Autologous bone marrow transplantation (BMT) was utilized as therapy for 23 patients with acute lymphoblastic leukemia (ALL) in second or greater remission. Bone marrow was treated in vitro with a combination of monoclonal antibodies, consisting of BA-1, BA-2, BA-3, and baby rabbit complement (BRC). All patients were prepared for transplantation with cyclophosphamide and fractionated total body irradiation. Engraftment occurred in all 23 patients. Seven of 23 patients remain relapse-free from six to 32 months (median, 21.4 months) posttransplant. Failures were due to relapse with the exception of one patient who died of infection. This study demonstrates that autologous BMT using in vitro marrow treatment with BA-1, BA-2, BA-3, and BRC is safe, allows engraftment, and results in prolonged survival for some patients with ALL in second or greater remission.

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The long-term survival for patients with acute lymphoblastic leukemia (ALL) following relapse has not improved over the past several years by use of various chemotherapeutic regimens. For patients with matched sibling donors, however, allogeneic bone marrow transplantation (BMT) offers improved survival. As only 30% to 40% of patients have a matched donor, alternative methods of transplantation of eligible patients are currently being explored. One approach involves the use of less than fully matched family donors or unrelated donors. A second approach has been to reinfuse the patient’s bone marrow after purging of any residual leukemic cells with drugs or monoclonal antibodies.

This article describes the experience of autologous BMT for patients with relapsed ALL at the University of Minnesota using autologous remission bone marrow treated in vitro with the monoclonal antibodies, BA-1, BA-2, BA-3, and rabbit complement.

MATERIALS AND METHODS

Patients. The current report concerns patients with ALL in second or subsequent remission who have received BA-1, BA-2, and BA-3-treated autologous marrow following intensive chemotherapy at the University of Minnesota. All 23 patients were transplanted between April 1982 and June 1984 with follow-up as of Dec 12, 1984. Patients were eligible for BMT on this protocol if in complete remission defined as: (1) <5% leukemic cells in a bone marrow aspirate, (2) no evidence of leukemic infiltration on marrow biopsy, and (3) no evidence of extramedullary leukemia. Leukemia cells at the time of diagnosis and/or relapse were all tested and found reactive with one or more of the monoclonal antibodies BA-1, BA-2, and the anti-common acute lymphoblastic leukemia antigen (CALLA) antibodies BA-3 or J5. Patients were eligible for this protocol only if they did not have an HLA-MLC matched sibling donor.

The patients ranged in age from 2.2 to 16.6 years, with a median age of 6.1 years at diagnosis of their leukemia and between 3.2 and 17.3 years at the time of BMT (median, 8.9 years). Nine patients were in second marrow remission, 13 patients were in third marrow remission, and one patient was in fourth marrow remission. Four patients had prior extramedullary disease, with two patients having had prior central nervous system disease, and two patients having had prior testicular disease. Other clinical characteristics of the study group are presented in Table 1.

All 23 patients had bone marrow leukemic cells analyzed at diagnosis and/or relapse for reactivity with at least one of the monoclonal antibodies BA-1, BA-2, and BA-3. In several cases, monoclonal antibody J5, which recognizes the same CALLA as BA-3, was used in lieu of BA-3. The details of this analysis are presented in Table 2. Leukemic cells were considered to be positive for the monoclonal antibody if >20% of the leukemic cells bound the antibody from a bone marrow that contained >50% leukemic cells. Monoclonal antibody binding was detected by indirect immunofluorescence as previously described. Binding of the most positive antibody ranged between 43% and 98%, with a median of 77%.

Transplant methodology. Bone marrow was harvested using hypocellular marrow aspirates and processed using the standard protocol. All patients received pretransplant conditioning, consisting of cyclophosphamide 60 mg/kg/d for two days (day −7 and day −6), one day of rest, and four days of total body irradiation given twice daily at a dose of 165 rad (10 rad/min) for a total dose of 1,320 rad (days −4, −3, −2, and −1). Radiation was given through right and left lateral portals, using 10 MeV x-rays. Males received a testicular boost of 200 rad on day −2 and day −1. Bone marrow was thawed on day 0 and immediately infused into the patient. Engraftment of marrow was defined as the third consecutive day of a peripheral white count >1 x 10^9/L. All patients received trimethoprim-sulfamethoxazole for pneumocystis prophylaxis, which was instituted on day −10, and broad-spectrum antibiotic therapy for febrile episodes.

In order to give therapy that was identical to that given patients...
receiving allogeneic marrow, an attempt was made to administer
methotrexate at a dose of 10 mg/m² weekly through day 100 once an
absolute neutrophil count of 10⁹/L was achieved. Patients were
randomized at day 100 if in complete remission to receive mainte-
nance chemotherapy consisting of 6-mercaptopurine (6MP), 50 mg/
m²/d orally and methotrexate 10 mg/m²/wk orally for two years. The
100-day randomization is identical to that currently ongoing for our
alloimmune ALL transplant patients treated during the same time
period. In accordance with DHSS guidelines, all patients signed
informed consents approved by the Committee on the Use of Human
Subjects in Research at the University of Minnesota.

Marrow harvesting and processing. Bone marrow cells collected
by multiple aspirations from the iliac crests were diluted into
heparinized medium (Hanks’ balanced salt solution, GIBCO, Grand
Island, NY; heparin 1,000 U/100 mL bone marrow suspension) to
total 5 x 10⁸ nucleated cells per kilogram. The suspension was
passaged through steel mesh filters and pooled. Plasma and red cells
were depleted by either inverted spin¹⁵ or hydroxyethyl starch
sedimentation¹⁶ (6% Hespian, American Critical Care, McGaw
Park, Ill; 1:7 Hesperan:marrow). An aliquot of plasma was set aside
and the marrow concentrate was resuspended in medium. Marrow
samples were then layered over Ficoll-Hypaque solution (Isolymph,
Gallard-Sehlesingen, Carle Place, NY; SG 1.077) in 30-mL conical
centrifuge tubes (Falcon, Oxnard, Calif) and centrifuged (Sorvall
RC-3 centrifuge) at 400 g for 45 minutes at room temperature.
Light-density cells were collected from the interface, washed with
medium twice, and resuspended to the appropriate concentration for
antibody and complement treatment.

Following antibody and complement treatment, the cells were
washed twice in antibiotic-free medium and resuspended to achieve
a final concentration of 50 to 100 x 10⁶ nucleated cells per milliliter
at the time of cryopreservation. Autologous irradiated or microper-
filtered (Millex-Gs 0.22-μ filters, Millipore Corp, Bedford, MA)
plasma and cold dimethylsulfoxide (Cryoserv, Research Industries
Corp, Salt Lake City) were rapidly added to give final volume
proportions of 10% for each in blood freezing bags (Delmed, Canton,
Mass). Bags were quickly transferred to a controlled-rate freezer
(Cryo-Med, Mt Clemens, Mich), brought to 0°C and begun on
programmed rate freezing at -1°C/min through -60°C and then
-3°C/min to -100°C. Bags were then in the liquid phase of liquid
nitrogen where they were kept until the time of infusion.

For administration, the purified marrow concentrates were taken
to the ward still in liquid nitrogen. Immediately prior to infusion, the
bag was transferred to a 37°C sterile saline bath, rapidly thawed,
drawn into a syringe, and injected over two to five minutes through
a central venous catheter into the recipient.

Bone marrow treatment with BA-1, BA-2, BA-3, and comple-
ment. Monoclonal antibodies BA-1, BA-2, BA-3 were purified and
supplied by Dr Richard Bartholomew, Hybridesics, Inc, San Diego. The
antibodies were purified from ascites by sodium sulfate precipitation
and diethyaminoethanol (DEAE) anion exchange chromatography,
adjusted to 0.5 mg/mL, and stabilized with 2 mg/mL of human
serum albumin (HSA). The antibodies were stable at 4°C and had a
shelf life of four to eight months, depending on the antibody and the
lot. Baby rabbit complement (BRC) was obtained from Pel-Freez
Biologicals, Rogers, Ariz. Three separate lots (0223, 0614, and
0105) were used during the course of this study. Each lot was
prescreened for lytic efficacy with BA-1, BA-2, and BA-3 and for
absence of CFU-GM inhibition. Antibodies and BRC were sterile-
filtered and endotoxin-free.

Two different marrow treatments were used during the course of
this study. In both treatments, Ficoll-Hypaque–isolated bone mar-
row cells were adjusted to the appropriate concentration in minimum
essential medium (MEM)–5% HSA, and aliquoted into Falcon
50-mL polypropylene tubes (30 mL per tube). BA-1, BA-2, and
BA-3 were each added to a final concentration of 10 μg/mL. BRC
was used at a final dilution of 1:4, 1:5, or 1:6, depending upon the
lot. Both treatments were carried out by incubating the cell suspensions
in a 37°C water bath. Tubes were inverted every ten minutes to
insure adequate mixing of cells, antibody, and BRC. The first 13
bone marrow samples were treated for 70 minutes with BA-1, BA-2,
BA-3 plus BRC at a final cell concentration of 5 x 10⁶/mL. These
conditions were initially selected based on preliminary studies using a
¹³Cr-release assay.¹⁷ The more recent ten bone marrows were
treated twice, for 35 minutes each, at a final cell concentration of
1 x 10⁷/mL. The double treatment protocol was incorporated into
the clinical trial based on a reanalysis of the marrow treatment
variables using a sensitive leukemic cell colony assay.¹⁸ Specifically,
we could achieve approximately 1 log of additional leukemic cell
killing using two cycles of treatment. Inclusion of deoxyribonuclease
(DNase I from bovine pancreas, No. D-0751, Sigma Chemical Co.
St Louis) at 18 IU/mL was a critical adjunct for minimizing cell
clumping in the double treatment protocol. After the initial 35-
minute treatment, the cells were centrifuged at 350 g for ten minutes
and then resuspended in the same initial concentration of antibody,
BRC, and DNase. After completion of the marrow treatments, the
cells were washed and cryopreserved as described above.

RESULTS

Engraftment and complications. Patients received a
median cell dose at transplant of 0.60 x 10⁸ nucleated cells
per kilogram with a range from 0.39 to 1.12 x 10⁹/kg. All 23 patients engrafted as defined by recovery of the white blood counts to >1 x 10⁹/L for three consecutive days. This occurred between 14 and 43 days, with a median of 24 days. This compares to a median time to engraftment of 27 days for 39 patients who received allogeneic transplants for ALL using the same preparative regimen. The absolute neutrophil count was greater than 0.5 x 10⁹/L ranging from 12 to 64 days (median, 22 days).

Patients received their last red cell transfusion at a median of 32 days, ranging from zero in one patient to 86 days posttransplant. Patients received their last platelet transfusion at a median of 34 days posttransplant with a range from seven to 78 days. Two patients who relapsed while still transfusion-dependent were excluded from the analysis of red cell and platelet transfusions.

Although there was an attempt to administer methotrexate weekly during the first 100 days once engraftment occurred, this was not always possible. Nine patients received no methotrexate because of low counts or early relapse. The remaining 14 patients received a median of 57% of the total calculated dose.

Complications following BMT included sepsis in 13 patients. The majority of the bacteremias were due to Streptococcus viridans infections. Because nine of the initial ten patients developed severe S viridans infections within seven days of transplant, certain procedures were instituted. Penicillin and streptomycin were added to the marrow in vitro, and all patients received prophylactic vancomycin before marrow reinfusion. Only one marrow, when cultured, was positive for S viridans. Subsequently, four of the next 13 patients developed sepsis. Three patients had meningitis and five patients had pneumonitis. Other complications included two patients who had poorly functioning grafts; both of these patients relapsed.

Relapse rate and survival. Fifteen of the 23 patients relapsed from 1.4 to 7.4 months posttransplant with a median time to relapse of 4.3 months (Fig 1). Nine of these patients have subsequently died. One patient who developed S viridans sepsis and pneumonitis died of nonleukemic causes 21 days posttransplant. The proportion of patients relapse-free is 30% ± 20% (95% confidence limits) at one year. Four of the 13 patients whose marrow received one cycle of BA-1, BA-2, BA-3, plus BRC' remain relapse-free and three of ten patients who received two cycles of BA-1, BA-2, BA-3, plus BRC' marrow treatment remain relapse-free. The number of antibodies that bone marrow leukemic cells reacted with did not influence the relapse rate; however, only 15 of 23 marrows were tested with all three antibodies. Of nine patients whose leukemic cells reacted with only one antibody, two remain disease-free. Two of seven patients whose cells reacted with two antibodies remain disease-free, and three of seven patients whose cells reacted with all three antibodies remain disease-free. Repeat phenotyping of the leukemic cells at the time of relapse was performed in ten patients. In six patients, it was identical to that obtained at diagnosis and/or relapse and in four patients, the phenotype of the leukemic cells differed from the evaluation prior to transplant (Table 3).

The Kaplan-Meier cumulative disease-free survival is 29% ± 19% (95% confidence limits) at one year, with seven patients alive without leukemic relapse six to 32 months (median, 21 months) (Fig 2). The cumulative survival rate is 49% ± 22% at one year with 12 patients still living with six to 32 months of follow-up (median, 15.5 months). Table 1 compares patient characteristics of patients transplanted in second versus third or fourth remission. As expected, the major difference between the groups is that second remission patients were followed a shorter time from diagnosis than were third and fourth remission patients. There was no significant difference in time to relapse-free or disease-free survival for patients transplanted in second or greater remission; however, one of nine patients transplanted in second...
remission is relapse-free and six of 14 patients transplanted in third or greater remission are relapse-free.

**DISCUSSION**

Although chemotherapy currently offers the possibility of cure for >50% of pediatric patients with ALL, the long-term survival for patients experiencing a relapse on therapy is extremely poor when treated with a variety of chemotherapeutic approaches. For this reason, allogeneic BMT has been utilized for these patients over the past several years. For patients with relapsed ALL who are transplanted in second or third remission, the two-year disease-free survival rates vary from 33% to 38% in two series. In another series, the disease-free survival at two years was greater for patients transplanted in second remission (62.5%) than for patients transplanted in third or greater remission (26.7%). The reasons for failure include deaths from infections and graft versus host disease (GVHD). In addition, recurrent leukemia remains a major reason for failure following allogeneic BMT in ALL. In spite of these problems, allogeneic BMT results in superior survival when compared to survival of patients with ALL receiving chemotherapy following a relapse. Allogeneic BMT, however, has been available almost exclusively to patients who have a sibling matched at the major histocompatibility complex. Several approaches are currently being investigated so that the 60% of otherwise eligible patients who lack a matched sibling may undergo BMT. Certain patients are being transplanted using marrow from less than fully matched family donors or matched unrelated donors. In this situation, the risks of graft rejection or lack of engraftment as well as the risk of GVHD may well be increased. Autologous transplantation is also being investigated as a means of transplanting patients who lack a matched donor. Initial studies of autologous transplantation used untreated remission marrow to reconstitute adult patients and demonstrated poor results. Because leukemic cells almost certainly contaminate remission bone marrow, current studies are investigating the use of remission bone marrow that has been treated with drugs or monoclonal antibodies in an effort to eliminate residual leukemic cells.

Most autologous transplants for patients with ALL currently use monoclonal antibodies plus complement to purge leukemia cells. At the University of Minnesota, our approach to the ex vivo elimination of leukemic cells is based on our experience in producing monoclonal antibodies that recognize distinct cell surface molecules expressed on ALL cells. Monoclonal antibodies BA-1, BA-2 (anti-p24), and BA-3 (anti-gp100/CALLA) bind to most non-T ALL cells but do not react with hematopoietic stem cells. Based on these considerations, and the realization that the immunologic phenotype of the clonogenic cell in ALL is unknown, we chose the combination of three antibodies for marrow treatment. Investigators at the Dana-Farber Cancer Center have evaluated the use of a single antibody J5 (anti-CALLA) plus complement for elimination of leukemic cells prior to autologous BMT.

In the current study, 23 patients with recurrent ALL who were in second or greater remission received autologous transplants following cytoreduction. The relapse rate of these patients is high. There are a number of factors that may contribute to the high relapse rate. First, the relapse rate following allogeneic BMT for ALL remains relatively high at our institution using the same conditioning regimen for
allogeneic patients (M.N., D.W., unpublished observations, May 1985). Because the preparative regimen at our institution is similar for patients undergoing either autologous or allogeneic transplantation for ALL, one would not expect the relapse rate following autologous BMT to be any lower. Until preparative regimens used to condition patients with ALL are more successful in eliminating residual leukemia from the patient, relapse will continue to be a significant problem. Alternative conditioning regimens used prior to transplantation for ALL may offer promise in achieving this objective.24 The second factor that may contribute to the high relapse rate in our series is that leukemic cells may have been inadequately removed by the ex vivo treatment with the monoclonal antibodies and complement. In our study, a change in the ex vivo treatment was made after the first 13 patients because of a high relapse rate and because in vitro studies demonstrating better leukemic cell kill.18 The relapse rate was not reduced following the change in marrow treatment. The third factor that may contribute to the high relapse rate in the patients receiving autologous BMT is the lack of any putative graft v leukemia effect as described by Weiden and colleagues.25 In addition, patients who receive autologous marrow grafts often do not receive any posttransplant immunosuppression such as methotrexate, which may provide additional antileukemic effect. At present, it is impossible to determine whether one or more of these factors accounts for the high relapse rate. Autologous transplant does, however, carry less morbidity and mortality from nonleukemic causes than does allogeneic transplantation. In our series of 23 patients, only one patient died due to nonleukemic causes.

Autologous BMT using marrow treated in vitro with a combination of monoclonal antibodies BA-1, BA-2, and BA-3 plus complement resulted in engraftment in 23 patients with poor-risk ALL. Mortality due to nonleukemic causes was low (1/23), but the relapse rate was high. It appears, however, that certain patients may have prolonged survival following such therapy. Improved methods of purging leukemic cells from bone marrow must be pursued, as well as better conditioning regimens for patients undergoing transplantation for ALL. In order to determine the efficacy of marrow purging as well as possible graft v leukemia effects, a comparison of comparable patients undergoing allogeneic, syngeneic, and autologous BMT using the same conditioning regimens will be essential.

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NOTE ADDED IN PROOF

As of June 1985, six patients remain disease-free with follow-up from 16 to 38 months (median, 28 months). The seventh patient relapsed at nine months after transplant.

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