasma was obtained. Horizontal lines indicate the normal range of blood monocyte concentration; solid squares, neutropenic-phase plasma; open squares, control plasma.

Fig. 5.—Eosinophil response to autologous plasma. Numbers in parentheses refer to the eosinophil concentration when the plasma was obtained. Horizontal lines indicate the normal range of blood eosinophil concentration; solid squares, neutropenic-phase plasma; open squares, control plasma.
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drawn simultaneously with plasma in patient R.S. were 16 μg/100 ml during the neutropenic phase and 15 μg/100 ml during the control phase (normal range 12–24 μg/100 ml).

**Discussion**

The blood neutrophil concentration is determined by the rate of input of neutrophils from the bone marrow, the rate of egress into the tissues, and the equilibration between the circulating and marginal granulocyte pools. An increase in blood neutrophil concentration accompanied by a marked rise in the ratio of nonsegmented to segmented neutrophils represents an increased rate of neutrophil release from the marrow; as shown by DFe32P labeling, neutrophilia following the administration of endotoxin or steroids in man, or the infusion of neutropenic-phase plasma or induction of infection in dogs, is due primarily to marrow release. In contradistinction, exercise-induced neutrophilia due to demargination is not associated with an increase in the ratio of nonsegmented to segmented neutrophils. The rapid rises in both total blood neutrophil concentration and ratio of nonsegmented to segmented cells observed following the administration of neutropenic-phase plasma in A.A. and R.S. are almost certainly, by analogy, due to rapid marrow release.

Features of the response in our patients and in the animal studies that suggest that endotoxin is not the cause of neutrophilia include: (1) the early appearance of neutrophilia as compared to that following endotoxin; (2) the absence of lymphopenia; and (3) the identical processing of control plasma and neutropenic-phase plasma.

The early neutrophilia and lymphocytosis in our studies qualitatively and kinetically are different from those reported following i.v., i.m., or oral administration of adrenal corticosteroids. Direct measurements in the postendotoxin plasma of dogs suggested that the neutrophilia-inducing activity was not due to steroids. In R.S. there was no significant difference between the cortisol concentration of the neutropenic-phase and control sera.

Various other humoral factors causing neutrophilia have been reported. Extracts of a mouse mammary tumor associated with leukocytosis caused neutrophilia within 3–7 hr in normal mice. Neutrophilia-inducing activity has been demonstrated in the plasma of rats with radiation-induced neutropenia, after leukopheresis and endotoxin, leukopheresis of one member of a pair of parabiotic rats produced neutrophilia in both members. Activity has been reported in postendotoxin rabbit serum, and in rabbit plasma following irradiation and leukopheresis. Postendotoxin dog serum or plasma produces neutrophilia in other dogs, which is not due to endotoxin itself. Relevant studies in man have been few. Human plasma injected into rabbits and rats produced neutrophilia within hours, but no clear distinction between normal and neutropenic-phase plasma could be made. The infusion of neutropenic-phase plasma from a patient with periodic neutropenia into a normal individual did not produce neutrophilia, however, no details regarding donor blood neutrophil concentration or the volume of plasma used were given.
Evidence for the existence of a humoral mechanism causing marrow neutrophil release in man following endotoxin injection, but distinct from it, has been presented. The kinetics of the neutrophilia induced by reinfusion of autologous plasma were similar to those found in the present studies.

Both plasmas with neutrophilia-inducing activity were also associated with increases in monocyte, eosinophil, and lymphocyte blood concentrations as well, although the timing and correlation with concentration of these cell types at the time of obtaining the plasmas is not clear. Further studies in normal individuals regarding marrow release of these elements as well as reticulocytes are needed, since the activity observed in our studies may not be specific for neutrophils.

Two reasons may explain the lack of any demonstrable neutrophilia-inducing activity in patient W.L.: (1) he received a somewhat smaller dose of neutropenic-phase plasma per unit of body weight than did the other patients (4.25 ml/kg versus 8.1 and 5.8 ml/kg); (2) his neutropenia was much less marked than that of the other patients (1130 cells/cu mm versus 128 and 215 cells/cu mm) and the concentration of neutrophilia-inducing activity may therefore have been much less. By analogy, the tibial content of mature neutrophils in mice 4 days after irradiation and in control animals is proportional to the logarithm of the blood neutrophil concentration. The normal response to endotoxin in this patient ruled out an inadequate marrow neutrophil reserve.

When factors causing release of neutrophils have been demonstrable, large amounts of plasma have been necessary: 5–60 ml/kg in various animals. In the only comparable human studies, 5–8 ml/kg post-endotoxin plasma was active. These quantities approximate those used in our patients. The source of neutrophilia-inducing activity is unknown. The spleen has been implicated in rats but not in dogs.

Neutrophilia-inducing activity, acting rapidly to cause release of young neutrophils from the bone marrow, cannot be referred to as “leukopoietin” since, as yet, no effect on neutrophil production has been demonstrated. An increase in production may well occur primarily or secondarily following release of neutrophils from the marrow. This material may be the final effector agent of such neutrophilia-inducing stimuli as infection, endotoxin, corticosteroids, leukapheresis, immunologically induced neutropenia, or pulmonary sequestration of neutrophils during hemodialysis.

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