Tis7 deletion reduces survival and induces intestinal anastomotic inflammation and obstruction in high-fat diet-fed mice with short bowel syndrome

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1Division of Gastroenterology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri; 2Division of Pediatric Gastroenterology, Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri; 3Department of Pediatric Surgery, Washington University School of Medicine, St. Louis, Missouri; 4Department of Medicine, Veterans Affairs St. Louis Health Care System; St. Louis, Missouri; and 5Department of Developmental Biology, Washington University School of Medicine, St. Louis, Missouri

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Garcia AM, Wakeman D, Lu J, Rowley C, Geisman T, Butler C, Bala S, Swietlicki EA, Warner BW, Levin MS, Rubin DC. Tis7 deletion reduces survival and induces intestinal anastomotic inflammation and obstruction in high-fat diet-fed mice with short bowel syndrome. Am J Physiol Gastrointest Liver Physiol 307: G642–G654, 2014. First published July 24, 2014; doi:10.1152/ajpgi.00374.2013.—Effective therapies are limited for patients with parenteral nutrition-dependent short bowel syndrome. We previously showed that intestinal expression of the transcriptional coregulator tetradeoxyacyl phorbol acetate-induced sequence 7 (tis7) is markedly increased during the adaptive response following massive small bowel resection and tis7 plays a role in normal gut lipid metabolism. Here, we further explore the functional implications of tis7 deletion in intestinal lipid metabolism and the adaptive response following small bowel resection. Intestinal tis7 transgenic (tis7tg), tis7−/−, and wild-type (WT) littermates were subjected to 50% small bowel resection. Mice were fed a control or a high-saturated-fat (42% energy) diet for 21 days. Survival, body weight recovery, lipid absorption, mucosal lipid analysis, and the morphometric adaptive response were analyzed. Quantitative real-time PCR was performed to identify tis7 downstream gene targets. Postresection survival was markedly reduced in high-fat, but not control, diet-fed tis7−/− mice. Decreased survival was associated with anastomotic inflammation and intestinal obstruction postresection. High-fat, but not control, diet-fed tis7−/− mice had increased intestinal IL-6 expression. Intestinal lipid trafficking was altered in tis7−/− compared with WT mice postresection. In contrast, high-fat diet-fed tis7−/− mice had improved survival postresection compared with WT littermates. High-fat diet feeding in the setting of tis7 deletion resulted in postresection anastomotic inflammation and small bowel obstruction. Tolerance of a calorie-rich, high-fat diet postresection may require tis7 and its target genes. The presence of luminal fat in the setting of tis7 deletion promotes an intestinal inflammatory response postresection.

intestinal adaptation; lipid metabolism; gut inflammation; short bowel syndrome

A DYNAMIC MORPHOLOGICAL and functional gut adaptive response occurs in rodents after loss of small bowel surface area due to surgical resection. The morphological response in the remnant intestine is characterized by increased villus height and crypt depth and increased crypt cell production rate, associated with an early surge in stem cell numbers and crypt fission (6, 10, 11). The functional adaptive response is characterized by increased nutrient, fluid, and electrolyte absorption (10). Morphological and metabolic adaptation also occurs in humans following loss of functional small bowel surface area, although the process is more variable and depends on the patient’s underlying disease(s) and overall morbidity (11). Elucidation of the molecular basis of this response, which is still poorly understood, has important potential therapeutic implications.

We have identified the immediate early gene tis7 (tetradeoxyacyl phorbol acetate-induced sequence 7) as a putative regulator of the gut adaptive response (15, 19, 23, 26). Tis7 is a transcriptional coregulator (21) that translocates into the nucleus, where it interacts with the SIN 3 protein complex that has histone deacetylase activity (21, 22). This complex binds to DNA-binding proteins that interact with specific promoter-binding sites to regulate gene transcription (21, 22). We have shown that tis7 mRNA expression increases up to eightfold in remnant mouse ileum after 50% midjejunooileal resection (15). This increase in tis7 mRNA expression occurs in adapting enterocytes, the principal absorptive cell of the gut, and in crypt cells (15).

To elucidate the function of tis7 in the normal gut, we generated transgenic mice in which promoter sequences from the rat liver fatty acid-binding protein gene were utilized to preferentially direct overexpression of tis7 in crypt and villus epithelium (23). These mice exhibited increased adiposity, despite reduced total body mass and weight, and increased rates of intestinal triglyceride (TG) absorption (23). In contrast, tis7−/− mice have normal weight and body fat but when fed a high-fat diet are protected from weight gain and have reduced adiposity compared with high-fat diet-fed wild-type (WT) mice (26). High-fat diet-fed tis7−/− mice had evidence of altered lipid metabolism, with reduced and delayed lipid absorption in the jejunum, with shunting of free fatty acids and TG to the ileum/distal gut. However, net lipid absorption as measured by fecal fat quantification methods was unchanged. The role of tis7 in gut adaptation was also studied using tis7−/− mice. After resection, tis7−/− mice had a blunted early (by 72 h) adaptive morphometric response, with reduced villus height and reduced crypt cell proliferative index, which resolved by 10 days postresection.

To further explore the functional implications of tis7 deletion on the metabolic gut adaptive response postresection, we performed 50% intestinal resections and fed mice a high-fat diet for 3 wk postresection. We examined survival, weight...
gain, and the adaptive response postresection. We show that tis7 deletion in high-fat, but not control, diet-fed mice results in increased mortality postresection, associated with intestinal obstruction, inflammation, and altered intestinal mucosal lipid concentrations, despite similar rates of body weight recovery postresection. In contrast, intestinal tis7 overexpression is protective in high-fat diet-fed mice postresection, with an improved survival rate compared with WT littermates and a small, but significant, increase in lipid absorption. We have generated a novel model of anastomotic inflammation and intestinal obstruction postresection. Small bowel obstruction following intestinal resection is a common clinical problem for which there are no preventive or effective medical therapies; this model may be used to understand how specific diets may contribute to the underlying pathogenesis and to the search for potential preventive and therapeutic strategies.

MATERIALS AND METHODS

Mice. The Washington University School of Medicine Animal Studies Committee reviewed and approved all animal experimentation. The tis7−/− mice were generated as described elsewhere (20). The mice are congenic on a C57BL/6 background; their respective WT C57BL/6 littermates were used as controls for all tis7−/− experiments. The tis7 transgenic (tis7+) mice were generated as described elsewhere (23) on a FVB/N background. WT FVB/N littermates were used as controls for all experiments with tis7+ mice. The liver fatty acid-binding promoter was used to overexpress tis7 specifically in villus and crypt enterocytes of the small bowel and in colonocytes of the proximal colon. All mice were bred and housed together in the same room of the Washington University School of Medicine mouse facility. The mice were fed a standard rodent chow diet (Picolab 20,Ralston Purina), except as described below, and were kept on a strict 12:12-h light-dark cycle.

Intestinal resection surgery. At 2 days prior to surgery, the animals were fed ad libitum a control liquid diet, which is equivalent to standard rodent chow (see below). On the day of surgery, mice were anesthetized with ketamine (87 mg/kg)-xylazine HCl (13 mg/kg) and isoflurane inhalation. As previously described (15), a 50% small bowel obstruction was made between the remaining jejunum and ileum, (obtained on the day of euthanization). Digital images were acquired for morphometry using Axiostarvation (version 4.7) software (Zeiss). Measurements were taken from ≥25 well-oriented crypt-villus units. Sections from the anastomotic site were also stained with hematoxylin-eosin for inflammatory cell analysis and processed for immunohistochemical analysis (see below). On the day of euthanization, all mice were given 5-bromodeoxyuridine (5-BrDU, 16 g/l, 2.5 mg/kg ip). Crypt cell proliferation was measured by 5-BrdU incorporation detected by an anti-BrdU antibody (1:1,000 dilution; Accurate Chemical & Scientific). Crypt cell proliferation rate was measured as percentage of labeled cells, which was calculated by counting the number of 5-BrdU-labeled cells per crypt and dividing by the total number of cells per crypt. At least 10 full-length crypts were evaluated in each sample.

Fecal fat analysis. The mice were placed into individual metabolic cages with mesh cage bottoms to allow collection and prevent ingestion of feces during days 19–21 of the experiment. Fat analysis was performed by Folch’s extraction, as described elsewhere (17). Briefly, feces were collected daily into separate sterile 15-ml conical tubes. One 24-h collection per mouse was used for fecal fat analysis. Feces were dried overnight in a 60°C oven, and 0.2 g of feces was removed. The dried feces were divided into two samples, each of which was added to a 2-ml microcentrifuge tube containing 0.2 ml of 1-mm glass beads, with 0.8 ml of deionized distilled water, incubated at room temperature for 2 h, and blended for 4 min. Then 0.5 ml of Folch reagent (2:1 chloroform-methanol) was added to each tube, and samples were vortexed. Samples were recombined, and an additional 4 ml of Folch reagent was added. Samples were vortexed, incubated for 30 min at room temperature, and centrifuged at 2,000 rpm for 5 min. The lower chloroform phase was transferred to a previously weighed 5-ml glass vial, and an additional 2 ml of Folch reagent were

feeding, as assessed by monitoring daily food intake, or were not moving freely and appeared distressed, as evidenced by a hunched posture or excessive weight loss, were euthanized.

Diets and monitoring postresection surgery. After resection, mice were housed in individual cages and fed ad libitum a liquid control or a liquid high-fat diet daily. The nutrient content of the control diet is equivalent to standard rodent chow and consists of 200 g of protein, 99 g of fat, and 47 g of fiber per kilogram of diet and water, providing 14.24 MJ/kg diet (4.5% of calories from fat) (AIN-93M Purified Liquid Diet-Control 7100080, Dyets, Bethlehem, PA). The high-saturated-fat (Western) diet contains 173 g of protein, 225 g of milk fat, and 1.5 g of cholesterol per kilogram of diet and water, providing 18.84 MJ/kg diet (modified, high-fat AIN-93M Purified Liquid Diet 710147, Dyets), with a total of 42% of energy derived from fat. This diet contains 0.47% ω-3, 2.56% ω-6, and 18% ω-9 (oleic acid) fatty acids. The amount of liquid diet consumed and the weight of each mouse were recorded daily.

Morphometric analysis. All histological studies were performed in the Advanced Imaging Tissue Analysis Core of the Digestive Disease Research Core Center. Sections were stained with hematoxylin-eosin, and villus heights and crypt depths were measured in preoperative ileal segments (obtained from the ileum adjacent to the site of resection) and in postoperative remnant ileum (obtained on the day of euthanization). Digital images were acquired for morphometry using Axiostarvation (version 4.7) software (Zeiss). Measurements were taken from ≥25 well-oriented crypt-villus units. Sections from the anastomotic site were also stained with hematoxylin-eosin for inflammatory cell analysis and processed for immunohistochemical analysis (see below). On the day of euthanization, all mice were given 5-bromodeoxyuridine (5-BrDU, 16 g/l, 2.5 mg/kg ip). Crypt cell proliferation was measured by 5-BrdU incorporation detected by an anti-BrdU antibody (1:1,000 dilution; Accurate Chemical & Scientific). Crypt cell proliferation rate was measured as percentage of labeled cells, which was calculated by counting the number of 5-BrdU-labeled cells per crypt and dividing by the total number of cells per crypt. At least 10 full-length crypts were evaluated in each sample.

Table 1. Primers used for quantitative RT-PCR

<table>
<thead>
<tr>
<th>Prime</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>IL-6</td>
<td>5'-AGA GCA AAG ACC CAG AGT CCT TCA GAG A-3'</td>
<td>5'-GGC ACT CCT TCT GTC ACT GCA GC-3'</td>
</tr>
<tr>
<td>KC</td>
<td>5'-GCA ACC AAA CCC AAG TCA TAG CCA-3'</td>
<td>5'-GGC TCC ACC AGA CAG TGC CCA-3'</td>
</tr>
<tr>
<td>IL-10</td>
<td>5'-GCC ACC CTT TCC CAG TGC GC-3'</td>
<td>5'-GGC TTG GCA ACC CAA GTA ACC CTP A-3'</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5'-ATG AGC ACA GAA AGC ATG ATG-3'</td>
<td>5'-TAC AGG CCT GTC ACT CTA ATT-3'</td>
</tr>
<tr>
<td>IL-18</td>
<td>5'-ATG AGC GCC AAA AGA TGA AG-3'</td>
<td>5'-TTG TCC ACA GCC AGA ATG AG-3'</td>
</tr>
<tr>
<td>18S</td>
<td>5'-CGG TGG CTT GAT TGG AA-3'</td>
<td>5'-GCC TTC ATT GCA CCT TGG TT-3'</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-GAG TGC CCT GGT CCT GGA CCT GA-3'</td>
<td>5'-GCT CCT TCA CGA CCT CTG TA-3'</td>
</tr>
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</table>
Fig. 1. Increased mortality in high-fat diet-fed H11005 mice after 50% small bowel resection. A: H11005 mice and wild-type (WT) littermates fed a control low-fat liquid diet have similar postoperative mortality rates following 50% intestinal resection (19% and 12%, respectively, P = not significant, n = 16 WT and 16 H11005 mice). B: when fed a high-saturated-fat liquid diet, H11005 mice had significantly increased mortality compared with WT littermates (61% vs. 6%, n = 17 WT and 41 H11005 mice). *P = 0.001 (by Gehan-Breslow-Wilcoxon survival test).

Table 2. Necropsy results for mice that died after resection prior to day 21 (end of experiment)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>Cause of Death</th>
<th>Postoperative Day of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT littermates of H11005</td>
<td>High-fat</td>
<td>Unknown (n = 1 of 17 operated)</td>
<td>Day 6 (n = 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Obstruction (n = 11)</td>
<td>Obstruction (n = 11); day 3 (n = 4), day 4 (n = 4), day 5 (n = 1), day 15 (n = 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dehiscence (n = 1)</td>
<td>Dehiscence: day 3 (n = 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anastomotic leak (n = 1)</td>
<td>Leak: day 4 (n = 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No obvious cause (n = 4)</td>
<td>No obvious cause; day 3 (n = 2), day 4 (n = 1), day 7 (n = 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;24 h (n = 1) (total n = 18 with early death of 24 operated)*</td>
<td></td>
</tr>
<tr>
<td>FVB/N</td>
<td>High-fat</td>
<td>Small bowel obstruction with hemorrhage (n = 4, hemorrhage (n = 2) (total n = 6 early death of 12 operated)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Obstruction with hemorrhage (n = 4): day 2 (n = 1), day 4 (n = 3)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Hemorrhage (n = 2): day 2 (n = 2)</td>
<td></td>
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<td></td>
<td></td>
<td>Obstruction with hemorrhage: day 3 (n = 1), day 4 (n = 1)</td>
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<tr>
<td></td>
<td></td>
<td>Hemorrhage: day 5 (n = 1)</td>
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</tr>
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n, Number of mice. *P = 0.0001 vs. WT. †P = 0.05 vs. WT.
PBS at 22,500 rpm for 5 s and incubated on ice for 5 min. Then 0.8 ml of homogenate was mixed with 5 ml of Folch reagent (2:1 chloroform-methanol) and 0.1 ml of 0.5% sulfuric acid, incubated at room temperature for 0.5 h, and then centrifuged at 2,000 rpm for 5 min. The organic phase containing lipids was isolated, and volume was recorded for calculation of total lipid. To prepare an aqueous lipid sample for the lipid concentration measurement, a mixture of 650 μl of the lipid extract and 100 μl of 0.5% Triton X-100-chloroform was blown dry with N₂ gas at 45°C for 15 min; then 100 μl of deionized distilled water were added, and the solution was incubated at 37°C for 15 min. The remaining homogenate was centrifuged at 10,000 g for 2 min, and the supernatant containing soluble cytosolic proteins was saved. Lipid extracts, aqueous lipid samples, and cytosolic protein samples were stored at −20°C until use.

Intestinal lipid and protein concentration measurements. The concentration of TG, total cholesterol, nonesterified fatty acids (NEFA), and free cholesterol in aqueous lipid samples was determined using the L-type TG M kit, Cholesterol E kit, HR Series NEFA-HR(2), and Free Cholesterol E kit (Wako Diagnostics, Richmond, VA), respectively, in a microplate reader (BioTek, Winooski, VT). For conversion of the measured TG and total cholesterol concentrations from mg/dl to mmol/l, the concentrations were multiplied by the coefficients 0.0113 and 0.0259, respectively, provided by the manufacturer. The cytosolic protein concentration was determined using the CB-X protein assay kit (G-Biosciences, St. Louis, MO) in a microplate reader. Total cytosolic protein concentrations were used to normalize the lipid content.

Assessment of neutrophil effector function. Neutrophil effector function was measured by examination of the respiratory burst in blood from tis7−/− mice and WT mice in collaboration with M. Dinauer (Washington University). Neutrophil superoxide production was quantified by flow cytometry using dihydrorhodamine 123 (DHR 123) (13). DHR 123 is used as a peroxidase substrate for the respiratory burst in neutrophils. DHR is oxidized to rhodamine by hydrogen peroxide, and rhodamine fluorescence is detected and measured by flow cytometry in the neutrophil gate.

Statistical analysis. Statistical analyses were performed using Prism 5.0 (GraphPad Software, La Jolla, CA). Survival analysis was performed utilizing the Gehan-Breslow-Wilcoxon test. Comparisons of daily body weight changes were analyzed by paired Student’s t-test. Differences in ability to achieve and exceed preoperative body weight were evaluated by χ² analysis. Changes in villus height, crypt depth, and gene expression levels were assessed by paired or unpaired Student’s t-test or by two-way ANOVA and Bonferroni’s post hoc analysis. Values are means ± SE. Differences were considered significant at P ≤ 0.05.

RESULTS

Increased mortality in tis7−/− mice fed a high-fat diet postresection. To elucidate the role of tis7 in the gut’s metabolic adaptive response following resection, tis7−/− mice and WT littermate controls were subjected to 50% small bowel resection.

Fig. 2. Increased anastomotic inflammation in tis7−/− mice fed a high-fat diet postresection. A–F: representative low-power views of histological sections of hematoxylin-eosin-stained surgical anastomotic sites (n = 4–5 mice per group). A and B: anastomoses harvested from high-fat diet-fed WT littermate mice that survived to postoperative day 21. Dashed arrow in A shows Peyer’s patch. Crypts and villi are present at the anastomotic site, with some distortion resulting from altered tissue orientation in paraffin. C and D: anastomoses from tis7−/− mice that died before day 21. Arrows indicate submucosal and serosal inflammation. E and F: persistent anastomotic inflammation in high-fat diet-fed tis7−/− mice that survived to day 21. Arrows indicate submucosal and serosal inflammation. Magnification: ×100 (A–C) and ×50 (D–F).
resection and fed a high-saturated-fat liquid diet (42% of calories from fat) or an isocaloric control liquid diet (4.5% calories from fat, nutritionally equivalent to standard rodent chow). Mortality rates were not significantly different between \( \text{tis7}^{-/-} \) and WT mice fed the control diet postresection (19% vs. 12% mortality, \( P = \) not significant; Fig. 1A). However, we observed 61% overall mortality in \( \text{tis7}^{-/-} \) mice compared with 6% mortality in WT littermate control mice fed the high-fat diet (\( P = 0.001 \); Fig. 1B). These mortality rates excluded mice that died within 24 h of surgery due to anesthesia or surgical mishaps (e.g., anesthesia overdose or bleeding). To determine the etiology of the increased mortality in \( \text{tis7}^{-/-} \) mice fed a high-fat diet, we performed postmortem analyses on a subset of these mice (\( n = 24 \) mice subjected to resection, with 18 early deaths; Table 2). We found intestinal obstruction at the anastomosis in 11 of 18 \( \text{tis7}^{-/-} \) mice fed the high-fat diet postresection. Of the additional seven deaths, one was a surgical mishap, in which death occurred at \( <24 \) h, for four of the deaths there was no obvious cause, one death was due to dehiscence (complete opening) of the surgical anastomosis, and one was due to a leak at the anastomosis. Only 1 of 17 WT mice died; this death occurred on postoperative \( \text{day} \) 6, and the cause of death could not be identified (\( P = 0.0001 \), deaths in \( \text{tis7}^{-/-} \) vs. WT mice, by \( \chi^2 \) analysis).

To determine whether increased mortality was dependent on gut resection or might be due to the stress of surgery in the setting of a high-fat diet, sham-operated \( \text{tis7}^{-/-} \) mice and WT littermates were fed a high-fat or a control diet for 21 days. There was no increase in mortality in either group compared with each other or with WT littermates fed the high-fat diet postresection (data not shown).

Increased anastomotic inflammation in \( \text{tis7}^{-/-} \) mice. To begin to elucidate the mechanisms regulating the increased mortality of high-fat diet-fed \( \text{tis7}^{-/-} \) mice postresection predominantly from small bowel obstruction, we analyzed the histology of hematoxylin-eosin-stained sections of intestine at the anastomosis and at sites distant from the anastomosis (\( n = 4–5 \) mice per group). High-fat diet-fed WT mice had normal-appearing anastomoses with intact crypts and villi (mild distortion is attributable to altered orientation of anastomotic tissue in paraffin blocks; Fig. 2, A and B, and Fig. 3A). In contrast, in high-fat diet-fed \( \text{tis7}^{-/-} \) mice, there was an increase in mucosal and submucosal lamina propria and serosal inflammatory cells at the anastomosis (Fig. 2, C–F, and Fig. 3, B–D). Increased inflammatory infiltrates were observed as early as postoperative \( \text{day} \) 4 (Fig. 2, C and D, and Fig. 3, B and C). In the \( \text{tis7}^{-/-} \) mouse that survived to \( \text{day} \) 21, a persistent, lymphocyte-predominant mucosal inflammatory cell infiltrate was seen at the anastomosis (Fig. 3D), with persistent serosal and submucosal inflammatory cell infiltrates (Fig. 2, E and F). Submucosal and serosal inflammatory cells were mainly composed of macrophages, as detected by F4/80 immunostaining (Fig. 4, A–C), and were increased in \( \text{tis7}^{-/-} \) compared with WT intestines. Mucosal infiltrates were composed of a predominance of B lymphocytes (CD45R+ ) with scattered T lymphocytes (CD3+) (Fig. 4, D and E). There was no histological evidence of inflammation in the control diet-fed \( \text{tis7}^{-/-} \) or WT mice (data not shown).

Although we did not observe an increase in neutrophils in the inflammatory infiltrate, \( \text{tis7} \) deletion has been shown to reduce neutrophil effector function, resulting in altered inflammatory responses in the lung (7). We examined neutrophil function in \( \text{tis7}^{-/-} \) vs. WT mice by measuring superoxide production using DHR 123, as described else-
where (13; see MATERIALS AND METHODS). No differences in neutrophil respiratory burst function were observed in WT compared with \(tis7^{-/-}\) mice (data not shown).

Because \(tis7\) regulates NF-\(\kappa\)B activation in muscle cells (12) and silencing \(tis7\) in myoblasts induces acetylation and nuclear import of p65 (12), we examined intestinal NF-\(\kappa\)B activation by detecting nuclear NF-\(\kappa\)B p65/RelA expression, which is specifically increased during NF-\(\kappa\)B activation (5). Intestinal anastomotic sites were analyzed by immunohistochemical analysis for nuclear p65 expression postresection in high-fat diet-fed WT and \(tis7^{-/-}\) mice (\(n=4\) in each group). Representative sections are shown in Fig. 5. Patchy areas of increased nuclear expression were noted in lamina propria and submucosal lymphocytes in \(tis7^{-/-}\) ileum (Fig. 5, B and D); many fewer immune cells were present in WT ileum, and rare cells showed nuclear p65 expression (Fig. 5, A and C). However, quantification of p65 expression by immunoblot revealed no significant differences between \(tis7^{-/-}\) and WT postoperative ileal or jejunal nuclear expression (data not shown). Although this may reflect the patchiness of nuclear p65 expression, the increase in nuclear p65 in \(tis7^{-/-}\) immune cells only suggests that this may be a nonspecific response secondary to the general increase in inflammation.

Histological analysis of remnant intestine beyond the anastomosis showed evidence of mucosal and submucosal inflammation in \(tis7^{-/-}\) mice that did not survive until postoperative day 21 (Fig. 6A). In contrast, \(tis7^{-/-}\) mice that survived to day 21 had generally normal-appearing mucosa beyond the anastomosis (Fig. 6B). Analysis of \(tis7^{-/-}\) mice that survived with partial intestinal obstruction revealed a marked increase in submucosal edema, with an increase in submucosal thickness compared with WT mice (Fig. 6, C and D, arrows). Trichrome stain to assess collagen expression and determine whether fibrosis was increased in \(tis7^{-/-}\) mice revealed no significant increase in collagen (Fig. 6, C and D).

The adaptive response is preserved in \(tis7^{-/-}\) mice that survive resection. The morphometric adaptive response in the remnant small bowel following massive resection is characterized by increased crypt depths, villus heights, and crypt cell proliferation. In the \(tis7^{-/-}\) mice that survived to postoperative day 21, the adaptive response was preserved, with the expected increase in ileal villus heights and crypt depths postresection, whether the
mice were fed a control or a high-fat diet (Fig. 7, A–D). This was similar to WT mice. Also, there were no differences in crypt cell proliferation rates in WT compared with tis7+/−/H11002 ileum postresection (Fig. 7E), as quantified by 5-BrdU incorporation.

Intestinal lipid metabolism is altered in tis7+/− mice postresection. We previously showed that, compared with WT littermates, (unoperated) tis7+/−/H11002 mice do not gain weight when chronically fed a high-fat diet (26) and tis7 deletion results in...
delayed lipid absorption and altered intestinal and hepatic lipid trafficking, with reduced intestinal TG, cholesterol, and free fatty acid mucosal levels in the jejunum but increased mucosal free fatty acid concentration in the ileum. We therefore examined mucosal lipid concentrations in the jejunum and ileum at postresection day 21 (Fig. 8). Similar to our previously published findings in unoperated mice (26), we observed reduced mucosal cholesterol and free fatty acid concentrations in post-op tis7−/− vs. WT jejunum (Fig. 8A) and a trend toward decreased TG concentration. Examination of tis7−/− vs. WT postoperative ileal lipid mucosal concentrations revealed no significant difference in mucosal cholesterol or TG concentrations, as observed in unoperated mice (Fig. 8B). However, in contrast to our previous observation that mucosal free fatty acid concentrations were increased in tis7−/− vs. WT unoperated ileum (21), we observed a significant decrease in mucosal free fatty acid concentration in tis7−/− vs. WT postoperative distal ileum (P = 0.04). Finally, total lipid absorption was measured by calculating fecal fat content and the percentage of ingested fat absorbed. As previously observed in unoperated mice, there were no significant differences in the percentage of lipid absorbed in high-fat diet-fed tis7−/− mice vs. WT mice postresection; however, there was a trend toward a decrease in fat absorption in tis7−/− mice (96.8% vs. 92% of ingested fat absorbed, P = 0.08). Thus mucosal lipid concentration is reduced in tis7−/− jejunum and ileum postoperatively follow-

ing massive resection; the compensatory increase in mucosal free fatty acid concentration in unoperated tis7−/− ileum was not preserved in the shortened postoperative ileum.

To determine whether tis7−/− mice had reduced weight gain resulting from altered lipid metabolism, as previously observed in unoperated high-fat diet-fed tis7−/− mice, we measured daily food consumption and compared daily weights, which had been measured for each mouse for the entire experiment, up to postoperative day 21. Daily food consumption was the same in tis7−/− and WT mice that survived to day 21 (data not shown). There were no significant differences in overall weight gain expressed as percentage of body weight recovery between surviving tis7−/− and WT mice fed the control diet (Fig. 8C) or the high-fat diet (Fig. 8D). However, tis7−/− mice that died prior to day 21 showed reduced body weight recovery compared with surviving mice (n = 14 per group, Fig. 8E).

High-fat diet-fed tis7−/− mice have increased intestinal IL-6 expression. To further explore the mechanism for the increase in anastomotic inflammation in tis7−/− mice fed a high-fat diet, we examined the pre- and postoperative ileal expression of inflammatory cytokines, including IL-6, KC (IL-8), TNF-α, and IL-10, by qRT-PCR. IL-6 expression did not change in high-fat diet-fed WT postoperative ileum compared with preoperative ileum (P < 0.03; Fig. 9A). However, tis7−/− mice that survived had increased ileal IL-6 mRNA expression compared with preoperative ileum (P < 0.001; Fig. 9B).
KC, TNF-α, and IL-10 was unchanged in all groups (data not shown). Also when mice were fed the control diet, there were no differences in ileal IL-6 mRNA levels between postoperative WT and postoperative tis7⁻/⁻ mice and between preoperative and postoperative mice within each genotype (Fig. 10B). Serum IL-6 levels were measured in tis7⁻/⁻ and WT mice by ELISA and were unchanged; thus the inflammation appears to be localized to the intestine and is not systemic.

**Intestinal tis7 overexpression protects mice from mortality induced by a high-fat diet postresection.** We next evaluated the postoperative survival of high-fat diet-fed tis7⁻/⁻ mice and WT littermates. The tis7⁻/⁻ mice are on an FVB/N background, in contrast to the tis7⁻/⁻ mice, which are congenic on a C57BL/6 background. To avoid confounding results due to strain differences, strain-matched WT littermates (also on a FVB/N background) were used as controls for all experiments with tis7⁻/⁻ mice. Interestingly, FVB/N WT mice tolerated the high-satu-
rated-fat diet following 50% small bowel resection less well, as shown by higher postoperative mortality rates, than high-fat diet-fed C57BL/6 WT mice (Figs. 11A and 1B). However, a protective effect of intestinal tis7 overexpression on survival was observed in mice fed the high-fat diet, as littermate FVB/N tis7<sup>tg</sup> mice fed the high-fat diet postresection had 31% mortality compared with 56% mortality in WT mice (P = 0.044; Fig. 11A). Early deaths at <24 h were due to an increased incidence of hemorrhage in both groups; these mice were removed from the survival analysis. Necropsy of a subset of these mice revealed the cause of death in WT and tis7<sup>tg</sup> mice to be obstruction with hemorrhage or hemorrhage alone (Table 2), with a reduced incidence of both complications in tis7<sup>tg</sup> mice. Sham-resected tis7<sup>tg</sup> and WT littermates showed no difference in mortality, and both tolerated sham resection when fed a high-fat or a control diet.

Histological analysis revealed evidence of an inflammatory response, as well as hemorrhage at the anastomosis, in high-fat diet-fed WT littermates that died early (Fig. 12, A and B). Similar findings were observed in tis7<sup>tg</sup> mice that died early, but the incidence of such findings was lower (Table 2). Both tis7<sup>tg</sup> and WT mice that survived to day 21 had normal-appearing anastomoses (Fig. 12, C and D).

We next used qRT-PCR to examine ileal IL-6 expression in tis7<sup>tg</sup> mice and WT littermates fed a high-fat diet postresection. Consistent with the higher mortality in high-fat diet-fed WT littermates postresection, IL-6 expression in WT tis7<sup>tg</sup> mice was increased postoperatively compared with preoperatively (P < 0.01; Fig. 11B). Conversely, IL-6 expression was not increased in high-fat diet-fed tis7<sup>tg</sup> mice postoperatively compared with preoperatively. Furthermore, postoperative ileal IL-6 mRNA levels were decreased in tis7<sup>tg</sup> mice compared with postoperative WT mice.

Our previous studies showed that intestinal tis7 overexpression resulted in an increased rate of small bowel TG absorption (23). To determine whether the tis7-mediated increase in TG absorption also occurs in the adapting gut postresection, we assessed lipid absorption by quantifying the percentage of ingested fat that was absorbed. Feces were collected from high-fat diet-fed tis7<sup>tg</sup> mice and WT littermates on days 19–21 postresection. There was a small 0.8% increase in the percentage of fat absorbed in tis7<sup>tg</sup> mice fed the high-fat diet compared with WT littermates fed the high-fat diet (P = 0.04).

We next assessed morphological adaptation following resection in tis7<sup>tg</sup> vs. WT mice (Fig. 13). The villus and crypt adaptive response, characterized by an increase in villus height and crypt depth postresection, occurred as expected in preoperative vs. postoperative ileum from tis7<sup>tg</sup> vs. WT littermates fed the control or the high-fat diet (P < 0.05; Fig. 13, A–D). We also observed the expected postresection increase in crypt cell proliferation: >30% of ileal crypt cells were positive for 5-BrdU expression in each group postresection, with no significant differences between tis7<sup>tg</sup> mice and their respective WT littermates fed either diet (Fig. 13E).

**DISCUSSION**

In the present study we have shown that tis7 plays a unique role in the intestinal response following loss of functional small bowel surface area. Compared with WT littermates, mice with tis7 deletion fed a high-fat diet but not a control diet following small bowel resection had a marked increase in mortality that was associated with increased gut inflammation and small bowel obstruction. The inflammatory changes were characterized by an influx of lymphocytes and macrophages, associated with submucosal edema and increased mucosal IL-6 expression. High-fat diet-fed tis7<sup>−/−</sup> mice postresection had altered jejunal and ileal lipid metabolism compared with WT mice, with reduced jejunal mucosal concentrations of free fatty acids and cholesterol and reduced ileal mucosal concentration of free fatty acids. These data suggest that tis7 deletion-induced changes in intestinal lipid metabolism and trafficking may result in increased susceptibility to an inflammatory response that predisposes to small bowel obstruction. In contrast, tis7<sup>tg</sup> mice had reduced mortality and reduced IL-6 expression compared with their WT littermates when fed a high-fat diet postresection. Increased intestinal tis7 expression also resulted in a small, but significant, increase in the percentage of lipid absorbed when mice were fed a high-fat diet postresection, again suggesting a possible link between changes in lipid metabolism induced by tis7, the inflammatory response to a high-fat diet, and postresection survival.

Our studies show that gut environmental factors (i.e., a high-fat diet) are required to induce postoperative intestinal inflammation in tis7<sup>−/−</sup> mice, since control diet-fed tis7<sup>−/−</sup> mice did not have postoperative anastomotic inflammation, increased IL-6 expression, or increased mortality (Fig. 5). Several studies have shown that a high-fat diet induces intestinal inflammation and barrier dysfunction. This occurred in association with alterations in gut microflora in obesity-prone rodents (1–3, 9) and in a colitis model (7), suggesting that...
Fig. 12. Histological analysis of surgical anastomoses from high-fat diet-fed WT and tis7tg mice. Representative hematoxylin-eosin-stained sections of intestinal anastomoses were obtained from WT mice that died before day 21 (A and B) or survived until completion of the experiment on day 21 (D) and from tis7tg mice that survived until day 21 (C). In mice that died early, there was an increase in submucosal and serosal inflammatory cells and hemorrhage in the serosa at the anastomosis (A and B, arrows). Mice that survived had normal-appearing anastomoses (C and D).

Fig. 13. Morphological adaptive response is preserved in high-fat diet-fed tis7tg mice postresection. WT littermates and tis7tg mice fed the control diet (A and C) or the high-fat diet (B and D) postresection show a preserved adaptive response, with increased villus heights (A and B) and crypt depths (C and D). E: no differences in postoperative crypt cell proliferation rates were noted between WT and tis7tg mice fed the control diet or the high-fat diet (n = 5–12 mice per group). *P < 0.05.
high-fat diet-induced changes in microflora are at least part of the mechanism by which high-fat diets result in gut inflammation. We speculate that the altered intestinal trafficking of fatty acids and cholesterol induced by tis7 deletion may also generate proinflammatory gut microflora. Further examination of the effects of tis7 deletion and overexpression on the gut microflora in the setting of a high-fat diet represents an intriguing avenue to be explored.

We observed strain-specific differences in survival of high-fat diet-fed FVB/N compared with C57BL/6 WT mice postresection. Thus all experiments were performed only using WT littersmates on the same genetic background as controls. The increased mortality of FVB/N WT compared with C57BL/6 mice occurs in part because of differences in their susceptibility to postoperative hemorrhage, in addition to inflammation induced by high-fat feeding. However, a specific role for tis7 in the inflammatory and obstructive process is further supported by our observation that high-fat feeding in tis7−/− mice induces a more robust inflammatory response, resulting in almost fivefold higher ileal IL-6 expression postresection than in high-fat diet-fed FVB/N WT mice postresection (Figs. 10A and 11B; note scale differences). Also, overexpression of tis7 in villus epithelial cells in tis7tg mice (FVB/N background) was partially protective, associated with a significant decrease in postoperative IL-6 expression, suggesting suppression of inflammation. Backcrossing of tis7tg mice into the C57BL/6 background shows that these mice retain their basal phenotype of increased adiposity (preliminary observations); future analyses of whether improved survival, adaptation, or body weight recovery following resection and high-fat feeding occurs in this background will be informative.

The tis7−/− mice have a global deletion of tis7 (20). Because tis7 is ubiquitously expressed, its deletion may result in increased postresection mortality due to other, unexamined extraintestinal effects. We were able to assign a cause of death to the majority of tis7−/− mice, but several mice had no obvious cause of death on necropsy (Table 2). Future experiments in mice with gut-specific deletion of tis7 are required to confirm that absence of intestinal tis7 is required for the increased mortality.

In addition to further defining the role of tis7 in the adapting gut, these studies have generated a novel murine model of inflammation and small bowel obstruction postresection. There is one previous report of a murine model of inflammation and fibrosis postresection (11) in which conventionally raised IL-10 null mice (with an intact enteric microflora) developed postsurgical inflammation and fibrosis after ileocecal resection (14). However, IL-10 null mice raised germ-free or WT conventionally raised mice did not develop inflammation or fibrosis. These mice are a widely used model of inflammatory bowel disease. Our postsurgical model of intestinal obstruction and inflammation is also dependent on the appropriate luminal environment and shares some features of inflammatory bowel disease, as intestinal inflammation is a hallmark of inflammatory bowel disease and intestinal obstruction is a common sequela of active inflammation in Crohn’s disease. The role of gut microflora and interactions with the luminal nutritional environment, e.g., comparing high-fat, low-fat, high-protein, or high-carbohydrate diets, can be readily explored in the present model. In addition, the tis7−/− mouse may prove useful for elucidating the mechanisms that regulate postoperative small bowel obstruction following resection in humans and possibly help identify preventive therapies.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.M.G., D.W., C.R., T.G., C.B., S.B., and E.A.S. performed the experiments; A.M.G., J.L., C.B., E.A.S., M.S.L., and D.C.R. analyzed the data; A.M.G., J.L., T.G., M.S.L., and D.C.R. interpreted the results of the experiments; A.M.G. drafted the manuscript; A.M.G., M.S.L., and D.C.R. edited and revised the manuscript; A.M.G., M.S.L., and D.C.R. approved the final version of the manuscript; B.W.W., M.S.L., and D.C.R. are responsible for conception and design of the research; D.C.R. prepared the figures.

REFERENCES