Involvement of Donor T-Cell Cytotoxic Effector Mechanisms in Preventing Allogeneic Marrow Graft Rejection

By Paul J. Martin, Yoshiki Akatsuka, Michael Hahne, and George Sale

DONOR CD8 CELLS PLAY a pivotal role in preventing allogeneic marrow graft rejection in mice and are substantially more effective than donor CD4 cells. Based on the known function and development of CD8 cells and CD4 cells in primary allospecific T-cell populations,2 we suggested that the generation of T-cell responses against major histocompatibility complex (MHC) class I alloantigens or class I-restricted peptides was the most likely mechanism by which donor T cells inactivate or eliminate the recipient effectors responsible for causing rejection. This hypothesis was supported by results demonstrating that T cells from nontolerant donors were more effective for preventing rejection than T cells from F11 or chimeric donors5 that could not recognize recipient alloantigens. We have now assessed the role of cytotoxic effector function as a mechanism by which donor T cells might prevent allogeneic marrow graft rejection. The cytotoxic effector function of T lymphocytes can be mediated by two well-characterized mechanisms.5,7 One of these involves cell surface interactions between Fas ligand expressed on activated cytotoxic lymphocytes (CTL) and Fas expressed on target cells. The other involves granule exocytosis, a process in which perforin facilitates the translocation of granzymes from CTL into target cells. Both mechanisms induce apoptosis in target cells. Additional less well-characterized mechanisms are involved in cytolytic effector function against certain types of target cells.8 In the present study, we evaluated the effect of mutations in the Fas-ligand and perforin/granzyme pathways on the ability of donor CD8 cells to prevent allogeneic marrow graft rejection caused by recipient CD8 cells. In further experiments, we evaluated the ability of Fas-ligand-defective perforin-deficient T cells to cause graft-versus-host disease (GVHD).

MATERIALS AND METHODS

Mice. C57BL/6J (B6; H2*, Ly5.2) males, B6.C-H2*,Ly5.2 (bm1; Ly5.2) males and females, and BALB/c (H2K) females were purchased from the Jackson Laboratory (Bar Harbor, ME). Founders for the Ly5.1 (Pfp tm1Sdz), granzyme B-deficient B6,129-GzmBtm1ly5.2;10 and perforin-deficient C57BL/6-Pfp tm1Ly5.2;10 strains (purified by Dr. Paul J. Martin, Fred Hutchinson Cancer Research Center, Seattle, WA; the Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland) were backcrossed to gld females, and the offspring were intercrossed to generate perforin-deficient gld progeny. In these mice, the neo cassette that disrupts perforin expression is inserted at the exon 3 Spfl site.11 Perforin-deficient, gld-homozygous mice were also generated by intercrossing the offspring from mating (gld × C57BL/6-Pfp tm1Ly5.2) F1 males with C57BL/6-Pfp tm1Ly5.2 females. In these mice, the neo cassette that disrupts perforin expression is inserted at the exon 3 BriII site.10 Mice were housed in groups of 5 under specific pathogen-free conditions with twice weekly cage changes and received sterilized chow and acidified water (pH 3.5) ad libitum. Four weeks after marrow transplantation, mice were transferred to conventional housing conditions. Experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the FHCRC.

Screening for gld and perforin mutations. For detection of gld and wild-type Fas or Moloney murine leukemia virus (MoMLV) gag sequences in the offspring, peripheral blood leukocyte (PBL) DNA was amplified respectively with 5′ primer TTGGAGAAATCTAAAGACC or TTGGAGAAATCTAAAGACCT and 3′ primer ATAGCTGACCTGT-TGGACC (94°C for 4 minutes, followed by 30 seconds at 94°C, 30 seconds at 58°C, and 45 seconds at 72°C for 35 cycles with 1.5 mmol/L MgCl2, 200 mmol/L dNTP, and 0.5 U Taq polymerase; AmpliTaq Gold; Perkin Elmer, Foster City, CA), each yielding a 200-bp product. For detection of neo insertions at the perforin exon 3 Spfl site11 and BriII site,10 PBL DNA was amplified, respectively, with 5′ primer ACCTC-CACTCCAGCTTAG and 3′ primer GATAGGTAGGCGCCCGCATG or with 5′ primer AGTGTGAGTGCAAGGATG and 3′ primer CGGAG-TTGGTTATGTC (94°C for 9 minutes, followed by 30 seconds at 94°C, 30 seconds at 60°C, and 90 seconds at 72°C for 35 cycles with 1.5 mmol/L MgCl2, 500 mmol/L dNTP, and 1.0 U Taq polymerase). The wild-type alleles, respectively, yield 115- and 199-bp products, whereas the mutated alleles yield 1,100- to 1,200-bp products.

From the Division of Clinical Research, the Fred Hutchinson Cancer Research Center, Seattle, WA; the Departments of Medicine and Pathology, University of Washington, Seattle, WA; and the Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland. Supported by US Public Health Service Grant No. HL-55257 awarded by the Department of Health and Human Services, National Institutes of Health.

Address correspondence to Paul J. Martin, MD, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, D2-100, Seattle, WA 98109-1024; e-mail: pamarin@fhcrc.org.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1998 by The American Society of Hematology.

Blood, Vol 92, No 6 (September 15), 1998: pp 2177-2181

2177

From www.bloodjournal.org by guest on September 24, 2017. For personal use only.
Marrow transplantation. Recipients 8 to 12 weeks of age were prepared by total body irradiation (TBI) in a single fraction from dual-opposed 60Co sources at an exposure rate of 20 cGy/min on the day before transplantation. Marrow obtained by femur flush was depleted of T lymphocytes by rabbit complement (1:10)-mediated lysis using a mixture of antibodies specific for Thy-1.2, CD4, and CD8, as previously described.1 Nylon wool-nonthenonadherent lymphocytes obtained from pooled mesenteric, retroperitoneal, femoral, axillary, and cervical lymph node suspensions were depleted of B cells by centrifugal immunodepletion on plastic petri dishes precoated with goat antibody against mouse Ig (10 µg/mL in pH 9.4 buffer, 18 hours at 4°C) or with CD45R/B220-specific antibody RA3-6B2 (rat IgG2a; PharMingen, San Diego, CA) or RA3-3A1/6.1 (rat IgM; hybridoma obtained from American Type Culture Collection [ATCC], Rockville, MD; 12.5 µg/mL bound to dishes coated with goat antibody against rat Ig). CD8-enriched T cells were isolated either by centrifugal immunoadherent depletion of CD4 cells on dishes precoated with antibody GK1.5 (rat IgG2b; ATCC; antibody from hybridoma culture supernatant bound to dishes coated with goat antibody against rat Ig) or by immunomagnetic positive selection with colloidal superparamagnetic microbeads conjugated with monoclonal rat antibody against murine CD8 (Miltenyi Biotec Inc, Sunnyvale, CA) used according to the manufacturer’s instructions. CD8-enriched populations isolated by negative selection contained 82% to 94% (mean, 88.5%) CD8 cells and 1.0% to 2.4% (mean, 1.5%) CD4 cells. CD8-enriched populations isolated by positive selection contained 83% to 96% (mean, 90.7%) CD8 cells and 3.8% to 10.6% (mean, 7.1%) CD4 cells. For experiments in which engraftment was an endpoint, graded numbers of CD8-enriched LN cells were added to grafts containing 5.0 × 10^6 T-cell-depleted marrow cells. For experiments in which GVHD was an endpoint, aliquots containing 1.0 × 10^7 CD3 cells were added to the graft. In this model, approximately 1.0 × 10^7 wild-type B6 CD8-enriched LN T cells added to the graft. The percentage of donor marrow-derived T cells in the lymphoid gate defined by two-color staining with CD3- and Ly5.1-specific antibodies. Data on days 28, 61, and 97 after transplantation was determined by thresholding for delineating positive and negative cells. For enumeration of B cells, PBL were stained with fluorescein isothiocyanate (FITC)-conjugated CD3-specific antibody and with biotinylated antibody specific for Ly5.1 or H2Kb and analyzed as previously described.1 For each experiment, thresholds for delineating positive and negative cells were determined by staining samples from appropriate positive and negative controls. In the T-cell and myeloid gates, respectively, negative control samples showed 0.15% to 1.56% (mean, 0.65%) and 0.56% to 14.0% (mean, 2.5%) background staining, whereas positive control samples showed 97.6% to 100% (mean, 99.3%) and 93.1% to 100% (mean, 98.0%) stained cells. For enumeration of B cells, PBL were stained with FITC-conjugated CD3-specific antibody and with biotinylated antibody specific for CD45RA (14.8, rat IgG2b; hybridoma obtained from ATCC) or with phycoerythrin (PE)-conjugated CD3-specific antibody (PharMingen) and FITC-conjugated CD45R/B220-specific antibody (PharMingen). B cells were enumerated as the percentage of CD45RA+ or CD45R−, CD3− cells in the lymphoid gate defined by forward and side scatter characteristics.

RESULTS

Ability of donor CD8 cells to prevent marrow graft rejection. Preliminary experiments demonstrated that TBI exposures ≥650 cGy were sufficient to prevent rejection in bm1 (Ly5.2) recipients transplanted with 5.0 × 10^6 T-cell-depleted marrow cells from MHC class I-disparate B6-Ly5.1 donors, but rejection occurred in recipients prepared with 550 cGy TBI (data not shown). The ability to overcome rejection by increased pretreatment TBI in this model is characteristic of a process mediated by T cells1,4 and not by natural killer (NK) cells13,14 of the recipient. Rejection of the B6-Ly5.1 marrow was prevented in some recipients prepared with 550 cGy TBI when as few as 5.0 × 10^6 CD8-enriched B6 (Ly5.2) LN cells were added to the graft, and rejection was prevented in all recipients when 8.0 × 10^6 CD8 cells were added to the graft (Table 1). Under these conditions, engraftment of the donor marrow depends on the presence of T cells in the graft. In most engrafted recipients, T cells in the blood on day 28 after transplantation were derived predominantly from Ly5.1 marrow progenitors and not from mature Ly5.2 LN cells added to the graft, and the proportion of donor marrow-derived T cells increased progressively during the first 3 months after transplantation. Thus, the B6 CD8 cells added to the graft did not prevent maturation of B6-Ly5.1 marrow progenitors in the bm1 recipient thymus.

Effect of cytotoxicity mutations on the ability of donor T cells to prevent marrow graft rejection. In a series of seven experiments, we evaluated the effect of mutations in the Fas-ligand and perforin/granzyme pathways on the ability of donor CD8 cells to prevent rejection of B6-Ly5.1 marrow in irradiated bm1 recipients. Each experiment included negative controls with no donor T cells added to the marrow and positive controls with wild-type B6 CD8-enriched LN T cells added to the graft. In this model, approximately 1.0 × 10^7 wild-type B6 CD8 cells prevent rejection in 50% of the recipients (Table 2). The defect in Fas-ligand-mediated cytotoxicity caused by the gld mutation had only a minimal effect on the ability of donor T cells to prevent rejection. Although rejection occurred in all 15 recipients transplanted with grafts containing 5.0 × 10^6 CD8 cells enriched by negative selection (P = .01 compared with wild-type B6 CD8 cells), rejection was prevented in all recipients transplanted with grafts containing 2.0 × 10^5 or 8.0 × 10^5 gld CD8 cells. The absence of granzyme B in donor CD8 cells also had a minimal effect on their ability to prevent rejection. Although rejection occurred in all 14 recipients transplanted with grafts containing 5.0 × 10^5 CD8 cells enriched by negative selection (P = .02 compared with wild-type B6 CD8 cells), rejection was prevented in all recipients transplanted with grafts containing 2.0 × 10^6 or 8.0 × 10^6 gld CD8 cells. The absence of granzyme B in donor CD8 cells also had a minimal effect on their ability to prevent rejection.

Table 1. Ability of Donor CD8 Cells to Prevent Rejection of Marrow in Recipients With MHC Class I Disparity

<table>
<thead>
<tr>
<th>Day</th>
<th>CD8 Cells Added</th>
<th>Percentage of Donor Marrow-Derived Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T Lymphocytes</td>
<td>Granulocytes</td>
</tr>
<tr>
<td>28</td>
<td>None</td>
<td>1, 0, 1, 0, 0</td>
</tr>
<tr>
<td></td>
<td>5.0 × 10^6</td>
<td>39, 89, 0, 1, 1</td>
</tr>
<tr>
<td></td>
<td>2.0 × 10^6</td>
<td>88, 0, 94, 94, 94</td>
</tr>
<tr>
<td></td>
<td>8.0 × 10^6</td>
<td>89, 87, 89, 86, 86</td>
</tr>
<tr>
<td>61</td>
<td>None</td>
<td>0, 0, 0, 3, 1</td>
</tr>
<tr>
<td></td>
<td>5.0 × 10^4</td>
<td>72, 99, 0, 0, 0</td>
</tr>
<tr>
<td></td>
<td>2.0 × 10^5</td>
<td>89, 3, 99, 99</td>
</tr>
<tr>
<td></td>
<td>8.0 × 10^6</td>
<td>99, 94, 84, 92, 96</td>
</tr>
<tr>
<td>98</td>
<td>None</td>
<td>0, 0, 0, 3, 1, 1</td>
</tr>
<tr>
<td></td>
<td>5.0 × 10^5</td>
<td>92, 99, 1, 0, 2</td>
</tr>
<tr>
<td></td>
<td>2.0 × 10^6</td>
<td>99, 0, 99, 99</td>
</tr>
<tr>
<td></td>
<td>8.0 × 10^6</td>
<td>97, 98, 100, 99, 99</td>
</tr>
</tbody>
</table>

Groups of 5 irradiated (550 cGy) bm1 (Ly5.2) recipients were transplanted with 5.0 × 10^6 T-cell-depleted B6-Ly5.1 marrow cells alone or with the indicated numbers of B6 (Ly5.2) CD8-enriched LN cells added to the graft. The percentage of donor marrow-derived T cells and granulocytes in the peripheral blood of recipients surviving on days 28, 61, and 97 after transplantation was determined by two-color staining with CD3- and Ly5.1-specific antibodies. Data indicate results for individual recipients.
regression, 6.8

Ly5.1 marrow in 550-cGy irradiated bm1 recipients could not

recipients survived and were engrafted with Ly5.1-positive cells

3

13 (15%), and in 0 of 5 (0%) recipients transplanted with grafts respectively containing 5.0

10^4 T cells. By logistic regression, 6.8 \times 10^4 LN CD8 cells were sufficient to prevent rejection in 50% of the recipients. In three experiments in which control CD8 cells were enriched by immunomagnetic positive selection, rejection occurred in 8 of 10 (80%), 4 of 10 (40%), and in 0 of 10 (0%) recipients transplanted with grafts respectively containing 5.0 \times 10^4, 2.0 \times 10^4, or 8.0 \times 10^4 T cells. By logistic regression, 1.66 \times 10^5 LN CD8 cells enriched by positive selection were needed to prevent rejection in 50% of the recipients (P = .04 as compared with LN CD8 cells enriched by negative selection). These results suggest that binding of immunomagnetic particles to CD8 molecules had a small effect on the ability of CD8 cells to prevent rejection, but this effect was easily overcome by increasing the number of CD8 cells added to the graft.

In four experiments in which control CD8 cells were enriched by immunoadherent negative selection, rejection occurred in 14 of 24 (58%), 2 of 13 (15%), and in 0 of 5 (0%) recipients transplanted with grafts respectively containing 5.0 \times 10^4, 2.0 \times 10^4, or 8.0 \times 10^4 T cells. By logistic regression, 6.8 \times 10^4 LN CD8 cells were sufficient to prevent rejection in 50% of the recipients. In three experiments in which control CD8 cells were enriched by immunomagnetic positive selection, rejection occurred in 8 of 10 (80%), 4 of 10 (40%), and in 0 of 10 (0%) recipients transplanted with grafts respectively containing 5.0 \times 10^4, 2.0 \times 10^4, or 8.0 \times 10^4 T cells. By logistic regression, 1.66 \times 10^5 LN CD8 cells enriched by positive selection were needed to prevent rejection in 50% of the recipients (P = .04 as compared with LN CD8 cells enriched by negative selection). These results suggest that binding of immunomagnetic particles to CD8 molecules had a small effect on the ability of CD8 cells to prevent rejection, but this effect was easily overcome by increasing the number of CD8 cells added to the graft.

†This strain had perforin expression disrupted by an insertion at the exon 3 BstEII site and was backcrossed to B6.  
‡This strain had perforin expression disrupted by an insertion at the exon 3 Sph I site and was fully backcrossed to B6.

Perforin-deficient donor CD8 cells were clearly impaired in their ability to prevent rejection. Results indicated that 2 to 3 \times 10^6 perforin-deficient CD8 cells enriched by positive or negative selection were needed to prevent rejection in 50% of recipients. With 1.0 \times 10^7 positively selected perforin-deficient CD8 cells added to the graft, rejection was prevented in all recipients. These results demonstrate that perforin-deficient CD8 cells have approximately 3% to 5% of wild-type activity in preventing marrow graft rejection in this model.

Prevention of marrow graft rejection by donor CD8 cells was tested with two strains having defects in both the Fas ligand and perforin pathways of cytotoxicity. Rejection was not prevented by as many as 2.0 \times 10^7 positively selected CD8 cells from doubly mutant donors with perforin expression disrupted by an insertion at the exon 3 Sph I site. Because this perforin-deficient strain was backcrossed to B6 for 8 to 10 generations, graft failure could have been caused through recognition of the donor B6-Ly5.1 marrow by the mutant T cells and not through failure to prevent rejection by the bm1 recipient. This issue was addressed in two ways. First, heavily irradiated (950 cGy) bm1 recipients were transplanted with grafts containing 5.0 \times 10^6 T-cell-depleted B6-Ly5.1 marrow cells alone (n = 4) or with 1.0 \times 10^7 CD8-enriched LN cells from (Sph I) perforin-deficient gld-homozygous donors added to the graft (n = 2). All recipients survived and were engrafted with Ly5.1-positive cells when tested at 28 and 61 days after transplantation (data not shown). These results demonstrate that rejection of the B6-Ly5.1 marrow in 550-cGy irradiated bm1 recipients could not have been caused by mutant T cells added to the graft. In a further experiment, we tested CD8 cells from doubly mutant donors generated from a perforin-deficient strain that was fully backcrossed to B6. Rejection was not prevented by 1.0 \times 10^7 positively selected CD8 cells from doubly mutant donors with perforin expression disrupted by an insertion at the exon 3 BstEII site (Table 2). Taken together, these results demonstrate that the doubly mutant T cells had less than 1% of wild-type activity in preventing marrow graft rejection in this model.

Ability of T cells with cytotoxicity mutations to cause GVHD. Results of a previous study showed that T cells from donors with defects in both the Fas ligand and perforin pathways of cytotoxicity did not cause GVHD in sublethally irradiated recipients.8 Our new findings demonstrating that donor T-cell-mediated cytotoxicity is necessary to prevent marrow graft rejection in sublethally irradiated recipients suggested that the absence of GVHD in the previous study could be explained by the lack of engraftment. Therefore, it was of interest to determine whether doubly mutant T cells could cause GVHD under conditions in which engraftment did not depend on the cytotoxic activity of these cells. For these experiments, lethally irradiated (1,100 cGy) BALB/c recipients were transplanted with 40 \times 10^6 T-cell-depleted B6 or B6-Ly5.1 marrow cells alone or with 1.0 \times 10^7 CD3 cells from doubly mutant donors added to the graft. All recipients were engrafted with donor cells, as demonstrated by H2 typing at 1 and 2 months after transplantation (data not shown). In each of three experiments, the perforin-deficient, Fas-ligand-defective T cells caused GVHD, as indicated by weight loss and B lymphopenia as compared with negative control recipients transplanted with marrow alone (Table 3). However, histopathologic examination

<table>
<thead>
<tr>
<th>T-Cell Phenotype</th>
<th>5 \times 10^6 CD8</th>
<th>2 \times 10^6 CD8</th>
<th>8 \times 10^6 CD8</th>
<th>2-3 \times 10^6 CD8</th>
<th>1-2 \times 10^7 CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>22/34 (65)</td>
<td>6/23 (26)</td>
<td>0/15 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fas-ligand-defective (gld)</td>
<td>15/15 (100)</td>
<td>0/5 (0)</td>
<td>0/5 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granzyme B-deficient</td>
<td>14/14 (100)</td>
<td>1/5 (20)</td>
<td>0/5 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perforin-deficient†</td>
<td>4/4 (100)</td>
<td>8/9 (89)</td>
<td>6/8 (75)</td>
<td>4/8 (50)</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>Perforin-deficient/gld</td>
<td>5/5 (100)</td>
<td>5/5 (100)</td>
<td>5/5 (100)</td>
<td>11/11 (100)</td>
<td></td>
</tr>
<tr>
<td>Perforin-deficient/gld†</td>
<td>5/5 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Irradiated (550 cGy) bm1 recipients were transplanted with grafts containing 5.0 \times 10^4 B6-Ly5.1 T-cell-depleted marrow cells and the indicated numbers of CD8-enriched LN cells from wild-type B6 donors or from donors with mutations in T-cell cytotoxicity. Data indicate the proportions (percentages) of survivors with less than 20% Ly5.1-positive granulocytes in the blood when last tested at 1 to 3 months after transplantation. All but 7 engrafted recipients were observed for at least 2 months after transplantation and had 35% to 100% (mean, 93.5%) donor marrow-derived granulocytes when last tested before being euthanized. Only 1 engrafted recipient had less than 50% donor marrow-derived granulocytes at the time of euthanasia. Recipients with rejection had 0% to 13% (mean, 2.52%) donor marrow-derived granulocytes when last tested before being euthanized. Rejection occurred in 39 of 40 (97%) recipients transplanted with marrow containing no added T cells. Data are summarized from a series of seven experiments.

*In four experiments in which control CD8 cells were enriched by immunoadherent negative selection, rejection occurred in 14 of 24 (58%), 2 of 13 (15%), and in 0 of 5 (0%) recipients transplanted with grafts respectively containing 5.0 \times 10^4, 2.0 \times 10^4, or 8.0 \times 10^4 T cells. By logistic regression, 6.8 \times 10^4 LN CD8 cells were sufficient to prevent rejection in 50% of the recipients. In three experiments in which control CD8 cells were enriched by immunomagnetic positive selection, rejection occurred in 8 of 10 (80%), 4 of 10 (40%), and in 0 of 10 (0%) recipients transplanted with grafts respectively containing 5.0 \times 10^4, 2.0 \times 10^4, or 8.0 \times 10^4 T cells. By logistic regression, 1.66 \times 10^5 LN CD8 cells enriched by positive selection were needed to prevent rejection in 50% of the recipients (P = .04 as compared with LN CD8 cells enriched by negative selection). These results suggest that binding of immunomagnetic particles to CD8 molecules had a small effect on the ability of CD8 cells to prevent rejection, but this effect was easily overcome by increasing the number of CD8 cells added to the graft.

‡This strain had perforin expression disrupted by an insertion at the exon 3 BstEII site and was backcrossed to B6.  
§This strain had perforin expression disrupted by an insertion at the exon 3 Sph I site and was backcrossed to B6 for 8 to 10 generations.

For personal use only.on September 24, 2017. by guest
of the skin, liver, gastrointestinal tract, and lungs with blinding of treatment groups showed no differences between mice transplanted with grafts containing mutant T cells and those transplanted with marrow alone. Recipients transplanted with grafts containing doubly mutant T cells did not have lymphadenopathy at the time of autopsy.

**DISCUSSION**

Results of the current study demonstrate that cytotoxicity mediated by either perforin- or Fas ligand-dependent mechanisms is necessary for enabling donor T cells to prevent allogeneic marrow graft rejection. Previous studies have shown that rejection of H-2K-disparate marrow is caused by recipient CD8 effectors that survive the pretransplant conditioning regimen. Our current results therefore indicate that these cells are sensitive to cytotoxicity mediated by both perforin or Fas ligand-dependent mechanisms. These results are consistent with observations that Con A blasts are sensitive to lysis by either mechanism but are not lysed by CTL in short-term (4 hours) assays when both mechanisms were absent. Even though activated CD8 cells are sensitive to apoptosis induced by ligation of TNFp75 receptors under certain conditions, cytokine-mediated mechanisms are not sufficient for the elimination of recipient CD8 cells that cause marrow graft rejection.

Comparison of results with Fas-ligand defective CD8 cells and perforin-deficient CD8 cells suggests that the ability of donor T cells to prevent marrow graft rejection depends predominantly on perforin-dependent mechanisms of cytotoxicity. The striking difference in results between granzyyme B-deficient and perforin-deficient T cells confirms that granzyme B is not essential for granule exocytosis mechanisms of cytotoxicity. In the absence of perforin, large numbers of donor CD8 cells were needed in order to prevent rejection, whereas Fas-ligand-defective CD8 cells were minimally impaired in their ability to prevent rejection. The relative importance of the perforin pathway as compared with the Fas-ligand pathway in cytotoxicity against T-cell targets has been demonstrated previously. Braun et al. showed that sublethally irradiated perforin-deficient H2d recipients could not reject allogeneic H2a BALB/c spleen cell grafts and were therefore susceptible to lethal acute GVHD, whereas identically treated Fas-ligand-defective recipients did not develop GVHD, suggesting that the ability to prevent allogeneic T-cell engraftment was not impaired. These results are consistent with in vitro observations that the absence of Fas on Con A blast targets has limited effects on their susceptibility to lysis by CTL.

Baker et al. have reported contrary results in experiments with B6 donors and lethally irradiated H2-compatible C3H.SW recipients. In their experiments, readily detectable numbers of host-derived myeloid cells, B lymphocytes, and thymocytes remained in recipients transplanted with grafts containing Fas-ligand-defective T cells, but few, if any, such host-derived cells were found in identically treated recipients transplanted with grafts containing perforin-deficient T cells. These results suggested that Fas-ligand-dependent mechanisms of cytotoxicity were predominantly responsible for eliminating recipient hematopoietic progenitors that survived the pretransplant conditioning regimen.

At least two explanations can be invoked to account for the differences in results among these studies. First, the targets killed by cytotoxic effectors were not identical in the two studies. T cells of the recipient or donor were the targets evaluated in our experiments, whereas hematopoietic progenitors of the recipient were the targets evaluated by Baker et al. Thus, T cells might be less susceptible to Fas-ligand-mediated cytotoxicity or more susceptible to perforin-mediated cytotoxicity as compared with hematopoietic progenitors. Second, the effectors evaluated in the two studies might not be identical. Although we have tested LN CD8 cells, the effectors responsible for elimination of recipient cells in the H2-identical B6 → C3H.SW transplant model have not been identified. If the minor histocompatibility antigens that cause GVHD in this model are presented by MHC-class II molecules, the effectors could be CD4 cells. This suggestion is consistent with observations that CD4 cells mediate cytotoxicity through Fas-ligand-dependent mechanisms and not through perforin-dependent mechanisms.

The availability of murine strains with induced mutations has facilitated considerable progress in dissecting the effector mechanisms contributing to GVHD. Previous studies have shown that perforin-deficient T cells have a decreased ability to induce GVHD as measured by the number of donor T cells required to cause mortality in recipients with disparity for major or minor histocompatibility antigens. Recipients transplanted with Fas-ligand-defective T cells from gld donors developed cachexia, but inflammatory lesions in the skin and liver were absent, and the profound B-lymphoid hypoplasia typically associated with GVHD did not occur. Our current results highlight the role of cytokine-mediated mechanisms by demonstrating that GVHD can occur in the absence of Fas-ligand- and perforin-mediated cytotoxicity. On the other hand, with a sufficient number of donor cells, lethal GVHD can also
occur in the absence of signals transduced by ligation of TNFp55 receptors.23 Taken together, these results suggest that GVHD can develop both through cell-mediated cytotoxicity and through cytokine-mediated mechanisms. Our results confirm the earlier report by Baker et al,22 who showed that inflammatory cell infiltrates and apoptotic epitelial damage are not prominent in the liver and skin when GVHD is caused by cells that lack Fas-ligand.

Our current results demonstrating the importance of perforin-mediated cytotoxicity as a mechanism enabling donor T cells to prevent allogeneic marrow graft rejection suggest possible approaches for future clinical trials. Recent studies have shown that cytotoxic CD8 cells can have either a type 1 or type 2 cytokine profile.24,25 Type 1 polarized CD8 cells mediate cytotoxicity through either Fas-ligand or perforin-dependent mechanisms, whereas type 2 polarized CD8 cells rely predominantly on perforin-dependent mechanisms.26 Type 2 polarized CD8 cells also have a reduced ability to cause GVHD as compared with type 1 polarized cells.27,28 Taken together, these results suggest that retention of type 2 polarized donor CD8 cells in the graft could avoid marrow graft rejection when T-cell depletion is used to prevent GVHD.

ACKNOWLEDGMENT

The authors thank Kelli McIntyre and Natasha Leman for technical assistance and animal husbandry, Dr Wendy Leisenring for statistical consultation, Drs Illona Rimm and Claudio Anasetti for critical reading of the manuscript, and Alison Sell for assistance in preparing the manuscript.

REFERENCES

17. Valverde DA, Taylor PA, Sprent J, Blazar BR: The role of host T cell subsets in bone marrow rejection directed to isolated major histocompatibility complex I versus class II differences of bm1 and bm12 mutant mice. Transplantation 57:249, 1994
Involvement of Donor T-Cell Cytotoxic Effector Mechanisms in Preventing Allogeneic Marrow Graft Rejection

Paul J. Martin, Yoshiki Akatsuka, Michael Hahne and George Sale