Tumor Necrosis Factor α Up-regulates Non-lymphoid Fas-ligand following Superantigen-induced Peripheral Lymphocyte Activation*

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Michael J. Pinkoski‡, Nathalie M. Droni, and Douglas R. Green
From the Division of Cellular Immunology, La Jolla Institute for Allergy and Immunology, San Diego, California 92121

Members of the tumor necrosis factor (TNF) and TNF receptor families play important roles in inducing apoptosis and mediating the inflammatory response. Activated T lymphocytes can trigger the expression of Fas-ligand on non-lymphoid tissue, such as intestinal epithelial cells (IEC), and this, in turn, can induce apoptosis in the T cells. Here, we examine the role of TNFα in this feedback regulation. Injection of TNFα into mice caused a rapid up-regulation of Fas-ligand mRNA in IEC. TNFα-induced activation of the Fas-ligand promoter in IEC requires NF-κB as this was blocked by an IκBαM super-repressor and by mutation of an NF-κB site in the Fas-ligand promoter. Activation of T cells by antigen induced Fas-ligand expression in IEC in vivo in wild type, but not in TNFα−/− or TNFR1−/− mice. These results define a novel pathway wherein TNFα, produced by activated T cells in the intestine, induce Fas-ligand expression in IEC. This is the first observation that one member of the TNF superfamily mediates the regulation of another family member and represents a potential feedback mechanism controlling lymphocyte infiltration and inflammation in the small intestine.

Apoptosis of intestinal epithelial cells can lead to gastric enteropathies such as ulcerative colitis, Crohn’s Disease, and other inflammatory bowel disorders (see Ref. 1). Destruction of villi is associated with infiltration of activated lymphocytes, which are believed to be the primary mediators of tissue destruction (2). Infiltrating lymphocytes in ulcerative colitis lesions express high levels of the death ligand, Fas-ligand (3), and Fas/Fas-ligand have been shown to be key mediators of epithelial destruction (4, 5). Additionally, apoptotic destruction of the lamina propria by transferred CD4+ T cells into SCID3 mice led to similar colitis lesions (6). Diseases resulting from autoimmune destruction of intestinal epithelium may be due to dysregulation of a protective mechanism utilized by the epithelium that contributes to peripheral lymphocyte deletion.

The epithelium of the small intestine encounters many lymphocytes that percolate through the region, possibly as part of maintenance of immunity at the mucosal barrier. In healthy tissue intestinal epithelial cells (IEC) have been shown to contribute to deletion of activated lymphocytes during clonal contraction (7). In a model similar to that observed in the thyroid (8), infiltrating lymphocytes induce the expression of non-lymphoid Fas-ligand, which in turn causes induction of apoptosis in Fas-bearing lymphocytes (7). Failure to do so results in autoimmune destruction of the epithelium induced by the activated lymphocytes (see (1)). An inability to regulate this process may also contribute to the mechanism of intestinal destruction in severe graft-versus-host disease (9).

It has been demonstrated that TNFα and its receptor, TNFRI (p55) are required for efficient deletion of activated lymphocytes (10–12). Taken with our previous observations that Fas-ligand expression is induced in the epithelium of the small intestine in response to peripheral lymphocyte activation (7) and the presence of a functional NF-κB site in the Fas-ligand proximal promoter (13) we investigated the possibility that TNFα produced by staphylococcal enterotoxin B (SEB)-activated T lymphocytes (14) is a mediator of Fas-ligand expression in the intestinal epithelium.

Consistent with this model we found that IEC, which constitutively express TNFRI (p55), respond in vitro and in vivo to TNFα with induction of Fas-ligand mRNA. TNFα-dependent expression of Fas-ligand in IEC was mediated through an NF-κB regulatory element in the proximal promoter of the Fas-ligand gene and was inhibited by an IκBα super-repressor of NF-κB. We also found that TNFα−/− and TNFR1−/− mice did not display an increase in IEC Fas-ligand following superantigen-induced lymphocyte activation observed in wild type animals.

**EXPERIMENTAL PROCEDURES**

Animals, Cell Culture, and Reagents—C57BL/6, C57BL/6-Tnf−/− (TNFRI−/−), B6129SF2, B6,129-Tnfr1tm1Mak (TNF−/−), BALB/cByJ, and BALB/cByJSmn-Prlhc.xmt (SCID) mice were obtained from Jackson Laboratories (Bar Harbor, ME) and housed under pathogen-free conditions at the La Jolla Institute for Allergy and Immunology. IEC were isolated from small intestines as previously described (15) and cultured in Dulbecco’s modified Eagle’s medium plus 10% fetal calf serum, supplemented with glutamine, penicillin, and streptomycin. Reporter constructs containing the 1.2-kb human Fas-ligand promoter were isolated from mice that constitutively express TNFRI (p55) and were labeled with anti-CD3-CyChrome™ (BD-Pharmingen, San Diego, CA) and anti-FasL (N-20) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and anti-actin from ICN (Aurora, OH). All other antibodies were obtained from BD-Pharmingen (La Jolla, CA) unless noted otherwise.

Lymphocyte Isolation and Flow Cytometry—Peripheral lymphocytes were isolated from spleens and inguinal lymph nodes as described previously (15). After blocking with CD16/CD32 (FcγRII/II receptor), lymphocyte preparations were labeled with anti-CD3-ECyChrome™ (145–2C11) and anti-Vβ8 T-cell receptor-PE (F23.1) (BD-Pharmingen, La Jolla, CA). Flow cytometry was performed on a Becton Dickinson FACScan and analyzed using the Cellquest™ software.

Protein Extraction and Western Blot Analysis—For protein extraction, cells were washed twice in PBS, lysed in buffer containing 150 mM NaCl, 50 mM, pH 8.0, Tris-HCl, 0.1% sodium-SDS, and 0.5% sodium-deoxycholate in the presence of protease inhibitors for 30 min at 4 °C.
and centrifuged (20 min; 15,000 × g), and supernatants collected. For Western blot, after SDS-PAGE and electrotransfer to nitrocellulose membranes (Amersham Biosciences), membranes were incubated for at least 1 h with 5% non-fat milk in TPBS (0.1% Tween 20, 1× PBS) and for 2 h at room temperature with primary antibodies. Horseradish peroxidase-conjugated goat anti-rabbit or anti mouse secondary antibodies were then added for 30–60 min, and proteins revealed by enhanced chemiluminescence reagent (Pierce, Rockford, IL) and autoradiography.

Reverse Transcription, RNase Protection Assay, and Real Time PCR—Total RNA was isolated from primary intestinal epithelial cells using Trizol (Invitrogen) according to the manufacturer’s instructions. RNA was reverse-transcribed by Moloney murine leukemia virus reverse transcriptase (Invitrogen) using random hexamers (Invitrogen). For Fas-ligand gene expression, primers were located in separate exons in genomic DNA to eliminate the possibility signal due to genomic DNA contamination. Sequence specific primers for murine Fas-ligand (forward primer: 5'-TGAATTACCATGGTCCCAG-3'; reverse primer: 5'-AACATGGACCTGAGGAGCC-3'); TNF (forward primer: 5'-ACAGAAACGATATTCCGCG-3'; reverse primer: 5'-GCCGGCAACTTCTTTG-3'); luciferase (forward primer: 5'-TGAATTACGTCGGACGTCGAAAGTG-3'; reverse primer: 5'-ACTCTCTCCGCCAACACTTTT-3'); β-actin (forward primer: 5'-TTGTCGCTGTTGAA-3'; reverse primer: 5'-GCGGGAATGAAACCTCTGATG-3') and 18 S (forward primer: 5'-ATGGTAGTTCGGCCTGCTGTA-3'; reverse primer: 5'-CCGGATAGAAACCTCTGATG-3') were used. Real time PCR was performed with AmpliTaq Gold™ polymerase in a PE Biosystems 5700 thermocycler using SyBr Green™ detection protocol as outlined by the manufacturer.

Expression Vectors and Transient Transfections—Murine TNFα cDNA was first cloned by RT-PCR from total RNA using TNFα forward primer (5'-atgagcacagaaagcatgatcc-3') and 18 S (forward primer: 5'-AAGATACTCCTGCTGCTGTA-3') as positive controls. Amplified fragment was cloned directly into pcDNA3.1-TOPO vector (Invitrogen) and sequenced according to the manufacturer’s instructions. Expression vector for LbO (18) was described previously (16).

Transient expression was performed using Superfect™ (Qiagen, Valencia, CA) according to the manufacturer’s instructions with 1 μg of each vector. Transfected IEC were incubated for 20 h at 37 °C before analysis by real-time PCR. Antagonist anti-TNFRI antibody (clone 55R-170) was used at a concentration of 2 μg/ml. Transfection efficiencies were measured by analysis of lacZ or luciferase reporter gene expression.

RESULTS

We reported previously that Fas-ligand expression in epithelial cells of the small intestine (IEC) is induced by activated T cells (7). In keeping with our previous observations, we observed a significant increase in Fas-ligand protein levels in response to superantigen-induced activation of peripheral lymphocytes (Fig. 1A). To study the induction of Fas-ligand expression, we developed a quantitative real time RT-PCR assay. Fig. 1B shows that the increases in Fas-ligand mRNA correlated with increases in Fas-ligand protein levels. Although this phenomenon has been observed in all mouse strains tested, C57BL/6 mice consistently showed a lower relative induction of Fas-ligand protein levels (Fig. 2A) than in Balb/c and related strains.

To identify a mechanism by which antigen-induced lymphocyte activation leads to Fas-ligand expression in IEC, we analyzed IEC for expression of receptors for factors produced by peripheral lymphocytes to which IEC respond. We performed RNase protection assays on primary IEC and found constitutive expression of TNFR1 (p55) (Fig. 1C), consistent with a previous observation of TNFR1 protein on the surface of IEC (19). Because TNFα is produced by activated lymphocytes infiltrating the small intestine (20), we investigated the possibility that TNFα acts through the TNFR1 on IEC to induce Fas-ligand in IEC following SEB-induced activation of peripheral lymphocytes.

TNFα is an inflammatory cytokine implicated in inflammatory bowel disease and celiac disease. In light of recent observations that TNFα is involved in both peripheral lymphocyte deletion (12, 21) and a number of enteropathies involving intestinal epithelia (22–24), we investigated the possibility that TNFα is a mediator of Fas-ligand induction in non-lymphoid tissue after peripheral lymphocyte activation.

To ascertain the effects of TNFα on IEC, we treated primary IEC ex vivo with various doses of TNFα (Fig. 2). At 2 h there was a significant induction of Fas-ligand mRNA expression at low doses of TNFα (Fig. 2A) and by 6 h a significant increase in Fas-ligand protein levels (Fig. 2B). To further analyze the effects of TNFα on IEC, we expressed TNFα in primary IEC by transient transfection and assayed the effects on Fas-ligand mRNA levels. IEC were isolated and transfected with TNFα-pcDNA, cells were harvested 20 h later, and RNA analyzed. Fas-ligand mRNA levels were measured relative to the internal control of 18 S RNA levels for each sample. TNFα mRNA was also measured as a transfection control. As shown in Fig. 2C, there was a significant, dose-dependent increase in endogenous Fas-ligand mRNA levels in TNFα-transfected IEC. Additionally, the TNFα-dependent effects on Fas-ligand were reduced to background levels when an anti-TNFRI antagonis-
Induction of Fas-ligand Expression by TNFα

**Fig. 2.** IEC up-regulate Fas-ligand expression in response to TNFα. A, primary IEC were isolated from Balb/c mice and treated *ex vivo* with TNFα for 2 and 6 h at 37 °C, after which Fas-ligand mRNA and protein levels were assessed by real time RT-PCR and Western blot analyses, respectively. B, primary IEC were prepared from Balb/c mice and transfected with a construct expressing TNFα. RNA was isolated from transfected IEC and analyzed for Fas-ligand mRNA by real time RT-PCR. Absolute amounts of Fas-ligand mRNA were determined, and the data shows Fas-ligand levels relative to empty vector-treated IEC controls. C, induction of Fas-ligand by transfected TNFα proceeds via extracellular interaction of TNFα with TNFR1. Primary IEC were transfected with TNFα-pcDNA or empty vector and analyzed for Fas-ligand expression. D, expression of TNFα resulted in increased Fas-ligand expression, which was reduced to background levels by addition of a neutralizing anti-TNFR1 antibody (TNFα+Ab).

Using this system, we performed co-transfections on primary IEC with TNFα in conjunction with a reporter construct containing the human Fas-ligand promoter driving the luciferase gene, hFasLpro-HsLuc. This system allowed us to perform simultaneous analyses on various Fas-ligand promoter constructs compared with activity of the endogenous Fas-ligand gene, 20 h after transfection, RNA populations were analyzed for luciferase, TNFα, and endogenous Fas-ligand expression by real time RT-PCR. Similar to its effects on endogenous Fas-ligand, expression of TNFα also caused a significant increase in luciferase expression mediated through the Fas-ligand promoter (Fig. 3). Luciferase mRNA was quantitated relative to luciferase DNA as a control of transfection efficiency for each sample.

When TNFα-pcDNA was co-transfected with the Fas-ligand promoter construct lacking the NFκB site, ΔNFκB-hFasLpro-HSLuc, the effects of TNFα were reduced to background levels similar to that observed with empty vector. This demonstrated that the effects of TNFα on the up-regulation of Fas-ligand mRNA in IEC were dependent NFκB sites in the Fas-ligand proximal promoter (Fig. 3). In the same experiments we found that TNFα-induced expression of endogenous Fas-ligand was not affected, demonstrating that ablation of the effects due to TNFα was the result of removing the NFκB site and not due to inhibition of some other aspect of TNFα signaling.

To further investigate the role of NFκB in TNFα-mediated Fas-ligand expression, we utilized a mutant IκB, IκBoM, which is not phosphorylated or degraded and thereby inhibits activation of NFκB (18). Expression of IκBoM by transient co-transfection in primary IEC blocked TNFα-mediated induction of Fas-ligand reporter construct (Fig. 3). Further, IκBoM also blocked expression of endogenous Fas-ligand mRNA in TNFα-transfected IEC, confirming that TNFα-mediated effects in IEC occur via NFκB activation, which in turn utilizes the NFκB site of the Fas-ligand proximal promoter (13). It is interesting to note that we observed a complete inhibition of Fas-ligand promoter activity despite a maximum transfection efficiency of 50%. This is likely due to autocrine effects of TNFα in co-transfected cells, which also accounts for the observation that activity of the endogenous Fas-ligand gene was inhibited by IκBoM.

To study the effects of TNFα on IEC Fas-ligand expression *in vivo*, we injected TNFα into mice to assess the response in the epithelium of the small intestine, with respect to induction of Fas-ligand expression. TNFα in PBS or PBS alone was administered intraperitoneal to Balb/c mice. Initial experiments were performed with a range of TNFα over a very short time period so as to minimize the possibility for indirect effects. Within 4 h we observed a significant increase in IEC Fas-ligand mRNA levels (Fig. 4A); however, in subsequent analyses, we utilized a lower dose of TNFα (2 μg/mouse) over a longer time frame (12 h) to reduce potentially toxic effects to the animals. IEC were isolated 12 h postinjection and analyzed by real time RT-PCR for Fas-ligand expression. As shown in Fig. 4B, injection of TNFα resulted in a significant increase in IEC Fas-ligand expression *in vivo*. These results do not exclude the possibility for indirect effects of TNFα on IEC, but clearly demonstrate that the effects occur very early after administration.

After demonstrating that TNFα is capable of inducing Fas-ligand expression in IEC, we asked if it is necessary for up-regulation of Fas-ligand in IEC after antigen-driven immune activation. To investigate this possibility, we utilized the superantigen SEB, which causes an increase in Fas-ligand in a number of non-lymphoid tissues, including the epithelium of the small intestine (7). TNFα−/−, TNFR1−/− and wild type...
controls (B6129SF2 and C57BL/6, respectively) were injected with SEB (75–100 μg intraperitoneally), and 3 days later IEC mRNA was isolated and assayed by real time RT-PCR for Fas-ligand expression. Control mice displayed a marked increase in Fas-ligand mRNA, but little or no increase in Fas-ligand expression was detected in TNFα−/− or TNFR1−/− mice (Fig. 5A). To rule out the possibility that TNFα−/− and TNFR1−/− mice had an abnormal response to superantigen, we analyzed peripheral lymphocytes from SEB-treated knock-out animals. As shown in Fig. 5B, we observed virtually identical expansion of Vβ8+ cells in both knockout strains compared with C57BL/6 control mice. Therefore, the knockout mice still developed signs of lymphocyte activation indicative of an appropriate response to SEB, but without the attendant increase in IEC Fas-ligand mRNA. Fas-ligand expression that we measured in SEB-treated wild type IEC correlated with Fas-ligand-specific killing activity toward Fas-bearing targets ((7) and data not shown). This demonstrates that Fas-ligand expression correlates with the presence of functional Fas-ligand on the epithelial cell surface.

To establish the role of lymphoid cells in the TNFα-mediated Fas-ligand induction in response to SEB, we compared Balb/c-SCID treated with SEB or TNFα. In these immune-deficient mice we did not observe any up-regulation of Fas-ligand following administration of SEB (Fig. 6). Balb/c-SCID recipients were injected with TNFα, and IEC were isolated 12 h later. IEC RNA was analyzed by quantitative real time RT-PCR and Balb/c-SCID displayed increased expression of IEC Fas-ligand in response to administration of TNFα, similar to Balb/c (Fig. 6). This rules out the possibility that the effects we observed in the small intestine were a direct action of SEB on the intestinal epithelium. In contrast, Balb/c-SCID treated with TNFα showed a rapid induction of Fas-ligand in IEC. These data provide strong support for the idea that TNFα acts directly on IEC in vivo to mediate the up-regulation of Fas-ligand mRNA and that induction of IEC Fas-ligand in response to superantigen requires peripheral lymphocytes.

Taken together, these data support the model in which TNFα, produced by lymphoid cells after activation by superantigen (14), interacts with TNFR1 (p55) on the surface of IEC to induce expression of Fas-ligand. Expression of Fas-ligand appears to be triggered by NF-κB, a well characterized component of TNF-R signaling (25, 26).

**DISCUSSION**

TNFα is produced in a variety of inflammatory diseases, including those of the intestine (5, 27, 28), and this cytokine has been implicated as a mediator of intestinal damage (22–24). In this paper we have shown that intestinal epithelial cells respond to TNFα via the expression of Fas-ligand, a protein implicated in apoptosis induction of Fas-expressing cells and the maintenance of immune privilege (29, 30).

We found that TNFα directly induces expression of the endogenous Fas-ligand gene in intestinal epithelial cells. Injection of the superantigen, SEB, into mice also induces Fas-ligand expression on intestinal epithelium (Ref. 7 and Figs. 1, 5, and 6), and this is not seen in animals lacking a functional TNFα gene (Fig. 5). IEC express TNFR1 (19), and animals lacking TNFR1 are similarly resistant to induction of IEC Fas-ligand expression following SEB injection.

TNFα signaling via TNFR1 induces the activation of NF-κB

![Fig. 4.](https://example.com/fig4.png) **TNFα induces Fas-ligand mRNA expression and increases in protein levels in the epithelium of the small intestine.** Balb/c mice were injected with TNFα at the indicated doses (panel A). IEC were harvested at 4 h to minimize possible indirect effect, and IEC Fas-ligand expression was measured by real time RT-PCR. B, to minimize toxicity, a low dose of TNFα (2 μg) was injected into animals, IEC harvested 12 h later, and analyzed for Fas-ligand expression. As described above, absolute mRNA values were determined and reported relative to the basal expression of IEC Fas-ligand in mock-treated (PBS) mice (panel B). C, Western blot analysis of IEC protein samples from the same mice described in panel A were performed, which shows that both Fas-ligand mRNA and protein levels are increased in IEC in response to TNFα. IEC lysates from three separate mice are shown to illustrate the experimental variation and variation between animals.

![Fig. 5.](https://example.com/fig5.png) **TNFα−/− mice or TNFR1p55−/− mice do not display an increase in IEC Fas-ligand in response to SEB.** A, TNFα−/−, TNFR1−/−, and corresponding wild type control mice (B6129SF2 and C57BL6, respectively) were injected with SEB to cause an expansion of peripheral Vβ8+ lymphocytes. IEC were isolated 3 days after SEB administration and analyzed for Fas-ligand mRNA as described above. Data shown is from a minimum of three separate mice per genotype and treatment. B, to exclude the possibility that TNF and TNFR1 knockout animals have abnormal responses to SEB, we quantified the number of Vβ8+ cells relative to the total CD3+ population in each mouse strain 3 days after administration of superantigen. Analysis of lymphocytes isolated from the inguinal nodes shows that the response of the two knockout animals (TNF−/− and TNFR1−/−) expanded Vβ8+ populations to a similar degree to wild type C57BL6 mice. At least three animals were used per treatment and strain. Error bars represent standard error between animals in each group.
**Induction of Fas-ligand Expression by TNF**

![Graph showing Fas Ligand mRNA levels](image)

**FIG. 6.** Exogenously administered TNFα does not require lymphoid cells to mediate Fas-ligand up-regulation. Balb/c and Balb/c-SCID mice were injected with TNFα (2 µg intraperitoneal) for 12 h or SEB for 3 days, after which IEC were isolated and analyzed for Fas-ligand mRNA by real time PCR as described above. TNF induced IEC Fas-ligand in wild type and SCID animals; however, SEB only elicited this effect in animals with peripheral T lymphocytes. Each experimental animal was analyzed individually, and the data shown were pooled from a minimum of three mice per type and treatment.

(26, 31, 32), and this transcription factor has been implicated in the control of Fas-ligand expression (16). We have found that co-expression of a non-degradable form of IκB (IκBαM) (18) inhibits endogenous Fas-ligand gene expression as well as activity of a human Fas-ligand promoter reporter construct upon co-expression with a TNFα expression plasmid (Fig. 3). A human Fas-ligand promoter lacking an NF-κB site (13) failed to respond to TNFα, confirming this role for NF-κB.

NF-κB has been implicated in the inflammatory response induced by TNFα and other inflammatory cytokines. The induction of Fas-ligand by NF-κB might contribute to inflammation by recruitment of neutrophils, which occurs under some conditions of enforced Fas-ligand expression (33–35). However, we have not observed neutrophil infiltration into the intestines of animals treated with either SEB or TNFα to induce Fas-ligand expression (not shown).

The primary function of Fas-ligand is the induction of apoptosis, particularly in activated T cells and other Fas-expressing cells (36, 37). We previously proposed that expression of non-lymphoid Fas-ligand in immunologically privileged sites inhibits immune responses by inducing apoptosis in infiltrating T cells (29, 30, 36). Similarly, we have provided evidence that inducible Fas-ligand expression in peripheral tissues during the response to superantigen contributes to the deletion of activated T cells following the peak of an immune response (7). It has been reported that activated peripheral T cells express TNFα (20) and also that TNF sensitizes activated T cells to Fas-mediated apoptosis (38). In this report we provide the first evidence for a link between TNF and epithelial cell Fas-ligand expression in the small intestine.

If Fas-ligand is expressed on T cells (which clearly is the case, see Ref. 37) why is this not sufficient to mediate peripheral deletion and thereby regulate the extent of T cell-mediated damage to peripheral tissues? Previous studies have shown that primary murine T cells and some T cell lines undergo activation-induced cell death only through “fratricide,” that is, they express Fas-ligand and induce apoptosis in neighboring T cells but not in themselves (7). Therefore, in peripheral tissues where individual activated T cells are dispersed, their damaging effects will not be effectively regulated by T cell Fas-ligand. Peripheral non-lymphoid Fas-ligand can perform this function.

Perhaps more importantly, the peripheral tissue acts as a monitor that functionally determines when an immune response can become harmful to the tissue. In the case of IEC, TNF produced by infiltrating T cells causes damage, and these cells respond by expressing Fas-ligand. The idea that this serves to protect the epithelium from the effects of the T cells is supported by a study of one model of inflammation, in which CD4+ T cells were observed to undergo Fas-dependent apoptosis in the lamina propria (5, 6). It is possible that the intestinal epithelium “senses” infiltration of lymphocytes by the presence of cytokines, in this case TNFα, produced by the lymphocytes. Induction of non-lymphoid Fas-ligand in this situation may represent a mechanism wherein the non-lymphoid compartment limits the number of lymphocytes that traffic through gut, thus providing a regulatory control on intestinal inflammation.

A similar scenario has been suggested for the control of immune responses in the thyroid. In this case, the cytokines produced by infiltrating lymphocytes can elicit opposing responses in the thyrocytes. Thyrocytes express a basal level of Fas-ligand (39), which has cytotoxic activity for infiltrating lymphocytes, however, IFN-γ secreted by T1 cells renders thyrocytes sensitive to Fas-ligand-mediated fratricide (8). Thyrocyte apoptosis induced in this manner is held as the underlying cause of tissue destruction in Hashimoto’s Thyroiditis. It had been proposed that thyrocyte Fas-ligand serves to limit the numbers of lymphocytes in the thyroid as a mechanism of both immune homeostasis and thyroid protection (40).

In a study of inflammation accompanying infection with cytomegalovirus, the clearance of infiltrating T cells from various peripheral tissues was found to depend on functional Fas and Fas-ligand, although the clearance of the virus was independent of either protein (41). Therefore, expression of Fas-ligand in non-lymphoid tissues, as we observed here, could help to limit lymphoid infiltration following the clearance of an infectious disease organism.

Infiltrating T cells have been observed in a number of models of inflammatory bowel disease (2, 20, 23, 42) and have been implicated as key mediators in the development of disease (2). Similarly, intestinal tissue damage during graft-versus-host disease has been related to increased infiltration and immune destruction by transplanted lymphocyte populations (15, 43–45). Consistent with our proposal that inducible expression of Fas-ligand contributes to the control of infiltrating lymphocytes, van den Brink et al. (9) have recently reported that Fas-ligand-deficient gld mice are more susceptible to acute graft-versus-host disease following allogeneic bone marrow transplant than wild type animals with functional Fas-ligand. Also, gld versus wild type recipients showed a significant increase in donor T cell expansion and intestinal pathology, which included massive lymphocyte infiltrates and crypt destruction (9).

Our observations indicate that TNF in the small intestine impacts its surroundings by inducing Fas-ligand expression, which is then proposed to induce apoptosis in infiltrating lymphocytes. TNF has been implicated in peripheral lymphocyte deletion based on the observations that TNF-dependent signaling is required for activation-induced cell death (10–12). However, these results did not exclude the possibility that TNF could contribute to peripheral deletion via an indirect (i.e. non-lymphoid) intermediate, and the connection between IEC Fas-ligand induction following peripheral lymphocyte activation and peripheral deletion is the focus of ongoing studies. The two proposed modes of action for TNF (direct/lymphoid and indirect/non-lymphoid) are not mutually exclusive, nor do we exclude the possible involvement of other factors that mediate
inducible expression of Fas-ligand and other death ligands in the intestinal epithelium and other involved non-lymphoid tissues.

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