Enhancement of Graft-Versus-Tumor Activity and Graft-Versus-Host Disease by Pretransplant Immunization of Allogeneic Bone Marrow Donors with a Recipient-derived Tumor Cell Vaccine

Larry D. Anderson, Jr., Demetrios Petropoulos, Linda A. Everse, and Craig A. Mullen

Departments of Experimental Pediatrics [L. D. A., D. P., L. A. E., C. A. M.] and Immunology [C. A. M.], The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

ABSTRACT

Allogeneic bone marrow transplantation (BMT) can be accompanied by a beneficial T cell-mediated antitumor immune response known as graft-versus-tumor (GVT) activity. However, BMT donor T cells are not exposed to target antigens of GVT activity until transfer to the host, where tumor antigen presentation may be suboptimal. This study tested in a murine model the hypothesis that immunization of MHC-matched allogeneic donors with a recipient-derived tumor cell vaccine would substantially increase GVT activity and extend survival of BMT recipients with preexisting micrometastatic tumor.

C3H/SLW and C57BL/10 mice were immunized against a C57BL/6-derived fibrosarcoma or leukemia, and they were used as BMT donors. Recipients were H-2-matched, minor histocompatibility antigen-mismatched C57BL/6 mice with previously established micrometastatic tumors. Donor immunization led to a significant increase in GVT activity that was T cell dependent and cell dose dependent. In some settings, donor immunization also prolonged survival of recipients with preexisting micrometastatic tumors. However, donor immunization significantly increased the incidence of fatal graft-versus-host disease such that long-term survival was uncommon. In vitro cytotoxicity assays indicated that donor immunization induced both tumor-selective and alloreactive cytolytic T-cell populations. In vivo cross-protection assays showed that a substantial portion of the GVT effect was mediated by alloreactive cells not specific for the immunizing tumor. In conclusion, immunization of allogeneic BMT donors with a recipient-derived whole tumor cell vaccine substantially increases GVT activity but also exacerbates graft-versus-host disease.

INTRODUCTION

Autologous tumor vaccines have been shown to induce antitumor immune responses in a number of preclinical models, but they often fail to produce significant responses in clinical trials. Therapeutic tumor vaccines require a substantial degree of host immunocompetence. However, patients with cancer often have significant impairment of immune competence induced by long-term exposure to tumor and/or high dose multitarget chemotherapy (1–5). In such settings, vaccine-induced antitumor activity may be very difficult to produce (6).

Allogeneic BMT is commonly used as a treatment for hematological malignancies and is under investigation as a treatment for several nonhematological tumors as well. In allogeneic BMT, donor lymphocytes that have not been tolerized by tumor and that have not been damaged by protracted chemotherapy are transferred to patients with minimal residual microscopic tumor burden. Such a setting may be conducive to successful cancer immunotherapy. Indeed, allogeneic BMT is associated with an unequivocal GVT effect, but the benefit of GVT activity is often offset by GVHD (7, 8).

It would be desirable to improve the therapeutic index of the immunological component of allogeneic BMT by causing the beneficial aspects of GVT activity to outweigh the toxicity of GVHD. In MHC-matched BMT, both tumor antigens and mHAg remain largely undefined, and thus it is not clear to what extent the donor T-cell populations mediating GVT activity and GVHD are overlapping or identical (9, 10). Although both recipient tumor and GVHD target organs are “recipient self,” tumors may have a different MHC/peptide epitope profile and thus may be capable of inducing different T-cell populations. Both clinical and experimental evidence suggests that some of the effector cells mediating GVT activity are distinct from those mediating GVHD (11–15). For instance, even allogeneic BMT recipients that do not develop GVHD have a lower incidence of leukemia relapse compared with syngeneic BMT recipients or to recipients of T cell-depleted allogeneic BMT (11). Furthermore, in vitro T-cell cloning and specificity studies have shown that some donor T cells that do not lyse normal recipient cells can lyse tumor targets (13–15). GVT activity has also been demonstrated in F1, into parent murine BMT models that do not evoke GVHD, proving that the two responses can occur independently (12).

In the course of a normal BMT, antigen-naive donor T cells are transferred to the tumor-bearing recipient, and development of GVHD represents a primary immune response to mHAg. Target organs of GVHD all contain substantial numbers of professional antigen-presenting cells, which may efficiently stimulate primary immune responses to tissue-specific antigens (16, 17). However, primary immune responses to antigens on tumor cells in the recipient may not be induced efficiently, because tumor cells usually lack the necessary costimulatory molecules for efficient antigen presentation and generally do not have a large and organized population of antigen-presenting cells (18, 19). Therefore, conditions after transplant may favor development of a primary GVHD response that is greater than the primary GVT response.

For the reasons noted, it is conceivable that the relative potency of GVT activity could be increased by activating and expanding donor T-cell populations capable of recognizing tumor cells. One approach to this is immunization of donors with a recipient-derived tumor cell vaccine before donor cell harvest and transplantation. The experiments described in this study tested the hypothesis that immunization of normal immunocompetent MHC-matched donors with a recipient-derived tumor cell vaccine would substantially increase GVT activity and extend survival of BMT recipients with preexisting micrometastatic tumor. Antitumor activity and GVHD were assessed both in vivo and in vitro.
MATERIALS AND METHODS

Animals. Female C57BL/6 and C57BL/10 mice were purchased from The National Cancer Institute (Frederick, MD). Female C3H.SW-H2bSnJ (C3H.SW) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). They were used for experiments at 6–12 weeks of age. Mice were housed in conventional rooms with food and water ad lib. From 2 or 3 days before BMT until day 14, water was acidified (pH 2.5) and supplemented with 2 g/l neomycin sulfate (Sigma Chemical Co., St. Louis, MO).

Cell Lines. 205 is a weakly immunogenic, methylcholanthrene-induced C57BL/6 fibrosarcoma cell line (18). This tumor is not spontaneously metastatic but reproductively forms multiple lung nodules when at least 1 × 10<sup>6</sup> cells are injected i.v. into C57BL/6 mice. 205 IL-2/TK is a 205 cell line modified to express both the IL-2 and the herpes simplex virus TK suicide gene using the LXSN and pBabePuro retroviral vectors, respectively. The pBabePuro vector has a puromycin resistance gene (6), and the LXSN vector contains a neomycin resistance gene (20). Transduced cells were selected in 2.5 μg/ml puromycin and/or 1 mg/ml G418. B16F10 is a spontaneously, weakly immunogenic C57BL/6 melanoma cell line (a gift from Dr. I. J. Fidler, M. D. Anderson Cancer Center). When 10<sup>5</sup> cells are injected i.v. into C57BL/6 mice, multiple lung metastases are formed. C1498 is a C57BL/6 myelomonocytic leukemia cell line of spontaneous origin (American Type Culture Collection, Rockville, MD). 10<sup>6</sup> cells is a uniformly lethal dose of cells when injected i.v.; animals die with gross evidence of leukemia, including hepatomegaly and splenomegaly. Cells were grown in tissue culture using DMEM or RPMI 1640 supplemented with 5% heat-inactivated fetal bovine serum (Biowhittaker, Walkersville, MD), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mm L-glutamine.

BMT Donor Immunization. Donors were immunized twice at 2-week intervals. Two to 3 weeks after the second immunization, mice were sacrificed for use as donors. For 205 tumor immunization, C3H.SW, C57BL/10, and C57BL/6 mice were injected s.c. in the flank with 3–5 × 10<sup>5</sup> 205 IL-2/TK cells in 0.2 ml of HBSS on day 0, and they received 1 mg of ganciclovir i.p. in 0.2 ml PBS on days 3–9. Ganciclovir-mediated ablation of 205 IL-2/TK cells has been shown to induce systemic immunity to unmodified 205 tumor but is not cross-protective against B16F10 in C57BL/6 mice (21). Live 205 cells do not form tumors in C3H.SW mice. For alloimmunization, C3H/SW mice were injected s.c. in the flank with 20 × 10<sup>5</sup> C57BL/6 spleen cells in 0.2 ml HBSS. For C1498 tumor immunization, C3H.SW mice were injected s.c. in the flank with 3–7 × 10<sup>5</sup> 30 Gy-irradiated C1498 leukemia cells in 0.2 ml of HBSS. Live C1498 cells do not grow progressively in C3H.SW mice.

In Vivo Tumor Inoculation. Micrometastatic lung tumors were established by injecting C57BL/6 mice with 0.5–2 × 10<sup>5</sup> 205 IL-2/TK cells or 10<sup>5</sup> B16F10 cells i.v. in 0.2 ml of HBSS 6 days before BMT (doses specified in text and legends). C1498 leukemia was established by injection of 1 × 10<sup>6</sup> i.v. cells i.d. 1 day before BMT (after TBI).

Bone Marrow Transplantation. BMT recipients received 850 cGy TBI using 46Co source 1 day before BMT. On the day of BMT, 2–4 × 10<sup>6</sup> bone marrow cells and 0–40 × 10<sup>6</sup> spleen cells were injected i.v. together in a total volume of 0.2 ml of HBSS. Bone marrow was isolated from donors by flushing each femur and tibia with RPMI 1640. Spleen cells were isolated by macerating the spleens between two frosted glass slides, followed by lysis of erythrocytes. Cell doses are specified in the text and legends. Early death from sepsis (in the first 2 weeks after BMT) was seen in some experiments in about 10% of recipients and was unrelated to treatment other than TBI. Such mice were excluded from analysis.

Enumeration of Pulmonary Tumor Nodules. At the time of death or sacrifice, lungs from mice injected with 205 tumor were stained black by sulfusion with India ink instilled through the trachea. Lungs were fixed, and in some experiments histological evidence corroborating the diagnosis of GVHD was obtained. Liver sections stained with H&E were examined for characteristic monoclonal infiltrates in portal triads.

Cytotoxicity Assays. For use as effector cells, spleen cells were cultured in 6-well plates at 1 × 10<sup>6</sup> cells/ml and 10 ml well in RPMI supplemented with 10% FBS (Summit Biotech, Ft. Collins, CO), 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mm l-glutamine, 100 μM sodium pyruvate, 0.1 mm nonessential amino acids, and 50 μM 2-mercaptoethanol (complete medium). In assays for allograft reactivity, 30 Gy-irradiated C57BL/6 spleen cells were used as stimulators at a concentration of 1 × 10<sup>6</sup> cells/ml. In assays for combined tumor reactivity and allograft reactivity, 250 Gy-irradiated 205 tumor cells were used as stimulators at a concentration of 5 × 10<sup>5</sup> cells/ml. After 4–5 days in culture with the appropriate stimulator cells, effector cells were harvested and plated in triplicate with 5 × 10<sup>3</sup> [35Cr]-labeled target cells per well at E:T ratios ranging from 300:1 to 19:1. Target cells were labeled by combining 5 × 10<sup>3</sup> cells in 0.1 ml of complete medium with 20 μl FBS (Summit) and 0.1 ml (100 μCi) sterile isotonic Na<sub>2</sub>CrO<sub>4</sub> (Amersham, Arlington Heights, IL) for 60 min at 37°C. Con A lymphoblast targets were generated by stimulating C57BL/6 spleen cells for 2 days with 2 μg/ml Con A at 2 × 10<sup>6</sup> cells/ml in complete medium. They were labeled with 35Cr as above for 45 min. Labeled targets were washed three times before plating with effectors in a total volume of 0.2 ml/well in 96-well, round-bottomed plates. Plated cells were incubated for 4 h at 37°C, after which 0.1 ml of supernatant was counted in a gamma counter (Wallac, San Francisco, CA). The percentage of lysis was calculated as: 100 × [([experimental cpm−spontaneous cpm])/maximum cpm−spontaneous cpm]]. Spontaneous release was usually <20% and always <30% of the maximum release.

Limiting Dilution Analysis. To determine the precursor frequency of allograft and tumor-reactive cytolytic lymphocytes, graded numbers of spleen cells were cultured in triplicates of 24–40 in 96-well, round-bottomed plates along with 250 Gy-irradiated 205 stimulator cells (500/well) or 30 Gy-irradiated C57BL/6 spleen cell stimulators (5 × 10<sup>5</sup>/well) in 0.2 ml. Cultures were fed on days 4 and 7 by replacing 0.1 ml of supernatant with fresh complete medium containing IL-2 (final concentration, 5 IU/ml; Chiron, Emnorryville, CA). On day 11, 5 × 10<sup>3</sup> target cells (35Cr-labeled C57BL/6 Con A lymphoblasts or 205 tumor cells) were added to each well, and cytotoxicity was measured as above. CTL precursor frequency was estimated based on the Poisson distribution and probability theory, which predicts that an average of one CTL precursor per well will result in the absence of lysis in 37% of the wells, as described (22, 23). Lysis was defined as 3 SD above the mean spontaneous 35Cr release.

Split-Well Specificity Assay. To determine the target specificity of cytolytic T cells, donor spleen cells were cultured using limiting dilution conditions as above. On day 11, the cells from each well were transferred in equal portions to two new wells and tested in parallel against two different 35Cr-labeled targets. Cytotoxicity was measured as above, and data were analyzed to determine whether particular clonal populations lysed both targets or only one of the targets.

T-Cell Subset Depletion. Prior to BMT, immune donor spleen cells were incubated on ice for 30 min with mAb to either CD4 (clone GK1.5; Pharmingen, San Diego, CA) or CD8 (clone 53-6.72; PharMingen) using a 1:100 dilution of antibody and a cell concentration of 60 × 10<sup>6</sup> cells/ml. They were then washed twice and incubated at 4°C for 15 min with a polyclonal F(ab')<sub>2</sub> secondary goat anti-rat IgG antibody coupled to a paramagnetic microbead (Miltenyi Biotec, Auburn, CA). The cells were next passed twice through a negative selection column using a SuperMACS separator (Miltenyi Biotec., Auburn, CA). The cell population that did not bind to the column contained essentially either no CD4<sup>+</sup> cells or no CD8<sup>+</sup> cells, as measured by flow cytometry (data not shown). As a control for BMT, some immune donor spleen cells were treated with labeled secondary antibody only and passed through a negative selection column in the same manner as the depleted cells ( sham-depletion). After T-cell subset depletion, 4 × 10<sup>6</sup> processed spleen cells were injected into BMT recipients along with 4 × 10<sup>6</sup> bone marrow cells. In other experiments, antibodies and complement were used to deplete cells. Immune donor spleen cells were incubated with monoclonal antibody to either CD4 (clone GK1.5) or CD8 (clone 116-13.1, a gift from Dr. M. Kripke, M. D. Anderson Cancer Center) as above. They were then washed twice and incubated at 37°C for 45 min with a 1:16 dilution of low-toxicity rabbit anti-mouse Ig. The cells were then washed twice, and the entire procedure was repeated. CD4<sup>+</sup> and CD8<sup>+</sup> cells were depleted to background levels as measured by flow cytometry (data not shown). Processed cells (10 × 10<sup>6</sup>) were injected into BMT recipients along with 4 × 10<sup>6</sup> bone marrow cells.
**RESULTS**

Vaccine-induced Tumor Immunity Can Be Effectively Transferred from Allogeneic BMT Donors to Cure Preexisting Micrometastatic Cancer in Recipients. Earlier work in our laboratory has shown that cellular tumor vaccines can protect syngeneic C57BL/6 mice against later challenge with 205 tumor (21, 24, 25). Experiments were conducted to test the hypothesis that progression of preexisting micrometastatic tumor in BMT recipients can be retarded by tumor immunization of normal, MHC-matched allogeneic BMT donors before BMT. Pulmonary metastases of the 205 tumor were established by tail vein infusion of tumor cells in syngeneic C57BL/6 mice. Six days later, these tumor-bearing animals underwent BMT using either 205-immune or naive (nonimmune) C3H.SW, C57BL/10, and C57BL/6 donors. Recipients of BMT from each of the three strains of 205-immune donors had significantly fewer lung nodules than recipients of BMT from naive donors of the same strain 1 month after BMT ($P < 0.001$; Table 1). Furthermore, 8 of 10 recipients of immune allogeneic donor cells and 5 of 5 recipients of immune syngeneic donor cells were tumor free ($P < 0.05$ for each strain). This GVT activity from 205-immune C3H.SW spleen cells was $T$ cell mediated; it was abolished by *in vitro* depletion of CD8$^+$ cells and reduced by depletion of CD4$^+$ cells in the graft before BMT (data not shown).

The GVT activity was cell dose dependent. C57BL/6 recipients with preexisting micrometastatic 205 were transplanted with a range of doses of 205-immune C3H.SW spleen cells ($0–10 \times 10^6$) in addition to bone marrow. Pulmonary surface tumor nodules were counted at the time of death. BMT recipients of $0.5 \times 10^6$ or more spleen cells exhibited a significant reduction in pulmonary tumor nodules compared with recipients of $T$-cell poor, marrow-only grafts ($P < 0.01$; Fig. 1A), and doses of $2, 5, \text{and} 10 \times 10^6$ spleen cells each afforded complete protection against growth of metastatic 205 pulmonary nodules.

The target specificity of 205-immune donor C3H.SW cytolytic T cells was assessed using a split-well microculture assay under limiting dilution conditions. Sixty-nine% (35 of 51) of the wells with lytic activity lysed 205 tumor cells but not nonmalignant C57BL/6 Con A lymphoblasts, whereas 31% (16 of 51) lysed both targets. No wells generated lysis of Con A lymphoblasts without lysis of 205 tumor cells. In an independent experiment, 73% (11 of 15) of wells with lytic activity lysed 205 tumor cells but not B16 tumor cells. Twenty-seven% (4 of 15) lysed both 205 and B16. No wells generated lysis of only B16 without lysis of 205.

Tumor-bearing BMT Recipients Exhibit Prolonged Survival after BMT Using Tumor-immune Allogeneic Donors. Because tumor-immune donors have both alloreactive and tumor-reactive $T$ cells in their repertoire, experiments were conducted *in vivo* to compare the relative magnitude of these activities. In the dose-response experiment described above, survival was slightly increased at all doses of 205-immune donor spleen cells compared with the group that received only bone marrow, and the increased was statistically significant at the dose of $1 \times 10^6$ spleen cells (mean, $39 \pm 2$ days *versus* $23 \pm 2$ days for the control group, $P < 0.01$; Fig. 1B).

This observation was extended in an experiment in which BMT recipients received a minimum tumor-curative dose of immune C3H.SW donor spleen cells ($2 \times 10^6$) or an equal dose of naive C3H.SW donor cells. In the group receiving immune donor cells, survival was significantly increased ($P < 0.05$; Fig. 2A), and the number of lung tumor nodules was significantly reduced (mean, $0.05 \pm 0.01$ for each group).

<table>
<thead>
<tr>
<th>Donors</th>
<th>Lung nodules (total per mouse)</th>
<th>Lung nodules (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No TBI/No BMT</td>
<td>$&gt;250, &gt;250, &gt;250, &gt;250, &gt;250$</td>
<td>$250 ± 0$</td>
</tr>
<tr>
<td>Naive C3H.SW</td>
<td>$25, &gt;250, &gt;250, &gt;250$</td>
<td>$194 ± 56$</td>
</tr>
<tr>
<td>Immune C3H.SW</td>
<td>0, 0, 0, 0, 1</td>
<td>$0.2 ± 0.2^a$</td>
</tr>
<tr>
<td>Naive C57BL/10</td>
<td>190, 195, 200, 200, &gt;250</td>
<td>$207 ± 11$</td>
</tr>
<tr>
<td>Immune C57BL/10</td>
<td>0, 0, 0, 0, 0</td>
<td>$10 ± 10^a$</td>
</tr>
<tr>
<td>Immune C57BL/6</td>
<td>142, 175, 185, 195, &gt;250</td>
<td>$189 ± 18$</td>
</tr>
</tbody>
</table>

$^a$ Immune C3H.SW, C57BL/10, and C57BL/6 donors were immunized twice with 205IL-2/TK and ganciclovir. Two weeks after the second vaccine, $2 \times 10^6$ bone marrow cells were transplanted along with a range of spleen cells into irradiated C57BL/6 recipients with preexisting pulmonary 205 micrometastases. Lung nodules were counted at the time of death for each recipient. Individual mouse lung nodule counts ($A$) and survival times ($B$) are shown as squares, and means are represented by lines. $^a P < 0.01$ compared with control recipients of “0 spleen cells.”
0.4 ± 0.2 compared with 50 ± 8 for controls, \(P = 0.0002\); Fig. 2B).

However, all recipients of immune donor cells ultimately died with GVHD.

The impact of allogeneic donor immunization was further tested in a second tumor model. C3H.SW donor mice were immunized with irradiated C57BL/6-derived C1498 leukemia cells. C57BL/6 mice with preexisting C1498 leukemia underwent BMT using C1498-immune or naive donors, and survival was monitored. Survival was significantly extended in recipients of C1498-immune donor cells \( (P = 0.003; \text{Fig. 3}) \). Although four of five recipients died with signs of both acute GVHD and leukemia, one recipient survived >100 days without evidence of leukemia or GVHD.

**Increased Alloreactivity after Tumor Immunization of Donors Contributes to the Potent GVT Activity.** Experiments were performed to characterize the alloreactivity resulting from donor immunization against recipient tumor cells. Alloreactivity was evaluated *in vivo* using non-tumor-bearing BMT recipients. Tumor-immune C3H.SW donor spleen cells were compared with naive C3H.SW spleen cells for their ability to induce fatal GVHD in C57BL/6 mice. BMT using either 1 \( \times 10^6 \) or 10 \( \times 10^6 \) tumor-immune or naive SW spleen cells into irradiated C57BL/6 recipients. Arrows, the number and type of spleen cells for each group. A, data were pooled from two identical experiments \((n = 10)\). B, data were from one experiment \((n = 5)\).

**Fig. 4. Tumor-immune donor spleen cells decrease survival in recipients without tumor.** C3H.SW (SW) donors were immunized twice with 205 IL-2/TK and ganciclovir (A) or irradiated C1498 cells (B). Two weeks after the second vaccine, 4 \( \times 10^6 \) bone marrow cells were transplanted along with either 1 \( \times 10^6 \) or 10 \( \times 10^6 \) tumor-immune or naive SW spleen cells into irradiated C57BL/6 recipients. Arrows, the number and type of spleen cells for each group. A, data were pooled from two identical experiments \((n = 10)\). B, data were from one experiment \((n = 5)\).
effect induced by tumor immunization of donors is mediated by alloreactive T cells that recognize tumor. If the antitumor activity induced by donor immunization is mediated in part by effector cells responding to widely distributed mHAgs, then immunization of donors against nonmalignant recipient cells should also increase GVT activity against 205. A related hypothesis is that immunization of donors against 205 should cross-protect recipients against growth of other C57BL/6 tumors, which likely express recipient mHAgs but do not share tumor antigens with 205. C3H.SW donors were immunized against 205 (205-immune) or against C57BL/6 spleen cells (alloimmune). BMT recipients were inoculated i.v. with either 205 or B16F10 cells before BMT. Both 205-immune and alloimmune donor cells significantly protected mice against growth of 205 and B16F10 metastatic lung nodules ($P < 0.05$; Fig. 5). In syngeneic C57BL/6 mice, 205 immunization prevents the growth of 205 but not B16F10 (21).

These results in vivo were consistent with the in vitro detection of substantial cross-reactive cytolytic activity in 205-immune C3H.SW mice. 205-immune donor cells efficiently lysed 205, B16F10, and to a lesser extent C57BL/6 Con A lymphoblasts, whereas naive C3H.SW cells lysed none of these targets ($P < 0.02$; Fig. 6).

**DISCUSSION**

This work tested the hypothesis that immunization of MHC-matched allogeneic donors with a recipient-derived tumor cell vaccine would substantially increase GVT activity and prolong survival of tumor-bearing BMT recipients. Tumor vaccination of donors produced substantial increases in GVT activity, which in many experiments completely eliminated preexisting micrometastatic tumor. The GVT effect was T cell dependent and related to transplanted cell dose. Survival was modestly prolonged in both tumor models examined. Although long-term survival was occasionally observed, most tumor-free mice died of GVHD. Donor vaccination exacerbated GVHD.

The simplest explanation of these findings is that donor immunization activated and expanded T-cell clones that recognized recipient mHAgs expressed on tumor cells and normal recipient tissues. According to this interpretation, GVT activity is simply another manifestation of GVHD. This is consistent with the observations that: (a) donor immunization with 205 tumor cells also protected against the syngeneic but immunologically unrelated B16F10 tumor; (b) alloimmunization with C57BL/6 spleen cells produced substantial protection against both tumors; and (c) increased GVHD mortality was seen in recipients that had never been challenged with tumor. Allogeneic donor cells have been shown to mediate GVT activity against an MHC-mismatched tumor (26). An alternative interpretation is that two populations of T cells were activated: one reactive with antigens predominantly found on tumor, and the other reactive with ubiquitously distributed mHAgs. The facts cited above provide unambiguous evidence for the second population. Induction of a tumor-selective population is compatible with the observations that: (a) under limiting dilution conditions, many tumor-reactive donor T cells did not lyse other C57BL/6 targets; and (b) antitumor activity was seen after BMT.
using tumor-immune C57BL/6 syngeneic donors. However, in allogeneic BMT experiments in vivo, we saw no compelling evidence of a tumor-specific effect.

These experiments show that although donor immunization with tumor cells induces powerful GVT activity, whole tumor cells are too complex to be used safely for donor sensitization strategies because of the unpredictable and uncharacterized nature of mHAg expression by tumors. Any method of stimulating allogeneic donor T cells with a patient’s tumor cells before BMT may lead to an increased risk of inducing severe GVHD in the patient. Although in some patients and models GVT activity may be separated from GVHD by depletion of CD8+ T cells (27, 28), such depletion was shown to abolish the GVT activity in this study.

There is substantial interest in finding methods that will dissociate GVT activity and GVHD in allogeneic BMT. Underlying many of these efforts is the assumption that tumor cells have some unique (or at least not widely expressed) immunogenic antigens. In some human malignancies, such as melanoma, antigens with relatively restricted tissue distribution have been identified (29, 30). Although our results show that immunization with complex allogeneic tumor vaccines induces GVHD, immunization of allogeneic donors with defined tumor antigens or immunodominant peptide fragments may selectively increase GVT activity. Preliminary studies in our laboratory show that donor immunization with a model tumor antigen does not exacerbate GVHD.

It is possible that in most cases there are no unique tumor targets for GVT activity or that any unique tumor antigens are overshadowed by immunodominant mHags. In this case, induction of GVHD may still have potential as an anticancer therapy. It may need to be used in a manner similar to chemotherapy, with careful titration of cell doses and immunosuppressive therapy, and application at times in which tumor cells are maximally sensitive to GVHD and critical organs are minimally sensitive to GVHD.

REFERENCES

Enhancement of Graft-Versus-Tumor Activity and Graft-Versus-Host Disease by Pretransplant Immunization of Allogeneic Bone Marrow Donors with a Recipient-derived Tumor Cell Vaccine


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/7/1525

Cited articles
This article cites 29 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/7/1525.full#ref-list-1

Citing articles
This article has been cited by 11 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/7/1525.full#related-urls

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, use this link
http://cancerres.aacrjournals.org/content/59/7/1525.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.