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Failure of Neutrophilia to Increase the Number of Neutrophils Entering a Peritoneal Exudate in Mice[1] (38775)

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It is well-known that the number of neutrophils entering an exudate is reduced in the presence of severe neutropenia (1). However, whether neutrophilia does or does not lead to an increased number of neutrophils entering an exudate has not been delineated clearly. In man, the only neutrophilic situation in which this has been evaluated to our knowledge is in chronic myeloid leukemia (CML). Boggs (2) considered the number and rate of appearance of cells in experimentally induced exudates to be normal in neutrophilic patients with CML. Other investigators have agreed with that observation (3, 4) while still others have reported delays in cell egress to be present (5, 6) or have reported an increased number of neutrophils in such exudates (7). Since the number of neutrophils appearing in exudates of patients with CML did not increase in proportion to the degree of blood neutrophilia, "decreased leukocyte clearance," it was suggested that this represented a defect in cell migration in CML (7, 8).

In studies of a transplanted tumor which produces marked neutrophilia in mice, we collected data suggesting the neutrophilia might be due to accumulation of leukocytes rather than increased neutrophil production (9). Studies of endotoxin-induced peritoneal exudates in such mice with a 20-fold or more increase in blood neutrophils revealed that they had no greater migration of neutrophils into an exudate than did controls (9). However, interpretation of this observation was difficult. The unanswered question in interpreting the above studies in CML or in a murine "leukemoid reaction" is; does an inflammatory signal call for a fixed number of neutrophils to enter an exudate perhaps depending on the degree of inflammation or does it call for a percentage of blood cells passing the inflammatory site in a fixed period of time? With either CML or the leukemoid reaction it could be argued that cells were abnormal.

To collect further data on this question the number of neutrophils entering endotoxin-induced peritoneal exudates in mice rendered neutrophilic by bleeding (10) or by infection with the intestinal nematode *Nematospiroides dubius* (11) was compared to normal controls.

Materials and Methods. Mice used in bleeding experiments were female $B_6D_2F_1$(C57BL/J6 female × DBA/$_2$ male) (Jackson Laboratory) which were approximately 7 mo old and weighed 30–42 g. Mice infected with parasites were outbred Swiss CD-1 (Charles River Laboratory) males which were approximately 7 mo old and weighed 40–50 g. Control and experimental groups were matched for age and weight.

In mice bled to induce neutrophilia, approximately 0.4 ml of blood was removed from the orbital sinus 2 hr before endotoxin injection (10). Blood leukocyte counts and smears for differential counts (300 cells) were from orbital sinus blood and leukocyte counts were determined electronically (12). Blood leukocyte counts were done just before endotoxin was injected to begin the exudate and 22 hr later, just before mice were killed.

Primary infections with the intestinal parasite *N. dubius* were produced by administering 200 third-stage infective larvae by stomach tube and secondary infections were produced by giving 200 larvae to previously infected mice. Both primary and secondary infection produces neutrophilia which lasts for 3 or more wk (11). Endotoxin (*E. coli*, Difco) was diluted to 100 μg/ml with

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TABLE I. FAILURE OF NEUTROPHILIA, PRODUCED BY BLEEDING OR INFECTION, TO ALTER THE NUMBER OF PERITONEAL NEUTROPHILS AFTER ENDOTOXIN INJECTION.[a]

	No. of mice/group	Blood neutrophil concentration $\bar{X} \pm$ SE (thousands)		Total number of neutrophils in peritoneal cavity 22 hr after endotoxin $\bar{X} \pm$ SE (millions)
		Before endotoxin injection	22 Hr after endotoxin injection	
Bleeding studies				
($B_6D_2F_1$ mice) Controls	8	1.7 ± .31	2.3 ± .17	3.1 ± .48
Bled group I	4	4.2 ± .84	2.0 ± .26	3.8 ± .41
Bled group II	5	3.6 ± .70	1.9 ± .24	2.9 ± .76
Bled group III	5	3.1 ± .58	2.5 ± .24	2.5 ± .12
Infection studies				
(Swiss mice) Controls	6	1.6 ± .26	3.1 ± .43	15.2 ± 3.22
Primary infection				
At day 5	4	6.1 ± 2.27	5.3 ± 1.04	15.5 ± 5.50
At day 8	5	19.4 ± 2.68	13.5 ± 3.69	8.4 ± .53
Secondary infection				
At day 5	5	10.8 ± 2.34	11.7 ± 2.65	13.0 ± 4.15
At day 8	5	19.8 ± 2.40	17.0 ± .50	10.6 ± 3.20

[a] Mice were bled 2 hr before 10 μg of endotoxin was injected ip or were infected with *N. dubius* 5 or 8 days before. The number of neutrophils in the peritoneal cavity was determined 22 hr after endotoxin was injected ip.

sterile pyrogen-free saline and 10 μg was injected ip. Pilot studies indicated that maximal numbers of neutrophils were present in the cavity at 20–24 hr after this dose so a 22-hr interval was chosen for study. Mice were killed by cervical dislocation and immediately afterward 12 ml of 1% K_2EDTA in normal saline was injected ip. The abdomen was massaged for 15–20 sec, opened, and a portion of the injected solution was aspirated. Leukocyte concentration of peritoneal fluid was measured, an aliquot was centrifuged at 100*g* for 10 min and the resultant cell pellet was resuspended in human sera and smears were made and Wright stained for 300 cell differential counts. Total neutrophils per peritoneal cavity was calculated from the percentage on smears, leukocyte concentration, and volume of fluid injected. Virtually no neutrophils were found in the peritoneal cavity of either normal or infected mice if endotoxin was not given.

Results. Normal mice, given endotoxin ip, have many neutrophils in the peritoneal cavity by 22 hr; an average of 3 million in $B_6D_2F_1$ mice and 15 million in Swiss (Table I). The two strains were used since the prior studies (10, 11) had been done in these strains. Some degree of neutrophilia is induced by the endotoxin injection as counts taken 22 hr after injection were slightly higher than when endotoxin was injected.

Neutrophil concentration in bled mice was approximately twice as high as that of controls when endotoxin was injected but there was no significant difference between blood neutrophils in bled as compared to control mice 22 hours later. The number of neutrophils in the peritoneum did not differ between bled mice and controls (Table I).

Blood neutrophil concentration in groups of infected mice ranged from 4 to 12 times that of controls when endotoxin was injected (Table I). In all groups neutrophil concentration still exceeded that of controls when the exudate was studied 22 hr later. There was no significant difference in the number of neutrophils in the peritoneal cavity of infected mice as compared to controls although the 8-day postprimary infection group tended to have less.

Linear regression analysis comparing individual blood neutrophil counts before endotoxin injection to peritoneal counts failed to yield a significant value in any experiment (r = .168 and .405). Blood neutrophil counts obtained when mice were killed and peritoneal counts did not correlate significantly (r = .186 and .257).

Discussion. These results suggest that the

number of neutrophils entering an exudate is not increased when blood neutrophils are increased above normal. Similar results were obtained in mice with a leukemoid reaction to a transplanted tumor (9). Pharmacologic doses of the adrenal glucocorticosteroids lead to decreased exudate cells in the presence of neutrophilia (1), but this observation may have no bearing on the current question. Leukocyte diapedesis is only one part of the complex and presumably interrelated series of events recognized as acute inflammation (13). While it is assumed that generation of chemotactic factors is the most important factor in attracting neutrophils to the inflamed area, changes in other factors such as blood flow and vascular integrity may also be important in neutrophil emigration. It is tempting to speculate that the number of neutrophils called for by an inflammation is directly proportional to the amount of chemotactic stimulus generated and that the amount of stimulus is in turn proportional to the severity of the inflammatory process. Our results would be compatible with such speculation but in view of the complexity of the inflammatory process this may be an oversimplification. Leukocytes entering the inflammed area might be capable of inactivating factors involved in chemotaxis, thus providing a self-limiting mechanism for cellular immigration. If there is a continuing need for neutrophils, as in a chronic bacterial abscess, migration continues for many days; but if the inflammatory stimulus is self-limited, as when killed bacteria are added to an induced exudate, the period of neutrophil migration is brief (1).

These studies do not prove that an increase in completely normal neutrophils might not yield different results. Although there is no reason to think that a neutrophil abnormality would be induced by bleeding or nematode infection, it can be argued that any of the methods producing neutrophilia might somehow alter blood neutrophils making them less likely to migrate into exudates or alter vessel walls, etc. Ideally, similar studies should be done in which enough normal blood neutrophils are transfused to produce neutrophilia, but this experiment is not practical with present neutrophil collection techniques (14).

Increased total egress of neutrophils from blood of man may be accomplished by increasing the fractional turnover rate of cells or by increasing the total number of neutrophils in the face of an actual decrease in fractional turnover rate (15). The current experiments suggest, but in no sense prove, that the fractional turnover rate is not the primary means of controlling cell loss; at least as loss is reflected by exudate migration and as long as neutrophils are above normal number.

These results suggest that studies of the number of neutrophils entering exudates in patients with CML may need to be reinterpreted. The suggestion that the failure of the number of exudate neutrophils to rise as blood neutrophils rise represents an abnormality of cells in patients with CML (7, 8), may not be valid.

Summary. Neutrophilia, produced by bleeding or by parasitic infection, did not result in a larger than normal number of cells migrating into endotoxin-induced peritoneal exudates. These results fail to support but do not disprove the concept that neutrophil loss from the blood may be controlled primarily by changes in the fractional turnover rate rather than by blood pool size. They indicate that the failure of exudate neutrophils to increase in proportion to blood neutrophils in patients with chronic myeloid leukemia may not be an abnormality as interpreted previously.

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