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At the end of an immune response, activated lymphocyte populations contract, leaving only a small memory population. The deletion of CD8⁺ T cells from the periphery is associated with an accumulation of CD8⁺ T cells in the liver, resulting in both CD8⁺ T cell apoptosis and liver damage. After adoptive transfer and in vivo activation of TCR transgenic CD8⁺ T cells, an increased number of activated CD8⁺ T cells was observed in the lymph nodes, spleen, and liver of mice treated with anti-TNF-α. However, caspase activity was decreased only in CD8⁺ T cells in the liver, not in those in the lymphoid organs. These results indicate that TNF-α is responsible for inducing apoptosis in the liver and suggest that CD8⁺ T cells escaping this mechanism of deletion can recirculate into the periphery. The Journal of Immunology, 2004, 173: 2402–2409.

A n important aspect of the immune response is the ability of specific lymphocyte populations to expand and contract in response to infection. Population shrinkage at the end of an effective immune response may be due to decreased levels of survival factors, causing a form of apoptosis termed passive cell death, or increased levels of proapoptotic signals, such as the ligation of death receptors, causing activation-induced cell death (AICD).³

TCR transgenic CD8⁺ T cells activated in vivo dispersed from the lymph nodes and spleen, and an increased number of activated CD8⁺ T cells were found undergoing apoptosis in the liver (1). No such evidence of apoptosis was detected in lymph nodes and spleen. This phenomenon appears to be specific for activated CD8⁺ T cells and is evident in various in vivo model systems (2–7). As there was an increased frequency of apoptotic cells in the liver compared with the peripheral lymphoid organs, we hypothesized that the liver was a site of CD8⁺ T cell disposal. Perfusion of the liver with lymphocyte mixtures showed that only activated CD8⁺ cells were retained in the liver, whereas resting or apoptotic cells passed through (8). Once trapped, the cells began to undergo apoptosis. These findings led us to hypothesize that the liver was a trap for activated CD8⁺ T cells, with intrahepatic death signals providing a basis for CD8⁺ T cell deletion.

The exact mechanism of CD8⁺ T cell death in the liver remained to be elucidated. In models of CD8⁺ deletion after liver transplantation, features of both active and passive cell death were reported, with blockade of either pathway resulting in rejection of the liver allograft (9, 10). In primary T cell activation by hepatocytes, CD8⁺ T cells died from “neglect” due to lack of costimulation (11). In contrast, viral infection of the liver using a replication-deficient adenovirus showed that CD8⁺ T cell apoptosis was dependent on Fas, because CD8⁺ T cell apoptosis was decreased in mice that lacked either Fas (lpr/lpr) or FasL (gld/gld) (12). However, when the SV40 large T-specific TCR transgene was crossed onto lpr/lpr mice, the frequency of apoptotic cells in the liver of TCR transgenic lpr/lpr mice was unchanged (13). Fas is not the only death receptor system. Both activated CD8⁺ T cells and liver-specific populations, such as Kupffer cells, NK-T cells, and liver sinusoidal endothelial cells, have the capacity to produce TNF-α. Both Fas and TNF-α have a documented role in systemic virus infection. Thus, HIV-specific CD8⁺ T cells from the peripheral blood of HIV-infected patients had an increased susceptibility to Fas-induced apoptosis compared with CD8⁺ T cells specific for CMV or EBV (14) and an increased sensitivity to apoptosis induced by signaling through both TNF receptor 1 (TNFR1) and TNFR2 (15). However, neither Fas, TNFR1, nor TNF-α was important in T cell deletion after lymphocytic choriomeningitis virus infection (16, 17). Most of the data are consistent with the concept that when an Ag is presented directly on hepatocytes, the outcome is passive cell death, whereas when T cells are activated elsewhere and accumulate in the liver due to adhesion mechanisms (8), the outcome is AICD.

In the present study we test the hypothesis that TNF-α is responsible for CD8⁺ T cell death in the liver. TNF-α has previously been associated with apoptosis of activated CD8⁺ T cells. In Con A- plus IL-2-activated T cells induced to undergo apoptosis through cross-linking of the TCR/CD3 complex, CD4⁺, but not CD8⁺, T cells from lpr/lpr or gld/gld mice were less susceptible to apoptosis. Conversely, anti-TNF-α treatment decreased the apoptosis of CD8⁺ T cells, but had no effect on CD4⁺ T cells. Staining for the TNFR suggested that the apoptosis induced by TNF-α was mediated through TNFR2, because TNFR1 was undetectable (18). High level presentation of peptide-MHC complexes on APCs also resulted in the apoptosis of CD8⁺ T cells through a TNF-α-mediated mechanism. These effects were again mediated by TNFR2, were caspase-dependent, and required the down-regulation of Bcl-2 (19, 20).

In CD8⁺ T cells, TNF-α also appears to have roles in activation and effector function. Upon activation with Ag, CD8⁺ T cells rapidly produce TNF-α (21), but this TNF-α production is short-lived and independent of the presence of Ag (22). The production of TNF-α at the initial stages of CD8⁺ cell activation suggests that
TNF-α can act as both an autocrine and a paracrine CD8+ T cell growth factor. Signaling through TNFR2 reduces the threshold for CD8+ T cell activation and increases the proliferative response (23). The effect of TNF-α on T cell proliferation in response to Ag or mitogen was direct and occurred in the absence of accessory cells. In the mouse, TNFR2-deficient CD8+ T cells required 5-fold more anti-CD3 Ab stimulation compared with wild-type cells (24). TNFR2-deficient cells also produced less IL-2 and IFN-γ, and showed delayed acquisition of cell surface activation markers.

Using OT-1 CD8+ T cells in an adoptive transfer model of T cell activation, we investigated the role of TNF-α in the peripheral deletion of activated CD8+ T cells. As TNF-α also has a role in CD8+ T cell activation, we chose the use of blocking Abs over a knockout model because this would allow us the flexibility to block TNF-α at a time when activation was complete. Mice treated with Abs against TNF-α had more CD8+ T cells in the lymph nodes, spleen, and liver. However, caspase activity was reduced only in the liver CD8+ T cell population. These results suggest that local TNF-α in the liver promotes intrahepatic CD8+ T cell apoptosis, and that this controls CD8+ T cell numbers not only in the liver, but also in the periphery.

Materials and Methods

Animals

C57B/6J and B6.SJL-Ptprc Pep3b BoyI mice were obtained from The Jackson Laboratory (Bar Harbor, ME). OT-1 mice express a transgenic TCR that recognizes the 8-mer SIINFEKL peptide derived from residues 257-264 of OVA. Transgens were identified by staining PBL with Abs against CD8 and the chains of the transgenic TCR, Vα2 and Vβ5. A colony of OT-1 mice was maintained on the B6.SJL (CD45.1-expressing) background. All animals were housed in a specific pathogen-free environment in accordance with institutional guidelines for animal care.

Adoptive transfer and in vivo activation

Donor OT-1 mouse spleen and peripheral lymph nodes CD8+ T cells were purified through depletion of B cells, dendritic cells, NK cells, and CD4+ T cells using primary Abs (clone 212.A1 specific for MHC class II molecules, clone 2.4.G2 specific for FcRs, clone GKL.1 specific for CD4, and clone HB.191 specific for NK1.1), followed by magnetic beads (Biomag, goat anti-mouse IgM, goat anti-mouse IgG and goat anti-rat IgG; Qiagen, Valencia, CA). Their purity was >87%. A suspension of 5 × 10^6 OT-1 cells was injected i.v. into each recipient. OT-1 T cells were activated after adoptive transfer by daily i.p. injections of 25 µM SIINFEKL peptide (New England Peptide, Fitchburg, MA) in PBS for 3 days starting 24 h after injection. The adoptively transferred OT-1 T cells were identified using light scatter gates characteristic of lymphocytes and staining for CD45.1 and CD8.

Apoptosis detection and surface staining

TNF expression was determined by staining cells with the Abs CD45.1-PE, CD8-PerCP, TNFR2-biotin (all from BD Pharmingen), and TNFR1-PE (Immunotech, Westbrook, ME), followed by streptavidin-allophycocyanin (BD Pharmingen) or the appropriate isotype controls. Caspase activation was determined using CaspTag caspase-3 (DEVD), caspase-8 (LETD), and caspase-9 (LEHD) activity kits following the manufacturer’s instructions (Intergen, Purchase, NY). Cells were then surface-stained with CD45.1-PE, CD62L-PerCP, and CD62L-allophycocyanin (BD Pharmingen), or Vγ2-allophycocyanin (Caltag Laboratories, Burlingame, CA). Cells were then analyzed using a BD FACSCalibur (BD Biosciences, Mountain View, CA).

Results

Accumulation of activated CD8+ OT-1 T cells upon TNF-α blockade

To determine whether TNF-α plays a role in the peripheral deletion of activated CD8+ OT-1 T cells, host mice were injected with a hamster IgG-blocking anti-TNF-α Ab. As TNF-α promotes the activation and proliferation of CD8+ T cells, the first Ab treatment was given 3 days after the initial peptide injection, by which time activation was complete. Ab was given on day 3 for mice analyzed on day 5 and on days 3 and 5 for mice analyzed on day 7. Hamster IgG1 against trinitrophenyl-keyhole limpet hemocyanin was used as an isotype control. Lymphocytes from the lymph nodes, spleen, and liver were harvested on days 3, 5, and 7 after the initial peptide injection. The adoptively transferred OT-1 T cells were identified using light scatter gates characteristic of lymphocytes and staining for CD45.1 and CD8.

Fig. 1 shows the percentages and absolute numbers of OT-1 cells in the lymph nodes, spleen, and liver from multiple mice (4–8), expressed as the mean ± SEM. On day 3 there was a significant increase in both the percentage and the absolute number of CD8+ OT-1 T cells in the lymph nodes, spleen, and liver of mice injected with SIINFEKL peptide compared with PBS controls. In the lymph nodes the increase was from 1.8 ± 0.09% in the PBS controls to 10.7 ± 3.2% in the SIINFEKL peptide-treated animals (p = 0.03), and the absolute number increased from 0.2 ± 0.03 × 10^6 in the PBS controls to 3.6 ± 1 ± 10^6 in the SIINFEKL group (p = 0.01). In the spleen the increase was from 0.6 ± 0.1% in the PBS controls to 4.4 ± 1.2% in the SIINFEKL peptide-treated animals (p = 0.02), and the absolute number increased from 0.5 ± 0.2 × 10^6 in the PBS controls to 4.1 ± 1.2 × 10^6 in the SIINFEKL group (p = 0.03). In the liver the increase was from 0.9 ± 0.07% in the PBS controls to 19.7 ± 5.4% in the
SIINFEKL peptide-treated animals ($p = 0.01$), and the absolute number increased from $0.01 \pm 0.004 \times 10^6$ in the PBS controls to $1.2 \pm 0.3 \times 10^6$ in the SIINFEKL group ($p = 0.02$). This expansion was similar to that in previous studies (2).

On day 5 the activated CD8$^+$ OT-1 T cells were undergoing peripheral deletion. The percentage of cells in the SIINFEKL peptide plus isotype control group remained significantly higher than those in the PBS group, and the absolute numbers remained significantly higher in the spleen and liver. However, upon treatment with SIINFEKL peptide and anti-TNF-α Ab, there was a significant additional increase in both the percentage and, with the exception of lymph nodes, the number of CD8$^+$ OT-1 T cells. In lymph nodes, the percentage of OT-1 T cells in the PBS group was $1.0 \pm 0.3\%$ compared with $2.2 \pm 0.3\%$ in the SIINFEKL peptide plus isotype control group ($p = 0.008$) and $5.5 \pm 1.7\%$ in the SIINFEKL peptide plus anti-TNF-α Ab group ($p = 0.03$ compared with SIINFEKL peptide plus isotype control; $p = 0.02$ compared with PBS). The absolute numbers were $0.2 \pm 0.08 \times 10^6$ in the PBS group compared with $0.3 \pm 0.05 \times 10^6$ ($p = 0.05$) in the SIINFEKL peptide plus isotype control group and $0.5 \pm 0.2 \times 10^6$ in the SIINFEKL peptide plus anti-TNF-α Ab group ($p = 0.2$ compared with SIINFEKL peptide plus isotype control; $p = 0.09$ compared with PBS). In the spleen, the percentage of OT-1 T cells in the PBS group was $0.5 \pm 0.1\%$ compared with $1.9 \pm 0.4\%$ in the SIINFEKL peptide plus isotype control group ($p = 0.008$) and $7.6 \pm 2.6\%$ in the SIINFEKL peptide plus anti-TNF-α Ab group ($p = 0.02$ compared with SIINFEKL peptide plus isotype control; $p = 0.02$ compared with PBS). The absolute numbers were $0.4 \pm 0.1 \times 10^6$ in the PBS group compared with $1.5 \pm 0.3 \times 10^6$ in the SIINFEKL peptide plus isotype control group ($p = 0.005$), and $10.2 \pm 3.6 \times 10^6$ in the SIINFEKL peptide plus anti-TNF-α Ab group ($p = 0.01$ compared with SIINFEKL peptide plus isotype control; $p = 0.02$ compared with PBS). In the liver the OT-1 T cell percentages were $1.9 \pm 0.7\%$ in the PBS group compared with $9.8 \pm 4.0\%$ in the SIINFEKL peptide plus isotype control group ($p = 0.002$) and $27.8 \pm 5.1\%$ in the SIINFEKL peptide plus anti-TNF-α Ab group ($p = 0.002$ compared with SIINFEKL peptide plus isotype control; $p = 0.0006$ compared with PBS). The absolute numbers were $0.05 \pm 0.02 \times 10^6$ in the PBS group compared with $0.7 \pm 0.2 \times 10^6$ in the SIINFEKL peptide plus isotype control group ($p = 0.006$) and $3.5 \pm 1.1 \times 10^6$ in the SIINFEKL peptide plus anti-TNF-α Ab group ($p = 0.008$ compared with SIINFEKL peptide plus isotype control; $p = 0.008$ compared with PBS).

By day 7, CD8$^+$ OT-1 T cells in the SIINFEKL peptide and isotype control Ab group had decreased to the level in the PBS control group. The number of CD8$^+$ OT-1 T cells in the SIINFEKL peptide- and anti-TNF-α Ab-treated group had decreased from day 5, but was still increased compared with the SIINFEKL peptide plus isotype control Ab treated group. The percentages were $1.2 \pm 0.8\%$ in the lymph nodes (0.2 ± 0.06%,), $3.0 \pm 2.0\%$ in the spleen (0.2 ± 0.07%), and $8.1 \pm 4.7\%$ (0.5 ± 0.2%) in the liver. The absolute numbers were also increased, but the increases were not significant ($p > 0.05$). The results from day 5 suggest that TNF-α is a major element in the peripheral deletion of activated CD8$^+$ OT-1 T cells.

**FIGURE 1.** Anti-TNF-α treatment increases both the percentage and the number of CD8$^+$ OT-1 T cells on day 5. After adoptive transfer of OT-1 cells, mice were injected with PBS (■), SIINFEKL peptide and isotype control (▲), or SIINFEKL peptide and anti-TNF-α Ab (●). Lymph nodes (LN), spleen (SPL), and liver (LVR) were harvested on days 3, 5, and 7. Bars represent the mean and SEM of at least four individual mice. The first column shows the percentage of OT-1 cells in each organ, and the second column shows the absolute number in millions.

**TREATMENT OF CELLS WITH ANTI-TNF-α HAS NO EFFECT ON CD8$^+$ OT-1 T CELL PROLIFERATION**

We hypothesized that the increase in CD8$^+$ OT-1 T cell number on day 5 was due to a decrease in apoptosis rather than an effect on cell proliferation. Our experimental model involves administration of anti-TNF-α on day 3, and we would expect proliferation to be complete by this time. To exclude an effect on proliferation, CFSE-stained lymphocytes were harvested on day 4 after the first peptide injection, i.e., 24 h after the first injection of anti-TNF-α. Fig. 2 shows that in the PBS-treated animals the CD8$^+$ OT-1 T cells had not divided and retained a high intensity of CFSE with a mean fluorescent intensity (MFI) of ~700. In comparison, OT-1 cells from the SIINFEKL peptide-treated mice had divided sufficiently to dilute out the CFSE signal, with an MFI of 8 in the lymph nodes and 11 in the spleen and liver. There was no difference in the animals treated with anti-TNF-α Ab. Thus, although TNF-α has been associated with T cell proliferation, there was no effect of anti-TNF-α Ab on clonal expansion in our model.

**LATE TREATMENT OF CELLS WITH ANTI-TNF-α HAD NO EFFECT ON ACTIVATION OF CD8$^+$ OT-1 T CELLS**

To ensure that the treatment with anti-TNF-α was not affecting the extent of CD8$^+$ OT-1 T cell activation, cells were stained for CD44 and CD62L and analyzed by flow cytometry. Fig. 3 shows the expression of activation markers on CD8$^+$ OT-1 T cells in the lymph nodes, spleen, and liver. The first panel within each group depicts the CD45.1$^+$CD8$^+$ gate that was used to identify the OT-1 CD8$^+$ T cell population. The upper panels show examples of the increased percentage of OT-1 T cells in the three organs after treatment with SIINFEKL peptide, compared with PBS, on day 3. Thus, in lymph nodes the percentage of OT-1 cells increased from 1.8 to 12.1%, in the spleen the increase was from 0.8 to 4.9%, and in the liver the increase was from 0.9 to 29.9% of all lymphocytes. These increases were consistent across multiple mice and in a total
of five independent experiments. The lower panels show the effects of anti-TNF-α Ab treatment, administered on day 3, on the abundance of T cells on day 5. As in the aggregate data in Fig. 1, anti-TNF-α Ab increased the frequency of OT-1 cells in all tissues. These differences were consistent across multiple mice and experiments and were also manifest in terms of absolute cell numbers (see Fig. 1). In all three organs, we determined the mean expression of CD44, based on MFI, and the down-regulation of CD62L, based on the percentage of cells that were CD62Llow. Although OT-1 cells in mice treated with SIINFEKL peptide showed an increase in CD44 and a decrease in CD62L staining in all tissues (upper panels), there was no effect of anti-TNF-α Ab on these markers (lower panels).

Treatment with anti-TNF-α inhibited apoptosis in liver, but not peripheral lymphoid organs

To detect the initiation of apoptosis in the CD8⁺ OT-1 T cells, we examined the activation of caspases 3, 8, and 9. We chose to detect caspases, because their activation is an intermediate event in the apoptotic pathway. In contrast, TUNEL staining documents DNA fragmentation, which is a late event, and is difficult to detect ex vivo because apoptotic cells are rapidly removed from the circulation by phagocytic cells (25). Phosphatidylserine exposure is also difficult to assess, because lymphocyte purification from the liver can disrupt the membrane, leading to loss of phosphatidylserine expression. Therefore, staining with fluorogenic caspase substrates is our current method of choice for documenting apoptosis in isolated liver lymphocytes.

Lymphocytes from the lymph nodes, spleen, and liver on day 5 were incubated with the caspase substrates, stained with Abs against CD45.1 and CD8, and analyzed by flow cytometry. Fig. 4A shows representative FACS profiles from the lymph nodes and liver of the caspase substrates plotted against CD8. As shown in Fig. 4B, OT-1 cells were identified by gating on lymphocytes using light scatter and on the CD45.1⁺ CD8⁺ population. The percentage of OT-1 cells with active caspase activity was then determined by histogram analysis of the gated population.

As shown in Fig. 4C, all three caspases were activated in CD8⁺ OT-1 T cells. Upon treatment with anti-TNF-α Ab, the percentage of cells showing caspase activation was unchanged in lymph nodes and spleen. However, the percentages of cells with activated
FIGURE 4. Anti-TNF-α Ab treatment decreases caspase activation in OT-1 CD8⁺ T cells from the liver, but not the lymphoid organs. Lymph nodes, spleen, and liver from mice on day 5 were stained with the fluorogenic caspase substrates, anti-CD45.1 and anti-CD8. Apoptosis was assessed by determining the percentage of OT-1 cells with active caspase-3 (C3), caspase-8 (C8), and caspase-9 (C9) from mice injected with SIINFEKL peptide and isotype control (OVA) and from mice injected with SIINFEKL peptide and anti-TNF-α (TNF). A, FACS profiles from the lymph nodes and liver of caspase-3, caspase-8, and caspase-9 plotted against CD8. B, OT-1 lymphocytes gated on light scatter, CD45.1⁺CD8⁺ cells, and caspase activation by histogram. C, Results were expressed as line graphs, with each line indicating the mean of two mice from an individual experiment. The significant p value from paired Student’s t test is expressed in the top corner of the liver graphs.
caspase-3, -8, and -9 in the liver were significantly decreased ($p = 0.006$, $p = 0.005$, and $p = 0.02$, respectively). These findings showed that TNF-α was an important mediator of CD8+ T cell apoptosis in liver, but not in lymphoid organs. The fact that the numbers of CD8+ OT-1 T cells on day 5 were also increased in the spleen and lymph nodes suggests that once CD8+ T cells escaped deletion in the liver, they recirculated into the periphery. Whether these cells were deleted elsewhere or survived to become memory cells has yet to be determined.

**Caspase activation correlates with apoptosis assessed by TUNEL**

To confirm that staining with fluorogenic caspase substrates was a valid indicator of programming for apoptosis, we adopted the method of TUNEL staining ex vivo after a brief period of culture (26). Fig. 5A shows representative histograms, from OT-1 cells in the lymph nodes and liver, of caspase-3 staining directly ex vivo compared with TUNEL staining from the same organs after a 4-h incubation. In the lymph nodes, 9.3% of OT-1 cells had activated caspase-3 at 0 h, which corresponded to the 9.3% of OT-1 cells that stained with TUNEL after a 4-h incubation. In the liver, 9.7% of OT-1 cells had activated caspase-3 at 0 h, and 9.7% were TUNEL positive at 4 h.

Mononuclear cells isolated from the lymph nodes, spleen, and liver of both PBS- and SIINFEKL-treated mice were either stained with fluorogenic caspase-3 substrates directly ex vivo or incubated for 4 h at 37°C and TUNEL stained. For each organ, the percentage of OT-1 cells with active caspase-3 was plotted against the percentage of TUNEL-positive OT-1 cells in a linear regression (n = 20). As shown in Fig. 5B, there was a direct linear correlation of caspase staining at 0 h with TUNEL staining at 4 h ($r^2 = 0.9719$). These results confirm that caspase-3 staining is a valid indicator of programming for apoptosis, as confirmed by TUNEL.

**Activated CD8+ T cells up-regulate the expression of TNFR2**

To determine whether TNF-α had the ability to act directly on OT-1 cells, we analyzed the cell surface expression of the TNFRs, TNFR1 (p55) and TNFR2 (p75), on OT-1 T cells activated in vivo. OT-1 cells from PBS-treated mice were negative for TNFR1 staining, with an MFI of 5.9 in the lymph nodes compared with 5.2 for the isotype control, 4.1 in the spleen compared with 3.6 for the isotype control, and 6.6 in the liver compared with 7.4 for the isotype control (Fig. 6). TNFR2 was expressed at low levels and had MFIs of 8.0 in the lymph nodes compared with 4.0 for the isotype control, 6.6 in the spleen compared with 3.3 for the isotype control, and 22.0 in the liver compared with 10.0 for the isotype control. Upon activation with SIINFEKL peptide, the level of TNFR2 on CD8+ T cells was up-regulated in all organs, with 2- to 7-fold changes in MFI. TNFR1 levels remained low (Fig. 6). The results shown are representative of multiple mice in three independent experiments. These results are consistent with those of previous studies, in both humans and mice, which show that activated T cells predominantly express TNFR2 (18, 21).

**Discussion**

TNF-α is a pleiotropic cytokine with diverse effects in multiple cell types. The effects of TNF-α are mediated by two distinct cell surface receptors, p55/TNFR1 and p75/TNFR2, which are ubiquitously expressed on nucleated cells. Both receptors lack recognizable enzymatic domains, and their ability to transduce signals is dependent on their interaction with adaptor proteins associated with their cytoplasmic tails. TNF receptor-associated factor 1 (TRAF1) and TRAF2 are critical signaling molecules that link both TNFR1 and TNFR2 to downstream signaling pathways (reviewed in Ref. 27). The generation of TRAF2-deficient mice has

![FIGURE 5](http://www.jimmunol.org/)

**FIGURE 5.** Caspase staining correlates with apoptosis. Lymphocytes from the lymph nodes, spleen, and liver were either stained for caspase-3 activity directly ex vivo or incubated at 37°C for 4 h and stained with TUNEL. A, Representative histograms of caspase-3 staining at 0 h and TUNEL staining at 4 h of OT-1 cells from the lymph nodes and liver. B, Linear regression of caspase-3-positive OT-1 cells plotted against TUNEL-positive OT-1 cells.

![FIGURE 6](http://www.jimmunol.org/)

**FIGURE 6.** Up-regulation of TNFR2 on CD8+ T cells after activation. Lymphocytes from lymph nodes, spleen, and liver were gated on the OT-1 population and analyzed for TNFR expression. The upper panels indicate TNFR1 expression on OT-1 cells isolated from a PBS control, and the middle panels show TNFR1 expression on OT-1 cells from a SIINFEKL-treated mouse (OVA). The lower panels indicate TNFR2 expression on PBS control- and SIINFEKL-treated mouse. Isotype control staining is expressed as a hatched line, and TNFR as a bold line. The data are representative of three experiments, with three mice per group in each.
highlighted the importance of TNFR signaling in the generation of a functional immune system. Thymocytes and hematopoietic progenitors in TRAF2−/− mice have an increased sensitivity to TNF-α-induced apoptosis, and the mice have severe atrophy of the thymus and spleen (28). In a transgenic mouse model expressing a dominant-negative form of TRAF2 only in lymphocyte populations, inhibition of TRAF2 resulted in lymphopenopathy, splenomegaly, and an abnormal expansion of B cells (29). In CD8+ T cells, TNF-α appears to have roles in activation, effector function, and apoptosis.

In the present study the blockade of TNF-α with neutralizing Abs, after activation was complete, resulted in an increase in the number of CD8+ T cells in the liver and peripheral lymphoid organs. This increase in CD8+ T cell number was accompanied by a decrease in the frequency of CD8+ T cells in the liver with activated caspases, suggesting that TNF-α is also involved in the peripheral deletion of CD8+ T cells. These findings support previous studies suggesting a role for TNF-α in AICD of CD8+ T cells (18–20).

TNFR1 contains a functional death domain, and its ligation with agonistic Abs implicated this receptor as the predominant mediator of TNF-α-induced cytotoxicity, whereas TNFR2 initiated signals for proliferation of thymocytes and CTL (30). However, both receptors had to be inhibited to negate the cytotoxic effects of TNF-α (31), and both were required to induce TNF-α-dependent apoptosis (32, 33). To explain the data implicating both receptors in cytotoxicity, it was suggested that TNFR2 enhances the association of TNF-α to TNFR1. Enhancement of TNFR1 signaling by TNFR2 may be required when the TNF-α concentration is low, because TNFR2 has a higher affinity (Kd = 100 pM), but also has a faster dissociation rate (t1/2 = 10 min) than TNFR1 (Kd = 500 pM; t1/2 = 3 h). It is argued that the fast off-rate of TNFR2 binding creates a locally high TNF-α concentration at the cell surface, which, in turn, facilitates binding to TNFR1 (34). TNFR2 has also been reported to confer sensitivity to Fas/FasL-induced apoptosis in CD8+ T cells (35, 36).

However, in the present study we found no TNFR1 expressed on the surface of activated CD8+ T cells, and Fas signaling was previously found to have no role (13). These results suggested that TNF-α-dependent apoptosis was either mediated directly through TNFR2 or through cooperation with another mechanism distinct from Fas and TNFR1. However, we have not directly assessed the function of TNFRs on OT-1 cells. Previous studies implicating TNF-α as a mediator of CD8+ T cell AICD have also identified a TNF-α/TNFFR2 signaling mechanism (18–20). The ability of TNFR2 to induce apoptosis is mediated through the adaptor molecule receptor-interacting protein (RIP), which is induced during IL-2-driven proliferation. In the absence of RIP, signaling through TNFR2 induces activation of NFκB, whereas in the presence of RIP, the apoptotic pathway is triggered (37). This apoptotic pathway occurs independently of NFκB activity. This suggests a model in which naive cells activate NFκB in response to TNF-α and TCR signaling, undergoing activation and proliferation. As the cells divide, RIP is induced, leading to apoptosis of the cell through engagement of the same receptors, thereby controlling the magnitude of the immune response.

The specific function of the liver in TNF-α-induced CD8+ T cell apoptosis has yet to be determined. The liver contains many potential sources of TNF-α. NK cells, NK-T cells, Kupffer cells, cholangiocytes, and liver sinusoidal endothelial cells all have the ability to produce TNF-α when activated, as do CD8+ T cells themselves. Kupffer cells produce TNF-α when triggered by signaling through Fas. During Jo2 (anti-Fas Ab)-induced liver damage, transfection of hepatocytes with an adenovirus producing a soluble inhibitory form of Fas resulted in only a partial decrease in liver damage and mortality. However, transfection with soluble Fas along with anti-TNFFR1 Ab treatment resulted in complete survival (38). Both the production of TNF-α and early mortality associated with Jo2 treatment were reduced after depletion of Kupffer cells using gadolinium chloride. This supports a model in which the ligation of Fas on Kupffer cells by FasL expressed on the surface of activated CD8+ T cells induces the secretion of TNF-α.

The role of TNF-α in intrahepatic T cell apoptosis supports the idea that the unique environment of the liver provides a safe location for the destruction of activated CD8+ T cells. The impact of inhibiting this mechanism on the numbers of T cells in the lymphoid organs suggests that the liver has a significant influence on systemic T cell numbers.

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