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Neutrophil Releasing Activity in Plasma of Normal Human Subjects Injected with Endotoxin* (32774)

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In dogs injected with endotoxin[2] or with vinblastine sulfate or nitrogen mustard (1) neutrophilia-inducing activity was demon-

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strable in plasma harvested during the period of drug induced neutropenia or during the period of recovery from neutropenia. Normal plasma or plasma harvested either before neutropenia developed or after recovery from neutropenia contained no such activity. The neutrophilia induced by infusion of such plasma resulted from an accelerated rate of release of neutrophils from the storage pool of the bone marrow to the blood. Studies were carried out which suggested that this activity was not due to endotoxin or elevated levels of cortisol or epinephrine in the plasma.

Other investigators have demonstrated neutrophilia inducing activity in the plasma of rats (2), rabbits, and dogs (3,4) injected with endotoxin and in rats rendered leukopenic by peritoneal lavage (5). Such activity may have been demonstrated in still other species and under still different conditions by other investigators as well (6).

We undertook the present study to determine if neutrophilia inducing activity was demonstrable in the plasma of normal human subjects injected with endotoxin.

Methods. The 16 normal human subjects were volunteers from the Utah State Prison. These male subjects ranged in age from 21 to 54 years and in weight from 52 to 92 kg.

The endotoxin used was Piromen (a lipopolysaccharide derived from Pseudomonas, 4 μg endotoxin/ml, Travenol Laboratories, distributed by Flint Laboratories). It was injected intravenously, undiluted.

Plasma was collected in the following fashion. Approximately 600 ml of whole blood was collected in plastic bags (Cutter; ACD-B, 65 ml/bag) by gravity after puncture of an antecubital vein. The bags were centrifuged at a force of at least 1000*g* for 1 hour at 4°C. Plasma was removed by suction into an empty evacuated glass bottle and care was taken not to transfer erythrocytes or buffy coat. The plasma was then frozen at —10°C for 1–3 days and thawed just before infusion. Erythrocytes were returned to the donor as soon as the plasma was separated. Sterile, pyrogen free glass and plastic were used in all procedures and all plasma transfers were carried out in a closed system. In 12 instances (4 normal and 8 postendotoxin plasma infusions) 2 units of plasma were infused sequentially. Such "double" plasmas were collected at 1- to 2-day intervals and plasma collection was begun each day at the same interval after injection of endotoxin or saline.

Plasma was infused beginning at 8 to 8:30 a.m. at a rate of approximately 15 ml/min. All infusions were with autologous plasma and the volume of plasma which was infused ranged from 250 to 630 ml. Leukocyte counts were done on 2 samples collected from an antecubital vein. Counts were done immediately before injection of endotoxin or saline; at 1,2,3,4,6,8,12, and 24 hours thereafter; immediately before and after plasma infusion and at 1,2,3,4,5,8,12, and 24 hours after completion of the infusion. Total leukocyte concentration was measured with a Coulter counter (7). Two hundred cell differential leukocyte counts were performed on Wright's stained coverslip smears from each sample. Neutrophils were differentiated as to segmented (a fine filament separating at least two of the lobes) and nonsegmented cells.

A control sample was obtained from each subject between 7:30 and 8:30 a.m. on each day that he was studied, so that from 3 to 9 control counts were made on each subject. In 15 of 16 subjects control counts (of either total neutrophil or nonsegmented neutrophil concentration) did not vary by more than 50% from the first control count. Results from one subject whose control counts varied by as much as 300%, were excluded from analysis of the data. In the remaining 15 subjects, individual control neutrophil concentration (per mm^3) ranged from 1200 to 13,600 (median of 4200) and % nonsegmented neutrophils (as percent of total neutrophils) from 5 to 39 (median of 15).

Results. The effect of graded doses of endotoxin upon blood neutrophils. The effect of 8, 12, 16 and 18 μg of endotoxin upon total and nonsegmented neutrophil concentration in blood is illustrated in Fig. 1.

Twelve, 16, and 18 μg induced more of an increase in total neutrophils than did 8 μg ($p = <.05$), but the degree of neutrophilia did not differ significantly between the three larger doses. In all subjects, at each dose level, peak neutrophilia was reached at either 3 or 4 hours but the time at which the increase began was rather variable. After the peak was

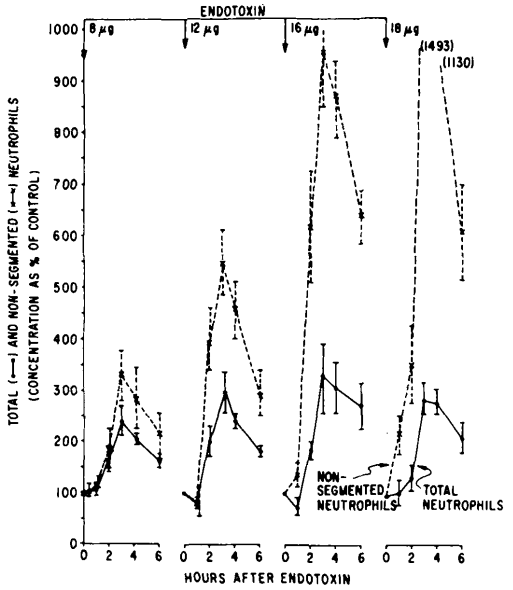

FIG. 1. The response of total and of nonsegmented blood neutrophils to graded doses of endotoxin. The mean and standard error for each point is illustrated. The number of subjects in each endotoxin group was 4, 4, 13, and 7, respectively.

reached, neutrophil concentration declined steadily and was usually normal by 24 hours after endotoxin.

An increment in the magnitude of increase in nonsegmented neutrophils was observed with each increase in the amount of endotoxin injected (Fig. 1). The peak value for nonsegmented neutrophils occurred at 3 hours in the average subject as did peak neutrophilia. The increase in nonsegmented cells usually was noted earlier and was of greater magnitude than the increase in total neutrophils.

Neutrophil releasing activity in postendotoxin plasma. Plasma collected from donors injected with endotoxin was compared to control plasma from donors injected with saline with respect to its effect upon blood neutrophils of recipients of autologous plasma. Plasma was harvested at intervals ranging from 0.5 to 2.5 hours after subjects were given 8–18 μg of endotoxin or from 1.5 to 2.0 hours after they were given saline.

Seven control plasmas were tested (Fig. 2). No significant increase was induced in total or nonsegmented neutrophil concentration.

The results following the infusion of postendotoxin plasma were analyzed on the basis of whether the donor was or was not releasing neutrophils from the marrow at a more rapid than normal rate when the plasma was collected. At the time when control plasma was collected the proportion of nonsegmented neutrophils ranged from 50 to 150% of control values. Therefore, donors injected with endotoxin were considered to be releasing at a normal rate if the proportion of nonsegmented neutrophils had not increased to more than 150% of control values at the time plasma was collected. Eleven of a total of 20 plasmas infused were included in this group. Donors with nonsegmented neutrophils in excess of 150% of control values at the time when postendotoxin plasma was collected were considered to show accelerated release. Nine of the total of 20 plasmas infused were included in this group.

The effect upon neutrophils of postendotoxin plasma obtained from donors releasing at a normal rate did not differ significantly from that of normal control plasma (Fig. 2). Postendotoxin plasma harvested from donors releasing neutrophils at an accelerated rate induced a significant increase in both total and nonsegmented neutrophils (Fig. 2). Immediately after infusion of this plasma there was a slight increase in total neutrophil concentration and this value was significantly higher than after control plasma infusion at 1, 2, 3, and 4 hours ($p = <0.05$ by t test). The mean and median for peak neutrophilia was 2 hours following completion of the infusion. The increase induced in the concentration of nonsegmented neutrophils was twice that induced in total neutrophils. Nonsegmented neutrophils were significantly increased at the end of the infusion, reached a peak 1 hour later and remained significantly increased until 6 hours after infusion.

Postendotoxin plasmas obtained from subjects who were or were not releasing neutrophils at an accelerated rate were compared with respect to the volume of plasma collected, the dose of endotoxin injected and the time of collection after endotoxin injection. The volume of control plasmas, postendotoxin plasmas collected during accelerated release or postendotoxin plasmas collected during normal

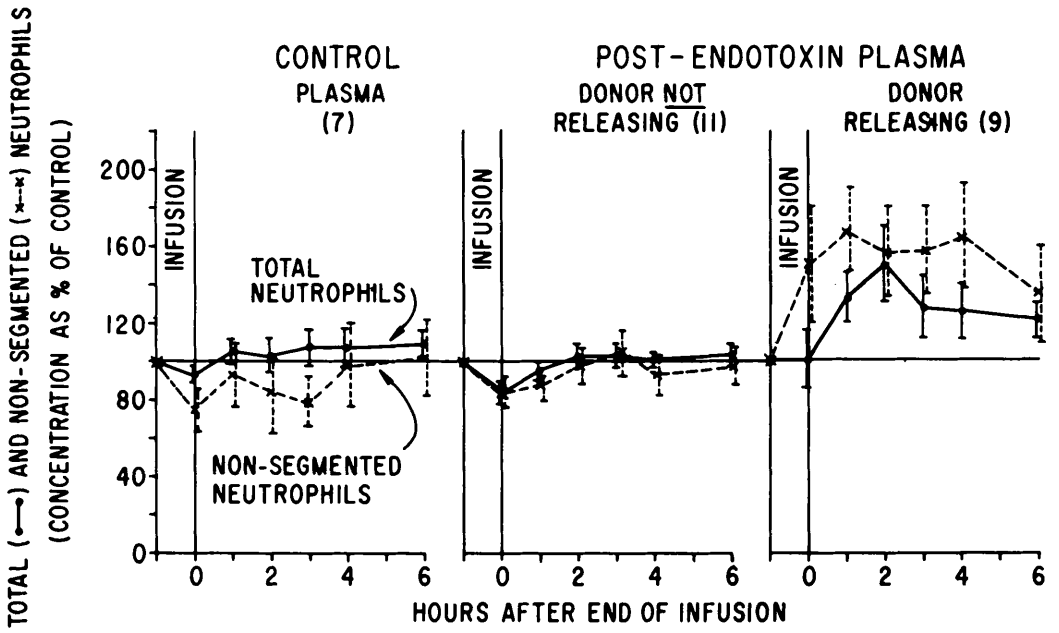

FIG. 2. The effect of control plasma and post-endotoxin plasma upon total and nonsegmented neutrophil concentration. The mean and standard error for each point are illustrated and the number of observations contributing to each point is indicated by the numbers in parentheses. "Donor not releasing" indicates that at the time the plasma was collected, the donors' nonsegmented neutrophils were within the normal range while "donor releasing" indicates infusions of plasma collected at a time when the donors' nonsegmented neutrophils were increased. See text for further details of this distinction as well as for details concerning dose of endotoxin, plasma volume, and time of plasma collection.

release was similar (mean of 440, 480, and 490 ml, respectively). Accelerated release plasma was collected at an average time of 1.8 hours after endotoxin (range of 1.0–2.5) and normal release plasma was collected at an average time of 1.5 hours after endotoxin (range of 1.0–2.0). Accelerated release was occurring during plasma collection in 8 of 12 instances after 12–18 μg of endotoxin was injected as compared to 1 of 8 after 8–12 μg. However, the effect of postendotoxin plasma upon neutrophils could not be explained merely on the basis of endotoxin dose. Results were analyzed separately for each dose of endotoxin and revealed a significant difference between accelerated release plasmas and plasmas obtained before accelerated release had occurred.

Discussion. These results suggest that in man, as in dogs (4),[2] rats (2), and rabbits (3), a factor appears in plasma following the administration of endotoxin which accelerates the rate of release of neutrophils from bone marrow when the plasma is infused.

That this is an endogenously produced factor rather than injected endotoxin persisting in the plasma at the time of harvest is suggested by certain observations. The onset of the effect of active plasma, whether measured by total or nonsegmented neutrophil concentration, occurred more rapidly than did the effect of endotoxin injection and the peak effect was also reached more rapidly. Furthermore, activity was demonstrable in post-endotoxin plasma only if harvested when the donor was releasing marrow neutrophils into the blood more rapidly than normal. Plasmas were inactive if harvested after endotoxin injection but before the donor began to release neutrophils at an abnormal rate or if harvested from donors who showed but a minimal response to endotoxin. If the activity was due to persisting endotoxin in plasma, then the activity should have declined steadily after

endotoxin injection instead of correlating with the donor's neutrophil releasing response. Thus, in man as in dogs,[2] if the activity in postendotoxin plasma is endotoxin, injected endotoxin must be cleared from plasma and then return in a form in which its biologic effects differ from those of the originally injected material.

In man as in the dog,[2] the changes induced in the percentage of nonsegmented neutrophils provide a more sensitive index for detecting a change in release rate from marrow than do changes in the total neutrophil concentration.

Whether neutrophil releasing activity in human subjects injected with endotoxin is due to a factor similar to that which appears in dogs (1,4),[2] rats (2,5), and rabbits (3) after injection of endotoxin, vinblastine sulfate, or nitrogen mustard, or after leukopheresis will require further study. At present it cannot be determined with certainty whether this factor(s) is a physiologic substance responsible for controlling the rate of release of neutrophils from bone marrow. However, the likelihood of the latter possibility is enhanced by the observation that the time at which activity appears in plasma is correlated with the occurrence of an accelerated rate of release in the donor, whereas plasma obtained at other times is inactive (1,3,4)[2] or at least less active (3,6).

Summary. Normal human subjects were injected with endotoxin and their plasma was harvested at various times thereafter. This plasma was later infused into the same subject, to determine if neutrophilia inducing activity was demonstrable in such plasma.

Infusion of normal control plasma induced no significant change in total neutrophil concentration and nonsegmented neutrophils tended to decline after such infusions.

Plasma collected after injection of endotoxin did not induce a significant change in blood neutrophils as compared to control plasma if obtained at a time when no significant increase in the rate of release had developed in the donor. Postendotoxin plasma, collected when the donor was releasing neutrophils at an abnormally rapid rate, induced a significant increase in both total and nonsegmented neutrophils.

Infusion of active postendotoxin plasma induced a more rapid onset of neutrophilia than did endotoxin injection. This observation and the inactivity of certain plasmas which were collected after the same dose of endotoxin yielding active plasma suggested that the activity of postendotoxin plasma was not representative of persistence of the injected endotoxin.

This study, in conjunction with similar studies in other species, suggests that the rate of release of neutrophils from the marrow to the blood may be controlled by a humoral factor.

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