Tumor Resistance Induced by Syngeneic Bone Marrow Transplantation and Enhanced by Interleukin 2: A Model for the Graft versus Leukemia Reaction

Larry W. Kwak, Michael J. Campbell, and Ronald Levy

Division of Oncology, Department of Medicine, Stanford University Medical School, Stanford, California 94305-5306

ABSTRACT

Lethally irradiated C3H/HeN mice reconstituted with normal syngeneic bone marrow survived significantly longer than unmanipulated control mice following challenge with a lethal dose of 38C13 lymphoma cells 2 to 3 weeks post-bone marrow transplantation (BMT). Although the magnitude of this effect was modest, it was highly reproducible. This resistance-producing effect of BMT could be enhanced by interleukin 2 administration and could be abrogated by anti-asialo-GM₁ antiserum treatment of recipients. These findings are consistent with the hypothesis that cells with a natural killer phenotype are activated by BMT and can mediate tumor resistance. These studies provide a model to explore the cellular basis, independent of donor alloreactivity, of the graft antitumor effect of BMT observed in humans.

INTRODUCTION

Bone marrow transplantation provides curative treatment for some patients with hematological malignancies. Undoubtedly, the high-dose radiation and chemotherapy used to prepare the host prior to the transplant are largely responsible for eradication of the patient’s tumor; nevertheless, immune effector mechanisms activated by the BMT may also contribute significantly to the antitumor effect. For example, GVL has been invoked as an explanation for the lower incidence of tumor recurrence observed in recipients of allogeneic marrow compared to recipients of syngeneic marrow grafts (1-3). However, the cellular basis of this clinical phenomenon is not well understood. The correlation of GVL with the severity of graft versus host disease in patients undergoing allogeneic BMT suggests that a mature alloreactive T-cell component may be involved, but recent laboratory studies indicate that nonspecific NK cells regenerating post-BMT can also have GVL activity (4-7). Indeed, graft versus host disease can be induced in animal models with syngeneic marrow and cyclosporine, a phenomenon that has recently been shown to be also associated with augmented NK cell activity (8, 9).

In the course of studies designed to induce specific antitumor immunity during syngeneic bone marrow transplantation (10), we observed that the control, nonimmunized, lethally irradiated mice reconstituted with syngeneic bone marrow survived significantly longer than normal mice after subsequent challenge with a lethal dose of a syngeneic lymphoma. This observation was reproducibly observed, and we have performed further studies suggesting that cells with NK phenotype activated post-BMT are also involved in this phenomenon of augmented host tumor resistance following BMT.

RESULTS AND DISCUSSION

Mice. Female C3H/HeN mice, 6 to 8 weeks old, were obtained from Simonsen Laboratories (Gilroy, CA).

Tumor. The carcinogen-induced 38C13 B-cell lymphoma has been previously described (11). A standard tumor transplantation protocol was developed that maximized the variability of tumor growth after transplantation into syngeneic mice. Tumor cells from a common frozen stock were passaged in vitro 3 to 4 days before use, and all injections for each experiment were made from the same suspension of tumor cells. Inoculation of 10⁶ 38C13 tumor cells i.p. into unmanipulated C3H/HeN mice resulted in progressive tumor growth and median survival times between 16 and 21 days. Nevertheless, it is important to note that differences in the absolute magnitudes of the median survival times between identical groups in separate experiments may reflect numerous variables and, as such, cannot be legitimately compared.

Syngeneic BMT. Donor female C3H/HeN mice, 8 to 10 weeks old, were euthanized, and both left and right femurs and tibiae were removed and cleaned of all soft tissue. The ends of each bone were removed and the bone marrow was collected in RPMI 1640 containing 1% penicillin/streptomycin using a 25-gauge needle and a 12-ml syringe. The bone marrow was filtered through a nylon mesh and washed three times before use. Viable cells were counted as determined by trypan blue exclusion.Recipient female C3H/HeN mice, 8 to 10 weeks old, were lethally irradiated with 950 R TBI in a Philips X-ray unit (Germany; 250 kV, 15 mA). Irradiated recipients were given i.v. injections of 15-20 x 10⁶ bone marrow cells in 0.5 ml RPMI plus penicillin/streptomycin in the lateral tail vein. We have established 950 R as a lethal dose of radiation for the C3H strain, which led to the death of all unprotected mice within 10 to 15 days.

Mice prepared in this manner were maintained in a sterile microisolator cage unit and received sterile food and water supplemented with tetracycline (400 mg/liter) for 2 weeks. Overall postoperative mortality was less than 5%, and all surviving mice were clinically healthy.

IL-2 Administration. IL-2 was a gift from Cetus Corporation (Emeryville, CA) and had a specific activity of 3 x 10⁶ Cetus units/mg. IL-2 treatment consisted of 50,000 units i.p. administered twice daily starting on the day of BMT for a total of four doses and a second course of 10,000 units i.p. daily for the 2 days prior to, but not including, the day of tumor challenge 2 to 3 weeks later. IL-2 excipient (provided by Cetus) was initially administered to control mice not treated with IL-2 according to the same schedule, but an equal volume of saline was subsequently substituted when experiments showed no difference in survival following tumor challenge between excipient- and saline-treated mice.

Anti-Asialo-GM₁ Treatment of BMT Recipients. Syngeneic BMT recipients were treated with either rabbit anti-asialo-GM₁ (WAKO Chemicals USA, Inc., Dallas, TX) or control normal rabbit immunoglobulin (Sigma Chemical Company, St. Louis, MO) at the time of tumor challenge 3 weeks post-BMT. Three serial doses of anti-asialo-GM₁ antiserum 0.1 ml, or an equal volume of normal rabbit immunoglobulin matched for protein concentration, were administered i.v. through the lateral tail vein at 4-day intervals starting 1 day prior to tumor challenge.

Analysis of Data. Mice were checked daily to determine the date of death. Statistical comparisons of survival were performed using the generalized Wilcoxon test of Gehan (12).

RESULTS AND DISCUSSION

The experiment shown in Fig. 1 is representative of five such experiments. Normal C3H/HeN mice treated with 950R TBI...
Fig. 1. Resistance-producing effect of syngeneic BMT against a syngeneic lymphoma. Lethally irradiated C3H/HeN mice reconstituted with normal syngeneic bone marrow survived significantly longer than nontransplanted littermate control mice following subsequent challenge (2 weeks post-BMT) with 1000 38C13 lymphoma cells from a single preparation of tumor. Survival is expressed as days following tumor challenge. This experiment is representative of 5 such experiments in which the logrank P was <0.05.

Guided by these results, we performed experiments designed to further elucidate the cellular mechanism underlying this phenomenon. In the experiment shown in Fig. 3A, lethally irradiated C3H/HeN mice reconstituted with normal syngeneic bone marrow and treated with IL-2 according to the schedule described above were randomly assigned to serial treatment with either anti-asialo-GM1 antiserum or control rabbit immunoglobulin at the time of tumor challenge 3 weeks post-BMT (0.1 ml antiserum administered i.v. every 4 days, starting on the day prior to tumor challenge, for a total of three doses). Nontransplanted littermate control mice treated with saline (instead of IL-2) were also randomly assigned to receive either anti-asialo-GM1 antiserum or control rabbit immunoglobulin according to the same schedule. Depletion of cells staining positively for anti-asialo-GM1 in the peripheral blood of mice treated with this antiserum was demonstrated by flow cytometry (data not shown). Anti-asialo-GM1 antiserum treatment of transplant-IL-2-treated mice was associated with a significant reduction in survival (median survival, 15 days) compared with transplant-IL-2-treated mice given normal immunoglobulin (median survival, 22 days; P = 0.016). Furthermore, the survival of only the latter group was significantly prolonged compared with that of the nontransplant-treated controls given normal rabbit immunoglobulin (median survival, 19 days; P = 0.046). Moreover, no effect of anti-asialo-GM1 antiserum treatment was observed on the survival of nontransplant-treated mice (median survival, 18 days; P = 0.703, compared with nontransplant-treated mice treated with normal immunoglobulin). Fig. 3B shows a second independent experiment using three of the four same groups of mice and reconstituted with 15 × 10^6 unfractionated bone marrow cells from normal syngeneic donors demonstrated significantly prolonged survival after i.p. challenge with 1000 38C13 lymphoma cells 2 weeks posttransplantation (median survival, 21 days) compared with nonirradiated, non-marrow-reconstituted littermates challenged with the same preparation of tumor (median survival, 17 days; P = 0.014). This enhanced resistance to tumor challenge was reproducibly observed over the course of multiple experiments, and although the magnitude of this phenomenon was modest, it should be noted that this is a virulent, widely metastatic tumor. The survival advantage conferred by syngeneic BMT against subsequent tumor challenge was especially surprising, because TBI is generally immunosuppressive, producing persistent deficits in host humoral and cellular immunity, despite gradual reconstitution of the immune system by transplanted marrow. However, this finding was consistent with a prior observation that lethal irradiation and syngeneic bone marrow reconstitution rendered mice more resistant to challenge with the P815 mastocytoma (13).

One explanation for this phenomenon is that BMT nonspecifically enhances a naturally occurring antitumor effector mechanism. The recovery of lymphoid cell subpopulations following BMT is asynchronous, favoring the early recovery of NK cells, in both experimental animals and humans (14–19). Thus, NK cells can represent the majority of peripheral blood lymphocytes during the first few weeks posttransplantation, and such cells appear to be more active in cytolysis, demonstrating the ability to lyse both NK-sensitive and -insensitive tumor targets in vitro (7). Such NK cells can be activated by IL-2 (5, 6, 20). We found that the administration of IL-2 to syngeneic BMT recipients further increased their resistance to challenge with tumor. In the experiment shown in Fig. 2, lethally irradiated C3H/HeN mice were reconstituted with normal syngeneic bone marrow and randomly assigned to receive either high-dose IL-2 (50,000 Cetus units twice daily for a total of 4 doses) or saline, starting on the day of BMT. Nontransplanted littermate control mice (“Normal”) were also randomly assigned to receive either IL-2 or saline according to the same schedule. All four groups of mice subsequently received a second course of either IL-2 (10,000 Cetus units daily for 2 days) or saline 2 weeks later prior to challenge with 1000 38C13 tumor cells. The survival of transplant-treated IL-2-treated mice was significantly prolonged (median survival, 22 days) compared with that of transplanted saline-treated mice (median survival, 18 days; P = 0.014). No such effect on survival was conferred by IL-2 treatment upon non-transplant-treated mice (median survival, 16 days, compared with 14.5 days in saline-treated mice; P = 0.304). Furthermore, both groups of transplant-treated mice demonstrated prolonged survival compared with their respectively treated non-transplant-treated controls (groups 1 versus 3, and 2 versus 4), confirming the protective effect of syngeneic BMT against the tumor. It should be noted that differences in the absolute magnitudes of the median survival times of identical groups in separate experiments may reflect numerous variables and, as such, cannot be legitimately compared. We conclude that the administration of IL-2 was synergistic with BMT in augmenting host tumor resistance.

Fig. 2. Resistance-producing effect of syngeneic BMT against the lymphoma is enhanced by IL-2 administration. Female C3H/HeN mice were randomly assigned to receive syngeneic BMT, with or without IL-2, or no BMT (“Normal”) with or without IL-2. IL-2 was administered as described in “Materials and Methods.” All four groups of mice were challenged with 1000 38C13 lymphoma cells i.p. 2 weeks post-BMT and followed for survival. These data are representative of two similar experiments.
mice (group 4 omitted). Again, prolonged survival of transplanted, IL-2-treated mice (given normal rabbit immunoglobulin, median survival was 24 days) compared with non-transplant-treated controls (given normal rabbit immunoglobulin, median survival was 20 days; $P = 0.008$) was reversed by anti-asialo-GM$_1$ antiseraum (median survival, 21 days; $P = 0.172$, compared with non-transplant-treated controls). Thus, in both experiments the treatment of recipient mice with anti-asialo-GM$_1$ antiseraum at the time of tumor challenge was associated with significant abrogation of the described phenomenon.

Taken together, these data demonstrate a reproducible effect of nonspecifically induced host resistance to a lymphoma resulting from lethal irradiation and syngeneic bone marrow reconstruction. The enhancement of this effect by IL-2 and the abrogation of it by anti-asialo-GM$_1$ antiseraum are consistent with the hypothesis that cells with the NK phenotype recovering post-BMT are involved. These latter observations also rule out the possibility that this tumor resistance by the host is explained by impaired establishment of implanted tumor in irradiated host tissue. Also, although not directly tested, the depletion of a radiosensitive precursor cell that functions normally to down-regulate antitumor responses also seems less likely as an explanation for the phenomenon in light of these findings. The lack of effect of IL-2 or anti-asialo-GM$_1$ antiseraum on tumor resistance in nontransplanted control mice argues that the activity of these agents was confined to a population of cells that had been uniquely activated by TBI and syngeneic marrow reconstitution. The lack of effect of these agents on the survival of nontransplanted controls challenged with tumor would be consistent with the low numbers of NK cells in normal mice; alternatively, this tumor may not be susceptible to normal effector cells not otherwise activated by BMT. As the spectrum of reactivity of anti-asialo-GM$_1$ is somewhat broad, the possibility remains that other cells, including activated macrophages, may also be involved in this phenomenon.

The therapeutic effect of syngeneic and autologous BMT in human clinical trials may be explained by this phenomenon. In addition, there is now considerable experimental data suggesting the participation of nonspecific effector systems such as NK cells in the GVL phenomenon following allogeneic BMT (5, 21). We propose that this novel tumor resistance-producing effect on the host may serve as a useful model to study the graft antitumor effect of BMT. In particular, by delaying tumor challenge until after BMT, when it then serves as an in vivo assay for an antitumor effector mechanism endogenously activated by BMT, one can dissociate this antitumor effect from any direct cytotoxic effect of BMT on the tumor. Furthermore, the use of a syngeneic transplant model would allow one to dissociate the cellular mechanism of the GVL reaction independent of any allogeneic T-cell component. Further studies are needed to explore the correlation of augmented tumor resistance post-BMT with cell-mediated lysis of a panel of tumor cell targets in vitro and then to characterize more precisely the phenotype of the effector cells involved (e.g., CD3 positive or negative). Also, modification of tumor cell dosage may serve to increase the magnitude of this phenomenon.

Finally, because IL-2 may serve to expand these effector cells activated post-BMT, this may independently provide a useful model to guide human studies already in progress exploring IL-2 therapy as a form of post-BMT immunomodulation (22–25). In particular, studies exploring different doses and schedules of IL-2 administration may have predictive value for clinical trials. In addition, other cytokines, either alone or in combination with IL-2, could be tested for their ability to further enhance tumor resistance. Models have been described previously for melanoma (26) and for other experimental tumors which require concurrent treatment with IL-2-activated marrow (27, 28); however, models of IL-2 administration alone post-BMT demonstrating antitumor efficacy against hematopoietic tumors are needed.

ACKNOWLEDGMENTS

We would like to thank Nancy G. Edwards for skillful preparation of the manuscript.

REFERENCES

4. Herend, T., Taktivian, T., Nowill, A., Tantravahi, R., Moingeon, P.,
AUGMENTED HOST TUMOR RESISTANCE FOLLOWING SYNGENEIC BMT


Tumor Resistance Induced by Syngeneic Bone Marrow Transplantation and Enhanced by Interleukin 2: A Model for the Graft versus Leukemia Reaction

Larry W. Kwak, Michael J. Campbell and Ronald Levy


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/52/15/4117

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.