Graft-versus-host disease (GVHD) is a major obstacle to successful bone marrow transplantation (BMT) from matched unrelated donor (MUD). Currently available HLA-A, -B, and -DR serologic typing may not be sensitive enough to detect clinically relevant donor/recipient (D/R) nonidentity. Better HLA matching of D/R pairs using molecular typing for class II antigens in combination with intensive GVHD prophylaxis may potentially reduce the incidence of GVHD and lead to an improved outcome of MUD transplantation. Between July 1991 and August 1993, thirty consecutive patients with hematologic malignancies underwent MUD transplantation from donors who were identical for HLA-A, -B, and -DR by serologic typing. Twenty-five D/R pairs were matched for DRB and DQB by molecular typing (restriction fragment-length polymorphism and sequence-specific oligonucleotide probe hybridization analyses), whereas five were allele mismatched at either DRB or DQB. All patients also received GVHD prophylaxis with the combination of cyclosporine (CSA), methotrexate (MTX), and prednisone (PSE). The median age was 35 years (range, 15 to 50). The diagnoses were: chronic myelogenous leukemia (CML) in chronic phase (CP) (16), CML in more than CP (3), acute leukemia in more than first complete remission (CR) (8), acute leukemia in first CR (1), and advanced high-grade lymphoma (2). The preparative regimen consisted of 1,320 cGy fractionated total body irradiation (FTBI) and 60 mg/kg cyclophosphamide (CY) daily for 2 days in 17 good-risk patients (CML/CP and acute leukemia first CR); and 1,320 cGy FTBI in combination with 60 mg/kg etoposide and 20 to 60 mg/kg CY in 13 patients with advanced leukemia and lymphoma. All patients received CSA, PSE, and MTX on days 1, 3, 6 for GVHD prophylaxis, and 10 patients also received day +11 MTX. All patients engrafted except one who died early of regimen-related toxicity. The incidence of grade III or IV acute GVHD was 24% (95% confidence interval [CI], 10% to 44%) and that of extensive chronic GVHD was 65% (95% CI, 43% to 84%). At a median follow-up of 13.6 months, 57% of the patients are alive in remission with a median Karnofsky performance status of 90%. The cumulative probability of 2-year disease-free survival for all patients was 53% (95% CI, 33% to 71%); for good-risk patients, 71% (95% CI, 46% to 87%) and for the poor-risk group, 34% (95% CI, 13% to 64%). Stepwise logistic regression analysis showed that status at BMT was the only significant prognostic variable associated with severe acute GVHD, whereas donor age greater than 30 predicted for extensive chronic GVHD. These results suggest that the utilization of both serologic and molecular typing for D/R matching with an intensive GVHD regimen may reduce the incidence of acute GVHD and may potentially improve the outcome of unrelated donor BMT. Further controlled clinical trials are warranted to confirm our results. © 1995 by The American Society of Hematology.

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Diagnosis

MATCHED UNRELATED BONE MARROW TRANSPLANTATION

Patient characteristics

Extended our experience with this GVHD prophylaxis regimen. Therefore, we have extended our experience with this GVHD prophylaxis regimen to our unrelated donor BMT patients. We report here the results of unrelated BMT using donor/recipient pairs matched at the HLA-A and -B loci by serology typing and -DR loci by both serology and molecular typing and the GVHD prophylaxis regimen of CSA, MTX, and PSE.

MATERIALS AND METHODS

Patient population. The study group consists of 30 consecutive recipients of unrelated donor BMTs between July 1991 and August 1993 at City of Hope National Medical Center. Written informed consent was obtained from each patient and the study was approved by the City of Hope Institutional Review Board.

The patient characteristics are shown in Table 1. The median age of the recipients was 35 years (range, 15 to 50). There were 17 males and 13 females. The diagnoses were chronic myelogenous leukemia (CML) in chronic phase (CP) (16), CML in more than CP (3), high-risk acute leukemia in first complete remission (CR) (1), acute leukemia in more than first CR (8), and lymphoblastic lymphoma (2). The median time from diagnosis to BMT was 18 months (range, 4 to 201).

Donor-recipient matching. The characteristics of donors and recipients are shown in Table 1. Serologic typing was used for both class I and class II antigens. Molecular typing of the class II HLA-DRB1, -DRB3, -DRB4, -DRB5 and -DQ antigens was done by both RFLP and SSOP hybridized to specific segments of DNA that have been amplified by polymerase chain reaction (PCR). Donors were selected based on matching at the HLA-A and -B by serologic testing and at -DRB and -DQB by both serologic testing and RFLP and SSOP/PCR. HLA-DP antigens were not tested. MLC was performed on the selected matched pair, but the results were not used to exclude a donor. In addition, donor selection was also based on donor/recipient sex matching, blood group, and cytomegalovirus (CMV) seropositivity.

Transplant preparative regimen. There were two preparative regimens used in this study. Fractionated total body irradiation (FTBI) 1,320 cGy at 120 cGy per fraction for a total of 11 fractions from day –8 to day –5 in combination with cyclophosphamide 60 mg/kg/d for 2 days on days –4 and –3 was used in 17 good-risk patients (CML in CP, and acute leukemia in first CR with high-risk features). Thirteen patients with advanced acute leukemia or CML in more than CP or refractory lymphoma received 1,320 cGy FTBI, 60 mg/kg etoposide on day –4 and escalating dose of 20 to 60 mg/kg cyclophosphamide on day –2 with day 0 as the day of donor marrow infusion. Ex vivo marrow T-cell depletion was not performed.

GVHD prophylaxis and treatment. All patients received a combination of CSA, MTX, and PSE with the following schedule: CSA 5 mg/kg/d by continuous intravenous infusion as a loading dose starting on day –2. The dose was reduced to 3 mg/kg/d on day 4, and increased to 3.75 mg/kg/d from day 15 to day 35. Thereafter, patients received 5 mg/kg of oral cyclosporine twice a day until day 83, followed by a tapering dose until day 180. Whole blood CSA levels were measured twice per week by immunosassay (TDX System, Abbott Laboratories, Abbott Park, IL). MTX was administered intravenously at a dose of 15 mg/m² on day 1 and 10 mg/m² on day 3 and day 6; subsequently MTX 10 mg/m² was also given on day 11 for the last 10 patients. MTX doses were reduced in patients who had renal dysfunction, hepatic dysfunction, or severe mucositis. Methylprednisolone was begun on day 7 at 0.25 mg/kg intravenously twice a day and doubled to 0.5 mg/kg intravenously twice a day on day 15. At the time of discharge, the patients were switched to oral PSE, which was then slowly tapered from day 29 until day 180. If acute GVHD of grade I1 to IV developed, methylprednisolone dose was increased to 2 mg/kg/d. Patients who had acute GVHD were maintained on small doses of CSA and PSE until 1 year posttransplant.

Acute GVHD was graded uniformly by clinical investigators according to previously described criteria. Retrospective chart reviews were also performed by different investigators. All patients with a rash suggesting GVHD underwent biopsy to document involvement with acute GVHD. Biopsies of the liver and gut were performed if clinically indicated. Chronic GVHD was classified as “limited” or “extensive” as described previously.

Supportive care. All patients were housed in single rooms with high-efficiency particulate air filtration systems. Gut decontamination was accomplished with oral nonabsorbable antibiotics (neomycin and vancomycin), oral trimethoprim-sulfamethoxazole, and a strict, low-bacteria diet all starting approximately on day 8. Reverse isolation technique with masks and gowns was used when neutrophil counts fell below 500/µL. Broad-spectrum antibiotics were used for the first febrile episode, and all patients received prophylactic low-dose amphotericin (0.15 mg/kg) starting on day 1. All blood products were irradiated with 2,500 cGy before infusion. Intravenous Ig
(Gammagard; Baxter Health Care Corp, Hyland Division, Glendale, CA or Gamimune-N; Miles Inc, Cutter Biologicals, Elkhart, IN) was administered to all patients at a dose of 500 mg/kg every other week from day −7 to day 100. Patients who were seropositive for cytomegalovirus (CMV) or who received a BM graft from a CMV-seropositive donor underwent bronchoalveolar lavage (BAL) on day 35. Those whose BAL specimens were positive for CMV, or those who had positive CMV blood culture received prophylactic ganciclovir as previously described by our group.14 Twenty-seven patients who were seropositive for herpes simplex virus also received acyclovir prophylaxis 250 mg/m² every 12 hours beginning on day −1 and continued until day 21 posttransplant. Twenty-four (80%) patients in this study were also given hematopoietic growth factors (rHu-G-CSF) 5 μg/kg/d intravenously beginning on day 7 and continued until absolute granulocyte count (ANC) > 0.5 × 10⁹/L for 3 consecutive days.

Statistical methods. Descriptive statistics were calculated and reported. The method of Kaplan-Meier was used to estimate overall survival, disease-free survival (DFS), and relapse, and 95% confidence intervals (CI) were determined using Greenwood’s variance and the logit transformation. Univariate logit regression was performed to evaluate whether covariates were predictive of acute GVHD and extensive chronic GVHD, and the odds ratios and 95% CI were estimated. Covariates tested included recipient age, donor age, recipient sex, donor sex, donor/recipient sex match, status at transplant (good risk [first CR/first CR] v all others), reduction in dose of CSA, reduction in dose of MTX, withholding of day 11 MTX, donor parity, HLA mismatch, ABO blood-type mismatch, MLC reactivity, donor CMV status, interval from diagnosis to BMT (<12 v ≥12 months), marrow mononuclear cells, marrow nucleated cells, acute GVHD, and extensive chronic GVHD. Any covariate significant in the univariate analysis at P < .20 was entered as a candidate variable in the stepwise logit regression; the P value to enter or leave the model was set at .20, and a significance level of .05 was used in the final model.

Univariate Cox regression was performed for time-to-event outcomes (overall survival, DFS, and relapse) using the same list of covariates as those given above. Risk ratios and 95% confidence limits were estimated using the method of logit limits. Stepwise Cox regression analysis was performed including any variables with univariate P values < .20; the P value to enter or leave the model was set at 0.20, and 0.05 was used as the significance level for the final model.

RESULTS

Unrelated BM donors. All donor/recipient pairs were 6/6 antigens matched for HLA-A, -B and -DR by serology typing. Twenty-five were matched for DRB and DQB, whereas five donor/recipient pairs were allele mismatched at either DRB or DQB. Two donor/recipient pairs also had bidirectional reactive MLC. The median interval from the initiation of donor search to identification of donor was 113 days (range, 25 to 835).

Engraftment and graft failure. Ten donor/recipient pairs were ABO blood group matched, 12 were major ABO mismatched, and 8 were minor ABO mismatched. The median number of marrow nucleated cells given was 3.56 × 10⁹/kg of recipient (range, 1.20 to 6.56) and the median number of marrow mononuclear cells given was 0.72 × 10⁹/kg (range, 0.32 to 1.80). All patients engrafted except one who died from regimen-related toxicity before engraftment. The median day to reach ANC > 0.5 × 10⁹/L was 19 (range, 11 to 30). Three patients developed severe acute GVHD and died before platelet transfusion independence. The median day to reach platelet count > 20 × 10⁹/L without transfusion was 27 days (range, 15 to 228) and the median day to red blood cell transfusion independence was 42 days (range, 15 to 228). One patient with CML who was transplanted in chronic phase developed late graft failure at 6 months posttransplant. Cytogenetic studies showed mixed hematopoietic chimeraism, and follow-up BM examination at 8 months post-BMT confirmed cytogenetic relapse with additional chromosomal abnormalities.

GVHD. One patient died at day 16 from regimen-related toxicity; therefore, twenty-nine patients were evaluable for acute GVHD. Eleven patients (38%; 95% CI, 21% to 58%) had grade I acute GVHD and the incidence of grade II-IV acute GVHD was also 38% (95% CI, 21% to 58%) (Fig 1). Severe grade III to IV acute GVHD developed in seven patients (24%; 95% CI, 10% to 44%); two of these patients died from acute GVHD and one other patient died from progressive acute to chronic GVHD. By univariate logistic regression analysis, disease status at BMT and recipient age (>30) were the only prognostic variables significantly associated with grade II-IV acute GVHD (P = .005 and .04, respectively). Only disease status at BMT remained significant in the stepwise logistic regression (P = .007; odds ratio, 9.33; 95% CI, 1.7 to 52.7).

At day 100 posttransplant, twenty-three patients were alive and, therefore, were evaluable for development of chronic GVHD. Limited chronic GVHD occurred in three patients (13%; 95% CI, 3% to 34%) and fifteen patients (65%; 95% CI, 43% to 84%) developed extensive chronic GVHD. The median time to onset of chronic GVHD was 140 days (range, 100 to 373). Two patients died from chronic GVHD or its complications including one from refractory progressive acute to chronic GVHD and the other from bronchiolitis obliterans and pulmonary aspergillosis. At the time of this analysis, the median Karnofsky scores of the 17 surviving patients is 90% (range, 60% to 100%). The distribution of Karnofsky scores is shown in Table 2. By univariate logistic regression analysis, two independent variables were
found to be significantly associated with extensive chronic GVHD: donor age > 30 (P = .02) and withholding day 11 MTX (P = .04). Only donor age remained significant in the stepwise logistic regression (P = .02; odds ratio, 10.8; 95% CI, 1.4 to 85.4).

Relapse. Four patients (13%) with refractory acute non-lymphocytic leukemia (ANLL) (1), CML in CP (2), and acute lymphoblastic leukemia (ALL) in second CR (1) relapsed at 2, 8, 8, and 22 months posttransplant, and two of these subsequently died from progressive leukemia. One with CML had late graft failure at 6 months; 2 months later, she developed cytogenetic relapse and eventually died from progressive pancytopenia. The other patient with CML had cytogenetic relapse at 8 months, but has achieved cytogenetic remission after withdrawal of immunosuppressive therapy and institution of interferon. This patient remains alive in clinical and cytogenetic remission at 2 years from cytogenetic relapse. The Kaplan-Meier estimate of the probability of relapse at 2 years was 19% (95% CI, 5% to 48%).

Overall and DFS. Seventeen patients (57%) are alive and in remission at a median follow-up post-BMT of 14 months (range, 0.5 to 33) for all patients and 21 months (range, 9 to 33) for the 17 surviving patients. The Kaplan-Meier estimate of overall survival at 2 years for all patients is 56% (95% CI, 37% to 74%) and is shown in Fig 2. The Kaplan-Meier estimate of DFS at 2 years is 53% (95% CI, 33% to 71%) for all patients, 71% (95% CI, 46% to 87%) for the good-risk patients, and 34% (95% CI, 13% to 64%) for the poor-risk group. The DFS curves stratified by disease status at BMT are shown in Fig 3. Univariate analysis of risk factors for survival did not identify any factor predicting for survival or DFS, possibly because of the small number of patients in this study.

Complications and cause of death. There were 13 deaths (43%). The principal causes of death are shown in Table 3. Infection was the major cause of death occurring in five patients: pulmonary aspergillosis (2), disseminated herpes simplex infection (1), bacterial sepsis (1), and CMV pneumonia and brain abscess (1). The remaining seven patients died from refractory acute and chronic GVHD (3), relapse (2), late interstitial pneumonitis (IP) (1), and graft failure and relapse (1), and chemoradiotherapy-induced toxicity (1).

DISCUSSION

Since the initial report of successful unrelated donor BMT in 1980, more than 3,000 unrelated donor BMTs have been performed through the development of the NMDP and other registries. Recent analysis of 462 unrelated donor BMTs from NMDP showed that the 2-year DFS was 40% for patients with good-risk leukemia compared with 19% for those with poor-risk leukemia. Similar results have been reported from other single institution series with a smaller sample size. Our results, albeit based on a smaller number of patients, appear improved when compared to the results reported previously. The 2-year DFS of 71% for the good-risk and 34% for the poor-risk group reported here is almost identical to the results obtained from matched sibling donor
transplantation. This favorable outcome in our study could be caused by patient selection, better donor/recipient matching, more intensive GVHD prophylaxis regimen, and improved supportive care.

It is known that the clinical outcome of BMT is directly dependent on the degree of HLA matching for HLA class I and class II specificities. With the introduction of molecular typing techniques, it is now apparent that many donors who appear to be HLA-identical serologically are disparate at the molecular level. Tieryc et al performed HLA oligotyping for DRB, DQB, and DPB using PCR-amplified DNA on 50 donor-recipient pairs that were serologically matched for HLA class I and class II, and found that 56% of the pairs were mismatched at either DRB or DQB loci.11 Furthermore, molecular typing can overcome the difficulties of typing patients with overt leukemia and provides a more refined HLA-DR, -DQ, - Dw, and -DP typing compared with serology. Therefore, oligotyping can be used to improve HLA class II typing and for a better matching for unrelated donor/recipient pairs. We have applied DNA typing to serology typing for the matching of unrelated donor/recipient pairs and report here the feasibility of using DNA typing for donor selection. Between January 1991 and August 1993, 133 patients entered unrelated donor search and 98 patients were found to have donors matched for HLA-A, -B, and -DR by serology typing. Among the 98 patients, 45 patients were found to have donors matched for DRB and DQB by DNA typing and then proceeded to BMT; the other 53 patients did not proceed with the search or BMT because of the following reasons: preliminary search only (11), transfer of the search to different transplant centers (9), autologous BMT/matched related BMTs (6), expired during search (13), and termination of the search because of medical conditions (14). The median duration of the donor search in our study was 113 days (range, 25 to 835), which was comparable to previous reports using donor matched for HLA-A, -B, and -DR only by serology and nonreactive MLC.11 These results suggest that it is possible to find donor/recipient pairs matched for HLA-A, -B, -DR by serology typing and for DRB and DQB by DNA typing methods within acceptable time frames.

Our results also suggest that the incidence of grade II to IV acute GVHD may be reduced in matched unrelated BMT using donor/recipient pairs matched at HLA class II by molecular typing. In a study reported by Beatty et al, the incidence of grade II-IV acute GVHD was 79% in 52 patients receiving grafts from HLA-A, -B, -DR, and -DW phenotypically matched, MLC-compatible, unrelated donors.10 These results suggest that it is possible to find molecularly matched unrelated donor/recipient pairs for DRB and DQB matched donors selected by DNA typing methods are necessary to confirm our observation.

Despite the use of donor/recipient pairs matched for HLA-A, -B, and -DRB, 38% of the patients in our study developed grade II-IV acute GVHD, suggesting that other factors such as class I polymorphisms as well as major histocompatibility complex (MHC) polymorphisms not yet recognized by current histocompatibility testing or the minor histocompatibility differences also play important roles in the development of GVHD. In a study reported by Santamaria et al, HLA class I, especially HLA-C, and HLA-DP mismatches are frequent in unrelated D/R pairs molecularly matched at -DRB, -DQA1, and -DQB1.18 The role of HLA-DP disparity in the development of acute GVHD remains unknown and is currently under investigation.19 Petersdorf et al reported that matching donor-recipient for HLA-A, -B, -DRB, and -DQB does not predict for matching at -DPB1.20 However, the risk of acute GVHD was not influenced by incompatibility for DPB1 alleles. In contrast, two other studies have suggested that HLA-DP antigen may function as a transplantation antigen like the other HLA class II molecules (DR, DQ) and contributes to the incidence of severe acute GVHD in unrelated BMT.21,22 Further study to determine the contribution of molecular class I and HLA-DP mismatches to the outcome of unrelated BMT is warranted.

The 38% (95% CI, 21% to 58%) overall incidence of grade II-IV acute GVHD in this study is generally less than the rate of 50% to 79% reported in other series of unrelated donor BMT.4,8,10 Several acute GVHD prophylaxis regimens have been used in unrelated BMT, and most use CSA and MTX, or T depletion alone or with CSA in donor/recipient pairs matched for HLA-A, -B, and -DR by serologic method with nonreactive MLC. The relative contribution of improved donor/recipient selection based on molecular typing versus an innovative steroid containing GVHD prophylaxis in achieving our reduction in the incidence of acute GVHD is uncertain. An update on the results of unrelated donor BMT in 97 patients with CML at the Fred Hutchinson Cancer Research Center was recently reported.23 Among these, 73 donor/recipient pairs were HLA-A, -B, -DR identical by serological testing and identical for -DRB1 alleles by SSOP/PCR. All patients received CSA and MTX for GVHD prophylaxis. The probability of developing acute GVHD grades II-IV was 75% for HLA-A, -B, and -D-identical transplant. These results would suggest that prednisone used in combination with CSA and MTX in our study may contribute to the lower incidence of acute GVHD in our molecularly matched unrelated BMT. A randomized study will be required to determine whether this GVHD regimen of CSA, MTX, and PSE is more effective than CSA and MTX in molecularly matched unrelated BMT.

Despite the reduction in the incidence of acute GVHD in our study, the incidence of chronic GVHD is similar to that reported by others.5-13 Increasing recipient age is a known predictive factor for chronic GVHD and may have contributed to the high incidence of chronic GVHD in our study, as the median age of our patients is relatively high. These results confirmed our previous observation that, although the combination of CSA, MTX, and PSE is very effective in preventing acute GVHD, it has no impact on the development of chronic GVHD.13 However, in this study, both CSA and prednisone were tapered off after day 180, and it is conceivable that extension of immunosuppressive therapy beyond day 180 may potentially reduce the incidence of
chronic GVHD. Prolonged CSA prophylaxis has been shown to reduce the incidence of chronic GVHD in a few studies.\textsuperscript{24,25} Currently we have extended our use of small doses of CSA and PSE to 1 year post BMT in an attempt to reduce the incidence of chronic GVHD in this patient group. Despite the high incidence of chronic GVHD in our study, only two patients died of complications associated with chronic GVHD. The majority of the patients have skin changes and buccal mucosa involvement, which responded to immuno-suppressive therapy, and their quality of life remains good with a median KPS of 90%.

Infectious complications have been reported to be higher in recipients of unrelated-donor BMTs and was the major cause of death in our patients, rather than GVHD per se. Our current approach to prophylaxis of CMV using preemptive BAL on day 35 and prophylactic ganciclovir has reduced the complications associated with CMV infection and only one patient in our study died from CMV pneumonia. Nevertheless, prolonged immunosuppressive therapy and a high incidence of acute and chronic GVHD predispose unrelated donor BMT recipients to opportunistic infections. Newer approaches for prevention and treatment of both infection and chronic GVHD may further improve the results of unrelated donor BMT.

Our results show that the outcome of unrelated donor BMT may be improved by using molecularly matched donor/recipient pairs, and suggest that molecular typing for HLA class II should be used for matching and selecting unrelated donor/recipient pairs. Further clinical studies with larger numbers of patients are also required to confirm our results and determine the importance of donor/recipient matching at the molecular level. Our results also show a reduction in the overall incidence of severe acute GVHD with the combination of CSA, MTX, and PSE in the molecularly matched unrelated-donor BMT. However, infection and chronic GVHD continue to be major problems. Continued efforts to eradicate or minimize these problems are necessary to further improve the results of matched unrelated-donor BMT.

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The outcome of matched unrelated donor bone marrow transplantation in patients with hematologic malignancies using molecular typing for donor selection and graft-versus-host disease prophylaxis regimen of cyclosporine, methotrexate, and prednisone

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