Long-Term Survival and Reversal of Iron Overload After Marrow Transplantation in Dogs With Congenital Hemolytic Anemia

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Severe hemolytic anemia in Basenji dogs secondary to pyruvate kinase deficiency was corrected by marrow transplantation from hematologically normal littermates. These dogs have now been followed for more than 5.5 yr. Essentially normal hematopoiesis has persisted, and the dogs remain in good health without cirrhosis or osteosclerosis. Furthermore, hepatic iron overload present before transplantation has gradually decreased. These results in dogs suggest that marrow transplantation could prevent the morbidity and mortality of severe hemolytic anemia and associated iron overload in man.

MATERIALS AND METHODS

Dogs

Seven dogs from five litters of the Basenji breed were studied. All had severe hemolytic anemia secondary to pyruvate kinase deficiency. Features of this autosomal recessive disease have been described by Searcy et al. and details of the hematologic status of the dogs that underwent marrow transplantation have been published. Briefly, affected dogs had hematocrits of 18%–27% (mean, 20%), uncorrected reticulocyte counts of 16%–67% (mean, 28%), 2–36 (mean, 14) circulating nucleated RBC/100 WBC and 51Cr RBC survivals of 1.6–6 (mean, 3.6) days. Since essentially normal values of enzyme activity are measured in vitro in dogs with hemolytic anemia because of the compensatory presence of the M2-type PK isozyme, pyruvate kinase deficiency was demonstrated in RBC from heterozygous parents or littermates of affected dogs.

EMOSIDEROSIS and cirrhosis of the liver contribute to the morbidity and mortality of individuals with severe, congenital hemolytic anemias, especially thalassemia major in man and anemia due to erythrocyte pyruvate kinase deficiency in dogs. Although the precise pathophysiologic relationships are not certain, anemia with erythroid hyperplasia is associated with increased intestinal iron absorption, hepatic parenchymal iron deposition, and eventual cirrhosis. We previously reported that transplantation of marrow from clinically normal littermates into dogs with severe hemolytic anemia secondary to pyruvate kinase deficiency resulted in normal hematopoiesis. These dogs have now been observed for more than 5.5 yr after transplantation. Their clinical course and hepatic iron concentrations will be described in this report.

RESULTS

Findings in Untreated Dogs With Hemolytic Anemia

Three dogs, 7–17 mo of age, with proven severe hemolytic anemia died of infection. In all three, there was histologically moderate to severe hepatic hemosiderosis. Hepatocytes, especially toward the lobular peripheries, displayed fine, cytoplasmic, iron-containing granules consistent with ferritin. Coarse aggregates of hemosiderin were present within Kupffer cells and in portal areas. There was no parenchymal or portal fibrosis. Splenic hemosiderosis was mild to moderate. Small amounts of stainable iron were present in aggregations of lymph node histiocytes. Marrow iron stores were not increased.

One dog with severe hemolytic anemia, approximately 3 yr of age, was noted to have radiographic evidence of osteosclerosis and clinical evidence of hepatic dysfunction with ascites and abnormal liver function.

Marrow Transplantation

Three dogs with hemolytic anemia were conditioned for marrow transplantation with 1200 R total body irradiation and given hematopoietic grafts from littermate donors compatible at the canine major histocompatibility complex. Allogeneic engraftment was confirmed by demonstration of donor type RBC enzyme allotypes in all three dogs and by resolution of the hemolytic anemia. Details of the initial 200 days post-transplant have been published.

Studies

Autopsies were performed on all dogs that died. Survivors underwent open liver biopsy and splenectomy, skin biopsy, and bone marrow aspiration and biopsy. Histologic stains included hematoxylin and eosin, periodic acid Schiff, Prussian blue, reticulin and Golgi montage. Peripheral blood hemostatic parameters and serum chemistry values were determined using standard techniques. RBC isoenzymes and 51Cr RBC survivals were determined by published methods. Mean RBC survival (t/2) in 14 normal dogs was 19.5 ± 2.0 days (± SD, range 17.2–24.2 days).

Liver iron concentrations were determined on tissue removed from paraffin blocks and rehydrated by passage through xylene and decreasing concentrations of ethanol into water. Specimens were then oven-dried, weighed, and solubilized overnight in concentrated nitric acid. The residue was redigested as needed (0–3 times) and dissolved in HCI. Iron was determined with the Ferro/Chem iron analyzer. Duplicate determinations generally differed by less than 10%.

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Fig. 1. Hematocrit values before and after allogeneic marrow transplantation in dogs PK16 (dark circles), PK18 (dark squares), PK90 (dark triangles), and normal dogs (small, open circles). Range and median values in normal dogs are indicated in upper left portion of figure and dotted line indicates lower limit of normal range. Note that before day 1600 after transplantation most values are above the lower limit of normal, while after day 1600 mild anemia is more frequent in all dogs.
found in all dogs with active hemolytic anemia (30.8–50.5 μg/mg dry wt). Hepatic iron concentration was substantially lower in PK18 1 yr after transplantation and even lower, although still above the normal range, in dogs PK90 and PK16 4.4–5.6 yr after transplantation.

DISCUSSION

We had previously shown that total body irradiation and marrow transplantation from nonanemic, histocompatible littermates could result in complete correction of severe, hereditary hemolytic anemia in Basenji dogs. These transplant recipients have now been followed for nearly 6 yr. They have no evidence of hemolytic anemia, and studies of RBC isoenzymes confirm persistence of donor hematopoietic cells. We have also followed normal canine recipients of allogeneic marrow grafts for over 10 yr after transplantation and observed stable persistence of donor hematopoietic and lymphoid cells. Equally important is the observation that, after elimination of the hemolytic anemia in these dogs, not only were further hepatic iron accumulation and scarring prevented, but, in addition, gradual loss of the excess hepatic iron resulted in eventual restoration of almost normal amounts of iron. It therefore appears reasonable to expect that tissue iron overload in patients might be reversed if the hemolytic anemia were corrected by marrow transplantation.

Allogeneic marrow transplantation from histocompatible siblings has generally been reserved for patients with a poor, short-term prognosis, e.g., those with severe aplastic anemia or end stage acute leukemia. Recently, however, improved results have been achieved by marrow transplantation earlier in the disease course. For example, 75% of 30 untransfused patients with aplastic anemia who received sibling marrow transplants within 3 wk (median) of diagnosis are projected to become long-term survivors. Most surviving patients are well and enjoy normal lives,
although some require therapy for chronic graft-versus-host disease. Similarly, 63% of patients with acute nonlymphoblastic leukemia in first remission transplanted after high dose chemoradiotherapy with marrow from histocompatible siblings are alive with a high probability of cure of their leukemia, results clearly superior to those achieved when marrow transplantation was delayed until patients had refractory disease. Comparable results have also been achieved in patients with acute lymphoblastic leukemia and by other transplant centers. It is nevertheless unlikely that equally favorable results would be obtained at the present time if marrow transplantation were undertaken in patients with thalassemia major, sickle cell disease or other severe hereditary hemolytic anemias who have been highly sensitized by multiple transfusions. The observations in Basenji dogs reported here suggest, however, that as the clinical results of allogeneic marrow transplantation continue to improve, this mode of treatment may eventually be applied to human patients with severe hereditary hemolytic anemias.

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