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

By Paul L. Weiden, Robert C. Hackman, H. Joachim Deeg, Theodore C. Graham, E. Donnall Thomas, and Rainer Storb

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.

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.

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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.

function tests: bilirubin = 1.4 mg/100 ml, alkaline phosphatase = 1434 IU/l and SGOT = 152 mU/ml (normals ± SD = 0.1 ± 0.0 mg/100 ml, 76 ± 60 mU/ml, and 58 ± 19 mU/ml, respectively). Spontaneous Clostridium sp. peritonitis resulted in death. Autopsy showed pleural effusions; ascites; an enlarged, fibrotic liver with apparent abscess cavities; an enlarged, firm spleen; and hard, brittle bones. Microscopic examination of the liver showed broad fibrous bands linking portal and centrilobular areas and containing massive hemosiderin deposits. Iron was also present in hepatocytes and in the endothelial cells of vessels in portal areas. Extramedullary hematopoiesis was prominent in the spleen. Iron was present within splenic red pulp histiocytes and in sinus histiocytes of some lymph nodes, in pancreatic islet cells and within the cytoplasm of renal tubular epithelial cells. The marrow was normocellular with myelofibrosis consisting of a diffuse network of coarse reticulin fibers associated with collagen.

Long-Term Post-Transplant Clinical Course

Three dogs, designated PK16, PK18, and PK90, received marrow grafts from histocompatible littermates as previously reported.6 These dogs were 7–9 mo old at the time of total body irradiation and hematopoietic grafting and were closely related to the three dogs described above that died at 7–17 mo of age. All three were severely anemic pretransplant (Fig. 1) and had markedly elevated reticulocyte counts and shortened 51Cr RBC survivals.6 Post-transplant, hematocrits ranged from 36%–44% and reticulocyte counts were less than 4%, indicating resolution of the hemolytic anemia.51Cr RBC survivals were 17–19.8 days in all three dogs 166–213 days post-transplant,6 and 16.5 and 18.5 days 1266 and 1307 days post-transplant in PK16 and PK90. Donor RBC esterase D allotypes were demonstrated repeatedly in all dogs, most recently on day 2007 post-transplantation in PK16 and on day 2048 in PK90. During the most recent 300–500 days, the hematocrits of PK16 and PK90 have decreased somewhat, but not outside the range observed in other long-term canine survivors with allogeneic marrow grafts (Fig. 1). In addition, no significant reticulocytosis has been observed in either dog (uncorrected reticulocyte counts ~ 0.7%–6%, mean, 3.1%). Clinically, the dogs remain well with stable body weight and normal serum values of bilirubin, alkaline phosphatase, SGOT, SGPT, LDH, iron and iron binding capacity.

Post-Transplant Histology

PK18 was killed 352 days post-transplant in an unrelated experiment. Histologic examination of the liver revealed increased iron in all areas, but markedly less than in the dogs that had not received hematopoietic grafts. There was no hepatic fibrosis. Examination of other tissues showed moderate splenic hemosiderosis, mild nodal lymphoid depletion, and a mildly hypercellular bone marrow with normal iron stores and no fibrosis.

Biopsies of liver, spleen, skin, and bone marrow were obtained from PK90 and PK16 on days 1616 and 2044, respectively, post-transplant. Iron stains of hepatocytes were negative. Histologically normal amounts of iron were present in the portal areas. There was no splenic or cutaneous hemosiderosis. The normocellular marrow contained normal iron stores and was not fibrotic.

Liver Iron Concentration

Hepatic iron concentrations are presented in Table 1. Markedly elevated tissue iron concentrations were
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-recipient dogs 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.12 Similarly, human patients with aplastic anemia or leukemia have now been observed for over 9 yr after allogeneic marrow transplantation with stable persistence of donor lymphohematopoietic cells without recurrence of either host type cells or the underlying disease for which they were transplanted.13,14 These observations indicate that allogeneic marrow transplantation from a hematologically normal, histocompatible sibling could result in the long-term and, most likely, permanent correction of congenital hemolytic anemia in man.

The high morbidity and mortality in individuals with severe, hereditary hemolytic anemia are not, however, due to anemia per se. Clinical manifestations vary with the hereditary defect. For example, patients with severe thalassemia develop complications that result from iron overloading of tissues, primarily the heart, liver, and endocrine glands.1,2,5,15 Iron overload-

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**Table 1. Liver Iron Concentrations in Normal Dogs and Dogs With Hemolytic Anemia Before and After Marrow Transplantation**

<table>
<thead>
<tr>
<th>Status of Dog(s)</th>
<th>No. of Dogs Studied</th>
<th>Hepatic Iron µg Fe/mg Liver dry wt</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Basenjis</td>
<td>5</td>
<td></td>
<td>3.7</td>
<td>1.6–5.0</td>
</tr>
<tr>
<td>Hemolytic anemia, died</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with hepatic dysfunction</td>
<td>1</td>
<td></td>
<td>39.0</td>
<td>—</td>
</tr>
<tr>
<td>Hemolytic anemia, died</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of infection</td>
<td>3</td>
<td></td>
<td>42.9</td>
<td>30.8–50.5</td>
</tr>
<tr>
<td>Hematologically normal,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 yr after marrow transplant</td>
<td>1</td>
<td></td>
<td>16.4</td>
<td>—</td>
</tr>
<tr>
<td>Hematologically normal,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.4–5.6 yr after marrow transplant</td>
<td>2</td>
<td></td>
<td>10.8</td>
<td>7.7–14.0</td>
</tr>
</tbody>
</table>

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ing results in part from RBC transfusions, but even in the absence of transfusions, from increased intestinal iron absorption.16 Hepatic iron accumulation begins in infancy in patients with thalassemia and hepatic fibrosis and scarring are observed in the first decade.1,2 The severity of hepatic fibrosis correlates both with hepatic iron concentration and patient age. Hereditary hemolytic anemia secondary to pyruvate kinase deficiency in the Basenji dog appears to share these clinical features with severe thalassemia syndromes, i.e., progressive tissue iron overload even in the absence of transfusions, leading to organ damage and, eventually, death.

Anemic patients with erythroid hyperplasia of the marrow and ineffective erythropoiesis generally have increased intestinal iron absorption, whereas equally anemic patients with hypoproliferative marrows do not.5,16 The nature of the “signal” relating marrow proliferation and intestinal absorption is unknown, raising the possibility that the genetic defect in thalassemia may result directly in the increased intestinal iron absorption. This possibility is strengthened by the observation that patients with other severe, hereditary hemolytic anemias, e.g., sickle cell disease, only rarely develop significant hepatic iron overload and cirrhosis.17–19 The present study, however, provides direct evidence that, at least in the Basenji dog with severe hemolytic anemia secondary to pyruvate kinase deficiency, the hepatic iron accumulation and subsequent cirrhosis characteristic of this disease could be avoided by elimination of the hemolytic anemia. Furthermore, none of the transplanted dogs developed osteosclerosis, another characteristic long-term sequel of this anemia in untreated dogs.3,4

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.21 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.22 Most surviving patients are well and enjoy normal lives,
although some require therapy for chronic graft-versus-host disease.\textsuperscript{23} 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,\textsuperscript{24} results clearly superior to those achieved when marrow transplantation was delayed until patients had refractory disease.\textsuperscript{25,26} Comparable results have also been achieved in patients with acute lymphoblastic leukemia\textsuperscript{25} and by other transplant centers.\textsuperscript{26,27} 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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