Depression of Erythrocyte Maturation as a Result of the Graft-vs.-Host Reaction[1] (34261)

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Recent experiments in this laboratory involving hybrid mice and one of their parental strains have shown the essential similarity of homologous disease and parabiosis intoxication (1–3). Both conditions are thought to be due to a graft-versus-host reaction, that is, an immunological attack of parental strain lymphoid cells on the tissues of the hybrid. Besides similar clinical and pathological features, anemia was present in both syndromes. Although multiple etiologic factors are responsible for the anemia in both syndromes (1), the earlier onset and greater severity of the anemia in intoxicated hybrid parabionts, as compared to that seen in homologous disease, was attributed to a superimposed shunting of blood from the intoxicated hybrid parabiont to the nonintoxicated parental strain partner (2, 4). The anemia in homologous disease was slowly progressive, with hematocrit values as low as 30%, yet the reticulocyte response was negligible. In the intoxicated hybrid parabionts the more severe anemia induced a mild reticulocyte response (1). This was followed by a gradual decrease in the reticulocyte count, despite a persistent anemia, to levels comparable to those seen in mice with homologous disease.

The present experiments represent a further investigation of the impaired reticulocyte response to anemia in the graft-versus-host reaction. When hybrid mice suffering from homologous disease were subjected to daily bleeding, the reticulocyte response was similar to that in the intoxicated hybrid parabionts. The interpretation of this response as probably being the result of an immunological depression of erythrocyte maturation was confirmed by bone marrow studies.

Materials and Methods. Inbred mice of the A strain and F_1 hybrids resulting from the cross between mice of the A and $C_{57}B1/1$ strains were used. Four groups were prepared as follows: (i) (A $\times$ $C_{57}B1/1$) F_1 hybrids injected with strain A spleen cells, followed by daily bleeding for 9 days beginning the day following cell injection (homologous disease plus bleeding), (ii) (A $\times$ $C_{57}B1/1$) F_1 hybrids injected with strain A spleen cells (homologous disease), (iii) (A $\times$ $C_{57}B1/1$) F_1 hybrids in parabiosis with strain A mice (parabiosis intoxication), and (iv) (A $\times$ $C_{57}B1/1$) F_1 hybrids subjected to daily bleeding for 20 days (bleeding controls). Homologous disease in groups i and ii was produced by the intravenous infusion of 400 $\times$ 10^6 strain A spleen cells into (A $\times$ $C_{57}B1/1$) F_1 hybrids. Suspensions of cells were prepared by a method previously described (5). Parabiosis in group iii was performed according to the technique reported by Cornelius *et al.* (1). The mice in groups i and iv were bled daily via the tail veins so as to simulate the hematocrit level of the intoxicated hybrid parabionts of group iii. After day 9 the mice in group i required no further bleeding to maintain the desired hematocrit level. Serial microhematocrit and reticulocyte determinations were made by standard methods (6) on blood samples withdrawn from the tail veins. Bone marrow smears were made from the femurs. The bone was crushed with forceps, the extruded marrow was suspended in 5% bovine serum albumin in lacate–Ringer's solution, smeared on glass slides and stained with Wright's stain. Tibias were also removed, decalcified, and sectioned

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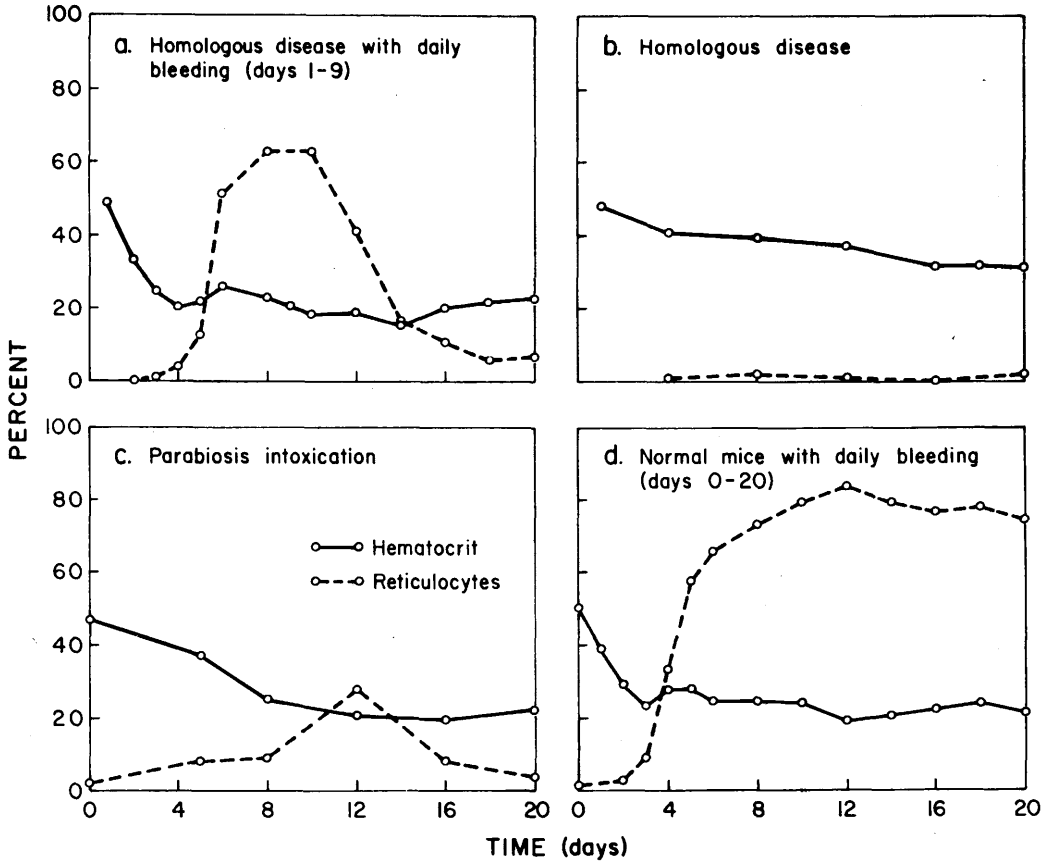

FIG. 1. Mean hematocrit and reticulocyte values for (a) 15 (A × C_{57}B1/1) F_1 hybrids suffering from homologous disease and subjected to daily bleeding from days 1–9 inclusive (group i), (b) 13 (A× C_{57}B1/1) F_1 hybrids suffering from homologous disease (group ii), (c) 25 (A× C_{57}B1/1) F_1 hybrids in parabiosis with A strain partners (group iii) and (d) 11 (A × C_{57}B1/1) F_1 hybrids subjected to daily bleeding from days 0–20 (group iv).

for evaluation of marrow cellularity.

Results. The mice suffering from homologous disease which had been bled from days 1–9 (Fig. 1a) showed a decrease in hematocrit from the initial prebleeding level of 49% on day 1 to 20% on day 4. Thereafter the hematocrit remained low, with a value of 23% on day 20. The reticulocyte count rose from 0.4% on day 2 to a peak value of 63% on days 8 and 10, then decreased progressively to 7% on day 20. The mice with homologous disease which had not been bled (Fig. 1b) showed a progressive decrease in the mean hematocrit value from 48% on day 1 to 31% on day 20. This progressive anemia was not accompanied by an increase in the reticulocyte count; on day 20 the reticulocyte count was 1.8%. Following parabiosis with parental strain mice, the mean hematocrit values for the intoxicated hybrid parabionts showed a progressive decrease from 47% on day 0 to 21% on day 12. (Fig. 1c). The hematocrit level remained constant thereafter. The reticulocyte count showed a progressive increase from the initial value of 2.4% on day 0 to a peak value of 28% on day 12. This was followed by a gradual decrease to a value of 3.4% on day 20, at which time the hematocrit value was 21.5%. Although the normal hybrid mice subjected to daily bleeding (Fig. 1d) had a hematocrit curve similar to that of the intoxicated hybrid parabionts and the homologous disease group subjected to bleeding the reticulocyte response

differed markedly: there was a rapid and persisting reticulocyte response to the anemia, reaching a peak value of 84% on day 12 and remaining at a high level thereafter, with a value of 77% on day 20 (when the hematocrit was 22%). In contrast, the mice with homologous disease which were also bled, as well as the intoxicated hybrid parabionts, showed an initial reticulocytosis, followed by a progressive and significant decrease in reticulocytes, despite a persistent anemia.

Bone marrow studies on 10 mice with homologous disease plus bleeding and on 10 mice of the homologous disease group sacrificed in the 16–20-day period showed cellular depletion with a paucity of normoblasts. Nine intoxicated hybrid parabionts sacrificed on day 10 of parabiosis with parental strain partners showed normal marrow cellularity with normoblastic hyperplasia; in contrast 4 intoxicated hybrids sacrificed on day 14 showed marked hypocellularity with depletion of normoblasts. Finally, 10 F_1 hybrid mice with anemia due to daily bleeding were sacrificed on day 13; all showed normal cellularity with marked normoblastic hyperplasia.

Discussion. Normoblastic hyperplasia of the bone marrow with impaired radioiron uptake was reported previously in mice undergoing graft-versus-host reactions (7). It was also shown that such animals exhibit a poor reticulocyte response to the anemia which is present in association with this reaction (1). In the present study, when F_1 mice, previously injected with parental strain lymphoid cells, were made severely anemic by repeated bleeding, the deficient reticulocyte response occurring in graft-versus-host reactions was demonstrated with great clarity. The bone marrow studies confirmed the interpretation of inhibition of erythropoiesis in this reaction. The etiology of this impaired response is not evident from these experiments. Decrease of red blood cell production in graft-versus-host reactions (1, 7) and in some strains of neonatally thymectomized mice (8) may be explained by immunological reactivity against hemopoietic tissue; possible mechanisms include a direct attack of antibody against red blood cells precursors (9) or erythropoietin (10). In this connection, it has been found that splenectomized, irradiated, spleen nodule cell-injected mice developed severe anemia, and for recovery from this anemia, the presence of the spleen was necessary, perhaps because of production of an erythrocyte maturation factor (11, 12). Lympho-hemopoietic cell transplants engaged in homograft reactions have shown deficient ^{59}Fe and ^{125}I deoxyuridine uptake at 7–10 days after grafting. This impairment of erythropoiesis and of cellular proliferation, respectively, was thought to be due to exhaustion of a pluripotent stem cell pool or to cytotoxic effects on hemopoietic elements present in the milieu of the immunological reaction (13).

In these experiments, a high degree of consistency was obtained in the results, because of the powerful immune response in the parent-F_1 hybrid strain combination used, and because of the large dose of cells employed for the induction of homologous disease. Inasmuch as immunological depression of the reticulocyte response to anemia is the net result of the opposing processes of cell destruction and cell proliferation in the bone marrow (9), it is probable that less clear-cut results might be obtained in other systems in which the immunological response is less pronounced.

Summary. In mice suffering from homologous disease and subjected to repeated bleeding, initial reticulocytosis due to blood loss was followed by a marked drop in the reticulocyte count, despite a persistent anemia. The bone marrow was hypocellular. The absence of a reticulocyte response to anemia in the course of a graft-versus-host reaction may be due to immunological depression of erythrocyte maturation.

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