Autologous Bone Marrow Transplantation for Patients With Acute Lymphoblastic Leukemia in Second or Subsequent Remission: Results of Bone Marrow Treated With Monoclonal Antibodies BA-1, BA-2, and BA-3 Plus Complement

By Norma Ramsay, Tucker LeBien, Mark Nesbit, Philip McGlave, Dan Weisdorf, Peter Kenyon, David Hurd, Anne Goldman, Tae Kim, and John Kersey

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 chemoradiotherapy 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 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 remission was confirmed in all patients prior to bone marrow harvest; a remission bone marrow contained less than 5% blasts. Cellularity was determined in all patients prior to harvest. Marrow was harvested under general anesthesia in volumes to obtain 5 x 10^8 nucleated cells per kilogram of recipient weight. The marrow was processed and treated with monoclonal antibodies and complement as described in the subsequent section.

Following harvest, patients were admitted to single isolation rooms with high-energy particulate (HEPA) filters on the bone marrow transplant ward. 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.
 AUTOLOGOUS TRANSPLANT FOR LEUKEMIA

Table 1. Summary of Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Bone Marrow Remission</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Second</td>
</tr>
<tr>
<td>No. of patients</td>
<td>9</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>5.4</td>
</tr>
<tr>
<td>Age at diagnosis (yr)</td>
<td>6.2(2-17)</td>
</tr>
<tr>
<td>Age at diagnosis (yr)</td>
<td>7 (3-17)</td>
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<tr>
<td>Duration of first remission (mo)</td>
<td>8.7 (4.0-35.7)</td>
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<tr>
<td>White blood count x 10^9/L at diagnosis</td>
<td>26.5 (1.2-134.0)</td>
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<tr>
<td>Prior extramedullary disease</td>
<td>12.9 (7.6-47.1)</td>
</tr>
<tr>
<td>Testicular</td>
<td>0</td>
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<tr>
<td>CNS</td>
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Table 2. Surface Marker Characteristics

<table>
<thead>
<tr>
<th>Antibody</th>
<th>No. Tested</th>
<th>No. Positive*</th>
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<tbody>
<tr>
<td>BA-1</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>BA-2</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>BA-3</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td>BA-1 and BA-2 and BA-3†</td>
<td>15</td>
<td>7</td>
</tr>
</tbody>
</table>

*Marrow samples at diagnosis or relapse were considered positive for a given antibody if >20% of the leukemic cells bound the antibody from a bone marrow that contained >50% leukemic cells. Analysis of leukemic cells revealed that the median percentage of positivity of the most reactive antibody was 77%, with a range from 43% to 98%.
†Tested as individual antibodies.

results

Engraftment and complications. Patients received a median cell dose at transplant of 0.60 x 10^8 nucleated cells.
per kilogram with a range from 0.39 to 1.12 x 10^9/kg. All 23 patients engrafted as defined by recovery of the white blood counts to >1 x 10^9/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 10^9/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 question of the value of maintenance chemotherapy beginning at day 100 cannot be addressed in this study as the relapses were early as well as frequent. Of the seven patients who remain free of relapse, four received maintenance therapy, and three have not received maintenance therapy.

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 v 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
Table 3. Phenotypic Change of Leukemia Cells at Relapse Following Bone Marrow Transplant Compared With Pretransplant Phenotype

<table>
<thead>
<tr>
<th>UPN</th>
<th>Pre-BMT BA-i</th>
<th>BA-2</th>
<th>BA-3</th>
<th>Post-BMT BA-i</th>
<th>BA-2</th>
<th>BA-3</th>
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<tbody>
<tr>
<td>288</td>
<td>+</td>
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<tr>
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<td>-</td>
<td>+</td>
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<td>ND</td>
</tr>
<tr>
<td>384</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>+</td>
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</tr>
</tbody>
</table>

Marrow at diagnosis or relapse were considered positive for a given antibody if >20% of the leukemic cells bound the antibody from a bone marrow that contained >50% leukemic cells. ND, not done.

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 vs 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. In addition to these preliminary published studies, numerous studies have recently been initiated at centers around the world.

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 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.19 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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Autologous bone marrow transplantation for patients with acute lymphoblastic leukemia in second or subsequent remission: results of bone marrow treated with monoclonal antibodies BA-1, BA-2, and BA-3 plus complement

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