Cyclic Hematopoiesis

EFFECTS OF ENDOTOXIN ON COLONY-FORMING CELLS AND COLONY-STIMULATING ACTIVITY IN GREY COLLIE DOGS

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A B S T R A C T   Cyclic changes in blood neutrophil counts of grey collie dogs with cyclic hematopoiesis can be eliminated by daily endotoxin injections. Studies were performed to determine the mechanism whereby endotoxin alters this disease. Bone marrow granulocyte-macrophage progenitor cells (colony-forming cells [CFUc]) showed cyclic variation in the untreated grey collie, which was eliminated by chronic endotoxin treatment (*Salmonella typhosa* lipopolysaccharide W, 5 μg/kg per day). Similar cyclic variation in blood CFUc was eliminated by this treatment. Tritiated thymidine suicide of the marrow colony-forming cells failed to show cyclic changes to explain the marked swing in CFUc numbers in untreated grey collies. The thymidine suicide rates were not significantly changed by chronic endotoxin treatment. Similarly, serum colony-stimulating activity did not show cyclic variation with the cyclic neutrophil counts in untreated grey collies and was not altered by chronic endotoxin treatment. We suggest that endotoxin eliminates neutrophil cycling in cyclic hematopoiesis by a direct effect on the flux of pluripotent stem cells into the committed stem cell compartment and that this occurs independent of changes in serum colony-stimulating activity.

INTRODUCTION

Cyclic hematopoiesis in grey collie dogs serves as a model for the study of the regulation of blood cell production by the bone marrow. The regular 11–12-d cycling of all the blood cell counts appears to be caused by a periodic change in marrow production rates (1–3), not an abnormality in the rate of destruction or sequestration of cells in the circulation (2, 4, 5). The cycle length for reticulocytes, platelets, neutrophils, monocytes, and lymphocytes is precisely the same but the pattern of cyclic variation for each cell line is different, implicating the pluripotent stem cell as the site of the defect in this disease (3). Marrow transplantation studies have shown that the disorder can be eliminated by total body irradiation and bone marrow transplantation from a normal dog (6), and that the blood cycling can be produced in previously normal dogs by irradiation and infusion of grey collie marrow cells (7). Therefore, alteration of the intrinsic regulation of the stem cell pool or pools is thought to produce this autosomal recessive disease (4, 8, 9).

In 1974, Maloney et al. (10) reported that progressively increasing daily doses of endotoxin abrogate the cycling of neutrophil counts in this disorder. We have confirmed this finding and observed that endotoxin eliminates the apparent cycling of the reticulocytes and platelets as well as the cycling of the neutrophil counts (11). Bone marrow differential count fluctuations were also abrogated (11). Thus, chronic endotoxin administration eliminates the remarkably stable oscillation of blood cell production typically seen in dogs with this disease.

To attempt to clarify the mechanism whereby endotoxin modifies cyclic hematopoiesis and to investigate further marrow regulation in this disease, we have studied blood neutrophil variation, blood and marrow granulocyte-macrophage precursor cells (colony-forming cells [CFUc]),[1] and serum levels of colony-stimulating activity (CSA) in grey collies with cyclic neutropenia and grey collies during chronic endotoxin treatment.

METHODS

Dogs. Grey collie dogs, male and female, aged from 2 to 12 mo and weighing from 5 to 15 kg, were housed in

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[1] *Abbreviations used in this paper:* CFUc, colony-forming cells; CSA, colony-stimulating activity; PEDS, postendotoxin dog serum; PMN, polymorphonuclear neutrophil(s); [³H]TdR, titrated thymidine.

individual cages in temperature-controlled quarters for the duration of these studies. Normal mongrel dogs served as controls. Blood specimens were routinely drawn from the cephalic vein of unanesthetized dogs between 8:30 and 9:30 a.m. and intravenous injections were given, when indicated, immediately after specimen withdrawal.

Blood counts. Leukocyte counts were performed on EDTA-anticoagulated blood specimens with a Coulter counter (Coulter Electronics Inc., Hialeah, Fla.). 100 cell differential counts were performed on air-dried, Wright's-stained coverslip smears made with an automated spinner (Plat Engineering Co., Cheltenham, Pa.).

Bone marrow aspirates. For bone marrow studies, dogs were anesthetized with intravenous thiamylal sodium (Surital, Parke, Davis & Co., Detroit, Mich.), from 15 to 20 mg/kg. Marrow was aspirated in sterile fashion by No. 14 or No. 16 Rosenthal-type needle from the long bones and iliac crests directly into syringes that contained 100–200 U of beef lung heparin (Upjohn Co., Kalamazoo, Mich.). 500 cell differential counts of Wright-Giemsa-stained coverslip smears were made. For serial marrow aspirate studies, the bone marrow sites were rotated so that no bone was aspirated more often than once per week.

In vitro bone marrow culture. Growth of granulocyte-macrophage precursor cells (CFUc) was performed by a modification of the method of Marsh et al. (12). Tissue culture medium 199 with Hanks' balanced salt solution and L-glutamine (Microbiological Associates, Walkersville, Md.) was supplemented with L-asparagine, 40 μg/ml (ICN Nutritional Biochemicals, Cleveland, Ohio), pyruvate, 10 μM (Microbiological Associates), penicillin, 20 U/ml, and streptomycin, 20 μg/ml (referred to hereafter as "supplemented media").

Freshly aspirated heparinized marrow was diluted in 5 ml of supplemented media, layered over 3 ml of Ficoll-Hypaque solution (Ficoll, Pharmacia Fine Chemicals, Piscataway, N. J.; Hypaque, Winthrop Laboratories, Sterling Drug Co., New York) (d 1.074–1.076, osmolarity 295–300 mosM) and centrifuged at 400 g for 35 min at 4°C. The cells at the interface zone were removed, washed three times, and resuspended in supplemented media. Cells were counted by hemacytometer and cell suspensions diluted to 2 × 10⁶ nucleated marrow cells/ml.

1 ml of a suspension that contained 2 × 10⁵ nucleated marrow cells, 0.1 ml of fetal calf serum (Irvine Scientific, Santa Ana, Calif. and Microbiologic Associates), 0.1 ml of a stimulatory material (CSA, see below), and 0.3% agar (Bacto-Agar, Difco Laboratories, Detroit, Mich.) in supplemented media were placed in 35-mm plastic dishes (Falcon Labware, Div., Becton, Dickinson & Co., Oxnard, Calif.), allowed to gel at room temperature, and placed in a 37°C, 5% CO_2, 100% humidity incubator (National Appliance Co., Portland, Oreg.) for 8–10 d. All assays were performed in triplicate. Colonies of >50 cells were counted with an inverted microscope and results expressed as colonies per plate or colonies per 2 × 10⁵ nucleated marrow cells. The optimal quantity of stimulator added and the optimal duration of incubation were determined in preliminary experiments. The 8–10-d incubation period represented the time of maximal colony number after which the colony numbers gradually decreased. The stimulator concentration was varied from 2.5 to 30% and found to be maximal at 10%. Colonies growing under these conditions were individually plucked from the agar and stained with aceto-orcein or with MacNeal's tetrachrome by the method of Lord and Testa (13) to confirm the cell type of the colonies grown.

Tritiated thymidine ([³H]TdR) suicide studies. The cell cycle status of CFUc was determined by a modification of the [³H]TdR suicide method of Iscove et al. (14). Marrow cell suspensions at 5–10 × 10⁶ cells/ml were incubated with 100 μCi of high specific activity [³H]TdR (>60 Ci/mM, New England Nuclear, Boston, Mass.) for 20 min at 37°C. The incorporation of labeled thymidine was stopped with large volume washes (×3) of supplemented media to which 5% fetal calf serum and 100 μg/ml unlabeled thymidine had been added. The pelleted, washed cells were finally resuspended at 2 × 10⁶ cells/ml and plated as above in supplemented media that contained unlabeled thymidine (10 μg/ml). Percent thymidine suicide was calculated by comparison of colony growth in the [³H]TdR-treated and control unlabeled thymidine-treated plates: percent suicide = control CFUc − [³H]TdR treated CFUc/control CFUc. Control plates to which both tritiated thymidine and excess unlabeled thymidine were added gave suicides of zero, demonstrating that nonspecific toxicity was not involved.

In vitro peripheral blood culture. Granulocyte-macrophage precursor cells (CFUc) were grown from blood cells prepared by Ficoll-Hypaque separations. Slight modifications of the above in vitro culture methods used for marrow cells were found to increase colony yields: blood CFUc were grown in single-layer plates that contained 20% fetal calf serum, 10% stimulator, 0.45% agar, and 2 × 10⁶ nucleated cells/ml in supplemented media. Blood CFUc were expressed as number of colonies per milliliter of whole blood.

In vitro colony-growth stimulators. Serum from various sources, feeder layers that contained blood cells from normal dogs, and conditioned media prepared by several different methods from murine, human, and canine cells were tested for stimulatory activity. Dog serum served as the best stimulator of colony growth. Normal dog blood was collected aseptically, allowed to clot at room temperature in glass tubes for 2 h, separated by centrifugation, and the serum stored at −10°C until use. Serum was also collected at various times after the intravenous injection of 0.1 μg/kg or 5 μg/kg *Salmonella typhosa* endotoxin into normal dogs and processed similarly. Maximum colony growth was shown to occur with sera collected between 3 and 6 h after the larger dose of endotoxin. All serial studies reported here were performed with a single pool of postendotoxin dog serum (PEDS) obtained 4 h after a 5-μg/kg dose.

Assay for CSA. CSA was determined by comparing the number of colonies stimulated to grow by a test serum to the number of colonies stimulated by the standard PEDS from a given marrow cell suspension. The "activity" of serum was normalized to allow comparison of different assay runs by the following formula: CSA = (colonies grown with test serum/colonies grown with PEDS in same assay) × mean number of colonies, grown with PEDS in the 20 test assays.

Endotoxin administration. Concentrated endotoxin (Lipopolysaccharide W from *S. typhosa* 0901, Lot 3124-25, Difco Laboratories, Detroit, Mich.) was diluted to 500 μg/ml in normal saline and stored at −10°C. Dilutions of <500 μg/ml were stored no more than 2 wk before use, and very diluted endotoxin doses (<1 μg/ml) were made up immediately before injection. Two grey collies and two normal dogs were given individual doses of endotoxin at 0.1 μg/kg to study the acute effects of this endotoxin dose (single-injection studies). Three other normal dogs were also given single endotoxin doses at 5 μg/kg. Endotoxin was administered intravenously once daily to two grey collies starting at 0.0005 μg/kg per d and increased geometrically to 0.1 μg/kg per d; the dose was subsequently increased arithmetically by 0.5 μg/kg per d increments to a maximum dose of 5 μg/kg per d in one dog (50 μg/d) and 30 μg/kg per d in the

other. This latter dog was later given a second course of endotoxin injections, to a maximum plateau dose of 5 μg/kg per d. Two normal dogs were given endotoxin in this same fashion to the plateau dose of 5 μg/kg per d and maintained there for 2 mo for parallel studies.

Statistical methods. CFUc and CSA values were expressed as arithmetic means of colony counts for triplicate plates. For comparison of data from cycle to cycle in each collie and for summarizing data for our group of cyclic dogs, the values were averaged by "cycle day," defining "cycle day 1" as the 1st d of a dog's cycle when the neutrophil count fell below 1,000/mm³. Each day was then numbered in sequence until the next occurrence of cycle day 1. Individual data points occurring on a given cycle day were averaged and standard errors calculated. During endotoxin treatment periods cycle day definition was derived in similar manner by extrapolation of prior and subsequent cycle periodicities. Group means were compared by the Student's *t* test.

RESULTS

Blood counts and endotoxin. The grey collies showed typical 11–13-d cycles of their neutrophil counts (Fig. 1, days 5–50). Single injections of endotoxin in grey collies during the neutrophilic phase (cycle days 6–7) and on normal dogs showed similar increments in polymorphonuclear neutrophil (PMN) counts at 4–6 h after intravenous endotoxin at 0.1 μg/kg and no difference in the pattern of count changes (Table I). When grey collies were neutropenic, increments were essentially zero as reported previously (2).

Chronic endotoxin treatment resulted in marked changes in the pattern of neutrophil counts. The periodic changes in total neutrophil count of the grey collies were replaced by stable counts, whereas in normal dogs the circulating neutrophil counts were increased to a level above their base-line values (Fig. 1, days 50–100). The mean neutrophil counts of grey collies given endotoxin chronically were not different from untreated grey collies, whereas normal dogs showed a 42% increase in mean neutrophil count (Fig. 1 and Table I). The increments in neutrophil counts at 4 and 6 h after the single endotoxin doses also were tabulated for both grey collies
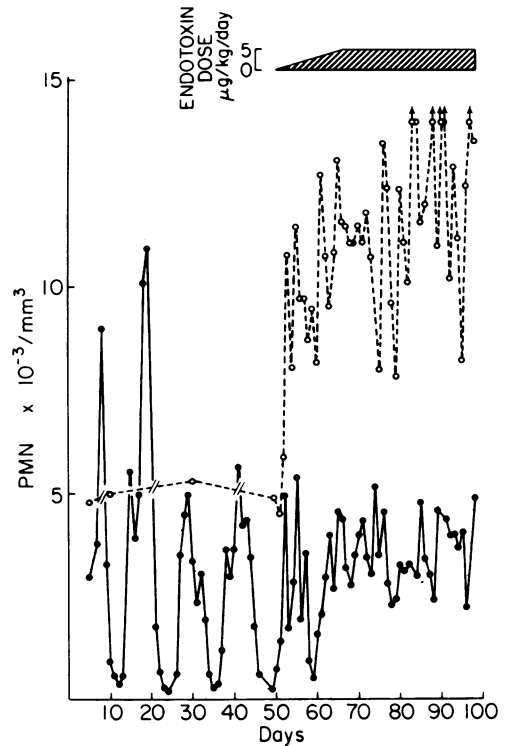

FIGURE 1   Circulating neutrophil counts in a grey collie (●) and a normal dog (○) before and during chronic endotoxin treatment (shaded bar). Only representative counts were taken from the normal dog data before endotoxin treatment.

and normals (Table I). Although the absolute values of the peak postendotoxin increments in the single-injection studies were greater in the grey collies than in the normals, the peak neutrophil counts at 6 h after endotoxin were very nearly the same. The postendotoxin increments in neutrophil counts during chronic endotoxin treatment, however, were much larger in the normals than in the grey collies (Table I). When these numbers are represented as proportionate changes, the increase in neutrophil counts after

TABLE I
Endotoxin Effects upon PMN Counts in Normal and Grey Collie Dogs

	PMN			PMN increment
	Morning	4 h after endotoxin	6 h after endotoxin	Maximum§
	counts/mm³			
Single injection, grey collie (4)*	4,402±762	10,810±1,599	13,577±2,485	9,175±2,314
Chronic treatment, grey collie (30)‡	3,656±154	6,043±422	—	2,370±410
Single injection, normal dog (4)*	6,840±1,407	7,963±1,910	12,409±3,111	4,701±2,461
Chronic treatment, normal dog (12)‡	9,710±586	31,095±561	—	20,938±700

* Number in parentheses is number of dogs given single endotoxin injections.
‡ Number in parentheses is number of injections evaluated in three different dogs.
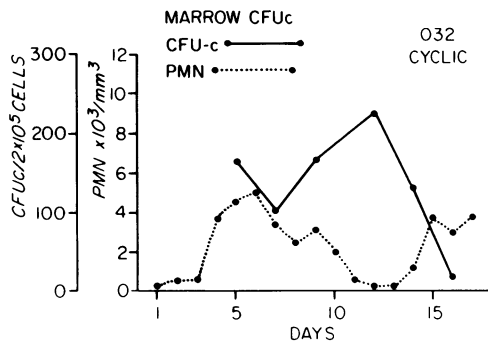§ Maximum increase in neutrophil count at 4 or 6 h after individual endotoxin doses.

FIGURE 2 Circulating neutrophil counts in a grey collie (– – –) are plotted with simultaneous bone marrow CFUc per 2 × 10⁵ nucleated bone marrow cells (——) for a single cycle.

chronic endotoxin injections appear qualitatively similar in grey collies and normals (70 and 200% increments, respectively).

Bone marrow and blood CFUc and endotoxin. The characteristic cycle of blood neutrophil counts in a grey collie was associated with an inverse rising and falling of simultaneous bone marrow CFUc (Fig. 2). When multiple cycles from five grey collies were averaged by cycle day and plotted with a representative neutrophil cycle, marrow CFUc numbers peaked on days 2–3 during the neutrophil nadir and decreased to a nadir on days 6–8 at the time of blood neutrophilia (Fig. 3, middle panel). Serial marrow studies on three normal dogs did not show evidence of cycling. The mean value for CFUc for 30 specimens in four normal dogs was 181 ± 9 colonies per 2 × 10⁵ nucleated marrow cells (Fig. 3).

Blood CFUc numbers in grey collies averaged by cycle day showed a definite nadir on cycle days 2–3 with a peak on days 6 and 7 (Fig. 3, lower panel). In normal dogs serial studies of blood CFUc do not show periodicity; the mean value for 22 specimens in three dogs was 42 ± 5 colonies/ml blood (Fig. 3).

In contrast to these data, the bone marrow CFUc numbers during chronic endotoxin treatment were markedly altered. In no case were cycles such as that seen in Fig. 2 noted. When averaged by cycle day the "peak-nadir" pattern in the grey collies was lost and the curve looked like a curve showing CFUc numbers in a normal dog over any 2-wk period (Fig. 4, middle panel). Mean marrow CFUc in grey collies on endotoxin were 139 ± 7.9 per 2 × 10⁵ cells, not different from untreated grey collies, 125 ± 10.5 ($P > 0.25$). When normal dogs given chronic endotoxin treatment were assayed, marrow CFUc levels were 208 ± 11 colonies per 2 × 10⁵ nucleated marrow cells (Fig. 4), slightly more than the 181 ± 9 colonies per

2 × 10⁵ cells (Fig. 3) in normal dogs not given endotoxin ($P < 0.05$).

The blood CFUc determinations during chronic endotoxin treatment showed that the wide swings in CFUc number seen in untreated grey collies (Fig. 3) were lost and replaced by more stable CFUc levels (Fig. 4). Individual animal curves showed no visible periodicity. Blood CFUc in normal dogs treated with endotoxin increased significantly compared to dogs not given endotoxin (111 ± 23 vs. 42 ± 5 colonies/ml blood, $P < 0.01$). Blood CFUc during endotoxin treatment was lower in the grey collies than in normal dogs similarly treated (grey collies = 60 ± 2 and normal dogs = 111 ± 23 colonies/ml blood, $P < 0.05$).

Colonies grown from normal dog marrow and blood all showed obvious macrophages or granulocytic cells. Colonies from grey collies were similar to those seen in normal dog cultures and appeared the same in all phases of the neutrophil cycle. The morphologic types of colonies grown in endotoxin-treated normal and grey collie dogs appeared in frequencies similar to those in untreated dogs.
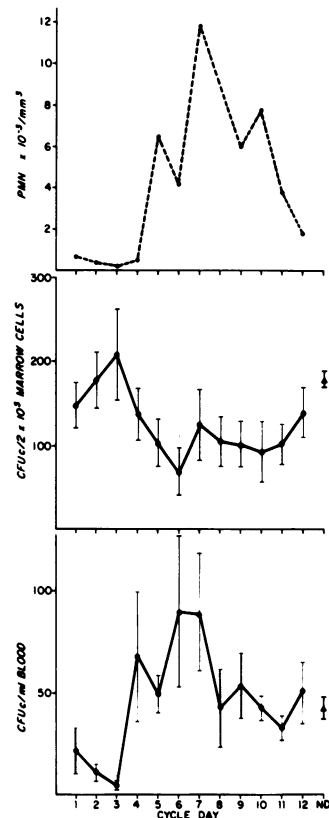

FIGURE 3 Circulating neutrophil counts for a representative grey collie (upper) are plotted with averaged data for bone marrow CFUc (middle) and blood CFUc (lower) in five grey collies. Normal dog values are indicated by ND (△), with bars representing standard errors.
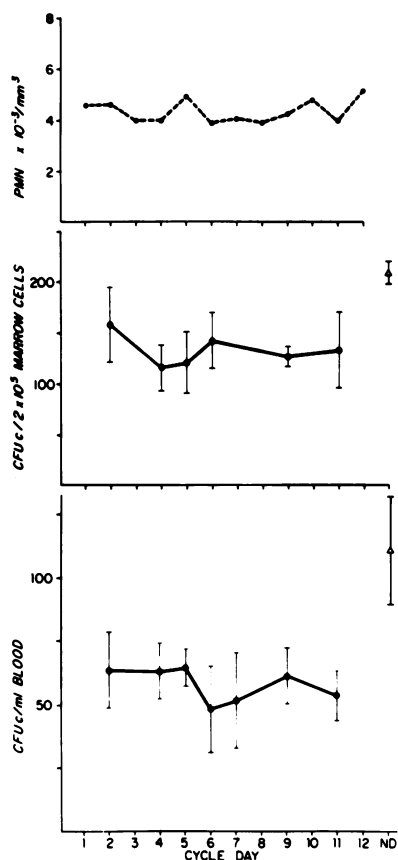
FIGURE 4 Circulating neutrophil counts for a representative endotoxin-treated grey collie (upper) are plotted with averaged data for bone marrow CFUc (middle) and blood CFUc (lower) in three grey collies. Normal dogs treated with endotoxin are indicated by ND (Δ) with bars representing standard errors.

FIGURE 5 Tritiated thymidine suicide percentages are shown averaged by cycle day. Individual points [(●)] are means for which only two data points were available for statistical analysis. The left panel shows data for un-manipulated grey collies with the corresponding normal dog suicide indicated ND (Δ). The right panel shows data on grey collies during chronic endotoxin injections, with corresponding endotoxin-treated normal dogs shown as ND (Δ).

[³H]TdR *suicide and endotoxin.* Thymidine suicide studies were used to determine the proportion of marrow CFUc in active cell cycle. The endotoxin treatment regimen did not significantly change the average CFUc [³H]TdR suicide rate in the grey collies (46±2% without endotoxin vs. 44±4% with endotoxin, $P > 0.30$) (Fig. 5). Similarly, normal dogs given chronic endotoxin did not alter their CFUc suicide rates (27±2.5% without endotoxin vs. 28±3% with endotoxin, $P > 0.30$) (Fig. 5). The mean [³H]TdR suicide of grey collie dogs CFUc was higher than the normal control dogs similarly treated (46±2 vs. 27±2.5% without endotoxin, $P < 0.001$; 44±4 vs. 28 ±3% with endotoxin, $P < 0.01$). Of particular note, the proportion of CFUc killed by [³H]TdR did not show any apparent cyclic change, and endotoxin treatment did not alter the suicide percentage in the grey collies (Fig. 5).

CSA *and endotoxin.* Sequential hourly changes of serum CSA in normal and grey collie dogs given single,
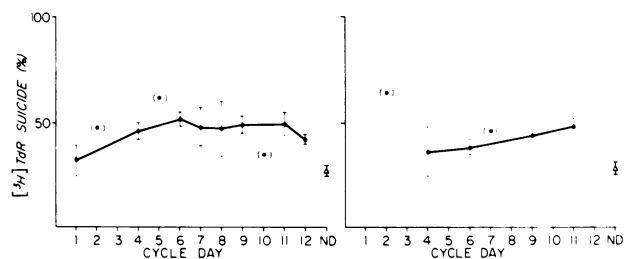
low-dose endotoxin injections (0.1 μg/kg) were similar. Normal dog CSA rose from 36±3 to 64±14 colonies/ plate at 4 h after endotoxin, whereas grey collie CSA rose from 58±13 to 78±17 colonies/plate (Table II). By 24 h after endotoxin injection, serum CSA had returned to base line. The increase in CSA produced by this low dose of endotoxin was 20±9 colonies/plate in the grey collies and 28±15 in the normals (Table II). High-dose endotoxin injections (5 μg/kg) in three normal dogs caused CSA to rise from 65±3 to 103±5 colonies/plate, an increment of 37±7 colonies/plate (Table II). Studies of single injections of

TABLE II
*Endotoxin Effects upon Serum CSA in Normal
and Grey Collie Dogs*

	CSA		
	Morning	4 h after endotoxin	Increment§
	colonies/plate		
Grey collie			
Single injection, 0.1 μg/kg (4)*	58±13.0	78±17.0	20±9.0
Chronic treatment, 5 μg/kg (30)‡	63±2.5	66±3.4	3±3.0
Normal dog			
Single injection, 0.1 μg/kg (4)*	36±3.0	64±14.0	28±15.0
Single injection, 5 g/kg (3)*	65±3.0	103±5.0	37±7.0
Chronic treatment, 5 μg/kg (12)‡	50±3.5	58±3.5	8±3.5

* Number in parentheses is number of dogs given single endotoxin injections.
‡ Number in parentheses is number of injections evaluated in three different dogs during chronic endotoxin treatment.
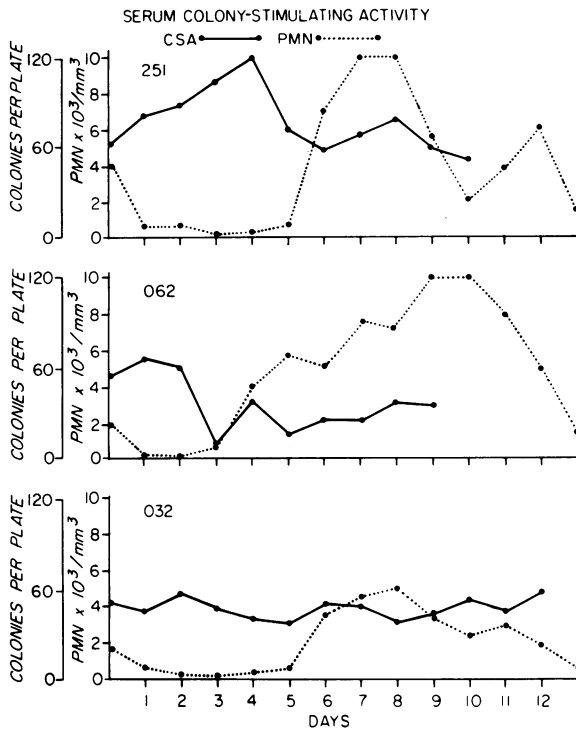§ Increase in CSA caused by endotoxin injection.

SERUM COLONY-STIMULATING ACTIVITY

CSA •——• PMN •·······•

FIGURE 6 Circulating neutrophil counts in three grey collies (- - -) are plotted with serum CSA (———) shown as colonies per plate over representative cycles.

high-dose endotoxin (5 μg/kg) were not performed in the grey collies.

Changes in serum CSA in successive phases of the neutrophil cycle in the grey collies were examined before and during chronic endotoxin administration. For three dogs studied before endotoxin treatment there was considerable variability of the serum CSA. In two dogs the values rose during neutropenia, whereas in another there was no change (Fig. 6). When combined as averages by cycle day, the apparent cycle of serum CSA in some animals is not seen (Fig. 7, left panel). Concurrent 2-wk periods of serum CSA in individual normal dogs showed no evident periodicity; mean CSA values in concurrently assayed normal dogs were 48±6 colonies/plate (Fig. 7, left panel).

Serum CSA in endotoxin-treated grey collies was determined for specimens obtained after the plateau dose of endotoxin was reached (5 μg/kg per d) and each value represented the CSA 24 h after the previous day's endotoxin dose. During endotoxin treatment none of the grey collies showed rising and falling values such as were sometimes seen in untreated grey collies (Fig. 6). When serum CSA values in endotoxin treated grey collies were averaged by cycle day, the curve was not significantly different

from that in untreated grey collies (Fig. 7). Normal dogs given chronic endotoxin did not show periodic changes in CSA; the mean serum CSA of endotoxin treated normal dogs was 50±3 colonies/plate, not different from the 48±6 colonies/plate of normal dogs not given endotoxin ($P > 0.30$) (Fig. 7).

To further examine these findings we determined the immediate effects of individual doses of endotoxin on serum CSA values during chronic endotoxin treatment. Serum CSA measured before and ≅4 h after doses is illustrated in Table II. For chronically treated normal dogs, although the postendotoxin CSA values are slightly increased compared to the pre-endotoxin values (58±3.5 vs. 50±3.5, $P < 0.05$), the postendotoxin CSA is markedly lower than the post-CSA in the normal dog given a single comparable dose endotoxin injection (58 vs. 103, $P < 0.01$) (Table II). The grey collie data show that in the chronically treated dog the CSA values 4 h after endotoxin are no different from pre-endotoxin values (66±3.4 vs. 63 ±2.5, $P > 0.10$). In addition, when increments in CSA are compared, the chronically treated grey collie and the chronically treated normal dog are not different (3±3 vs. 8±3.5 colonies/plate) (Table II).

DISCUSSION

Early studies of cyclic hematopoiesis in grey collie dogs showed that marrow production of neutrophils is interrupted periodically at 10–13-d intervals (2, 3). Bone marrow transplantation experiments showed that this production defect is manifest in transplanted hematopoietic tissue, implicating the pluripotent stem cell as the locus of the defect (6, 7). Endotoxin, a potent stimulator of granulopoiesis, was recently shown to alter the cycling of neutrophil counts in grey collies (10) and served as a probe to dissect the mechanisms of cyclic hematopoiesis in our studies.

We have confirmed the observation by Maloney et al. (10) that daily endotoxin administration will eliminate the cyclic changes of neutrophil counts in
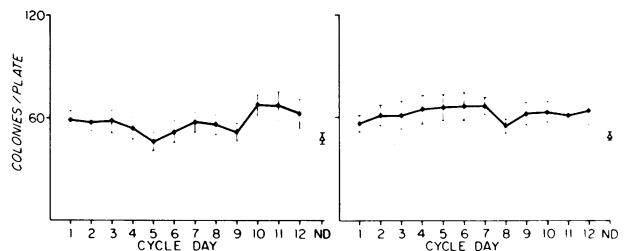

FIGURE 7 Serum CSA averaged by cycle day in untreated grey collies (left) and in grey collies during chronic endotoxin injections (right). Corresponding CSA values are shown for normal dogs as ND (△).

the grey collie. We also showed that once a dose of 5 μg/kg per d was reached, maintenance of dosage at that level resulted in elimination of the cycling of neutrophil counts, without the need to continually escalate the endotoxin dose. This allowed evaluation of the characteristics of endotoxin tolerance in this setting and a more detailed study of the effects of chronic endotoxin administration upon cyclic hematopoiesis.

The studies reported here comparing the neutrophil count responses and CSA increases of normal and grey collie dogs closely parallel data in mice (15). When grey collie or normal dogs are given single, isolated doses of endotoxin (0.1 μg/kg) they respond with increments in blood neutrophils and a rise in serum CSA at 4–6 h after the dose. When grey collie or normal dogs are subjected to repeated endotoxin challenge, up to 5 μg/kg per d, they show a normal response of neutrophil count, but become hyporesponsive to the CSA-elevating effect of the endotoxin. These data suggest that the grey collie has normal responsiveness to endotoxin challenge and is capable of developing a normal degree of endotoxin tolerance.

When repeated doses of endotoxin are given to normal or grey collie dogs at doses of 5 μg/kg per d, normal dogs showed an increase in circulating neutrophil counts, whereas the grey collies showed abolition of neutrophil cycling. This occurs with a plateau dose and is reversible. When endotoxin administration is stopped, neutrophil cycles promptly reappear.

Our data show that untreated grey collies have wide periodic fluctuations in both blood and marrow CFUc numbers (Fig. 2 and 3). Although the proliferative status of marrow CFUc (measured by [^{3}H]TdR suicide) remains fairly stable (Fig. 5) the proportion of marrow cells comprising that pool of progenitor cells shows marked swings not seen in normal dogs. The significance of the rise of blood CFUc on days 4–6, instead of on days 2–3 at the time of peak bone marrow CFUc numbers is not known but may reflect a difference between the two CFUc pools (16). Our data are in general agreement with the report by Dunn et al. (17) showing a pattern of rise and fall in marrow CFUc numbers though our numbers are fivefold higher. However, our data on thymidine suicide percentage are quite different. They report a marked swing from very low suicide rates (0–20%) during neutrophilia to very high rates (90–100%) during neutropenia, suicide values being correlated with the total number of marrow CFUc reported (17). We find much greater stability of the suicide rate, varying from 32% (cycle day 1) to 50% (cycle day 6) in the grey collies. Because cell-cycle status is highly dependent upon the level of CSA stimulation in the system (18), we believe this difference in suicide rates and the higher rate of colony yield in our sys-

tem may reflect differences in the stimulators used. Therefore, from our observation, we do not believe the fluctuations in the CFUc pool in the grey collies can be explained solely from changes in cell-cycle status as has been suggested previously (17).

Because we (19) and others (20) have previously found cyclic changes in CSA in urine and serum from dogs with cyclic hematopoiesis, the results of the serum CSA assays reported here were not expected. Although some cycles showed rising CSA during neutropenia, such a pattern was not uniform. Cycles showing such rises tended to be associated with more severe illness in the dog, suggesting that endotoxemia secondary to severe neutropenia might be contributing to the CSA rise. Our data provide support for a similar hypothesis by Greenberg et al. (21) to explain changing CSA levels in human cyclic hematopoiesis.

The data presented here support the concept that cyclic hematopoiesis in grey collie dogs is a disorder of the stem cell regulatory system in which committed stem cell pool sizes fail to maintain homeostatic balance, and consequently produce widely fluctuating numbers of differentiated progeny. The disease process appears not to be mediated by serum CSA for two reasons. One is that in the untreated grey collie, cyclic fluctuations in CSA are not required for fluctuations in CFUc numbers. The second is that endotoxin treatment fails to alter serum CSA levels when cyclic changes in CFUc and blood neutrophils are abolished. Although intramedullary levels of CSA could conceivably be mediating this effect of chronic endotoxin treatment without being reflected in serum CSA, we have no data to suggest such an effect.

The [^{3}H]TdR suicide data reported here suggest that cyclic hematopoiesis is not a manifestation of simple switching on and off of proliferation within committed stem cell compartments. The proliferative capacity of the precursor cell pool (measured as percent suicide for CFUc) does vary somewhat during the cycle period, but not to the degree that the CFUc pool size appears to change. We infer from these data that the size of the committed granulocyte-macrophage progenitor cell pool is more dependent upon the flux of pluripotent stem cells in and more differentiated progeny out than it is upon the self-replication of committed stem cells within the pool. Because quantitation of total marrow stem cell pools have not been made, however, such an inference remains speculative.

That repeated daily endotoxin injections in these dogs eliminate the cyclic changes in blood neutrophil counts, stabilize the bone marrow progenitor cell pool, and cause the marrow neutrophil reserves to resemble that of normal dogs suggests that endo-

toxin acts at a key regulatory site early in hematopoiesis. Our data show that this is not associated with changes in serum CSA or in the [³H]TdR suicide rate of the CFUc. These findings suggest that endotoxin's effect is to increase the flux of cells into the committed stem cell compartment from the pluripotent stem cell pool.

ACKNOWLEDGMENTS

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