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Allografting in Patients With Severe, Refractory Aplastic Anemia Using Peripheral Blood Stem Cells and a Fludarabine-Based Conditioning Regimen: The Mexican Experience

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We studied the effectiveness of a fludarabine/cyclophosphamide-based conditioning regimen without anti-thymocyte globulin in 23 aplastic anemia patients who had no response to previous conventional pharmacologic treatment. Patients received oral busulphan 4 mg/kg/day/2 days, IV cyclophosphamide 350 mg/m^2/day/3 days, and fludarabine 30 mg/m^2/day/3 days. For GVHD prophylaxis, patients received MTX 5 mg/m^2 days +1, +3, +6, and +11 and oral cyclosporin A (CyA) 5 mg/kg/day, starting on day −1. Peripheral blood stem cell products were used with a median dose of 5.5×10^6 CD34$^+$/kg. The patients were followed for an average of 25 months. By a median of day +11, an ANC > 0.5×10^9/L was reached; and by day +12, the platelet count had reached >$20{,}000 \times 10^9$/L. Acute grade I–II GVHD occurred in 4 patients, whereas limited chronic GVHD presented in 6 cases. Twenty-one patients (91.3%) achieved engraftment. Two patients failed to engraft, and 4 developed late rejection; 2 of these individuals died, 2 have survived with high transfusion requirements, whereas 2 received a second peripheral blood stem cell infusion and achieved sustained engraftment. Currently 21 (91%) of the 23 patients are alive, whereas 19 of 21 (90%) remain in complete remission. The average cost was about USD 15,000 for this kind of reduced-intensity allotransplant. Reduced-intensity stem cell transplantation represents an affordable alternative to traditional more cytotoxic conditioning for severe aplastic anemia (SAA) patients. Long-term effects however, remain to be evaluated. Am. J. Hematol. 81:157–161, 2006. © 2006 Wiley-Liss, Inc.

Key words: aplastic anemia; marrow transplantation; reduced-intensity; fludarabine

INTRODUCTION

Aplastic anemia (AA) is a syndrome characterized by insufficient production of blood cells and empty bone marrow. The best treatment available for severe cases is allogeneic hematopoietic stem cell transplantation (HSCT). However, mortality, transplant-related complications, and graft rejection remain important obstacles to a successful transplantation, especially in patients who have received multiple transfusions [1]. Addition of antithymocyte globulin (ATG) and/or total lymph node irradiation to cyclophosphamide (CY) has been recommended in order to avoid graft rejection [2–5] but results in higher cost and complications.

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Mexico is a country with a large number of cases of AA [6]; however, due to financial and idiosyncratic factors, the elapsed time from diagnosis to hematopoietic cell transplantation is usually long, leading to AA patients being multiply transfused and highly sensitized to HLA antigens before transplant is undertaken. We have studied the effectiveness of peripheral blood stem cells and a fludarabine/cyclophosphamide-based conditioning regimen without ATG or lymph node irradiation in 23 AA patients who had no response to previous conventional pharmacologic treatment, including androgens and immunosuppressive therapy, and who had been heavily transfused.

MATERIAL AND METHODS

Patients

The population studied consisted of 23 patients with severe refractory AA, recruited in four Mexican institutions: the Hospital Universitario in Monterrey, Nuevo León; the Centro de Hematología y Medicina Interna in Puebla, Puebla; Centro Médico La Raza; and the Instituto Nacional de Cancerología in Mexico City, after January 2000. Patients met the criteria for the diagnosis of severe AA [1,6]. All patients had failed to respond to conventional treatment with immunosuppression using ATG and/or cyclosporin and had been heavily transfused (see Table I). Karnofsky scores were 100% when the procedure was performed. Donors were HLA-identical (6 out of 6) siblings in all instances. Institutional review board approval and written consent from all the individuals were obtained.

Hematopoietic Stem Cell Mobilization and Apheresis

The method has been described previously [7]. Briefly, filgrastim (10 μg/kg/day) was delivered to the sibling donors on days −4 to −1. Apheresis was performed on days 0 to 2, according to cell count, by means of a Haemonetics V-50 PLUS machine (Haemonetics Corporation, Braintree, MA) or a Baxter CS-3000 PLUS machine (Baxter Healthcare, Deerfield, IL), using the Spin–Nebraska protocol. During each apheresis, 5000–7000 mL of blood/m^2 was processed, in order to obtain at least 5×10^8 mononuclear cells and/or $(3–6) \times 10^6$ CD34 cells/kg of the recipient. Enumeration of total white blood, mononuclear, and CD34-positive cells was done by flow cytometry in a FACSCalibur (Becton Dickinson, San Jose, CA) or EPICS Elite ESP apparatus, using the anti-CD34 monoclonal antibody HPCA-2 (Becton Dickinson). No purging procedures were performed.

TABLE I. Salient Features of the 23 Patients With SAA Who Received Allogeneic Stem Cell Allografts From HLA-Identical Siblings

Patients	23
Age, median (range)	25 years (4–65)
Sex (M/F)	9/14
Immunosuppression/androgens	23 patients
Transfusions (range)	20 (5–56)
Follow-up, months (range)	24 (2–77)
Early deaths	2
Engraftment	21
Failure to engraft	2
Late rejection	4
Engrafted after second BMT	2
Acute GVHD, grades I and II	4
Chronic GVHD, limited	6

Conditioning Regimen, Grafting, and GVHD Prophylaxis

A low-intensity conditioning regimen based on busulfan, fludarabine, and cyclophosphamide (CY) without ATG [7] was used. Oral busulfan 4 mg/kg/day on days −6 and −5, intravenous CY 350 mg/m^2/day on days −4, −3, and −2 and fludarabine 30 mg/m^2/day on days −4, −3, and −2. In cases with an absolute neutrophil count below 250, busulfan was not used and CY dose was doubled. Ondansetron at 8 mg IV everyday was used on days −6 to −2. After chemotherapy, ciprofloxacin (1.0 g/day), fluconazole (100 mg/day), and acyclovir (1200 mg/day) were used in all patients, until more than 500 granulocytes/μL were present. The products of peripheral blood apheresis were re-infused on days 0, 1, and 2, according to the $CD34^+$ yield. For graft-versus-host disease (GVHD) prophylaxis, patients received methotrexate at a dose of 5 mg/m^2 IV on days 1, 3, 6, and 11 after cell infusion. Oral cyclosporine (CyA) was started on day −1 at a dose of 5 mg/k/day in two divided doses and was continued for 6 months with adjustments according to serum levels; if GVHD data were present, CyA was tapered over longer periods. GVHD was graded according to the clinical assessment using modified Seattle Criteria [7]. Engraftment was defined as an absolute neutrophil count $>0.5 \times 10^9$/L for at least 3 consecutive days, and platelet engraftment was defined as the first of 7 consecutive days with a platelet count $>20 \times 10^9$/L without platelet transfusion. Graft failure was defined as failure to reach an absolute granulocyte count of $>0.5 \times 10^9$/L on day +30. Graft rejection was defined as a progressive decrease in blood counts after initial engraftment. All blood products used in supportive care were irradiated and leukocyte-depleted. Overall survival was estimated using the Kaplan–Meier method.

RESULTS

Twenty-three patients, 14 females, received transplants from HLA identical sibling donors (Table I), with an age range of 4–65 years (median age, 25 years). All patients had received multiple transfusions, with a median of 20 blood cell units (range, 5–56 units). Only 3 individuals received the conditioning regimen as outpatients, whereas 20 were admitted to the hospital at the time of the grafting as a consequence of their clinical conditions. Peripheral blood stem cells were used in all instances and were infused on days 0, +1, and +2, with a median dose of 5.5×10^6 $CD34^+$/kg of body weight. Patients were followed for a median of 25 months (range, 2–77 months). At day +11 (range, day 0–17), an ANC $>0.5 \times 10^9$/L was reached; by day +12 (range, day 0–20), the platelet count reached $>20{,}000 \times 10^9$/L. Acute grade I–II GVHD occurred in 4 patients (17.3%), whereas limited chronic GVHD was present in 6 (26%). Twenty-one patients (91.3%) achieved engraftment (see Table I), 2 failed to engraft, and 4 developed late rejection; 2 of these individuals died as a result of graft failure, 2 survive with high transfusion requirements, and 2 received a second peripheral blood stem cell infusion after a second conditioning regimen (as described above), achieving sustained engraftment. Currently, 21 of the 23 patients are alive, and 19 of those 21 (90.4%) remain in complete remission (Table I). The 1500-day overall survival was 90% for patients not receiving busulfan and 87% for those receiving it ($P > 0.01$). We could not demonstrate a relation between donor and recipient sex disparity and graft failure or late rejection. The median post-transplant survival has not been reached, being above 4 years, whereas the 1500-day overall survival is 88% (Fig. 1).

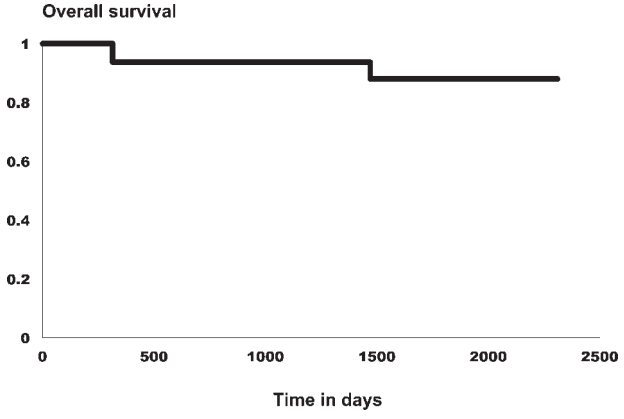

Fig. 1. Overall survival according to Kaplan-Meier analysis of the 23 individuals with severe, refractory aplastic anemia.

DISCUSSION

Allogeneic hematopoietic cell transplantation from an HLA-identical sibling donor is the initial treatment of choice for newly diagnosed patients with SAA, especially if they have failed immunosuppressive therapy with ATG and/or cyclosporin. Survival after an HLA-identical sibling transplant has increased from 48% in 1976 to 88% in recent years [8–11]. However, graft failure, GVHD, and infections remain considerable barriers to success. Three major forms of pretransplant immunosuppression have been used to prevent graft rejection: single-agent CY, CY plus ATG, and CY plus radiotherapy. Changes in conditioning regimens, like intensification with radiotherapy, have not been found to be related with improved survival. Decreased graft failure with more intensive conditioning has generally been offset by increased mortality from interstitial pneumonia and GVHD and a higher incidence of secondary cancers [12–15]. Furthermore, the long-term effects of these toxic conditioning regimens on fertility, hormone production, and physical growth are also of concern. The addition of ATG to cyclophosphamide during the conditioning regimen has been shown to improve results without the use of radiotherapy and is considered to be the most effective and reliable regimen to prevent graft rejection [9], but the high cost of ATG is beyond the majority of our mostly uninsured patients.

We have had previous experiences using high-dose peripheral blood stem cell (PBSC) transplant for multiply transfused, severe aplastic anemia patients without ATG in the conditioning regimen [14]. The presumed advantages of using mobilized PBSCs include rapid hematologic recovery and earlier immune system reconstitution, because PBSC grafting contains 3–4 times more CD34-positive cells and 1–2 log units more T cells. In the present study, we coupled these advantages with the use of fludarabine in the conditioning regimen in order to maximize immunosuppression, reduce toxicity, and avoid graft rejection. We found a cumulative rejection incidence of 26% in heavily transfused patients; in contrast, in a recent study from Brazil, a graft rejection of 43% was observed in 48 polytransfused patients conditioned with cyclophosphamide and low-dose busulfan without ATG and grafted with bone marrow-derived cells [16]. On the other hand, GVHD has a significant impact on survival; in fact, it has been considered in some studies as the major cause of morbidity and mortality [11]. Acute GVHD occurs in 5–30% of SAA patients, and chronic GVHD occurs in 25–60% of patients [17,18]. In our study, despite the use of peripheral blood as the source of stem cells, an incidence of acute GVHD of only 17.3% was observed, as was, until now, a relatively low incidence of chronic GVHD (26%).

The conditioning regimens that we have used were able to control both GVHD and prevent graft rejection through suppressing the host-versus-graft reaction. Fludarabine, a purine analogue, is endowed with potent immunosuppressive activity [19], and this effect has been demonstrated by its inducing lymphopenia, the prolonged depression of $CD4^+$ and $CD8^+$ lymphocyte counts, and the occasional development of transfusion-associated GVHD when non-irradiated blood products are administered. Fludarabine also inhibits the mechanism of alkylator-induced DNA repair, and the combination of fludarabine + CY has been used in the treatment of several lymphoid malignancies, including patients refractory to each drug alone; moreover, this combination has been used by us and others in pretransplant immunosuppression. The addition of ATG to cyclophosphamide during the conditioning regimen is aimed at decreasing the risk of graft rejection. This agent has also been used in combination with fludarabine and cyclophosphamide in cases of SAA [19]. This regimen has been successfully used in patients with SAA, but to our knowledge, the use of reduced doses of cyclophosphamide, with or without busulfan, in combination with fludarabine without ATG is unique to the group of patients in the present analysis. The role of busulfan in the conditioning regimen is not clear, since the differences in outcome of patients given or not the drug were not significant and the number of patients precludes serious statistical comparisons.

The regimens that we have used were found to have acceptable toxicity, low cost, and allow durable engraftment, using unmanipulated peripheral blood hematopoietic stem cells from HLA-identical siblings in patients with SAA. Lowering treatment costs for patients with severe hematological diseases is critical in developing countries, where few individuals have access to these sophisticated treatments [20–23].The standard Cy/ATG conditioning regimen is in fact non-myeloablative and is considered the "gold standard" for conditioning in SAA patients. Three doses of fludarabine are less costly in Mexico than a course of ATG [23]; accordingly, the use of a fludarabine-based conditioning regimens with peripheral blood stem cell transplantation represents a viable alternative to traditional more expensive and more cytotoxic conditioning for SAA patients who receive stem cell allografts. Long-term effects however, remain to be evaluated.

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