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 syngeneic 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 been 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.

MATERIALS AND METHODS

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 minimized 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 1000 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 with 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. Viability 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 × 10^6 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 × 10^6 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-GMI Treatment of BMT Recipients. Syngeneic BMT recipients were treated with either rabbit anti-asialo-GMI (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-GMI 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...
cells from normal syngeneic donors demonstrated significantly bone marrow survived significantly longer than nontransplanted littermate con-

Thus, NK cells can represent the majority of peripheral blood specifically enhances a naturally occurring antitumor effector challenge with the P815 mastocytoma (13).

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

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 in 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.

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 were challenged with tumor. In the experiment shown in Fig. 3A, lethally irradiated C3H/HeN mice reconstituted with normal syngeneic bone marrow and treated with IL-2 were challenged with tumor. In the experiment shown in Fig. 3A, lethally irradiated C3H/HeN mice reconstituted with normal syngeneic bone marrow and treated with IL-2 were challenged with tumor. In the experiment shown in Fig. 3A, lethally irradiated C3H/HeN mice reconstituted with normal syngeneic bone marrow and treated with IL-2 were challenged with tumor. In the experiment shown in Fig. 3A, lethally irradiated C3H/HeN mice reconstituted with normal syngeneic bone marrow and treated with IL-2 were challenged with tumor.
AUGMENTED HOST TUMOR RESISTANCE FOLLOWING SYNGENEIC BMT

Fig. 3. Two experiments demonstrating that the resistance-producing effect of syngeneic BMT plus IL-2 against the lymphoma is significantly abrogated by anti-asialo-GM1 antiserum treatment of the recipients. In A, female C3H/HeN mice receiving syngeneic BMT combined with IL-2 treatment as described in Fig. 2 were randomly assigned to receive treatment with either rabbit anti-asialo-GM1 or control normal rabbit immunoglobulin, as described in “Materials and Methods,” at the time of tumor challenge with 1000 38C13 lymphoma cells 3 weeks post-BMT. Nontransplant-treated littermate control mice (Normal) treated with saline (to control for IL-2 injections) were also randomized to receive anti-asialo-GM1 antiserum or normal rabbit immunoglobulin according to the same schedule and were challenged with the same preparation of tumor as transplant-IL-2-treated mice. B, same as in A, including groups 1–3 only.

ACKNOWLEDGMENTS

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

REFERENCES

4. Herend, T., Takvorian, 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.