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*J Immunol* 2000; 164:656-663; doi: 10.4049/jimmunol.164.2.656
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The p55 TNF-α Receptor Plays a Critical Role in T Cell Alloreactivity

Geoffrey R. Hill,*† Takanori Teshima,‡ Vivienne I. Rebel,* Oleg I. Krijanovski,* Kenneth R. Cooke,* Yani S. Brinson,* and James L. M. Ferrara‡

TNF-α is known to be an important mediator of tissue damage during allograft rejection and graft-vs-host disease (GVHD), but its role in supporting T cell responses to allogeneic Ags is unclear. We have studied this question by comparing normal mice with those lacking the p55 (p55 TNFR-/-) or p75 (p75 TNFR-/-) TNF-α receptors as donors in well-defined bone marrow transplant (BMT) models. Recipients of p55 TNFR-/- cells had significantly reduced mortality and morbidity from GVHD compared with the other two sources of T cells. In vitro, T cells lacking the p55 (but not the p75) TNF-α receptor exhibited decreased proliferation and production of Th1 cytokines in MLC. This defect was only partially restored by exogenous IL-2 and affected both CD4+ and CD8+ populations. CD8+ p55 TNFR-/- proliferation was impaired independently of IL-2 whereas CTL effector function was impaired in an IL-2-dependent fashion. Inhibition of TNF-α with TNFR:Fc in primary MLC also impaired the proliferation and Th1 differentiation of wild-type T cells. BMT mixing experiments demonstrated that the reduced ability of p55 TNFR-/- donor cells to induce GVHD was due to the absence of the p55 TNFR on T cells rather than bone marrow cells. These data highlight the importance of TNF-α in alloreactive T cell responses and suggest that inhibition of the T cell p55 TNF-α receptor may provide an additional useful therapeutic maneuver to inhibit alloreactive T cell responses following bone marrow and solid organ transplantation. 

*Abbreviations used in this paper: LT, lymphotoxin; GVHD, graft-vs-host disease; BMT, bone marrow transplant; TBI, total body irradiation.
mice, we demonstrate that T cells lacking the p55 TNF-α receptor have impaired responses to alloantigen in vitro, including reduced proliferation, type 1 cytokine production, and cytolytic function. In vivo, T cells lacking the p55 TNF-α receptor have a reduced capacity to induce GVHD, which is also characterized by impaired IFN-γ and TNF-α production, reduced CD8$^+$ expansion, and impaired cytolytic function. These data suggest that TNF-α plays a critical role in T cell responses to alloantigens.

Materials and Methods

Mice

Female C57BL/6 (B6, H-2$^b$), B6C3F1 (H-2$^{b\times k}$), B6SJLF1 (H-2$^{b\times s}$), and B6D2F1 (H-2$^s$) mice were purchased from The Jackson Laboratories (Bar Harbor, ME). B6 mice deficient in the p55 TNF-α receptor and p75 TNF-α receptor were supplied by Immunex (Seattle, WA) and have been previously described (10, 16–18). The age of mice used as bone marrow transplant (BMT) recipients ranged between 9 and 15 wk and the age of donors was >24 wk. Mice were housed in sterilized microisolator cages and received filtered water and normal chow, or autoclaved hyperchlorinated drinking water for the first 2 wk after BMT.

Bone marrow transplantation

Mice were transplanted according to a standard protocol described previously (19, 20). On day 0, mice received 1300 cGy total body irradiation (TBI) ($^{137}$Cs source), split into two doses separated by 3 h to minimize gastrointestinal toxicity. A total of $5 \times 10^5$ bone marrow cells and $2 \times 10^6$ nylon wool purified splenic donor T cells were resuspended in 0.25 ml of Leibovitz’s L-15 medium (Life Technologies, Gaithersburg MD) and injected i.v. into recipients. In some experiments, bone marrow was T cell depleted with anti-Thy 1.2 and rabbit complement. Survival was monitored daily, and recipient’s body weights and GVHD clinical score were measured weekly.

Assessment of GVHD

The degree of systemic GVHD was assessed by a scoring system which sums changes in five clinical parameters: weight loss, posture (hunching), activity, fur texture, and skin integrity (maximum index = 10) (21). Individual mice were ear-tagged and graded weekly from 0 to 2 for each criterion without knowledge of treatment group.

FACS analysis

FITC-conjugated mAbs to mouse H-2$^d$, CD3, CD4, and CD11b, and PE-conjugated CD4, CD8, CD44, CD62L, CD45RB, and B220 were purchased from Pharmingen (San Diego, CA). Cells were first incubated with mAb 2.4G2 for 15 min at 4°C, then with the relevant FITC- or PE-conjugated mAb for 30 min at 4°C. Finally, cells were washed twice with PBS/0.2% BSA, fixed with PBS/1% paraformaldehyde, and analyzed by FACSscan (Becton Dickinson, San Jose, CA). Donor T cell expansion was determined by multiplying the fraction of CD4$^+$ and CD8$^+$ cells that were H-2$^d$ negative in the spleen by the total number of spleen cells 2 wk after BMT.

Cell cultures

All culture media and incubation conditions were as previously described (19, 20). In studies of naive mice, splenic T cells were enriched by passage through nylon wool. T cell fractions after this process ranged from 60 to 70% and were similar in each group. These responder T cells were then plated in 96 flat-bottom plates (Falcon, Lincoln park, NJ) at a concentration of 1 or 2 $\times 10^5$ T cells (CD4$^+$ plus CD8$^+$) well with 0.5 or 1 $\times 10^6$ irradiated (2000 rad) peritoneal macrophages lavaged from naive B6D2F1 (allogeneic) or B6 (syngeneic) animals. At 72 h, cultures were pulsed with $[^3]$H)thymidine (1 $\mu$Ci/well), and proliferation was determined 20 h later on a 1205 Betaplate reader (Wallac, Turku, Finland). Purified CD4$^+$ and CD8$^+$ T cell subsets were obtained by selecting CD4$^+$ and CD8$^+$ cells from nylon wool purified splenic cells using minimacs columns (Miltenyi Biotech, Bergisch Gladbach, Germany). Supernatant was removed at 48 h for IL-2 determination and 72 h for IFN-γ and IL-4 determination. To block TNF-α, 100 $\mu$g/ml of TNFR-Fc (Immunex) or control human Ig was added to MLC. In experiments analyzing T cell responses after BMT, splenocytes were removed from animals 14 days after transplant, and three to six spleens were combined from each group. Single cell suspensions were then layered over Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ) and centrifuged at 800 $\times g$ for 15 min. Cells were collected from the interface and washed twice before suspension in supplemented 10% FCS/RPMI. Cells were plated in the same concentrations as above and were pulsed with $[^3]$H)thymidine at 48 h; plates were harvested 20 h later.

FIGURE 1. p55 TNFR$^{-/-}$ cells have a reduced capacity to induce GVHD mortality and morbidity. B6C3F1 (A) or B6D2F1 (B) recipients were transplanted after 1300 cGy of TBI with bone marrow and splenic T cells from wild-type ($n = 20$ and 10), p55 TNFR$^{-/-}$ ($n = 20$ and 10), or p75 TNFR$^{-/-}$ animals ($n = 11$ and 11). Bone marrow and T cells from syngeneic B6D2F1 donors was used as nonsGVHD controls ($n = 10$ and 5). GVHD severity in surviving animals was determined by clinical score as detailed in Materials and Methods. Mortality was significantly reduced in B6C3F1 recipients of p55 TNFR$^{-/-}$ cells ($\ast$, $p < 0.03$) compared with recipients of wild-type or p75 TNFR$^{-/-}$ cells. Due to the smaller sample sizes, the reduction in mortality in B6D2F1 recipients of p55 TNFR$^{-/-}$ cells did not reach statistical significance ($p = 0.17$). However, GVHD severity was significantly reduced in both B6C3F1 and B6D2F1 recipients of p55 TNFR$^{-/-}$ cells ($\ast\ast$, $p < 0.04$, from the time marked onward) compared with recipients of wild-type or p75 TNFR$^{-/-}$ cells. GVHD severity was increased in B6D2F1 animals receiving p75 TNFR$^{-/-}$ cells compared with wild-type and p55 TNFR$^{-/-}$ cells ($\ast$, $p < 0.03$ from day 49 onward).
Supernatant was removed 48 h after culture for determination of cytokines. Mitogenic responses were determined by stimulating 10^5 T cells with 10 μg/ml of plate-bound CD3 (145-2C11, PharMingen) or 2.5 μg/ml of Con A. In some cultures, CD28 (37.51, PharMingen) was added at a concentration’s protocol. All assays were performed according to the manufacturer’s protocol.

**Cytokine ELISA**

The Abs used in the TNF-α assay were purchased from Genzyme (Cambridge, MA). Abs used in the IFN-γ, IL-2, and IL-4 assays were purchased from PharMingen. All assays were performed according to the manufacturer’s protocol.

**51Cr release assays**

A total of 2 × 10^6 P815 (H-2b) or EL4 (H-2b) tumor targets were labeled with 100 μCi of 51Cr for 2 h. After washing three times, labeled targets were plated at 10^4 cells per well in U-bottom plates (Costar, Cambridge, MA). Splenocytes from allogeneic BMT recipients (prepared as described above) were added to quadruplicate wells at varying E:T ratios and incubated for 5 h. Maximal and background release was determined by the addition of Triton X-100 (Sigma, St. Louis, MO) or medium alone to targets, respectively. 51Cr activity in supernatants taken 5 h later were added to quadruplicate wells at varying E:T ratios and incubated for 5 h. Maximal and background release was determined by the addition of Triton X-100 (Sigma, St. Louis, MO) or medium alone to targets, respectively.

**Statistical analysis**

Survival curves were plotted using Kaplan-Meier estimates. The Mann-Whitney U test was used for the statistical analysis of cytokine data, LPS levels, clinical scores, and weight loss, whereas the Mantel-Cox log-rank test was used to analyze survival data. A p value of <0.05 was considered statistically significant.

**Results**

**Bone marrow and T cells from donor p55 TNFRp55R−/− animals have an impaired ability to induce GVHD after allogeneic BMT**

To study the responses of TNFRp55R−/− T cells to alloantigen in vivo, we used well-established murine models of GVHD that are directed to both major and minor histocompatibility Ags (B6 → B6D2F1 and B6 → B6C3F1). TNF-α production in these BMT models is high and neutralization of TNF-α at the time of BMT with recombinant human TNF-α receptor:Fc (TNFR:Fc) effectively prevents GVHD (19). We transplanted bone marrow and nylon wool purified T cells from wild-type, p55 TNFRp55R−/−, or p75 TNFRp75R−/− B6 animals into B6D2F1 or B6C3F1 recipients after 1300 CGy of TBI. Fig. 1A shows that GVHD mortality was significantly reduced in B6C3F1 recipients of p55 TNFRp55R−/− cells compared with recipients of wild-type and p75 TNFRp75R−/− cells (day 70 survival: 90% vs 55% and 40%, p < 0.05). Although moderate GVHD (clinical scores between 2 and 4) was present in animals transplanted with p55 TNFRp55R−/− cells, this was significantly less than recipients of wild-type or p75 TNFRp75R−/− cells. Interestingly, recipients of p75 TNFRp75R−/− cells in this model tended to have more severe GVHD than recipients of wild-type cells. In the B6 → B6D2F1 strain combination, recipients of p55 TNFRp55R−/− cells had less mortality from GVHD (survival at day 70: 80% vs 45%) and an impressive reduction in the severity of GVHD was seen (clinical scores <2) in surviving animals (Fig. 1B).

<table>
<thead>
<tr>
<th>Table II. Splenocyte phenotypesa</th>
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<tr>
<td>CD3+</td>
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<td>CD44mph</td>
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<td>CD62Llow</td>
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<tr>
<td>CD45RBlow</td>
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<tr>
<td>CD4+</td>
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<tr>
<td>CD8+</td>
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<tr>
<td>B220+</td>
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<tr>
<td>Mac-1+</td>
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</table>

a Splenocytes from five to six animals in each group were phenotyped. Results are total cells (×10^6) per spleen or % of CD3+ cells (CD44, CD62L, and CD45RB) or total spleen cells (CD3, CD4, CD8, B220, Mac-1) and represent mean ± SE.

b p < 0.05.

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Table I. T cell responses in primary culturea

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type T Cells</th>
<th>p55R−/− T Cells</th>
<th>p75R−/− T Cells</th>
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<tbody>
<tr>
<td>MLC (anti-B6D2F1)</td>
<td>cpm (×10^3)</td>
<td>95.7 ± 11.1</td>
<td>6.0 ± 2.2**</td>
</tr>
<tr>
<td>IL-2 (U/ml)</td>
<td>2.4 ± 0.3</td>
<td>UD**</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>MLC (anti-B6D2F1) + IL-2</td>
<td>0 U/ml cpm (×10^3)</td>
<td>82.6 ± 5.9</td>
<td>23.9 ± 2.3</td>
</tr>
<tr>
<td>10 U/ml cpm (×10^3)</td>
<td>129.4 ± 8.1 (1.6)</td>
<td>82.0 ± 2.9 (3.4)</td>
<td>132.2 ± 7.1 (1.6)</td>
</tr>
<tr>
<td>50 U/ml cpm (×10^3)</td>
<td>239.2 ± 9.1 (2.9)</td>
<td>156.6 ± 6.6 (5.3)</td>
<td>ND</td>
</tr>
<tr>
<td>Mitogen</td>
<td>Con A (cpm ×10^3)</td>
<td>115.7 ± 10.4</td>
<td>130.7 ± 7.8</td>
</tr>
<tr>
<td>Con A + CD82 (cpm ×10^3)</td>
<td>538.7 ± 14.4</td>
<td>421.8 ± 26.9</td>
<td>537.5 ± 9.5</td>
</tr>
</tbody>
</table>

a Nylon wool-purified splenic T cells were cultured in MLC with B6D2F1 peritoneal macrophages or Con A (2.5 μg/ml) ± CD82 (1 μg/ml) as detailed in Materials and Methods. Results represent mean ± SD of quadruplicate wells in one of seven experiments in which the range of p55R−/− to wild-type proliferative responses was 6–33% for alloantigen (***p < 0.01 vs wild and p75R−/− T cells) and 78–150% for Con A. Proliferative responses (×10^3) to alloantigen are also shown in the presence of exogenous IL-2. Numbers in parentheses show the fold increase over baseline; UD, under level of detection; ND, not done.
T cells lacking the p55 TNF-α receptor proliferate poorly to alloantigen

We next tested splenic T cells from mice lacking either the p55 TNF-α receptor or p75 TNF-α receptor for responses to alloantigen in primary mixed lymphocyte cultures. T cells from p55 TNFR−/− animals had markedly impaired proliferation and IL-2 production to H2bxd (B6D2F1) stimulators, whereas responses from p75 TNFR−/− T cells were equivalent to wild type (Table I). Exogenous IL-2 only partially restored the proliferative response however, suggesting that the impaired production of IL-2 was not completely responsible for the impaired proliferation (Table I). The proportion of apoptotic cells was not increased in MLC using p55 TNFR−/− responder T cells compared with wild-type or p75 TNFR−/− cells (4% vs 6% and 17%, respectively). Despite the reduced response to alloantigen, responses of TNFR−/− T cells to Con A and CD28 were normal (Table I). In addition, the numbers of T cells in the spleens of naive p55 TNFR−/− animals were normal although there was a significant increase in the proportion of memory T cells (CD44high, CD62Llow, CD45RBlow) compared with wild-type animals (Table II). Conversely, there was a significant reduction in the proportion of memory T cells in p75 TNFR−/− spleens compared with those from wild-type animals (Table II), suggesting that the p55 and p75 TNF-α receptors may play opposing roles in the generation of T cell memory. The most pronounced abnormality in the spleens of p55 TNFR−/− mice was a reduction in the number of B cells (B220+), consistent with the known role of TNF-α in B cell proliferation and differentiation (23, 24).

Proliferation to alloantigen is impaired in both CD4+ and CD8+ p55 TNFR−/− T cell subsets

To study whether the impairment in proliferation to alloantigen in p55 TNFR−/− T cells was restricted to either the CD4+ or CD8+ subsets, splenic T cells were purified as detailed in Materials and Methods and stimulated with alloantigen or CD3 and CD28. These experiments demonstrated that proliferation and IL-2 production to alloantigen were significantly impaired in both the CD4+ and CD8+ subsets whereas responses to mitogen were normal (Table III). Given that IL-2 was detectable in culture supernatant from wild-type CD8 cells in MLC, the apparent absence of IL-2 production from mitogen stimulated wild-type CD8 cells is likely to reflect the sampling of supernatant after autocrine consumption had exhausted IL-2 in the culture medium. Surprisingly, the production of IFN-γ to mitogen was dramatically increased in both the CD4+ and CD8+ p55 TNFR−/− T cell populations (6-fold and 100-fold, respectively). This increase was not associated with changes in the production of the type 2 cytokine IL-4. Enhanced proliferation to low concentrations of anti-CD3 was observed in both CD4+ and CD8+ p55 TNFR−/− cells compared with wild-type cells (data not shown). Taken together, these data show that CD4+ and CD8+ T cells lacking

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**Table III. CD4+ and CD8+ responses in primary culture**

<table>
<thead>
<tr>
<th></th>
<th>CD4+</th>
<th>CD8+</th>
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<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>p55 TNFR−/−</td>
</tr>
<tr>
<td>Proliferation (×10^3)</td>
<td>30.2 ± 4</td>
<td>19.8 ± 1.2*</td>
</tr>
<tr>
<td>IL-2 (U/ml)</td>
<td>0.42 ± 0.08</td>
<td>0.17 ± 0.08*</td>
</tr>
<tr>
<td>CD3 + CD28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proliferation (×10^3)</td>
<td>243 ± 12</td>
<td>211 ± 24</td>
</tr>
<tr>
<td>IL-2 (U/ml)</td>
<td>6.1 ± 0.9</td>
<td>5.8 ± 1.2</td>
</tr>
<tr>
<td>IFN-γ (U/ml)</td>
<td>31 ± 3</td>
<td>194 ± 18*</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>259 ± 53</td>
<td>361 ± 95</td>
</tr>
</tbody>
</table>

*CD4 and CD8 populations were purified from wild-type or p55R−/− spleens as detailed in Materials and Methods and stimulated with B6D2F1 peritoneal cells in primary MLC or CD3 (10 μg/ml) and CD28 (1 μg/ml). Results represent mean ± SD of quadruplicate wells from one of three similar experiments in which the range of p55R−/− to wild-type responses in MLC in the CD4 fraction was 11–66% and CD8 fraction was 29–61%.

* p < 0.05 vs respective wild-type T cell population.

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**FIGURE 2.** TNF-α promotes proliferation and IFN-γ production to alloantigen in MLC. B6 nylon wool-purified splenic T cells from wild-type, p55 TNFR−/−, or p75 TNFR−/− animals were cultured with B6D2F1 peritoneal macrophages in MLC. Proliferation (A) and IFN-γ (B) was determined in MLC with control Ig (■) or TNFR:Fc (□) as detailed in Materials and Methods. *, p < 0.02. Results represent one of four experiments. The range of reductions (1 - (TNFR:Fc/control) × 100) in proliferation from cultures with TNFR:Fc were as follows: wild-type cells, 28–38% (p < 0.02); p55 TNFR−/−, 39–95% (p < 0.03); and p75 TNFR−/−, 0–51%. The range of reductions in IFN-γ production were: wild-type cells, 12–59% (p < 0.02); p55 TNFR−/−, 13–66% (p = NS to <0.04); and p75 TNFR−/−, 15–25% (p = NS to <0.03), NS = not significant.
the p55 TNF-α exhibit specific defects to allogeneic APC even though the TCR and CD28 signaling pathways are intact.

**TNF-α enhances T cell proliferation and Th1 differentiation to alloantigen**

TNF-α enhances T cell proliferation to both mitogenic (25) and allogeneic stimuli (15), independently of its effects on APC (15), and TNF-α may therefore function as an autocrine growth factor for T cells. Mitogenic responses can be enhanced by p75 TNF-α receptor agonists (4), but the specific TNF-α receptors involved in responses to alloantigen have not been elucidated. To study the role of TNF-α in the defects seen in p55 TNF-α−/− T cells, splenic T cells from wild-type, p55 TNF-α−/−, and p75 TNF-α−/− animals were cultured in primary MLC with a TNF-α inhibitor (recombinant TNF-α receptor linked to the human Fc Ig, TNFR:Fc) or control human Ig. As shown in Fig. 2, the neutralization of TNF-α in these cultures impaired proliferation of wild-type T cells, p55 TNF-α−/− T cells, and p75 TNF-α−/− T cells. TNFR:Fc did not affect IL-2 production (data not shown) but inhibited IFN-γ production of T cells from all animals (Fig. 2). Importantly, the inhibition of TNF-α in these cultures did not reduce T cell proliferation or IFN-γ production in wild-type T cells down to the levels of p55 TNF-α−/− cells, suggesting a component of the defect in the p55 TNF-α−/− T cells was independent of exogenous TNF-α. As shown in Fig. 3, the defect in proliferation and cytokine production to three allogeneic stimuli was similar, confirming the general nature of the deficit. To investigate whether these defects also affected T cell expansion and cytolytic function, respective CD8+ cells were examined after primary MLC. The addition of exogenous IL-2 to the primary MLC restored defective CTL lysis by p55 TNF-α−/− CD8+ cells but did not improve their poor expansion (Table IV). Interestingly, T cells from both old (>24 wk) and young (<12 wk) p55 TNF-α−/− animals had impaired proliferation and T cell expansion in vitro. T cells from old p55 TNF-α−/− animals also had marked impairment in cytokine production and cytotoxicity to alloantigen, which was only mild in T cells from young p55 TNF-α−/− animals. The reasons for the more pronounced defect in T cells from older animals is not clear but is likely to relate to a progressive T cell defect as a result of the prolonged absence of TNF-α signaling. In support of this we have also noted a progressive age-related stem cell defect in p55 TNF-α−/− animals (26).

**p55 TNF-α−/− T cell responses to allogeneic Ags are impaired in vivo**

Given the defects of p55 TNF-α−/− T cells to alloantigens in vitro, we measured this response in vivo by analyzing systemic IFN-γ levels after allogeneic BMT. All animals received wild-type T cell-depleted bone marrow together with either wild-type, p55 TNF-α−/−, or p75 TNF-α−/− T cells. No IFN-γ was detectable in the sera of recipients of p55 TNF-α−/− T cells 4 days after BMT, and it remained significantly depressed 7 days after BMT (Fig. 4). We next examined donor T cell expansion and function 2 wk after BMT, a time of maximal T cell expansion in this model. The total number and percentage of splenic CD4+ cells from p55 TNF-α−/− donors were not different from wild-type donors 14 days after BMT, although there was a significant decrease in the number and percentage of CD8+ cells (Table V). By 2 wk after BMT, donor p55 TNF-α−/− T cells proliferated normally and produced equivalent amounts of the type I cytokines IL-2 and IFN-γ to wild-type.
cells in vitro (Table V). In view of the impaired CD8+ T cell expansion, we next studied the effector function of these cells in standard 51Cr assays. As shown in Fig. 5, in two separate experiments there was a consistent reduction in the capacity of p55 TNFR2−/− T cells to lyse host type P815 targets after allogeneic BMT. Because IFN-γ is important for priming mononuclear cells to secrete TNF-α after BMT, we were interested in whether the reduced production of IFN-γ following transplantation of p55 TNFR2−/− T cells would effect subsequent TNF-α production. As shown in Fig. 4, the levels of TNF-α 7 days after BMT were 10- to 20-fold lower following transplantation of p55 TNFR2−/− T cells than those seen in recipients of wild-type or p75 TNFR2−/− T cells. These data confirm that p55 TNFR2−/− CD8+ T cells expand poorly and function suboptimally in vivo, and demonstrate that T cells lacking the p55 TNF-α receptor are unable to amplify the proinflammatory cytokine cascade that is known to be critical in the pathophysiology of GVHD (27).

The reduced ability of p55 TNFR2−/− cells to induce GVHD is due solely to defects in the T cell compartment

To study whether the reduction in GVHD seen after transplantation of p55 TNFR2−/− donor bone marrow and T cells (Fig. 1) was due primarily to defects in donor T cell function, we performed mixing studies. As shown in Fig. 6, survival was significantly lower in recipients of p55 TNFR2−/− bone marrow and wild-type T cells than in those receiving p55 TNFR2−/− bone marrow and p55 TNFR2−/− T cells (50% vs 100%, p < 0.01), confirming that p55 TNFR2−/− mononuclear cells did not play a role in reducing GVHD. Furthermore, the severity of GVHD in surviving recipients of p55 TNFR2−/− T cells was minimal (clinical scores, <2), regardless of the origin of the bone marrow. By contrast, recipients of wild-type T cells had moderately severe GVHD (clinical scores, >3), regardless of the origin of the bone marrow. Therefore the expression of the p55 TNF-α receptor on donor T cells was critical to the induction of acute GVHD, confirming the earlier in vitro studies.

Discussion

This study demonstrates that T cells lacking the p55 TNF-α receptor have impaired responses to alloantigen, characterized by reduced proliferation, type I cytokine production, and cytolytic function, despite the normal response of these cells to mitogen and CD28. In vivo, T cells lacking the p55 TNF-α receptor have a reduced capacity to induce GVHD, characterized by impaired IFN-γ and TNF-α production, reduced CD8+ expansion, impaired cytosis of host tissues, and milder systemic disease. The interpretation of the effects of TNF-α neutralization on donor T cell function is complex because TNF-α is known to induce cytokine production by APCs (such as IL-12 (28)), which may effect T cell responses indirectly. Indeed, we cannot exclude this as a mechanism for the reduced proliferation and IFN-γ production by T cells in the presence of TNF-α inhibitors that we observed in vitro. For these reasons the direct effects of TNF-α on T cells can best be studied by the use of T cells lacking appropriate TNF-α receptors.

Agonists of the p75 TNF-α receptor are known to enhance T cell proliferation in response to mitogen (29, 30). TNF-α has been shown to enhance proliferative responses in MLC (15), probably through increased IL-2 expression (31). The role of the p55 receptor during autocrine T cell stimulation is unclear because the surface expression of p75 is dominant following T cell activation (15, 32). However, the reduced apoptosis of p55 TNFR2−/− CD8+ cells following activation in vivo (33) and the synergistic effect of both receptors on T lymphoblast apoptosis (34) confirms a role for the...
p55 TNF-α receptor on T cells. TNF-α is also known to play a role in the survival of activated T cells (35, 36), although normal negative selection induced by superantigen has been shown in p55- and p75-deficient mice (10, 17). The p55 receptor appears to control the deletion of peripheral lymphocytes following their activation by peptide in vivo (33). The prolonged persistence of activated p55 TNFR−/− T cells, associated with resistance to activation-induced cell death (33), is consistent with the increased numbers of memory T cells seen in the spleen of the p55 TNFR−/− animals in this study.

To our knowledge, abnormalities in the proliferative function of p55 TNFR−/− T cells have not been previously described. In this study we noted an increased sensitivity of p55 TNFR−/− T cells to activation by mitogen, which is consistent with the lower activation threshold of memory T cells compared with naive T cells (37, 38). The differences seen between responses to alloantigen and to mitogen may relate to differences in the intensity of TCR signaling or alternatively may reflect enhanced sensitivity to inhibitory signals from the APC itself. In support of this latter possibility, p55 TNFR−/− T cells also respond to mitogen suboptimally in the presence of syngeneic APCs (data not shown). Supernatants taken from primary MLC of p55 TNFR−/− T cells and allogeneic APC do not inhibit responses of naive wild-type or p55 TNFR−/− T cells to alloantigen, suggesting that this process requires cell-cell contact. Studies have shown that memory CD4+ T cells have an increased expression of CTLA-4 and impaired proliferative responses in the presence APC bearing B7 (39). However the proliferative defect in p55 TNFR−/− T cells was not rescued by CTLA-4 inhibition or simultaneous CD28 stimulation and B7 inhibition with CTLA-4Ig (data not shown). Further studies are in progress to delineate the mechanisms by which APCs inhibit the responses of T cells lacking the p55 receptor.

TNF-α is also required for the optimal generation of human CTL in vitro; when added to the sensitizing phase of the primary MLC, TNF-α causes a selective up-regulation of the IL-2R on CD8-positive T cells (40). TNF-α can also increase granzyme activity induced by IL-2 as determined by benzoyloxy carbonyl-L-lysine thiobenzylester-esterase (BLT-E) activity (40). Our data are consistent with the ability of TNF-α to amplify CTL activity by increasing IL-2 production. This study demonstrates that TNF-α signaling through the p55 receptor controls both the generation and the function of CTL in vivo. In agreement with this observation, we and others have recently confirmed that TNF-α neutralization inhibits the graft-vs-leukemia effect after allogeneic BMT (41, 42).

FIGURE 6. The reduced capacity of donor p55 TNFR−/− cells to induce GVHD is due to a defective T cell function. Wild-type T cells were transplanted with wild-type bone marrow cells (n = 10) or p55 TNFR−/− bone marrow cells (n = 10). p55 TNFR−/− T cells were transplanted with wild-type bone marrow cells (n = 10) or p55 TNFR−/− bone marrow cells (n = 10). Survival was significantly improved in recipients of p55 TNFR−/− T cells (*, p = 0.05) regardless of the bone marrow source, and GVHD was significantly reduced from day 14 onward (+, p < 0.01) in these recipients.

The current study suggests that TNF-α may be required in the differentiation of CTL from precursors rather than as an effector molecule by mature CTL. We noted a more profound reduction in CTL generation in vitro than in vivo, and we believe this is likely to reflect the ability of other compensatory factors to support CTL generation in the absence of TNF-α in vivo. It is likely that IL-12 is important in this regard (43) because the production of IL-12 in MLC after BMT was high and similar between animals transplanted with wild-type or p55 TNFR−/− T cells. Interestingly, preliminary data suggest that the addition of exogenous IL-12 to primary MLC also improves the proliferative and cytokine defects in p55 TNFR−/− T cells in secondary cultures. This may, in part, explain the apparent equivalent proliferation and cytokine production of p55 TNFR−/− T cells and wild-type T cells after BMT (Table V). Unfortunately, interpretation of T cell function at this time is complicated by the presence of moderately severe GVHD and its associated immunosuppression which is absent in p55 TNFR−/− T cells. This impairment in function of wild-type T cells in mice with GVHD will therefore mask differences in relation to p55 TNFR−/− T cells in mice without GVHD.

The role of TNF-α as an effector of host cytotoxicity during acute GVHD is well established (19, 44), and the presence of the p55 TNF-α receptor on recipient tissues has been shown to be critical for the induction of GVHD (45). The present study confirms that the p55 receptor plays a critical role in T cell responses to alloantigens. The fact that donor p55 TNFR−/− T cells failed to expand and produce IFN-γ in response to alloantigen suggests that TNF-α also alters T cell alloreactivity directly. The reduced levels of TNF-α produced in vivo despite a normal wild-type bone marrow compartment confirm the importance of IFN-γ from donor T cells in the priming of mononuclear cells to produce inflammatory cytokines (46). This study delineates an additional mechanism by which neutralization of TNF-α early after allogeneic BMT may reduce acute GVHD. The apparent requirement for TNF-α in the differentiation of cytotoxic T cells suggests that complete neutralization of TNF-α early after BMT may impair the graft-vs-leukemia (GVL) effect and highlights the complexity of inflammatory cytokine involvement in both GVHD and GVL.

References


