1985

..ects of In Vitro Depletion of T Cells in HLA-Identical Allogenei.. Marrow Grafts

By Paul J. Martin, John A. Hansen, C. Dean Buckner, Joan E. Sanders, H. Joachim Deeg, Patricia Stewart, Frederick R. Appelbaum, Reginald Clift, Alexander Fefer, Robert P. Witherspoon, Michael S. Kennedy, Keith M. Sullivan, Nancy Flournoy, Rainer Storb, and E. Donnall Thomas

We report results of a pilot study designed to evaluate the effects of in vitro depletion of T lymphocytes from donor marrow in patients receiving HLA-identical marrow grafts for treatment of hematologic malignancies. Twenty patients aged 31 to 50 years were prepared for transplantation with cyclophosphamide (120 mg/kg) and fractionated total body irradiation (12.0 or 15.75 Gy). All received cyclosporine after grafting. The donor marrows were treated with a mixture of eight murine monoclonal antibodies and rabbit serum complement in a manner that achieved a 2- to 3-log depletion of T cells in most patients. Initial engraftment occurred promptly in 19 of the patients, and only three had clinically significant acute graft-versus-host disease. Depletion of donor T cells, however, was associated with an increased incidence of graft failure, which occurred as late as 244 days after transplantation. Graft failure was transient in one patient but apparently was irreversible in seven others. Three of the seven patients had cytogenetic but not morphological evidence of leukemic relapse at the time of graft failure. All seven patients with irreversible graft failure have died, six after receiving second bone marrow transplants. Seven of the eight cases of graft failure occurred among the 11 patients prepared for transplantation with 12.0 Gy of total-body irradiation, and only one occurred among the nine patients with advanced malignancies who received 15.75 Gy of total-body irradiation. This association with irradiation dose suggests that host factors were partly responsible for the graft failures. Because graft failure seldom occurs in irradiated recipients of unmodified HLA-identical allogeneic marrow transplants, it appears that T cells in the donor marrow may serve a beneficial function in helping to maintain sustained engraftment possibly by eliminating host cells that can cause graft failure. Optimal application of in vitro manipulation of donor marrow as a method for preventing graft-versus-host disease will require more effective immunosuppression of the recipient in order to assure sustained engraftment and function of donor stem cells.

ALLOGENEIC BONE MARROW TRANSPLANTATION can provide curative therapy for patients with acute leukemia or aplastic anemia.[1,2] However, clinically significant acute graft-versus-host disease (GVHD) occurs in 35% to 50% of patients who receive an allogeneic marrow graft from a genotypically HLA-identical sibling even though these patients are treated after transplantation with immunosuppressive agents such as methotrexate, cyclosporine, corticosteroids or antithymocyte globulin.[3-5] The development of moderate (grade II) or severe (grade III or IV) acute GVHD after marrow transplantation is associated with decreased survival.[6-10] At least 15% of marrow transplant patients die of complications related to acute or chronic GVHD,[8-10] indicating a clear need for more effective methods for preventing or treating this complication.

In numerous rodent models it has been demonstrated that mature T lymphocytes are responsible for causing GVHD after allogeneic marrow or spleen cell grafting.[11-13] GVHD does not occur in irradiated animals when the graft is devoid of mature T cells. Transplanted fetal liver cells[11] or spleen cells from neonatally thymectomized mice[12] can reconstitute normal hematopoiesis without causing acute GVHD. Furthermore, removal of mature T cells from allogeneic donor spleen cells with the use of anti-Thy-1 heteroantisera or monoclonal antibodies can prevent GVHD.[13] As few as 3×10^4 total T cells can cause GVHD in recipient mice with disparity only in non-H-2 minor histocompatibility antigens.[14] Prevention of GVHD by removal of mature T cells has also been demonstrated in other species.[15,16]

Removal of T cells from human donor marrow was first attempted with the use of a carefully titrated heterologous rabbit anti-T cell antiserum that was extensively absorbed in order to remove antibodies that were cross-reactive with hematopoietic precursors.[17] Physical separation of T cells with the use of lectin agglutination and E rosetting has also been investigated.[18,19] More recent attempts to remove T cells from human donor marrow have used murine monoclonal antibodies specific for surface molecules carried by human T lymphocytes. Previous studies have demonstrated that treatment of marrow with unmodified murine monoclonal antibodies in the absence of exogenous complement does not prevent GVHD.[20-22]

We report here results of a pilot study designed to evaluate the effects of in vitro depletion of T lymphocytes from donor marrow with the use of anti-T cell monoclonal antibodies and complement. The objectives of this study were to determine (a) the toxicity of marrow treatment, in terms of delay or failure of engraftment; and (b) the efficacy of T cell depletion as a method of preventing acute GVHD. Patients in this study received genotypically HLA-identical marrow grafts for treatment of hematologic malignancies, and results for engraftment and acute GVHD were compared with those for similar patients who received unmodified donor marrow.

From the Fred Hutchinson Cancer Research Center, the Puget Sound Blood Center, and the Division of Oncology, University of Washington, Seattle.

Supported by contract No. N00014-82-K-0660 from the office of Naval Research, Department of Defense, and by grants No. CA18029, CA30924, CA29548, CA18221, and CA15704 awarded by the National Cancer Institute. E.D.T. is the recipient of Research Career Award AI02425 from the National Institutes of Allergy and Infectious Diseases.

Submitted Nov 6, 1984; accepted March 18, 1985.

Address reprint requests to Dr Paul J. Martin, Fred Hutchinson Cancer Research Center, 1124 Columbia St, Seattle, WA 98104.

0006-4971/85/6503-0030$03.00/0

HLA-Identical Marrow Transplantation During Accelerated-Phase Chronic Myelogenous Leukemia: Analysis of Survival and Remission Duration

1988

By Paul J. Martin, Reginald A. Clift, Lloyd D. Fisher, C. Dean Buckner, John A. Hansen, Frederick R. Appelbaum, Kristine C. Doney, Keith M. Sullivan, Robert P. Witherspoon, Rainer Storb, and E. Donnall Thomas

Results of HLA-identical allogeneic marrow transplantation were analyzed for 66 patients with accelerated-phase chronic myelogenous leukemia (CML). Multivariate proportional hazards regression models were used to determine disease-related and transplant-related factors associated with posttransplant mortality and relapse. The projected 5-year survival rate was estimated at 18% by the product-limit method. The major causes of death were interstitial pneumonia, infection, and relapse. Splenomegaly at initial diagnosis and longer time interval from diagnosis to transplant were associated with decreased overall survival and event-free survival. Sixteen patients have relapsed between 17 and 1,569 days (median, 486) posttransplant. The use of T-cell–depleted marrow and older age of the donor or recipient were associated with an increased probability of leukemic relapse. Ten of the 16 relapses occurred among the 15 patients who received T-cell–depleted marrow. The actuarial relapse risk 2.5 years posttransplant was 100% in patients administered T-cell–depleted marrow as compared with 26% in patients administered unmodified marrow. The data in this report emphasize the increased risks and relatively poor results that occur when marrow transplantation is deferred until after signs of acceleration appear. When compared with results for patients who received transplants during chronic phase, the poor results seen here in patients administered unmodified marrow stem primarily from increased transplant-related mortality rather than increased relapse risk. The strikingly increased relapse rate associated with the use of T-cell depletion would discourage its use for graft-v-host disease prevention in patients who receive transplants for CML.

HIGH-DOSE CHEMORADIOTHERAPY with marrow rescue by allogeneic or syngeneic transplantation represents the only treatment thus far demonstrated to be capable of curing patients with chronic myelogeneous leukemia (CML).[1-3] Prolonged disease-free survival can be achieved by marrow transplantation during any phase of the disease, but best results have occurred when transplantation is carried out during the chronic phase (CP). The probability of survival at 5 years after allogeneic transplantation from HLA-identical donors is 60% for patients who receive transplants in CP, 22% for patients in accelerated phase (AP), and 13% for those in blast phase (BP).[4] Transplantation during AP and BP has been associated with increased transplant-related mortality and increased leukemic relapse. These observations encourage early marrow transplantation for patients with newly diagnosed CML who have suitable donors. On the other hand, transplantation during CP is associated with 20% mortality during the first 100 days and 30% mortality during the first year, which is greater than would be expected without transplantation.[7] The early mortality associated with marrow transplantation and the long duration of CP in some patients might advocate delay of transplantation until signs of acceleration appear. In this study we analyzed the results of HLA-identical allogeneic marrow transplantatation for 66 patients with CML in AP and identified disease-related and transplant-related factors associated with posttransplant mortality and relapse to clarify the optimal approach for treating patients with CML.

From the Division of Clinical Research, the Fred Hutchinson Cancer Research Center, the Puget Sound Blood Center, and the Department of Medicine, University of Washington, Seattle.

Submitted April 11, 1988; accepted July 28, 1988.

Supported by Contract No. N00014-82-K-0660 from the Office of Naval Research and by Public Health Service Grants CA 18029, CA 29548, CA 18221, CA 15704, and CA 09515 awarded by the National Cancer Institute, National Institutes of Health, DHHS. E. Donnall Thomas is the recipient of Research Career Award AI 02425 from the National Institute of Allergy and Infectious Diseases.

Presented in part at the 29th annual meeting of the American Society of Hematology, Washington, DC, December 5 through 8, 1987.

Address reprint requests to Paul J. Martin, MD, Fred Hutchinson Cancer Research Center, 1124 Columbia St, Seattle, WA 98104.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

0006-4971/88/7206-0010$3.00/0

METHODS

Patient accrual. From August 1973 through December 1985, a total of 66 patients received HLA genotypically identical allogeneic marrow transplants for treatment of Philadelphia chromosome (Ph)-positive CML in AP. A diagnosis of AP was assigned retrospectively after a complete review of the pretransplant data. At least one of the following findings was necessary to confirm the diagnosis of AP: anemia (hematocrit <25%), leukocytosis (WBC count, >500,000/μL), or thrombocytopenia (platelet count, <100,000/μL) uncontrolled by conventional therapy with busulfan or hydroxyurea; progressive splenomegaly or lymphadenopathy; CNS or other extramedullary disease; the presistent presence of 10% to 30% blasts in the blood or marrow; the presence of cytogenetic abnormalities other than a single Ph; or the development of constitutional symptoms (fever, weight loss, fatigue) or bone pain without other explanation. Patients persistently having more than 30% blasts in the blood or marrow and BP patients successfully treated with chemotherapy pretransplant were excluded from this study. Splenomegaly was considered to be clinically significant when the spleen was palpable more than 4 cm below the left costal margin. Cytogenetic abnormalities were categorized according to Przepiorka and Thomas.[8]

Marrow transplant regimens. Treatment was determined according to nonrandomized or randomized protocols under investigation at the time of transplant. All patients received cyclophosphamide, 60 mg/kg body weight intravenously on each of two successive days, followed by total body irradiation administered from opposing