Selective Depletion of Bone Marrow T Lymphocytes With Anti-CD5 Monoclonal Antibodies: Effective Prophylaxis for Graft-Versus-Host Disease in Patients With Hematologic Malignancies

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Seventy-one patients with hematologic malignancies received bone marrow from a histocompatible sibling (n = 48) or a partially matched relative (n = 23) that had been depleted of CD5$^+$ T cells with either an anti-CD5 monoclonal antibody (MoAb) plus complement (anti-Leu1 + C) or an anti-CD5 MoAb conjugated to ricin A chain (ST1 immunotoxin [ST1-IT]). These patients received intensive chemoradiotherapy consisting of cytosine arabinoside, cyclophosphamide, and fractionated total body irradiation. Both anti-Leu1 + C and ST1-IT ex vivo treatments effectively depleted bone marrow of T cells (97% and 95%, respectively). Overall, primary and late graft failure each occurred in 4% of evaluable patients. The diagnosis of myelodysplasia was a significant risk factor for graft failure ($P < .001$), and if myelodysplastic patients were excluded, there were no graft failures in major histocompatibility complex (MHC)-matched patients and 2 of 23 (8.7%) in MHC-mismatched patients. The actuarial risk of grade 2 to 4 acute graft-versus-host disease (GVHD) was 23% in MHC-matched patients and 50% in MHC-mismatched patients. In MHC-matched patients, acute GVHD tended to be mild and treatable with corticosteroids. Chronic GVHD was observed in 6 of 36 (17%) MHC-matched patients and none of 11 MHC-mismatched patients. There were no deaths attributable to GVHD in the MHC-matched group. Epstein-Barr virus-associated lymphoproliferative disorders were observed in 3 of 23 MHC-mismatched patients. The actuarial event-free survival was 38% in the MHC-matched patients versus 21% in the MHC-mismatched patients. However, if outcome is analyzed by risk of relapse, low-risk patients had a 62% actuarial survival compared with 11% in high-risk patients. These data indicate that the use of anti-CD5 MoAbs can effectively control GVHD in histocompatible patients, and that additional strategies are required in MHC-mismatched and high-risk patients.

ALLOGENEIC bone marrow transplantation (BMT) has been widely applied to patients with aplastic anemia, hematologic malignancies, myelodysplastic syndromes, and congenital immunodeficiency syndromes. Successful transplantation has been largely confined to those patients who are young and have major histocompatibility complex (MHC)-matched sibling donors. The major impediment to allogeneic marrow grafting is the development of severe acute and chronic graft-versus-host disease (GVHD).

Much attention has focused on the prevention of GVHD rather than on the elimination of established disease. Transplants performed without GVHD prophylaxis may result in severe "hyperacute" GVHD.[1] Immunosuppressive drugs such as methotrexate, cyclosporine, cyclophosphamide, and corticosteroids, either alone or in combination, have been used with varying degrees of success. Even the most optimistic studies only show a reduction in acute GVHD from 60% to 100% without prophylaxis to approximately 30%.[2] Persistent immunosuppression and intrinsic toxicities of the drugs are major problems with both the prophylaxis and treatment of GVHD. Furthermore, the inherent toxicity of methotrexate and cyclosporine may limit our ability to increase the intensity of the transplant conditioning regimen.

Studies in murine models of allogeneic marrow grafting have shown that the major cell responsible for acute GVHD is a mature T lymphocyte, and that acute GVHD can be abrogated by in vitro depletion of the marrow of mature T cells.[3] In these models, increasing genetic disparity between donor and recipient mice increases the risk of both graft rejection and of GVHD. The incidence of graft rejection is reduced either by increasing the cell dose of marrow infused or by intensifying recipient immunosuppression.[4] Based on these murine models, clinical trials in humans were initiated to determine whether T-cell depletion of donor marrow would prevent acute GVHD.[5-7] The incidence and severity of acute GVHD were markedly reduced after T-cell depletion. Unfortunately, graft rejection[8] and loss of the graft-versus-leukemia (GVL) effect[9] offset the reduction in mortality from GVHD. It appears that improvements in disease-free survival from the reduction in GVHD achieved by T-cell depletion will depend on the prevention of graft rejection and the maintaining of an antileukemic effect. These goals may be achieved by using conditioning regimens that are sufficiently cytotoxic both to prevent graft rejection and to compensate for the loss of GVL.[10,11] Moreover, because residual host T cells can mediate graft rejection,[12,13] it may be preferable to leave some alloreactive

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T cells or natural killer (NK) cells in the donor marrow that can offset the residual host lymphocytes. These donor T cells and NK cells may be able to reduce graft failure and maintain GVL.[14-16]

This study was initiated to assess the toxicity of removing $CD5^+$ T cells from the BM and to determine the efficacy of this treatment in controlling GVHD. The cell surface protein CD5 is found on 95% of peripheral blood (PB) T cells. Thus, treatment of the donor BM with anti-CD5 monoclonal antibodies (MoAbs) decreases the total number of T lymphocytes but leaves $CD5^-$ T cells. Furthermore, NK cells, which are not recognized by anti-CD5 MoAb, remain in the marrow. We hoped that the residual NK cells and some T cells in the marrow would sustain a GVL effect. We also intensified the conditioning regimen for leukemic patients in an effort to reduce the graft failure rate and increase antileukemic efficacy. Anti-CD5 reagents have been used for T-cell depletion alone[17] and in combination with other anti-T-cell reagents,[18,19] but with less intensive conditioning regimens.

MATERIALS AND METHODS

Patients

Between 1983 and 1989, 71 patients with hematologic malignancies were entered into one of two consecutive studies. In the first study (1983 to 1986), 29 donor marrows were depleted of $CD5^+$ cells using the murine MoAb anti-Leu1 plus newborn rabbit complement (anti-Leu1 + C). In the second study (1986 to 1989) marrow from 42 donors was purged with ST1 immunotoxin (ST1-IT), an $F(ab')_2$ fragment of a murine anti-CD5 IgG2a MoAb conjugated to ricin A chain. Patient characteristics are described in Table 1. The anti-Leu1 MoAb was obtained from Becton Dickinson Immunocytometry Systems, Inc (Mountain View, CA). It is an IgG2a antibody that fixes rodent complement. Newborn rabbit complement (Pel-Freez Biologicals, Rogers, AR) was screened for toxicity against hematopoietic progenitors and for lytic activity before use. ST1-IT (SR44163) was obtained from Sanofi Research, Inc (Montpelier, France). It is an $F(ab')_2$ fragment of an IgG2a MoAb coupled to an average of 2 ricin A chains. The immunotoxin has an average molecular weight of 160 Kd. Ricin B chain contamination is less than 0.006%.

These studies were conducted under the guidelines of the institutional review boards of the Brigham and Women's Hospital, The Children's Hospital, and the Dana-Farber Cancer Institute.

Conditioning Regimens

The conditioning regimen differed between the two studies.

Anti-Leu1 + C. Patients received cytosine arabinoside (ara-c) 500 mg/m²/d by continuous intravenous infusion (day −11 to −5), cyclophosphamide 60 mg/kg/d or 1,800 mg/m²/d (day −4 to −3), and total body irradiation (TBI), either 12 Gy total midline dose in six equal fractions over 3 days or 14 Gy total dose in eight equal fractions over 4 days (day −4 to −1). Radiation was administered in a dedicated facility at 0.05 to 0.1 Gy/min with opposing anteroposterior/posteroanterior fields on a dual-headed 4 million eV linear accelerator with a 5 to 6 hour minimal interfraction interval. Two patients did not receive ara-c because of prior drug-related toxicity. All of the MHC-mismatched patients and 10 of 19 of the MHC-matched patients received methotrexate 10 mg/m² on days 1, 3, 6, and 11 after the transplant, but no other GVHD prophylaxis.

Table 1. Patient Characteristics

	MHC-Match		MHC-Mismatch	
	Anti-Leu1 + C (n = 19)	ST1-IT (n = 29)	Anti-Leu1 + C (n = 10)	ST1-IT (n = 13)
Median patient age (range) (yr)	32 (12-48)	39 (27-51)	15 (4-29)	7 (1-41)
Median donor age (range) (yr)	31 (13-44)	37 (23-56)	40 (1-50)	33 (17-73)
Sex (M/F)	6/13	13/16	7/3	7/6
Sex compatibility	MHC-Matched		MHC-Mismatched	
M → F	17		6	
F → M	10		5	
M → M	9		9	
F → F	12		3	
Diagnosis				
ANLL				
CR1	6	8	0	2
CR2	0	1	1	2
CR ≥ 3	0	1	0	1
Relapse	2	1	2	3
ALL				
CR1	1	0	0	0
CR2	1	0	3	3
CR ≥ 3	1	0	2	0
Relapse	1	0	0	0
CML				
SP	1	12	0	1
AP	2	2	0	0
2nd SP	1	1	1	0
BC	0	0	0	0
JCML	0	0	0	1
MDS				
RA	0	1	0	0
RAEB	2	2	0	0
RAEB-IT	1	0	1	0

Abbreviation: MDS, myelodysplastic syndrome.

ST1-IT. Patients received ara-c 3 g/m² twice per day (day −6 to −4), cyclophosphamide 1,800 mg/m²/d (day −3 to −2), and TBI 14 Gy total dose in eight equal fractions over 4 days (day −4 to −1 or −3 to 0). Radiation was administered at 0.1 Gy/min as described above. The MHC-mismatched patients received etoposide 300 mg/m²/d (days −8, −6, and −4) in addition to the above regimen; however, etoposide was eliminated after the first four patients were treated due to severe skin toxicity. No patients received methotrexate.

The transplants were performed in either laminar air flow rooms or positive-pressure reverse isolation rooms. All patients received oral antibiotics for gut sterilization and trimethoprim-sulfamethoxazole or inhaled pentamidine before the transplant and for 1 year after the transplant for prophylaxis against pneumocystis carinii. Trimethroprim-sulfamethoxazole (trimethroprim dose 5 mg/kg in divided doses three times a week) was administered when granulocytes were greater than 1,000/μL and platelets $\geq 10^5$/μL.

BM Harvest and Treatment

Fifteen to 20 mL/kg recipient weight of donor marrow was harvested and the leukocytes concentrated on an IBM 2991 blood cell processor (Pharmacia, Piscataway, NJ). The marrow was diluted 1:3 in modified Dulbecco's medium or Hank's balanced salt solution and divided into 30-mL aliquots. Each aliquot was sedimented over 10 to 12 mL of Ficoll-hypaque solution (specific gravity, 1.077 to 1.080 g/mL) and centrifuged at 400*g* for 30 to 40

minutes at 10°C. The cell interfaces were collected and washed three times.

Anti-Leu1 + C. To treat the marrow with anti-Leu1 + C, the cells were resuspended at a concentration of 2×10^7/mL in modified Dulbecco's medium without Ca^{+2} and Mg^{+2}, with 5% donor autologous serum. Cells were treated with anti-Leu1, 10 μg/mL per 2×10^7 cells and incubated at 4°C for 30 minutes with regular, gentle shaking. A 0.5 to 1.0-mL aliquot of treated marrow was removed to determine antibody binding. Cells were then washed and resuspended at 2×10^7 cells/mL into complement diluted 1:4 in warm (37°C) RPMI 1640. The mixture was gently stirred and incubated in a dry incubator at 37°C for 45 minutes with regular, gentle mixing. A 1- to 2-mL aliquot was obtained before and after treatment to perform a cell count, assess cell viability, perform BM phenotyping and limiting dilution analysis of T-cell precursors, and assess progenitor growth. The remaining cells were centrifuged at 250*g* for 25 minutes at 18°C to 20°C and the supernatant removed. The anti-Leu1 + C treatment and evaluation were repeated unless either the number of cells remaining was less than 1×10^7/kg recipient body weight or the initial marrow harvest was below 15 mL/kg of recipient body weight. After completion of the antibody/complement treatment, the cells were washed three times as detailed above, resuspended in medium, and administered intravenously by rapid infusion. T-cell depletion with anti-Leu1 + C was discontinued after 29 patients were treated because degradation of the antibody was noted on a one-dimensional polyacrylamide gel electrophoresis (PAGE) of a sample of the antibody stored at 4°C (data not shown). There was no change in high performance liquid chromatography (HPLC) pattern or in the antibody titer. However, a small change in molecular weight (2 to 4 Kd) and indirect evidence from the pattern of binding of MoAb directed against the constant region of murine antibodies suggested that cleavage of a small segment of the constant region had occurred. At the time of investigation, approximately 50% of antibody was affected. The number of patients treated with the altered antibody is uncertain, but suboptimal (<90%) depletion of T lymphocytes was observed in one patient.

ST1-IT. Human serum albumin and tromethamine (THAM) were each added to the marrow mononuclear cells to a final concentration of 1% to 5% wt/vol and 5% vol/vol, respectively. Ammonium chloride was added to a final concentration of 20 mmol/L and the pH was adjusted with THAM buffer to pH 7.65 to 7.95. ST1-IT was then added to a final concentration of 10^{-8} mol/L (ricin A chain content). The marrow was gently rocked at 37°C for 2 hours and infused without washing. An aliquot was taken before and after ST1-IT addition for cell count, limiting dilution analysis (LDA) of T-cell precursors,[20] fluorescence-activated cell sorter (FACS) analysis of T cells, and progenitor cell assays.

Determination of Residual T Cells

Anti-Leu1 + C. BM cells were labeled before and after each cycle of anti-Leu1 + C treatment with combinations of fluorescein-conjugated (FITC) and phycoerythrin-conjugated (PE) MoAb as described previously.[21] Aliquots of marrow were labeled with the following combinations of MoAb (all obtained from Becton Dickinson): PE-Leu1/FITC-HLE, PE-Leu2/FITC-HLE, PE-Leu3/FITC-HLE, biotin-Leu4/PE-avidin/FITC-HLE, PE-Leu5/FITC-HLE (HLE = CD45, Leu1 = CD5, Leu2 = CD8, Leu3 = CD4, Leu4 = CD3, Leu5 = CD2.) For control labeling, PE-mouse IgG irrelevant MoAb/FITC-mouse IgG irrelevant MoAb-labeled marrow was analyzed on a FACS 440 or FACS analyzer (Becton Dickinson). Volume threshold was set to include all cells labeling with the pan leukocyte MoAb anti-HLE. This threshold assured that all viable nucleated cells were analyzed and that the percentage of T cells was calculated with a denominator of "all nucleated cells." Right angle scatter and forward angle scatter (or electronic volume) gates were set to include all labeled cells. Data was displayed as dual-parameter contour plots of FITC versus PE fluorescence. In all cases the percentage of nonspecifically labeled cells was less than 10% of specifically labeled cells. Mixing experiments showed the ability to detect 0.3% residual T cells (data not shown).

ST1-IT. Because ST1-IT does not kill $CD5^+$ cells immediately, we used three techniques to measure the efficacy of T-cell depletion: (1) treated and untreated aliquots of BM cells were put into short-term culture (3 to 5 days) with phytohemagglutinin (PHA) and interleukin-2 (IL-2) and then labeled with a cocktail of anti-CD5, anti-CD3, anti-CD8, and anti-CD4 MoAb (provided by Sanofi Research or Becton Dickinson) plus an FITC-conjugated goat antimouse antibody; (2) proliferation of treated and untreated cells in short-term culture by PHA/IL-2–stimulated [^{3}H]-thymidine incorporation; and (3) limiting dilution analysis of T-cell precursors proliferating to PHA. The total number of T cells infused was calculated by multiplying the proliferating T-cell precursors determined in the LDA by the total number of nucleated cells infused.

Determination of Marrow Progenitors

Aliquots of marrow mononuclear cells before and after treatment with either anti-Leu1 + C or ST1-IT were cultured in methylcellulose by standard techniques.[22]

Determination of Engraftment and Chimerism

Engraftment was defined as a granulocyte count of ≥ 500/μL for 3 consecutive days, reticulocyte count of ≥ 1%, and platelet count of ≥ 50,000/μL for 3 consecutive days without transfusion support. To determine levels of mixed chimerism, MHC-mismatched patients were HLA typed by standard methods. All other patients had sex chromosome determination, red blood cell typing, and/or DNA polymorphism analysis.[23,24]

Assessment of Acute GVHD

All patients were evaluated for the presence of clinical acute GVHD by standard criteria and graded according to the system of Glucksberg et al.[25] Clinically significant acute GVHD was considered ≥ grade 2. All patients with clinically suspect skin lesions and all patients at 30 to 60 days after the transplant had 3-mm punch biopsies performed and evaluated by a dermatopathologist who was not aware of the clinical state of the patient.

Statistics

Event-free survival and time to acute GVHD probabilities were calculated by the Kaplan-Meier method.[26] Standard errors were estimated using Greenwood's formula[27] and the log-rank test[28] was used to compare differences in survival distributions. Fischer's exact test[29] was used to test the association of patient characteristics with grade of acute GVHD. Logistic regression[30] was used to investigate prognostic factors associated with grades 2 to 4 acute GVHD. A step-up procedure was used to find the most parsimonious model, with *P* value for entry of .1 and a value for dropping of .05. All *P* values are two-sided.

RESULTS

Efficacy of T-Lymphocyte Depletion

Anti-Leu1 + C. Pretreatment marrow contained from 3% to 15% T cells by anti-CD3 staining. After treatment,

an average of 2.9 ± 3.7 × 10^5 T cells/kg were infused based on LDA data. This number of T cells represents the 97.2% depletion based on functional assessment of precursor proliferating T cells by LDA and 97.8% depletion by FACS analysis (Table 2).

ST1-IT. Similarly, ST1-IT reliably resulted in the removal of T cells and the average number of T cells infused was 5.6 ± 7.7 × 10^5 T cells/kg by LDA. The number of infused T cells with ST1-IT–treated marrow was not significantly different than the anti-Leu1 + C–treated marrow. Because of difficulties in the assessment of background proliferation when PHA-stimulated marrow was used to assess T-cell numbers, it was felt that the LDA gave more reliable estimates of T-cell depletion. Assessment of proliferating T cells by LDA showed 95.4% depletion (Table 2). Phenotypic analysis of proliferating cells in short-term marrow culture was felt to be reliable in 10 patients. PHA and IL-2–stimulation of treated and control marrow shows that after 5 days, $CD5^+$ cells were depleted and the marrow was relatively enriched for T cells expressing the γ/δ T-cell receptor (γ/δ T cells) and NK cells (Fig 1). B cells were also relatively enriched (data not shown). Neither anti-Leu1 + C nor ST1-IT treatment affected the in vitro growth and differentiation of committed progenitor cells.

Engraftment

Anti-Leu1 + C. The average nucleated cell dose administered was 0.64 ± 0.3 × 10^8/kg. Among patients who engrafted, median time to ≥500 polymorphonuclear cells (PMN)/μL for 3 consecutive days was 20 days (range, 15 to 36) in the MHC-matched patients and 26 days (range, 16 to 36) in the MHC-mismatched individuals. Platelets attained ≥50,000 cells/μL without transfusion support for 3 consecutive days in 30 days (range, 21 to 110) and 36 days (range, 25 to 104), respectively (Table 3). This result may be explained by the additional methotrexate administered to these patients. One of 18 evaluable MHC-matched patients (6%) failed to engraft. This individual had a myelodysplastic syndrome with monosomy 7, but no significant marrow fibrosis. There were no late graft losses in the MHC-matched patients. Two of eight (25%) evaluable MHC-mismatched patients failed to engraft. One of these patients, who had myelodysplasia with marrow fibrosis secondary to therapy for Hodgkin's disease, rejected a paternal transplant followed by two unsuccessful attempts at transplant from a parental donor. Host T cells were observed that responded to donor MHC class I antigens and inhibited in vitro hematopoietic colony formation, suggesting that the rejection was mediated by residual host T cells.[10] All patients that engrafted had complete lympho-hematopoietic chimerism.

ST1-IT. The average nucleated cell dose administered was 1.15 ± 0.51 × 10^8/kg. All patients initially engrafted. The median time to ≥500 PMN/μL for 3 consecutive days was 18 days (range, 13 to 33) in the MHC-matched patients and 18 days (range, 12 to 67) in the MHC-mismatched individuals. Platelets reached ≥50,000 cells/μL without transfusion support for 3 consecutive days in 33 days (range, 14 to 82) and 28 days (range, 12 to 138), respectively (Table 3). Two of 27 (7%) MHC-matched and 1 of 12 (8%) MHC-mismatched patients had late graft failures. Graft failure was observed at days 73 and 153 in two patients with myelodysplasia who received MHC-matched marrow. Although both of these events were evaluated as graft failures, one patient had the persistence of his original chromosome abnormality [t(3;3)] and the other had host hematopoiesis demonstrated by restriction fragment length polymorphism (RFLP) analysis. One patient with acute nonlymphocytic

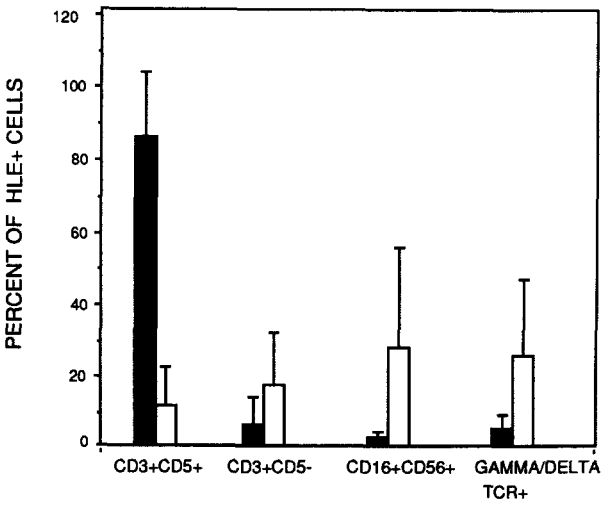

Fig 1. Flow cytometric analysis of residual lymphoid populations in the donor BM (n = 10). Marrow treated with ST1-IT (□) and control (■) marrow were cultured for 5 days in PHA and IL-2. Depletion of $CD5^+$ T cells occurred in association with relative enrichment of NK cells, $CD5^-$ T cells, and T cells expressing the γ/δ T cell receptor.

Table 2. T-Cell Depletion

Analysis	Anti-Leu1 + C	ST1-IT
FACS	97.8%	95.2%
Proliferation	—	92.3%
LDA	97.2%	95.4%
T-cell dose/kg* (mean ± SD × 10^{-5})	2.85 ± 3.7	5.6 ± 7.7†

*T-cell dose/kg was defined as the product of T-cell precursor frequency in LDA and total nucleated cells infused divided by weight in kilograms.

†There was no significant difference in T-cell dose between ST1-IT and anti-Leu1 + C or between MHC-matched and MHC-mismatched. There was also no significant correlation relationship between T-cell dose and the development of GVHD.

Table 3. Time to Engraftment

	MHC-Match		MHC-Mismatch	
Engraftment*	Anti-Leu1 + C	ST1-IT	Anti-Leu1 + C	ST1-IT
PMN	20 (15-36)	18 (13-33)	26 (16-36)	18 (12-67)
Platelets	30 (21-110)	33 (14-82)	36 (25-104)	28 (12-138)
Graft failure†	1/18	2/27	2/8	1/12

*Engraftment was considered to be the first of 3 consecutive days with PMN ≥500 cells/μL and platelets ≥50,000 cells/μL. Data are expressed as median day (range).

†Expressed as number of patients with graft failure/number of evaluable patients.

leukemia (ANLL) in second remission who received a single antigen-mismatched transplant from his mother developed late graft failure at day 50 in association with cytomegalovirus (CMV) hepatitis.

Overall, primary graft failure occurred in 3 of 71 (4%) patients and late graft failure occurred in 3 of 68 (4%) evaluable patients. Four of the six patients with graft failure had myelodysplasia with myelofibrosis, and three of four patients with myelodysplasia and myelofibrosis had graft failures compared with 2 of 67 patients with other diagnoses. The diagnosis of myelodysplasia was significantly associated with graft failure ($P < .001$ by logistic regression). Marrow cell dose, T-cell dose, donor age, donor sex, and MHC compatibility were not risk factors for graft failure by univariate or multivariate analysis.

Control of GVHD

Patients were considered evaluable for acute GVHD if they had sustained engraftment. Chronic GVHD was evaluated in patients who survived at least 100 days posttransplant. The results of our experience with anti-Leu1 + C are summarized in Tables 4 and 5.

Anti-Leu1 + C. Acute GVHD (grade 2 and 3) developed in 4 of 18 (22%) evaluable histocompatible patients. There were two cases of grade 3 but no grade 4 GVHD. Only 3 of 14 (21%) evaluable histocompatible recipients have developed chronic GVHD. In contrast, four of eight (50%) evaluable MHC-mismatched individuals developed acute GVHD (grade 2 to 4) and none of three evaluable patients developed chronic GVHD.

ST1-IT. Acute GVHD (grade 2 and 3) was observed in 7 of 28 (25%) evaluable histocompatible patients. There was no grade 4 GVHD and no deaths attributable directly to GVHD. In contrast, 6 of 13 (46%) patients with MHC-mismatched donors had grade 2 to 4 GVHD and death was attributed to GVHD in 4 of 13 (31%) patients. Data from both protocols were pooled for the analysis of chronic GVHD. Chronic GVHD was observed in only 3 of 36 (17%) evaluable MHC-matched patients. Chronic GVHD was not observed in 11 evaluable MHC-mismatched patients.

Because there was no difference in the actuarial rates of

Table 5. Degree of MHC-Mismatch and GVHD

Patient No.	Donor Relationship	Mismatched MHC Antigens: GVHD Vector	Mismatched MHC Antigens: Graft Rejection Vector	Acute GVHD Grade
One antigen mismatch				
154	Sister	A	—	2
320	Sister	MLC	—	4
380	Brother	DR	—	0
160	Sister	—	B	0
407	Brother	—	MLC	1
437	Mother	—	DR	0
162	Sister	DR	DR	0
216	Mother	B	B	2
236	Father	B	B	NE
332	Father	DR	DR	3
439	Sister	DR	DR	1
Two antigen mismatch				
194	Brother	B, DR	A, B	1
202	Father	A, A	A, A	1
217	Father	A, DR	A, B	4
321	Father	B, DR	B, DR	1
327	Father	B	B, DR	2
359	Mother	A, B	B	0
370	Father	A, B	A, B	3
373	Father	A, B	B	4
392	Father	A, DR	A, B	1
Three antigen mismatch				
204	Father	A, B	A, B, DR	NE
205	Father	A, B, DR	A, DR	3
378	Father	A, B	A, B, DR	1

Abbreviations: A, HLA-A; B, HLA-B; DR, HLA-DR; MLC, reactive mixed lymphocyte culture; NE, not evaluable.

Table 4. GVHD

	MHC-Match			MHC-Mismatch		
	Anti-Leu1 + C (n = 19)	ST1-IT (n = 29)	Overall (n = 47)	Anti-Leu1 + C (n = 10)	ST1-IT (n = 13)	Overall (n = 23)
Acute GVHD Grade*						
0-1	14 (78%)	21 (75%)	35 (78%)	4 (50%)	7 (54%)	11 (50%)
2	2 (11%)	5 (18%)	7 (16%)	2 (25%)	1 (8%)	3 (14%)
3	2 (11%)	2 (7%)	4 (9%)	1 (13%)	2 (15%)	3 (14%)
4	0 (0%)	0 (0%)	0 (0%)	1 (13%)	3 (23%)	4 (18%)
Unevaluable	1	1	2	2	0	2
Days to onset						
Median (range)	27 (9-71)	31 (18-55)		12 (9-22)	27 (19-35)	
Chronic GVHD						
None	11 (79%)	19 (86%)	30 (83%)	3 (100%)	8 (100%)	11 (100%)
Limited	0 (0%)	2 (9%)	2 (6%)	0 (0%)	0 (0%)	0 (0%)
Extensive	3 (21%)	1 (5%)	4 (11%)	0 (0%)	0 (0%)	0 (0%)
Unevaluable	5	7	12	7	5	12

There was no statistical difference in GVHD incidence between ST1-IT and anti-Leu1 + C protocols.

*Percentages are calculated based on evaluable patients.

acute GVHD between protocols, we combined the data from the anti-Leu1 + C study and the ST1-IT study. The actuarial risk of acute GVHD was 23% (95% confidence interval [CI], 11% to 36%) in the MHC-matched patients compared with 50% (95% CI, 28% to 72%) in the MHC-mismatched patients. As shown in Fig 2, the development of acute GVHD occurred faster in patients with MHC-mismatched donors than with MHC-matched donors, but after 5 weeks there were no new cases of acute GVHD in the MHC-mismatched group. In contrast, patients with MHC-matched donors developed acute GVHD as late as 10 weeks after the transplant, but fewer patients developed significant acute GVHD. No patient characteristics (patient age or sex, donor age or sex, diagnosis, or sex mismatch) had a significant association with grade 2 to 4 acute GVHD in the logistic regression model, and there was no relationship between the number of infused T cells and the development of grade 2 to 4 acute GVHD.

Survival

There were no significant differences between the anti-Leu1 + C and ST1-IT patients in the rate of GVHD, graft failure, survival, or event-free survival. Therefore, data from both protocols were pooled and survival was analyzed based on MHC compatibility and risk group.

MHC-matched transplants. Of the 48 MHC-matched individuals, two (4%) died of interstitial pneumonitis, one (2%) died of hepatic veno-occlusive disease, nine (19%) died of relapse or progressive disease, two (4%) died of graft failure, one (2%) died of bronchiolitis obliterans, seven (15%) died of infections, and one (2%) died of renal failure. The actuarial event-free survival is 38% (95% CI, 22% to 53%) (Fig 3).

MHC-mismatched transplants. Of the 23 patients with MHC-mismatched grafts, three (13%) died of interstitial pneumonia, two (9%) died of infection, two (9%) died of graft failure, four (17%) died of acute GVHD including

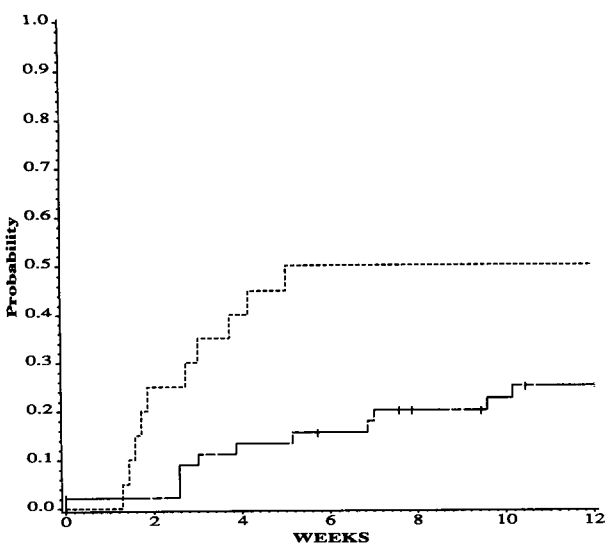

Fig 2. Actuarial risk of developing grade 2 to 4 acute GVHD. (—) MHC-matched; (- - - -) MHC-mismatched. Log rank test, $P = .02$.

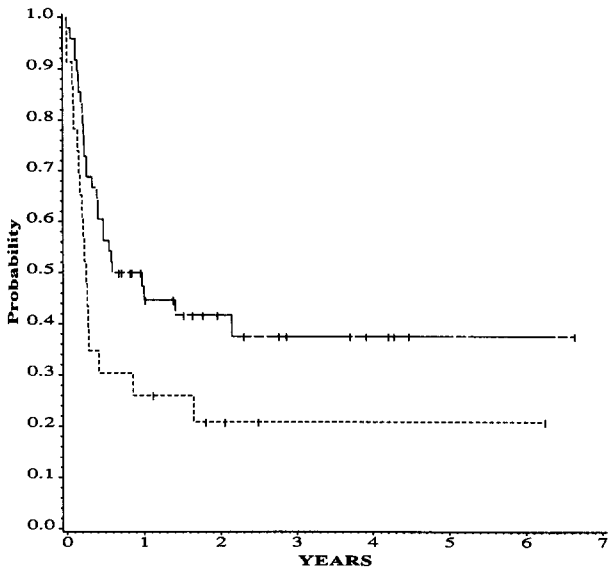

Fig 3. Actuarial event-free survival. (—) MHC-matched; (- - - -) MHC-mismatched. Log rank test, $P = .036$.

three with B-cell lymphomas, four (17%) died of progressive disease, and one (4%) died of congestive heart failure. The actuarial event-free survival is 21% (95% CI, 4% to 38%) (Fig 3).

As described in Table 1, we chose a cohort of patients that was at very high risk of relapse and of GVHD. Because of the heterogeneous diagnoses of patients enrolled in this study, we were unable to analyze survival as a function of diagnosis. However, we split the patients into two groups based on the broad disease categories. Patients at "low risk" had ANLL or acute lymphocytic leukemia (ALL) in first remission, chronic myelogenous leukemia (CML) in stable phase, or refractory anemia. All other patients were considered "high risk." High risk patients were more heavily represented in the MHC-mismatched group. When the MHC-matched patients were evaluated separately, the data were similar. However, age, sex, sex mismatch, donor age, and T-cell dose were comparable. Actuarial event-free survival is 62% (95% CI, 42% to 81%) for low-risk patients and 11% (95% CI, 8% to 21%) for the high-risk group ($P < .001$, Fig 4). This survival difference is associated with a lower rate of GVHD (23% *v* 35%), relapse/progressive disease (3% *v* 29%), death rate (20% *v* 85%), and a difference in treatment-related toxicity (17% *v* 66%).

Lymphoproliferative Disorders

Three of the 71 patients in this trial developed lymphoproliferative disorders that were unrelated to their original disease. All three of the patients were MHC-mismatched with their donor. One received marrow from a donor mismatched at a single MHC locus, and two received marrow from haploidentical donors. All three of the patients had developed clinically significant acute GVHD and were being heavily immunosuppressed (Table 6). The lymphomas were diagnosed soon after transplant, at 2 months in two cases and 153 days in the other case. Growth

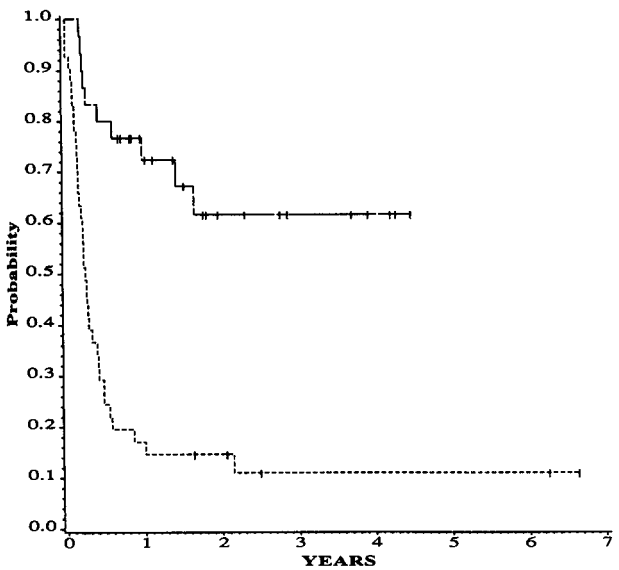

Fig 4. Actuarial event-free survival. (—) Low-risk patients; (- - - -) high-risk patients. Log rank test, $P < .001$.

of the lymphomas was impressively rapid, with death resulting within 2 weeks in the two cases diagnosed during life. All were diffuse large cell lymphomas with immunoblastic characteristics, and by immunophenotyping all were of B-cell origin. At autopsy, the distribution of the lymphomas was widespread, involving not only lymph nodes and BM but also spleen, liver, and gut, as well as a variety of unusual sites including thyroid, adrenals, pancreas, submandibular glands, and gall bladder.

The two patients whose lymphomas were diagnosed during life had a monoclonal B-cell proliferation as assessed both by surface light chain Ig expression and by Southern blot analysis of the heavy and light chain Ig genes (data not shown). Moreover, Epstein-Barr virus (EBV) RNA was shown in the malignant B lymphocytes. One lymphoma diagnosed at postmortem was a polyclonal B-cell proliferation, as assessed by surface Ig staining, although it had comparable light microscopic morphology compared with the other two.

DISCUSSION

This study was designed to determine whether T-cell depletion of marrow with anti-CD5 MoAb was a safe and effective method to prevent GVHD. Both methods of T-cell depletion effectively depleted the marrow of greater than 95% of $CD3^+$ T cells and reduced the incidence of severe GVHD in MHC-matched transplants and across MHC barriers. We found that the ST1-IT was more convenient to use and required fewer manipulations of the marrow. A major benefit of ST1-IT was the avoidance of rodent complement. Complement lots are very variable in efficacy and toxicity and require careful screening. ST1-IT allowed a more standardized approach. The control of GVHD was similar using either MoAb, and it was particularly encouraging given the ages of the patients and the proportion of patients that are sex-mismatched. In addition, the increased intensity of the conditioning regimen was well-tolerated. The graft failure rate was very low for a trial of T-cell–depleted marrow grafting in both MHC-matched and MHC-mismatched transplants.

GVHD was not entirely eliminated with anti-CD5 MoAb. One explanation for the occurrence of acute GVHD is suggested by our previous observation that normal individuals have from 0% to 27% (5% ± 6%; mean ± SD) circulating $CD3^+CD5^-$ T cells.[31] Studies of these $CD3^+CD5^-$ T cells on a clonal level have shown that they are able to initiate a cytotoxic and proliferative response to allo-antigens, suggesting that they may have the capacity to cause GVHD in vivo. Subsequently, we and others have shown that the proportion of these cells in the circulation is increased after allogeneic BMT and that they are associated with acute GVHD.[32,33] It was noteworthy that the onset of acute GVHD appeared to be delayed in many of our patients receiving MHC-matched transplants. This finding suggests that the small number of T cells infused may require several weeks to expand to a large enough number of cells to cause clinical GVHD. In contrast, GVHD occurred sooner after marrow infusion in MHC-mismatched transplants, suggesting that the MHC disparity may have been a more potent stimulus to the expansion of small numbers of T cells.

Chronic GVHD was also diminished compared with reported rates as high as 62% to 85% in patients receiving pharmacologic prophylaxis.[34] The improvement in chronic GVHD may be related to a reduction in acute GVHD per se, or more likely to the infusion of fewer T cells that mediate chronic GVHD. Other investigators have observed similar findings with T-cell depletion.[6,7,15]

While T-cell depletion can reduce or eliminate severe acute GVHD, this improvement comes at the expense of an

Table 6. Lymphoproliferative Disorders

UPN	MHC-Mismatch	GVHD Grade	GVHD Treatment	Day of Diagnosis	Day of Death	Lymphoma
194	3 antigen	2	Methylprednisolone Azathioprine	+143	+153	Monoclonal B cell +EBV genome
216	2 antigen	3	Methylprednisolone In vivo anti-Leu1*	+61 (autopsy)	+61	Polyclonal B cell EBV not tested
217	3 antigen	4	Methylprednisolone In vivo anti-Leu1 ATG, cyclosporine	+52	+57	Monoclonal B cell +EBV genome

*Four patients (one MHC-matched and three haploidentical) in this trial with severe acute GVHD received intravenous anti-Leu1 MoAb 0.1 mg/kg/d infused over 6 hours for 5 days. Antibody excess was achieved in all patients.

increase in the graft failure rate from ≤3% to 10% to 30%.[5,6,10,15,35] There may also be an increase in the leukemic relapse rate, especially in patients with CML.[36] The increase in graft failure and the loss of GVL have offset the reduction in GVHD-related mortality, and there is no current evidence that T-cell depletion improves survival.[5,36] It is possible that graft rejection and loss of GVL could be overcome in two ways. First, selective depletion of a subset of mature T cells might allow residual $CD5^-$ T cells, including T cells expressing the γ/δ T-cell receptor, and NK cells to maintain a GVL effect and reduce the likelihood of graft failure. Second, we used conditioning regimens that were designed to provide enhanced antineoplastic activity and immunosuppression. We increased the intensity of the conditioning regimen by using ara-c, administering cyclophosphamide and radiation simultaneously, and using 14 Gy fractionated TBI at 0.10 Gy/min. These increases were possible because toxicity from conventional posttransplant immunosuppression was avoided. Thus, by increasing the immunosuppressive and antileukemic capacity of the conditioning regimen and leaving $CD3^+CD5^-$ T cells, γ/δ T cells, and NK cells in the marrow, we hoped to control GVHD while avoiding some of the known pitfalls of T-cell purging.

We observed a low incidence of graft failure (8.5%). This low incidence was particularly true for transplants from MHC-mismatched donors, where the graft failure rate was substantially lower than reported rates in T-cell–depleted transplants of 50% to 75%.[10,37] Residual host T cells are probably responsible for graft rejection.[8,11] Most prior studies attempted to remove as many T cells from the donor marrow as possible, leaving residual host T cells unopposed to reject the graft.[16,35,38] Selective T-cell depletion of BM $CD5^+$ T cells may have been beneficial if residual NK cells, γ/δ T cells, and $CD3^+CD5^-$ T cells contributed to the suppression or elimination of these residual host lymphocytes. Furthermore, these residual lymphocytes may also produce lymphokines that are important for engraftment. Although they probably participate in the induction of acute GVHD, the small numbers of $CD3^+CD5^-$ T cells infused did not result in high-grade GVHD, and the trade-off between graft rejection and GVHD was acceptable. In addition, we used a conditioning regimen that was more intensive than many previous trials. It is likely that GVHD prophylaxis with cyclosporine and/or methotrexate contributes to the prevention of graft rejection. If pharmacologic GVHD prophylaxis is not used, the intensity of the conditioning regimen must be increased to compensate. The increased immunosuppression expected with the regimen used in this trial may have eliminated the residual host T cells that are thought to mediate graft rejection. Previous trials of T-cell depletion may have observed more graft failures, because they often used the same conditioning regimens that were used with pharmacologic GVHD prophylaxis. Further analysis of our data has suggested that patients with myelodysplasia and marrow fibrosis are at particularly high risk of graft failure[39] and by excluding these individuals from analysis, the graft failure rate for MHC-matched transplants is very low (0%) for a trial of T-cell depletion. Patients with marrow fibrosis may have microenvironment abnormalities that affect engraftment, because graft failure in such patients has been reported even with pharmacologic GVHD prophylaxis.[40]

The trend in previously reported clinical trials toward a higher leukemic relapse rate in patients whose marrows have been depleted of T cells suggests there has been a loss of GVL.[9] Although an effect of selective $CD5^+$ T-cell depletion on relapse rate cannot be detected in this trial, it is possible that the sparing of donor NK cells and $CD5^-$ T cells contributes to tumor cell kill. It has been recently shown that lymphokine-activated killer cells (LAK cells) persist after T-cell depletion.[41] The increased relapse rate may be more related to effective control of GVHD than to T-cell depletion per se. The use of combined cyclosporine and methotrexate is associated with better control of GVHD and a higher relapse rate than either drug used alone.[42] Clift et al have recently shown that increasing the cytotoxicity of the conditioning regimen in acute leukemia results in a lower risk of relapse.[43] In that study, GVHD was more prevalent in patients with the intensified conditioning regimen, perhaps because the higher irradiation dose resulted in toxicity and prevented the effective administration of GVHD prophylaxis. In our trial, the use of T-cell depletion allowed us to intensify the conditioning regimen, as limited or no posttransplant GVHD prophylaxis was administered.

The development of secondary lymphoproliferative disorders is a life-threatening complication of transplantation that appears to be more common after T-cell depletion of donor marrow.[44,45] As in our study, it occurs primarily, but not exclusively, in MHC-mismatched patients and in patients receiving T-cell depletion. The development of secondary lymphoproliferative syndromes in this group may have been due to the uncontrolled proliferation of EBV-infected B cells, because T-cell control of B-cell proliferation had been abrogated. An additional four cases of lymphoproliferative disorders occurred in patients transplanted after the study closed. Three occurred after MHC-matched sibling-donor transplants and one after phenotypically matched parental transplant. To determine the overall risk of lymphoproliferative disorders after anti-CD5 MoAb-purged transplants, we analyzed data derived from all patients reported here as well as patients with immunodeficiencies, congenital disorders of metabolism, and additional patients with malignancies that received similar transplants after this study was closed to patient entry (unpublished data). The actuarial probability of developing a lymphoproliferative disorder in 87 patients was 12% at 7 years. This value is less than the 24% estimate of the risk using anti-CD3 MoAb,[45] but still causes serious concern. Four patients received in vivo anti-Leu1 MoAb infusions for the treatment of acute GVHD (data not shown). Two of the four patients developed lymphoproliferative disorders. Anti-CD5 MoAbs have been shown to be mitogenic for T cells[46] and such T-cell activation may result in stimulation of B cells.[47] T-cell activation would not be expected after infusion of an anti-CD5 immunotoxin that kills the stimu-

lated cells. Similar lymphoproliferative disorders have been observed after infusions of anti-CD3 MoAb for the treatment of GVHD[48] or organ transplantation,[49] but not after the use of anti-CD5 immunotoxin.[50] Other strategies will be required to avoid this life-threatening complication.

We have shown that T-cell depletion with anti-CD5 MoAb reduces the incidence and morbidity of GVHD. Use of anti-CD5 MoAb and intensive conditioning regimens resulted in a low graft failure rate and a low relapse rate, especially in good-risk patients. New approaches may be needed to prevent the development of lymphoproliferative disorders. To show an effect of T-cell depletion on survival would require a large randomized study. It is possible that as the problems of graft rejection and leukemic relapse are solved, selective or subtotal T-cell depletion will be preferable to immunosuppressive prophylaxis, particularly in older patients and patients with liver or renal dysfunction. The results presented here are encouraging and the use of anti-CD5 MoAb warrants further study.

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