Canine Cyclic Hematopoiesis: Effects of Chronic Endotoxin Administration

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Endotoxin was given to grey collie dogs to investigate the mechanism of cyclic hematopoiesis. Over prolonged periods of daily administration, low doses of endotoxin (0.1 μg/kg/day) failed to change the cycling of blood neutrophils or reticulocytes. However, higher doses of endotoxin maintained at a plateau level (5 μg/kg/day) eliminated the cyclic changes in neutrophils and stabilized the reticulocyte and platelet counts in the normal range. Administration of endotoxin at this dose also eliminated the cyclical marrow differential count fluctuations characteristic of this disease. We infer from these results that endotoxin ameliorates the cyclical changes in blood cell counts by regulating hematopoietic proliferative activity at the stem cell level.

Cyclical hematopoiesis in grey collie dogs is an autosomal recessive disease characterized by a periodic decrease in bone marrow production of blood cells. Bone marrow transplantation studies showing abolition of cycling in affected puppies infused with normal marrow and development of cycling in previously normal dogs given marrow from affected dogs strongly suggest that this hematologic disorder originates within the stem cell compartment. The primary defect presumably lies in the regulation of stem cell proliferation, but the specific abnormality is not yet known.

Recently, Maloney et al. showed that progressively increasing doses of endotoxin ameliorate the neutrophil cycles in canine cyclic hematopoiesis. Whether or not endotoxin treatment alters the reticulocyte and platelet count cycling seen in these dogs was not determined, and bone marrow examinations were not reported.

To further investigate the mechanism of cyclic hematopoiesis we studied the effects of three regimens of endotoxin treatment on the blood and bone marrow differential counts in grey collie dogs.

MATERIALS AND METHODS

Dogs. Grey collie dogs, 2-8 mo old, weighing 5-12 kg, were individually caged in a temperature-controlled room and cared for as described previously.

Blood counts. White blood cell and platelet counts were performed by Coulter counter, total PMN were determined by 100-cell differential counts of Wright-stained blood smears, and reticulocyte counts were performed in standard fashion as described previously.

Bone marrow aspirates. Marrow was aspirated by a 16-gauge Rosenthal needle from the long bones and iliac crests, and direct smears of visible spicules were evaluated by 500-cell differential counts.

Endotoxin. Escherichia coli 127:B8 endotoxin (Difco, Detroit, Mich.) was diluted in saline and injected intravenously (i.v.) at 0.1 μg/kg/day in two dogs in the first series of experiments. In
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Fig. 1. Circulating neutrophil counts in grey collie given E. coli endotoxin 0.1 μg/kg/day i.v. for 3 wk. •, morning baseline neutrophil counts; ○, neutrophil counts 6 hr after endotoxin. On days 38–40 and 50 there was no increase in count after endotoxin.

Three other dogs Salmonella typhosa endotoxin (Difco, batch 3124 25) was given i.v. starting at 0.0005 μg/kg/day, increasing rapidly to 0.1 μg/kg/day; the dose was subsequently increased by increments of 0.5 μg/kg/day to a maximum dose of 30 μg/kg/day in two dogs (200 μg/day). One of these latter two dogs and the third dog were later given courses of endotoxin injections, beginning in the same fashion, to a maximum plateau dose of 5 μg/kg/day.

RESULTS

Blood counts. Daily doses of 0.1 μg/kg/day of E. coli endotoxin for 3 wk failed to alter the neutrophil cycle (Figs. 1 and 2) in two dogs. The increment in blood neutrophils 6 hr after endotoxin (Figs. 1 and 2), a measure of bone marrow neutrophil reserve, continued to show the cyclic changes seen in untreated grey collies.1
Higher doses of *S. typhosa* endotoxin markedly altered cycling of neutrophils, reticulocytes, and platelets (Figs. 3–5). In the first two dogs so treated, the endotoxin dose was progressively increased to 200 μg/day by 6 wk and was then discontinued (Figs. 3 and 4). One of these dogs developed azotemia and died of renal failure, possibly because of the endotoxin or chronic aminoglycoside
antibiotic therapy. The second dog tolerated the endotoxin well, except for failure to gain as much weight as a littermate; she resumed her cycling following discontinuation of the endotoxin and then responded to a second course of endotoxin by discontinuation of cycling again (Fig. 4). The third dog was given gradually increasing amounts of endotoxin up to 50 μg/day (5 μg/kg/day) and then maintained at that dose for 3 mo (Fig. 5).

*Marrow aspirates.* Bone marrow aspirates were not performed on dogs whose blood cell cycles were not altered by the endotoxin. In grey collie 032, given long-term endotoxin (Fig. 5), serial marrow aspirate differentials were obtained prior to institution of endotoxin (days 41–52) and after reaching the plateau dose of 50 μg/day (days 85–99) when cycling had apparently ceased. The sequential changes in marrow cell proportions are shown (Fig. 6) for each period. In the preendotoxin period the typical waves of granulocytic and normoblastic proliferation previously documented can be seen. In the endotoxin-treatment period these waves were eliminated; this is seen most clearly in the stable number of postmitotic neutrophils.

**DISCUSSION**

Cyclic hematopoiesis in dogs results in highly predictable changes in blood neutrophils, reticulocytes, and platelets. Previous studies have shown normal blood half-lives of both red cells and neutrophils, suggesting that periodic peripheral destruction does not account for the cyclic phenomenon. Marrow
studies have shown a wavelike proliferation of granulocytic and erythroid cells with short periods of almost absent precursors, implying a periodic failure of precursor cell proliferation. Transplantation of the disorder to irradiated normal dogs indicated that cyclic hematopoiesis is probably not a disease of the marrow microenvironment.

From these studies it has been inferred that the basic defect in cyclic hematopoiesis is a regulatory abnormality within the stem cell compartment. Several studies indicated that erythropoietin (ESF) and colony-stimulating activity (CSA) levels vary predictably with the cycle period, but whether these changes precede or follow the marrow changes is not known. Hypertransfusion completely eliminated reticulocyte cycling and presumably completely suppressed circulating ESF but did not alter the blood neutrophil counts; cycling of the reticulocytes reappeared following the gradual return of the hematocrit to prior levels. Thus although long-range humoral factors could be playing some role in the pathogenesis of this disease, it appears that some property of the stem cell itself or its relationship to other cells within the marrow space may cause the cyclic blood changes.

Endotoxin, derived from the cell walls of gram-negative organisms, has multiple effects upon the hematopoietic system. It has been shown to activate complement, to generate the potent chemotactic factors C3a and C5a, and to promote margination of circulating neutrophils in the capillary bed. In addi-
tion, endotoxin induces a humoral factor that releases mature PMN from storage in the marrow and a separate factor, CSA, that is thought to stimulate granulopoiesis. Finally, endotoxin acts to release committed granulocytic progenitor cells from the marrow into the circulation and may directly trigger resting stem cells into active cycle. Since any of the above effects could alter granulocyte production, attempts to modify the cycle in collies with cyclic hematopoiesis by chronic endotoxin administration were made.

Maloney et al. first reported that progressively increasing doses of endotoxin (4–31 μg/kg) could abrogate the neutrophil cycle in canine cyclic neutropenia. They observed, as did we using the same E. coli endotoxin, that this effect was not observed at very low doses. Reasoning that constant, low-dose endotoxin might induce tolerance, they gave progressively increasing doses of endotoxin. They observed that reticulocyte fluctuations tended to be less during the period of endotoxin treatment and absolute reticulocyte counts were lower in both affected and normal animals. However, their observation periods were relatively short, and it was unclear from their data whether endotoxin did or did not clearly eliminate reticulocyte cycling. They did not measure platelet counts or report bone marrow examinations.

In our studies we found that high doses of endotoxin eliminated cyclic neutropenia in grey collies. We observed that progressively increasing doses of endotoxin are not necessary for cycle elimination, suggesting that tolerance to this effect of endotoxin did not develop at the doses used and allowing us to observe the collies given endotoxin for longer periods. Visual inspection of the data presented shows an initial period of apparently damped oscillation followed by a stable count pattern not recognizably different from normal dog blood counts. The prominent swings of neutrophil counts to less than 1000/μl and of platelets to more than 600,000/μl are completely eliminated, while the pattern of reticulocyte counts is sharply altered at the beginning and end of endotoxin treatment periods. Statistical studies including periodogram analysis as performed previously confirmed the elimination of neutrophil cycling and showed that the phase relationships of the neutrophil, reticulocyte, and platelet count cycles prior to endotoxin treatment are reestablished after treatment (Hoffman HJ, Alling DW, Hammond WP, Dale DC: Unpublished data). This change in the blood is paralleled by a major shift of the marrow differential count. Before endotoxin treatment, there are large cyclic fluctuations in all types of marrow cells; with endotoxin the marrow differential count is constant throughout the cycle.

In an earlier report Patt et al. proposed that a restricted stem cell pool with an oscillatory efflux of cells to granulopoiesis and erythropoiesis caused cyclic hematopoiesis. This changing efflux to committed stem cell pools was thought to involve stem cell competition. The observation that platelets as well as neutrophils and reticulocytes cycle in this condition cannot be readily explained by simple competition between neutrophilic and erythroid cell lines. The observations by Adamson et al. that hypertransfusion and phlebotomy affected reticulocyte cycles but not neutrophil cycles also suggests that stem cell competition does not occur. The present report also stands against the original concept of stem cell competition, since at least three cell lines were affected by endotoxin simultaneously.
Several investigators have suggested that short-range cell-cell interactions in the marrow may govern the commitment of stem cells to the granulocytic pathway. \(^6\) Abnormal sensitivity to the regulatory signal in such interactions might well result in cyclic hematopoiesis. When combined with previous work, the endotoxin studies reported here support the view that endotoxin acts via an intramedullary effect upon stem cell proliferation. We believe that endotoxin most likely acts to change the cycle characteristics in canine cyclic hematopoiesis by triggering increased proliferative activity at the pluripotent stem cell level. This mechanism best accounts for the parallel changes observed for all types of blood cells in this report. Whether this mechanism involves a direct effect on these cells or may occur through short-range cell-cell interaction or secondary mediators should be the subject of further studies.

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