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Neutrophil-Releasing Activity in Plasma of Normal Human Subjects Injected with Etiocholanolone (39904)

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Introduction. Leukocytosis, characteristically a neutrophilia, occurs commonly in the acute phase of many infectious and inflammatory diseases. This increase in blood neutrophils has been attributed primarily to the accelerated release of mature neutrophils from the bone marrow reserves (1). Numerous animal experiments (2-5) and a few studies in man (6) have determined that a humoral factor, called "leukocytosis-inducing factor," "leukocyte-mobilizing factor," "neutrophil-releasing factor," or other similar names, is measurable in the plasma after an inflammatory stimulus.

Etiocholanolone injection induces fever and an acute inflammatory reaction in man (7). It has been widely used to measure the bone marrow granulocyte reserves because a predictable granulocytosis occurs 12 to 18 hr following its intramuscular injection (7). To study the mechanism of the neutrophilia induced by etiocholanolone and to develop a model for investigating the mechanisms of inflammation-induced neutrophilia in man, studies were undertaken to determine if neutrophil-releasing activity is present in the plasma of normal subjects injected with etiocholanolone.

Materials and methods. Sixteen normal subjects of both sexes (ages 19 to 25 years) were used. Informed consent was obtained. The subjects were taking no medications during this study. They were given etiocholanolone (0.3 mg/kg) intramuscularly (prepared in propylene glycol by the Pharmaceutical Development Service of the Clinical Center, NIH). At 1.5, 5, 8, 10, 12, or 17 hr after the etiocholanolone injection, a plasmaphoresis was performed. Two subjects received 2.0 ng/kg of endotoxin (Lipexal, Dorsey Laboratories, Lincoln, Neb.) intravenously instead of etiocholanolone and were plasmaphoresed at 3 hr after the endotoxin injection to confirm previous studies, indicating that normal human subjects have a neutrophilia-inducing activity of their plasma under these circumstances (6).

The plasmaphoreses were performed in a standard fashion in the Blood Bank of the Clinical Center at the National Institutes of Health. At the specified time intervals following the injection, approximately 450 ml of venous blood was collected through a large-bore needle into a plastic bag (Fenwal Laboratories, Morton Grove, Ill.) containing 2250 USP units of heparin in 30 ml of buffered normal saline (pH 7.4) (final heparin concentration 5 units/ml). Clear plasma was obtained by centrifugation of the heparinized whole blood and the plasma was removed sterilely to a satellite plastic bag. A small amount of normal saline was added to the blood cell fraction and the cells were returned to the donor prior to performance of a second plasmaphoresis. With these methods, approximately 500 ml of heparinized plasma was obtained from each donor and stored at $-70°$ in the plastic bag for 5 to 10 days prior to reinfusing the donors with their own plasma.

The heparinized plasma was thawed in a water bath at room temperature and reinfused into the donors in a standard fashion. At approximately 8:00 AM, baseline leukocyte counts were obtained from the resting subjects, and they then remained at bed rest for the study period except for brief intervals to eat regular meals and for bathroom privileges. The plasma was returned to the subjects as rapidly as possible through a large vein, usually over a period of 5 to 10 min. The reinfusion of plasma in no instance was accompanied by any significant side effects. Samples of venous blood were obtained from the antecubital veins at 0.5, 1,

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2, 3, 4, 5, and 6 hr after the reinfusion to determine total white blood cell and differential counts by standard methods.

Results were expressed as the difference between the maximum neutrophil count for the 6 hr after the plasma infusion minus the baseline count. This simple method for expressing results was used because previous studies have established an excellent correlation of these values with the areas under the neutrophil response curves for agents causing a neutrophilic leukocytosis in man (8).

Results. Two control subjects who received no injection of either etiocholanolone or endotoxin did not have any substantive change in their blood neutrophil count when they were reinfused with autologous heparinized plasma which had been frozen for 5 days (Fig. 1). In contrast, two subjects given endotoxin 3 hr prior to plasmaphoresis both developed neutrophilia within 1 hr of reinfusing autologous plasma (Fig. 2), similar to the findings of previous studies (5).

Etiocholanolone regularly induced a blood neutrophilia by 12 to 18 hr after injection. The mean increase was 6750/mm^3 (range 3500 to 10,700 mm^3). After reinfusion of the autologous plasma obtained following etiocholanolone injection, the subjects generally developed an increase in blood neutrophils within 2 to 4 hr. Figures 3 and 4 illustrate typical curves for pairs of subjects whose neutrophil counts were measured over the 6 hr following the reinfusion of their plasma. For the group of 12 subjects given etiocholanolone, the responses became, in general, progressively larger as the time interval between the injection of etiocholanolone and the performance of the plasmaphoresis was increased (Fig. 5). Neither the etiocholanolone injection nor the infusion of postetiocholanolone plasma caused an increase in band neutrophils in the blood. None of the subjects developed a fever or any other symptoms as a consequence of the reinfusion of their own plasma.

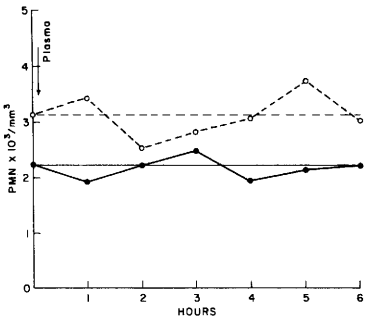

FIG. 1. Neutrophil counts following reinfusion of autologous plasma in two control subjects given no etiocholanolone or endotoxin injection.

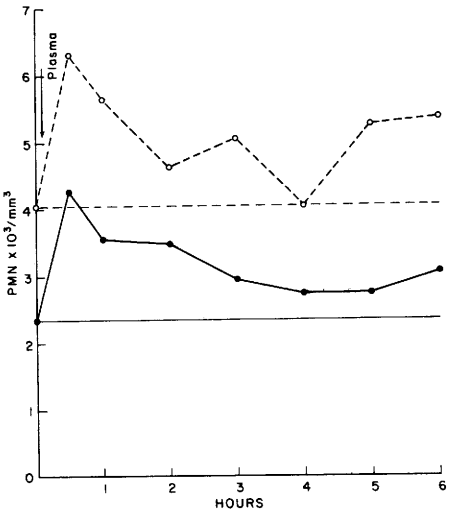

FIG. 2. Neutrophil counts for two subjects given postendotoxin plasma. The plasma was obtained 3 hr after the endotoxin injection.

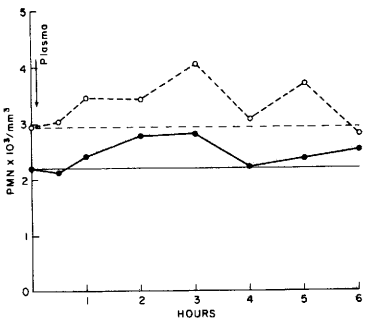

FIG. 3. Neutrophil counts for two subjects given postetiocholanolone plasma, obtained 1.5 hr after the etiocholanolone injection.

Discussion. There are two major classes of regulatory substances which are thought to govern the blood neutrophil count, substances affecting cell production and those affecting release of cells from the bone marrow into the blood (9). In this study, a

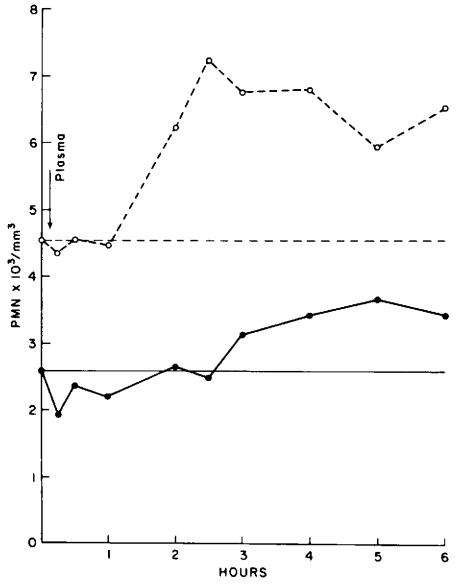

FIG. 4. Neutrophil counts for two subjects given postetiocholanolone plasma, obtained 12 hr after the etiocholanolone injection.

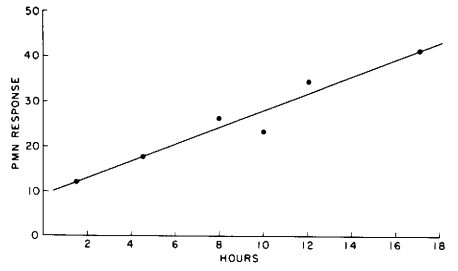

FIG. 5. Mean neutrophil responses to postetiocholanolone plasma for six pairs of subjects who had received the etiocholanolone injection from 1.5 to 17 hr prior to the plasmaphoresis. Neutrophil responses are expressed as the mean percentage increase of the maximum count over the baseline count.

neutrophil-releasing activity has been demonstrated in the plasma of human subjects given etiocholanolone. The occurrence of this type of a substance in man after this inflammatory stimulus corresponds to previous studies indicating the presence of a neutrophil-releasing activity in human plasma after endotoxin administration (5) and to animal studies which demonstrated neutrophil-releasing activity after leukaphoresis (2), the injection of endotoxin (10), antigen-antibody complexes (11), typhoid vaccine (2), and turpentine (12). Although some recent experiments suggest that etiocholanolone may also affect hematopoietic precursor cells to simulate cell division (13), only the acute effects of etiocholanolone to accelerate the release of mature neutrophils from the bone marrow were investigated in these present studies.

The most compelling evidence for the occurrence of neutrophil-releasing activity comes from the studies of Gordon and his colleagues in rats subjected to leukophoresis (2). These investigators and others (4, 14) have found that normal and parabiotic animals subjected to leukaphoresis by peritoneal lavage developed a plasma factor which will cause a neutrophilic leukocytosis when transferred to normal animals. In some animal experiments, endotoxin administration and leukophoresis will produce a profound and prolonged neutropenia. It has been postulated that it is the reduction in the blood neutrophil count with endotoxin and leukophoresis which stimulates the rise of neutrophil-releasing factors. Other investigations of this factor using triamcinolone (15) and turpentine (12), which do not cause neutropenia but which may stimulate neutrophil-releasing activity, suggest that the development of the plasma factor is not necessarily linked to the neutropenic response. Both animal and human studies suggest that this activity probably is due to activation of the complement system (11, 17).

The present experiments, although somewhat limited in scope, relate well to these earlier investigations. Etiocholanolone induces inflammation in man probably corresponding to the inflammation induced in animals by turpentine. Etiocholanolone, like endotoxin, is rapidly cleared from the blood after intramuscular or intravenous administration (18) and, therefore, the neutrophil-releasing activity of the plasma samples examined in these experiments is probably not due to residual etiocholanolone. Etiocholanolone, in contrast to endotoxin, does not cause leukopenia and does not raise serum cortisol levels (7), further supporting the view that neither neutropenia nor an intact adrenal gland is necessary for the development of neutrophil-releasing activity. Presently, it is not known if etiocholanolone administration alters complement

levels or activates the complement system. Because it has been amply demonstrated that etiocholanolone injections are well tolerated in man, further delineation of the physiologic role for neutrophil-releasing factor should be possible through investigations of this potent inflammatory agent.

Summary. Etiocholanolone was administered to normal subjects and heparinized plasma was collected at varying time intervals from 1.5 to 17 hr afterwards. Control subjects received either no injection or endotoxin, an agent known to increase neutrophil-releasing activity. Readministration of the autologous plasma to the control subjects who received no injection prior to the plasmaphoresis caused no neutrophilic leukocytosis. The plasma obtained after endotoxin and etiocholanolone administration showed neutrophil-releasing activity and the mean activity for pairs of subjects given etiocholanolone was progressively greater through 17 hr after the injection. This study, along with similar studies in human subjects and experimental animals, suggests that the release of neutrophils from the bone marrow is subject to humoral regulation.

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