Enterocyte apoptosis after enterectomy in mice is activated independent of the extrinsic death receptor pathway

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Knott, Andrew W., David P. O’Brien, Russell J. Juno, Yufang Zhang, Jodi L. Williams, Christopher R. Erwin, and Brad W. Warner. Enterocyte apoptosis after enterectomy SBR in mice is activated independent of the extrinsic death receptor pathway. Am J Physiol Gastrointest Liver Physiol 285: G404–G413, 2003. First published April 30, 2003; 10.1152/ajpgi.00096.2003.—Intestinal adaptation following small bowel resection (SBR) is associated with increased rates of enterocyte apoptosis by unknown mechanism(s). Because postresection adaptation is associated with increased translocation of luminal bacteria, we sought to characterize the role for the extrinsic, death receptor pathway for the activation of enterocyte apoptosis after massive SBR. We first performed SBR or sham operations in mice, and the temporal expression of caspases 8, 9, and 3, death receptors tumor necrosis factor receptor-1 (TNFR1) and Fas and corresponding ligands (TNF and Fas ligand) was determined in the remnant intestine at various postoperative time points. Ileal TNFR1 and Fas expression were then measured after SBR in the setting of increased (waved-2 mice) or decreased (exogenous EGF administration) apoptosis. Finally, intestinal adaptation and apoptosis were recorded in the remnant ileum after SBR in TNFR1-null and Fas-null mice. The expression of death receptor family proteins and caspases demonstrated only modest changes after SBR and did not correlate with the histological appearance of apoptosis. In the setting of accelerated apoptosis, TNFR1 and Fas expression were paradoxically decreased. Apoptotic and adaptive responses were preserved in both TNFR1-null and Fas-null mice. These results suggest that the mechanism for increased enterocyte apoptosis following massive SBR does not appear to involve the extrinsic, death receptor-mediated pathway.

tumor necrosis factor receptor; Fas; intestinal mucosa; short bowel syndrome; intestinal adaptation

INTESTINAL ADAPTATION FOLLOWING massive small bowel resection (SBR) is a crucial mitogenic signal, which primarily involves the intestinal mucosa (39). Increased enterocyte proliferation contributes to the altered intestinal morphology that characterizes adaptation, which includes taller villi, deeper crypts, and greater luminal caliber and length. These morphological changes are an important compensatory response that serves to augment the intestinal absorptive and digestive surface area following massive intestinal loss. In addition to increased rates of enterocyte proliferation, enhanced rates of programmed cell death (apoptosis) have been measured in enterocytes of the crypt and villus tips after SBR (15, 57). Whereas increased rates of apoptosis may be a passive response to counterbalance the elevated levels of proliferation and thereby maintain homeostasis, the mechanism and/or mediators of postresection apoptosis are presently unknown.

Apoptosis is executed by a family of cysteine proteases, which cleave after aspartate residues and are referred to as caspases (38). Although some overlap exists, caspase activation occurs via two general canonical pathways (23, 45). The intrinsic or mitochondrial pathway is regulated by such factors as chemotherapeutic agents and growth factor or nutrient withdrawal and is mediated primarily by active caspase 9. In the extrinsic pathway, caspase 8 is considered to be the initiator caspase and is activated by ligation of specialized cell-surface receptors termed death receptors. Among the growing list of death receptors, the tumor necrosis factor receptor-1 (TNFR1) and CD95 (Fas) are the best characterized. The role for the extrinsic pathway in the genesis of increased enterocyte apoptosis following massive SBR has not previously been determined.

A significant cause of morbidity and mortality in patients requiring massive intestinal resection is sepsis (35, 46). Indeed, translocation of bacteria from the lumen of the adapting intestine has been considered to be an important origin of these septic episodes (26). The incidence of liver injury, cholestasis, and septic episodes may be reduced when measures are taken to reduce or modify bacterial levels in the gut lumen (25, 36, 59). In animal models of intestinal resection, an increased incidence of bacteria cultured from mesenteric lymph nodes has been demonstrated (40, 44).

Increased luminal bacteria and translocation after intestinal resection may either contribute toward or occur as a result of a proinflammatory milieu within the intestinal mucosa. After bacterial entry, intestinal

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epithelial cells rapidly upregulate the expression of an array of proinflammatory cytokines, including TNF-α (22). Furthermore, apoptosis may be induced in intestinal epithelial cells by the exogenous administration of both IFN-γ and TNF-α (24) or by ligation of the Fas receptor (1, 2). The purpose of the present study was to characterize the role for the extrinsic, death receptor-mediated pathway in resection-induced enterocyte apoptosis. Because TNFR1 and Fas are the best characterized, we specifically tested the hypothesis that these death receptors regulate apoptosis in enterocytes following massive SBR.

MATERIALS AND METHODS

Experimental design. The protocol for this study was approved by Cincinnati Children’s Hospital Research Foundation Institutional Animal Care and Use Committee (Cincinnati Children’s Hospital Medical Center, Cincinnati, OH). In the first series of experiments, the expression of TNF-α, TNFR1, IFN-γ, Fas, and Fas ligand (Fasl) and caspases 3, 8, and 9 was measured in the remnant ileum in either ICR or C57BL/6 mice (control mice) at 12 and 24 h and at 3 and 7 days following either a 50% proximal SBR or sham operation. In the next series of experiments, TNF-α and Fas expression were monitored in the remnant ileum under conditions of inhibited (exogenous administration of EGF) or enhanced postresection apoptosis (waved-2 mice with perturbed EGF receptor signaling) (19). In the final series of experiments, parameters of intestinal adaptation, enterocyte proliferation, and enterocyte apoptosis were determined in the remnant ileum of TNFR1-null and Fas-null mice.

Animals. Male mice weighing 25–29 g were used for each experimental group. The strains used in the study included ICR (Harlan Laboratory, Indianapolis, IN), C57BL/6 (The Jackson Laboratory, Bar Harbor, ME), waved-2 (The Jackson Laboratory), C3H (The Jackson Laboratory), Fas-null (Fas knockout; The Jackson Laboratory), and TNFR1-null (The Jackson Laboratory). Mice were housed in groups of 4 at 21°C on 12:12-h light-dark cycles (6 AM-6 PM). The mice were allowed to acclimate for at least 5 days before experimentation. One day before operation, the diet was changed from regular chow to a liquid rodent diet (Micro-Stabilized Rodent Liquid Diet LAD 101/101A, Purina Mills, St. Louis, MO). Operative procedure. The details of the murine model for SBR and sham operation have been described previously (20). Briefly, the mice were anesthetized using a balanced mixture of inhaled 2% isofluorane, oxygen (90%), and carbon dioxide (4%) that was kept constant and measured with an Ohmeda 5250 gas analyzer (Ohmeda, Louisville, CO). The abdomen was clipped, prepped with providone-iodine solution, and draped in a sterile manner. The operations were performed using an operating microscope (×6.25 magnification). Through a midline incision, the ileocecal junction was identified. Sham operations consisted of division and reanastomosis of the bowel ~12 cm proximal to the ileocecal junction. In mice undergoing SBR, the bowel was divided ~12 cm proximal to the ileocecal junction and again 2–3 cm distal to the ligament of Treitz. The mesentry of the resected intestine was ligated, and ~12 cm of the intervening small intestine were removed (50% resection). Intestinal continuity was restored with an end-to-end, single-layered anastomosis using interrupted 9–0 monofilament sutures. After closure of the abdomen, the mice were resuscitated with a 3-ml intraperitoneal injection of warm saline and allowed to recover in an incubator (30°C). Water was provided ad libitum for the first 24 h. Thereafter, the mice from all experimental groups were fed with the liquid rodent diet. Whereas animals were not specifically pair fed, the differences in food intake between the various groups after either sham or SBR were negligible.

Tissue harvest. Mice were killed by cervical dislocation after an intramuscular injection of ketamine/xylazine/acemazine (4:1:1). Six centimeters (~1 cm from the anastomosis) of ileum were excised, the luminal contents were gently expressed with cotton swabs, and the wet weight was recorded. The first centimeter of proximal intestine was immediately fixed with 10% neutral buffered formalin and used for histology; the remaining 5 cm were weighed and frozen in liquid nitrogen and stored at −80°C until further use.

Histology. Formalin-fixed specimens of ileum were embedded in paraffin and oriented to provide cut sections parallel with the longitudinal axis of the bowel. Five-micrometer tissue slices were mounted on poly-L-lysine slides and stained with hematoxylin and eosin or subjected to PCNA immunohistochemistry provided in kit form (Zymed Laboratories, San Francisco, CA) following the instructions of the manufacturer. With the use of DAB and brown nuclear sections, villus heights were recorded using computer-aided video microscopy (Image Tool 2.02, University of Texas Health Sciences Center, San Antonio, TX). Only villi with intact central lymphatic channels were measured. These sections were also used to establish rates of apoptosis by scoring the number of apoptotic bodies identified within the crypts. Apoptotic bodies were defined by the presence of pyknotic nuclei, condensed chromatin, and nuclear fragmentation (15). An apoptotic index was defined as the number of apoptotic bodies per crypt, as determined by the blinded scoring and averaging of 20 crypts per sample.

An index of crypt cell proliferation was derived in PCNA-stained sections by calculating the ratio of crypt cells staining positively for PCNA to the total number of crypt cells. Fifteen representative crypts were counted per sample. All histological analyses were performed without knowledge of the source of the ileal tissue.

Measurement of protein and TNF-α and IFN-γ levels. Individual ileum samples were thawed and homogenized (PowerGen, Fisher Scientific, Pittsburgh, PA). Ileal protein content was determined for each sample using a modified Lowry assay (32). TNF-α and IFN-γ protein expression was quantified using a commercially available ELISA kit according to the manufacturer’s protocol (Endogen, Woburn, MA). Samples were normalized by loading equal amounts of protein (80 μg) into each well of a 96-well plate.

Western blot analysis. Ileum samples were thawed, homogenized (PowerGen, Fisher Scientific), and total protein was measured as described above. The protein from each sample was pooled into the appropriate group. Seventy-five micrograms of protein were added to an equal volume of 2× protein sample buffer. The samples were resolved on a 4–20% gradient polyacrylamide gel (Invitrogen, Carlsbad, CA) at 4°C with standard protein running buffer. Protein was transferred to a polyvinylidene difluoride plus membrane (Micron Separations, Westboro, MA) and, after blocking in 5% nonfat milk, exposed for 1 h at room temperature to a 1:2,000 dilution of the specific mouse antibody against TNFR1, Fas, Fasl, or caspases 3, 8, and 9 (pro- and cleaved caspase forms; Santa Cruz Biotechnology, Santa Cruz, CA). After six washings, antibody detection was accomplished by incubating the membrane for 1 h at room temperature in a 1:10,000 dilution of horseradish peroxidase-avidin anti-mouse IgG (Transduction Laboratories, Lexington, KY) followed by use of a chemiluminescence system (Renaissance, New England Nuclear
Life Science Products, Boston, MA) and exposure to autoradiography film (Biomax ML, Eastman Kodak, Rochester, NY).

Statistical analyses. Results are presented as mean values ± SE. When experiments included only two groups, an unpaired Student’s t-test was used. When the experiments included more than two groups, statistical differences were identified using a one-way ANOVA followed by the Student-Neuman-Keuls test. The SigmaStat statistical package (SPSS, Chicago, IL) was used for all statistical analyses. A P value of <0.05 was considered significant.

RESULTS

Intestinal adaptation and temporal expression of death receptor proteins. Survival in all groups was >80%, and all mice were healthy and vigorous at the time of death. In the first set of experiments, male ICR and C57BL/6 mice underwent SBR or sham operation and were killed at 12 and 24 h and at 3 and 7 days after operation. Intestinal adaptation in the SBR group was confirmed grossly as early as 3 days by measuring ileal wet weights (Fig. 1). In these mice, there were no differences in TNF-α production at early time points; however, there was a modest but significant decrease in intestinal TNF-α levels in the SBR animals by 7 days (Fig. 2A). Compared with sham, TNFR, expression was increased after SBR in the early (12 h) and late (day 7) postoperative periods, whereas the lowest expression was observed on day 3 (Fig. 2B). Fas expression was highest at the earliest time point after SBR (12 h), decreased slightly, and was then equivalent with sham-operated mice by postoperative day 7 (Fig. 3). In contrast, FasL expression peaked on postoperative day 3 in the SBR mice, with declining levels observed on postoperative day 7. The different expression at these time points between the two groups is more likely due to reduced expression of FasL in the sham-operated mice rather than increased expression in the SBR mice. The expression of a surrogate marker for inflammation (IFN-γ) was increased slightly at 24 h after SBR, but there were no significant differences at other time points (Fig. 4). Together, the death receptor family proteins surveyed in these experiments revealed time-related expression changes following SBR. However, the general pattern of expression of these proteins did not coincide with the usual histological appearance of apoptosis following SBR (Table 1) (15).

Temporal expression of caspases. Caspase 8, which is primarily involved in the extrinsic pathway of apoptosis via death receptor activation, demonstrated a modest increase in expression for the native form from 12 to 24 h after SBR (Fig. 5A). This was followed by a reduction in intestinal levels of procaspase 8 at later time points relative to sham. Interestingly, the cleaved form of caspase 8 (activated moiety) demonstrated a mild, early reduction in expression over time, with
minimal differences compared with sham. On the other hand, caspase 9, the main caspase involved in the intrinsic pathway for activation of apoptosis, demonstrated a mild increase in both pro- and cleaved forms early, with reduced levels relative to sham after 7 days (Fig. 5B). Finally, the expression of caspase 3, the main executer of apoptosis via both intrinsic and extrinsic pathways, was measured. Procaspase 3 showed minimal change after SBR. However, cleaved (activated) caspase 3 showed a gradual decrease, followed by a dramatic increase (roughly 4-fold) in caspase activity by 7 days (Fig. 5C). This pattern of expression correlated with the histological appearance of apoptosis observed between postoperative days 3 and 7 in these mice (data not shown).

TNFR1 and Fas expression during accelerated or attenuated postresection apoptosis. To further define the relationship between death receptor family protein expression and enterocyte apoptosis, alterations in TNFR1 and Fas expression were determined in the context of known experimental conditions for perturbed postresection apoptosis. In a strain of mice with defective EGF receptor signaling capacity (waved-2), SBR results in an attenuated adaptation response (16) and apoptosis is markedly elevated when compared with nonmutant mice (19). Alternatively, we have previously demonstrated that exogenous administration of EGF results in enhanced intestinal adaptation (18) and reduced apoptosis following SBR (19). Both waved-2 and EGF-treated mice were studied on postoperative day 3, because we have previously observed that waved-2 mice do not consistently survive beyond this time point after SBR (16), and alterations in apoptosis are obvious in both experimental paradigms by this time interval. In the current study, waved-2 mice showed an expected increase in apoptosis, whereas EGF-treated mice did not increase apoptosis following SBR on postoperative day 3 (Table 2). When compared with the EGF-treated groups, the baseline expression of TNFR1 and Fas was very high in the waved-2 mice. The expression of both was reduced after SBR (Fig. 6). Alternatively, TNFR1 and Fas expression were increased after SBR in the EGF-treated mice. In general, these alterations in TNFR1 and Fas expression are somewhat paradoxical and do not coincide with the pattern of enhanced or inhibited postresection apoptosis.

Postresection adaptation and apoptosis in TNFR1-null and Fas-null mice. TNFR1-null, Fas-null, and respective control mice were subjected to SBR and sham operation to directly test the role of the individual death receptors in the activation of enterocyte apoptosis after SBR. Intestinal adaptation occurred normally after 3 days in the TNFR1-null mice as gauged by postresection increases in remnant ileal wet weight, villus height, and enterocyte proliferation (Fig. 7, A–C). Furthermore, absent expression of TNFR1 did not affect the expected increase in enterocyte apoptosis after SBR (Fig. 7D).

Ileal wet weight was significantly increased in the control and Fas-null mice after SBR (Fig. 8A). In con-
Contrast, ileal villus height did not change appreciably in the Fas-null mice and was significantly lower compared with the control mice after SBR (Fig. 8B).

Whereas the rates of enterocyte proliferation were not different after SBR in either group of mice (Fig. 8C), the trend was an increase in a similar magnitude as the TNFR1-null group. Finally, absent expression of Fas did not have an effect on the usual increase in postresection enterocyte apoptosis (Fig. 8D).

DISCUSSION

In the present study, we first delineated the expression of several key death receptor family proteins and caspases in the remnant ileum after SBR. Despite alterations in the expression of individual proteins over time, the expression profile did not directly correlate with the histological appearance of apoptosis following SBR. Alterations in the expression and active cleavage of both caspases 8 and 9 were modest, occurred relatively early, and were not sustained during the adaptation period. Proteolytic cleavage of caspase 3 dramatically increased between postoperative days 3 and 7, which coincided with the histological evidence for the increase in apoptosis.

Table 2. Apoptosis index within ileal crypts after EGF receptor manipulations and SBR

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<tr>
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<th>Sham</th>
<th>SBR</th>
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<tr>
<td>Control</td>
<td>0.100 ± 0.018</td>
<td>0.375 ± 0.025*</td>
</tr>
<tr>
<td>Waved-2</td>
<td>0.317 ± 0.04</td>
<td>0.650 ± 0.032*†</td>
</tr>
<tr>
<td>EGF</td>
<td>0.081 ± 0.016</td>
<td>0.0750 ± 0.025†</td>
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Values are means ± SE. *P < 0.05 vs. corresponding sham group. †P < 0.05 vs. control SBR and EGF SBR. ‡P < 0.05 vs. control SBR.
for increased postresection apoptosis. In the next series of experiments, we demonstrated that SBR paradoxically induced the expression of the death receptors TNFR1 and Fas under conditions of decreased apoptosis. Alternatively, both TNFR1 and Fas expression were reduced under conditions of exaggerated apoptosis following SBR. Finally, we confirmed that the usual increase in enterocyte apoptosis was preserved in TNFR1-null and Fas-null mice following massive SBR. These results support the hypothesis that the induction of enterocyte apoptosis by massive SBR is mediated independently of the extrinsic death receptor pathway.

Intestinal epithelial cells are capable of TNF-α production in response to multiple factors, including pathogenic bacteria (22), endotoxin (43), and ischemia-reperfusion (60). Because we have demonstrated increased translocation of bacteria to mesenteric lymph nodes and liver following SBR in our murine model (40), we would have anticipated a postresection increase in intestinal TNF-α levels. However, the postresection expression pattern in the remnant ileum demonstrated only modest changes and did not coincide with the histological appearance of apoptosis. It is possible that greater changes occurred within enterocytes that were undetected due to confounding protein from other cells within the whole bowel homogenate. As such, determination of protein expression within enterocyte populations isolated by mechanical dissociation (8) or navigated laser capture microdissection microscopy (49) might have revealed more profound changes. On the other hand, failure to identify altered expression of these factors does not exclude their potential significance in the genesis of increased apoptosis. Local intestinal TNF-α production may not change, despite significant increases in serum levels (58). Along these lines, administration of a neutralizing antibody to TNF-α was shown to prevent the increased enterocyte apoptosis following burn injury, despite the fact that no changes were identified in intestinal TNF-α mRNA expression (48). Although serum levels were not measured after SBR in the present study, no significant alterations in circulating levels of TNF-α were revealed in a previous report of rats undergoing a 70% SBR (31). Fas and FasL are important candidates to be considered in the regulation of enterocyte apoptosis during several immune-mediated pathological gastrointestinal conditions.

Fig. 6. Western blots of ileal TNFR1 and Fas expression at 3 days following either a 50% proximal SBR (R) or sham operation (S; transection and reanastomosis only). EGF corresponds to mice given exogenous epidermal growth factor twice daily by orogastric gavage (50 μg·kg⁻¹·day⁻¹), whereas waved-2 denotes the strain of mice with defective EGF receptor signaling.

Fig. 7. Ileal wet weight (A), villus height (B), apoptotic index (C), and proliferative index (D) from control (C57BL/6) and TNFR1-null mice 3 days following either a sham operation (transection and reanastomosis only) or 50% proximal SBR. The apoptotic index was derived by counting the number of apoptotic bodies per crypt with characteristic abnormal morphology (pyknotic nuclei, condensed chromatin, and nuclear fragmentation). The proliferative index was derived by the ratio of crypt cells staining positively for PCNA compared with the total number of crypt cells. *P < 0.05 C57BL/6 and TNFR1-null sham vs. SBR, n = 5–7 per group.
nal conditions including graft-vs.-host disease (30), celiac disease (4), and ulcerative colitis (53, 63). Infiltration of lymphocytes or neutrophils in histological sections of the intestine is not a usual characteristic of postresection adaptation. Furthermore, the differences between sham and SBR groups with regard to intestinal expression of IFN-γ were slight and would argue against a significant inflammatory response within the remnant intestine after massive intestinal resection. We found that the expression of FasL was more than twofold greater at the 3-day time point after SBR when compared with sham-operated mice. It is therefore tempting to propose that resection-induced expression of this proapoptotic ligand may contribute to the increased enterocyte apoptosis that is typically observed at this same time point. However, careful review of the Western blot (Fig. 3) revealed that this difference was probably more likely a result of reduced FasL expression in the sham-operated mice rather than increased expression in the mice undergoing SBR. Similarly, the difference in Fas expression between sham and SBR groups was fairly modest (50% increase after SBR) at the 3-day time interval but was absent at 7 days, during which time increased enterocyte apoptosis is still ongoing (15). Collectively, these data do not strongly endorse the concept that postresection enterocyte apoptosis is regulated by alterations in the tissue expression of Fas or FasL.

The expression and cleavage of caspases 8 and 9 after SBR were slightly increased in the early postoperative period, with a reduced expression for the remainder of the study period. Although we are presently unclear as to its significance, this could have been a nonspecific response to the stress of surgery. This pattern was followed by a major increase in caspase 3 cleavage, which corresponded with the appearance of apoptosis on histological sections. Although the expression profiles for caspases 8 and 9 between sham and SBR were modest, these findings do not exclude the possibility that either pathway for caspase activation was potentially operative as an early signal for increased postresection apoptosis. Alternatively, it must be considered that although the extrinsic and intrinsic pathways are capable of operating independently, accumulating evidence suggests that cross-talk between the two pathways may exist (13). For example, the link between death receptor signaling and the intrinsic pathway comes from the finding that the BH3 domain-containing protein Bid is cleaved by active caspase 8, which then translocates to mitochondria and triggers cytochrome c release (28, 34). Cytosolic cytochrome c forms an essential part of the apoptosis complex “apoptosome,” ultimately activating caspase 9 (29). Once caspase 9 is activated, there is a hierarchical activation of specific caspases for the execution of apoptosis (47). Thus early activation of caspase 8 by death receptor ligation could potentially have resulted in increased cleavage of caspase 9.

Intact signaling through the EGF receptor (EGFR) affects postresection apoptosis (19) and appears to be critical for a robust adaptation response to massive SBR (50). Along these lines, we recorded TNFR1 and Fas expression after SBR in waved-2 mice, which harbor a spontaneous point mutation within the tyrosine kinase domain of the EGFR, resulting in a reduced (greater than 7-fold) tyrosine kinase activity in vivo (9, 33). When subjected to massive SBR, we have previously documented that these mice demonstrate an attenuated adaptation response (16) and accelerated enterocyte apoptosis (19). It was interesting that in the context of perturbed EGFR signaling, the constitutive expression of both receptors was markedly elevated, possibly due to an ongoing proinflammatory state of the intestine, although we saw no obvious histological
evidence for this. We have previously observed elevated basal rates of enterocyte apoptosis in sham-operated waved-2 mice (19). Thus it is possible that TNFR1 and/or Fas are involved in baseline, as opposed to resection-induced apoptosis. Alternatively, the reduced expression of these death receptors after SBR could have been due to increased ligand/receptor interaction with compensatory downregulation of receptor production. Direct measurement of elevated TNF-α and FasL levels in the intestine and/or serum in these mice might have supported this concept. On the other hand, the expression of TNFR1 and Fas was significantly and paradoxically elevated in the intestine of EGF-treated mice with attenuated apoptosis after SBR. It is possible that EGFR and death receptor signaling are inversely related. In other cell systems, EGFR stimulation bestows resistance, whereas EGFR inhibition confers vulnerability to TNF-α or Fas-induced apoptosis (10–12, 21). Because we have previously demonstrated increased intestinal EGFR expression after SBR (7) and with exogenous EGF (17), it is possible that the increased TNFR1 and Fas expression observed after SBR in the EGF-treated groups was a compensatory response to an overall increased resistance to apoptosis.

It has been previously established that mice lacking TNFR1 are resistant to TNF-α-induced apoptosis and cell detachment from the villi of the duodenum and small intestine (42). Therefore, TNFR1-null mice were employed to test the relevance of this receptor toward enterocyte proliferation and apoptosis following SBR. Intestinal adaptation and increased enterocyte apoptosis occurred normally in both TNFR1 and Fas-null mice. These observations add strength to the notion that postresection apoptosis is not regulated by either pathway. It must also be considered that in these null mice, one receptor may be capable of taking over the absent function of the other. The TNF-receptor superfamily is complex and comprised of multiple members, with overlapping functions. In addition to TNFR1, signaling via the related death receptors DR-3, DR-4, DR-5, TNFR2 (37), or the TNF apoptosis-inducing ligand (TRAIL) (61) may contribute to the induction of enterocyte apoptosis following SBR.

The magnitude of the adaptation response in the remnant ileum is significantly enhanced under conditions in which the relative ratio of enterocyte proliferation to crypt cell apoptosis is increased. Indeed, increases in enterocyte proliferation, coupled with reduced rates of apoptosis, have been described under several conditions of enhanced adaptation after SBR by exogenous administration of such factors as EGF (19), glucagon-like peptide-2 (6), and the combination of glutamine and growth hormone (14). Alternatively, when the ratio of enterocyte proliferation to apoptosis is reduced, an attenuated adaptation response to SBR has been observed under several experimental paradigms, including waved-2 mice (19), p21<sup>cip1</sup>-null mice (51), or pharmacological EGFR blockade (41). On the other hand, adaptation after SBR is inhibited when the rates of enterocyte proliferation and apoptosis are both reduced. This has been described with administration of lipopolysaccharide (55) or somatostatin analog (54), during vitamin A deficiency (56), or malnutrition (5).

The most compelling evidence to suggest that enterocyte apoptosis exerts a major influence on the postresection adaptation response is the recent demonstration of enhanced adaptation in transgenic mice overexpressing the antiapoptotic protein bcl-2 (62).

The mechanism for postresection enterocyte apoptosis is probably regulated independently of factor(s) that control proliferation. Evidence to support this disassociated response includes two experimental paradigms. In the first, the expected postresection increase in apoptosis was completely blocked in mice unable to express the proapoptotic bcl-2 family member bax (52). Despite the blocked increase in apoptosis, proliferation and other parameters of adaptation normally increased after SBR. In the second paradigm, mice lacking the gene for the cyclin-dependent kinase inhibitor p21<sup>cip1</sup> demonstrated no induction of proliferation or other adaptive parameters after intestinal resection (51). This finding was somewhat paradoxical, because the absence of a cell cycle inhibitor would be expected to promote proliferation. On the other hand, at low levels, p21<sup>cip1</sup> is found structurally to reside within catalytically active cyclin-dependent kinases (64) and to act as an adaptor molecule to promote assembly and nuclear targeting of ckdk4/cyclin D complexes to stimulate cell proliferation (3, 27). Despite absent induction of enterocyte proliferation and adaptation, the usual postresection increase in enterocyte apoptosis occurred normally in the p21<sup>cip1</sup>-null mice. Thus it would appear that two separate candidate genes are critical for the apoptotic (bax) and proliferative (p21<sup>cip1</sup>) response of the enterocyte to massive SBR. Further studies to more completely elucidate the function of these genes during adaptation are crucial in the design of novel therapy to enhance this important response.

DISCLOSURES

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REFERENCES


