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Accelerated Onset and Increased Severity of Acute Graft-Versus-Host Disease Following Adoptive Transfer of DR6-Deficient T Cells

Jinqi Liu, Josef G. Heuer, Songqing Na, Elizabeth Galbreath, Tonghai Zhang, Derek D. Yang, Andrew Glasebrook, and Ho Yeong Song

DR6 is a recently identified member of the TNFR family. In a previous study, we have shown that DR6 KO mice have enhanced CD4+ T cell proliferation and Th2 cytokine production. Acute graft-vs-host disease (GVHD) results from the activation and expansion of alloreactive donor T cells following bone marrow transplantation. In this article, we demonstrate that the transfer of donor T cells from DR6 KO mice into allogeneic recipient mice in a parent into an F1 model of acute GVHD results in a more rapid onset of GVHD with increased severity. Recipients of DR6 KO T cells exhibit earlier systemic symptoms of GVHD, more rapid weight loss, earlier histopathological organ damage in the thymus, spleen, and intestines, and earlier mortality. The rapid onset of GVHD in these mice may be attributable to the enhanced activation and expansion of DR6 KO CD4+ and CD8+ T cells. Our findings support the hypothesis that DR6 serves as an important regulatory molecule in T cell immune responses. The identification and use of DR6 ligands and/or agonistic Abs to DR6 may represent useful therapeutics in the treatment of T cell-mediated diseases such as GVHD. The Journal of Immunology, 2002, 169: 3993–3998.

A llogeneic Bone marrow transplantation is increasingly being used clinically to treat hematological malignancies and various other genetic and hematological disorders (1). Although patients are typically put on posttransplant immunosuppressive regimens, graft-vs-host disease (GVHD) continues to be a significant problem associated with this procedure (2, 3). Acute GVHD presents itself as a progressive systemic disease characterized by cachexia and substantial organ damage, including the liver, intestines, and skin, and typically concludes with death.

Acute GVHD is caused by the allogenic recognition of host tissue by mature donor T cells present in the graft. This has been demonstrated in both experimental animal models of GVHD and in clinical studies through the depletion of mature T cells in the donor inoculum with the use of anti-T cell Abs (4, 5). Although the depletion of T cells from donor grafts can prevent the onset of GVHD, this procedure is offset by the increased risk of graft failure in patients and the loss of a graft-vs-leukemia effect (6–8).

The recognition of foreign host tissue by alloreactive T cells results in their initial activation, proliferation, and subsequent production of cytopathic amounts of cytokines (9, 10). Cytokine production is typically exacerbated by the high-dose chemotherapy and radiotherapy that typically precludes a bone marrow transplant. These pretransplant conditioning regimens result in damage to the intestinal epithelium and leakage of endotoxin from the gut into the host’s system, which can trigger monocyte and macrophage cells to release proinflammatory cytokines (11, 12). The migration of activated monocytes, macrophages, and autoreactive T cells into host target organs results in substantial organ damage through direct cytotoxic injuries mediated through proinflammatory cytokines, Fas/Fas ligand (L), or perforin/granzyme pathways (13–16). Further identification of important molecules employed by T cells in the processes of T cell activation, expansion, and cytotoxicity during GVHD will facilitate our ability to develop better therapeutic treatments for this disease.

DR6 is a recently identified death domain containing the orphan receptor of the TNFR superfamily (17). Several of these family members and their ligands are involved in T cell activation and effector functions (18). Our previous study described the generation and characterization of DR6 KO mice (19). We demonstrated that DR6 plays an important regulatory role for both T cell activation and differentiation. The purpose of this study was to determine the potential function of DR6 in acute GVHD. A murine parent-into-F1 model of acute GVHD was used to study the ability of DR6 KO T cells to induce GVHD and to gain a better understanding of DR6 function in the physiological setting of a T cell-mediated disease.

Materials and Methods

Mice
Six- to 8-wk-old male C57BL/6 (B6) and B6 × DBA/2 F1 (BDF1; H-2b + d) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). BALB/c mice were purchased from Harlan (Indianapolis, IN). The details of the generation of DR6 KO mice are described in the study of Liu et al. (19). Chimeric mice (H-2b), produced by injection of targeted mutant embryonic stem cells (129) into B6 blastocysts, were crossed to B6 mice (H-2b) to generate B6 × 129 F1 mice. Heterozygous DR6+/− mice from this cross were interbred to generate F2 mice that were subsequently analyzed for the presence or absence of the DR6 allele by Southern blotting.
Offspring identified as homozygous DR6 null (−/−) or wild type (+/+) were interbred to generate subsequent generations. Donor mice for the induction of GVHD were randomly selected from F2 or F3 mice identified as either null or wild type. All mice were housed in microisolator cages and fed autoclaved chow and acid water ad libitum.

Cell lines and reagents
All tissue culture media, reagents, and FBS were obtained from Life Technologies (Gaithersburg, MD). The P815 mastocytoma cell line was purchased from the American Type Culture Collection (Manassas, VA) and was grown in IMDM supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. All fluorochrome-labeled Abs for flow cytometry were purchased from BD Pharmingen (San Diego, CA). Cytokine ELISA kits were purchased from R&D Systems (Minneapolis, MN) and used according to the manufacturer’s instructions.

Isolation of T cells
Splenocytes were obtained by macerating spleens in IMDM supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin with a Dounce homogenizer and then passing the cells through a mesh filter (Falcon; BD Biosciences, Franklin Lakes, NJ). Cells were then layered over Histopaque 1119 (Sigma-Aldrich, St. Louis, MO) and centrifuged for 45 min at 750 rpm without brake. Cells at the interface were removed and washed three times with cold PBS supplemented with 2% BSA. T cells were then isolated by positive selection with anti-Thy1.2-conjugated microbeads and MACS (Miltenyi Biotec, Auburn, CA) according to the manufacturer’s instructions. Cells not selected for anti-Thy1.2 were collected and used as a T cell-depleted control group. A fraction of isolated T cells was assayed by immunostaining with a FITC-conjugated anti-CD3 Ab and flow cytometric analysis on a FACScan machine (BD Biosciences, San Jose, CA). The purity of T cells was typically >94%.

Induction of GVHD
Isolated T cells were washed in HBSS twice and resuspended at 5 × 10⁶ cells/ml. Recipient male BDF1 mice were weighed and then sublethally irradiated with 500 rad of a 137Cs source at a rate of 107 rad/min. Irradiated mice were injected with 2.5 × 10⁷ T cells in 0.5 ml of HBSS via the tail vein within 1 h of irradiation. Control mice received 2.5 × 10⁷ T cells-depleted (TCD) (3) splenocytes, which consisted mainly of B cells. Mice were monitored daily for symptoms of GVHD such as ruffled fur, hunched posture, inactivity, eye lesions, and snout swelling and were weighed every 2 days. Some mice were sacrificed at 5, 7, or 10 days posttransplant for analysis of various parameters. All animal experiments were approved and conducted according to Guidelines of the Animal Care and Use Committee at Eli Lilly (Indianapolis, IN).

Flow cytometry analysis
Splenocytes and thymocytes were analyzed with fluorescent-conjugated Abs against CD4, CD8, and CD19 to determine donor T cell expansion and host B cell depletion. Abs specific to the mouse MHC marker H-2Kd were used to distinguish host cells from donor cells. Donor cells were identified in histograms by the absence of H-2Kd staining. Flow cytometric analysis was conducted by resuspending cells in PBS/1% BSA at 1 × 10⁶ cells/ml in a volume of 200 µl/sample. FITC-conjugated anti-mouse H-2Kd and PE-conjugated anti-mouse CD4, CD8, and CD19 were used for staining. CD4⁺ CD8⁻ T cells in the thymus were also analyzed with a FITC-conjugated anti-mouse CD4 Ab and a PE-conjugated anti-mouse CD8 Ab. Activation of donor T cells by alloantigens in vivo was assessed with FITC-conjugated anti-mouse CD25, PE-conjugated anti-mouse CD28, PE-conjugated anti-mouse CD40L, and PE-conjugated anti-mouse FasL Abs. Cells were analyzed on a fluorescence activated cell sorter purchased from BD Biosciences.

Histopathological analysis and TUNEL assay
Transplanted mice were necropsied and tissues were fixed in fresh 4% paraformaldehyde and transferred into 70% ethanol. The tissues were subsequently processed by dehydration, clearing, and embedding in paraffin according to standard histological procedures. Blocks were sectioned at 5 µm and prepared for immunohistochemistry by mounting of sections onto electrostatically charged slides (ProbeOn Plus; Fisher Scientific, Pittsburgh, PA). Tissue sections were rehydrated, quenched of endogenous peroxidase, and blocked in normal serum. For TUNEL, tissue sections were permeabilized with proteinase K and labeled with digoxigenin-linked dUTP (Intergen, Purchase, NY). Incorporated dUTP was located with a biotinylated anti-digoxigenin Ab and visualized with the streptavidin complex with diaminobenzidine as the chromagen.

Mixed leukocyte reaction
Splenocytes from BALB/c mice (H-2Kb) were irradiated with 3000 rad of a 137Cs source at a rate of 250 rad/min and used as allogeneic stimulator cells. Responder splenocytes from either wild-type or DR6 KO mice (H-2Kb) were mixed with the stimulator cells to initiate the reaction. Both cell populations were seeded at 4 × 10⁵ cells/well of a 96-well tray in 0.2 ml of IMDM supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. After 72 h of stimulation, wells were pulsed with 1 µCi of [3H]thymidine and allowed to incubate another 6 h before harvesting the cells and counting.

CTL assays
Cytotoxic activity was measured directly (without in vitro restimulation) from splenocytes of transplanted mice. Purified splenocytes were obtained and seeded into wells of a 96-well U-bottom plate at different E:T ratios. Target cells were P815 mastocytoma cells (H-2Kb) with EL-4 thymoma cells (H-2Kb) as a syngeneic control. CTL assays were set up and conducted with a Cytotox 96 nonradioactive kit purchased from Promega (Madison, WI) according to their instructions.

Statistical analysis
Statistical analysis of survival was done by a Kaplan-Meier survival analysis. Values for in vitro experiments and cell numbers are expressed as the mean ± SEM, unless indicated otherwise. Statistical comparisons between groups was made using Student’s unpaired t test. Values of p < 0.05 were considered to be significant.

Results
Recipients of DR6 KO donors rapidly lose weight and exhibit earlier mortality
To determine the effect of DR6 deficiency on the development of GVHD, we used a well-characterized murine parent-to-F1 model of acute GVHD (20, 21). In this model, wild-type donor T cells (H-2Kb) are engrafted into sublethally irradiated BDF1 recipient mice (H-2Kd). Although the mice do not receive a bone marrow transplant in this model, the amount of sublethal irradiation administered to the mice is not sufficient by itself to induce mortality by bone marrow aplasia. Mice that are irradiated and receive either no graft or TCD grafts recover hemopoietically within 2–3 wk postirradiation and survive beyond 60 days (J. G. Heuer, unpublished observations). However, recipients of T cells acutely develop GVHD that leads to pronounced weight loss beginning around 14–16 days posttransplant and 100% mortality by 22–26 days posttransplant (Fig. 1 and J. G. Heuer, unpublished observations). The weight loss and mortality characteristic of acute GVHD are not observed in TCD grafts (Fig. 1). BDF1 recipients of DR6 KO donor T cells (H-2Kb) exhibited a much more rapid weight loss, beginning around 5 days posttransplant (Fig. 1A). The most rapid reduction in weight was observed between days 7 and 9. This earlier onset of rapid weight loss resulted in a significant increase in mortality of these recipients compared with wild-type T cell recipients (p < 0.0001) and reached 100% by 16 days posttransplant (Fig. 1B).

Greater donor T cell expansion and host B cell depletion in recipients of DR6 KO donor cells
To identify the potential mechanism resulting in the earlier onset of GVHD in the recipients of DR6 KO donor T cells, we analyzed spleen cells with fluorescent-labeled Abs and flow cytometry to determine donor T cell engraftment and host B cell depletion at 7 days posttransplant. Our historical experience with this model of acute GVHD has shown that initial donor T cell expansion occurred by 5 days posttransplant and peaked at 12 days posttransplant (J. G. Heuer, unpublished observations). We chose the day 7...
time point as the body weight loss and mortality observed in recipients of DR6-deficient T cells occurred much sooner compared with wild-type recipient T cells. Body weight change of recipients was measured every 2 days. Recipients of DR6 KO donor T cells exhibited an earlier onset of rapid weight loss compared with wild-type donor T cells from week 1. Values represent the average weight of 10 animals from each group ± SD. Results were similar from a second independent experiment. B, Recipient animals were monitored for survival every 2 days. BDF1 recipients of DR6 KO donor T cells developed significantly higher mortality from week 2. Data are combined from two independent experiments. Each group consisted of 10 recipient mice per experiment.

**FIGURE 1.** BDF1 recipients of DR6 KO T cells have increased morbidity and mortality of GVHD. A, BDF1 mice were sublethally irradiated (5 Gy) and transplanted with T cells from either DR6 KO or wild-type mice or splenocytes from TCD wild-type donors. Body weight change of recipients was measured every 2 days. Recipients of DR6 KO donor T cells exhibited an earlier onset of rapid weight loss compared with wild-type donor T cells from week 1. Values represent the average weight of 10 animals from each group ± SD. Results were similar from a second independent experiment. B, Recipient animals were monitored for survival every 2 days. BDF1 recipients of DR6 KO donor T cells developed significantly higher mortality from week 2. Data are combined from two independent experiments. Each group consisted of 10 recipient mice per experiment.

**FIGURE 2.** Engraftment of donor T cells in BDF1 recipient mice. Purified T cells (2.5 × 10⁶) from splenocytes of wild-type and DR6 KO mice were injected i.v. into irradiated BDF1 mice. The group of BDF1 mice that received wild-type TCD splenocytes was used as a negative control. A, On day 7 after transplantation, splenocytes of recipient mice were examined for engraftment of donor CD4⁺ and CD8⁺ T cells. B, Depletion of host B cells was also measured by two-color immunofluorescent staining and flow cytometry. The data for both A and B are shown as the mean ± SEM for three mice from each group. Results were similar in two other replicate experiments.

Destruction of host thymus is pronounced with DR6 KO donors

Thymus is another target organ in acute GVHD. To determine whether DR6-deficient donor T cells could accelerate damage to the thymus, we analyzed recipients at 7 days posttransplant. Our observations indicated that the thymus in mice receiving DR6 KO grafts was extremely small (data not shown). We confirmed this by counting thymocytes from these mice (Fig. 3). Although TCD recipient mice had, on average, around 4–5 × 10⁷ thymocytes, recipients of wild-type grafts were 2-fold lower, indicating some loss in thymic cellularity. Strikingly, recipients of DR6 KO grafts had around 5 × 10⁵ thymocytes, indicating significant thymic GVHD in these mice. The loss of thymocytes in these mice predominantly represented CD4⁺CD8⁻ double-positive T cells, as determined by flow cytometry analysis (data not shown). We observed a small reduction in double-positive T cells for recipients of wild-type grafts as expected (77% vs 88%). However, the number of thymic double-positive T cells in recipients of DR6 KO grafts was remarkably lower compared with that observed with wild-type grafts (12% vs 77%).
DR6 KO recipients exhibit more severe intestinal GVHD pathology

The onset of GVHD, as determined by outward physical symptoms such as hunched posture, ruffled fur, inactivity, eye lesions, and swelling of the skin around the snout, was apparent in mice receiving DR6 KO grafts at a much earlier time posttransplant than in mice receiving wild-type grafts (data not shown). Physical symptoms of GVHD were apparent as early as 5 days posttransplant in DR6 KO recipients, whereas these symptoms appeared in wild-type recipients at a much later time, around 14–18 days posttransplant. We examined specific GVHD target organ pathology in recipients at 10 days posttransplant. Upon necropsy, skin, liver, spleen, and intestine including terminal ileum and ascending colon were preserved and embedded in paraffin for histological examination and TUNEL assays. Although other target organs exhibited ongoing GVHD pathology, the severity of GVHD was most pronounced in the small intestine of mice receiving DR6 KO grafts. To detect apoptotic cells, TUNEL assays were done and showed prominent cell death in the crypt epithelium of the small intestine, extending along the villous epithelium for both wild-type and DR6 KO grafts (Fig. 4). However, the frequency of apoptotic cells was visibly higher in mice receiving DR6 KO donors compared with wild-type donors (Fig. 4, Band C), whereas very few apoptotic cells were observed in mice receiving TCD donors (Fig. 4A). Apoptotic and necrotic intestinal epithelial cells were observed being sloughed into the lumen predominantly in recipients of DR6 KO donors.

Increased T cell activation in spleens of DR6 KO recipients

Because we observed greater donor T cell expansion and target organ pathology in recipients of DR6 KO grafts, we then decided to examine the percentage of activated T cells in these mice. Spleens were removed at 5 or 7 days posttransplant and cells were analyzed by flow cytometric analysis for the T cell activation markers CD25, CD28, CD40L, and FasL as shown in Table I. The percentages of T cells expressing these markers were significantly higher in mice receiving DR6 KO grafts vs wild-type or TCD at this early time point posttransplant, whereas wild-type vs TCD groups showed no significant differences at this time point. Since we also determined that the frequency of DR6 KO T cells in the spleens of recipient mice was >5-fold compared with wild type and the percentage of T cells that express activation markers is roughly 2-fold higher for DR6 KO recipients, the total number of activated T cells is roughly 10-fold higher in recipients of DR6 KO donors compared with that of wild type.

The response of DR6 KO T cells to alloantigens is greater in MLR and CTL assays

DR6 KO recipient mice exhibited a much faster onset and more severe GVHD pathology, as well as greater T cell activation than wild-type recipient mice. To further determine whether DR6 deficiency affects activation and effector functions of T cells in response to alloantigens, MLR and CTL assays were done. In the MLR, splenocytes from DR6 KO or wild-type mice (H-2Kb) were stimulated with irradiated splenocytes from BALB/c mice (H-2Kb) and then pulsed with tritiated thymidine at 72 h to measure proliferation. Proliferative responses were 2-fold higher for DR6 KO cells compared with those of wild-type cells (Fig. 5A). For the CTL assay, BDF1 mice were transplanted with either TCD cells,

<table>
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<th>DR6 KO</th>
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<td>8.5 ± 1.7</td>
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<td>CD28</td>
<td>4.4 ± 0.8</td>
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<tr>
<td>CD40L</td>
<td>4.6 ± 1.0</td>
<td>7.4 ± 1.4</td>
<td>15 ± 1.8</td>
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<td>FasL</td>
<td>9.77 ± 5.5</td>
<td>11.5 ± 2.8</td>
<td>20.7 ± 6</td>
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FIGURE 3. Enhanced destruction of thymocytes by transplantation of DR6-deficient T cells in allogeneic recipients. On day 7 after transplantation, the size of the thymus from each transplantation group was compared and cell numbers of recipient thymi were counted. The data are expressed as the mean ± SEM for three mice per group. Results were similar in two other independent experiments.

FIGURE 4. BDF1 recipient mice transplanted with DR6 KO T cells developed increased intestinal GVHD pathology. At 10 days posttransplantation, three animals per group were sacrificed and organs were removed and processed for histology. Tissue sections were prepared for histology and TUNEL assays. Histological examination by TUNEL indicated more severe apoptosis in the small intestine of DR6 KO mice. Since results were similar among mice in a particular group, only one representative mouse is shown per group. A–C, In the small intestine of DR6 KO mice, apoptosis was most predominant in the crypt epithelium, extending along the villous epithelium (C). Apoptotic and necrotic cells were sloughed into the lumen. The intestines of the TCD group were essentially normal (A), and there was some beginning evidence of GVHD in the intestines of the wild-type group (B).
The Journal of Immunology

A

FIGURE 5. DR6 KO T cells show increased T cell proliferation in a MLR and cytotoxicity in a CTL assay compared with wild-type T cells. A. Splenocytes from BALB/c mice (H-2d) were used as allogeneic stimulators and were irradiated with 3000 rad before the cells were mixed with responder splenocytes from wild-type or DR6 KO mice (H-2d). Both stimulator and responder cells were seeded at 4 × 10^5/well. Cells were incubated for 72 h and [3H]thymidine was used to pulse the cells for the last 6 h of incubation. Proliferative responses are shown as cpm from incorporation of [3H]thymidine. The data are depicted as the mean ± SEM for three replicates per group. Similar results were obtained in two other independent experiments. B. BDF1 recipients were transplanted as described previously. At 10 days posttransplantation, animals were sacrificed and spleens were harvested. After RBC lysis, CTL activity of splenocytes from transplanted recipients was examined for cytotoxicity against host Ags (P815 mouse mastocytoma, H-2d). CTL activity of splenocytes from transplanted recipients was also examined for cytotoxicity against syngeneic Ag (EL-4 thymoma cells, H-2d) with no apparent cytotoxicity observed (data not shown). The data are shown as the mean ± SEM of three replicates per group. A second independent experiment also yielded similar results.

Discussion

This study reports that T cells lacking the DR6 receptor generated a much more severe form of acute GVHD. Recipients of DR6 KO T cells exhibited earlier mortality, a more rapid onset of weight loss and systemic symptoms, increased expansion of both donor CD4^+ and CD8^+ T cells, enhanced T cell activation, more severe thymic atrophy, and increased apoptosis in intestinal epithelium. Although the increased pathologies we observed in these mice are probably multifactorial in nature, we propose that a major contributory factor is the increased expansion of alloreactive DR6 KO T cells vs wild-type T cells. Although the same number of T cells were transplanted from either wild-type or DR6 KO donors on day 0, there was roughly 10-fold more activated T cells in mice transplanted with DR6 KO donors vs wild type at 7 days posttransplantation. This observation suggests that alloreactive T cells lacking the DR6 receptor increase in number more rapidly than wild-type T cells in response to in vivo Ag stimulation. The more rapid increase in alloreactive T cell numbers for the DR6 KO donors could lead to more severe pathology through a simple mass effect. In support of this idea is the observation that increasing the donor T cell inoculum in a transplant leads to more severe GVHD with greater morbidity and mortality (Ref. 22; J. G. Heuer, unpublished observations).

A more rapid expansion in vivo could be due to either increased proliferation, improved survival, or both. We suggest that the increased expansion is due to increased proliferation as this idea is supported by our previous observations in vitro (19) and the increased proliferation in DR6 KO T cells in response to alloantigens in a MLR assay (Fig. 5A). We have previously reported that CD4^+ T cells lacking DR6 exhibited increased proliferation in response to in vitro anti-TCR stimulation. This effect was not due to alterations in cell survival, but to an increased sensitivity of these cells to IL-2 through up-regulation of CD25 (IL-2Rα) and CD28 and the down-regulation of CTLA-4 (19). DR6 KO T cells taken from mice with ongoing GVHD also expressed significantly higher levels of CD25 and CD28 as well as enhanced expression of the costimulatory molecule CD40L. The importance of CD28-B7 interactions, in particular, has been well characterized in models of GVHD. Blockade of CD28-B7 interactions, either through the use of CD28 KO donors (23, 24), anti-CD28 Abs (24), or anti-B7 Abs (25, 26), has been shown to inhibit donor T cell expansion and decrease GVHD-induced mortality. Thus, the distinct expression profile of DR6 on activated DR6 KO T cells may contribute to their rapid expansion in vivo.

Both CD4^+ and CD8^+ donor T cells from DR6 KO donors expanded much more rapidly than the cells of wild-type donors during the course of GVHD. In our previous study, DR6 deficiency did not affect CD8^+ T cell proliferation in vitro (19). However, the highest expression of DR6 was observed in resting CD8^+ T cells, while the level of DR6 decreased after the activation of CD8^+ T cells (J. Liu, unpublished data), indicating a potential role of DR6 in mediating CD8^+ T cell immune responses. The observation of increased CD8^+ T cell proliferation for DR6 KO donors during GVHD could be explained by enhanced cytokine production from DR6 KO CD4^+ T cells that would provide support for CD8^+ T cell proliferation in vivo. It is also possible that the effect of DR6 deficiency on CD8^+ T cells in vivo is different from that in vitro. For instance, the DR6 ligand may normally be expressed only on CD4^+ T cells, thus the effects due to DR6 deficiency would be observed in vitro only for CD4^+ T cells. Nevertheless, the effects of DR6 deficiency on CD8^+ T cells would be more apparent under in vivo physiological conditions. Identification of the ligand for DR6 will allow for a better understanding of the effects of DR6 signaling on CD8^+ T cells vs CD4^+ T cells. We have now demonstrated that in addition to the enhanced proliferative response of DR6 KO T cells in vitro, the absence of DR6 also enhanced alloanigen-specific T cell proliferation in vivo. The property of increased expansion for DR6 KO T cells suggests a role for DR6 in vivo limiting T cell immune responses and agrees very well with our initial hypothesis that DR6 is a negative regulator of T cell activation and expansion (19).

The observation that transfer of DR6 KO T cells resulted in more severe thymic, splenic, and intestinal GVHD pathology is
intriguing. It has been reported that intestinal GVHD is mediated through TNF-α and IFN-γ (15, 27–31) and that TNF-α also contributes to lymphoid atrophy during GVHD (31). We did observe increases in serum IFN-γ and TNF-α levels in DR6 KO recipients vs wild-type recipients at 7 days posttransplant (data not shown). The increase in serum cytokines could simply be attributed to the increased numbers of activated DR6 KO donor T cells vs wild-type donors. Thus, the enhanced lymphoid and intestinal pathologies in our study may also be due to the increased production of these cytokines resulting from the greater expansion of DR6 KO T cells. The increased allospecific in vitro cytotoxic activity of DR6 KO splenocytes at 7 days posttransplant that we observed could also help explain the more severe organ pathologies. The increased cytotoxicity in vitro is most likely due to the greater number of activated DR6 KO T cells present in the spleens of recipients at 7 days posttransplant. The greater percentage of FasL expression on DR6 KO T cells from GVHD mice could also play a role. The thymus is a well-known target of acute GVHD and constitutively produces a multitude of chemokines that could attract transplanted T cells (32). The dendritic cells in the thymic medulla provide an optimal niche for the activation and expansion of allosecretive T cells (33) and CD4/CD8 double-positive thymocytes are known to express Fas and are sensitive to FasL killing (34). The increased thymic GVHD seen with DR6 KO donors could simply be due to the increased numbers of activated DR6 KO T cells expressing FasL compared with wild-type T cells. The explanation for the absence of more severe hepatic GVHD in the recipients of DR6 KO donor cells is unclear. Because we examined GVHD target organ pathology at only a single time point posttransplant, it is possible that increased hepatic pathology might be observed at a later time point posttransplant.

Our study demonstrated that T cells derived from DR6 KO mice exacerbated GVHD, and although we cannot rule out other factors responsible for the greater pathologies in these mice, we suggest that the increased expansion and activation of allosecretive DR6 KO T cells has a major role. The ligand for DR6 remains unidentified, yet from our analysis of DR6 KO mice, we have concluded that DR6 and its ligand have an important regulatory role in limiting T cell immune responses. Identification of the DR6 ligand or development of agonistic Abs to DR6 may have potential therapeutic utility in the treatment of T cell-mediated diseases such as acute GVHD.

Acknowledgments

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