Graft-versus-host disease after allogeneic hematopoietic stem cell transplantation induces a CD8+ T cell–mediated graft-versus-tumor effect that is independent of the recognition of alloantigenic tumor targets

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Cure of hematologic malignancies after allogeneic hematopoietic stem cell transplantation is partially attributable to immunocellular antitumor reactions termed graft-versus-tumor (GvT) effect. GvT effects are heterogeneous with respect to effector cell populations, target antigens, and their interrelation with graft-versus-host disease (GvHD). In the present study, allogeneic parent-into-F$_1$ murine transplantation models (BALB/c or C57BL/6 → [C57BL/6 × BALB/c]F$_1$) with different tumors derived from either parental strain were used to evaluate tumor-specific GvT effects. Compared with syngeneic F$_1$-into-F$_1$ controls, significant CD8+ T cell–mediated GvT effects occurred in both allogeneic transplantation models, even in the absence of histoincompatibilities between donor cells and host tumor. Identical genetic background of donor and tumor precluded allorecognition of tumor cells, indicating that tumor-associated antigens (TAAs) were targeted. With allowance made for selective major histocompatibility complex (MHC) disparities between donor cells and normal host tissue, GvHD was identified as a driving force for TAA-specific GvT effects. Adoptive transfer of the effector cells into secondary tumor-bearing recipients confirmed sustained antitumor activity and specificity of the T-cell response. The results provide experimental proof of a donor CD8+ T cell–mediated TAA-specific antitumor response in vivo that is driven by GvHD. It may represent one of the mechanisms contributing to GvT effects observed in allogeneic transplant recipients. (Blood. 2004;104:1210-1216)

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Introduction

An immunologically mediated antitumor effect, referred to as graft-versus-leukemia (GvL) or graft-versus-tumor (GvT) reaction, is considered essential for the curative potential of allogeneic hematopoietic stem cell transplantation (HSCT) in hematologic malignancies and may be effective after stem cell allografting in selected solid tumors.[1-5] This observation has prompted fundamental re-evaluation of allogeneic HSCT.[3,4,6] To diminish the procedural toxicity and optimally exploit GvL/GvT effects, conditioning regimens of reduced intensity and posttransplantation adoptive immunotherapy using donor lymphocytes or lymphocyte subsets as well as tumor-targeted vaccination following allografting are currently being explored.[7-11]

As of now, the precise immunologic mechanisms of the GvL/GvT effects have not been fully elucidated. They may involve the activation of different effector populations, including CD4+ and CD8+ T-cell subsets as well as natural killer cells,[12-14] and probably depends on the recognition of distinct sets of target antigens. Besides disparate major histocompatibility complex (MHC) molecules, 2 categories of antigens are candidates as targets for GvL/GvT effects: polymorphic minor histocompatibility antigens (mHAs)[15-18] and tumor-associated antigens (TAAs). The latter category includes leukemia- or tumor-specific antigens, such as the breakpoint cluster region/Abelson leukemia virus (BCR-ABL) fusion protein,[19] and nonmutated antigens selectively or aberrantly expressed by malignant cells, such as proteinase 3 in myeloid leukemias.[20]

In addition to the diversity in effectors and targets, it is still a matter of conjecture whether GvL/GvT effects are closely intertwined with the development of graft-versus-host disease (GvHD)[1,21] or whether the 2 phenomena are separable.[22,23] Thus, in theory, it would appear likely that different immunocellular antitumor effects after allogeneic HSCT may be operative in a given disease and even in an individual patient. They may vary in their dependence on alloantigenic target recognition, be subject to different tolerance mechanisms, and synergize in short-term and long-term tumor control to account for what is observed as GvL/GvT effects.

Here we present a murine transplantation model that allows us to variably direct the immune response of donor cells against alloantigens or TAAs. With this model, we provide direct experimental proof of a CD8+ T cell–mediated antitumor response in vivo that is driven by GvHD, but that targets TAAs.

Materials and methods

Experimental animals

C57BL/6, BALB/c, and [C57BL/6 × BALB/c]F$_1$ were bred at the animal facility of the University of Münster (Münster, Germany). C57BL/6-Pfp^{tm1Sdz} (perforin-defective [pfp$^{-/-}$]) and B6Smn.C3Tnfsfg^{gld} (Fas ligand

[FasL]–deficient) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). B6.129S2-Cd4[tm1Mak] (CD4^{−/−}) and B6.129S2-Cd8a[tm1Mak] (CD8^{−/−}) were kindly provided by T. Blankenstein (Max-Delbrück-Center [MDC], Berlin, Germany). Only female mice between 12 and 18 weeks of age were used. Donor and recipient mice were kept in laminar flow racks under pathogen-free conditions. Transplant recipients received sterilized food and sterilized water supplemented with cotrimoxazole (200 mg/L). All animal care and procedures were in accordance with European regulations and were approved by the regional governmental review board.

Tumor cell lines

MethA and CMS5 are transplantable 3-methylcholanthrene–induced sarcomas of BALB/c (H-2[d]) origin with distinctly different tumor antigenicities.[24] CMS5 was kindly provided by B. Gaensbacher (Institut für Experimentelle Onkologie und Therapieforschung der Technischen Universität München, Germany). MCA205, a 3-methylcholanthrene–induced sarcoma of C57BL/6 (H-2[b]) origin, was kindly provided by S. A. Rosenberg (National Cancer Institute [NCI], Bethesda, MD).

Cell transplantation and assessment of GvHD

In transplantation experiments, recipient mice received 9 Gy total body irradiation (TBI) from a ^{60}Co source at a dose rate of 128 cGy/minute 1 day before transplantation (day −1). Bone marrow cells obtained by flushing tibias and femurs of killed donors were given as a single intravenous injection via the tail vein at 1.0×10^6/g body weight, either alone or mixed with splenic lymphocytes (0.5×10^6/g body weight) as indicated. In all experiments with CD8^{−/−}, CD4^{−/−}, perforin^{−/−}, and FasL-deficient donors, 0.5×10^5 donor splenic lymphocytes per gram body weight were coinjected with the bone marrow cells to ensure complete donor chimerism. GvHD was monitored by the loss in total body weight and confirmed by histology of the skin, gut, liver, and lung.

Tumor inoculation and monitoring

Recipient mice were subcutaneously inoculated with tumor cells (1.0×10^6) 11 to 14 days after transplantation. In initial experiments, 1.0×10^6 tumor cells irradiated with 30 Gy were coinjected intraperitoneally. Since the additional intraperitoneal application of tumor cells did not affect the observed GvT effects (data not shown), it was omitted in subsequent experiments. Tumor size was measured with a microcaliper and is presented as maximum tumor diameter or volume. Tumor volumes were calculated with the following formula: volume [mm^3] = squared shortest diameter (mm)^2 × longest diameter (mm) divided by 2.

Adoptive transfer of spleen cells

Spleen cells were harvested from MethA tumor–bearing or non–tumor-bearing [C57BL/6 × BALB/c]F$_1$ recipients 25 to 28 days after transplantation from BALB/c parental donors. Cell subset depletion was performed by incubation of spleen cells with CD4 (L3T3), CD8a (Ly-2), or natural killer (NK) cell (DX5) MicroBeads, and negative magnetic separation (Miltenyi Biotec, Bergisch Gladbach, Germany). For adoptive transfer, 3.5×10^7 depleted or nondepleted spleen cells were given as a single injection via the tail vein into lethally irradiated naive BALB/c mice (8 Gy TBI). Immediately afterward, 1.0×10^6 tumor cells were administered subcutaneously.

Tumor reactivity and specificity were confirmed by adoptive transfer experiments using splenic lymphocytes alloreactive against F$_1$ recipients, but MHC identical with the tumor from either MethA-bearing, CMS5-bearing, or non–tumor-bearing F$_1$ transplant recipients. Splenic lymphocytes (3.5×10^7) of these F$_1$ transplant recipients were intravenously transferred into lethally irradiated naive BALB/c mice simultaneously inoculated with MethA or CMS5 tumor cells (1.0×10^6).

Histopathologic analysis and immunostaining

Tumor pathology and GvHD organ pathology for bowel, liver, skin, and lung were assessed in a blinded fashion on hematoxylin and eosin (H&E)–stained tissue. Infiltration of tumor tissue with CD8$^+$ lymphocytes was confirmed by immunohistology with the use of antimouse CD8a antibody (53-6.7) (Becton Dickinson Biosciences, Heidelberg, Germany). Tumor-infiltrating cells were identified as donor derived by immunostaining of tumor sections with monoclonal antibodies against H-2K^d (SF$_1$-1.1) and H-2K^b (AF6-88.5) (both from Becton Dickinson Biosciences).

Antibodies and flow-cytometric analyses

Flow-cytometric analysis of peripheral blood or spleen cells for chimerism and control of cell subset depletion was performed with fluorescein isothiocyanate (FITC)–, phycoerythrin (PE)–, and PE-cyanine 5 (Cy5)–conjugated antibodies to mouse CD3 (1 μg/mL) (145.2C11); CD4 (1 μg/mL) (H129.19); CD8 (1 μg/mL) (53-6.7); CD49b/Pan-NK (1 μg/mL) (DX5); H-2K^b (1 μg/mL) (AF6-88.5); or H-2K^d (1 μg/mL) (SF1-1.1). Cells were labeled according to the manufacturer's instructions and were analyzed on a FACSCalibur with CellQuest software. All antibodies and the FACSCalibur were purchased from Becton Dickinson Biosciences.

Cell-killing assay

Before the measurement of cytotoxic activity, spleen cells from transplant recipients were restimulated in vitro with MethA tumor cells irradiated with 36 Gy at a ratio of 10:1 for 5 days. For the killing assay, MethA target cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) (Molecular Probes, Leiden, The Netherlands) at a concentration of 5 μM for 5 minutes and were washed afterward 3 times with RPMI 1640 (Biochrom, Berlin, Germany) containing 10% fetal calf serum (FCS) (Gibco, Karlsruhe, Germany). After 24 hours of incubation of target and effector cells at the ratios indicated, propidium iodide (PI) (Sigma, Taufkirchen, Germany) was added to identify killed target cells. Cytotoxic activity was measured by flow-cytometric analysis comparing CFSE$^+$PI$^+$ cells (killed targets) with CFSE$^+$PI$^-$ cells (vital targets).

Statistics

Data are presented as means ± standard deviation (SD). The 2-tailed Mann-Whitney U-test was used for the statistical analysis of in vitro and in vivo data. P values less than .05 were considered statistically significant.

Results

Tumor-specific and alloreactive GvT effects associated with GvHD

To study the role of GvHD in eliciting GvT effects and to determine their specificity and dependence on alloantigens, we employed murine models of syngeneic or allogeneic bone marrow transplantation with subsequent tumor cell inoculation. Tumor cell lines were chosen to share the complete genetic background of the marrow donors or to differ from donor mice in MHC antigen expression. Both tumors used in this set of experiments, MCA205 (H-2^b) and MethA (H-2^d), were methylcholanthrene-induced fibrosarcomas; the tumors originated from C57BL/6 (H-2^b) or BALB/c (H-2^d) mice, respectively. Donors and tumors of both C57BL/6 and BALB/c background were used to account for potential strain-specific differences in GvHD and GvT activities. Transplantation and tumor models are summarized in Table 1.

After total body irradiation (TBI) with 9 Gy, [C57BL/6 × BALB/c]F$_1$ (H-2$^{b/d}$) mice received transplants of whole bone marrow from either syngeneic F$_1$ donors (negative controls) or allogeneic parental C57BL/6 (H-2^b) and BALB/c (H-2^d) donors. Engraftment of the bone marrow with peripheral blood leukocyte counts exceeding 1000/μL occurred on days 11 to 14 after transplantation. When mice were inoculated with the MethA tumor at the day of transplantation, rapidly progressive tumor growth up to a volume of 1000 mm^3 was observed until the time of engraftment, while

Table 1. Experimental models

Transplantation setting, donor, model	Tumor inoculum
Syngeneic	
[C57BL/6 × BALB/c]F$_1$	
F$_1$ → F$_1$: MCA205	MCA205 (C57BL/6 derived)
F$_1$ → F$_1$: MethA	MethA (BALB/c derived)
F$_1$ → F$_1$: CMS5	CMS5 (BALB/c derived)
Allogeneic	
BALB/c	
BALB/c → F$_1$: MCA205	MCA205 (C57BL/6 derived)
BALB/c → F$_1$: MethA	MethA (BALB/c derived)
BALB/c → F$_1$: CMS5	CMS5 (BALB/c derived)
C57BL/6*	
C57BL/6* → F$_1$: MCA205	MCA205 (C57BL/6 derived)
C57BL/6 → F$_1$: MethA	MethA (BALB/c derived)
C57BL/6 → F$_1$: CMS5	CMS5 (BALB/c derived)

All recipients are [C57BL/6 × BALB/c]F$_1$.

*MHC background of CD8$^{-/-}$, CD4$^{-/-}$, perforin$^{-/-}$, and FasL-deficient donors used in the experiments was identical to that of C57BL/6.

engraftment and GvHD activity were not impaired (data not shown). However, with this tumor burden and growth kinetics, it was not possible to detect GvT effects. Therefore, in the experiments shown, tumor inoculation was delayed until engraftment, which allowed monitoring of GvT effects at a lower tumor burden.

In the syngeneic F$_1$-into-F$_1$ transplantation models with either tumor inoculate (F$_1$ [H-2$^{b/d}$] → F$_1$: MCA205 [H-2^b], or F$_1$ [H-2$^{b/d}$] → F$_1$: MethA [H-2^d]), rapidly progressive tumor growth occurred (Figure 1A,C-D, open triangles). The MethA (H-2^d) tumor showed the same progressive growth pattern in additional control experiments comparing the syngeneic F$_1$ model (F$_1$ → F$_1$: MethA) with an MHC-identical setting using parental mice as donors and recipients (BALB/c → BALB/c: MethA) (data not shown). As expected, GvT activity with significant reduction of MCA205 and MethA tumor growth was evident when recipients and tumors were MHC disparate from donors: BALB/c (H-2^d) → F$_1$: MCA205 (H-2^b) (Figure 1A, open circles); C57BL/6 (H-2^b) → F$_1$: MethA (H-2^d) (Figure 1C-D, open circles).

Surprisingly, we observed a similar GvT effect in MCA205 (H-2^b)–inoculated F$_1$ recipients of transplants from C57BL/6 (H-2^b) donors (Figure 1A, closed circles). Since tumor and donor cells share the same MHC background in this allogeneic transplantation model, the observed tumor control was probably not due to an alloreactive mechanism. Rather, this observation gave rise to the hypothesis of target recognition through TAAs. When MethA, which shares the same MHC background as BALB/c donor cells, was used as tumor target, a less pronounced, but significant reduction in tumor growth was evident in F$_1$ recipients of transplants from BALB/c donors (Figure 1C, closed circles). This GvT effect was markedly augmented when naive splenic lymphocytes from the donors were coinjected with the marrow graft (Figure 1D, closed circles). Of note is the considerable difference in the growth kinetics between MCA205 and MethA (Figure 1A,C-D). The more aggressive growth pattern of MethA may account for the requirement of additional donor lymphocyte transfer in MethA-bearing F$_1$ recipients to achieve immunologic tumor control via targets other than alloantigens.

The engraftment kinetics, as measured by leukocyte counts after transplantation, were similar in all models studied and therefore are unlikely to influence the reduction in tumor growth (data not shown). In addition, donor cell chimerism of splenic CD3$^+$ T lymphocytes was consistently greater than 90% at day 28 after allogeneic transplantation (Figure 2J-L).
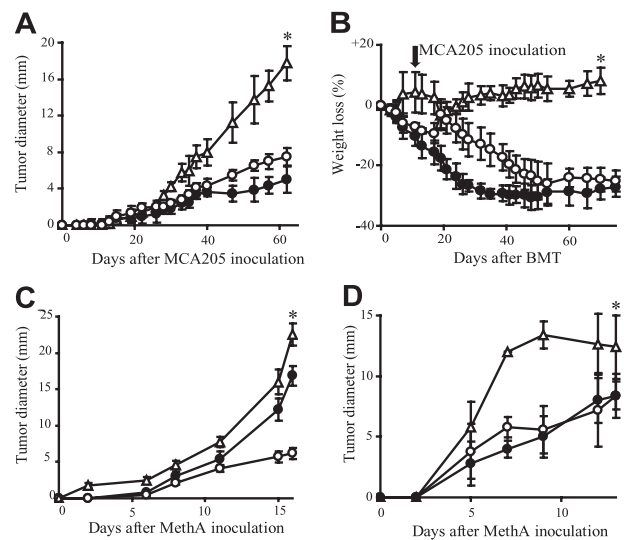
In both allogeneic transplantation models, GvHD of comparable severity occurred as measured by body weight loss (Figure 1B). GvHD was verified by macroscopic skin changes (Figure 2A-C, inserts) and by histopathologic signs such as tissue infiltration with lymphocytes and single cell apoptosis in the gut, skin, and liver (not shown). Lethality from GvHD during the observation time ranged from 0% to 16%. GvHD was consistently associated with reduced macroscopic tumor growth (Figures 1 and 2A-C) as well as with tumor necrosis and mononuclear cell infiltration on microscopic examination of tumor sections (Figure 2D-F).

Immunohistochemical analysis of the tumor sections on day 11 (MethA) (Figure 2G-I) or 22 (MCA205, not shown) after tumor inoculation revealed that the mononuclear cell infiltrates consisted predominantly of CD8$^+$ lymphocytes in F$_1$ recipients of transplants from allogeneic donors. This was true for both allogeneic models, with tumors sharing (Figure 2I) or not sharing (Figure 2H) the MHC background of the donors. In either model, the donor origin of tumor-infiltrating cells was verified by the presence or absence of immunostaining with antibodies against the parental MHC class I molecules H-2K^d and H-2K^b (not shown). In contrast, no tumor cell necrosis or lymphocyte infiltration could be observed in the syngeneic control group (Figure 2G). Thus, the GvT effects observed appeared to be mediated by donor-derived CD8$^+$ lymphocytes in both allogeneic settings.

Adoptive transfer of tumor-specific GvT activity

To prove a cellular GvT effect directed against tumor targets other than alloantigens and to verify the type of effector cells, we measured GvT

Figure 1. Graft-versus-tumor (GvT) and graft-versus-host (GvH) activity in tumor-bearing mice after syngeneic or allogeneic bone marrow transplantation (BMT). (A) Growth of MCA205 fibrosarcoma in the syngeneic (negative control) and both allogeneic transplantation models. [C57BL/6 × BALB/c]F$_1$ (H-2$^{b/d}$) mice received transplants from either [C57BL/6 × BALB/c]F$_1$ (H-2$^{b/d}$) donors (△, syngeneic model); BALB/c (H-2^d) donors (○, allogeneic model); or C57BL/6 (H-2^b) donors (●, allogeneic model) (n = 5 per group). Transplant-recipient mice were inoculated with MCA205 tumor cells of C57BL/6 (H-2^b) origin. (B) Weight loss after BMT as a measure of GvH disease (experiments and symbols as in panel A). (C) Growth of MethA fibrosarcoma (BALB/c [H-2^d] origin) in the syngeneic and both allogeneic transplantation models. [C57BL/6 × BALB/c]F$_1$ (H-2$^{b/d}$) mice received transplants from either [C57BL/6 × BALB/c]F$_1$ (H-2$^{b/d}$) donors (△, syngeneic model) (n = 8), BALB/c (H-2^d) donors (●, allogeneic model) (n = 8), or C57BL/6 (H-2^b) donors (○, allogeneic model) (n = 7). Transplant-recipient mice were inoculated with MethA tumor cells of BALB/c (H-2^d) origin. Weight loss as measure of GvH activity was absent in the syngeneic model and comparable to the data shown in B in both allogeneic models. (D) Same experiment as in C with additional transfer of splenic T cells from the donor at the day of BMT (n = 5 per group). Note augmented GvT effects in allogeneic transplant recipients in which donor and tumor share the same MHC background (●). Data are presented as means ± SD. The results are representative of at least 3 independent series of experiments. *P < .05 for syngeneic donors (△) versus allogeneic BALB/c and C57BL/6 donors, respectively (●, ○).
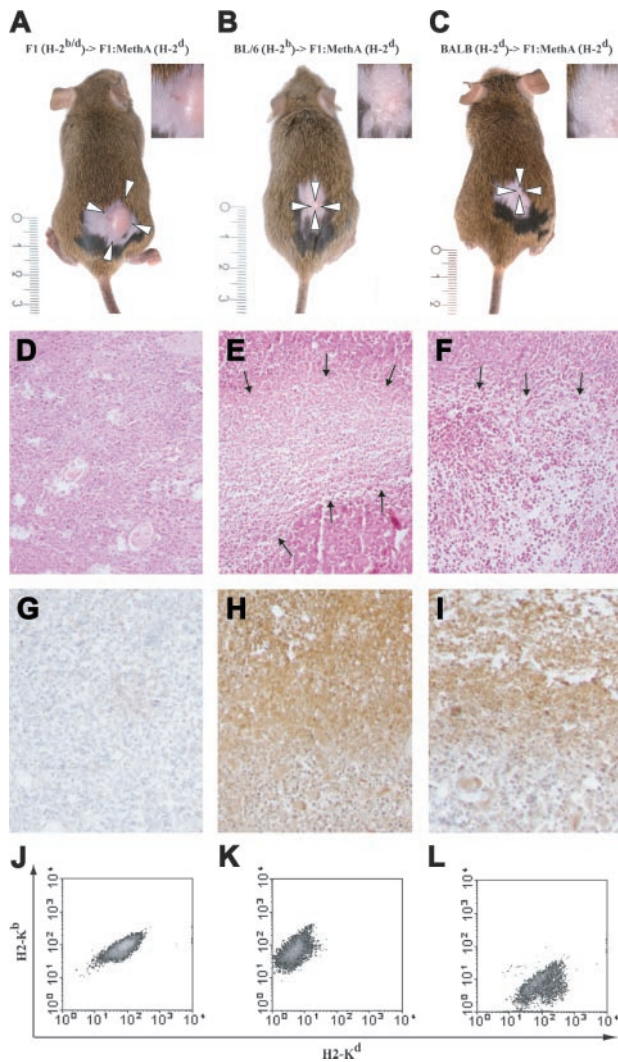
Figure 2. Reduced tumor growth correlating with graft-versus-host disease (GvHD). [C57BL/6 × BALB/c]F_1 (H-2b/d) mice received transplants of bone marrow from syngeneic F_1 donors (panels A,D,G,J, negative control group); allogeneic C57BL/6 (H-2b) donors (panels B,E,H,K); or allogeneic BALB/c (H-2d) donors (panels C,F,I,L), respectively. Original magnification × 100 used for histology (D-I). (A-C) Outgrowth of the BALB/c (H-2d)–derived MethA fibrosarcoma (H-2d) in the respective transplantation models (tumor size indicated by arrows). Inserts show manifestations of cutaneous GvHD with squamous skin changes in both allogeneic models (panels B-C). (D-F) Histology of tumors (H&E-staining) showed marked necrosis and adjacent mononuclear cell infiltrates (indicated by arrows) in recipients of allogeneic transplants (panels H-I). (G-I) Immunohistochemistry for CD8 of MethA tumor sections identified massive CD8+ cellular infiltrates in the border area of tumor necrosis (brown staining) in allogeneic transplant recipients (panels H-I). (J-L) Donor cell chimerism of CD3+ T cells on day 28 after BMT revealed by fluorescence-activated cell sorter (FACS) analysis after immunostaining for H-2Kd and H-2Kb.

reactions in adoptive transfer experiments with splenic lymphocytes selectively depleted of cellular subsets from either tumor-bearing or non–tumor-bearing transplant recipients. Intravenous transfer of spleen cells from MethA-bearing F_1 mice that received transplants from BALB/c donors (BALB/c [H-2d] → F_1: MethA [H-2d]) into lethally irradiated secondary BALB/c recipients significantly reduced MethA tumor growth as compared with adoptive transfer of spleen cells from non–tumor-bearing F_1 mice (Figure 3A). Similar results were obtained with adoptive transfer of splenic lymphocytes from MCA205-bearing versus non–tumor-bearing F_1 mice that received transplants from C57BL/6 donors (C57BL/6 [H-2b] → F_1: MCA205 [H-2b]) into lethally irradiated secondary C57BL/6 recipients (data not shown). No signs of GvHD occurred in secondary parental recipients after adoptive transfer

of spleen cells from primary F_1 recipients of transplants from the respective parental donors.

To identify the antitumor-reactive cell subpopulation, splenic lymphocytes from BALB/c (H-2d) → F_1: MethA (H-2d) recipients were depleted with either anti-CD8, anti-CD4, or anti–NK-cell antibodies and transferred into naive BALB/c recipients. Depletion of spleen cells from the CD8 antigen–expressing cellular subset abolished adoptive tumor protection in the secondary recipients (Figure 3B). In contrast, NK cells and CD4 antigen–expressing lymphocytes did not contribute to tumor immunity in our models, since their removal from spleen cells did not alter adoptive inhibition of tumor growth (Figure 3C).

Tumor-specific GvT effects are mediated by CD8+ cells

To further verify that CD8+ lymphocytes were the effector cells of the GvT effect not targeting alloantigens on tumor cells, we repeated the allogeneic transplantation experiments shown in Figure 1A (C57BL/6 [H-2b] → F_1: MCA205 [H-2b]) using genetically engineered donor mice deficient for either CD4 or CD8 antigen–expressing lymphocytes. Again, the donor knockout mice had the same MHC H-2b background as the MCA205 tumor. Wild-type (wt) C57BL/6 and F_1 mice were used as donors in control experiments. In a comparison with the wt donors, we observed complete abrogation of the GvT effect in MCA205 (H-2b)–bearing F_1 recipients of transplants from allogeneic CD8−/− (H-2b) donors (Figure 4A). In contrast, the antitumor effect was completely sustained in analogous experiments using allogeneic CD4−/− (H-2b) donor mice.

Major cytotoxic pathways of effector cells are granule- or Fas-mediated.[25-27] To determine the relative importance of these cytotoxic mechanisms for the observed tumor-specific reaction, we used donors defective for either perforin (pfp−/−) or FasL (gld) in the transplantation model with alloreactivity of donor cells against the host, but not against the tumor. Both deficiencies weakened the antitumor response in primary recipients (data not shown). Adoptive transfer experiments using spleen cells from MCA205 (H-2b)– bearing F_1 recipients of transplants from pfp−/− (H-2b), gld (H-2b), or C57BL/6 wild-type (H-2b) donors demonstrated a significant dependence of the transferable tumor-specific GvT reaction on both cytotoxic pathways (Figure 4B).

Specificity of the alloantigen-independent antitumor response in vitro

To define the specificity of this antitumor response, we performed in vitro cytotoxicity assays with splenic lymphocytes from MethA (H-2d)– bearing and non–tumor-bearing F_1 recipients of transplants from BALB/c (H-2d) donors. Since significant antitumor reactions could not be detected directly, splenic lymphocytes from these animals were restimulated once with irradiated tumor cells (secondary mixed lymphocyte tumor culture). When spleen cells from non–tumor-bearing F_1 recipients were used, a cytotoxic activity against MethA (H-2d) tumor cells was not observed (tumor cell lysis of 6.6% ± 1%; effector-target ratio, 25:1; n = 3). In contrast, spleen cells from MethA (H-2d)–bearing F_1 recipients of transplants from BALB/c (H-2d) consistently induced significant lysis of MethA tumor cells, indicating the existence of specifically primed T cells in tumor-bearing recipients (tumor cell lysis of 95.5% ± 3%; effector-target ratio, 25:1; n = 4; $P < .05$).

Specificity of the alloantigen-independent antitumor response in vivo

Since the target antigens of the tumor-specific graft response were unknown, we proceeded to confirm its specificity in vivo by adoptive
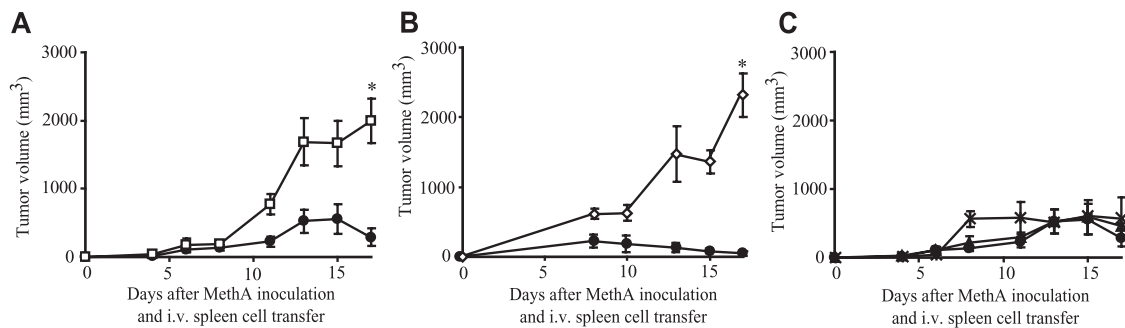
Figure 3. Specific GvT effect mediated by CD8+ cells. Spleen cells of [C57BL/6 × BALB/c]F$_1$ (H-2$^{b/d}$) recipients of transplants from BALB/c (H-2^d) donors were intravenously transferred into naive, lethally irradiated BALB/c mice simultaneously subjected to subcutaneous MethA (H-2^d) tumor cell inoculation. (A) MethA tumor growth after adoptive transfer of spleen cells from non–tumor-bearing (□) or MethA-bearing (●) primary transplant recipients (n = 5 per group). (B) Transfer of CD8-depleted (◇) or nondepleted (●) spleen cells from MethA-bearing primary transplant recipients (n = 5 per group). (C) Same experiments as in panel B with NK cell–depleted (△), CD4-depleted (×), or nondepleted (●) spleen cells from MethA-bearing primary transplant recipients (n = 5 per group). Notably, no GvHD was observed in secondary recipients after adoptive transfer of spleen cells. Data are presented as means ± SD. The results are representative of at least 2 independent series of experiments. *$P < .05$ versus respective control groups.

transfer experiments using the target tumors MethA (H-2^d) and CMS5 (H-2^d). Both tumors are sarcomas of BALB/c (H-2^d) origin with distinctly different tumor antigenicity.[24] As shown for MCA205 and MethA tumors in Figure 1, a significant antitumor effect after allogeneic HSCT could also be demonstrated for CMS5 (Figure 5A). Again, GvT effects in primary recipients were associated with GvHD (Figure 5B) and were of similar activity regardless of whether tumors and donors were MHC disparate or shared the same genetic background.

To verify the specificity of the non–alloantigen-targeted GvT effects, spleen cells of MethA-bearing, CMS5-bearing, or non–tumor-bearing F$_1$ recipients of transplants from BALB/c donor mice were adoptively transferred into lethally irradiated BALB/c mice inoculated subcutaneously with either MethA or CMS5. As shown in Figure 5C-D, the adoptively transferable GvT effect was specific and strictly dependent on priming with the target tumor in the F$_1$ transplant recipients. Induction of an antitumor effect in F$_1$ mice by MethA conferred a significant transferable antitumor response against MethA (Figure 5C), but not against CMS5 (Figure 5D). Conversely, adoptively transferred spleen cells from CMS5-bearing F$_1$ recipients inhibited the growth of CMS5 tumor cells (Figure 5D), but had no effect on MethA growth (Figure 5C).

Discussion

A close association has been described clinically between both acute and chronic GvHD and the occurrence of GvL/GvT ef-

fects.[1,28,29] The coincidence of the 2 phenomena may simply reflect a disparity in major or minor histocompatibility antigens between donor cells and both normal and tumor tissue of the host.[30,31] On the other hand, clinical and experimental observations suggest that some effector cells mediating GvHD may be distinct from those mediating GvL/GvT activity.[13,20,32] Hence, antitumor and antihost responses may in part be separable.[23]

In our models, GvHD was required for the induction of a TAA-reactive GvT effect. In primary transplant recipients, GvHD and, for example, the involved inflammatory cytokines may have contributed to the observed antitumor response. Since the skin is a target organ of GvHD, it may also be argued that changes caused by GvHD in the environment of the subcutaneous experimental tumors may have inhibited tumor growth. One might further speculate that despite MHC homology of donor and tumor, the recognition of mismatched host alloantigens expressed by endothelial cells in nutritive tumor vessels may have caused tumor necrosis by devascularization. Thus far, GvHD and antitumor effects are interrelated phenomena, and their relative contributions to the observed GvT effects cannot be discerned in our models. However, once primed in the presence of GvHD, the CD8+ T cell–mediated antitumor response is transferable into naive secondary recipients of the parental strain, where it is active in the absence of allorecognition, GvHD, and related cytokines. On the basis of these results, we conclude that GvHD targeting alloantigens is a driving force for eliciting a TAA-specific GvT reaction that is mediated by T cells not involved in alloantigenic target recognition.
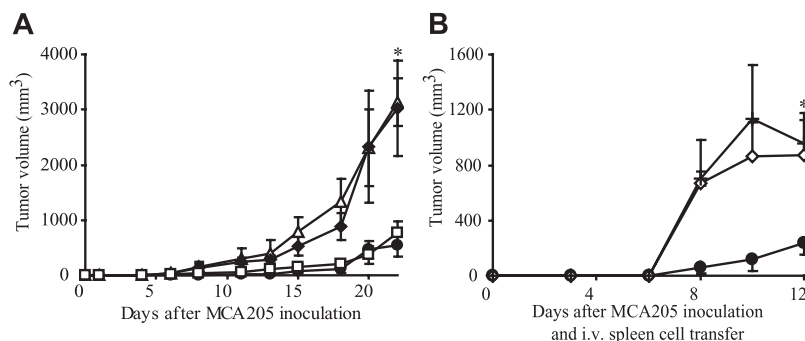
Figure 4. Dependence of non–alloantigen-mediated graft-versus-tumor activity on CD8 T cells and on perforin and FasL-mediated cytotoxicity. (A) MCA205 (H-2^b) tumor growth in [C57BL/6 × BALB/c]F$_1$ (H-2$^{b/d}$) recipients of transplants from syngeneic F$_1$ donors (△, negative control) or allogeneic wild-type (●), CD8$^{-/-}$ (◆), and CD4$^{-/-}$ (□) C57BL/6 (H-2^b) donors (n = 6 per group). (B) Splenic lymphocytes of MCA205 tumor–bearing F$_1$ recipients of transplants from either wild-type (●), perforin$^{-/-}$ (+), or FasL-deficient (◇) C57BL/6 donors were adoptively transferred into irradiated naive C57BL/6 mice. These secondary recipients were simultaneously subjected to subcutaneous MCA205 (H-2^b) tumor cell inoculation. The transferable GvT effect was significantly impaired by both perforin and FasL deficiency (n = 3 per group). Data are presented as means ± SD. *$P < .05$ for C57BL/6 wild-type donors versus syngeneic F$_1$ donors and allogeneic CD8$^{-/-}$, perforin$^{-/-}$, and FasL-deficient C57BL/6 donors, separately.
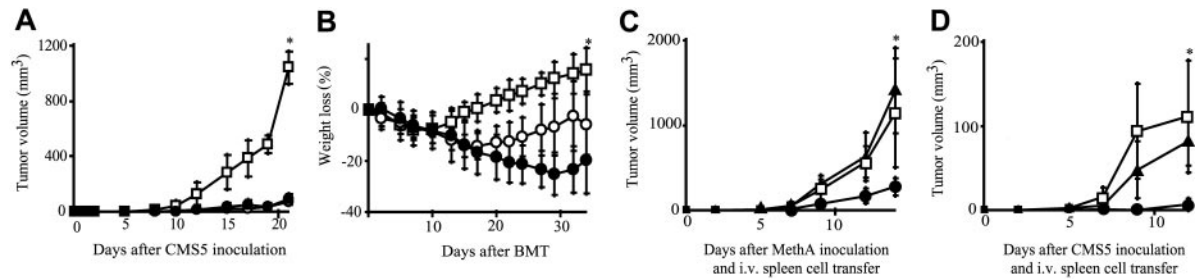
Figure 5. Tumor-specific priming and reactivity of the T-cell response in transfer experiments. Specificity of the priming and reactivity of the T-cell response was tested in cross-over transfer experiments using 2 different fibrosarcomas of BALB/c (H-2^d) origin: MethA and CMS5. (A) Antitumor effects against CMS5 fibrosarcoma in [C57BL/6 × BALB/c]F$_1$ (H-2$^{b/d}$) recipients of transplants from either [C57BL/6 × BALB/c]F$_1$ (H-2$^{b/d}$) donors (□, syngeneic control); BALB/c (H-2^d) donors (●, allogeneic model); or C57BL/6 (H-2^b) donors (○, allogeneic model) (n = 5 per group). Mice were subcutaneously inoculated with CMS5 tumor cells on day 14 after transplantation and showed a significantly reduced growth in both allogeneic groups (compare similar experiments with MCA205 and MethA tumor in Figure 1). (B) Weight loss as measure of GvHD was observed in both groups receiving transplants from allogeneic donors and was absent in the syngeneic model. (C) Growth of MethA (H-2^d) tumors in lethally irradiated naive BALB/c (H-2^d) mice after adoptive transfer of spleen cells from non–tumor-bearing (□, n = 10); MethA (●, n = 9), or CMS5-bearing (▲, n = 7) F$_1$ recipients of transplants from BALB/c donors. Inhibition of MethA tumor growth was observed only after transfer of spleen cells from MethA-bearing primary transplant recipients. (D) Growth of CMS5 (H-2^d) tumors in naive secondary BALB/c recipients after transfer of spleen cells from non–tumor-bearing (□, n = 6), CMS5-bearing (●, n = 5), or MethA-bearing (▲, n = 7) F$_1$ mice receiving transplants from BALB/c donors. A transferable GvT effect against CMS5 was evident only with spleen cells derived from CMS5-bearing primary transplant recipients. Data are presented as means ± SD. *$P < .05$ versus respective control groups.

To date, evidence for a TAA-targeted GvL/GvT effect after allogeneic HSCT is based on the detection of tumor-specific or tumor-reactive T cells in the blood of patients who had received transplants for hematologic malignancies.[23,33,34] In patients with chronic myelogenous leukemia, Molldrem et al[20] were able to identify circulating cytotoxic T cells specific for PR1: a peptide derived from proteinase 3, which is overexpressed in myeloid leukemias. The authors described a correlation between the presence of PR1-specific T cells and clinical responses after allogeneic HSCT, suggesting that a TAA-specific GvL effect may contribute to the elimination of leukemic cells. However, at least in patients with GvHD, the possibility cannot be excluded that an allogeneic T-cell response to mismatched mHAs or MHC antigens has added to the observed GvL effects.[35,36]

Our murine parental-into-F$_1$ transplantation models were designed to separately study GvT effects secondary to recognition of alloantigens from those targeting TAAs. In the allogeneic recipients with experimental tumors expressing the same histocompatibility antigens as the donor strain, GvT effects attributable to the recognition of alloantigens on tumor cells could be excluded. Nonetheless, we observed a significant retardation of tumor growth in these mice. This antitumor response was mediated entirely by TAA-specific CD8$^+$ donor cells. Priming and target reactivity of this T-cell response were highly tumor-specific and non–cross-reactive, even between related tumors. Taken together, our data provide the experimental link between the detection of tumor-specific T cells ex vivo as mentioned in the preceding paragraph and their biologic contribution to GvL/GvT effects.

The allogeneic HSCT models enabled us to distinguish between GvT effects targeting TAAs and those targeting alloantigens and to study their biologic activity and interrelation with GvHD. Under the conditions of MHC-identical tumor and donor cells, alloantigen-

targeted GvT effects were unlikely to occur. It can be argued, however, that tumor cell lines differ genetically from the mouse strains they are derived from, which may also result in differences in mHAs. In this case, the discriminating antigens would be presented by the tumor and most probably not by the normal tissue of the F$_1$ hosts. Consequently, these mHAs could be considered TAAs.

The experimental setting employed in this study is suited to identify a CD8$^+$ T-cell response targeting TAAs and triggered by GvHD as one of the fundamental mechanisms contributing to GvT effects in allogeneic transplantation recipients. It allows segregating this T-cell response from GvT effects mediated by alloreactive T cells. However, in clinical allogeneic HSCT, it is likely that both types of T cell–mediated GvL/GvT effects can add to tumor control. Owing to limitations of our model, such as the requirement for delayed tumor inoculation at the time of engraftment and active GvHD, it is difficult to draw conclusions on the relative importance of either type of T-cell effects, particularly with respect to the clinical setting. Nonetheless, our results suggest that the stimulatory activity of GvHD could be used to enhance the efficiency of tumor-targeted cellular immunotherapy or tumor vaccination after allogeneic HSCT.[37,38] The data might also support the rationale for current activities that aim at extending allogeneic cell therapy to patients with immunogenic solid tumors.

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