Smooth muscle overexpression of IGF-I induces a novel adaptive response to small bowel resection

Andrew W. Knott, Russell J. Juno, Marcus D. Jarboe, Sherri A. Profitt, Christopher R. Erwin, Eric P. Smith, James A. Fagin, and Brad W. Warner. Smooth muscle overexpression of IGF-I induces a novel adaptive response to small bowel resection. Am J Physiol Gastrointest Liver Physiol 287: G562–G570, 2004. First published May 13, 2004; 10.1152/ajpgi.00438.2003.—Prior studies of intestinal adaptation after massive small bowel resection (SBR) have focused on growth factors and their effects on amplification of the gut mucosa. Because adaptive changes have also been described in intestinal smooth muscle, we sought to determine the effect of targeted smooth muscle growth factor overexpression on resection-induced intestinal adaptation. Male transgenic mice with smooth muscle cell overexpression of insulin-like growth factor I (IGF-I) by virtue of an α-smooth muscle actin promoter were obtained. SMP8 IGF-I transgenic (IGF-I TG) and nontransgenic (NT) littermates underwent 50% proximal SBR or sham operation and were then killed after 3 or 28 days. NT mice showed the expected alterations in mucosal adaptive parameters after SBR, such as increased wet weight and villus height. The IGF-I TG mice had inherently taller villi, which did not increase significantly after SBR. In addition, IGF-I TG mice had a 50% postresection persistent increase in remnant intestinal length, which was associated with an early decline and later increase in relative mucosal surface area. These results indicate that growth factor overexpression within the muscularis layer of the bowel wall induces significant postresection adaptive intestinal lengthening and a unique mucosal response. IGF-I signaling within the muscle wall may play an important role in the pathogenesis of resection-induced adaptation.

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derived growth factor expression on the adaptive response of the intestine to massive SBR.

METHODS

Experimental design. The protocol for this study was approved by the Children’s Hospital Research Foundation Institutional Animal Care and Use Committee (Cincinnati Children’s Hospital Medical Center, Cincinnati, OH). Mice were randomized to undergo either a sham operation or 50% proximal SBR, as described below. Animals from all groups (n = 6–8) were killed on the 3rd or 28th postoperative day, and parameters of adaptation were determined in the remnant ileum, as outlined below.

Animals. The characterization and phenotypic analysis of the SMP8 IGF-I transgenic mouse has been described previously (30). Briefly, the transgene is composed of a murine smooth muscle α-actin promoter fragment SMP8 cloned upstream of a rat IGF-I cDNA. The transgene is highly expressed postnatally, uniquely in SMCs, and is released in a paracrine fashion in tissues with an elevated concentration of smooth muscle, such as arteries, veins, uterus, bladder, and the muscular compartment of the gastrointestinal tract. For the purposes of this experiment, FVB/N SMP8 IGF-I transgenic animals were backcrossed to a C57BL/6 background for seven generations (weight range 25–29 g; Jackson Laboratories, Bar Harbor, ME). The backcrossings were done because we have observed that the FVB/N strain is less tolerant of SBR procedures when compared with C57BL/6 crossings were done because we have observed that the FVB/N strain is less tolerant of SBR procedures when compared with C57BL/6 mice (unpublished data). Male mice were used for the current study. The mice were housed in groups of four at 21°C on 12:12-h day-night cycles (6:00 AM to 6:00 PM) and 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-least 5 days before experimentation. One day before operation, the diet was changed from regular chow to a liquid rodent diet (Micro-

Operative procedure. The murine model for SBR and sham operation has been described by our group previously (12). In short, the mice were anesthetized using a balanced mixture of inhaled 2% isoflurane, oxygen (90%), and carbon dioxide (4%). Under sterile conditions and with the aid of an operating microscope (6.25× magnification), the ileocecal junction was identified through a midline incision. Sham operations consisted of division and reanastomosis of the bowel ~12 cm proximal to the ileocecal junction in nontransgenic mice and ~15 cm in SMP8 IGF-I transgenic mice. The difference in site of transection between the nontransgenic and SMP8 IGF-I transgenic mice was because of the previously documented 23% greater bowel length of the transgenic mice (30). Likewise, in mice undergoing SBR, the bowel was divided ~12 cm proximal to the ileocecal junction in nontransgenic and ~15 cm in SMP8 IGF-I transgenic mice. In all animals undergoing SBR, the bowel was transected proximally 2–3 cm distal to the ligament of Treitz. The mesentry of the resected intestine was ligated, and either 12 (nontransgenic) or 15 cm (SMP8 IGF-I transgenic) of the intervening small intestine were removed to afford a 50% resection. Intestinal continuity was restored using an end-to-end, single-layered anastomosis with 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 both experimental groups were fed with a liquid diet. Food intake differences between groups of mice in the postoperative period were negligible.

Tissue harvest. Mice were killed by cervical dislocation after an intramuscular injection of ketamine-xylazine-acepromazine (4:1:1 proportion). The distal ileal remnant between the anastomosis and cecum was removed. Intestinal contents were gently expressed with cotton swabs, and the length of the remnant ileum was recorded. The postoperative change in length of the ileal remnant was calculated by determining the percentage difference between the pre- and postoperative lengths [(preop length in cm − postop length in cm) ÷ (preop length in cm) × 100]. From the remaining remnant, 6 cm (~1 cm from the anastomosis) of ileum were excised, and the wet weight was recorded. The proximal first centimeter of ileum was immediately fixed with 10% neutral buffered formalin and used for histology; the remaining 5 cm was frozen in liquid nitrogen and stored at −80°C until further use.

Histology. Formalin-fixed and paraffin-embedded ileum specimens were cut into 5-μm-thick tissue slices parallel with the longitudinal axis of the bowel. Tissue slices were mounted on poly-L-lysine slides and stained with hematoxylin and eosin or subjected to Ki-67 immunohistochemistry using a kit (ABCam, Cambridge, UK) following the instructions of the manufacturer. With the hematoxylin- and eosin-stained sections, villus heights and muscle cellularity were recorded using computer-aided video microscopy (Image Tool 2.02; University of Texas Health Sciences Center, San Antonio, TX). At least 20 villi were counted and averaged for each sample, and only villi with an intact central lymphatic channel were considered. Muscle cellularity was calculated by counting the number of nuclei within an ~3,500-μm² area of the muscle layer. These sections also were 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 (11). An apoptotic index was defined as the number of apoptotic bodies per crypt determined by the blinded scoring and averaging of 20 crypts/sample.

An index of crypt cell proliferation was derived in Ki-67-stained sections by calculating the ratio of crypt cell counts positively for Ki-67 to the total number of crypt cells. Fifteen representative crypts were counted per sample. All histological analyses were performed in a blinded manner, without knowledge of the source of the ileal tissue.

Muscle thickness. Sections of remnant ileum were collected and preserved as described above. Total, as well as longitudinal and circular muscle layer, thickness was measured at 10 different points along the longitudinal axis of each sample using computer-aided video microscopy (Image Tool 2.02; University of Texas Health Sciences Center, San Antonio, TX). Only sections containing an intact crypt-villus axis were counted and averaged to avoid sampling errors in areas not cut perpendicular to the longitudinal axis of the bowel.

cDNA synthesis and quantification. Individual ileal samples were thawed and homogenized (PowerGen; Fisher Scientific, Pittsburgh, PA). Total RNA was isolated by using TRIzol reagent (GIBCO-BRL), following the instructions of the manufacturer (4). The concentration of total RNA was determined spectrophotometrically at 260 nm absorbance. An RT reaction was performed by using 5 μg total RNA and RTase (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer. The cDNA concentration was then quantified using OligoGreen (Molecular Probes, Eugene, OR) and a fluorometer (Turner Deign, Sunnyvale, CA), following the instructions of the manufacturer. Equal amounts of cDNA were used for subsequent PCR reactions.

Real-time RT-PCR. Real-time PCR was performed using a SmartCycler (Cepheid, Sunnyvale, CA), and the Quantitect SYBR Green PCR kit (QIAGEN, Valencia, CA). Total IGF-I was determined using oligonucleotides, synthesized by the University of Cincinnati DNA Core (forward: 5′-TGG ATG CTC TTC AGT TCG TGT-3′; reverse: 5′-CAA CAC TCA TCC ACA ATG CCT G-3′; 112 bp), which were validated as follows: serial dilution of cDNA template revealed an amplification efficiency of 1.89; melt curve of amplified product revealed a unique product; ethidium-stained agarose gel electrophoresis of amplified product revealed a single species at the expected size of ~100 bp; and cDNA templates from SMP8-IGF-I transgenic tissues with smooth muscle predominance (e.g., aorta and bladder) displayed significantly lower threshold cycle numbers. Transgenic IGF-I was determined using SYBR oligonucleotides, as previously described (30). Samples from each tissue were analyzed in triplicate. The relative abundance of IGF-I mRNA expression was determined by deriving a ratio of expression to β-actin (30) using the Microsoft
Excel-based software Q-Gene from the Biotechniques software library (23).

Statistical analysis. Results are presented as mean values ± SE. When the experiments included more than two groups, statistical differences were identified using a one-way ANOVA followed by the Student-Newman-Keuls test. The SigmaStat statistical package (SPSS, Chicago, IL) was used for all statistical analyses. A P value < 0.05 was considered significant.

RESULTS

Mucosal morphology is unaffected by SBR in SMP8 IGF-I transgenic mice. Postoperative survival in all groups was 78%, and all surviving mice were healthy and vigorous at the time of death. Intestinal adaptation after SBR was measured grossly by recording wet weight per centimeter of the harvested ileum. Consistent with our previous study (32), baseline intestinal wet weight of the SMP8 IGF-I transgenic mice (undergoing sham operation) was significantly higher than nontransgenic mice (Fig. 1A). Intestinal resection resulted in a significant increase in specific wet weight of the remnant ileum in nontransgenic mice. Although the SMP8 IGF-I transgenic mice demonstrated a trend toward increased ileal wet weight after SBR, this difference did not achieve statistical significance when compared with the sham-operated transgenic mice. At the 28-day postoperative time, there was a continued increase in ileal wet weight after SBR in the nontransgenic mice; however, there were no significant differences observed between sham and SBR in the SMP8 IGF-I mice. In a similar manner, there was the expected increase in villus height after SBR was observed in the nontransgenic mice (Fig. 1B). The sham-operated SMP8 IGF-I transgenic mice had significantly higher baseline villus heights when compared with sham nontransgenic and had levels similar to those in the nontransgenic group that underwent SBR. Intestinal resection did not further increase villus height in the ileum of the transgenic mice. At the later 28-day time, there was continued villus growth seen in the nontransgenic, but no significant changes were observed in the SMP8 IGF-I transgenic mice. Representative photomicrographs of the ileal remnant for each group are depicted in Fig. 2.

Enterocyte turnover in SMP8 IGF-I transgenic mice is similar to nontransgenic mice after SBR. Intestinal resection induced a significant increase in enterocyte proliferation in both nontransgenic and SMP8 IGF-I transgenic mice (Fig. 3A). In contrast to the baseline taller villi and greater wet weight of the ileum in sham-operated SMP8 IGF-I transgenic mice, transgenic IGF-I expression in the muscularis did not result in increased basal rates of enterocyte proliferation. With regard to rates of enterocyte apoptosis, there were no differences between the nontransgenic and SMP8 IGF-I transgenic sham-operated mice (Fig. 3B). Furthermore, intestinal resection resulted in significantly greater rates of enterocyte apoptosis in both nontransgenic and transgenic mice to the same extent.

Expression of total IGF-I is elevated in SMP8 IGF-I transgenic mice after SBR. SBR did not significantly affect IGF-I mRNA expression in the ileum of the nontransgenic mice, as measured by real-time quantitative PCR. The expression levels of IGF-I transcript in the transgenic mice undergoing sham operation were similar to the nontransgenic mice undergoing sham or SBR (Fig. 4A). However, SBR resulted in a significant increase in total IGF-I mRNA in the SMP8 IGF-I transgenic mice. On specific analysis of transgenic IGF-I, there was minimal change after SBR (Fig. 4B). Overall, the transgenic IGF-I accounted for ~10% of the total. Therefore, the postresection increase was probably derived from the native, not transgenic, IGF-I.

Postresection adaptive intestinal length is greater in SMP8 IGF-I transgenic mice. Although the ileal villus heights of SMP8 IGF-I transgenic mice showed little change in response to SBR, the SMC compartment was markedly altered. First, and consistent with earlier observations (2), the baseline muscle thickness within the ileum was greater in the sham-operated transgenic mice compared with the nontransgenic mice (Fig. 5A). Surprisingly, intestinal resection in the transgenic mice resulted in a significant decrease in muscle thickness to the same thickness as in resected nontransgenic mice. In contrast, there was no change in the muscle thickness of the ileum in the nontransgenic mice after SBR. At the later 28-day time point, this trend had reversed for the nontransgenic mice, as they demonstrated the expected increase in muscular thickness after SBR. In the SMP8 IGF-I transgenic mice, the pattern of thinner muscle in the SBR animals persisted. Further analysis of the longitudinal (Fig. 5B) and circular (Fig. 5C) muscle layers...
revealed that the greatest postresection diminution in muscle thickness occurred in the longitudinal muscle of the SMP IGF-I transgenic mice. It was interesting that the circular layer accounted for the greatest increase in thickness after SBR in the nontransgenic mice.

SMC cellularity, derived by counting the number of nuclei per square centimeter, was greater in the sham-operated SMP8 IGF-I transgenic mice, suggesting a hyperplasia response to transgenic muscular IGF-I expression (Fig. 6). Intestinal resection resulted in a significant reduction in the number of nuclei per unit area in the IGF-I TG mice, suggesting that the early adaptive response of the SMCs within the ileal wall consists of longitudinal stretching. The same decline in nuclei per square micrometer took place after SBR in the nontransgenic mice; however, this difference did not achieve statistical significance. In the nontransgenic mice at the 28-day time point, smooth muscle cellularity was significantly lower after SBR when compared with sham operation. In face of resection-induced thicker muscle cellularity in these mice, this measurement of cellularity would suggest that the muscle cell response to SBR is primarily hypertrophy. In the SMP8 IGF-I transgenic mice, the earlier decrease after SBR was no longer present at 28 days, since there were no differences between sham or SBR mice. In the context of decreased muscle thickness after SBR in the transgenic mice at the later postoperative time interval, the lack of change in number of smooth muscle nuclei would offer the possibility that hyperplasia occurred.

To determine whether the possible longitudinal myocyte stretching within the intestinal wall contributed toward the postresection increase in intestinal length, we measured the change in small bowel length after SBR in both nontransgenic and SMP8 IGF-I transgenic mice. No appreciable change in intestinal length was observed in the nontransgenic mice at 3 days after SBR. In contrast, intestinal resection in the SMP8 IGF-I transgenic mice resulted in a marked increase in length of the ileal remnant, particularly when expressed as a percentage change (Fig. 7). This same pattern persisted into the later 28-day time point (data not shown).

Because the postresection increase in intestinal length in the transgenic mice was associated with significantly greater rates of enterocyte proliferation but unaltered villus heights, the effect of resection on mucosal surface area was measured according to a standardized formula (Fig. 8A; see Ref. 13). As expected, the increased enterocyte proliferation and resultant taller villi in the nontransgenic mice after SBR resulted in a significant increase in mucosal surface area (Fig. 8B). In contrast, mucosal surface area was significantly reduced in the remnant ileum in the SMP8 IGF-I transgenic mice after SBR. With regard to the later postoperative time (28 days), mucosal surface area continued to increase in the nontransgenic mice and began to increase also in the SMP8 IGF-I transgenic mice.
The differential response to intestinal resection with regard to intestinal length, smooth muscle thickness, and mucosal surface area between the nontransgenic and transgenic mice is summarized in Table 1.

DISCUSSION

In the present study, we have characterized the effect of IGF-I overexpression in SMCs on the adaptive intestinal response to massive SBR. Under basal conditions after sham operation, the intestinal muscle wall thickness was greater, and the mucosa demonstrated taller villi within the ileum of SMP8 IGF-I transgenic mice. Intestinal resection resulted in an attenuation of muscle wall thickness and was associated with significant adaptive lengthening of the remnant bowel. This response is unique, since it occurred relatively early during the adaptation phase (72 h) and, in contrast with the nontransgenic mice, was not associated with an additional increase in villus height but with a reduction in relative mucosal surface area. The mechanism for early postresection intestinal lengthening appears to be because of longitudinal stretching of the baseline.

The differential response to intestinal resection with regard to intestinal length, smooth muscle thickness, and mucosal surface area between the nontransgenic and transgenic mice is summarized in Table 1.

Fig. 4. Real-time PCR for total IGF-I (A) and transgenic (SV-40) IGF-I (B) mRNA using whole bowel homogenates from SMP8 IGF-I transgenic (IGF-I TG) and nontransgenic control (NT) mice harvested 3 days after either sham operation (transection and reanastomosis only) or 50% proximal SBR. *P < 0.05, SBR vs. sham (n = 5 animals/group). Samples were run in triplicate.

Fig. 5. Measurement of total (A), longitudinal (B), and circular muscle thickness (C) of the remnant ileum from SMP8 IGF-I transgenic (IGF-I TG) and nontransgenic control (NT) mice 3 and 28 days after either sham operation (transection and reanastomosis only) or 50% proximal SBR. *P < 0.05, ANOVA (3-day and 28-day done separately), sham vs. SBR (n = 2–7 animals/group).
expanded numbers of individual SMCs in the transgenic mice rather than resection-induced SMC proliferation. Taken together, these observations highlight the significant involvement of the intestinal SMC compartment in the genesis of the adaptive response to massive SBR. Because most previous research has focused on stimulation of enterocyte proliferation, these findings suggest that the intestinal SMC may provide a novel target to direct therapy designed to amplify the adaptation response.

The IGF-I transgenic mice used in this study have been well characterized, demonstrating SMC hyperplasia in multiple organs and tissues (30). In addition to the intestine, transgenic IGF-I expression has been detected by in situ hybridization within the SMC compartment of arteries, veins, urinary bladder, stomach, and uterus and not expressed in skeletal muscle, heart, or liver. A paracrine effect of transgenic SMC expression of IGF-I has been shown to induce intestinal mucosal growth in nonperturbed mice (32). This effect seems to be most pronounced in the ileum when compared with the jejunum and the colon, as demonstrated by greater increases in mucosal weight and DNA and protein content per unit length. Therefore, this transgenic mouse is ideally suited for our SBR model in which adaptation is studied in the remnant ileum after a proximal jejunal resection (12). Sham-operated SMP8 IGF-I transgenic mice had significantly taller villi at baseline when compared with sham-operated nontransgenic mice and were equivalent to the resected nontransgenic group. Our findings of taller villi at baseline differ from studies by Williams et al. (32) in which villus heights were similar between IGF-I transgenic and nontransgenic mice. It is possible that sham operation induced some type of signal for mucosal growth in our transgenic mice. Alternatively, the differences could have been

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**Fig. 6.** Muscle cellularity (nuclei/μm²) of the remnant ileum from SMP8 IGF-I transgenic (IGF-I TG) and nontransgenic control (NT) mice 3 and 28 days after either sham operation (transection and reanastomosis only) or 50% proximal SBR. *P < 0.05, ANOVA (3-day and 28-day done separately), sham vs. SBR (n = 2–7 animals/group).

**Fig. 7.** Percent increase of remnant length [((postop length — preop length)/preop length) × 100] from SMP8 IGF-I transgenic (IGF-I TG) and nontransgenic control (NT) 3 days after either sham operation (transection and reanastomosis only) or 50% proximal SBR. *P < 0.05, ANOVA, SBR vs. sham (n = 5–7 animals/group).

**Fig. 8.** Relative mucosal absorptive surface area for remnant mouse ileum. A: representation of measurements taken from hematoxylin- and eosin-stained sections and formula (from Ref. 14) used to calculate relative mucosal surface area. VH, villus height; VW, villus width; CW, crypt width; M, mucosal surface area. For each group, 10 measurements for each variable were taken from 2–8 individual animals. B: relative mucosal surface area (M) from nontransgenic control (NT) and SMP8 IGF-I transgenic (IGF-I TG) mice 3 and 28 days after either a sham operation (transection and reanastomosis only) or 50% proximal SBR. *P < 0.05, ANOVA (3-day and 28-day done separately), SBR vs. sham.
because of different strains of mice or variation in techniques for mounting and/or processing of the tissue sections.

It was interesting that there was no significant increase in villus height after intestinal resection in the SMP8 IGF-I transgenic mice. This suggests that there may be a maximal level of morphological compensation of the intestinal mucosa in response to massive SBR. Alternatively, IGF-I appears to have a much greater effect on mucosal growth when administered systemically (15, 24, 38). In fact, serum IGF-I levels in the SMP8 IGF-I transgenic mice used in the present study are the same as in nontransgenic mice (30). It is therefore possible that the paracrine effect of transgenic SMC-derived IGF-I was insufficient to signal for postresection mucosal growth. Finally, the finding that the villus heights were sustained at a taller level in the SMP8 IGF-I transgenic mice despite a significant postresection increase in intestinal length endorses the concept that there indeed was a profound induction of mucosal growth. Along these lines, we might have anticipated the villi to shorten in response to longitudinal intestinal lengthening if compensatory mucosal growth had not transpired.

SBR induced enterocyte proliferation in nontransgenic but was not different from in the SMP8 IGF-I transgenic mice. In other studies, systemic administration of IGF-I (20, 24) or in transgenic mice, in which IGF-I overexpression is widespread and driven by a metallothionein promoter (31), has resulted in stimulation of enterocyte proliferation. In addition, exogenous IGF-I has been shown to enhance enterocyte proliferation in a model of parental nutrition-induced mucosal atrophy (5) and after distal SBR and cecectomy (6, 8). It is possible that the paracrine effects of SMC-derived IGF-I were not adequate to persuade this parameter of enterocyte turnover or that locally expressed transgenic expression might have downregulated the IGF-I receptor. Furthermore, it must be considered that the SMP8 IGF-I transgenic mice used in our experiments might have displayed altered rates of proliferation at either earlier or later time points after intestinal resection. Another possibility is that the factors that regulate proliferation in the ileum are different from what has been observed in the jejunum (5, 6, 8).

Enterocyte apoptosis was significantly increased in nontransgenic mice after SBR, and an equivalent increase occurred after resection in the SMP8 IGF-I transgenic mice. As with proliferation, it is possible that the paracrine effects of IGF-I in the transgenic mice was insufficient to affect postresection apoptosis. The regulation of enterocyte apoptosis by IGF-I is presently unclear. In one report, systemic IGF-I significantly reduced enterocyte apoptosis in the jejunum of parenterally fed rats with intact bowel (5). Similarly, in transgenic mice with IGF-I overexpression resulting in elevated serum levels, rates of apoptosis are reduced (31). On the other hand, systemic administration of IGF-I to rats undergoing SBR did not affect postresection rates of apoptosis (6). In that study, rates of apoptosis were noted to decrease in the jejunum of parenterally fed rats at 1 wk after a 70% midjejunoileal resection. This is in contrast with other reports in which rates of enterocyte apoptosis are noted to be elevated in the remnant bowel after intestinal resection (11, 27, 28). These disparate results might be explained on the basis of several factors, including different animals (rats vs. mice), magnitude and site of resection, presence of luminal or parenteral nutrient, and time course after SBR. Despite the fact that postresection rates of apoptosis were similar between the SMP8 IGF-I transgenic and nontransgenic mice in the present study, it is interesting that transgenic IGF-I overexpression (and elevated plasma levels of IGF-I) has been shown to attenuate irradiation-induced enterocyte apoptosis along with reduced abundance of the proapoptotic bax protein in the crypt stem cell region (31). These results fit well with the proposed critical role for bax in the pathogenesis of increased postresection apoptosis (25, 26).

When compared with nontransgenic mice, intestinal resection in the transgenic animals was associated with a modest increase in the expression of IGF-I. In prior studies, intestinal resection in rats has been shown to result in either increased IGF-I mRNA abundance (9, 38) or no change (1). Because intestinal IGF-I expression has not previously been described after SBR in mice, our findings in the nontransgenic mice were not necessarily incongruent. We distinguished between native and transgenic IGF-I expression and found that the increase was primarily contributed by native IGF-I in response to SBR.

Postresection intestinal adaptation characteristically involves all layers of the intestinal wall. More specifically, SMC hyperplasia has been well documented after intestinal resection in rats (16, 21) and dogs (19). Hyperplasia may occur in face of no change in muscle thickness, possibly because of increased caliber (21) and/or length of the intestine. Our finding of insignificant postresection increases in muscle thickness in the nontransgenic mice at the early (3-day) postoperative time point is therefore not unexpected. The anticipated increase in muscle thickness was identified at the later time point (28 days) in these mice. Interestingly, the profile of reduced muscular thickness in the SMP8 IGF-I transgenic mice persisted into the later period of adaptation. Looking at the specific muscle layers revealed that the greatest thinning in bowel of the transgenic mice took place in the longitudinal layers, whereas the greatest thickening in the nontransgenic mice occurred in the circular layer. At the present time, we do not have a good explanation for this differential effect. Because prior work has established that smooth muscle-targeted overexpression of IGF-I results in enhanced vascular contractility (36), it is possible that the transgenic IGF-I selectively affected contractility of smooth muscle layers within the intestinal wall. Alternatively, the thinning of the longitudinal layers in the transgenic intestine could have represented a unique response to intraoperative manipulation, including the development of a paralytic ileus.

| Table 1. Relative changes in intestinal length, smooth muscle thickness, and mucosal surface area in nontransgenic and SMP8 IGF-I transgenic mice after small bowel resection |
|-----------------------------------------------|-----------------------------------------------|
| Nontransgenic                               | Smooth muscle thickness                        |
| Intestinal length                            | Early                                         |
|                                              | Late                                          |
| Smooth muscle thickness                      | NC                                            |
| Mucosal surface area                         | NC                                            |
| SMP8 IGF-I transgenic                       | ↑ (circular)                                  |
| Intestinal length                            | ↑                                             |
| Smooth muscle thickness                      | ↓ (longitudinal)                              |
| Mucosal surface area                         | ↓                                             |

Early represents measurements taken at 3 days after intestinal resection compared with measurements in sham-operated mice. The late period corresponds with measurements taken at 28 days after intestinal resection and compared with the early period (e.g., ↑, ↑↑, or NC would indicate an increase, further increase, or no appreciable change compared with sham-operated mice in the early period or early vs. late periods).
Finally, the stimulus for mucosal growth in the transgenic mice might have been the primary initiator of longitudinal stretching of the intestine.

The implication for decreased numbers of SMC nuclei per unit area after SBR is unclear. Perhaps the stimulus for mucosal growth precedes and serves as a signal for muscular growth. Alternatively, the muscular stretch may serve as a stimulus for mucosal growth. Additional studies at later time points will be necessary to link the temporal pattern for postresection mitotic stimulation to the intestinal length increase. The intestinal length had increased by 15–20% at this same time point, the intestinal length had increased by 80% in these mice, with a greater postresection intestinal length. The degree of surface mucosal surface area in the transgenic mice was countered by response to SBR.

It is possible that the muscular stretch may serve as a stimulus for mucosal growth. Alternatively, the muscular stretch may serve as a stimulus for mucosal growth. Additional studies at later time points will be necessary to link the temporal pattern for postresection mitotic stimulation to the intestinal length increase. The intestinal length had increased by 15–20% at this same time point, the intestinal length had increased by 80% in these mice, with a greater postresection intestinal length. The degree of surface mucosal surface area in the transgenic mice was countered by response to SBR.

At the early (3-day) postoperative time point, the reduced mucosal surface area in the transgenic mice was countered by a greater postresection intestinal length. The degree of surface area reduction after SBR was roughly 10–20%. This contrasts with the modest (roughly 20%) increase in mucosal surface area with no significant change in intestinal length in the control mice.

In patients with short bowel syndrome, intestinal length remains as the most predictive indicator for the need of long-term parenteral nutritional supplementation (18). The significance of greater longitudinal intestinal growth in transgenic mice with growth factor expression targeted to the smooth muscle is therefore clear. Future experiments using inducible transgenic mouse strains will be necessary to delineate whether the IGF-I-induced longitudinal thinning that we observed was the result of greater numbers of SMCs at baseline or represents a specific response of SMCs to chronic IGF-I exposure.

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