

## Canine Cyclic Neutropenia

### A STEM CELL DEFECT

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**ABSTRACT** Two normal collie dogs were given 1,200 R total body irradiation followed by successful marrow grafts from their grey collie littermates with cyclic hematopoiesis. During observation periods of 97 and 41 days after grafting, both previously normal recipients showed regular cyclic fluctuations of their granulocyte and reticulocyte counts similar to those observed in their donors. These findings suggest that canine cyclic neutropenia is due to a defect in the marrow stem cell.

### INTRODUCTION

Cyclic neutropenia is an autosomal recessive disorder of grey collie dogs characterized by cyclic fluctuations of all peripheral blood cells (1-3). Recent studies have indicated that the granulocyte cycles are due to regularly recurring failure of neutrophil production (2, 3),

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but the mechanism of this failure remains unknown. In general, two possibilities exist: (a) these cycles could be the result of a defect in the marrow stem cell, or (b) these cycles could be the result of "environmental" factors in the affected dogs.

Canine marrow grafting after 1,200 R total body irradiation (TBI)<sup>1</sup> provides an experimental tool to discriminate between these two possibilities, since the marrow in grafted dogs is exclusively repopulated by donor cells (4, 5). In the present study, two hematologically normal collie dogs were given successful marrow grafts from their grey collie littermates with cyclic hematopoiesis, and hematologic parameters were studied for up to 97 days after grafting.

### METHODS

*Dogs.* A litter of four pups was the product of a mating between a female known to be a carrier of the grey collie syndrome and a male collie with the syndrome in his pedigree. Two of the pups (both male) were of normal coat color (tricolor) while two (both female) had the characteristic grey coloring of collies with cyclic neutropenia. The litter was obtained when six wk old, at which time all pups were clinically well, although the greys were smaller

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<sup>1</sup> Abbreviations used in this paper: DL-A, dog leukocyte antigen; MLC, mixed leukocyte culture; TBI, total body irradiation.

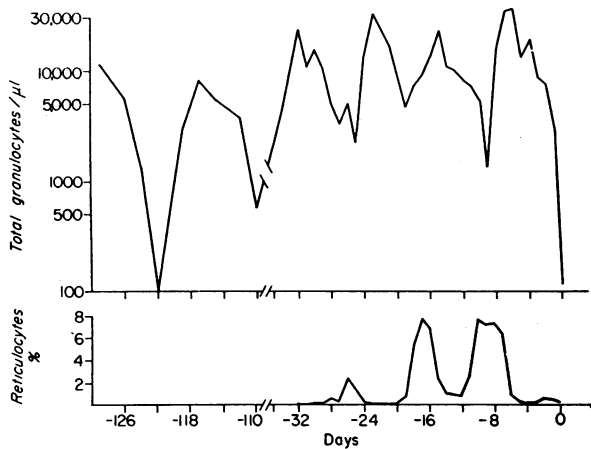


FIGURE 1 Daily granulocyte and reticulocyte counts in the grey collie G1, who was the marrow donor for the normal littermate N1. Day 0 was the day of marrow donation.

(1.25 and 1.65 kg) than the normals (2.5 and 3.1 kg). The two greys are designated G1 and G2, and the normal littermates are designated N1 and N2. They were raised under identical conditions for 18 wk, during which the discrepancy in weight between the greys and the normals persisted (5.5 and 6.5 vs. 15.0 and 12.5 kg). One of the greys developed purulent rhinorrhea and was maintained on antibiotics for four weeks before transplantation.

**Blood counts.** 1 ml of blood was removed from a peripheral vein daily at 9 a.m. White blood cell, 100-cell differential, platelet, and reticulocyte counts were performed by standard, nonautomated hematologic methods. Total granulocyte counts are the product of the white blood cell count and the sum of the percent polymorphonuclear leukocytes and band forms. Cycle lengths are the number of days between granulocyte nadirs or reticulocyte peaks.

**Histocompatibility matching.** Dog leukocyte antigen (DL-A) groups were determined by typing with over 150

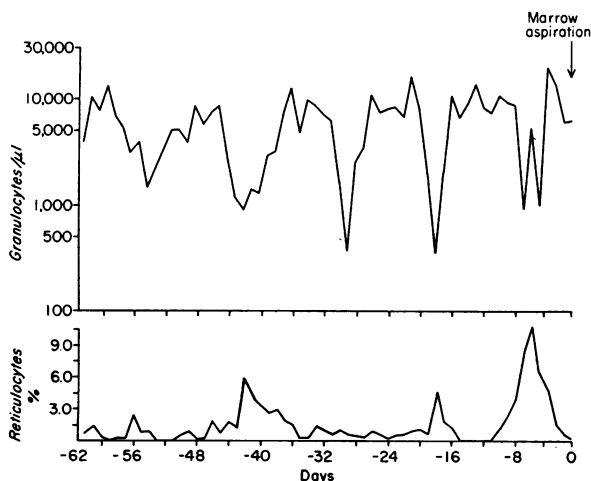


FIGURE 2 Daily granulocyte and reticulocyte counts in the grey collie G2, who was the marrow donor for the normal littermate N2 in the second transplantation experiment.

lymphocytotoxic antisera that recognize 23 DL-A groups (6). Mixed leukocyte culture (MLC) was performed as recently described (7).

**Marrow transplantation.** Recipients were conditioned by 1,200 R TBI administered by opposing  $^{60}\text{Co}$  sources at 9.3 R/min (midpoint tissue dose approximately 1,000 rads). Donor marrow was obtained and processed as previously described (4, 5), and infused intravenously into the recipient within 3 h of irradiation; day of irradiation and marrow transplant is designated day 0. Parenteral fluid and antibiotic support were given for 5 days and then as clinically indicated. No platelet transfusions were necessary in the first transplantation experiment (G1 donor, N1 recipient), but in the second experiment, the recipient N2 received two transfusions from unrelated dogs during the post-graft period when his platelet count fell below 10,000/ $\mu\text{l}$ . No post-transplant immunosuppression was given to either recipient. Karyotype analyses were performed on aspirates of recipient marrow periodically as previously described (8).

## RESULTS

All four littermates and their mother were DL-A identical, although only two DL-A groups could be unequivocally identified. All five dogs were mutually non-stimulatory in MLC, although all were stimulated normally by lymphocytes of unrelated dogs and by phytohemagglutinin.

Fig. 1 shows the granulocyte and reticulocyte counts in the grey collie G1 who served as the marrow donor for her normal littermate N1. Initially (from 7 to 9 wk of age), a 12-day granulocyte cycle was evident. During the 36 days before marrow donation (from 21 to 25 wk of age) when the dog had a severe respiratory tract infection, 8.8 day granulocyte cycles were present. 8-day cycles of reticulocytosis were also clearly evident during this period. The other grey collie, G2, had regular 11.8-day granulocyte and 12.5-day reticulocyte cycles as shown in Fig. 2. Fig. 3 shows the granulocytes and reticulocyte counts in the normal collie N1. No cyclic fluctuations of daily granulocyte and reticulocyte counts were evident in the 26 days before marrow grafting. Similar findings were made in the other normal collie, N2, who was observed for 55 days before grafting (Fig. 4).

N1 was given  $4 \times 10^8$  marrow cells/kg obtained from the grey collie G1 at the nadir of her granulocyte cycle (Fig. 1). The donor's granulocyte zeniths had occurred 6, 15, 23, and 32 days before marrow aspiration. After recovery from the TBI-induced depression of peripheral blood counts, regular 11.4 days fluctuations of granulocyte counts were now seen in N1 (Fig. 3). The initial granulocyte zeniths in the recipient occurred 2, 11, and 22 days after transplantation, remarkably close to the days predicted by extrapolating the donor's 8.8-day cycle beyond the day of transplantation (i.e. zeniths predicted on days 3, 11, and 20). Regular 11.6 day cycles of reticulocytosis were also seen in the recipient

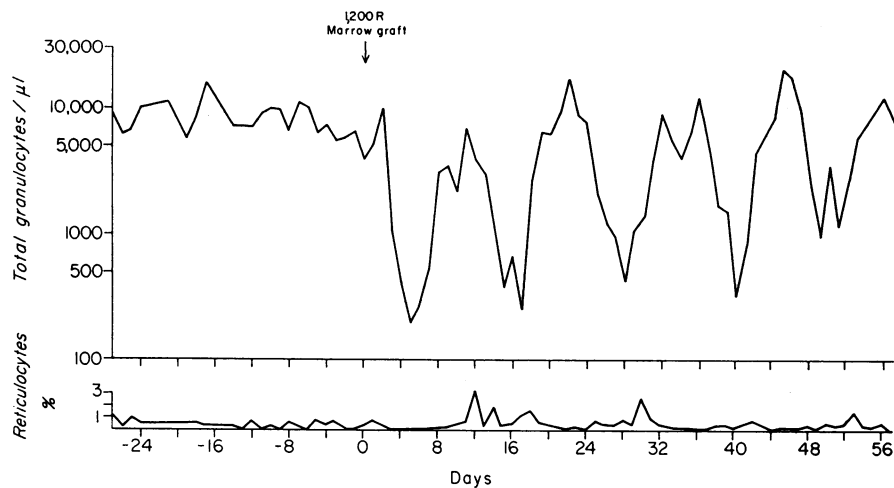


FIGURE 3 Daily granulocyte and reticulocyte counts in the normal dog N1, given 1,200 R TBI and marrow from the grey collie G1.

N1 (Fig. 3), although these were less pronounced than in the donor before marrow aspiration. Graft-versus-host disease was not observed. Regular granulocyte and reticulocyte cycles continued throughout the 97 days of observation after grafting. Proof for allogeneic marrow engraftment was obtained by karyotype analyses of marrow cells, carried out 15, 38, 50, and 85 days after grafting: all 39 cells analyzed showed the donor female sex karyotype.

Essentially identical observations were made in N2 given  $0.7 \times 10^8$  marrow cells/kg obtained from the grey collie G2 at the expected zenith of the granulocyte count. After recovery from the TBI-induced depression of peripheral counts, regular granulocyte cycles of 8.0 days were observed, and regular cycles of reticulocytosis

were also noted (Fig. 4). Karyotype analyses performed on day 21 after grafting showed all 19 analyzable metaphases to be of donor female type.

## DISCUSSION

This study clearly shows that a successful and sustained marrow graft from grey collie dogs with cyclic hematopoiesis into lethally irradiated normal dogs results in transfer of the cyclic hematopoiesis defect. This result suggests that the defect underlying canine cyclic neutropenia is associated with the marrow stem cell. It is not possible to exclude the remote possibility that some other, regulatory cell is actually responsible for the observed cycles, and that this hypothetical regulatory cell

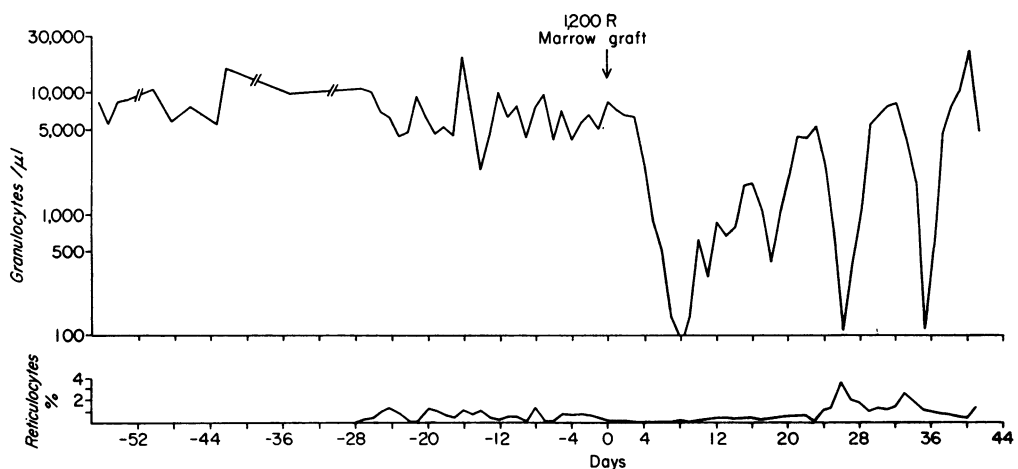


FIGURE 4 Daily granulocyte and reticulocyte counts in the normal dog N2 that was given 1200 R TBI and marrow from the grey collie G2 on day 0.

is transplanted with a marrow graft. It is possible to exclude the possibility, however, that the "environment" of the grey collies is responsible for cyclic hematopoiesis. The present data are in agreement with recent findings that transplantation of marrow from a normal into a grey collie after TBI results in abolition of cyclic neutropenia (9). This finding, however, could have been the result of some alteration by the supralethal irradiation of "environmental" factors in the grey collie recipient. The current study therefore provides direct, confirmatory evidence that the cyclic hematopoiesis defect is in the marrow stem cell.

It is difficult to speculate about the nature of the stem cell defect responsible for cyclic hematopoiesis in these dogs. The autosomal recessive inheritance pattern suggests a single genetic defect; this defect has protean manifestations, including grey coat color, increased perinatal mortality (1), and decreased weight gain even when uninfected, in addition to cyclic hematopoiesis. Certainly much information regarding control of hematopoiesis awaits an understanding of the mechanism of the stem cell defect in these dogs.

In view of the many similarities between canine and human cyclic neutropenia (10) and in view of recent demonstrations of successful human marrow transplants in other nonmalignant hematologic diseases (11-13), the results of the marrow transplantation experiments of canine cyclic neutropenia presented in this report and those of Dale and Graw (9) suggest that a patient with cyclic neutropenia may well benefit from transplantation of normal marrow.

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