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Endotoxin-Induced Release of Colony-Stimulating Activity in Man[1] (38911)

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Granulocyte and monocyte colony formation in softgel bone marrow culture is dependent on the presence of colony-stimulating activity (CSA), a heterogeneous substance believed to be a humoral regulator of granulopoiesis (1). In mice, CSA has been isolated from many tissues (2). In adult man, however, cells of the monocyte/macrophage complex (3–5) and lymphocytes responding to mitogenic or antigenic stimuli (6) appear to be the important sources of this regulatory material.

Although factors modulating the production and release of CSA are poorly understood, endotoxin from gram-negative bacteria is known to stimulate the release of CSA from mononuclear cells *in vitro* (7, 8). Injections of endotoxin in mice result in markedly augmented levels of CSA in the serum and increased granulopoiesis (9–11). Also, murine postendotoxin serum causes increased granulocyte production when administered to normal mice (12). The present report describes experiments showing that endotoxin administration in man results in the generation of increased serum CSA levels.

Materials and Methods. Six healthy adult volunteers gave informed consent for endotoxin administration. Fasting subjects were admitted to the Clinical Research Center at UCLA and remained at rest drinking only clear fluids during the first four hours of the experimental procedure. Piromen, a purified Pseudomonas endotoxin, was obtained from Flint Laboratories and injected intravenously in a dose of 0.1–0.15 μg/kg. Complete blood counts and differentials were performed prior to endotoxin administration and at intervals thereafter to 24 hr. Serum samples were obtained at appropriate intervals and assayed for CSA while fresh or after less than 3 weeks' storage at −20°.

Serum was assayed for colony-stimulating activity *in vitro* against normal human bone marrow obtained from unrelated healthy volunteers. A standard double-layer agar technique was employed (4). The double-layer system minimizes the effect of poorly diffusible inhibitors of colony formation present in the lipoprotein fraction of human serum (13, 14). Untreated serum (0.2 cc) was incorporated into a 0.5% agar underlayer and 2×10^5 normal human bone marrow cells were dispersed in a 0.3% agar overlayer. McCoy's 5A medium supplemented with 15% fetal calf serum and antibiotics was used throughout. Plates were incubated in duplicate or triplicate at 37° in a humidified atmosphere of 7.5% CO_2 in air. In selected assays, colony-stimulating cells were removed from the target bone marrow by an adherence procedure prior to culture (15). Control cultures contained either no stimulator in the underlayer or they contained 1×10^6 normal peripheral blood leukocytes or monocyte-conditioned medium (4).

Colonies of 20 cells or more were counted with a dissecting microscope in 10-day-old cultures. The minimum size criteria for a colony was reduced to 20 cells because colonies forming in response to serum were substantially smaller than those forming with white cell underlayers. Selected colonies were picked with a finely drawn pipette and smeared and stained with Giemsa for morphological examination.

Results. The administration of endotoxin produced variable symptomatology in the subjects. Some were aware of no changes, while others had shaking chills, suffused conjunctivae, and headache. Most noted

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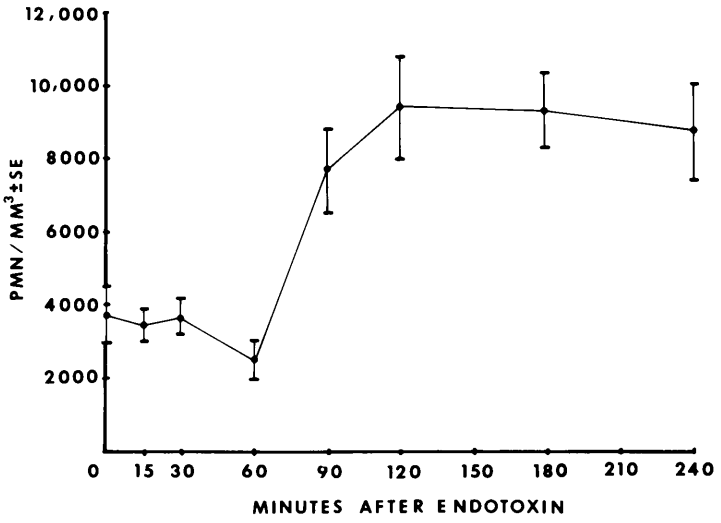

FIG. 1. Absolute neutrophil counts (PMN) in five subjects after endotoxin administration (0.1 μg/kg).

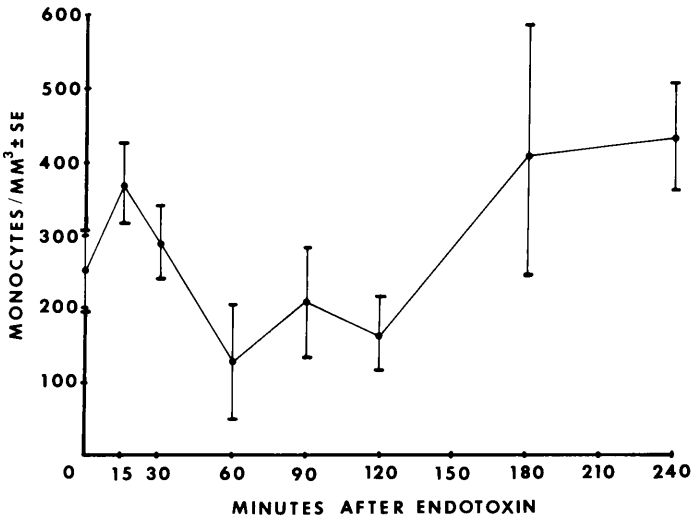

FIG. 2. Absolute monocyte counts in five subjects after endotoxin administration (0.1 μg/kg).

malaise and flu-like symptoms. Injection of endotoxin resulted in leukopenia followed by a rebound leukocytosis. Granulocytopenia was maximal at 60 min, and by 90 min there was a marked granulocytosis (Fig. 1). Monocytopenia was also produced by the endotoxin but the rebound monocytosis was of relatively small magnitude (Fig. 2).

Assay of CSA in serum showed a marked increase after endotoxin administration. Figure 3 gives composite data on six subjects. Maximum levels of CSA were achieved by 60 minutes and high levels persisted to 180 min. By 24 hr, serum CSA returned to base line levels. Post-endotoxin serum also stimulated colony formation in bone marrow from which adherent cells had been removed (Table I), suggesting that the mode of action of the serum stimulator was not absolutely dependent on the presence of colony-stimulating cells in the bone marrow. The colonies formed in response to serum were smaller than those seen in cultures with leukocyte feeder layers. Colonial morphology, however, appeared nor-

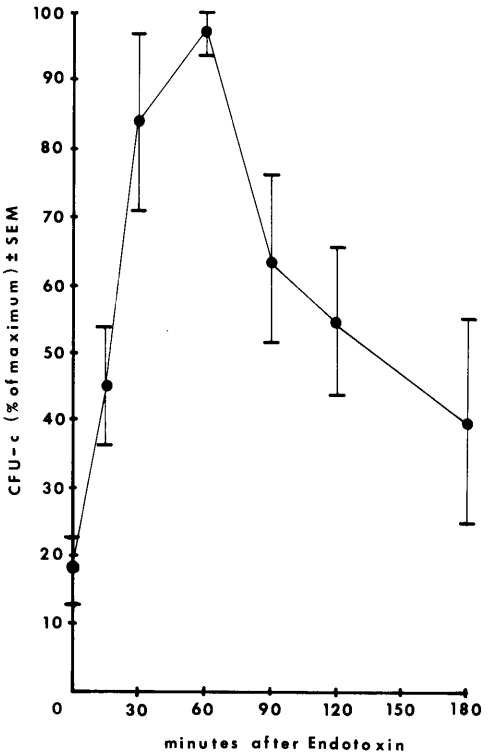

FIG. 3. Colony-stimulating activity in postendotoxin serum expressed as a percentage of maximum (6 studies). Maximum colony counts (CFU-C) for each target bone marrow ranged between 25–60 CFU-C/2 × 10^5 nucleated cells.

mal and the distribution of granulocyte and monocyte colonies was appropriate for the age of the cultures.

Discussion. Products of gram-negative bacteria are known to have potent effects on hematopoiesis in mammals. When endotoxin preparations are injected into animals, they result in a rise in serum colony-stimulating activity (9–11, 13), increased granulopoiesis (10, 12), and increased numbers of colony-forming cells (CFU-C) in the spleen and peripheral blood (16). Endotoxin also causes augmentation in total numbers and mobilization of pluripotent stem cells (CFU-S) and the fraction of such cells in DNA synthesis (13, 17, 18).

Endotoxin injections in man result in granulocytopenia consequent to margination and nonrecirculation of neutrophils. This is followed by a rebound granulocytosis associated with the release of immature neutrophils into the peripheral

TABLE I. ASSAY OF POSTENDOTOXIN SERUM ON BONE MARROW DEPLETED OF ADHERENT CELLS.

Time after endotoxin (minutes)	Colonies/2 × 10^5 cells
0	3 (0–6)
30	37 (37–43)
60	36 (35–37)
90	16 (14–18)
180	2 (1–3)

circulation (19). The degree of rebound neutrophilia has been taken as a measure of bone marrow reserve (20). Boggs and colleagues have provided good evidence for the appearance of a neutrophil-releasing activity in the serum of man after endotoxin administration (21). Others have shown in rats that the endotoxin-induced neutrophil-releasing activity is clearly separable from CSA and that both substances are endogenous products and not endotoxin itself (9).

The present studies were conducted to determine if endotoxin injected into man could produce a measurable rise in serum colony-stimulating activity. The assay of CSA from human sources is fraught with difficulty. Most assays have utilized murine bone marrow as a target cell population. Recently, however, it has been demonstrated that assays on mouse and human bone marrow lead to disparate results and that such measurements are not equivalent (22). Assays performed on human bone marrow present difficulties in standardization and comparability, and human bone marrow is rich in cells which produce CSA (1, 15). Furthermore, potent inhibitors to colony-stimulating activity have been found in human serum (1, 14). We have attempted to circumvent some of these problems by assaying untreated serum against human bone marrow from healthy subjects in a double-layer system which minimizes the effect of poorly diffusable inhibitors (4, 13).

Endotoxin administered to normal volunteers resulted in the previously described alteration in level of neutrophils and monocytes (19). The postendotoxin serum was found to stimulate granulocyte/monocyte colony formation in agar culture. Colonies also formed in cultured bone marrow which had been depleted of adherent cells (colony-

stimulating cells), indicating that the presence of such cells was not a prerequisite for stimulation by postendotoxin serum. Normal human serum has been reported to stimulate cluster formation (≥ 5 cells) from human bone marrow containing adherent cells (23).

The data presented indicate that in man, as in animals, endotoxin adminstration leads to elevated levels of serum CSA. The CSA levels were usually highest during the phase of leukopenia. It is not certain that the postendotoxin increase of CSA in the serum is derived from mononuclear cells although endotoxin stimulates production or release of CSA from these cells *in vitro* (8, 9). The present studies, however, provide evidence that granulopoietic humoral factors can be modulated in man by endotoxin administration.

Summary. Six healthy adult volunteers were injected intravenously with Pseudomonas endotoxin (Piromen) to determine effects on serum colony-stimulating activity (CSA). Serum was assayed with a double-layer agar cloning technique using normal human bone marrow as the target cell preparation. A marked increase in serum CSA was noted after endotoxin administration lasting up to 180 min. The colonies formed in response to postendotoxin serum were smaller than those seen with leukocyte feeder layers but were otherwise morphologically normal. These studies suggest that granulopoietic humoral factors may be modulated in man by endotoxin administration.

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