Bone Marrow Transplantation With Interleukin-2–Activated Bone Marrow Followed by Interleukin-2 Therapy for Acute Myeloid Leukemia in Mice

By Bishan S. Charak, Russell K. Brynes, Susan Groshen, Su-Chiu Chen, and Amitabha Mazumder

We have investigated approaches to induce graft-versus-leukemia (GVL) effect in allogeneic bone marrow transplantation is contributed by graft-versus-host disease (GVHD). The high relapse rate after autologous bone marrow transplantation (ABMT) has been related to the absence of GVHD and thus poorer GVL effect. Because ABMT is the only option for many patients with leukemia, new strategies to improve the GVL effect of ABMT need to be explored. Interleukin-2 (IL-2) has been used after ABMT in patients with acute myeloid leukemia and found to induce antileukemic activity in vitro. However, the effect of this therapy on remission status remains to be defined.

We have shown previously that IL-2–activated bone marrow (ABM) is superior to spleen lymphokine activated killer (LAK) cells in its cytotoxic activity both in vitro and in vivo against murine natural killer (NK)-resistant solid tumors. Our studies have also shown that bone marrow transplantation (BMT) with ABM followed by IL-2 therapy is superior to BMT with fresh syngeneic bone marrow (FBM) in controlling the dissemination of melanoma and sarcoma in mice. However, long-term survival and cure were not examined in those studies. The present investigation studies the role of BMT with ABM and IL-2 therapy as a means of biotherapy in a virulent leukemia model. Our results show that BMT with ABM followed by IL-2 therapy posttransplant was superior to BMT with FBM (with or without IL-2 therapy) in terms of long-term survival and cure of mice with acute myeloid leukemia. BMT with ABM and IL-2 therapy also resulted in normal reconstitution of the hematopoietic system.

MATERIALS AND METHODS

Leukemic cells. C1498 (murine acute myeloid leukemia cell line) cells were obtained from the American Type Culture Collection (Rockville, MD), propagated in complete medium (CM) consisting of RPMI 1640, 0.1 mmol/L nonessential amino acids, 1 mmol/L sodium pyruvate, and 0.03% L-glutamine (Irvine Scientific, Santa Ana, CA); 5 x 10^-7 mol/L 2-mercaptoethanol; penicillin (100 U/mL); streptomycin (100 μg/mL); and 10% heat-inactivated fetal calf serum (FCS) (JR Scientific, Woodland, CA); and cryopreserved. Before use, the cells were grown in culture for 24 hours, washed three times with RPMI, and suspended appropriately in RPMI to infuse 2 x 10^6 cells per mouse in a volume of 0.5 mL via the tail vein. (In our preliminary studies, this dose of leukemic cells was found lethal for 100% of the animals.) Morphologically these cells are myeloblasts (Wright-Giemsa stain) and are positive for chloroacetate esterase (CAE).

Animals. The animals used in this study were C57BL/6 female mice (each weighing approximately 20 g) between the ages of 8 and 12 weeks obtained from Charles River Laboratories (Wilmington, MA).

IL-2. Recombinant IL-2 was a generous gift of Cetus Corporation (Emeryville, CA). It had a specific activity of 3 x 10^8 U/mg (1.2 mg/vial), a purity of 98%, and an endotoxin level <0.01 ng/vial.

Bone marrow (BM): Collection and activation. BM collection and IL-2 activation were performed as described earlier. BM cells (1 x 10^6/mL) were incubated with IL-2 (1,000 U/mL) in CM for 24 hours at 37°C in a humid atmosphere containing 5% CO2. The ABM was harvested with dispo cell scrapers (American Scientific Product, McGaw Park, IL), washed three times with RPMI, and resuspended appropriately in RPMI. FBM was collected in RPMI immediately before transplantation.

Design of study. Ten experiments were performed, each with four to six treatment arms; each treatment arm was replicated 4 or 10 times (Table 1). There were always four mice under a treatment arm in each experiment. A control group that received no treatment was included in every experiment.

Treatment/BMT. Treatment comprising of IL-2 alone/ABM alone (5 x 10^6 cells per mouse)/a combination of IL-2 and ABM/BMT, with or without IL-2 therapy, was started 3 days after infusion of leukemic cells. Conditioning therapy for BMT consisted of cyclophosphamide (C) (Bristol-Myers, Evansville, IN) 1 mg/mouse intraperitoneally (ip) and 500 rads of total body irradiation given at a rate of 150 rads/min from a Gammacel Cesium irradiator (Atomic Energy of Canada, Ottawa, Ontario) 4 hours after C. BM (5 x 10^6 cells in a volume of 0.5 mL/mouse) was infused 8 to 10 hours after C. IL-2, 10 U ip, twice a day was started immediately after infusion of BM and was continued for 7 days.

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Table 1. Survival and Cure Rates With Different Treatment Schedules

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Median Survival (d)</th>
<th>Cure Rate (%) ± SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control†</td>
<td>22 (18-25)</td>
<td>0</td>
</tr>
<tr>
<td>2. IL-2†</td>
<td>23 (18-26)</td>
<td>0</td>
</tr>
<tr>
<td>3. ABM‡</td>
<td>22 (19-27)</td>
<td>0</td>
</tr>
<tr>
<td>4. ABM + IL-2‡</td>
<td>23 (19-25)</td>
<td>0</td>
</tr>
<tr>
<td>5. BMT with FBM†</td>
<td>30 (23-43)</td>
<td>16.7 ± 6.2</td>
</tr>
<tr>
<td>6. BMT with ABM‡</td>
<td>28 (24-36)</td>
<td>12.5 ± 8.3</td>
</tr>
<tr>
<td>7. BMT with FBM + IL-2†</td>
<td>29 (23-60)</td>
<td>12.5 ± 5.2</td>
</tr>
<tr>
<td>8. BMT with ABM + IL-2†</td>
<td>40 (26-45)</td>
<td>36.1 ± 8.0</td>
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</table>

*P values comparing treatment groups: 1 v 2 v 3 v 4, P = .41; 1 v 5, P < .001; 5 v 6 v 7, P = .72; 5 v 8, P < .001.
†Data pooled from 10 experiments (four mice each) for each group.
‡Data pooled for four experiments (four mice each) for each group.

Hematologic evaluation. Mice were killed by cervical dislocation and blood was obtained by cardiac puncture for analysis of hematologic parameters using an ELT-8 counter (Ortho Diagnostics Inc, Raritan, NJ). BM smears were made by flushing the femurs with FCS. The smears were stained with Wright-Geimsa and CAE stains and examined for hematopoietic process and leukemic infiltration.

Survival monitoring. The mice were observed daily until death or up to 100 days for their general appearance, signs of GVHD (erythroderma, alopecia), and survival. Autopsies were performed on all mice (dead or near death) under treatment groups 2, 3, 4, and 6, and on all mice in the first six experiments with treatment groups 1, 5, 7, and 8 of Table 1, to examine internal organs and to confirm the absence of leukemia in BM. Touch imprints were made from spleens and granulocytic sarcomas from autopsied mice and stained for cytologic confirmation of leukemia.

Statistical analysis. P values were calculated by two-sided log-rank test.7 Survival curves and median survival were computed by the method of Kaplan and Meier.8 Survival days were calculated using the day of infusion of leukemia as day 0. Mice living ≥100 days were considered as cured, because in preliminary experiments no deaths were seen after 60 days and autopsy examination on mice living ≥100 days did not show evidence of leukemia.

RESULTS

The data have been pooled from 10 experiments. Analysis of the results showed consistency from experiment to experiment for every treatment arm (P = .53) based on a stratified log-rank test to evaluate inter-experiment variability.

Disease course. Table 1 shows the outcome of various treatment groups. None of the mice from the control group survived; they became sick at the end of 3 weeks and died in the next 2 to 3 days.

All the dying mice (from various groups) that were autopsied showed splenomegaly (1.5 to 2 times the normal size). BM harvested from dying animals from all groups showed myeloblasts (52% to 75%), marked decrease in megakaryocytes, and dyserythropoiesis. Myeloblasts were also seen on touch imprints from the granulocytic sarcomas and spleens of these mice.

Nontransplant therapy. Treatment with IL-2 alone, ABM alone, or a combination of IL-2 and ABM did not influence the cure and the survival as compared with the control group (P = .41).

BMT with FBM, with and without IL-2 therapy posttransplant. Figure 1 shows the effect of different modifications of BMT on survival. BMT with FBM significantly improved the survival over the control group (P < .001) and achieved cure in 16.7% ± 6.2% mice. However, addition of IL-2 therapy posttransplant did not improve the survival or cure rate further (P = .98). Delaying the institution of IL-2 therapy 1 to 3 weeks posttransplant also did not influence the results (data not shown).

BMT with ABM, with and without IL-2 therapy posttransplant. BMT with ABM without IL-2 therapy improved the survival over the control group (P < .001); however, it was not different from BMT with FBM (P = .42). BMT with
ABM followed by IL-2 therapy resulted in a higher cure rate and improved survival as compared with BMT with FBM (P < .001). Similar results were seen when cryopreserved BM was thawed, activated with IL-2, and used for BMT, and was followed by IL-2 therapy (data not shown).

Hematologic analysis. Data in Table 2 show that untreated mice had evidence of frank leukemia and BM failure when they were terminally ill. A similar picture was seen in mice undergoing treatment with IL-2 alone, ABM alone, and a combination of ABM and IL-2.

A peripheral blood picture of mice undergoing BMT with various modifications was similar at the end of 3 weeks. BM from mice undergoing BMT with ABM and IL-2 therapy did not show myeloblasts; however, myeloblasts were seen in the BM harvested from mice in other groups of BMT. Hematologic parameters 45 days after BMT with FBM or with ABM followed by IL-2 therapy were comparable with those of normal mice (Table 3). Mice in both of these groups had normal-sized spleens and did not show myeloblasts either in the BM or on touch imprints of spleens.

Signs of GVHD were not seen in any mice, whether they were or were not treated with IL-2.

DISCUSSION

This is the first report of induction of GVL effect in ABMT by manipulation of the BM. It summarizes our observations on BMT with FBM or ABM with and without posttransplant IL-2 therapy, respectively, in mice with acute myeloid leukemia. Preliminary studies have been performed for optimization of time of starting treatment after infusion of leukemic cells and IL-2 administration schedule (once v twice a day; immediately, 1, 2, or 3 weeks posttransplant).

Deficient B- and T-cell functions and decreased IL-2 secretion account in part for deranged immune functions and possibly for early relapses in patients undergoing ABMT. IL-2 has been used after ABMT in patients with acute myeloid leukemia and has been found to induce cells with antileukemic activity in vitro; however, its long-term effect on the disease status is not known.14 The present study shows that IL-2 used after ABMT with FBM does not improve long-term survival or cure in mice with acute myeloid leukemia. Nevertheless, IL-2 therapy after BMT with ABM significantly improved the survival as compared to BMT with FBM. This suggests that higher levels of IL-2 than could be achieved by in vivo therapy might be necessary to generate killer cells with optimum antileukemic activity from the BM.

On the other hand, when ABM had been generated in vitro and used for BMT, IL-2 therapy maintained higher antileukemic activity than it did when FBM was used for BMT. Because BMT with ABM not followed by IL-2 therapy did not improve the survival as compared to BMT with FBM, IL-2 therapy in the posttransplant period seems crucial to maintain higher antileukemic activity. Absence of response to IL-2 alone, ABM alone, or a combination of ABM and IL-2 suggests that such therapy would not be effective against florid disease that has not been decreased by chemoradiotherapy. However, a combination of ABM and IL-2 therapy after conditioning therapy for BMT could possibly eradicate minimal residual disease.

Improved survival after BMT with ABM and IL-2 therapy could be attributed to better hematologic reconstitution reducing the mortality related to marrow aplasia (sepsis and hemorrhage) and/or higher antileukemic activity, as compared to BMT with FBM. Our data show that hematologic reconstitution in the two groups of BMT was similar. IL-2 therapy has been reported to reduce the septic death in mice,10 but IL-2 after BMT with FBM did not improve the survival in our study. In addition, all the dying mice that were autopsied showed evidence of leukemia irrespective of the treatment administered. These observations favor the interpretation that improved survival following BMT with ABM and IL-2 therapy was related to higher antileukemic effect rather than better hematologic reconstitution following this form of treatment.

It could be argued that we used a fixed dose of BM cells (approximately 2.5 × 10^8/kg of body weight) and that the GVL effect of BMT could possibly be enhanced by simply increasing the FBM cell dose supplemented with exogenous IL-2 therapy rather than using ABM. We have shown that fresh human BM cells do not have antileukemic activity in

<p>| Table 3. Hematologic Analysis of Mice With Acute Myeloid Leukemia 45 Days After BMT |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Hematologic Parameter</th>
<th>Normal Controls*</th>
<th>BMT With FBM</th>
<th>BMT With ABM + IL-2 Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>8.0 ± 0.7</td>
<td>8.6 ± 1.7</td>
<td>8.2 ± 1.3</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>32.6 ± 1.4</td>
<td>33.8 ± 2.1</td>
<td>33.2 ± 1.8</td>
</tr>
<tr>
<td>WBC count (× 10^3/μL)</td>
<td>17.4 ± 2.3</td>
<td>16.9 ± 1.5</td>
<td>18.3 ± 2.1</td>
</tr>
<tr>
<td>Platelet count (× 10^3/μL)</td>
<td>191.0 ± 16.0</td>
<td>186.0 ± 13.0</td>
<td>190.0 ± 21.0</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM of values obtained from four mice surviving from different experiments.

*Untreated mice, 21 days after induction of leukemia.
vitro, and that the antitumor effect of BMT with $2 \times 10^8$, $5 \times 10^8$, or $10^9$ FBM cells (supplemented with IL-2 therapy) in NK-resistant solid tumors in mice was not different (Charak BS, Mazumder A: unpublished data, July 1989). However, controlled studies are necessary to establish whether the antileukemic effect of BMT with ABM and IL-2 therapy is therapeutically superior to BMT with a larger dose of FBM cells combined with IL-2 therapy.

LAK cells have been proposed as adjuvant therapy with ABMT to clear the residual leukemic cells. However, delaying the LAK cell therapy until the patient recovers normal blood cell counts after ABMT might allow proliferation of residual disease. In addition, the dose of IL-2 to maintain optimum LAK cell activity in vivo is highly toxic. We have recently shown that the cytotoxic activity of ABM is superior to that of LAK cells against various human hematologic tumors in vitro. Our murine studies showed that the IL-2 requirement of ABM is 10-fold less than that of LAK cells to maintain optimum antitumor activity in vivo.

Lower doses of IL-2 soon after ABMT have been reported to result in acceptable clinical toxicity. Thus, ABMT with ABM followed by IL-2 therapy may abrogate the need for, and may be superior to, additional LAK cell therapy.

Initially, IL-2 therapy after BMT was seen with skepticism based on reports that it could compromise engraftment by stimulating LAK cells that were found to inhibit the BM progenitor cells in vitro. Subsequent studies showed that BM purged with LAK cells led to normal reconstitution of lethally irradiated rats. The present study shows that mice undergoing BMT with ABM followed by IL-2 therapy achieved normal hematopoiesis. We have made similar observations in solid tumor models. In a clinical trial, IL-2 used after ABMT did not impede the engraftment and actually led to an increase in the circulating neutrophils.

We have shown that human BM contaminated with leukemic cells can be activated with IL-2 to eradicate the leukemia without losing progenitor cell activity in vitro. IL-2 therapy has been reported to induce secretion of interferon-γ and tumor necrosis factor, both of which have antileukemic activity; both of these cytokines synergize with IL-2 in the generation of ABM. These observations and the results of the present study suggest that BMT with ABM followed by IL-2 therapy may be a novel approach to ABMT in the context of preventing leukemic relapse by inducing a potent GVL effect.

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REFERENCES

Bone marrow transplantation with interleukin-2-activated bone marrow followed by interleukin-2 therapy for acute myeloid leukemia in mice

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