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The Blood and Bone Marrow Neutrophil Response to Graded Doses of Endotoxin in Mice.* (32598)

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A change in neutrophil concentration in venous blood can be effected by a change in the rate at which neutrophils are released to the blood from the marrow, by a change in the rate of outflow of neutrophils from the blood, by a change in the proportion of neutrophils in blood which are marginated along vessel walls rather than circulating freely, or by any combination of these 3 mechanisms.

It has been suggested that endotoxin administration is associated with an increase in the rate at which neutrophils are released from the marrow to the blood(1,2), that it influences the proportion of neutrophils which are marginated along vessel walls(3,4) and also effects the rate at which neutrophils leave the blood(5).

That the effect of endotoxin upon neutrophil kinetics is somewhat complex is suggested by studies in which the response of the blood neutrophil concentration has been compared after different doses of endotoxin. Neutropenia is the 1st change observed after the injection of endotoxin in dogs(6) and the degree and duration of the neutropenia increase as the dose of endotoxin is increased. After relatively small doses of endotoxin, neutropenia is followed by neutrophilia and the height of the neutrophilia increases as the dose of endotoxin is increased. However, Sheagren *et al*(7), who did not follow counts beyond 5 hours, failed to observe neutrophilia in the monkey after very large doses of endotoxin had been given. Neutropenia was not observed after small doses in the rabbit(8,9) or monkey(7). A clear relationship of neutrophil changes to the dose of endotoxin administered also was not observed in the mouse(10) or in man(11,12).

With the development of technics for determining the total number of neutrophils in the marrow of a mouse(2,13), it is now possible to correlate changes occurring in blood and marrow neutrophils simultaneously. Such correlations were used in the present study in an attempt to more definitively characterize the effect of endotoxin upon neutrophil kinetics. For this purpose, mice were injected with increasing amounts of endotoxin and the effect of endotoxin upon the total number of neutrophils in the marrow and their concentration in blood was determined at various times thereafter.

Materials and methods. Female mice (C57B1 ♀ × DBA ♂)F_1, bred in our laboratory from parent stock purchased from Jackson Laboratory, weighing 18-22 grams and approximately 8 weeks of age were used in all experiments. Mice were housed 8-10 per cage and Purina Lab Chow and water were available *ad libitum*. Animals were injected intraperitoneally with either 0.01 μg, 0.1 μg, 0.2 μg, 5.0 μg, or 25.0 μg of *S. typhosa* endotoxin (Lipopolysaccharide B-0901, Difco Laboratories) in a volume of 0.1 cc isotonic saline.

Blood for leukocyte counts was collected from the orbital sinus in heparinized micro-

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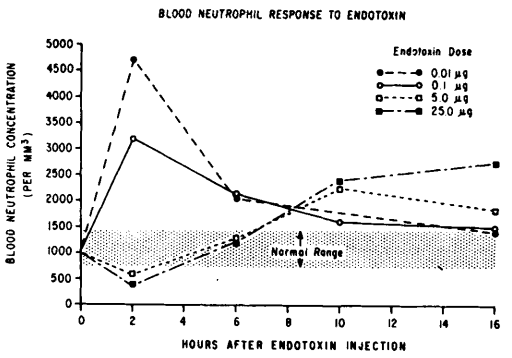


FIG. 1. Blood neutrophil response to endotoxin. Each point represents the mean neutrophil count of 4 - 14 mice.

hematocrit tubes as described previously(13). 7 consecutive tubes were collected from each mouse and leukocyte counts were done on blood from the 1st and 7th tube. 40 lambda of blood were diluted 1:100 in 1% centrimide solution and counted electronically(14) Coulter Electronics—Model B, Hialeah, Florida). The percent of leukocytes which were neutrophils was determined from a 200 cell differential count of a smear stained with Wright's stain.

6 and 16 hours after the injection of endotoxin, animals were sacrificed and the humeri were removed and processed as described previously(13) to determine the total number of nucleated cells. Percent of neutrophils was determined from the % of peroxidase positive cells in smears of femoral bone marrow and the absolute count calculated from this percentage and the total nucleated cell count.

Results. Blood neutrophil response to endotoxin. Fig. 1. Following the injection of small doses of endotoxin (0.01 µg, 0.1 µg) neutrophilia, as measured in the 1st sample obtained from the orbital sinus appeared earlier and was of greater magnitude than that which occurred after 5 µg or 25 µg. The neutrophilia observed after 0.01 µg was slightly higher than after 0.1 µg, but the difference was not statistically significant ($p>0.1$). The response after large doses was quite different. Not until 6-8 hours following injection of 5 µg or 25 µg did neutrophilia become apparent, a time when the neutrophilia related to smaller doses was declining. By 16 hours neutrophils returned to near normal levels after the smaller doses, but remained elevated after the larger doses. Neutropenia was not observed following the smaller doses, but was marked and persisted for several hours after the larger doses.

In another experiment, neutrophil determinations were done at 15, 30 and 60 minutes following the injection of 0.2 µg of endotoxin; neutropenia was not detected.

In normal mice the leukocyte count from the 1st tube of blood obtained from the orbital sinus is twice as high as is the count in the 7th consecutive tube of blood from the same sinus (13). However, differential leukocyte counts are the same in both samples(13).

Counts in the 7th sample were compared to counts in the 1st sample in mice which had been injected with 0.1 µg, 5.0 µg, or 25.0 µg of endotoxin or with saline (Fig. 2). After endotoxin administration, the degree of decrease of leukocytes in the 7th sample was less than is normally observed. 2 hours after endotoxin, the proportional degree of decrease was inversely related to the dose of endotoxin, but at longer intervals this dose relationship was no longer present.

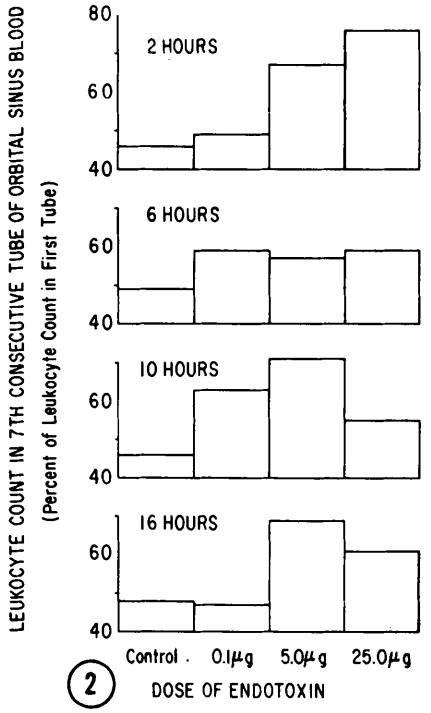
Bone marrow neutrophil response. In order to correlate changes occurring in the marrow with those observed in the blood the total number of neutrophils in a mouse humerus was determined 6 and 16 hours following injection of endotoxin. The absolute number of neutrophils remaining in the marrow after various doses of endotoxin is seen in Fig. 3. The absolute number of neutrophils in the humerus of control animals averaged 2.9×10^6. Significant reductions ($p<0.05$) occurred following each dose of endotoxin administered. The degree of decrease paralleled the increase in dose. 6 hours following 0.01 µg or 0.1 µg, respectively, the doses which resulted in an earlier and greater degree of blood neutrophilia, 14% and 20% of neutrophils had been released from the marrow. 6 hours after 5 µg, or 25 µg, when blood neutrophilia was not yet apparent, 33% and 38% of neutrophils respectively, had been released. At 16 hours neutrophils tended to be higher in the marrow than at 6 hours, but only the change noted after 5 µg was statistically significant ($p< 0.02$).

Discussion. In response to increasing doses

of endotoxin, a progressive decrease in the number of neutrophils was observed in the marrow six hours following endotoxin administration. A dose response relationship between the concentration of neutrophils in the blood and the dose of endotoxin was also evident at 6 hours, but this was the reverse of that observed for the number of cells released from the marrow. As the dose of endotoxin was increased, the concentration of neutrophils in the blood decreased.

We suggest that these relationships are explained as follows (Fig. 4). There are at least 2 dose related kinetic events in response to endotoxin; the rate at which cells leave the blood and the rate at which cells leave the bone marrow. With small doses of endotoxin, the increase in rate of release from the marrow exceeds the increase in rate of loss from the blood. As the dose is increased, both rates are accelerated, but the rate of acceleration of outflow from the blood is greater than is the rate of acceleration of release from the marrow. Consequently with larger doses, more cells are lost from the blood than are released to the blood from the marrow and neutropenia results. An alternative possibility, but 1 for which no supporting data are available is that cell destruction occurs in the marrow and/or within the circulating blood stream and may in part be responsible for the neutropenia.

An hypothesis which suggests that the direct effect of endotoxin is to increase outflow



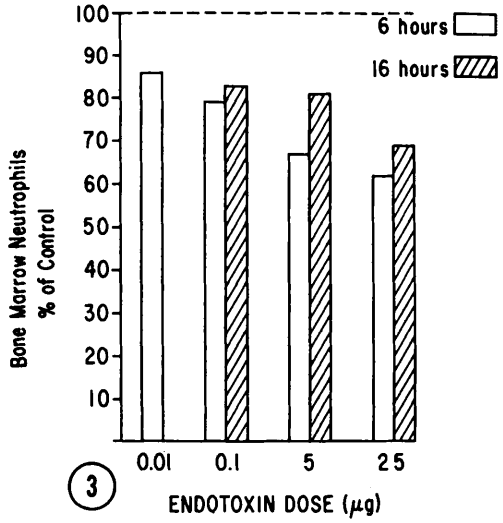


FIG. 2. Leukocyte count in 7th consecutive sample of orbital sinus blood. Values are expressed as percent of leukocyte count in first orbital sinus sample.

FIG. 3. Bone marrow neutrophil response to endotoxin.

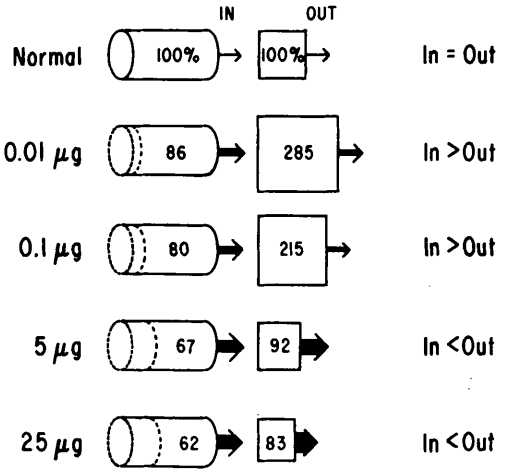


FIG. 4. Neutrophil kinetics following endotoxin. Summary of events in first 6 hours. The numbers in the boxes indicate the change induced by endotoxin in marrow and blood as % of the control. *In* and *Out* refer to input to the blood from the bone marrow and outflow from the blood, respectively.

from the blood and that release from the marrow is a secondary phenomenon can be supported. After endotoxin administration a substance has been demonstrated in the plasma or serum of rats(15), rabbits(16) and dogs(17,18) which accelerates the rate of release of neutrophils from the marrow of normal animals and which appears not to be residual endotoxin. Furthermore, after the administration of large doses of tritiated endotoxin, the label appears in large numbers of neutrophils in the capillaries of the lung although very few labeled cells are present in the circulating blood(19). These observations permit the following tentative hypothesis concerning the mediation of the effect of endotoxin upon neutrophil kinetics. Injected endotoxin is taken up by blood neutrophils. Endotoxin damages the cells, perhaps by acting as a lysosome labilizer(20) and these damaged cells are removed from the circulation. The reduced number of blood cells, by some mechanism triggers accelerated release from the marrow, probably by means of a humoral releasing factor. With small doses of endotoxin, the compensatory increase in release exceeds the rate of loss and neutrophilia develops. In contrast, with larger doses release fails to compensate adequately for cell loss and a prolonged period of neutropenia is observed. This would explain the failure to detect neutropenia after smaller doses of endotoxin.

Our data are also compatible with the possibility that the administration of endotoxin is associated with a change in the proportion of neutrophils which are marginated along vessel walls. Athens *et al*(1), studying human subjects injected with endotoxin whose neutrophils had been labeled with radioactive diisopropylfluorophosphate, observed that the marginal granulocyte pool was increased at a time when the number of circulating cells was slightly decreased. Smith *et al*(10) interpreted a change in the relationship of tail counts to orbital sinus counts in endotoxin injected mice as being most compatible with an increase in the relative proportion of marginated cells.

We are as yet uncertain how the decrease in concentration of leukocytes in successive tubes of blood from the orbital sinus of a mouse is to be correctly interpreted. However, the change in relationship between the 1st and 7th tube after endotoxin suggests that some type of change has occurred in the relative proportion of neutrophils in the circulating and marginal blood pools.

Summary. Studies were undertaken to characterize more definitively the effect of endotoxin upon neutrophil kinetics. The data presented herein indicate that the neutrophil response to endotoxin in the mouse is dose related. With relatively small doses of endotoxin, neutrophilia appeared earlier, was of greater magnitude and was not preceded by neutropenia. After larger doses, neutropenia persisted for several hours and was later followed by neutrophilia.

The response in marrow neutrophils was determined at 6 and 16 hours following the injection of endotoxin in order to correlate changes occurring in the marrow with those observed in the blood. Smaller doses of endotoxin resulted in an earlier and greater degree of blood neutrophilia than did larger doses but fewer cells were released from the marrow after small doses than after large doses.

At least 2 dose related kinetic events explain the neutrophil changes occurring after the administration of endotoxin. With small doses of endotoxin, the acceleration of release rate from the marrow exceeds the rate of loss from the blood. Larger doses have a more profound effect than small doses in accelerating the rate of release of neutrophils from the marrow but they have an even greater effect on acceleration of the outflow of neutrophils from the blood.

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Heterotypic Antibody Responses in Man Following Vaccination with Adenovirus Type 1 Hexon Antigen. (32599)

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Recent evidence suggests that a rise in serum neutralizing antibody following immunization with either fiber or hexon antigens of adenovirus types 1 and 4 is protective(1,2). Furthermore, volunteers vaccinated with fiber antigen of type 1 virus showed a high relative frequency of neutralizing and hemagglutination-inhibition (HI) antibody rises to other adenovirus serotypes(3). An analysis of these responses according to the immunologic classification of adenoviruses and the status of pre-existing antibody revealed that all rises in heterotypic antibody were confined to group 3, the group to which the vaccine type belonged. This report will describe the heterotypic antibody responses to adenoviruses that developed in adult volunteers after administration of an adenovirus type 1 hexon antigen vaccine.

Materials and methods. Vaccine preparation and immunization schedule. Procedures for the preparation, assay an safety-testing of the adenovirus type 1 hexon antigen vaccine administered to the participants in this study have been previously described(4). In brief, the hexon antigen was obtained by fractionation of virus suspensions by column chromatography with DEAE-cellulose. The fraction containing the bulk of the antigen was rechromatographed twice.

The volunteers received the hexon antigen preparation as follows: 8 received 1.0 ml intramuscularly on the 1st and 8th day; 2 received a single injection of 2.0 ml on the 1st day; one man received 2 ml initially and 1 ml on the 8th day.

Source of serum specimens. Paired serum specimens from 11 adult male volunteers who were immunized with an adenovirus type 1 hexon antigen vaccine preparation were used in this study(4). The 1st serum specimens were obtained before vaccine administration and the 2nd specimens were collected 28 days after vaccination. All of the individuals were antibody negative ($<1:4$) to type 1 adenovirus before vaccination but developed a 4-fold or greater rise in serum neutralizing antibody titer by 28 days.

Serologic tests. Neutralization and hemagglutination-inhibition (HI) tests were done