Bone Marrow Transplantation in Fanconi Anemia Using Matched Sibling Donors

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Eighteen patients with Fanconi anemia (FA) with evidence of bone marrow (BM) aplasia underwent allogeneic BM transplants (BMT) from matched sibling donors (MSD). Median age at BMT was 7.6 years. Conditioning consisted of low-dose cyclophosphamide (CY; 5 mg/kg x 4 days) and thoracoabdominal irradiation (TAI; 400 cGy). Graft-versus-host disease (GVHD) prophylaxis included cyclosporin A and prednisone. In addition antithymocyte globulin (ATG) was administered in the pretransplant period to promote engraftment and in the posttransplant period for additional GVHD prophylaxis. Engraftment occurred rapidly (median, 12 days for an absolute neutrophil count ≥0.5 x 10^9/L; median, 22 days for platelet count ≥50 x 10^9/L). Seventeen patients have sustained engraftment and are transfusion-independent, with Lansky scores of 100% at median follow-up of 27 months. One patient developed graft failure 4 months after initial engraftment and required a second BM infusion. None of the patients developed acute GVHD; 3 patients (16%) developed chronic GVHD. BMT is a feasible option for FA patients having an MSD and should be performed at a young age and early in the course of the disease, before the development of complications. We believe the addition of ATG to the transplant regimen of low-dose CY, TAI, and cyclosporin was responsible for improvement in the survival of FA patients undergoing BMT. The regimen was well tolerated and was associated with a low incidence of complications including GVHD.

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**MATERIALS AND METHODS**

**Patients.** Of the 18 patients transplanted, 12 were boys and 6 were girls. The median age at diagnosis of FA was 7 years (range, 1.5 to 10 years). Thrombocytopenia (or pancytopenia) with clinical bleeding or routine testing was the most common initial presentation (16 of 18 patients); the remaining 2 were investigated for FA because of their short stature and congenital abnormalities and were subsequently discovered to be pancytopenic. Of the patients, 14 had short stature (height below the 5th percentile for age); 10 had associated thumb, toe, or radial abnormalities; and 8 patients had renal abnormalities including fused or pelvic kidneys. Other associated abnormalities included café-au-lait spots (7), external ear abnormalities (4), and a variety of other malformations. Problems before BMT included repeated bacterial infections and hemorrhagic episodes. Confirmation of diagnosis of FA was made in all patients by documenting increased spontaneous and DEB-induced chromosomal breaks.

A total of 8 patients had not received any transfusions or medications for their aplasia before BMT; 7 patients had received transfusions as well as androgens with or without steroids, and 3 patients had received only platelet transfusions pre-BMT. All except 1 of the patients had received less than 20 transfusions. None of the patients were refractory to blood products.

Median age at BMT was 7.6 years (range, 2.7 to 12.6 years). At the time of BMT, all patients had evidence of BM aplasia confirmed by BM examination. The median pretransplant values (with ranges) were as follows: Hemoglobin (Hb), 8.4 g/dL (3.3 to 12.8); white blood cell count (WBC), 3.5 x 10^9/L (1.6 to 7.5); absolute neutrophil count (ANC), 0.65 x 10^9/L (0.19 to 3.45); and platelets, 51 x 10^9/L (8 to 78). Pretransplant cytogenetic analysis of the BM did not show any clonal abnormality, and none of the patients had malignant transformation.

**Donors.** All donors were HL-A-identical siblings (matched at HL-A loci A, B, and DR) with nonreactive mixed lymphocyte culture. The median donor age was 8.7 years (range, 0.9 to 19 years), excluding the case where HL-A-matched sibling cord blood was used as a...
source of stem cells.13,14 All donors were screened for increased chromosomal breaks by DEB testing and found to be normal.

There were 11 male-to-male, 6 male-to-female, and 1 female-to-male transplant. In 9 transplants, the donors and recipients were cytomegalovirus (CMV)-seronegative, and in 7 instances, donors were CMV-seropositive with CMV-seronegative recipients. In another 2 cases, the donors were CMV-seronegative with CMV-seropositive recipients.

Serpology indicated presence of past Epstein-Barr virus (EBV) infection (IgG) in 8 of 13 patients and in 9 of 13 donors. None of the donors or recipients tested had evidence of recent EBV infection (negative IgM).

The BM (and the cord blood) were not T-cell-depleted. The BM dose consisted of a median number of 3 × 10^8 nucleated cells/kg (range, 2.0 to 7.0 × 10^8/kg). The patient transplanted with cord blood received a lower dose of 0.26 × 10^9 nucleated cells/kg.15

Preparative regimen. All patients, including the patient receiving the cord blood transplant, received a preparative regimen consisting of 20 mg/kg (CY) [5 mg/kg/d for 4 days (days 5, 4, 3, and 2 pretransplant)]. TA1 was administered 1 day before transplant, extending from the angle of the mandible to the upper third of the thighs with shielding of lungs and shielding of kidneys posteriorly and of bladder anteriorly. The single dose of 400 cGy was administered at a dose of 200 cGy/min.

GVHD prophylaxis. GVHD prophylaxis consisted of cyclosporin A administered from day 2 pretransplant to day 100 posttransplant with trough levels maintained between 300 and 350 ng/mL and then tapered to discontinue by 6 months. Prednisone was administered at a dose of 10 mg/M^2 per day from day 2 pretransplant to day 14 posttransplant and then tapered over the next 2 weeks.

ATG. ATG was administered during pretransplant and posttransplant periods for engraftment and as part of GVHD prophylaxis in the following manner: 40 mg/kg on days 6, 4, and 2 pretransplant and 20 mg/kg on days 2, 4, 6, 8, 10, and 12 posttransplant.

Supportive care. All patients were maintained in protective isolation (laminar air flow for 17 patients and high efficiency particulate air filter with positive pressure for 1 patient) until their ANC was greater than 0.5 × 10^9/L for 3 consecutive days. Weekly intravenous Ig (500 mg/kg) was administered for 3 months after BMT. All CMV-seronegative patients received CMV-seronegative blood products. Herpes simplex virus-seropositive patients received acyclovir, 750 mg/M^2/d, during transplant and until 3 months after transplant.

RESULTS

Toxicity. There were no deaths related to the toxicity of the preparative regimen. Mucositis was the only significant complication, and oral mucositis was scored from grade 1 to 3, depending on redness, ulceration, and ability to eat.15 It was of mild to moderate severity (grade 1 to 2). One patient developed hemorrhagic cystitis. None developed veno-occlusive disease of the liver or interstitial pneumonitis. One patient had a seizure of undetermined etiology. All patients received broad spectrum antibiotic coverage after transplant for febrile episodes, although bacteremia was documented in only 3 patients (Staphylococcus epidermidis, Escherichia coli, nonhemolytic streptococci). Adenovirus was isolated from the urine of 1 patient.

All patients except 1, were supported with parenteral nutrition (PN) during BMT. Glycosuria, not always accompanied by hyperglycemia, was observed in 14 patients while receiving PN, and 2 patients required insulin. The patients became normoglycemic when PN was discontinued. A total of 10 patients developed hypertension (blood pressure greater than the 95th percentile for age) during BMT, requiring short-term (less than 4 weeks) antihypertensive treatment. A total of 7 patients developed fever (greater than 101°F) during ATG infusion, and 2 patients developed chills and rigors that responded to antipyretics and antihistamines. Elevation of blood pressure was also noted in 4 patients during ATG administration. None of the side effects were severe enough to warrant discontinuation of ATG therapy. Serum sickness or anaphylactic reaction was not observed in any patient.

Engraftment. Engraftment was defined as an increase in ANC greater than 0.5 × 10^9/L for 3 consecutive days. It was confirmed by BM aspiration and biopsy along with cytogenetic studies, ABO typing, and neutrophil typing wherever applicable.

Engraftment was rapid, with a median time of 12 days (range, 9 to 37 days). The median time to achieve a self-sustaining platelet count of 50 × 10^9/L was 22 days (range, 11 to 60 days). One patient developed delayed graft failure (described under follow up).

GVHD. GVHD was graded according to the published criteria.10 Acute GVHD was not observed in any patient. A total of 3 patients developed chronic GVHD (16%); 2 patients developed limited chronic GVHD of the skin, and 1 patient developed chronic GVHD involving the liver, gastrointestinal tract, and lungs. All 3 patients responded to treatment with steroids and cyclosporin.

Follow-up. All patients are alive with Lansky scores of 100% at a median follow-up of 27 months (range, 6 to 75 months). A total of 17 patients have sustained grafts and are transfusion-independent with the following median blood counts (with ranges): Hb, 13.1 g/dL (11.6 to 16.1); WBC, 6.8 × 10^9/L (3.5 to 14.5); ANC, 3.52 × 10^9/L (1.1 to 9.3); platelets, 255 × 10^9/L (170 to 329). A total of 2 patients (donor and recipient CMV-seropositive in both cases) developed asymptomatic CMV viruria post-BMT. They were treated with ganciclovir with resolution of viruria.

One patient had delayed graft failure at day 120 after successful engraftment. She also had evidence of chronic GVHD involving the skin. About 25% of the peripheral mononuclear cells were of donor origin. She received a repeat BM infusion from the same donor 10 months after her first BMT, after reconditioning with ATG (30 mg/kg/d for 3 days). Engraftment was documented by day 13, and she is gradually becoming less transfusion-dependent. At her most recent follow-up on day 60, postboost, almost all of the nonlymphocyte white blood cells are donor-derived, although 50% of the lymphocytes are of host origin.

Immune reconstitution. Of 15 patients evaluated for immune reconstitution 1 year after BMT, 12 had attained normal Ig levels (IgA, G, and M), and 3 patients had low levels of one or two Igs.

Total numbers of T cells and their subsets were normal in 13 and reduced in 2 patients. The T-cell helper-suppressor ratio was normal in 13 patients and low in 2 patients. Mitogen stimulation response to phytohemagglutinin, concanavalin A, and pokeweed mitogen were normal in 5 patients; 6 patients had diminished response to one and 2 patients to
two of the three mitogens tested. Only 1 patient had poor response (20% to 34% of controls) to all three agents. Mitogen studies were not performed on 1 patient.

DISCUSSION

The natural history of FA is characterized by progressive BM failure, with mean age of 6.24 years at the diagnosis of aplasia. These patients are at an increased risk for development of malignancies, especially leukemias and carcinomas. The mean age at diagnosis of leukemia is 14.8 years, whereas carcinomas develop at a much later age.

In the early reports, FA patients undergoing BMT had a poor outcome primarily because of preparative regimen-related toxicity, GVHD, and infections. The initial conditioning regimens were similar to those used in aplastic anemia, using CY at a dose of 200 mg/kg, which led to severe toxicity that often resulted in death. The French group showed that lowering the dose of CY to 20 mg/kg over 4 days along with TAI (500 cGy) resulted in successful engraftment and was associated with acceptable toxicity. Using this regimen, Gluckman reported an overall survival of 75% in 39 patients with FA transplanted from sibling donors (BM or cord blood). However, GVHD still remained a major problem, contributing significantly to overall morbidity and long-term survival of FA patients undergoing BMT. Acute GVHD was observed in 14 (47%) patients. Chronic GVHD was observed in 12 of 26 patients surviving for more than 120 days. A total of 9 patients died because of graft rejection, severe acute GVHD, interstitial pneumonia or infection associated with chronic GVHD, and late complications including liver cirrhosis and adenocarcinoma of the tongue.

Other groups have modified the Gluckman regimen mainly by altering the radiation and the GVHD prophylaxis. Hows et al reported 10 FA patients transplanted from HLA-identical sibling donors using the same low dose of CY. Total body irradiation (TBI; 200 cGy × 3) was used instead of TAI. GVHD prophylaxis consisted of cyclosporine in all patients, and BM was T-cell–depleted in 3 of 10 patients. One patient died very early, and one patient had graft failure. Clinically significant acute GVHD (grade II–IV) occurred in 6 of 8 (75%), and chronic GVHD developed in 3 of 8 (35%) evaluable patients. The Italian group also used TBI at a lower dose (167 cGy × 3) along with low-dose CY in the MSD transplants. All 5 patients are alive, with full donor engraftment 18 to 67 months after BMT. Cyclosporin alone or with methotrexate was used for GVHD prophylaxis. One patient developed acute GVHD.

The Seattle group used a nonradiation-containing regimen consisting of high-dose CY (140 to 200 mg/kg) in 12 FA patients (donors were HLA-identical siblings in 11 and an HLA-identical mother in 1 case). All patients engrafted, including 1 who required two additional BM infusions. Methotrexate with or without cyclosporin was used for GVHD prophylaxis. Regimen-related toxicity contributed to 4 deaths. A total of 4 patients (33%) developed significant acute GVHD (grade II–IV), and extensive, chronic GVHD occurred in 4 of 8 (50%) patients at risk; 1 patient died of squamous cell carcinoma. A total of 7 (65%) patients were alive at a median of 5 years after BMT.

The International Bone Marrow Transplant Registry reported better survival of patients who received low-dose CY (15 to 25 mg/kg) versus those who were transplanted using higher doses. The incidence of graft failure was not significantly different between the groups conditioned with TBI or limited field radiation along with low-dose CY. Among 89 patients transplanted from MSD, the overall actuarial probability of 2-year survival was 61% ± 11%. The probability of acute GVHD was 49% ± 11%, and of chronic GVHD was 51% ± 13%.

We report our experience with 18 FA patients with BM failure who were successfully transplanted using HLA-MSD. We believe that addition of ATG to the Gluckman regimen was a major factor in improving the survival of patients in our study. Based on animal models, ATG was administered at much higher dosage (40 mg/kg in the pre-BMT phase, followed by 20 mg/kg in the post-BMT period) than used in other clinical trials in BMT patients. By using ATG pre-transplant, the radiation dose could be lowered by 20% without compromising the chances of successful engraftment. ATG, at appropriate concentration, is effective in eliminating natural killer cell activity, and this together with its anti–T-cell activity could facilitate engraftment when used before the infusion of hematopoietic progenitor cells. The conditioning regimen provided adequate immunosuppression and myeloablation of the recipient BM and was associated with minimal toxicity. The use of limited field radiation avoided TBI and used a lower dosage of radiation than other series. Seventeen patients are well, have fully engrafted, and are transfusion-independent, whereas 1 patient required an additional BM boost and is in the early posttransplantation phase. The GVHD prophylaxis was intensified by the addition of ATG in the posttransplant period. The incidence of GVHD was very low in our patients. None of the patients developed acute GVHD, and only 3 (16%) had chronic GVHD. Although over half of the patients experienced side-effects, the high dose of ATG was generally well tolerated, and none of the patients had serious life-threatening problems, including severe infections. ATG is not routinely used in other centers in FA patients undergoing BMT.

The influence of ATG was analyzed from the data reported to the Fanconi Anemia Transplant Registry. There was a significant improvement in survival of patients who received ATG/antilymphocyte globulin (ALG) (90%) as part of the preparative regimen compared with that of those who did not (69%; P = .05), although both groups had otherwise received identical conditioning (low-dose CY and limited field radiation) and GVHD prophylaxis (cyclosporin).

A major concern associated with the use of ATG has been the development of secondary malignancies, especially EBV-related lymphomas and solid tumors, and ATG has been related to failure of immune surveillance. Most of the lymphomas in this context were observed very shortly after intense immunosuppression (less than 100 days after transplantation). They most often followed the use of ATG for the treatment of acute GVHD, frequently in the setting
of T-cell depletion and HLA mismatch. These risks must be considered because FA patients are already prone to development of malignancies. Although a significant proportion of the donors and the recipients had positive EBV serology, this complication has not been observed in our patients. Our group of patients are well beyond the above risk period. They received non-T-cell-depleted graft from fully matched sibling donors, and their immune reconstitution after BMT was very comparable with other patients transplanted at our institution for indications other than FA (data not shown).

Other factors could have also contributed to the improved survival of our patients. The younger age of patients may be an important factor, because the incidence and severity of GVHD is much lower in children. Only 2 of our patients were older than 10 years of age. However, the median age of patients at BMT in the French, Seattle, and English series was 9, 8, and 8.5 years, respectively, and was comparable with that of our patients (7.6 years). In these reports, there was a significant incidence of GVHD and toxicity even in patients younger than 10 years of age that influenced the overall survival. Thus, the low incidence of GVHD in our patients cannot totally be accounted for by their relatively younger age.

The young age of the donors, the low numbers of patients who had received pretransplant transfusions or had transplanta- tion-related sequelae, and the absence of malignant transformation are other significant factors that may have influenced the outcome of our patients. Prednisone was incorporated in the first 4 weeks with an intent to diminish the side-effects of ATG, such as serum sickness. Although used in very low dose, the use of the steroid may also be a factor in diminishing the incidence of GVHD. The regular use of Igs may have contributed to the low incidence of infectious complications. The use of protective environment could considerably diminish the incidence of GVHD and infections.31

Currently, the follow-up is too short to assess the efficacy of BMT in FA patients in reducing the incidence of hematologic malignancies. The risk of carcinomas and other nonhematologic malignancies, especially in the radiation field after BMT, needs further evaluation. The use of nonradiation-containing regimens should be considered in an attempt to diminish the risk of secondary malignancies. Long-term follow-up is continuing in these patients to address these and other questions related to delayed regimen-related toxicity.

The phenomenon of glycosuria and hyperglycemia in FA patients undergoing BMT is interesting and needs further investigation. Swift and Sh01man reported a high mortality in heterozygous females from causes associated with diabetes mellitus. Currently, we are evaluating this observation in FA patients.

If an HLA-matched sibling is available for BM donation, we suggest that BMT be performed early in the course of the disease, when BM aplasia first becomes evident, preferably by 10 years of age. BMT at a young age would help in diminishing risk factors that may jeopardize a successful transplantation at a later date, such as limitation of transplanta- tion complications and adverse effects of alternative forms of therapy including androgens and steroids. Because a significant number of FA patients develop complications in the second decade of life, early transplantation could theoretically reduce the incidence of hematologic malignancies.7 The incidence and severity of GVHD is also lower in the younger age group. The Cincinnati regimen containing ATG is well tolerated and is associated with a low incidence of complications including GVHD. Early diagnosis of patients will have a significant impact towards transplantation at a younger age.8

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