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# Modification of Canine Cyclic Hematopoiesis by Endotoxin (38312)

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Recent studies with marrow transplants (1) have provided direct evidence for the supposition that the genetically determined blood cell periodicity of the grey collie reflects a stem cell problem (2). Although the dramatic observation that periodicity can be eliminated by a marrow transplant from a normal collie (1) or induced by a marrow transplant from a grey collie (3) clearly implicates the stem cell, the nature of the defect remains an open question. It is not known, for example, where the intermittent failure of hemic cell production is expressed in the developmental pathway. Conceivably, the basic defect could be restricted to the neutrophil system with the periodicity of reticulocytes and other blood cells as a secondary manifestation. We have suggested that the predisposing condition in the grey collie is a functionally restricted stem cell pool with an oscillatory efflux governed primarily by a neutrophil feedback loop operating against a background of stem cell competition (2). If this were so, a protracted demand for neutrophils might abolish their periodicity and that of reticulocytes as well. Experiments to test this hypothesis are the subject of the present paper.

*Materials and Methods*. This study was performed with three grey and three normal adult collies weighing 13–21 kg. Peripheral blood counts were made on venous blood samples obtained daily between 8:00 and 11:00 a.m. Blood samples were anticoagulated with EDTA, total leukocyte and erythrocyte counts were made with a Coulter Counter, differential counts of a minimum of 200 leukocytes were determined on Wright's-stained smears and the number of reticulocytes per 2000 erythrocytes was scored after staining with new methylene blue. Blood counts were made for at least 1 mo before initiation of daily iv injections of endotoxin (*Escherichia coli* lipopolysaccharide-0127, Difco). The dose of endotoxin was increased progressively from 1 to 16 $\mu$g/kg of body wt over 55 days in one grey collie (designated G-20), from 4 to 16 $\mu$g/kg over 28 days in a second grey collie (G-21), and from 4 to 31 $\mu$g/kg over 28 days in a third collie (G-22). The average daily doses were 8.3, 13.5, and 18.8 $\mu$g/kg, respectively. Normal collies were treated similarly. The step-wise increase in dosage was intended to prevent the development of endotoxin tolerance with repeated administration (4). Daily blood counts were also made just before each endotoxin injection and for 30 days after treatment was completed.

The blood count pattern for each dog during the entire period of study was determined by computing the moving average for three consecutive daily points to minimize random fluctuations due to sampling, counting, etc. Periodicity was considered to occur if the resulting blood count curve revealed a regular oscillatory pattern upon visual inspection with nadir-to-apogee variation of at least 50%. The results obtained by this method of analysis are generally concordant with a more formal treatment by a $\chi^2$ test based on expected and observed runs (2). Cycle duration was determined by the mean interval between consecutive minima and consecutive maxima.

*Results*. Peripheral blood counts in the grey collies before endotoxin treatment were similar to those reported previously (2, 5). These dogs presented a 10- to 14-day periodicity with the reticulocyte level usually elevated when the neutrophil count was depressed, and vice versa. In confirmation of earlier studies with collies (2, 5) periodicity as defined here was not apparent in the control animals. In each of the grey collies studied, the characteristic cyclic behavior of

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blood neutrophils disappeared during the course of endotoxin treatment and reappeared upon its completion (Figs. 1 and 2). Disappearance of cycling was preceded by muted oscillations with about a 25% decrease in mean cycle duration. Treatment with the higher average daily endotoxin doses was begun in one dog (G-22) at the nadir of the neutrophil count and in another (G-21) when the neutrophil count was declining (Fig. 1). In the first instance, there was a 100-fold increase in the neutrophil level within a few days, while in the second instance, the customary profound neutropenia failed to develop. In both cases, there was a subsequent increase of 30–50% in the mean neutrophil count over that seen during an equivalent preendotoxin interval. Control dogs receiving the same endotoxin dose schedule (N-21, N-22) exhibited an early sharp neutrophilia followed by a sustained increase in the mean neutrophil count of some 200–350% (Fig. 3). When treatment was begun with the lower endotoxin dose in the third dog (G-20), the mean neutrophil count was slightly depressed during the absence of obvious cycling, whereas that of the corresponding control (N-20) was slightly elevated (Table I). In all three grey collies the coefficient of variation of the mean neutrophil counts decreased strikingly during endotoxin treatment and returned to preendotoxin levels when the injections were terminated.

The reticulocyte pattern was also altered in the endotoxin-treated grey collies (Figs. 1 and 2). The oscillations were muted but generally tended to persist. As shown in Table I, the mean reticulocyte level and its coefficient of variation were depressed in the grey collies during endotoxin treatment. The oscillatory amplitude appeared to increase during the immediate post-endotoxin period in the dog receiving the lowest average daily endotoxin dose (Fig. 2), but a definite periodicity was not apparent at this time in the two grey collies receiving the higher daily doses even though neutrophil cycling was restored (Fig. 1). In these dogs, the most prominent feature of the early postendotoxin period was a rise in reticulocyte count. This was also

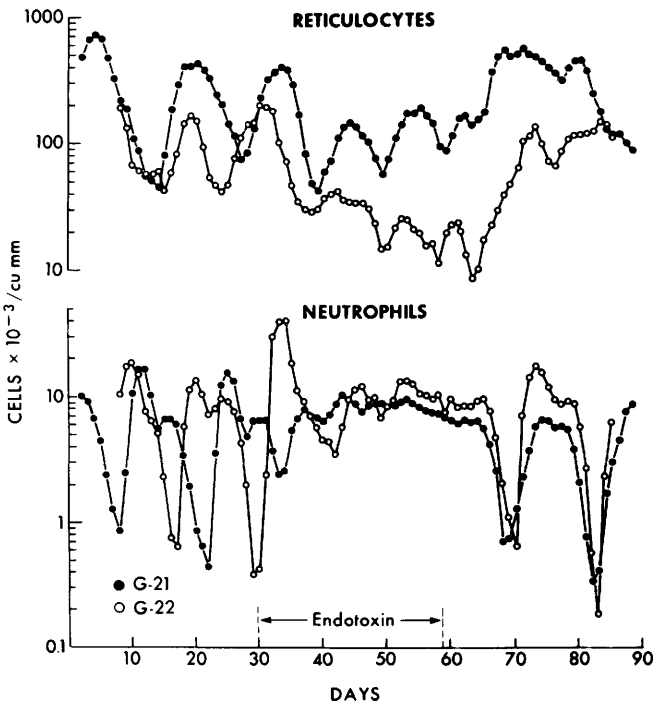


FIG. 1. Changes in peripheral neutrophil and reticulocyte levels with endotoxin treatment in two grey collies. Average daily doses in μg/kg body wt: 13.5 (G-21), 18.8 (G-22).

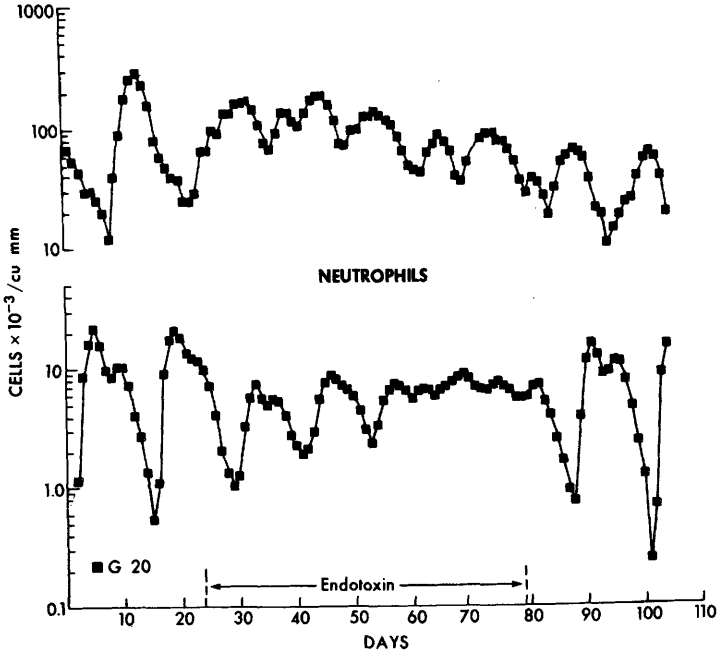


FIG. 2. Changes in peripneral neutrophil and reticulocyte levels with endotoxin treatment in a grey collie. Average daily dose in μg/kg body wt: 8.3 (G-20).

observed in the corresponding normal controls (Fig. 3). The grey collie presents a moderate anemia, *i.e.*, a 25% reduction in erythrocyte count, which is not exacerbated during endotoxin treatment.

*Discussion.* It is clear from bone marrow transplantation studies (1, 3) that cyclic hematopoiesis of the grey collie is basically a stem cell disease. It is also evident from the endotoxin studies described here that the cyclic behavior can be manipulated by agents extrinsic to the system, such as endotoxin.

Endotoxin is known to act at several levels. It not only promotes the release of the more mature neutrophilic elements from marrow to blood (6), but it also increases the turnover and pool of presumptive granulocyte-committed stem cells as assayed in agar cultures (7) and of pluripotential stem cells as assayed by the spleen colony technique (8). Although a single injection of endotoxin can release a considerable number of neutrophils during specific phases of the cycle in grey collies (9), the basic periodicity persists regardless of time of administration in the cycle. Apparently, a protracted stimulation, presumably of the committed stem cell compartment, is necessary to abolish the cyclic neutropenia.

With a step-wise increase of endotoxin dose from 1 to 16 μg/kg of body wt over 55 days, the amplitude of neutrophil oscillation was diminished progressively until cycling finally disappeared after some 30 days only to reappear again soon after endotoxin treatment was ended. Although the amplitude of reticulocyte oscillation was also muted and the mean reticulocyte level was depressed during daily endotoxin injections, cycling persisted despite the disappearance of neutrophil periodicity. At this dose level, there was comparatively little change in the neutrophil and reticulocyte counts in a normal collie. With higher initial and average daily endotoxin doses, neutrophil periodicity in the grey collie was eliminated within 1–2 weeks and was restored rather promptly upon cessation of treatment. Reticulocyte fluctuations were again muted and even tended to disappear in the dog treated with the highest average daily dose. Significantly, the reticulocyte level was elevated and cycling was seemingly absent during the several-week period of observation after endotoxin. It is noteworthy that the higher average daily endotoxin doses led to a considerable elevation of the neutrophil count and depression of the reticulocyte count in the control collies. The reticulocyte effect is no doubt a reflection of stem cell diversion to the neutrophil pathway. It

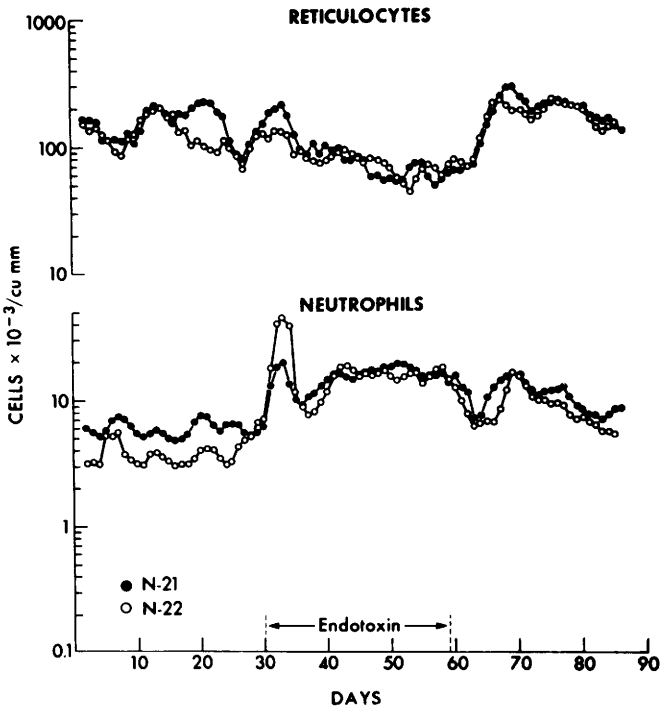


FIG. 3. Changes in peripheral neutrophil and reticulocyte levels with endotoxin treatment in normal collies. Average daily dose in μg/kg body wt: 13.5 (*N*-21, *N*-22).

is known that even in a normal situation selective changes in hematopoietic activity may occur at the expense of other blood elements during the response to specific stresses (10, 11).

It would seem from these results that the characteristic periodicity and phase difference of blood neutrophils and reticulocytes in the grey collie is not a simple consequence of a peripherally determined competition for a limiting stem cell pool as we have suggested (2). If successive activation and deactivation of a peripheral neutrophil feedback loop played an essential role in determining the periodicity as well as the competitive pressure for stem cells, a sustained demand for neutrophil production should have led to the disappearance of reticulocyte cycling along with a depression of the reticulocyte count *pari passu* with the absence of neutrophil cycling. Since the reticulocyte data did not conform to this expectation, another explanation must be sought for the alternating ebb and flow of neutrophil and reticulocyte produc-

TABLE I. Mean Neutrophil and Reticulocyte Counts Before, During, and After Endotoxin Treatment.[a]

| | Neutrophils (Mean and coefficient of variation) | | | Reticulocytes × $10^{-3}$ (Mean and coefficient of variation) | | |
|---|---|---|---|---|---|---|
| Dog | Pre-endotoxin | During endotoxin | Post-endotoxin | Pre-endotoxin | During endotoxin | Post-endotoxin |
| G-20 | 8830 (75) | 7279 (11) | 7750 (60) | 112 (86) | 60 (29) | 42 (44) |
| N-20 | 6162 (15) | 7245 (12) | 6216 (12) | 149 (19) | 180 (24) | 131 (42) |
| G-21 | 6360 (86) | 8230 (10) | 3706 (64) | 219 (69) | 123 (36) | 422 (21) |
| N-21 | 5996 (16) | 16966 (10) | 11318 (25) | 185 (21) | 60 (14) | 204 (15) |
| G-22 | 6941 (59) | 10500 (18) | 8653 (63) | 111 (48) | 20 (28) | 107 (21) |
| N-22 | 3552 (12) | 15970 ( 7) | 10154 (30) | 145 (27) | 66 (17) | 187 (12) |

[a] Based on daily counts (cells/mm³) over a 12- to 14-day period; the endotoxin period corresponds to a similar interval without neutrophil cycling.

tion in the grey collie.

We suggest that the basic oscillation may be inherent in the limiting stem cell pool itself, the limitation being due to an abnormality of short-range cell–cell interactions governing the transition to neutrophil commitment. Thus, a decreased sensitivity for the "turning on" of pluripotential stem cell determination without an appropriately increased sensitivity for its "turning off" could lead to wide fluctuations of the pool of neutrophil-committed stem cells, resulting in oscillatory neutrophil production. Viewed in this context, a peripheral neutrophil feedback could contribute to the overall response but it would not be a necessary condition. Moreover, the oscillation of erythrocytopoiesis, and of thrombocytopoiesis as well, might simply represent a manifestation of a regularly recurring stem cell competition coinciding with the periodic turning on of a substantial outflow to neutrophil commitment as the pool of committed cells reached its nadir. Studies in the mouse indicate that there is normally a 3- to 4-day supply of granulocyte stem cells and perhaps a 4- to 5-day supply of erythrocyte stem cells (12). Although we are not aware of comparable data for the dog, fluctuations in pool size consistent with a 12-day periodicity would not seem to be unreasonable.

Within this framework, endotoxin treatment would abolish blood neutrophil periodicity by maintaining an efflux from committed stem cells even when this pool was greatly decreased because of the depressed control setting for pluripotential stem cell determination. The committed stem cell compartment would continue to oscillate, but with some decrease in amplitude and cycle duration owing to the increased recruitment by endotoxin. Periodic diversion of pluripotential stem cells to neutrophil commitment, and hence reticulocyte cycling, should also continue, although at a reduced rate. In this connection, it will be recalled that reticulocyte cycling became muted as neutrophil oscillation disappeared. With an increasing endotoxin dose the committed stem cell compartment would tend to stabilize at a low level, thus permitting a progressively steadier inflow from the pluripotential stem cell compartment. This would minimize or even remove reticulocyte cycling and lead to further depression of the reticulocyte count depending upon the magnitude of the compensatory adjustment of pluripotential stem cell pool size. Indeed, the reticulocyte increase after endotoxin treatment along with the absence of a definite periodicity at a time when neutrophil cycling had reappeared probably reflects an increased availability of uncommitted stem cells. Although the foregoing concept of a functionally restricted stem cell pool in the grey collie seems to be compatible with the endotoxin effect, its validity remains to be established by direct assay of the stem cell dynamics before, during, and after endotoxin treatment.

*Summary.* Studies were performed with three grey collies to determine whether a protracted demand for neutrophils resulting from daily endotoxin injections would influence their cyclic hematopoiesis. In each case, the characteristic profound oscillations of the neutrophil blood count were eliminated during a 1- or 2-mo course of endotoxin treatment and were restored rather promptly upon cessation of treatment. In contrast, reticulocyte cycling generally tended to persist, although at a reduced rate. However, during the immediate postendotoxin period, there was a sharp rise in reticulocyte count without a readily discernible cycling pattern in the two grey collies that had received the higher average daily endotoxin doses. Thus, the reticulocyte data did not conform to expectation based on the assumption that a peripheral neutrophil feedback provided the alternating competitive pressure for production of one or another blood cell type by a functionally restricted stem cell pool. It is possible that cyclic hematopoiesis in the grey collie may be inherent in the nature of the stem cell defect *per se, e.g.*, an abnormality of the mechanism governing the transition to neutrophil commitment could lead to an oscillating committed stem cell compartment with a resulting regularly recurring competition for pluripotential stem cells. The effects of endotoxin in the grey collie can be understood within this postulated framework.

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