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T Cell TRAIL Promotes Murine Lupus by Sustaining Effector CD4+ Th Cell Numbers and by Inhibiting CD8 CTL Activity

Violeta Rus, Vinh Nguyen, Roman Puliaev, Irina A. Puliaeva, Valentina Zernetkina, Irina Luzina, John C. Papadimitriou, and Charles S. Via

T cells play an essential role in driving humoral autoimmunity in lupus. Molecules such as TRAIL exhibit strong T cell modulatory effects and are up-regulated in lupus, raising the possibility that they may influence disease severity. To address this possibility, we examined the role of TRAIL expression on pathogenic T cells in an induced model of murine lupus, the parent-into-F1 (P→F1) model of chronic graft-vs-host disease (GVHD), using wild-type or TRAIL-deficient donor T cells. Results were compared with mice undergoing suppressive acute GVHD. Although chronic GVHD mice exhibited less donor T cell TRAIL up-regulation and IFN-α-inducible gene expression than acute GVHD mice, donor CD4+ T cell TRAIL expression in chronic GVHD was essential for sustaining effector CD4+ Th cell numbers, for sustaining help to B cells, and for more severe lupus-like renal disease development. Conversely, TRAIL expression on donor CD8+ T cells had a milder, but significant down-regulatory effect on CTL effector function, affecting the perforin/granzyme pathway and not the Fas ligand pathway. These results indicate that, in this model, T cell-expressed TRAIL exacerbates lupus by the following: 1) positively regulating CD4+ Th cell numbers, for sustaining help to B cells, and for more severe lupus-like renal disease development. Thus, although much evidence supports a role for TRAIL in T cell-driven responses in vivo, its precise effect is unclear.

Systemic lupus erythematosus (SLE) is characterized by T cell-driven B cell hyperactivity resulting in the production of pathogenic autoantibodies. Our group and others have demonstrated previously that SLE patients exhibit striking elevations in TRAIL gene expression (27, 28). Moreover, both T cell-associated membrane TRAIL and release of soluble TRAIL are increased in SLE patients with active disease (7, 29, 30). These results strongly support a role for TRAIL in SLE pathogenesis; however, as with many of the immune abnormalities associated with lupus, it is not clear whether increased TRAIL expression reflects a compensatory mechanism aimed at limiting active disease or instead represents a mechanism central to disease exacerbation.

To address the functional in vivo role of TRAIL in mediating lupus pathology, we used an induced model of murine lupus, the parent-into-F1 (P→F1) model of chronic graft-vs-host disease (GVHD). As a control, we also tested the role of TRAIL in mediating a strong cytotoxic T cell response using the P→F1 model of acute GVHD. The transfer of TRAIL-deficient donor cells into TRAIL-intact recipients demonstrates a dichotomous role for the expression of TRAIL on Ag-specific T cells. Specifically, TRAIL expression is important in sustaining effector CD4+ Th cell numbers, which in turn provide help for B cell production of autoantibodies; yet TRAIL expression also in vitro data indicate that TRAIL can either suppress or costimulate T cell responses depending on whether it acts as a ligand or as a receptor, respectively (17–19).

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3 Abbreviations used in this paper: DR, death receptor; FasL, Fas ligand; GVHD, graft-vs-host disease; KO, knockout; MCF, mean channel fluorescence; MxA, myxovirus (influenza virus) resistance 1; OAS1a, oligoadenylate synthetase; SLE, systemic lupus erythematosus; WT, wild type; Ct, cycle threshold.

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down-regulates CTL responses that could possibly limit B cell hyperactivity and autoantibody production.

Materials and Methods

Mice

TRAIL KO mice were generated by gene targeting, as described (31), and have been backcrossed to C57BL/6 mice for >10 generations. All mice used in this study were housed and bred at the University of Maryland Animal Care Facility. Genetic stability of the mutation in breeder mice was verified by genotyping from ear punch DNA. B6 and BDF1 mice were purchased from The Jackson Laboratory. All procedures were preapproved by the University of Maryland and Baltimore Veterans Administration Institutional Animal Care and Use Committee.

Induction of GVHD

Donor splenocytes were prepared and GVHD induced, as described (32). Unless otherwise noted, acute GVHD was induced with 4 × 10^8 unfractionated splenocytes and chronic GVHD was induced with 10^7 CD4^+ (CD8^− T cell-depleted) splenocytes. Flow cytometry was used before injection to confirm that equal numbers of CD4^+ and CD8^+ T cells were injected into recipient F1 mice. Donor CD8^+ T cells were depleted using Dynabeads Mouse CD8 (Ly2) (Invitrogen Life Technologies). Flow cytometric analysis demonstrated <1% contaminating CD8^+ T cells. Controls consisted of un.injected age- and sex-matched F1 mice.

Flow cytometric analysis

Staining of splenocytes was performed, as previously described (32, 33), and analyzed on a FACSscan flow cytometer (BD Biosciences). mAb against CD4, CD8, B220, H-2K^d, I-Ad, Pgp-1 (CD44), Fas (CD95), and Fas ligand (Fasl) (CD178) were purchased from BD Pharmingen. Intracellular staining for granzyme B and perforin was performed according to the manufacturer’s recommendations (Caltag Laboratories). Fluorescence data were collected for 10,000 gated cells. Studies of donor T cells were performed on 5,000 CD4^+ or CD8^+ T cells that did not stain positively for MHC class I of the uninjectected parent H-2K^d.

Preparation of CFSE-labeled donor cells

CFSE (Molecular Probes) labeling of donor splenocytes and analysis of donor cell proliferation by flow cytometry were performed as previously described (34). Briefly, cells were adjusted to 5 × 10^6/ml in PBS/0.1% BSA, then incubated in the dark for 10 min at 37°C with CFSE stock solution (10 mM in DMSO to a final concentration of 5 μM). Staining was quenched with 5 times the initial volume of ice-cold RPMI 1640/10% FBS, after which cells were washed three times in 1× PBS before injection into F1 mice. Proliferating CFSE plus donor CD4^+ or CD8^+ T cells were distinguished by multiparameter flow cytometry. The percentages of cells under each proliferation peak were calculated using CellQuest software.

BrdU incorporation

On day 7, 10, or 14 after donor cell transfer, mice received one dose of 1 mg of BrdU i.p. At 1 h after BrdU administration, mice were sacrificed and splenocytes were stained with anti-CD4 or anti-CD8 PE and anti-H-2K^d CyChrome (BD Pharmingen). Proliferating donor T cells were defined as staining positively for BrdU and CD4^+ or CD8^+, but negatively for H-2K^d.

 Determination of apoptotic donor CD4^+ and CD8^+ T cells

At days 6–10 after donor cell transfer, splenocytes from acute or chronic GVHD mice were stained for CD4 and H-2K^d, as described above, and then stained with Apo-annexin V (BD Pharmingen), according to the manufacturer’s instructions.

ELISA for ssDNA

Serum was tested for the presence of anti-ssDNA IgG Abs, as described (32).

In vivo cytokine capture assay

In vivo production of IFN-γ was measured by serum in vivo cytokine capture assay, as previously described (35, 36).

In vivo cytotoxicity assay

B6 and DBA/2 splenocytes were labeled with either 0.5 μM CFSE (B6-CFSElow) or 5 μM CFSE (B6-CFSEhigh), as described (37). Cell
suspensions were irradiated at 2000 rad, and 5 × 10^6 cells of each population were mixed together (1:1 ratio) and injected i.v. into control F1 and GVHD mice. Mice were sacrificed after 5 h, and CFSE-labeled cells were analyzed by flow cytometry. Percent specific lysis of DBA/2 spleen cells was calculated as follows (38): percentage of lysis = 100 − ((percentage of CFSE<sup>high</sup> in GVHD/percentage of CFSE<sup>low</sup> in GVHD)/ (percentage of CFSE<sup>high</sup> in control F1/percentage of CFSE<sup>low</sup> in control F1)) × 100.

**FIGURE 2.** Acute GVHD is enhanced in mice injected with suboptimal numbers of TRAIL KO spleen cells. A. Mice were injected with 50, 40, or 30 × 10^6 splenocytes from WT or TRAIL KO B6 mice, and mean number of host B cells (±SEM; n = 5 mice/group) is shown at 14 days after parental transfer for WT→F1 (□) or TRAIL KO→F1 (■). B and D. Acute GVHD kinetics using 40 × 10^6 splenocytes from WT or TRAIL KO mice. Kinetics are shown for host B cell numbers (B), engraftment of donor CD8<sup>+</sup> (C) and CD4<sup>+</sup> (D) T cells for WT→F1 (solid line, ○), or TRAIL KO→F1 (dotted line, ●). For all groups, values represent mean ± SEM (n = 5 mice/group; *, p < 0.05). Similar results were obtained in two additional experiments.

**FIGURE 3.** TRAIL KO donor CD8<sup>+</sup> T cells exhibit enhanced granzyme B/perforin pathway activity. Acute GVHD was induced using 40 × 10^6 splenocytes from WT or TRAIL KO B6 mice and recipient splenocytes examined at day 7 (A–C) or 10 (D–F) after cell transfer. A, Flow cytometric determination of intracellular granzyme B expression on gated naive and engrafted donor CD8<sup>+</sup> cells. B, Mean percentage of granzyme B-positive donor CD8<sup>+</sup> cells. C, Mean perforin mRNA expression from unfractionated splenocytes (mean fold increase over uninjected control mice). D, Flow cytometric determination of intracellular perforin in engrafted donor CD8<sup>+</sup> cells (mean MCF fold increase over naive donor). E and F, Representative histogram of intracellular perforin expression in engrafted WT (E) and TRAIL KO (F) donor CD8<sup>+</sup> cells compared with naive donor CD8<sup>+</sup> cells. G, Mean in vivo donor antihost CTL activity performed on day 10, as described in Materials and Methods. B–D and G, Results represent group mean ± SEM (n = 5 mice/group).
**Figure 4.** TRAIL KO donor cells exhibit enhanced donor CD8⁺ T cell proliferation, but no changes in initial proliferation or apoptotic rates. Acute GVHD was induced with 40 × 10⁶ WT or TRAIL KO donor cells. Proliferation of donor CD8⁺ T cells was determined using CFSE-labeled donor cells on day 3 (A) and by using in vivo BrdU labeling on days 7 and 10 (B), as described in Materials and Methods. Proliferating CFSE⁺ and BrdU⁺ cells are shown for gated donor CD8⁺ T cells. A, Percentage of proliferating cells (mean ± SEM) in each division peak on day 3. B, Percentage of BrdU⁺ cells (mean ± SEM) on days 7 and 10. C and D, Apoptotic rates (mean ± SEM) of donor CD8⁺ (C) and CD4⁺ (D) T cells are shown as assessed ex vivo by annexin V staining at days 7 and 10 after parental cell transfer (n = 5 mice/group). Controls (day 0) consisted of un.injected naive WT or TRAIL KO donor CD8⁺ T cells. Similar data were obtained in an additional experiment.

**Ex vivo cytotoxicity assay**
Responder cells from uninjectected F₁ mice, WT→F₁, and TRAIL KO→F₁ mice were cultured with irradiated (3000 rad) EL-4 (H₂b) or P815 (H-2d) tumor target cells in a 4-h Cr release assay, as described (39). Effectors were tested in triplicate at four E:T ratios. The percentage of lysis was calculated according to the formula: (cpm spontaneous – cpm maximum – cpm spontaneous)/cpm spontaneous) × 100%.

**Real-time PCR**
Total RNA isolation, quantitation, and reverse transcription were performed, as described (32). For myxovirus (influenza virus) resistance 1 (Mx-1) and oligoadenylate synthetase (OAS1a), the RT-PCR was conducted on an ABI 7500 Real-Time PCR System using TaqMan Gene Expression Assays (Applied Biosystems). The TaqMan probe/primer sets were as follows: Mx-1 Mn00487796_m1; OAS1a Mn00836412_m1; primers for the Mx-1 model of GVHD is a useful approach for determining the role of surface molecules on Ag-specific T cells. Acute GVHD is induced by the transfer of both CD4⁺ and CD8⁺ donor T cells and is characterized by near-complete elimination of host B cells by donor antihost CTL at 2 wk after transfer. In contrast, chronic GVHD is induced by the transfer of donor CD4⁺ T cells only and is characterized by donor CD4⁺ T cell-driven B cell hyperactivity, elevated serum anti-ssDNA Ab by 2 wk, and lupus-like renal disease at >2 mo (32, 42). Previous work in both mice and humans demonstrating that TCR-mediated T cell activation induces TRAIL up-regulation (43, 44) suggests that donor T cell TRAIL expression may contribute to the induction of either form of GVHD. To determine the kinetics of TRAIL up-regulation, acute and chronic GVHD were induced and TRAIL up-regulation was assessed by flow cytometry at days 7, 10, and 14 after donor transfer. At day 7, both donor and host CD4⁺ and CD8⁺ T cells from acute GVHD mice exhibited significant TRAIL up-regulation (Fig. 1, A, B, D, and E), whereas no detectable up-regulation was seen for donor or host CD4⁺ T cells from chronic GVHD mice (data not shown). Analysis of mean channel fluorescence (MCF) from day 7 acute GVHD mice demonstrated an increase in TRAIL expression by ~8-fold for donor CD4⁺ T cells and ~4-fold for donor CD8⁺ T cells when compared with naive, uninjectected donor cells, but no increase in TRAIL expression on host T cell from injected mice compared with uninjectected normal F₁ mice. Conversely, MCF

**Kidney histopathology**
H&E, immunohistochemistry, and control staining were performed, as described (41). All slides were blindly scored semiquantitatively by two independent observers (J. Papadimitriou and I. Luzina). For H&E slides, the following glomerular features were graded: mesangial hypercellularity, neutrophilic exudate, membrane thickness, crescents, and glomerular cell apoptosis. A cumulative glomerular score was calculated for each individual mouse. For immunohistochemistry staining, slides were evaluated semiquantitatively using the following scale: 0 = normal/negative; 1⁺ = mild; 2⁺ = moderate; and 3⁺ = severe. The level of Ig deposition was graded using the same scale.

**Statistical analysis**
Mice were tested individually, and mean values ± SEM were calculated. Data were examined for normality and equal variance (Kolmogorov-Smirnov). If satisfactory, groups were compared by two-tailed Student’s t test.

**Results**
**T cell TRAIL up-regulation is characteristic of acute GVHD, but not chronic GVHD**

The P→F₁ model of GVHD is a useful approach for determining the role of surface molecules on Ag-specific T cells. Acute GVHD is induced by the transfer of both CD4⁺ and CD8⁺ donor T cells and is characterized by near-complete elimination of host B cells by donor antihost CTL at 2 wk after transfer. In contrast, chronic GVHD is induced by the transfer of donor CD4⁺ T cells only and is characterized by donor CD4⁺ T cell-driven B cell hyperactivity, elevated serum anti-ssDNA Ab by 2 wk, and lupus-like renal disease at >2 mo (32, 42). Previous work in both mice and humans demonstrating that TCR-mediated T cell activation induces TRAIL up-regulation (43, 44) suggests that donor T cell TRAIL expression may contribute to the induction of either form of GVHD. To determine the kinetics of TRAIL up-regulation, acute and chronic GVHD were induced and TRAIL up-regulation was assessed by flow cytometry at days 7, 10, and 14 after donor transfer. At day 7, both donor and host CD4⁺ and CD8⁺ T cells from acute GVHD mice exhibited significant TRAIL up-regulation (Fig. 1, A, B, D, and E), whereas no detectable up-regulation was seen for donor or host CD4⁺ T cells from chronic GVHD mice (data not shown). Analysis of mean channel fluorescence (MCF) from day 7 acute GVHD mice demonstrated an increase in TRAIL expression by ~8-fold for donor CD4⁺ T cells and ~4-fold for donor CD8⁺ T cells when compared with naive, uninjectected donor cells, but no increase in TRAIL expression on host T cell from injected mice compared with uninjectected normal F₁ mice. Conversely, MCF
values for TRAIL expression on donor CD4$^+$ T cells in chronic GVHD mice did not differ significantly from either uninjected donor or host CD4$^+$ T cell TRAIL expression (data not shown).

The kinetic analysis of TRAIL expression showed that although the percentage of TRAIL-positive cells for both donor and host T cells was significantly elevated at all three time points in acute GVHD, CD8$^+$ T cells exhibited a striking peak at day 7 for both donor (Fig. 1C) and host (Fig. 1F) populations. Chronic GVHD mice exhibited no significant changes in the percentage of TRAIL-positive CD4$^+$ T cells at any of these time points for donor or host cells (data not shown).

**TRAIL-deficient donor cells exacerbate acute GVHD**

The foregoing results support a functional role for T cell TRAIL expression in acute GVHD, particularly at day 7. To address this question, acute GVHD was induced using TRAIL KO donor cells and TRAIL-intact BDF$^1$ hosts. Thus, only the alloantigen-activated donor T cells are TRAIL deficient. In all experiments, flow cytometric analysis was performed on the donor inocula before transfer to ensure that similar numbers of WT and TRAIL KO donor CD8$^+$ T cells were transferred. Using a dose of 50 × 10$^6$ donor splenocytes that consistently induces acute GVHD (32), we observed no differences in acute GVHD phenotype at 2 wk as assessed by host B cell elimination; however, at subthreshold doses (40 × 10$^6$ and 30 × 10$^6$), TRAIL KO donor cells exhibited significantly greater host B cell elimination at day 14 than did WT donor cells (Fig. 2A). Kinetic analysis demonstrated that compared with WT→F$^1$ mice, TRAIL KO→F$^1$ mice exhibited significantly greater elimination of host B cells at both day 10 and day 14 (Fig. 2B), significantly greater donor CD8$^+$ T cell engraftment at day 10 (Fig. 2C), and a small reduction in donor CD4$^+$ T cell engraftment seen at day 14 (Fig. 2D).

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**FIGURE 5.** TRAIL KO donor CD4$^+$ T cells exhibit reduced CD4$^+$ effector/helper numbers and reduced help to autoreactive B cells. Chronic GVHD was induced, as described in Materials and Methods, using either WT or TRAIL KO CD4$^+$ T cells, and mice were assessed at the indicated times by flow cytometry. A, Kinetics of donor CD4$^+$ T cell engraftment. B, Initial proliferation rates of donor CD4$^+$ T cells determined at day 3 using CFSE-labeled donor cells. Shown is percentage of proliferating cells (mean ± SEM) in each division peak. C, Percentage of BrdU$^+$ donor CD4$^+$ T cells at day 10. D, Kinetics of donor CD4$^+$ T cell apoptosis shown as mean annexin-positive cells. E, Kinetics of percentage of donor CD4$^+$ CD44$^{high}$ T cells. F, A representative histogram of CD44 expression on naive, WT, and TRAIL KO CD4$^+$ donor cells at day 10 after donor cell transfer. G–I, Chronic GVHD kinetics. The kinetics of host B cell numbers (G), MHC II up-regulation (I-A$^d$) on host B cells (H), and serum IgG anti-ssDNA Ab (I) were determined, as described in Materials and Methods. For all panels except B, results represent group means ± SEM (n = 5 mice/group); *, p < 0.05 between WT→F$^1$ and KO→F$^1$ experimental groups. D and E, Controls (day 0) consist of naive WT or KO donor cells. A, G, H, and I, Day 0 controls consist of uninjected F$^1$ mice. Similar data were obtained in two additional experiments.
FIGURE 6. Donor CD4+ T cell TRAIL expression promotes more severe lupus-like glomerulonephritis. Chronic GVHD was induced with 2 × 10^7 donor CD4+ cells, and animals were sacrificed at 10 wk of disease (n = 5 mice/group). A and C, H&E-stained kidney sections of uninjected F1 mice (A) and chronic GVHD mice induced with WT (B) or TRAIL KO donor cells (C). Shown are capillary loop thickening (thin arrowhead), mesangial hyperproliferation (thick arrowhead), and mesangial matrix deposition (+). D–F, Immunohistochemistry staining of IgG deposits in normal (D), WT→F1 (E), or KO→F1 (F), demonstrating less IgG deposited in TRAIL KO compared with WT-injected mice (original magnification, ×40). G, Glomerular damage was scored by two independent observers, as described in Materials and Methods. Data represent group means ± SEM.

Greater B cell elimination in TRAIL KO→F1 acute GVHD mice is accompanied by greater perforin/granzyme, but not Fas/FasL pathway activity

The severity of host B cell elimination in acute GVHD mice at 2 wk of disease is a surrogate marker of in vivo antihost CTL activity (36) and is mediated primarily by both perforin/granzyme and Fas pathways (36, 45, 46). We sought to determine whether the enhanced host B cell elimination shown for TRAIL KO→F1 mice (Fig. 2B) is due to alterations in either of these cytolytic pathways. Increased donor CD8+ granzyme B expression in donor CD8+ T cells was observed as early as day 7 in TRAIL KO→F1 mice compared with WT→F1, mice as measured by changes in MCF (118.3 ± 4.8 KO→F1 vs 95.3 ± 2.9 WT→F1; p = 0.003) (Fig. 3A) or percentage of positive cells (Fig. 3B). Significant elevations of granzyme B in donor CD8+ T cells from TRAIL KO→F1 mice persisted through day 10, but by day 14 were no longer detectable (data not shown). We also observed a small (~3-fold), but significant increase in perforin mRNA expression for TRAIL KO→F1 mice vs WT→F1 mice at day 7 (Fig. 3C) and a small, but statistically significant increase in perforin protein (Fig. 3, D–F) as determined by intracellular flow cytometry at day 10 (MCF fold increase over naïve donor for KO CD8+ T cells = 1.8 ± 0.08 vs WT CD8+ T cells over control = 1.3 ± 0.03; p = 0.001). Regarding the Fas/FasL pathway, we did not detect differences in peak (day 10) expression of FasL on donor CD4+ T cells, FasL on donor CD8+ T cells, and Fas expression on host B cells, or in peak (day 7) serum IFN-γ levels, a major correlate of Fas/FasL up-regulation (data not shown) (36, 45, 46). To determine whether the greater intracellular granzyme B expression in KO→F1 mice is functionally significant, we measured in vivo antithost CTL killing of normal (Fas-dull), irradiated DBA/2 splenocytes, as described in Materials and Methods. TRAIL KO→F1 mice exhibited a small, but significant increase in peak (day 10) in vivo antithost CTL activity (Fig. 3G). Although the differences in antihost CTL activity are relatively small at day 10, they are biologically significant in that they result in greater host B cell elimination by day 14 for TRAIL KO→F1 mice (Fig. 2B). Similar results have also been observed in an ex vivo assay using Fas-negative P815 target cells that demonstrated an increase in TRAIL KO→F1 cytotoxicity (35 ± 3.1% lysis) compared with WT→F1 (18 ± 4.6% lysis; p = 0.02) at an E:T ratio of 100:1. Taken together, our results support the conclusion that in submaximally induced acute GVHD, the modest, but significant enhancement of donor CTL elimination of host B cells seen in TRAIL KO→F1 mice reflects a modest, but significant enhancement of the perforin/granzyme B pathway and not the Fas/FasL pathway.

Increased engraftment of TRAIL KO donor CD8+ cells in acute GVHD is due to increased proliferation, rather than decreased apoptosis

Given the dual role of TRAIL in both proliferation and apoptosis (11, 13, 14), the increased engraftment of TRAIL KO donor CD8+ T cells at day 10 (Fig. 2C) could be the consequence of increased proliferation and/or decreased apoptosis. To distinguish between these two possibilities, we assessed the proliferation rate of donor CD8+ cells using CFSE-labeled donor cells at an early time point (day 3) and in vivo BrdU injection at later time points. At day 3 after GVHD induction (Fig. 4A), the majority of donor CD8+ T cells from WT and TRAIL KO mice have undergone ≥5 cell divisions. The percentage of WT and TRAIL KO donor CD8+ T cells in each proliferating peak did not differ significantly. However, at both day 7 and day 10, the percentage of proliferating donor CD8+ cells was significantly higher for TRAIL KO donor cells compared with WT donor cells (Fig. 4B). No significant differences in apoptotic rates at day 7 or 10 were observed for donor CD8+ cells at day 10 (Fig. 4C) or CD4+ T cells (Fig. 4D). These data support the idea that greater engraftment of TRAIL KO donor CD8+ T cells at...
day 10 is due to greater in vivo proliferation at the time of peak CTL activity.

**TRAIL-deficient donor cells attenuate chronic GVHD**

Our results demonstrating significant TRAIL up-regulation in acute GVHD, but not in chronic GVHD (Fig. 1), raise the possibility that T cell TRAIL expression may be less important in chronic GVHD pathogenesis. To test this idea, we compared the ability of donor T cells from either WT or TRAIL KO mice to induce chronic GVHD. As shown in Fig. 5A, engraftment of donor CD4+ T cells did not differ between groups at day 7 after donor cell transfer; however, by 2 and 4 wk, the number of donor CD4+ T cells declines dramatically in the spleens of TRAIL KO→F1 mice compared with WT→F1 mice (data not shown). No significant differences in the initial donor CD4+ T cell proliferation as determined by CFSE labeling were detected (Fig. 5B); however, by day 10, the percentage of proliferating donor CD4+ cells as determined by BrdU incorporation was significantly lower for TRAIL KO compared with WT donor cells (Fig. 5C) consistent with the subsequent reduction in TRAIL KO CD4+ T cell engraftment seen after day 7 (Fig. 5A). No significant differences were detected in donor CD4+ T cell apoptotic rates at either 7 or 10 days after GVHD induction (Fig. 5D).

Importantly, significantly fewer TRAIL KO donor CD4+ T cells were positive for the activation marker CD44 compared with WT→F1 mice after the first week of disease (Fig. 5, E and F), indicating reduced numbers of donor CD4+ T cells with effector phenotype and raising the possibility of reduced help for host B cells. Supporting this idea, critical parameters of chronic GVHD severity, such as host B cell number (Fig. 5G), MHC class II up-regulation (Fig. 5H), and serum ssDNA autoantibody levels (Fig. 5I), were significantly decreased (~2-fold) in TRAIL KO→F1 mice compared with WT→F1 mice at time points ≥1 wk of disease. These data indicate that in the setting of T cell-driven polyclonal B cell activation, CD4+ T cell TRAIL expression does not contribute significantly to the initial activation of donor CD4+ T cells (days 0–7), but instead has a major role in sustaining CD4+ effector/Th cell numbers and function after the first week.

**Impaired donor CD4+ Th cell function in TRAIL KO→F1 chronic GVHD mice is associated with milder lupus-like renal disease**

To determine whether the attenuation of chronic GVHD parameters shown in Fig. 5 for TRAIL KO→F1 mice is biologically significant and alters the severity of lupus-like renal disease, we examined kidneys from TRAIL KO→F1 and WT→F1 mice at 10 wk after GVHD induction. Using a donor cell inoculum of 10^7 CD4+ T cells, a mild glomerulonephritis was observed in WT→F1 mice, as evidenced by glomerular enlargement, mesangial hypercellularity, focal perivascular inflammation, and a mean glomerular score of 1.2 ± 0.45, whereas no features of glomerulonephritis were detectable in the kidneys of TRAIL KO-injected F1 mice, and they were indistinguishable from those of normal F1 mice (data not shown). To assess whether this effect is maintained in the setting of more severe renal involvement, F1 mice received 2 × 10^7 purified WT or TRAIL KO CD4+ cells. As shown in Fig. 6, WT→F1 mice exhibited more severe histological evidence of renal disease (Fig. 6, A–C), more glomerular deposition of IgG (Fig. 6, D–F) (WT→F1 = 2.3–3.5 vs KO→F1 = 1.0–2.0), and significantly greater glomerular scores compared with KO→F1 mice (Fig. 6G).
These results confirm that the reductions in chronic GVHD surrogate markers (B cell numbers, B cell MHC II up-regulation, and serum anti-ssDNA Ab) seen in TRAIL KO→F1 mice are biologically significant and result in less severe renal disease. Taken together, these experiments demonstrate that donor CD4 TRAIL expression critically modulates lupus-like disease severity by positively regulating the survival of donor CD4+ T cell, which in turn results in sustained T cell help to B cells, increased autoantibody production, and more lupus-like glomerulonephritis.

**Donor T cell TRAIL expression is not required for DR5 up-regulation in acute or chronic GVHD**

A prerequisite for TRAIL activity is up-regulation of its signaling receptor DR5, the only signaling receptor in mice, and is typically expressed at low levels on resting splenocytes (47). To determine whether the alterations in GVHD phenotype seen above in TRAIL KO→F1 mice reflect changes in DR5 up-regulation, the kinetics of DR5 expression were assessed by flow cytometry in TRAIL KO→F1 and WT→F1 mice. As shown in Fig. 7, WT→F1 acute GVHD mice exhibited strong up-regulation of DR5 on donor CD4+ (Fig. 7A), donor CD8+ T cells (Fig. 7B), and host B cells (Fig. 7C) at day 14. No defects in kinetics of DR5 up-regulation were detected for TRAIL KO→F1 compared with WT→F1 acute GVHD mice for either donor CD4+ (Fig. 7F), donor CD8+ T cells (Fig. 7G), or host B cells (Fig. 7H), and in some cases were actually greater than in WT→F1 mice. By contrast, neither WT→F1 nor TRAIL KO→F1 chronic GVHD mice exhibited significant increase in donor CD4 expression of DR5 over un.injected controls (Fig. 7D, E, and F). Host B cells from TRAIL KO→F1 and WT→F1 chronic GVHD mice exhibited similar DR5 up-regulation kinetics with the exception of day 10, which demonstrated a greater percentage of DR5-positive cells for KO→F1 mice. These results demonstrate that the alterations in GVHD phenotype seen with TRAIL KO donor cells are due to lack of T cell TRAIL expression and do not reflect impaired up-regulation of TRAIL ligand (DR5).

**Donor T cell TRAIL expression is not required for IFN-α gene expression in acute or chronic GVHD mice**

Type I IFNs are critical for up-regulation of TRAIL protein on T cells following TCR-mediated activation (43, 44); however, TRAIL expression has also been shown to induce type I IFNs (48). To determine whether differential TRAIL up-regulation seen in acute and chronic GVHD mice (Fig. 1) reflects differential IFN-α production or whether TRAIL KO donor cells alter host IFN-α induction, we measured the expression of two IFN-α-inducible genes, Mx-1 and OAS1α (49, 50). As shown in Fig. 8, both WT→F1 and TRAIL KO→F1 acute GVHD mice exhibited significant elevations of both Mx-1 (Fig. 8A) and OAS1α (Fig. 8B) gene expression, which peaked on day 7, as did donor CD8+ T cell TRAIL expression (Fig. 1C). Chronic GVHD mice exhibited low level, but significant increases in Mx-1 and OAS1α over uninjectected controls at day 7 (Fig. 8C and D). We did not detect a significant difference in IFN-α-inducible gene expression for TRAIL KO→F1 vs WT→F1 in either acute or chronic GVHD at either time point, demonstrating that in the GVHD model, type I IFN induction is independent of donor T cell TRAIL expression.

**Discussion**

The present study demonstrates that the in vivo role of TRAIL expression on naive T cells differs depending on whether a cell-mediated or Ab-mediated response is induced. Specifically, using acute and chronic GVHD as models of cell-mediated (i.e., CTL) or Ab-mediated responses, respectively, the transfer of TRAIL-deficient donor T cells into TRAIL-intact recipients enhances acute GVHD and attenuates chronic GVHD. In acute GVHD, TRAIL-deficient donor T cells exhibit greater killing of host B cells consistent with a down-regulatory role for TRAIL. The mechanism of enhanced killing by TRAIL KO cells involves both greater donor CD8+ T cell proliferation and greater granzyme/perforin pathway activity. We did not detect significant differences in FasL up-regulation or in serum IFN-γ, an important prerequisite for FasL...
up-regulation in this model (46), supporting the idea that T cell-expressed TRAIL acts primarily as a negative regulator of both CD8\(^{+}\) T cell numbers and perforin/granzyme-mediated CD8\(^{+}\) CTL killing best observed for subthreshold numbers of donor cells. In contrast, chronic GVHD mice receiving TRAIL-deficient donor CD4\(^{+}\) T cells exhibited impaired host B cell expansion, reduced autoantibody production, and an attenuated lupus-like renal disease long-term. The reduction in host B cell activation and renal disease appears secondary to a reduced proliferation and expansion of TRAIL-deficient donor CD4\(^{+}\) T cells exhibiting effector phenotype. The decline in donor CD4\(^{+}\) Th effector cells begins after the first week of disease and most likely accounts for the reduction in host B cell activation. These results indicate Ag-specific T cell TRAIL expression can also act as a positive regulator by sustaining effector CD4\(^{+}\) T cell help for autoreactive B cells by promoting their continued proliferation, which in turn increases disease severity.

Given the well-documented role of TRAIL in mediating cytotoxicity against tumors and virally infected cells (31, 51–56), the increase in antihost CTL in the absence of donor T cell TRAIL was toxic against tumors and virally infected cells (31, 51–56), the ease severity.

moting their continued proliferation, which in turn increases disease long-term. The reduction in host B cell activation and renal autoantibody production, and an attenuated lupus-like renal disease appear secondary to a reduced proliferation and expansion of TRAIL-deficient donor CD4\(^{+}\) T cells exhibiting effector phenotype. The decline in donor CD4\(^{+}\) Th effector cells begins after the first week of disease and most likely accounts for the reduction in host B cell activation. These results indicate Ag-specific T cell TRAIL expression can also act as a positive regulator by sustaining effector CD4\(^{+}\) T cell help for autoreactive B cells by promoting their continued proliferation, which in turn increases disease severity.

Taken together, our results in both acute and chronic GVHD advance our understanding of the role of T cell-expressed TRAIL in human lupus. In humans, we and others have previously identified TRAIL as part of the up-regulated genes belonging to the IFN-\(\alpha\) signature in PBMC from SLE patients (27, 28). Additionally, we have observed increased membrane-associated TRAIL on CD4\(^{+}\) and CD8\(^{+}\) T cells from lupus patients, which correlated with disease activity (30). Interestingly, in the present study, we observed the induction of lupus-like disease in chronic GVHD mice was associated with low-level membrane TRAIL up-regulation on CD4\(^{+}\) T cells and low-level elevations of IFN-\(\alpha\) genes compared with values in acute GVHD mice. These seemingly conflicting observations support a novel paradigm regarding the role of TRAIL in the pathogenesis of human lupus. In the P→F\(_1\) model, perforin-mediated CTL play a critical role in eliminating activated autoreactive B cells and preventing lupus-like renal disease (45). Not only may TRAIL directly contribute to severity of lupus by promoting sustained CD4\(^{+}\) T cell help for B cells, but it may also exacerbate disease by down-regulating perforin-mediated CTL activity, which in turn allows activated autoreactive B cells to escape deletion. The impaired elimination of autoantibody-producing host B cells in TRAIL-intact (WT→F\(_1\)) compared with KO→F\(_1\) acute GVHD mice is consistent with reports in human lupus of increased membrane TRAIL on CD8\(^{+}\) T cells (29, 30) and defective in vitro CTL activity of CD8\(^{+}\) T cells (62, 63). Our results in chronic GVHD mice indicate that although a functional TRAIL molecule is required on CD4\(^{+}\) T cells for sustaining lupus pathogenesis, it need not exhibit significant up-regulation for lupus-like renal disease to develop. Similarly, our demonstration that low-level increases in IFN-\(\alpha\)-inducible gene expression in chronic GVHD mice that develop lupus-like renal disease supports the idea that increased IFN-\(\alpha\) and CD4\(^{+}\) TRAIL up-regulation are not absolutely required for disease expression, but rather serve to amplify disease expression. These results lead us to postulate that CD4\(^{+}\) TRAIL expression, perhaps in conjunction with increased IFN-\(\alpha\), exacerbates lupus by not only sustaining CD4\(^{+}\) Th cell numbers and help for autoreactive B cells, but also by impairing CD8\(^{+}\) CTL elimination of activated autoreactive B cells. Moreover, increased T cell TRAIL expression exacerbates lupus and is not a compensatory mechanism acting to limit lupus severity. These studies suggest that in vivo impairment of TRAIL-expressing CD4\(^{+}\) T cells may be a useful therapeutic approach in human lupus.
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Disclosures

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References


