Alloantigen Affinity and CD4 Help Determine Severity of Graft-versus-Host Disease Mediated by CD8 Donor T Cells

Xue-Zhong Yu, Michael H. Albert and Claudio Anasetti

*J Immunol* 2006; 176:3383-3390; doi: 10.4049/jimmunol.176.6.3383

http://www.jimmunol.org/content/176/6/3383

References
This article cites 32 articles, 21 of which you can access for free at:
http://www.jimmunol.org/content/176/6/3383.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Alloantigen Affinity and CD4 Help Determine Severity of Graft-versus-Host Disease Mediated by CD8 Donor T Cells

Xue-Zhong Yu,2* Michael H. Albert,3* and Claudio Anasetti4*†

TCR affinity dictates T cell selection in the thymus and also has a high impact on the fate of peripheral T cells. Graft-vs-host disease (GVHD) is a pathological process initiated by activation of donor T cells after adoptive transfer into an allogeneic recipient. How TCR affinity affects the potential of alloreactive T cells to induce GVHD is unclear. Using alloreactive CD4\(^+\) and CD8\(^+\) TCR transgenic (Tg) T cells, GVHD models are presented that allow for the visualization of how CD8\(^+\) alloreactive T cells behave in response to alloantigens with different TCR affinity in the absence or presence of CD4 help. In a nonmyeloablative transplant model where GVHD lethality is due to marrow aplasia, alloreactive CD8\(^+\) TCR Tg T cells induced significantly more severe GVHD in the recipients that express an intermediate-affinity alloantigen than in the recipients that express a high-affinity alloantigen. In a myeloablative transplant model where GVHD lethality is due to epithelium injury, CD8\(^+\) TCR Tg T cells were also more pathogenic in the recipients with an intermediate-affinity alloantigen than in those with a high-affinity alloantigen. The presence of alloreactive CD4\(^+\) TCR Tg cells enhanced the potential of CD8\(^+\) TCR Tg cells to cause GVHD in recipients with an intermediate-, but not with a high-, affinity alloantigen. These findings underscore that alloantigen affinity and CD4 help control the fate and pathogenicity of alloreactive CD8\(^+\) T cells in vivo. The Journal of Immunology, 2006, 176: 3383–3390.

Graft-vs-host disease (GVHD)\(^5\) is a pathological process initiated by activation of donor T cells after adoptive transfer into an allogeneic recipient. The types and degree of histocompatibility disparities between the donor and recipient, host conditioning regimes, and ongoing inflammation control alloreactive T cell responses and GVHD manifestations (1). It is assumed that CD4 and CD8 T cell cooperation is required for the induction of lethal GVHD. Understanding the interactions between grafted CD4 and CD8 T cells is important for developing better strategies to prevent GVHD. However, donor T cells that recognize recipient alloantigens lack specific markers and are only present at a low frequency in vivo, making it difficult to monitor and characterize them directly in the host. The availability of an experimental model in which identifiable T cell populations with known alloantigen specificity cause GVHD would make it feasible to study the fate of T effector cells and the development of GVHD in vivo. With such a model, it would be possible to test strategies designed to eliminate or inactivate T cells responsible for GVHD while preserving other T cell populations that do not recognize recipient alloantigens thereby preserving anti-infectious or antitumor immunity.

The use of TCR transgenic (Tg) T cells has allowed the visualization of Ag-specific T cell population dynamics and function in vivo. Previous attempts to develop a clinical model of GVHD using alloreactive Tg T cells have met with limited success. Transfer of L\(^d\) (2, 3) or H-Y-specific (4) CD8\(^+\) Tg cells into Ag-expressing hosts results in a short burst of T cell expansion, followed by apoptosis and the development of anergy in the residual cells. Those Tg cells did not cause overt GVHD in these models. However, a polyclonal CD4\(^+\) T cell population, when transferred together, increased the severity of GVHD initiated by anti-L\(^d\) Tg T cells (5). Interaction of CD4 and CD8 T cells in the development of GVHD has been addressed by adoptive transfer of TCR Tg CD4\(^+\) and CD8\(^+\) cells into a recipient that expresses specific alloantigens for both T cell subsets (6). However, this study was limited to sublethal conditioning of the host, which does not adequately represent the clinical situation of GVHD because recipient death is caused by destruction of recipient hemopoiesis and not epithelial damage in typical GVHD target organs such as gut, skin, and liver.

To address these limitations, we developed a novel system in which CD4\(^+\) D10 and CD8\(^+\) 2C TCR Tg populations alone or together were transferred in combination with donor marrow cells into lethally irradiated recipients that express alloantigens to be recognized by either T cell population. The D10 TCR is positively selected by IA\(^b\) in the thymus and recognizes the IA\(^b\) alloantigen (7). The 2C TCR is positively selected by H-2K\(^b\) and negatively selected by H-2L\(^d\) in the thymus (8, 9). The 2C TCR binds to a natural peptide p2Ca presented by H-2L\(^d\) as an alloantigen with a high affinity (10). A naturally occurring mutant of H-2K\(^b\), termed H-2K\(^{bm3}\) (Asp\(^77\) to Ser, Lys\(^80\) to Ala) is also an alloantigen to 2C (11). The 2C TCR binds to a natural peptide dEV8 presented by H-2K\(^{bm3}\) with an intermediate affinity and to the same peptide presented by H-2K\(^b\) with a low affinity, which is lower by factors of ~20 and 30, respectively, than the p2Ca/H-2L\(^d\) complex (12–14). Using the D10 and 2C TCR Tg systems, we were able to track the fate and pathogenicity of CD4\(^+\) or CD8\(^+\) effector cells alone.

\(^{2}\)Current Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; and \(^{1}\)Department of Medicine, University of Washington, Seattle, WA 98195

\(^{3}\)Received for publication June 7, 2005. Accepted for publication January 6, 2006.

\(^{4}\)The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\(^{5}\)This work was supported by National Institutes of Health Grants CA 84132 (to X.-Z.Y.), CA 18029, AI 51693 (to C.A.), and by a grant from Deutsche Krebshilfe (to M.H.A.).

\(^{6}\)Address correspondence and reprint requests to Dr. Xue-Zhong Yu at the current address: Experimental Therapeutics Program, SRB-2, H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, 12902 Magnolia Drive, Tampa, FL 33612. E-mail address: yuxz@moffitt.usf.edu

\(^{7}\)Current address: Dr. von Haunersches Children’s Hospital, Ludwig-Maximilians-University, Munich, Germany.

\(^{8}\)Current address: H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL 33612.

\(^{9}\)Abbreviations used in this paper: GVHD, graft-vs-host disease; Tg, transgenic; BM, bone marrow; TCD, T cell depleted.
or in combination in recipients that express specific alloantigens. Furthermore, we demonstrated that alloantigen affinity and CD4 help are the key factors that control GVHD development induced by CD8 alloreactive T cells.

Materials and Methods

Mice

C57BL/6 (B6), B6.SJL-Ly5.1 Ptpre· Pep3b (B6.Ly5.1), C3H, B10.BR, B6.C-H2b/H9-K/H2dJ/E3 (B6 bm3), (B6 × C3H)F1, (BALB/c × B6)F1 (C57BL), and (B6 × C3H)F1 mice were purchased from The Jackson Laboratory. Founders of 2C TCR Tg mice were provided by D. Loh (Nippon Roche Research Center, Kamakura, Japan). Founders of D10 TCR-Tg mice were provided by D. Sant’Angelo (Memorial Sloan-Kettering Cancer Center, New York, NY) (7). (B6 × bm3)F1 mice were bred in our facility. Mice were housed in microisolator cages at the Fred Hutchinson Cancer Research Center (Seattle, WA). Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee.

Isolation of T cells and bone marrow (BM) cells

CD4+ and CD8+ cells were purified from pooled spleen and lymph node cells by positive selection with a magnetic cell separation system (Miltenyi Biotec) as described previously (15). T cell purity ranged from 95 to 99%. BM was harvested from tibia and femurs, and T cells were depleted through complement lysis of Thy1.2+ cells.

Transplantation

In nonmyeloablative models, CB6F1, (B6 × bm3)F1, or (B6 × C3H)F1 mice were exposed to 750 cGy of irradiation at 20 cGy/min, and freshly isolated CD8+ or CD4+ cells were injected within 24 h after irradiation. In myeloablative models, CB6F1, (B6 × bm3)F1, or (B6 × C3H)F1 mice were exposed to 1000–1200 cGy of irradiation at 20 cGy/min, and T cell-depleted (TCD)-BM cells alone or in combination with purified CD4+ or CD8+ cells from indicated donors were injected via the tail vein to recipients within 24 h after irradiation. Recipient mice were monitored every other day for mortality and clinical signs of GVHD, such as ruffled fur, hunched back, inactivity, or diarrhea. Body weight was measured two times a week. Animals judged to be moribund (i.e., unable to take food or water) were sacrificed and counted as GVHD lethality.

Immunofluorescence analysis

Peripheral blood samples or spleens were collected from recipients at the time points indicated. Cells were stained and analyzed using a FACScan flow cytometer and CellQuest software (BD Biosciences). Anti-CD4-FITC, anti-CD8-PE, anti-H2b-biotin, anti-CD8-CyChrome, anti-CD4-CyChrome, streptavidin-PE, and streptavidin-CyChrome were purchased from BD Pharmingen. Biotin-labeled Ab specific for Ly5.1, 2C TCR (1B2), and D10 TCR (3D3) were prepared in our laboratory.

CTL assay

Cytotoxic activity of 2C cells was measured directly without in vitro restimulation as previously described (3). Briefly, spleen cells from each recipient were used as effectors against 51Cr-labeled P815 (Ld+) targets with a E:T at 40:1. Chromium release was measured after 4.5 h of incubation, and percent cytotoxicity was calculated as (experimental release − spontaneous release)/(maximal release − spontaneous release) × 100%.

Statistical analysis

The log-rank test was used to detect statistical differences in recipient survival in GVHD experiments. The Student t test was used to compare percentages or numbers of donor T cells and host B cells.

Results

The effect of alloantigen affinity on GVHD induced by TCR Tg CD8+ T cells

2C TCR Tg cells induce acute GVHD in alloantigen (Ld)-bearing CB6F1 recipients, where the target of GVHD is primarily host lymphoid tissues (3). However, 2C cells rapidly die shortly after their expansion in Ld+ recipients (2, 5). We hypothesized that the abortive response of 2C effector cells might be due to clonal deletion resulting from TCR engagement with a strong Ld alloantigen. To test this hypothesis, we compared the pathogenicity of 2C cells in CB6F1, and (B6 × bm3)F1 recipients that expressed a high-(Ld) and intermediate-affinity (Kbm3) ligand to the 2C TCR, respectively. After adoptive transfer, 2C cells engrafted, expanded, and eliminated host B cells and thymocytes in sublethally irradiated CB6F1 recipients (Ref. 3 and data not shown), but most of those recipients survived long-term without clinical signs of GVHD (Fig. 1). 2C cells not only engrafted and expanded, but also caused death in the majority of (B6 × bm3)F1 recipients (Fig. 1). The GVHD lethality was significantly higher in (B6 × bm3)F1 than in CB6F1 recipients (p = 0.029).

Because CB6F1 recipients express completely incompatible MHC Ags as compared with donor B6 mice, it is possible that the different fate and GVHD-inducing potential of 2C cells in the two different types of hosts resulted from hybrid resistance mediated by NK cells in the CB6F1 recipients. Considering that hybrid resistance can be overcome by high doses of donor cells (16), we tested whether higher doses of 2C T cells could induce the level of GVHD in CB6F1 recipients comparable to that in (B6 × bm3)F1 recipients. By titrating up the number of 2C cells, we found that

![FIGURE 1](http://www.jimmunol.org/)

**FIGURE 1.** The fate and pathogenicity of 2C cells in allogeneic recipients. A, CB6F1 and (B6 × bm3)F1 mice were exposed to irradiation at 750 cGy, and then transferred with 5 × 10⁸ purified CD8+ cells from 2C Tg mice. A group of irradiated F1 mice that were not transferred with donor T cells were used as controls without GVHD. Data were pooled from three separate experiments with the same setting. B, A total of 5 × 10⁶ purified CD8+ cells from 2C Tg mice were transferred to sublethally irradiated CB6F1 mice. A group of CB6F1 mice were NK-depleted with 50 µg/mouse asialo GM1 Ab on days −1 and −2 before cell transfer. Irradiated F1 mice that were not transferred with donor T cells were used as controls without GVHD. Two weeks after cell transfer, blood samples were collected and blood cells were strained for the presence of 2C cells and host B cells. The percentage of 2C cells (1B2+) and host B cells (B220+) in total WBC were presented as the average ± 1 SD of each group (n = 6).
increased cell doses (up to $15 \times 10^6$ cells/recipient) did not enhance engraftment and GVHD lethality in CB6F1 recipients (data not shown). To rule out the role of NK-mediated hybrid resistance against 2C cells in CB6F1 recipients, we depleted NK cells in the recipients before cell transfer with anti-NK cell Ab asialo GM1. We found that 2C cells were unable to cause GVHD lethality in CB6F1 mice regardless of NK depletion, and 2C expansion and host B cell elimination were comparable in both groups (Fig. 1B). Taken together with our previously published work that 2C cells have a higher level of expansion and a lower level of apoptosis in (B6 $\times$ bm3)F1 recipients than in CB6F1 recipients (17), the current data on recipient survival indicate that pathogenicity of CD8 effector cells activated by a high-affinity alloantigen is mitigated by rapid clonal deletion.

**Pathogenicity of CD4$^+$ TCR Tg cells in a nonmyeloablative model**

We first evaluated the capability of CD4$^+$ cells alone to induce GVHD. To visualize the CD4$^+$ effector cells, we chose to test the response of D10 Tg cells in recipients that express a specific alloantigen (IA$^b$) to the D10 TCR (7). We transplanted purified CD4$^+$ D10 cells at various doses into sublethally irradiated syngeneic C3H or allogeneic (B6 $\times$ C3H)F1 mice. A group of irradiated (B6 $\times$ C3H)F1, mice were used as no transplant controls. All (B6 $\times$ C3H)F1, mice that received $0.1$–$2.5 \times 10^6$ D10 cells/mouse rapidly lost their body weights (data not shown) and died within 4 wk after transplantation (Table I). In contrast, with rare exception, the irradiation controls, syngeneic recipients, and allogeneic recipients of $0.02 \times 10^6$ or fewer donor cells survived long-term without significant signs of GVHD. Furthermore, survival increased after transplantation of $5 \times 10^6$ or more cells/mouse (Table I), which is consistent with prior data on transplantation of non-TCR Tg CD4$^+$ T cells (18).

To further evaluate GVHD in vivo, peripheral blood samples were obtained from each mouse at multiple time points to detect donor T cells and host B cells. The data indicate that D10 cells engrafted in both C3H and (B6 $\times$ C3H)F1 recipients (Fig. 2A). D10 cells expanded extensively at early time points and then rapidly disappeared 2 wk after transplantation (Fig. 2B, upper panel), and prevented host B cells from recovery (Fig. 2B, lower panel). In contrast, D10 cells expanded slowly but continuously in irradiated syngenic recipients without impairing host B cell reconstitution (Fig. 2B). The number of D10 cells in the recipient circulation was lower when $5 \times 10^5$ or more D10 cells/mouse were transferred into (B6 $\times$ C3H)F1 recipients (Fig. 2B), suggesting that fratricide of D10 cells occurred in allogeneic recipients at a high concentration of donor cells. These results are consistent with prior data on transplantation of non-TCR Tg CD4$^+$ T cells.

**Cooperation of D10 and 2C cells in GVHD**

We then studied the effects of TCR-Tg CD4 cells on GVHD mediated by 2C cells. Lethally irradiated CB6F1 mice were used as recipients, which express L$^b$ to be recognized by 2C cells and express IA$^b$ to be recognized by D10 cells. CB6F1 mice that were transplanted with TCD-BM from B10.BR donors were used as controls without GVHD. B10.BR mice were used as BM donors in this myeloablative GVHD model, because neither D10 nor 2C cells react with the BM graft (H2b) whereas D10 cells react against B6 mice (H2b). In separate experiments where B6 mice were used as the source of donor BM, 2C cells alone also failed to induce GVHD lethality in CB6F1 recipients (data not shown), excluding a potential effect of MHC disparities between alloreactive T cells and the allograft on the development of GVHD.

Recipient of TCD-BM plus 2C cells survived long-term without obvious signs of GVHD. A subset of recipients of TCD-BM plus D10 cells or plus D10 and 2C cells died (Fig. 4), but the survival rate of either group was not significantly different from the group with BM alone ($p > 0.05$). The results indicate that 2C cells were unable to induce GVHD lethality in CB6F1 recipients even with CD4 help.

<table>
<thead>
<tr>
<th>Table I</th>
<th>TCR-Tg D10 cells induce lethal GVHD in (B6 $\times$ C3H)F1 recipients$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient</td>
<td>No. of Mice</td>
</tr>
<tr>
<td>C3H</td>
<td>9</td>
</tr>
<tr>
<td>(B6 $\times$ C3H)F1</td>
<td>8</td>
</tr>
<tr>
<td>(B6 $\times$ C3H)F1</td>
<td>4</td>
</tr>
<tr>
<td>(B6 $\times$ C3H)F1</td>
<td>5</td>
</tr>
<tr>
<td>(B6 $\times$ C3H)F1</td>
<td>10</td>
</tr>
<tr>
<td>(B6 $\times$ C3H)F1</td>
<td>10</td>
</tr>
<tr>
<td>(B6 $\times$ C3H)F1</td>
<td>9</td>
</tr>
<tr>
<td>(B6 $\times$ C3H)F1</td>
<td>3</td>
</tr>
<tr>
<td>(B6 $\times$ C3H)F1</td>
<td>4</td>
</tr>
</tbody>
</table>

$^a$ C3H or (B6 $\times$ C3H)F1, mice were irradiated at 700 cGy and then transferred with purified CD4$^+$ D10 cells at the cell dose indicated. Data were pooled from three separate experiments with the same experimental setting.
To evaluate the fate and pathogenicity of TCR-Tg donor cells, we measured 2C, D10, and B cells in recipient spleens on day 15 after transplantation (Fig. 5A). The number of 2C cells was significantly higher in the recipients transplanted with 2C plus D10 cells than 2C cells alone \((p < 0.005)\) (Fig. 5B, left panel), indicating that D10 cells facilitated 2C cell expansion. The number of D10 cells was also increased 2-fold when 2C cells were coadministered (Fig. 5B, middle panel, \(p < 0.005\)). A small proportion of host B cells (B220\(^{+}\)H2d\(^{+}\)) was still present in the recipients of TCD-BM alone, but not in the recipients of additional 2C or D10 cells (Fig. 5A, right panels), indicating that either type of donor T cells could eliminate host B cells.

The effect of alloantigen affinity on GVHD induced by 2C cells in the presence of CD4 help

The 2C TCR Tg cells induced a significantly higher rate of GVHD lethality in (B6\(\times\)C3H)F\(_1\) than in CB6F1 recipients in a nonmyeloablative model (Fig. 1). Therefore, we tested how alloantigen affinity affects the GVHD lethality mediated by 2C cells in the presence of CD4 help

The absolute numbers of CD4\(^{+}\)/3D3\(^{+}\) T cells (upper panel) or B220\(^{+}\) B cells (lower panel) in the blood were calculated from the WBC counts multiplied by the percentage of either population among WBC based on flow cytometric analysis.

FIGURE 3. D10 cell expansion and host B cell recovery in allogeneic recipients that were lethally irradiated. (B6 \(\times\) C3H)F\(_1\) mice were exposed to irradiation at 1100 cGy, and then transferred with purified CD4\(^{+}\) cells from D10 mice at the numbers indicated. On day 15 after transplantation, a peripheral blood sample was collected from each mouse and stained for expression of CD4, D10 TCR (3D3), and B220. The numbers shown are percentage of CD4\(^{+}\)/3D3\(^{+}\) T cells (left panels) or percentage of B220\(^{+}\) B cells (right panels) in total WBC. B. Peripheral blood samples were collected from each recipient on the days indicated. The absolute numbers of CD4\(^{+}\)/3D3\(^{+}\) T cells (upper panel) or B220\(^{+}\) B cells (lower panel) in the blood were calculated from the WBC counts multiplied by the percentage of either population among WBC based on flow cytometric analysis.
myeloablative model. Lethally irradiated (B6 × bm3)F1 mice were used as recipients, which express H-2Kbm3 to be recognized by 2C cells and express IAb to be recognized by D10 cells. Irradiated F1 mice that were transplanted with TCD-BM from B10.BR donors were used as controls without GVHD. B10.BR mice were used as BM donors in this myeloablative GVHD model, as neither D10 nor 2C cells react with the BM graft (H2k) whereas D10 cells react against B6 mice (H2b).

The survival rate of recipients that were transplanted with TCD-BM plus D10 cells was not different from the recipients with TCD-BM alone (p = 0.174). In contrast, all the recipients that were transplanted with TCD-BM plus 2C cells died, significantly different from the recipients of TCD-BM alone (p = 0.001). Furthermore, GVHD was significantly more severe in the recipients with both 2C and D10 cells than those with 2C (p = 0.001) or D10 (p < 0.001) cells alone (Fig. 6). These results indicate that 2C cells but not D10 cells were able to induce GVHD lethality in (B6 × bm3)F1 recipients, but D10 cells provided help to enhance the pathogenicity 2C cells.

In comparing two types of recipients (Fig. 4 vs Fig. 6), D10 cells induced GVHD lethality similarly in CB6F1 and (B6 × bm3)F1 mice (p = 0.82). The results were expected because D10 cells recognize the same alloantigen (IAb) expressed on either recipient. In sharp contrast, 2C cells caused 70% lethality in (B6 × bm3)F1 recipients but 0% lethality in CBF1 recipients in the absence of CD4 help (p = 0.0002). In the presence of CD4 help, 2C cells also...
caused significantly more severe GVHD in (B6 × bm3)F1 recipients than in CB6F1 recipients (p < 0.0001).

To further analyze the cooperation between D10 and 2C cells in (B6 × bm3)F1 recipients, cytokine levels (TNF-α, IFN-γ, and IL-5) in the serum as well as expansion of D10 and 2C cells and CTL activity of 2C effectors in the spleen were evaluated 7 days after transplantation. The levels of TNF-α, IFN-γ, and IL-5 were comparable in the serum from recipients of D10 plus 2C cells and from recipients of 2C cells alone (p > 0.1), suggesting that cytokine production did not contribute to acute mortality of the recipients of D10 plus 2C cells. When D10 cells were coadministered, the expansion level of the 2C cells remained the same, but their CTL activity was increased (p = 0.07) (Fig. 7). Considering most of the activated CD8+ T cells presumably migrated into the target organs (i.e., gastrointestinal mucosa), this increase, although marginally significant, suggests that the D10 cells enhanced the CTL activity of 2C cells. Collectively, these data demonstrate that alloantigen affinity plays a key role in the development of GVHD and that CD4 help further enhances GVHD pathogenicity of alloreactive CD8+ T cells.

**Discussion**

Transfer of 2C cells into Ld-bearing recipients results in a burst of expansion of 2C cells followed by apoptosis and anergy in the residual 2C cells, but no overt GVHD development in the recipient (2, 3, 5, 6). This study has shown that 2C cells induced GVHD lethality in Kbm3 recipients with a significantly higher rate than in Ld recipients. The outcome was observed in sublethally (Fig. 1) as well as in lethally irradiated recipients (Figs. 4 and 6), and in the absence or presence of CD4 help (Figs. 4 and 6). These results indicate that alloantigen affinity plays a critical role in the fate and pathogenicity of donor CD8+ T cells.
Why do 2C cells have a limited expansion capacity and why are they unable to induce lethal GVHD in recipients that express high-affinity alloantigens? Lethal GVHD could be induced in L<sup>+</sup> recipients by 2C cells that were deficient for IFN-γ (19). Because IFN-γ has been reported to play an important role in regulating the death of activated CD<sup>4+</sup> and CD<sup>8+</sup> cells (20–23), the augmentation of GVHD in L<sup>+</sup> recipients of IFN-γ-deficient 2C cells is likely due to decreased cell death of those alloreactive CD<sup>8+</sup> T cells. Furthermore, our recent work directly showed that 2C cells had a higher level of expansion, associated with a higher level of apoptosis and more severe injury of the lymphoid compartment in L<sup>+</sup> recipients than in B<sub>b<sup>+</sup></b> recipients (17). Deletional deletion of CD<sup>8+</sup> T cells in response to high-dose or high-affinity TCR ligation is not restricted to 2C Tg cells. In an experimental autoimmune encephalomyelitis model with CD8<sup>+</sup> Tg cells specific for myelin basic protein, immunization with wild-type Ag expanded the high-affinity T cells which was required to induce encephalomyelitis. In contrast, immunization with strongly antigenic analogs led to the elimination of T cells bearing high-affinity TCRs by apoptosis, thereby preventing disease development (24). In an autoimmune diabetes model with CD8<sup>+</sup> Tg cells specific for OVA, a high dose of Ag led to tolerance of OVA-reactive T cells through deletion (25, 26). In the current study, we demonstrated that ligation of high-affinity T cells triggers death of alloreactive CD<sup>8+</sup> T cells and thus facilitates transplantation tolerance.

The lack of CD4 help is another reasonable explanation for the inability of CD8<sup>+</sup> T cells to mount an effective anti-host response. In support of this hypothesis, Gonzalez et al. (6) showed that the development of lethal GVHD occurred only when alloreactive CD8<sup>+</sup> (2C) plus CD4<sup>+</sup> (D10) Tg cells, but not CD8<sup>+</sup> cells alone, were transferred into sublethally irradiated recipients. In this study, we found that CD4<sup>+</sup> D10 cells enhanced 2C-mediated GVHD severity in lethally irradiated recipients that express either high- or intermediate-affinity alloantigens. These findings extend the existing knowledge about the role of CD4<sup>+</sup> T cells in controlling the fate and function of CD8<sup>+</sup> T cells in GVHD. The level of 2C expansion was significantly higher with CD4<sup>+</sup> donor T cells than without (Fig. 5). Enhanced expansion of CD8<sup>+</sup> T cells can result from an increased rate of cell division induced by cytokines secreted by CD4<sup>+</sup> cells, and/or CD4 help may also prevent rapid deletion of CD8<sup>+</sup> cells in those recipients (27). In addition, CD4 help can enhance the CTL activity of CD8<sup>+</sup> cells, presumably through CD154-induced maturation of host dendritic cells (28–30).

2C cells cause rejection of L<sup>3</sup>-expressing grafts in skin or heart transplantation (31, 32), but our work and others (19) showed that 2C cells fail to induce GVHD in L<sup>4</sup>-expressed recipient. Rejection of skin or heart grafts is a hyperacute process (<14 days), while causing GVHD lethality takes longer. We surmise that 2C cells become tolerant before they can produce lethal GVHD. This assumption is supported by the observation that pre-exposure of L<sup>4</sup> Ag to the recipient-bearing 2C cells results in a long-term L<sup>4</sup> skin allograft acceptance (31).

In the current report, we also compared the fate and pathogenicity of CD4<sup>+</sup> vs CD8<sup>+</sup> donor T cells in GVHD. In nonmyeloablative models, either CD4<sup>+</sup> (Table I) or CD8<sup>+</sup> (Fig. 1) TCR Tg cells alone are able to induce lethal GVHD in the recipients that express the appropriate alloantigen (i.e., one with intermediate affinity). Under these circumstances, Tg CD4<sup>+</sup> cells will not cause GVHD lethality when given in large doses (Table I). This phenomenon was also observed with non-Tg CD4<sup>+</sup> cells and was termed as prozone (18). The prozone effect likely results from Fas-mediated fratricide of activated CD4<sup>+</sup> T cells at high density in vivo. Such a phenomenon has not been observed for CD8<sup>+</sup> cells, which might be due to the relative insensitivity of CD8<sup>+</sup> cells to Fas-mediated killing.

Although Tg CD4<sup>+</sup> cells were capable of inducing lethal GVHD in sublethally irradiated F<sub>1</sub> recipients (Table I), those cells were not able to do so consistently in lethally irradiated F<sub>1</sub> recipients (Figs. 4 and 6). We noticed that donor CD4<sup>+</sup> cells were very capable of suppressing growth of lymphoid cells derived either from host or donor (Fig. 3), yet clinical GVHD was not overt in those recipients (data not shown). It is possible that cytotoxicity mediated by CD4 T cells may be sufficient to eliminate hemopoietic cells, but not sufficient to cause lethal injury in epithelial GVHD target organs.

The findings from this study underscore the importance of alloanigen affinity and CD4 help in controlling the fate of alloreactive CD8<sup>+</sup> T cells in vivo. We developed a new system to visualize the fate and interactions of alloreactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the pathogenesis of GVHD, facilitate studies of GVHD immunopathology, and assess potential strategies to prevent GVHD.

**Acknowledgments**

We thank Dr. Paul Martin and Michael Bevan for helpful discussion of this project, and Sasha Mayer, Lisa Rapalus, Melissa Makris, Yaming Liang, and Kelli McIntyre for their technical assistance. The founder of D10 TCR Tg mice was kindly provided by Dr. Derek Sant’Angelo at the Memorial Sloan-Kettering Center.

**Disclosures**

The authors have no financial conflict of interest.

**References**


