TFF2 deficiency exacerbates weight loss and alters immune cell and cytokine profiles in DSS colitis, and this cannot be rescued by wild-type bone marrow

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Submitted 9 May 2014; accepted in final form 1 October 2014

TREFOIL FACTORS (TFFs) constitute a three-member family of small, secreted peptides with a unique disulfide-bound tripartite structure. TFF1 and TFF2 have been shown to be expressed primarily in the stomach and TFF1 and TFF3 in the intestine and colon (15, 20, 23, 38, 49, 52). The three TFF genes are present together on a short region of chromosome 21 in humans (and on mouse chromosome 17), and, given the overlapping distribution of their derived proteins, it is not surprising that they have common regulatory elements and cross-regulatory roles. For instance, genetic depletion of TFF2 can induce TFF1 expression in the thymus, can inhibit TFF expres-

ion in the spleen, and is without effect in the stomach (23), and its effect in the colon has not been determined, while application of TFF2 to stomach cells increases transcription of TFF2 in a MAPK-dependent fashion (5). TFF3, the main colonic TFF and a major epithelial homeostatic regulator and repair factor, is negatively regulated by proinflammatory colitis-associated cytokines (8), although cross-regulation of TFF3 by the other TFFs is yet to be determined.

More recently, the expression pattern of the TFFs has expanded to incorporate other mucosal sites, including the lung (29, 56), breast (18, 37, 42), and eye (24), with expression at many of these sites ectopically induced following induction of inflammatory pathology or tissue damage. In the gut, ectopic expression of TFF2, in particular, occurs in human Crohn’s disease (25) and in gastric intestinal metaplasia (20). In addition, TFF2 and other TFFs have been detected at low levels in rodent lymphoid tissues, including the spleen (7, 23) and thymus (7), and in extracts from peritoneal macrophages (13, 23), suggesting a potential immunomodulatory role.

Coincident with the site of expression of the TFFs, is their main purported role, which is the protection and restorative repair of mucosal surfaces at which they are secreted (10, 16, 32, 54, 57). TFFs primarily mediate these roles by promoting motogenesis (4, 16), enhancing differentiation (12, 19, 27, 46), and inhibiting inflammation throughout the gut (13, 23, 34). TFFs are secreted luminally but are also secreted into the circulation and, thus, appear to act systemically as well (53). We showed that TFF2, when applied intrarectally in a rat model of hapten colitis, accelerates mucosal healing and reduces inflammatory indexes (51). Similar protective effects of TFF2 have been demonstrated in dextran sodium sulfate (DSS)-induced colitis in the mouse (36), likely mediated in part by reduced leukocyte recruitment coincident with inhibition of endothelial VCAM1 expression (43). An additional target of TFF2 in the inflammatory cascade appears to be macrophage-derived nitric oxide (NO). TFF2 can inhibit inducible NO synthase to reduce proinflammatory NO and the production of free radicals (13). Together, these findings suggest that TFF2 is required for maintenance of mucosal integrity throughout the gut and that reduced TFF2 expression increases susceptibility to inflammatory disease in the colon in particular.
ity, especially downstream epithelial and immune gene targets of TFF2, in wild-type or TFF2-deficient mice, and 2) to determine whether mucosal or infiltrating immunocyte-derived TFF2 is more important in mediating the anti-inflammatory and repair functions of this TFF.

**MATERIALS AND METHODS**

**Mice.** TFF1- and TFF2-deficient mice were developed as previously described (11, 50). All mice were backcrossed for a minimum of eight generations onto a C57BL/6 background, and age-matched wild-type littermates were used as controls. Mice were housed in a...
specific pathogen-free (SPF) animal facility. Mice were genotyped by multiplex PCR, as previously described (34). For all mouse experiments, equivalent numbers of males and females were used in the wild-type and deficient groups, and the values for genders were averaged. All animal experiments were performed with the approval of the Murdoch Children’s Research Institute Animal Ethics Committee (AEC A713).

**Induction of DSS colitis.** Acute and chronic models of DSS-induced colitis were used. Because the standard acute DSS protocol was lethal in TFF2-deficient mice, it was modified as follows. On days 1–5, 3% DSS (mol wt ~40,000; TdB Consultancy) in drinking water was provided ad libitum for TFF1-deficient, TFF2-deficient, and matched wild-type mice, and plain drinking water was provided for controls. On days 6–8, standard drinking water was provided for all mice, and mice were celled on day 8. In the chronic DSS protocol, on days 1–5, 3% DSS in drinking water was provided ad libitum for TFF2-deficient and matched wild-type mice, and plain drinking water was provided for controls. On days 6–12, standard drinking water was provided for all mice. This protocol was repeated four times, with mice celled on day 44. All mice were weighed daily.

**Macroscopic and histological assessment.** Colonies were removed from mice from the cecum to the rectum and photographed with a Coolpix 4500 digital camera (Nikon Instruments, Melville, NY). Length of the colon was measured from the images using ImageJ software for Windows version 1.3 (http://rsb.info.nih.gov/ij/index.html). Subsequently, the colon was cut open and bisected longitudinally; half was frozen in liquid nitrogen, and the other half was fixed in 4% buffered paraformaldehyde before processing. Paraffin sections (4 μm) were stained with hematoxylin-eosin.

**Microscopic morphometry.** Images were captured with a Coolpix 4500 digital camera, and morphometric analysis was performed using ImageJ software for Windows version 1.3. For damage measurements, images of the entire colon mucosa were captured, and a digital line was drawn along the muscularis mucosa in all regions where the epithelium was no longer intact (termed damaged), and the software was used to quantify this. The length of the damaged mucosa was expressed relative to the total length of the mucosa.

**Analysis of leukocyte infiltration into the colon.** Single cell suspensions were isolated from the colon as previously reported (55). Briefly, colon samples were incubated with shaking in Hanks’ balanced saline solution (HBSS) containing 5 mM EDTA and 1 mM DTT. The resultant cell solution contained epithelial cells and intraepithelial leukocytes (IELs). The remnant tissue was rinsed in HBSS to remove EDTA and DTT and digested with shaking in HBSS containing 0.4 mg/ml collagenase type IV. The resultant cell suspension contained lamina propria mononuclear cells (LPMCs). IELs and LPMCs were purified from contaminating epithelium, stroma, and dead cells by centrifugation on a 40%-80% Percoll gradient. Purified IELs and LPMCs were stained with a cocktail of leukocyte cell surface markers (CD45 (catalog no. 560468), CD8a (catalog no. 561967), CD11b (catalog no. 57397), CD11c (catalog no. 550261), CD45R (catalog no. 553087), and Gr-1 (catalog no. 552093); all from BD Biosciences). CountBright beads were used to determine total cell number per sample, and cell surface staining was analyzed on a flow cytometer (model LSR II, BD Biosciences) using FACS Diva (BD Biosciences). On the basis of forward- and sidescatter, propidium iodide staining, and staining on unutilized wavelengths, the following cell populations were eliminated: dead cells, aggregates, debris, red blood cells, and autofluorescent cells. Leukocytes (CD45+ E-cadherin+ ) and epithelial cells (CD45+ E-cadherin−) were isolated from these preparations. Unstained cells did not receive any antibody and demonstrated the background staining present in these cell preparations. RNA was extracted from the sorted cell populations and subjected to qRT-PCR analysis of TFF2, CD4, CD8, epcam, cytokeratin 18, and the housekeeping gene L32. Results are expressed as fold change compared with unsorted single cell preparations. Bars show mean values; n = 5/group. B: wild-type mice were housed in specific pathogen-free (SPF) or conventional facilities. RNA was extracted from spleens of mice and subjected to qRT-PCR analysis. iNOS, inducible nitric oxide synthase. Values are means ± SE.

**Isolation of purified colonic cell populations.** LPMCs were prepared as described above and stained for CD45 (catalog no. 561487, BD Biosciences) and E-cadherin (catalog no. FAB7481P, R&D Systems). On the basis of forward- and sidescatter, propidium iodide staining, and staining on unutilized wavelengths, the following cell populations were eliminated: dead cells, aggregates, debris, red blood cells, and autofluorescent cells. Leukocytes (CD45+ E-cadherin+ ) and epithelial cells (CD45+ E-cadherin−) were isolated from the remaining cells on an Influx sorter (BD Biosciences) on a 100-μm nozzle at 22 psi.

**Bone marrow transplants.** Bone marrow was prepared from male TFF2-deficient or wild-type mice as follows. Mice were culled, femurs were removed, and bone marrow was flushed with HBSS and a 20-gauge needle, strained to a single cell suspension, counted, and stored on ice for injection into recipient mice. Bone marrow chimeras were generated as described elsewhere (44). Briefly, recipient female TFF2-deficient mice were lethally irradiated (2 doses, 5.5 Gy, 3 h apart) and reconstituted with 5 × 106 donor cells by tail vein injection.Recipient mice were treated with prophylactic antibiotics (2 mg/ml neomycin sulfate; Sigma) for 4 wk to allow for complete reconstitution. Mice were then challenged with the acute DSS protocol as described above.

**Isolation of peritoneal macrophages.** Peritoneal macrophages were recovered from mice after injection of 5 ml of HBSS into the peritoneal cavity. To enrich for macrophages, cells were resuspended in RPMI with 10% FCS and plated onto uncoated plastic. After a 10-min incubation, nonadherent cells were removed and plated onto six-well dishes. Adherent macrophage-enriched cells were incubated for 16 h in 10% FCS, stimulated with LPS (100 ng/ml), and harvested 3 h later. RNA was purified using the RNeasy MiniKit (Qiagen).

**Analysis of mRNA expression.** Total RNA was harvested using TRIzol reagent (Life Technologies) or RNeasy columns (catalog no. 74104, Qiagen). (RNA 3 μg, n ≥ 5 animals/group) was reverse-transcribed into cDNA using Moloney’s murine leukemia virus reverse transcriptase (Promega) primed with oligo(dT). Primer Express (Applied Biosystems) was used to design quantitative RT-PCR (qRT-PCR) primers: IL-1β (CAGGCAGTATCACCTATGTTGG and GTGCGATTGTCTAAATGGGAACG), myeloperoxidase (MPO: CGGGCATGTCAGTGAATA and TGTAGCGCTGAGGATGATAACC), IL-1β (TAAAGGAAATACGACATTGCG and CACCTGACATGCTCGACAG), IL-10 (TGATGCCTCAATGCGACACA and CACCAGGGATTCAAATGC), IL-6 (AAGAAGCAGTATCACCATTTTACGAAAAC and AAGAAGGCACTGTTAGGATGAGT), wild-type TFF2 (TATCCGAGGACCCGTCTACACC and CTGTTGGTTTTTGACCTGAGAC), and mutant TFF2 (TATCCGAGGACCCGTCTACACC and TAGGTGAGCACTGATCAGAC), SYBR Green chemistry was used with rL32 as the internal reference gene. qRT-PCR conditions were 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 15 s (AB7500, Applied Biosystems). Results were analyzed using sequence detector software, and relative fold differences were determined using the cycle threshold (ΔΔCT) method.

**Statistical analysis.** Values are means ± SE. Statistical analysis was performed using one-way analysis of variance and the appropriate parametric or nonparametric statistical test using Sigmasstat (Jandel Scientific, San Rafael, CA). P ≤ 0.05 was considered statistically significant.
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COLITIS SUSCEPTIBILITY IN TFF2-DEFICIENT MICE

AJP-Gastrointest Liver Physiol • doi:10.1152/ajpgi.00172.2014 • www.ajpgi.org
RESULTS

Expression of TFF peptides in the colon is cross-regulated. mRNA was prepared from the colon, spleen, and thymus of wild-type and TFF2-deficient mice that had been housed in SPF microisolators and analyzed for expression of TFF1, TFF2, and TFF3 (Fig. 1; n = 5/genotype with data from 2 representative animals shown). TFF1 was expressed at low levels in the colon and spleen and at a significantly higher level in the thymus. Intriguingly, TFF2 genetic deficiency led to an increase in TFF1 expression in the spleen but a decrease in the colon and thymus (Fig. 1A). TFF2 was expressed in the colon and thymus of wild-type mice but, under these housing circumstances, was not detectable in the spleen. TFF2-deficient mice did not express TFF2, as we expected. TFF3 was expressed in the colon, spleen, and thymus of wild-type mice. Interestingly, expression of TFF3 was modulated by knockout of the TFF2 gene, such that, in its absence, TFF3 expression was decreased in the colon and increased in the spleen and thymus (Fig. 1A). We confirmed by qRT-PCR that TFF1 expression was decreased in the colon of TFF2-deficient mice (2.97 ± 0.56 fold in TFF2-deficient relative to wild-type mice) and TFF3 mRNA expression was also decreased in the colon of TFF2-deficient mice (1.78 ± 0.15 fold in TFF2-deficient relative to wild-type mice) and increased in the spleen (30.14 ± 3.46 fold in TFF2-deficient relative to wild-type mice) and thymus (7.74 ± 0.85 fold in TFF2-deficient relative to wild-type mice; Fig. 1A).

Acute DSS colitis was induced in wild-type mice, and expression of the TFF family members was analyzed by qRT-PCR. Expression of all three TFFs was decreased in the colon of mice with DSS-induced colitis compared with water-treated controls (Fig. 1B; −2.56 ± 0.71, −195.67 ± 79.72, and −4.39 ± 1.00 fold for TFF1, TFF2, and TFF3, respectively), with expression of TFF2, in particular, most profoundly decreased. Note: DSS-induced colitis is an inflammatory condition, reduced TFF expression in colitic colons suggests that the TFFs are not expressed by infiltrating inflammatory cells. To clarify whether colonic epithelial cells or leukocytes express TFF2, a single cell suspension of the colon was prepared, and the cells were sorted on the basis of CD45 (leukocyte) or E-cadherin (epithelial) expression (Fig. 2A). The CD45+ population additionally expressed the leukocyte markers CD4 and CD8 and exhibited negligible expression of the epithelial markers epcam and cytokeratin 18. In contrast, the E-cadherin+ population additionally expressed epcam and cytokeratin 18 and showed negligible expression of CD4 and CD8 (Fig. 2A). Expression of TFF2 was quantified in each population and found to be enriched in the E-cadherin+ cells and negligible in the CD45+ cells (Fig. 2A), providing further evidence that, in the colon, TFF2 is predominantly expressed by epithelial cells, and not by leukocytes.

Since modest TFF2 mRNA expression has previously been detected in rodent spleen (4, 19), we compared splenic expression of TFF2 in mice housed in SPF or conventional (open-top cage) facilities (Fig. 2B). As previously reported, TFF2 mRNA was near the lower limit of detection in spleens from animals housed in SPF conditions, but expression was strongly induced in spleens of conventionally housed mice. To better understand the stimulatory conditions of different housing environments, spleens were also analyzed for a range of pro- and anti-inflammatory moderators (Fig. 2B). Notably, the T helper (Th) type 2 (Th2) cell cytokines IL-13 and IL-4, as well as IL-1β, inducible NO synthase, and IL-10, were significantly upregulated in spleens from conventionally housed wild-type mice, while the Th1 cell cytokines IFNγ and IL-12 were unchanged. Together, these findings suggest that TFF2 mRNA expression in the spleen is very low basally but can be readily induced when mice are exposed to inflammatory stimuli, particularly those that promote Th2 cell immunity.

TFF1 expression does not influence susceptibility to DSS-induced colitis. Among the TFF family members, TFF1 and TFF2 are predominantly expressed in the stomach, with limited expression in the lower gut (Fig. 1) (47). However, TFF2 deficiency has been shown to moderate the response to DSS-induced colitis (23). Here we demonstrate that this effect is specific to TFF2, since TFF1-deficient and wild-type mice show the same response to acute DSS. Weight loss in TFF1-deficient mice was equivalent to that in wild-type mice (Fig. 3Ai), as was colon damage (Fig. 3Aii).

TFF2 protects mice from acute and chronic DSS-induced colitis. As previously reported (23), TFF2-deficient mice are more susceptible to acute DSS-induced colitis, showing more severe weight loss (~5% on days 6–8) than wild-type mice (Fig. 3Bi) but, interestingly, not greater colon shortening (Fig. 3Bi) or damage (Fig. 3Bii). Additionally, in regions of the colon from DSS-treated mice that were comparatively unaffected by ulceration, the number and distribution of goblet cells were not affected by the TFF2 null mutation (Fig. 3Biii). TFF2-deficient mice are also more susceptible to chronic DSS-induced colitis, displaying more weight loss throughout the experiment, including at its completion (Fig. 3Ci; 88.0 ± 3.5 vs. 96.9 ± 1.4%), again without greater colon shortening (Fig. 3Cii). Interestingly, in these longer-term studies, changes in body weight became most apparent beginning on day 10, suggesting a significant time lag in the peak of systemic
observed recruitment of Th (CD4+ IELs and submucosal LPMCs. After DSS treatment, we mice subjected to the acute (8-day) DSS protocol: mucosal two regions of the colons of wild-type and TFF2-deficient phages from TFF2-deficient mice appeared to be hypoacti- macrophages were isolated from wild-type and TFF2-deficient inflammatory cells were isolated from DSS-induced colitis indexes of TFF2-deficient mice (2, 6, 14). However, this does not translate into increased TFF2-deficient macrophages, and changes in expression of IL-33 and IL-19 in deficient mice are hyperresponsive; however, this is the first previously published report that macrophages from TFF2- TFF2-deficient mice express reduced levels of IL-33, basally and with LPS stimulation (Fig. 4C). These data corroborate the previously published report that macrophages from TFF2-deficient mice are hyperresponsive; however, this is the first demonstration of altered expression of IL-33 and IL-19 in TFF2-deficient macrophages, and changes in expression of these cytokines have been implicated in susceptibility to colitis (2, 6, 14). However, this does not translate into increased DSS-induced colonic damage indexes of TFF2-deficient mice compared with wild-type mice in this acute model.

TFF2 deficiency promotes recruitment of inflammatory cells in DSS-induced colitis. Inflammatory cells were isolated from two regions of the colons of wild-type and TFF2-deficient mice subjected to the acute (8-day) DSS protocol: mucosal IELs and submucosal LPMCs. After DSS treatment, we observed recruitment of Th (CD4+), CTL (CD8+), and B cells (CD45R+), macrophages (CD11c−CD11b+Ly6G/C−), neutrophils (CD11b+Ly6G/C+), and dendritic cells (CD11c+) into the IEL and LPMC populations (Fig. 5). Inflammation was more pronounced in the DSS-treated TFF2-deficient than wild-type mice, with moderately increased numbers of Th cells in the IELs and LPMCs and increased numbers of macrophages and dendritic cells in the LPMCs (Fig. 5). These results support the notion that TFF2 has anti-inflammatory activity, as a deficiency in TFF2 caused a modestly elevated inflammatory response following DSS treatment. However, as previously noted, slightly elevated inflammation did not translate into increased colonic damage indexes in this acute model.

TFF2 expression by bone marrow-derived cells does not compensate for TFF2 deficiency in DSS-induced colitis susceptibility. TFF2-deficient mice were lethally irradiated and transplanted with bone marrow derived from wild-type or TFF2-deficient donor mice. Transplanted mice were left for 8 wk to allow for complete repopulation of the immune system and then subjected to the acute DSS protocol. Reconstitution of the immune system was verified by PCR analysis. Spleens of mice transplanted with bone marrow from TFF2-deficient mice contained mRNA only for the TFF2 mutant allele, and spleens of mice transplanted with bone marrow from wild-type mice contained mRNA for the wild-type and the TFF2 mutant allele (Fig. 6A). Additionally, fluorescence-activated cell sorting analysis of the blood of the recipient animals at the time of culling showed numbers of leukocytes that were normal and in the correct proportions in all animals. No differences were noted between mice that received a transplant from TFF2-deficient mice and those that received a transplant from wild-type mice (data not shown).

After acute (8-day) DSS colitis induction, TFF2-deficient mice that received a bone marrow transplant lost the same amount of weight as the nontransplanted TFF2-deficient mice, regardless of whether they were transplanted with bone marrow from wild-type or TFF2-deficient mice (Fig. 6B). The extent of colonic damage following DSS treatment was also not affected by TFF2 expression in bone marrow-derived cells (Fig. 6Bii).

An incidental finding throughout all these experiments is a significantly greater weight loss in the TFF2-deficient water-treated control than wild-type mice. This result was consistent, regardless of other interventions, including bone marrow transplantation (Fig. 6C). Also consistent with this subtle, but consistent, weight loss in TFF2-deficient water-treated controls, was a decrease in TFF3 expression in the colons of TFF2-deficient mice (−1.78 ± 0.15 fold; Fig. 1) and a small increase in expression of inflammatory mediators [IL-19 (5.61 ± 3.24 fold), IL-33 (2.01 ± 0.24 fold), and MPO (1.83 ± 0.15 fold); Fig. 4B]. These changes in mRNA did not reach statistical significance, but the fact that they are all trending upward in conjunction with the increased weight loss suggests low-level basal inflammation coincident with some weight loss over the course of the experiment in colons of the TFF2-deficient mice.

To further evaluate the outcome of TFF2-deficient or -replete bone marrow transplantation, relevant cytokine mRNA from the colitic colon of bone marrow-transplanted mice was quantified. Expression of IL-6 and MPO was increased in TFF2-deficient mice transplanted with bone marrow from TFF2-deficient mice following acute DSS treatment and further augmented in mice transplanted with bone marrow from
Fig. 4. TFF2 deficiency alters expression of cytokines in inflamed colonic mucosa and isolated peritoneal macrophages. Effect of TFF2 expression on induction of cytokine mRNA in DSS-induced colitis in TFF1-deficient mice (A), TFF2-deficient mice (B), and isolated peritoneal macrophages (C) was assessed by qPCR and standardized against the housekeeping gene rL32. Values are means ± SE. *P ≤ 0.05 vs. wild-type water-treated or as indicated.
wild-type mice [Fig. 7; IL-6 (103.7 ± 23.1 and 455.7 ± 132.8 fold) and MPO (1.6 ± 0.58 and 6.5 ± 1.1 fold)]. However, expression of other inflammatory moderators such as IL-1β, which was increased in colons of TFF2-deficient mice transplanted with bone marrow from TFF2-deficient mice following DSS treatment, was not further increased in mice transplanted with bone marrow from wild-type mice (Fig. 7). This finding demonstrates that TFF2 deficiency in bone marrow-derived cells causes an attenuated, not exaggerated, inflammatory response in the DSS-treated colon in this acute model. Our data further support the notion that TFF2 deficiency in bone marrow cells is not essential for driving increased susceptibility of TFF2-deficient mice to acute DSS-induced colitis.

DISCUSSION

TFF2 is a key regulatory factor secreted by the gastrointestinal epithelium. While TFF2-deficient mice maintained in low-pathogen conditions have a comparatively mild gastric pathology, deficiency of TFF2 in the stomach dramatically exacerbates the severity of Helicobacter pylori infection and accelerates gastric tumorigenesis in mouse models (34). Similarly, we demonstrate here that, in acute and chronic models of DSS colitis, TFF2 deficiency potentiates weight loss associated with susceptibility to colonic inflammation. The mechanism by which DSS induces colitis is most likely multimodal but primarily represents a damage-induced model of injury and repair. DSS is directly toxic to the colonic epithelium and, therefore, compromises the intestinal barrier and increases the permeability of the tissue (33). This is evidenced by the resistance of germ-free or antibiotic-treated mice to DSS-induced pathologies. The susceptibility to DSS-induced colitis can be manipulated by further alteration of the intestinal barrier function or the immune response following compromise to the barrier (45). The TFFs, in particular TFF2, are known to have important roles in the maintenance of epithelial barrier function and repair and restitution of mucosal surfaces, as well as in modulation of the immune response over the long term, thus underscoring the importance of TFF2 in ameliorating colitis. Here we show that, in the colon, TFF2 is preferentially expressed by the epithelial cells, which is crucial for maintenance of body weight during DSS-induced colitis but has no effect on colonic shortening or ulceration. This suggests that TFF2 is required to maintain barrier function and likely limits dehydration from the colon upon insult but does not influence damage to the colon response per se.

An important finding of the current study is that expression of the main colonic trefoil TFF3 is regulated by TFF2 in colonic and immune tissues. Colonic TFF3 expression is strongly inhibited after deletion of the TFF2 gene, suggesting that TFF2 normally promotes TFF3 colonic expression, thereby contributing to enhanced barrier function. This may be a key local regulatory role for TFF2. Colonic TFF3 deficiency leads...
Increased severity of radiotherapy- or chemotherapy-induced mucositis (3), chemically induced colitis due to impaired mucosal repair (28, 36), and reduced resistance to damage-induced apoptosis (48). On the other hand, thymic and, to a lesser extent, splenic TFF3 is induced in the absence of the TFF2 gene, implying that TFF2 normally represses TFF3 expression in these organs. TFF3 has previously been identified as an intrathymic medullary antigen that can be upregulated by the autoimmune regulatory gene Aire (22), suggesting that its role in the thymus is to instruct the T cell repertoire of...
extrathymic self-antigens. As TFF2 is also expressed in the thymus and expression of TFF3 in the spleen and thymus appears to be similarly regulated by TFF2, further analysis of the actions of TFF2 and TFF3 on lymphocyte development and migration to lymph nodes and the gut mucosa is warranted. It is also possible that key TFF3 regulatory elements are located within the TFF2 locus and that deletion of these elements (in the TFF2 knockout mice) leads to the alterations in TFF3 expression. This seems unlikely, however, because TFF3 expression is suppressed in the colon, but stimulated in spleen and thymus, of TFF2-deficient mice.

TFF2 has been traditionally viewed as an exclusively stomach protein, albeit with expression in the colon associated with colitis induction. We first showed that TFF2 was expressed by rat lymphoid tissues outside the gut, including the spleen and the thymus, most likely in macrophages in the former (7), raising the prospect that TFF2 may have immunomodulatory activity. These observations have been extended by Kurt-Jones et al. (23), who showed that TFF2 deficiency markedly increased the IL-1β responsiveness of mouse macrophages but that LPS sensitivity was not different from wild-type cells. Conversely, we demonstrate here that peritoneal macrophages from TFF2-deficient mice are hyperresponsive to LPS stimulation, showing elevated levels of IL-6 and IL-1β and reduced levels of IL-10 and IL-19, a pattern of cytokine expression that matches that of the colon during DSS-induced colitis. This difference may be explained in part by the differing genetic backgrounds of the mice in each study: cells from mixed-lineage TFF2-deficient (C57BL/6 × 129Sv) mice were used by Kurt-Jones et al., while the mice used in the current study were backcrossed more than eight generations onto C57BL/6. Additionally, we demonstrate here that TFF2 expression in the spleen is strongly induced by variable environmental stimuli, and we would therefore predict that peritoneal macrophages sourced from different laboratories may behave differently in vitro.

Although epithelial mucous cells and immunocytes express TFF2, in the colon it appears that TFF2 is expressed by epithelial cells, rather than leukocytes. Bone marrow transfer experiments show that TFF2-deficient mice transplanted with bone marrow from wild-type or TFF2-deficient mice were equally susceptible to weight loss after DSS-induced colitis at early time points (day 8). This suggests that neither resident nor infiltrating leukocyte-derived TFF2 contributes substantially to the resistance to acute DSS-induced colitis, which is consistent with this epithelial damage model. Therefore, it seems likely that epithelial TFF2, produced in the stomach and secreted luminally or from the colon or both, carries out this function. Both sources are plausible, since secreted TFF2, by virtue of its disulfide-bound tertiary structure, is highly stable in gastric juice.

There are several caveats and limitations to our conclusions. First, most of our analysis was done after short-term (8 days) follow-up, so potentially important, but more slowly developing, immunomodulatory effects of TFF2 may not have been detected. Second, while our previous dosing experiments (4) and the results presented here appear to point to a luminal role for TFF2, other studies (1, 53; T. C. Wang et al., unpublished data) have shown that TFF2 is also secreted, presumably from different laboratories may behave differently in vitro.

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require epithelial-specific deletion of TFF2 in gastric and colonic mucosa or overexpression of TFF2 in the hematopoietic system, with acute and chronic DSS challenge, and is outside the scope of the present study. Additionally, intrarectal administration of TFF2 or TFF3 (if available) to TFF2-deficient mice with DSS-induced colitis would assist in demonstrating the therapeutic potential of these peptides in inflammatory bowel disease and, additionally, demonstrate if luminal trefoils alone are sufficient to modify pathologies generated by DSS-induced colitis.

The cytokine IL-33 has recently been described as an alarmin in epithelial tissues, the function of which is to mobilize the innate immune system in the very early stages of epithelial tissue damage to initiate inflammatory and repair processes (26, 30, 31, 41). In the lung, expression of IL-33 is dependent on TFF2 (56). Here we show a similar interdependence between TFF2 and IL-33 expression in the colon and in peritoneal macrophages. The role of IL-33 in colitis is controversial, being very dependent on the timing of its activation or expression relative to the damaging insult. Exogenous IL-33 administered simultaneously with DSS leads to exacerbation of DSS-induced colitis; when administered after DSS, however, IL-33 can lead to improved recovery (9, 14) and reduced goblet cell loss (17). IL-33-deficient mice showed increased weight loss with DSS-induced colitis but, surprisingly, also showed increased survival rates (30, 39). We found that colonic IL-33 mRNA increased with DSS-induced colitis and that this increase was dependent on TFF2. The possibility remains that the attenuated IL-33 response in TFF2-deficient mice partly contributes to the DSS-induced phenotype.

In this study we have confirmed that TFF2-deficient, but not TFF1-deficient, mice have increased susceptibility to DSS-induced colitis. In contrast to earlier suggestions, the increased susceptibility to acute (8 days) DSS-induced colitis is not primarily due to expression of TFF2 in leukocytes. Our data support the possibility that TFF2 regulates expression of TFF3 and IL-33 in the colonic epithelium, and, therefore, TFF2-deficient colons have compromised barrier function and Th2 immune responses, resulting in increased susceptibility to DSS-induced colitis. The significance of direct TFF2 modulation of the immune response is still not entirely apparent and requires further investigation.

REFERENCES


