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In Vitro Treatment with Monoclonal Antibody Prior to Autologous Bone Marrow Transplantation in Acute Lymphoblastic Leukemia *

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A. Introduction

Monoclonal antibodies which are specific for surface antigens of leukemic cells have become useful diagnostic reagents and have been used to dissect the heterogeneity of leukemia in man [1, 2]. In addition, it is apparent that large quantities of homogeneous antibody which primarily react with leukemic cells may become useful therapeutic reagents. Previous trials of serotherapy with various monoclonal antibodies in patients with multiply relapsed acute lymphoblastic leukemia (ALL) or lymphoma have demonstrated that intravenously administered antibody can rapidly bind to tumor cells in peripheral blood and bone marrow and that relatively large numbers of malignant cells can be eliminated in vivo [3–6]. In one patient with B-cell lymphoma, a complete remission was achieved following intravenous infusion of monoclonal anti-idiotype antibody [7]. In general, however, these studies have not produced clinically significant responses and have clearly identified several specific factors such as presence of serum-blocking factors, antigenic modulation, and inefficiency of natural effector mechanisms, which limit the therapeutic activity of monoclonal antibody in vivo (reviewed in [8]).

One approach which circumvents several of the obstacles to effective serotherapy in vivo is the utilization of monoclonal antibody in vitro. Thus, in a controlled in vitro environment, extracellular blocking factors can be removed, incubation with monoclonal antibody at 4 °C can effectively inhibit antigenic modulation, and multiple treatments with heterologous complement can be used to ensure the lysis of all tumor cells. In addition, potential cross reactivity of monoclonal antibodies with non-hematopoietic tissues can be avoided. A previous report has presented our preliminary experience with the use of the J5 monoclonal antibody and rabbit complement to treat bone marrow in vitro to remove residual leukemic cells prior to autologous transplantation [9]. This report summarizes the current results of this clinical study.

B. Methods

I. J5 Monoclonal Antibody

The method for generation and characterization of J5 monoclonal antibody specific for the common acute lymphoblastic leukemia antigen (CALLA) has been described previously [10]. J5 antibody (murine IgG2A) is reactive with leukemic cells from 80% of patients with non-T cell ALL and 40% of patients with chronic myelocytic leukemia in blast crisis. In addition, lymphoma cells from almost all patients with B-cell nodular poorly differentiated lymphocytic lymphoma and Burkitt's lymphoma, and 45% of patients with T-cell lymphoblastic lymphoma, are reactive with

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J5 antibody [11]. Within normal bone marrow, approximately 1% of cells also express CALLA, but previous studies have shown that these normal CALLA-positive cells are not myeloid precursor cells (CFU-C, BFU-E, CFU-E, and CFU-G/E) [12].

More recent studies have indicated that CALLA is expressed during early lymphoid cell differentiation, but it appears that the earliest lymphoid stem cells do not express this antigen [13]. In addition to hematopoietic cells, it has been demonstrated that J5 antibody is reactive with various nonhematopoietic tissues including cells from renal glomerulus and proximal tubules [14]. Recently, it has also been found that J5 antibody is reactive with cultured fibroblasts from normal bone marrow (J. Ritz, unpublished observation) as well as cell lines established from various solid tumors (H. Lazarus, personal communication). These findings are of particular importance for the therapeutic application of CALLA-specific antibodies since these normal cells would also be potential targets for antibody-directed therapy.

Our method for obtaining large quantities of purified J5 monoclonal antibody and our method for in vitro treatment of bone marrow have been previously described [9, 15, 16]. Briefly, J5 antibody was obtained aseptically from ascitic fluid of Balb/c mice that had been primed with pristane followed by intraperitoneal inoculation of J5 hybridoma cells. Bone marrow was harvested from anterior and posterior iliac crests under general anesthesia, and mononuclear cells were isolated using discontinuous Ficoll-Hypaque density gradients. Bone marrow cells were then treated three times with J5 antibody and rabbit complement and cryopreserved in the vapor phase of liquid nitrogen in media containing 10% DMSO and 90% autologous seurm. Prior to infusion, cryopreserved marrow was rapidly thawed and cells were diluted in medium which contained DNAase to prevent clumping.

C. Results

I. Clinical Protocol

All patients with ALL who had relapsed following standard chemotherapy and whose leukemic cells expressed the common ALL antigen (CALLA) were considered eligible for the protocol that is outlined in Fig. 1. Patients who had normal identical twins or histocompatible siblings were ineligible for this study and received either syngeneic or allogeneic bone marrow transplantation. In addition, patients in whom a complete remission could not be induced with chemotherapy alone were excluded.

Following induction of second or subsequent remission, patients received intensive chemotherapy with the following agents: VM-26, cytosine arabinoside (araC), and *L*-asparaginase (Fig. 1). CNS repropylaxis with intrathecal araC and hydrocortisone was also adminstered at that time. After recovery from intensification, patients underwent bone marrow harvest under general anesthesia. Mononuclear cells were isolated and treated three times with J5 antibody and rabbit complement prior to cryopreservation. A separate aliquot of marrow was also cryopreserved without antibody treatment. These cells constituted a "back-up marrow" which could be used in the event that antibody-treated marrow failed to engraft but was not used in any of our patients. In patients 1, 2, and 3, "back-up marrow" was harvested separately just prior to intensification and cryopreserved without antibody treatment.

One day after marrow harvest, patients began receiving ablative treatment consisting of VM-26, araC, cyclophosphamide, and total body irradiation (TBI) (Fig. 1). Approximately 12 h after TBI, cryopreserved marrow which had previously been treated in vitro was rapidly thawed and reinfused through a central venous catheter. Patients did not receive any additional chemotherapy.

Thus far, six patients have been treated under this protocol and have been followed for more than 4 months. The clinical history of these patients and their current status is summarized in Table 1. Patient 1 had relapsed in bone marrow 20 months after elective cessation of therapy and now continues in unmaintained remission 20 months after autologous transplantation. Patient 2 had relapsed in both testes 2 months after completion of chemotherapy.

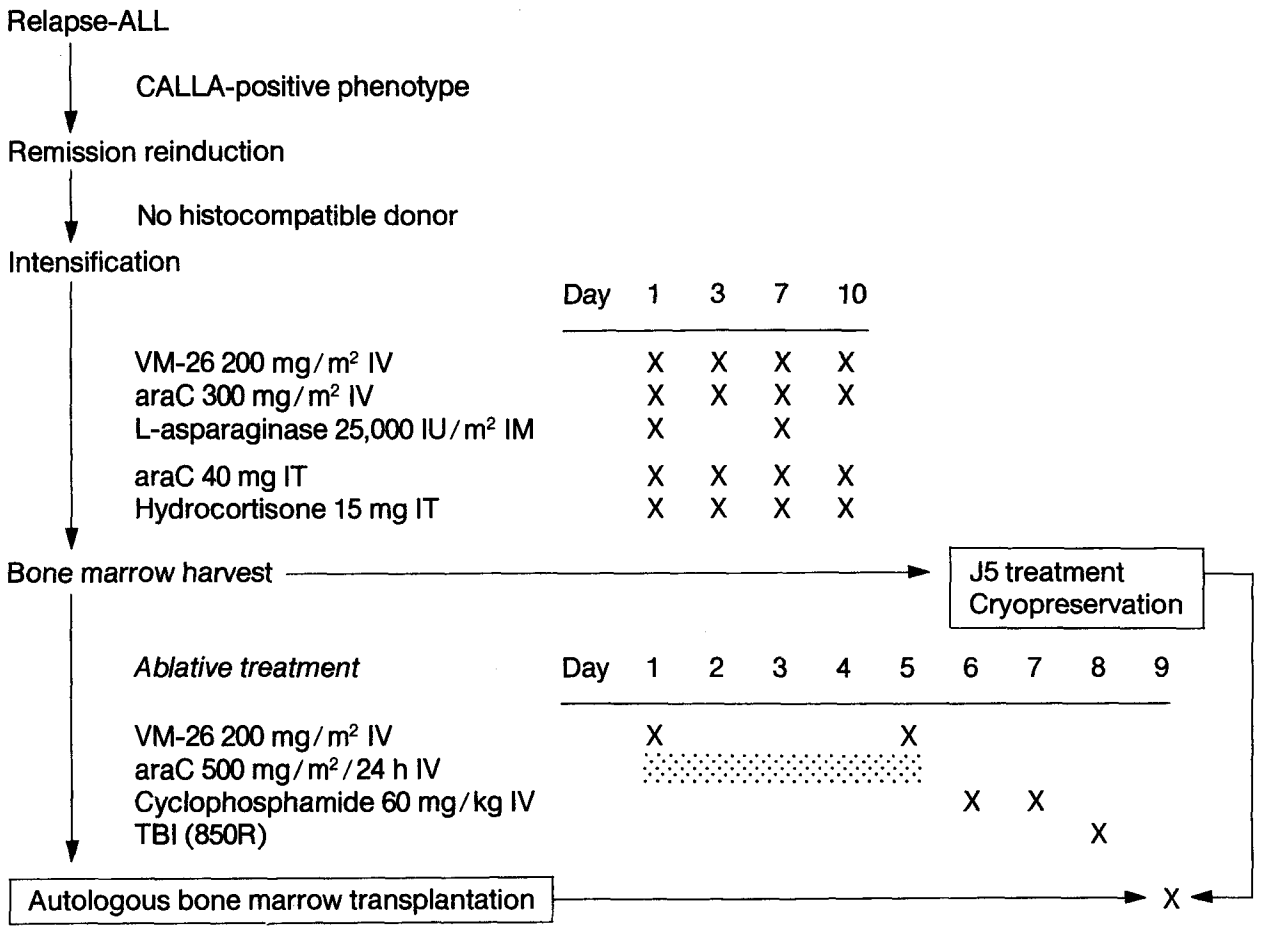

Fig. 1. Clinical protocol for autologous transplantation with J5 antibody and complement-treated bone marrow

Bone marrow at that time contained 7% lymphoblasts. He continues in unmaintained remission 18 months after autologous bone marrow transplantation. Patient 3 first relapsed in the CNS while receiving systemic chemotherapy and later relapsed in the bone marrow as well. A second bone marrow remission was difficult to achieve and was only attained after 4 months of intensive chemotherapy. He relapsed with CALLA-positive lymphoblasts 7 weeks after transplantation. Patient 4 relapsed in the bone marrow 3 months after initial diagnosis. He tolerated the ablative regimen well but subsequently developed interstitial pneumonitis, which was probably secondary to cytomegalovirus infection and expired 3 months after transplantation. Pneumonitis was also complicated by intrapulmonary hemorrhage secondary to persis-

Table 1. Clinical characteristics of patients treated with autologous bone marrow transplantation

Patient	Age	Sex	Initial WBC/mm³	Duration of 1st remission	Relapse site	Postautologous transplant status
1	10	M	2,000	50 months	BM	CR 20 months
2	5	M	150,000	32 months	Testes	CR 18 months
3	3	M	22,000	15 months	BM/CNS	Rel 7 weeks
4	4	M	31,000	2 months	BM	Exp 3 months in remission
5	14	M	98,000	29 months	BM/testes	5 months
6	11	M	5,900	7 years	BM/CNS/ testes	4 months

tent thrombocytopenia. At autopsy, there was no evidence of leukemic relapse. Patient 5 was transplanted in third remission. He first relapsed in the bone marrow 30 months after initial diagnosis. and subsequently continued on chemotherapy for an additional 4 years until therapy was electively stopped. Testicular relapse with CALLA-positive cells occurred 8 months later. Morphologic examination of bone marrow at this time demonstrated 3% blasts but immunofluorescence analysis of purified mononuclear cells demonstrated 19% CALLA-positive cells. He was subsequently entered onto our protocol and continues in remission 5 months after transplantation. Patient 6 received chemotherapy for 5 years after initial diagnosis but relapsed simultaneously in the bone marrow, CNS, and testes 2 years after elective cessation of therapy. Following reinduction of a second complete remission, he received the intensification and ablative therapy outlined in Fig. 1. He continues in remission 4 months after autologous transplantation.

II. Hematopoietic Reconstitution

Hematopoietic engraftment in six patients following autologous transplantation with J5-treated bone marrow is summarized in Table 2. In patient 1, the first evidence of marrow engraftment was seen 11 days after marrow infusion, and subsequent recovery of granulocytes, reticulocytes, and platelets occurred promptly. In patient 2, the first evidence of marrow engraftment was seen 9 days after transplant, but subsequent hematopoietic recovery occurred slowly. Although complete recovery did eventually occur, severe thrombocytopenia persisted for 3 months.

In patient 3, hematopoietic reconstitution proceeded gradually after marrow infusion, but bone marrow relapse became evident before complete recovery of peripheral counts had occurred. Seven weeks posttransplant, bone marrow aspirate demonstrated engraftment of granulocytic, erythroid, and megakaryocytic precursors but also contained approximately 40% CALLA-positive lymphoblasts. Patient 4 exhibited prompt recovery of granulocytes but reconstitution of both platelets and reticulocytes was much slower. Although megakryocytes were present in bone marrow aspirates and at autopsy, circulating platelet counts remained $< 20{,}000$ mm^3.

Patients 5 and 6 have been followed for relatively short periods, but hematopoietic recovery in both of these patients appears to be comparable to that seen in previous patients.

III. Immunologic Reconstitution

The appearance of B cells in peripheral blood and bone marrow was detected by reactivity with monoclonal antibody B1 which identifies a unique antigen expressed by normal B cells [17]. In patients 1 and 2, serum immunoglobulin levels gradually increased following the appearance of B1-positive cells.

In all six patients, T-lymphocytes were the first cells to engraft following transplantation. These cells expressed T3, T10 [18], and Ia antigens [19]. Although T cells from

Table 2. Hematopoietic recovery following autologous bone marrow transplantation

	Day S/P transplant					
Patient	1	2	3	4	5	6
Hematologic						
1st granulocyte	11	9	9	11	10	10
Granulocytes >1000/mm^3	49	78	54	56	43	63
Platelets $>25{,}000$/mm^3	22	92	–	–	62	49
Reticulocytes $>0.9\%$	22	36	25	69	45	47
Immunologic						
B cells (B1+)	63	128	–	61	45	64
T cells (T3+, T10+, Ia+)	11	9	10	12	9	11

peripheral blood normally express T3 antigen, both T10 and Ia antigens are normally expressed only after cell activation [20, 21]. Both T4 cells (T-inducer phenotype) and T8 cells (T-suppressor phenotype) were present, but the relative percentage of these cells in peripheral blood varied during engraftment. In almost all patients, the T4/T8 ratio of circulating T cells was abnormally low. In patients who initially had normal percentages of T4-positive cells (patients 1 and 5), peripheral T cells also later became predominantly T8 positive. The number of T cells which were Ia positive gradually decreased during the first 4 months following engraftment. Expression of T10 antigen also gradually decreased following engraftment but persisted much longer. At no time during engraftment was T6 antigen expressed by peripheral blood cells.

D. Discussion

Autologous bone marrow transplantation has previously been used in patients with various malignant diseases in an effort to circumvent marrow toxicity and to allow the administration of otherwise lethal doses of chemotherapy. Unfortunately, in most patients with solid tumors, higher doses of chemotherapy have not resulted in more effective eradication of malignant cells. In contrast, it has been demonstrated that leukemia and lymphoma cells can be eradicated with intensive chemotherapy and total body irradiation (TBI) even when these tumors are resistant to conventional doses of chemotherapy. This has led to the successful treatment of acute leukemia with ablative chemotherapy and TBI in conjunction with bone marrow transplantation from identical twins or allogeneic histocompatible siblings [22–25]. Unfortunately, the utilization of ablative therapy in leukemia is restricted to approximately 40% of patients who have normal histocompatible marrow donors, and autologous marrow transplantation is limited by the fact that residual leukemia is present in the patient's marrow, even during complete remission, Previous studies have attempted to circumvent this problem through the use of physical separation techniques [26] or treatment with conventional rabbit heteroantisera [27, 28] to eliminate leukemic cells prior to autologous transplantation. High-titer monoclonal antibodies which activate complement and specifically react with leukemic cells and not with hematopoietic stem cells are potentially very useful reagents which can be used to eliminate small numbers of leukemic cells in the presence of a large excess of normal marrow. The utilization of these reagents in vitro may therefore allow the application of autologous bone marrow transplantation to patients who do not otherwise have histocompatible donors.

In the present study, six patients with relapsed ALL received ablative therapy with VM-26, araC, cyclophosphamide, and TBI followed by infusion of autologous remission bone marrow which had been treated in vitro with J5 antibody and rabbit complement to remove residual leukemic cells. Hematopoietic engraftment with J5-treated bone marrow occurred in all six patients. Reconstitution of B cells and immunoglobulin production occurred after reconstitution of myeloid cells. Since all of our patients have engrafted with J5 antibody-treated bone marrow and two patients have been in unmaintained remission for more than 18 months, our study suggests that this approach may be a feasible alternative to conventional chemotherapy in patients with relapsed ALL. Treatment of additional patients and longer follow-up periods will be necessary to determine if in vitro antibody treatment is a clinically effective therapeutic modality.

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