Neutrophil Releasing Activity in Rats Injected with Endogenous Pyrogen (36334)

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The elaboration of an endogenous plasma factor which accelerates the rate of release of marrow neutrophils to the blood has been demonstrated in the plasma of rats (1, 2), dogs (3), and humans (4). The nature and origin of this endogenous factor has not been established. The rate of release of neutrophils from marrow has also been shown to be affected by the administration of endotoxin (5–7). Although precautions have been taken to eliminate endotoxin from the effect produced by the endogenous plasma factor, there would still appear to be some possibility of an interrelationship (8).

In some of our recent studies we found that many of the effects produced in the host by administering endotoxin are acting through an endogenous factor from leukocytes (9–11). Thus, the production of acute phase globulin (9), lowering of plasma iron (10) and plasma zinc (11) can result from a protein identical with, or similar to, endogenous pyrogen. The results of this investigation suggest that neutrophil releasing activity can be added to the list of effects of this low molecular weight protein(s).

Methods. Measurements of blood leukocytes were made in female Holtzman rats weighing approximately 200 g. Heart blood, collected with a heparinized syringe from anesthetized rats, was used for total and differential leukocyte counts. Samples of blood were diluted 1:200 with Turk's diluting fluid; and total leukocyte numbers were determined in a hemocytometer. The percentage of leukocytes that were neutrophils was determined from a 200-cell differential count of a smear stained with Wright's stain. Individual rats were used only once in the time course experiments.

Endogenous pyrogen was prepared from rabbit and rat peritoneal leukocytes by the method of Kaiser and Wood (12). Partial purification was achieved by the modified butanol–methanol method described by Rafter *et al.* (13). The special precautions used to avoid bacterial or endotoxin contamination have been previously described (9, 10, 14). Prior to use in these experiments this partially purified extract produced a febrile response in rabbits made refractory to endotoxin and lowered plasma iron in endotoxin-tolerant rats (10). After partial purification the protein content of the extracts from rabbit cells averaged 126 μg from 1×10^8 peritoneal leukocytes.

Results. The peripheral blood neutrophils at various times after an ip injection of endogenous pyrogen are shown in Fig. 1. The endogenous pyrogen obtained from 1×10^8 rabbit peritoneal leukocytes was injected into each rat. There was a significant lowering of the blood neutrophils 30 min after injection. After 45 min, the neutrophils started to increase; and by 1 hr, they had increased 3-fold. The number of neutrophils remained at this increased level for several hours but had almost returned to normal by 16 hr. Endogenous pyrogen prepared from rat peritoneal leukocytes also produced about a 3-fold increase above normal at 6 hr after injection of the extract from 1×10^8 cells/rat.

The changes in blood lymphocytes and total blood leukocytes with time after injection of endogenous pyrogen are shown in Fig. 2. The total number of blood leukocytes increased at 45 min and remained slightly above normal for 24 hr. The number of lymphocytes was depressed below normal between 1 and 6 hr after injection of endogenous pyrogen.

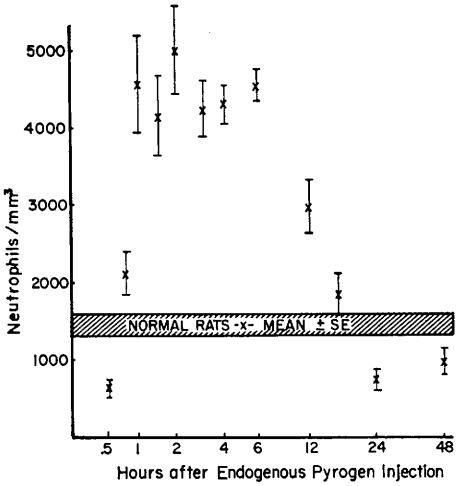

FIG. 1. Time course change in number of rat neutrophils after an ip injection of the endogenous pyrogen obtained from 1 × 10^8 rabbit leukocytes: Each point is the average value for groups of 6–20 animals with the brackets showing the standard error.

The effects of varying the dose of endogenous pyrogen are shown in Fig. 3. There was a direct relationship between the dose and the number of neutrophils in the peripherial blood at 6 hr after injection.

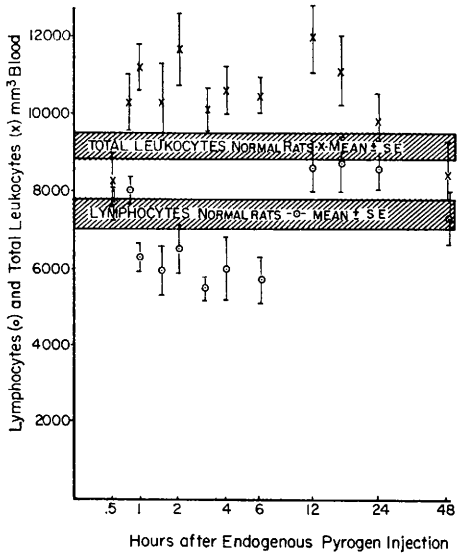

FIG. 2. The effect of endogenous pyrogen on the number of blood lymphocytes and total leukocytes in rats at various times after an ip injection of the extract from 1 × 10^8 PMN rabbit leukocytes.

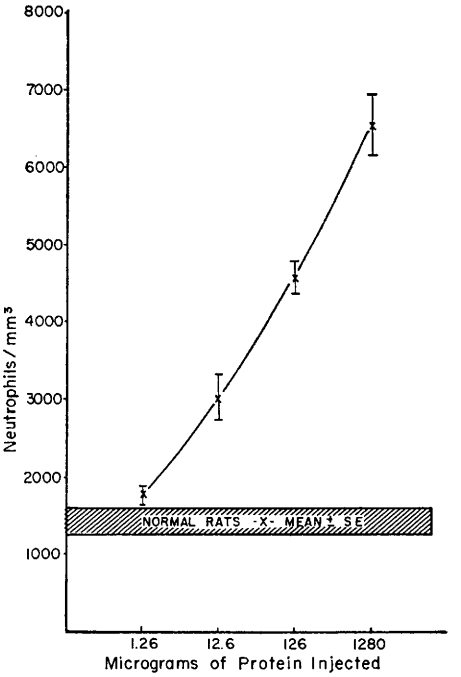

FIG. 3. The effect of varying doses of endogenous pyrogen upon the number of blood neutrophils in the rat: The measurements were made in groups of 12 animals 6 hr after an ip injection of endogenous pyrogen prepared from rabbit PMN leukocytes. The partially purified endogenous pyrogen from 1 × 10^7 leukocytes had a protein content of approximately 12.6 μg.

Discussion. It seems unlikely that the neutrophil release described in this investigation was due to endotoxin. In addition to the usual precautions taken to avoid contamination (12), the extracts were checked for febrile response in refractory rabbits (10). The activity could be destroyed by heat, proteolytic enzymes, and reagents that oxidize sulfhydryl groups; whereas these treatments are ineffective against endotoxin. The linear response to increasing dosage shown in Fig. 3 is quite different from the response to graded doses of endotoxin (6). The onset of the increase (Fig. 1) with endogenous pyrogen is faster than we or others (6) had observed with low doses of endotoxin.

Endotoxin injection not only accelerates the release of neutrophils from the marrow but also accelerates the loss of neutrophils from the blood (6, 15). A tentative hypothe-

sis of neutrophil kinetics after endotoxin has been suggested by Chervenick *et al.* (6). This hypothesis indicates that endotoxin is taken up by, and then damages, the blood neutrophils. The damaged cells are removed, and the reduced number of cells somehow triggers the humoral neutrophil releasing factor. Our investigation suggests that, after the endotoxin is taken up by the neutrophils, the endogenous pyrogen that would be formed acts as a neutrophil releasing factor.

The effect that endogenous pyrogen has on neutrophil release from bone marrow fits in quite well with exisiting theories of neutrophil kinetics after endotoxin or during periods of inflammation (4, 6, 15). Less clear, however, are the possible interrelationships of endogenous pyrogen to neutrophil kinetics in the normal animals. It is unlikely that endogenous pyrogen would be involved as a humoral releasing factor under normal conditions. However, in the search for a normal humoral releasing factor, if there is one, care must be taken to avoid not only endotoxin (8), but the more difficult to distinguish endogenous pyrogen. Evidence for a humoral neutrophil releasing factor not associated with endogenous pyrogen was shown in studies by Katz *et al.* (2). They found neutrophil releasing activity in the plasma of leukocytapheresed rats, but it did not induce a febrile reaction in rabbits. Other experiments where interference by endogenous pyrogen would seem improbable are those of Boggs *et al.* (3). They observed a neutrophilia activity in plasma or serum of dogs during the period of recovery from neutropenia induced with vinblastine sulfate or nitrogen mustard.

Summary. Partially purified endogenous pyrogen from rabbit polymorphonuclear leukocytes, when injected in rats, will cause neutrophil release from bone marrow. It is suggested that the production of this protein by endotoxin-damaged (activated) leukocytes is responsible for the accelerated release of marrow neutrophils during periods of infection or inflammation.

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