Mesenchymal IGF-I overexpression: paracrine effects in the intestine, distinct from endocrine actions

KRISTEN L. WILLIAMS,¹,² C. RANDALL FULLER,¹ JAMES FAGIN,³ AND P. KAY LUND¹
Departments of ¹Cell and Molecular Physiology and ²Medicine, University of North Carolina, Chapel Hill, North Carolina 27599-7080; and ³Division of Endocrinology and Metabolism, University of Cincinnati, Cincinnati, Ohio 45267

Received 4 March 2002; accepted in final form 3 June 2002

Williams, Kristen L., C. Randall Fuller, James Fagin, and P. Kay Lund. Mesenchymal IGF-I overexpression: paracrine effects in the intestine, distinct from endocrine actions. Am J Physiol Gastrointest Liver Physiol 283: G875–G885, 2002. First published June 5, 2002; 10.1152/ajpgi.00089.2002.—Local IGF-I expression is frequently increased in intestinal mesenchyme during adaptive growth of intestinal epithelium, but paracrine growth effects of IGF-I in vivo are not defined. We tested whether overexpression of IGF-I in intestinal mesenchyme increases epithelial growth and if effects are distinct from known effects of circulating IGF-I. SMP8-IGF-I-transgenic (TG) mice overexpress IGF-I driven by an α-smooth muscle actin promoter. Mucosal and muscularis growth were assessed in the jejunum, ileum, and colon of SMP8-IGF-I-TG mice and wild-type littermates. Abundance of the SMP8-IGF-I transgene and IGF binding protein (IGFBP)-3 and -5 mRNAs was determined. Mucosal growth was increased in SMP8-IGF-I-TG ileum but not jejunum or colon; muscularis growth was increased throughout the bowel. IGFBP-5 mRNA was increased in SMP8-IGF-I-TG jejunum and ileum and was specifically upregulated in ileal lamina propria. Overexpression of IGF-I in intestinal mesenchyme has preferential paracrine effects on the ileal mucosal epithelium and autocrine effects on the muscularis throughout the bowel. Locally expressed IGF-I has distinct actions on IGFBP expression compared with circulating IGF-I.

insulin-like growth factor-I; mucosa; intestinal growth; insulin-like growth factor binding protein-3; insulin-like growth factor binding protein-5

IGF-I mediates linear body growth as well as growth of multiple organs, including the intestine (reviewed in Ref. 21). IGF-I stimulates proliferation of cultured intestinal epithelial cells, fibroblasts, myofibroblasts, and smooth muscle cells (9, 16, 17, 35, 36). Effects of circulating IGF-I to increase mass of small intestinal mucosa and muscularis propria in vivo are well documented (18, 29, 30, 38). The liver is a primary source of IGF-I in circulation (42), but IGF-I is also expressed locally in most, if not all, other tissues, including the intestine (22). Hepatocyte-specific deletion of the IGF-I gene results in normal body growth and normal growth of a number of organs despite a 75% reduction in circulating IGF-I (42). These findings indicate that IGF-I derived from nonhepatic sources has a greater role in mediating postnatal body and organ growth than has previously been suspected. Defining the role of increased local expression of IGF-I in intestinal growth in vivo independent of altered circulating IGF-I requires appropriate cell type-specific experimental manipulations of IGF-I expression.

Available evidence suggests that mesenchymal cells, including α-smooth muscle actin (α-SMA)-positive myofibroblasts and smooth muscle cells, are primary sources of locally expressed IGF-I in the intestine (33, 41). In the normal intestine, IGF-I is expressed in scattered cells in the pericryptal regions and the lamina propria (30, 33, 41). Several situations of increased growth of the mucosal epithelium, including refeeding and adaptive growth following bowel resection, are accompanied by increased local IGF-I mRNA expression in mesenchymal cells in the lamina propria or muscularis propria (23, 41, 43, 47). These findings provide correlative evidence for paracrine actions of mesenchymal cell-derived IGF-I on the intestinal epithelium. Recent studies (36) of the contribution of IGF-I in conditioned medium from intestinal myofibroblasts to growth of cultured epithelial cells provide support for this possibility but do not provide the important in vivo evidence for paracrine actions of mesenchymal cell-derived IGF-I on the intestinal epithelium.

SMP8-IGF-I transgenic (SMP8-IGF-I-TG) mice express rat IGF-I under transcriptional control of the murine α-SMA promoter. SMP8-IGF-I-TG mice have normal levels of circulating IGF-I but have been shown to exhibit high levels of transgene expression in smooth muscle in the small intestine and several other tissues (40). Expression of the transgene would be predicted in subepithelial myofibroblasts in the intestine because these cells are α-SMA positive and are proposed to be major mediators of epithelial-mesenchymal interactions (reviewed in Refs. 31 and 32). We used SMP8-IGF-I-TG mice as a model to test the hypothesis that...
IGF-I overexpressed in intestinal mesenchymal cells in vivo in the absence of altered circulating IGF-I exerts paracrine actions on growth or function of the mucosal epithelium. Increased length and weight of the small intestine of SMP8-IGF-I-TG mice have been previously reported, associated with increased thickness of the muscularis propria (40). The present study evaluates mucosal mass, crypt cell proliferation, and the activity of sucrase, a marker of differentiated epithelial cells, to define whether mesenchymal cell-derived IGF-I overexpression increases mucosal growth and whether observed effects are intestinal region specific. Muscularis growth was assessed in more detail than previously reported (40) in the small intestine as a positive control and in the colon to define whether there are segmental differences in the autocrine actions of locally expressed IGF-I in the intestinal muscularis.

Locally expressed IGF binding proteins (IGFBPs) are known to modulate IGF-I action. Of six known high-affinity IGFBPs, IGFBP-3 and IGFBP-5 have been of interest with respect to a role in regulating intestinal growth. IGFBP-3 mRNA is expressed primarily in the lamina propria of the normal intestine of the rat (41) and mouse (27). Decreases in intestinal IGFBP-3 expression accompany increased mucosal growth following small bowel resection (1). In vitro, IGFBP-3 can inhibit or potentiate IGF-I action, depending on the cell system (reviewed in Refs. 2 and 5). IGFBP-3 can inhibit IGF-I action on human chondrocytes when secreted or added to cell culture medium in conjunction with IGF-I (24). IGFBP-3 can potentiate IGF-I action when associated with the cell surface of some IGF-I-responsive cells (6) and has been implicated as a mediator of reduced proliferation and differentiation of colon cancer epithelial cell lines (15, 26). IGFBP-5 has been localized primarily to the muscularis propria of normal rat and human small intestine (46). IGFBP-5 appears to potentiate the growth-promoting effects of IGF-I in cultured smooth muscle cells derived from the intestine (4) or other organs (8, 28).

Systemically administered IGF-I is known to increase the expression of both IGFBP-3 and -5 in rat small intestine (30) and colon (11), although there are segmental differences in the effects of circulating IGF-I on locally expressed IGFBPs, which correlate with segment-specific growth effects (11, 30). After jejunocolic resection in rats, systemic IGF-I stimulates growth of the ileum but not the jejunum (44). In the same model, systemic IGF-I increased IGFBP-3 mRNA expression in the jejunum but not in the ileum (44). Interestingly, IGF-I-dependent induction of IGFBP-5 in the pericryptal regions of the lamina propria, probably in myofibroblasts, has been linked to more potent growth-promoting effects of systemic IGF-I on the small bowel epithelium (11, 30). IGFBP-5 is also upregulated in hyperplastic regions of the muscularis propria at sites similar to those of elevated IGF-I expression in animal models of enterocolitis (47) or patients with Crohn’s disease (46). Altered IGFBP-3 or -5 expression could therefore impact on the growth-promoting actions of locally expressed IGF-I in SMP8-IGF-I-TG mice. Furthermore, the SMP8-IGF-I-TG mice provide a model to directly assess whether local IGF-I overexpression in intestinal mesenchymal cells leads to changes in expression of IGFBP-3 or -5 that are similar to or distinct from the effects of circulating IGF-I on these molecules. In the present study, we therefore examined IGFBP-3 and -5 mRNA expression by Northern blot hybridization and in situ hybridization histochemistry to assess whether they are regulated by locally expressed IGF-I and whether any alterations in expression correlate with observed growth effects. Our data provide definitive in vivo evidence for autocrine and paracrine actions of mesenchymal cell-derived IGF-I on intestinal smooth muscle and epithelium. These studies also point to some interesting differences in paracrine effects of locally expressed IGF-I in the ileum compared with the jejunum and colon and demonstrate different effects of locally expressed IGF-I on intestinal IGFBP expression compared with effects of circulating IGF-I.

**MATERIALS AND METHODS**

**Animal care and genotyping.** SMP8-IGF-I-TG founder mice on the inbred FVB/N genetic background were derived as previously described (40). A colony was established in the University of North Carolina animal facility by breeding hemizygous male transgenic founder mice with wild-type (WT) FVB/N females. Mice were housed and fed in accordance with guidelines established by the University of North Carolina at Chapel Hill Institutional Animal Use and Care Committee. Mice were genotyped by Southern blot analysis of tail DNA as previously described (40).

**Sample collection.** Sex-matched pairs of SMP8-IGF-I-TG and WT littermates were killed at 50–60 days of age. The entire small intestine and colon were dissected on ice, and the contents were flushed with ice-cold 0.9% saline. Wet weight of the entire small intestine was measured. The jejunum, ileum, and colon were then separately measured and weighed. Length of each segment was determined using a 5-g weight to provide constant tension. Care was taken to assess comparable regions of the bowel from each animal; the small intestine was dissected from the ligament of Treitz to the ileocecal valve. The most proximal third was dissected to provide a sample of jejunum, and the most distal third was dissected to provide ileum. Anatomically comparable 0.5-cm segments of jejunum, ileum, and colon from each animal were subsequently fixed in formalin for histological analyses, frozen in OCT embedding compound (Tissue-Tek, Torrance, CA) for in situ hybridization histochemistry, fixed in Carnoy’s reagent for analysis of crypt cell mitosis, or snap frozen in liquid nitrogen for subsequent analyses of bowal mass or extraction of total RNA. In an additional four pairs of SMP8-IGF-I-TG and WT mice, the entire bowel was dissected and the jejunum, ileum, and colon were snap frozen for RNA isolation to permit analyses of regional expression of the transgene or IGFBP mRNAs.

**DNA, protein, and sucrase assays.** Mucosa was scraped from the samples of jejunum, ileum, and colon collected for evaluation of bowel mass as previously described (27). Briefly, tissue samples were thawed on ice and opened longitudinally. The mucosa was then removed by gently scraping a glass microscope slide over the tissue segment several times, resulting in a mucosal fraction and a submucosal/muscularis fraction. Each fraction was weighed and then
sonicated in 5 mM sodium phosphate (pH 7.2). DNA content in homogenates of mucosa and muscularis was determined by using a fluorescent dye microassay as previously described (27). Protein content in mucosa and muscularis was determined by the method of Lowry et al. (20). Sucrase activity in mucosal homogenates was measured by standard methods (39).

Crypt cell mitoses. Whole crypts were dissected from Carry’s fixed samples of jejunum, ileum, and colon and stained with Schiff’s reagent. Mitotic cells were counted in microdissected crypts by a single blinded observer. Eight to twelve crypts were counted per sample, and samples were counted by a single blinded observer. Eight to twelve

Crypt cell mitoses. Whole crypts were dissected from Carry’s fixed samples of jejunum, ileum, and colon and stained with Schiff’s reagent. Mitotic cells were counted in microdissected crypts by a single blinded observer. Eight to twelve crypts were counted per sample, and samples were counted by a single blinded observer. Eight to twelve crypts were counted per sample, and samples were counted from ten to twelve mice of each genotype.

Histological analyses. Small (0.5-cm) segments of jejunum, ileum, and colon were opened longitudinally, placed on a strip of filter paper to maintain orientation, and fixed in formalin for 4 h. The tissue blocks were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Crypt depth, villus height, and thickness of the muscularis propria were measured by a single blinded observer using a Nikon Microphot-FXA microscope with an Optronics DEI 750 three-chip charge-coupled device camera for digital imaging and morphometry.

In situ hybridization histochemistry. Tissue blocks were oriented in OCT embedding compound and then frozen in isopentane on dry ice. Sections (10 μm) were cut, and slides were stored frozen at –80°C until use. Sections were fixed in 4% paraformaldehyde for 30 min and then washed in sterile PBS. In situ hybridization was performed as previously described by using antisense RNA probes (27). A rat IGF-I RNA probe (27) was used to localize transgene-derived rat IGF-I mRNA and endogenous mouse IGF-I mRNA. Antisense probes for IGFBP-3 and -5 have been described previously (41). Corresponding sense RNA probes were used as negative controls. All probes were labeled with 35S-UTP (Amersham, Arlington Heights, IL) as previously described (27).

IGF-I radioimmunoassay. Blood was collected by cardiac puncture at the time of death. Plasma was separated by centrifugation and extracted with Sep-Pak C18 cartridges (Millipore, Bedford, MA) to remove IGFBPs. IGF-I concentration in extracted samples was determined by radioimmunoassay as previously described (27).

Total RNA extraction and Northern blot analysis. Frozen tissues were homogenized in 4 M guanidine thiocyanate. Homogenates were layered over 5.7 M cesium chloride and centrifuged overnight to isolate total RNA. Total RNA was collected by ethanol precipitation and resuspended in sterile water. Concentration of RNA was determined by absorbance at 260 nm, and the quality of each sample was examined by gel retardation of 18S and 28S ribosomal RNAs on ethidium bromide-stained agarose gels. Northern blot hybridization was performed as previously described (27) using 32P-UTP-labeled antisense cRNA probes specific for IGFBP-3, IGFBP-5, or the SMP8-IGF-I transgene. The transgene-specific probe is a 351-bp fragment containing the rat IGF-I 3’ untranslated region and sequences up to the SV40 early polyadenylation signal (40). To control for RNA loading differences, blots were reprobed with a probe specific for PL7 mRNA, which encodes a constitutively expressed ribosomal protein (34). Blots were scanned on a phosphoimager, and abundance of specific RNAs was quantified using Image Quant software for Macintosh. Abundance of each RNA examined was normalized to the abundance of PL7 RNA for comparisons of RNA abundance.

Statistical analysis. All growth data and mRNA abundance of IGFBP-3 and -5 were analyzed by converting the value obtained in each transgenic mouse to a ratio of the value in its WT littermate. This eliminates any effects of interlitter variation, which is a significant issue in assays of intestinal growth because litter size can affect food intake, and this, in turn, affects bowel mass. Ratios were analyzed by the nonparametric Wilcoxon signed rank test by using SyStat software for PC. Comparisons of absolute data such as morphometric analyses in SMP8-IGF-I-TG and WT mice were compared by using the Mann-Whitney t-test. The abundance of the SMP8-IGF-I transgene, IGFBP-3, and IGFBP-5 in jejunum, ileum, and colon was assessed by one-way ANOVA. Anova value of <0.05 was considered statistically significant.

RESULTS

Body weight and plasma IGF-I do not differ in SMP8-IGF-I-TG and WT mice. Mean body weight of SMP8-IGF-I-TG mice was 27.0 ± 1.2 g, which did not differ significantly from the mean body weight of WT littermates (27.3 ± 1.1 g). Plasma levels of IGF-I in SMP8-IGF-I-TG mice (351 ± 23 ng/ml) did not differ significantly from levels in WT littermates (321 ± 37 ng/ml; P = 0.40).

Levels and sites of expression of the SMP8-IGF-I transgene in small and large intestine. We first examined jejunum, ileum, and colon for levels of SMP8-IGF-I transgene expression by Northern blot hybridization (Fig. 1). The transgene mRNA was detected in each bowel segment, but the ileum and colon exhibited higher levels of expression than the jejunum (Fig. 1).
Since the transgene is expressed at high levels in smooth muscle cells in the muscularis propria, this may reflect a larger relative mass of the muscularis layers compared with the mucosal layers in the ileum and colon (see Fig. 4). In situ hybridization revealed high levels of transgene expression in the muscularis propria of jejunum, ileum, and colon (Fig. 2). Transgene expression was also evident in the pericryptal and villus regions of the jejunal and ileal mucosa and in the muscularis mucosa and lamina propria of the colon (Fig. 2).

Local overexpression of IGF-I increases bowel growth. SMP8-IGF-I-TG mice had increased wet weight and length of small intestine relative to WT littermates (Table 1). Wet weight per unit length was increased in SMP8-IGF-I-TG jejunum, ileum, and colon (Table 1), indicating an increase in thickness of the bowel wall. SMP8-IGF-I-TG mice had increased mass (weight per unit length), DNA content, and protein content of the ileal mucosa but not of the jejunal or colonic mucosa (Fig. 3A). Sucrase activity was assessed in the mucosa of the jejunum and ileum as a measure of differentiated epithelial cell function. Sucrase activity was increased in the ileum but not the jejunum of SMP8-IGF-I-TG mice (Fig. 3A). Crypt cell mitoses were measured in microdissected crypts to assess IGF-I transgene effects on crypt cell proliferation. Consistent with the data on mucosal DNA and protein content, increased crypt cell mitoses were observed in the ileum but not jejunum or colon of SMP8-IGF-I-TG mice (Fig. 3A). Mass, DNA content, and protein content were significantly increased in the submucosa/muscularis layer throughout the small intestine and the colon, as shown in Fig. 3B. Histological evaluation supported these biochemical findings. There was no significant difference in crypt depth or villus height in the jejunum and colon (Table 2), which is consistent with the lack of effect of transgene expression on mucosal growth in these bowel segments. An increase in crypt depth and/or villus height might have been anticipated in the ileum. However, the observed increase in crypt cell proliferation was relatively modest, and the increased DNA and protein content and sucrase activity could reflect greater numbers of villi and crypts per unit length rather than an increase in size of the crypt/villus axis.

Qualitative histological evaluation revealed major increases in the thickness of the muscularis propria in

### Table 1. Wet weight and length of intestine in SMP8-IGF-I-TG and WT mice

<table>
<thead>
<tr>
<th></th>
<th>Small Intestine</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight, g</td>
<td>Length, cm</td>
<td>Ratio, mg/cm</td>
<td>Jejunum, mg/cm</td>
</tr>
<tr>
<td>TG</td>
<td>1.68 ± 0.08*</td>
<td>58.58 ± 1.8*</td>
<td>28.45 ± 0.7*</td>
<td>35.47 ± 1.6</td>
</tr>
<tr>
<td>WT</td>
<td>1.25 ± 0.03</td>
<td>46.47 ± 0.6</td>
<td>26.54 ± 0.5</td>
<td>34.86 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. TG, transgenic; WT, wild-type. *P < 0.05 SMP8-IGF-I-TG vs. WT mice; n = 12 pairs of SMP8-IGF-I-TG and WT littermates.
analyses confirmed this observation (Table 2). The intestinal segments (Fig. 4); quantitative morphometric analyses show. In each case, values in SMP8-IGF-I-TG mice compared with WT mice in all age-matched WT littermates. Values are means ± SE; n = 6 SMP8-IGF-I-TG and WT pairs. *P < 0.05 vs WT.

SMP8-IGF-I-TG mice compared with WT mice in all bowel segments (Fig. 4); quantitative morphometric analyses confirmed this observation (Table 2). The increased muscle thickness combined with parallel increases in DNA and protein content in the muscularis propria of all bowel segments suggests increases in cell number rather than in cell size.

Local IGF-I overexpression modulates IGFBP-5 but not IGFBP-3. Expression of IGFBP-3 and -5 mRNAs was assessed by both Northern blot hybridization and in situ hybridization. In situ hybridization controlled for the possibility that differences in the relative mass of IGFBP-3- and IGFBP-5-expressing cells could affect our ability to accurately detect differences in IGFBP expression in SMP8-IGF-I-TG and WT mice by Northern blot and allowed us to detect altered expression at the cellular level.

By Northern blot hybridization, IGFBP-3 mRNA expression was significantly increased in the colon of WT mice compared with the jejunum in the same WT mice (Fig. 5). An overall aboral gradient was observed such that the ileum and colon had generally higher levels of expression of IGFBP-3 and -5 mRNAs than jejunum in both SMP8-IGF-I-TG and WT mice (Figs. 5 and 6). Within a given bowel segment, there were no major differences in levels of expression of IGFBP-3 mRNA between SMP8-IGF-I-TG vs. WT mice (Fig. 5).

Table 2. Villus height, crypt depth, and muscularis thickness in SMP8-IGF-I-TG and WT mice

<table>
<thead>
<tr>
<th></th>
<th>Jejunum, μm</th>
<th>Ileum, μm</th>
<th>Colon, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>338 ± 11</td>
<td>234 ± 6</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>331 ± 13</td>
<td>241 ± 8</td>
<td></td>
</tr>
<tr>
<td>Crypts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>117 ± 5</td>
<td>111 ± 6</td>
<td>195 ± 5</td>
</tr>
<tr>
<td>WT</td>
<td>104 ± 4</td>
<td>104 ± 5</td>
<td>186 ± 7</td>
</tr>
<tr>
<td>Muscularis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>71 ± 3*</td>
<td>45 ± 1*</td>
<td>165 ± 7*</td>
</tr>
<tr>
<td>WT</td>
<td>37 ± 2</td>
<td>31 ± 2</td>
<td>104 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE for each parameter. For each animal, 8–12 measurements were taken and averaged; n = 10 pairs of SMP8-IGF-I-TG and WT littermates in total. *P < 0.05 for SMP8-IGF-I-TG vs. WT mice.
locally derived IGF-I. Wang et al. (40) have previously shown that transgene expression in SMP8-IGF-I-TG mice is localized to the muscularis propria layer of the small intestine. By in situ hybridization histochemistry, we have localized IGF-I overexpression in SMP8-IGF-I-TG mice to cells in the pericryptal and midvillus regions of the lamina propria as well as the muscularis propria in small intestine and colon. This is an expected but important finding because α-SMA-positive subepithelial myofibroblasts and smooth muscle cells are known to reside in these regions (31, 32). Intestinal subepithelial myofibroblasts are particularly important mediators of growth and differentiation of the intestinal epithelium (31, 32, 37). Expression of the SMP8-IGF-I transgene in subepithelial cells of the lamina propria places IGF-I overexpression in an ideal location for potential paracrine growth or differentiative effects on neighboring epithelial cells. In addition, the pattern of IGF-I overexpression in the intestine of SMP8-IGF-I-TG mice reflects sites of documented IGF-I upregulation in models of adaptive intestinal growth (23, 41, 43, 44). This model was thus ideal for testing whether IGF-I expressed in the muscularis and lamina propria mesenchymal cells can exert local actions on growth of intestinal epithelial or mesenchymal cells.

The observed increase in crypt cell mitoses, sucrase activity, and mucosal mass in the ileum of SMP8-IGF-I-TG mice provides new and definitive evidence that local IGF-I overexpression in intestinal mesenchymal cells has paracrine actions in vivo to stimulate growth of the mucosal epithelium. However, it is intriguing that this paracrine effect was limited to the ileum and was not observed in the jejunum or colon. Several studies have shown that the jejunal and colonic epithelium are capable of responding to IGF-I (11, 23, 30). Thus it is unlikely that the lack of effect of mesenchymal cell-derived IGF-I on the jejunal and colonic epithelia is due to an inability of these tissues to respond to IGF-I. Recent reports in a rat model of jejunoileal resection and subsequent total parenteral nutrition support the concept that endogenous, locally expressed IGF-I may have segment-specific effects on mucosal growth (10, 11). In SMP8-IGF-I-TG mice, transgene expression was lower in the jejunum than in the ileum. However, the colon, which did not exhibit increased mucosal growth, showed higher levels of SMP8-IGF-I transgene expression than the levels observed in the ileum. Thus the specific mucosal growth effect in the ileum of SMP8-IGF-I-TG mice cannot be attributed simply to higher levels of transgene expression.
In the jejunoileal resection model, increased local IGFBP-5 expression within the lamina propria as well as increased local IGF-I expression occurred in those bowel segments that exhibited enhanced mucosal growth (11). IGFBP-5 is known to potentiate IGF-I action in a number of settings (8, 28), and its induction within the lamina propria correlates with growth of intestinal mucosa in response to elevated circulating IGF-I in several models (10, 11, 30). This has led to the concept that coinduction of mucosal IGFBP-5 may be an important determinant of mucosal growth responses to circulating or locally expressed IGF-I (10, 11, 30). Our findings that IGFBP-5 is specifically increased in the villus and pericryptal regions of the ileal lamina propria of SMP8-IGF-I-TG mice support a hypothesis that interaction between transgene-derived IGF-I and locally induced IGFBP-5 may contribute to the specific paracrine actions of IGF-I on the mucosal epithelium in the ileum.

In vitro studies have demonstrated that IGF-I induces IGFBP-5 in enteric smooth muscle cells (45). In vivo studies have reported coinduction of IGF-I and IGFBP-5 in the muscularis propria in models of intestinal inflammation (47). Despite high levels of transgene expression in muscularis layers of SMP8-IGF-I-TG mice, we observed only modest induction of IGFBP-5 in scattered cells within the muscularis propria and no evidence that locally expressed IGF-I induced widespread increases in IGFBP-5 expression within the bulk of the enteric smooth muscle. We...

---

**Fig. 5. IGF binding protein (IGFBP)-3 mRNA expression in SMP8-IGF-I-TG and WT intestine.**

**A:** representative Northern blot of IGFBP-3 and control PL7. J, jejunum; I, ileum; C, colon. **B:** IGFBP-3 mRNA abundance in SMP8-IGF-I-TG and WT intestine. Values are means ± SE represented as absolute densitometry units of IGFBP-3 mRNA expression normalized to PL7 as a loading control. *P < 0.05 for WT colon vs. WT jejunum. **C:** IGFBP-3 mRNA abundance in SMP8-IGF-I-TG mice expressed as a ratio of values in paired WT littermates. Values are means ± SE; n = 4 littermate pairs.

In the jejunoileal resection model, increased local IGFBP-5 expression within the lamina propria as well as increased local IGF-I expression occurred in those bowel segments that exhibited enhanced mucosal growth (11). IGFBP-5 is known to potentiate IGF-I action in a number of settings (8, 28), and its induction within the lamina propria correlates with growth of intestinal mucosa in response to elevated circulating IGF-I in several models (10, 11, 30). This has led to the concept that coinduction of mucosal IGFBP-5 may be an important determinant of mucosal growth responses to circulating or locally expressed IGF-I (10, 11, 30). Our findings that IGFBP-5 is specifically increased in the villus and pericryptal regions of the ileal lamina propria of SMP8-IGF-I-TG mice support a hypothesis that interaction between transgene-derived IGF-I and locally induced IGFBP-5 may contribute to the specific paracrine actions of IGF-I on the mucosal epithelium in the ileum.

In vitro studies have demonstrated that IGF-I induces IGFBP-5 in enteric smooth muscle cells (45). In vivo studies have reported coinduction of IGF-I and IGFBP-5 in the muscularis propria in models of intestinal inflammation (47). Despite high levels of transgene expression in muscularis layers of SMP8-IGF-I-TG mice, we observed only modest induction of IGFBP-5 in scattered cells within the muscularis propria and no evidence that locally expressed IGF-I induced widespread increases in IGFBP-5 expression within the bulk of the enteric smooth muscle.
cannot exclude the possibility that the transgene induces IGFBP-5 expression at earlier times in development and that feedback responses, such as IGF-I receptor downregulation, prevent maintained increases in IGFBP-5 within the muscularis. Though we cannot definitively identify the IGFBP-5-expressing cells that lie between the circular and longitudinal muscle layers, their location suggests that these cells are likely enteric ganglia, neurons, or interstitial cells of Cajal.

Interestingly, the expression of IGFBP-3, an IGFBP that is known to be induced in the intestine by elevated circulating IGF-I (30), was not increased in SMP8-IGF-I-TG mice relative to WT mice. In vitro studies and in vivo evidence suggest that IGFBP-3 has predominantly inhibitory effects on crypt cell proliferation but may play a role in enterocyte differentiation (15, 26). Sites of expression of IGFBP-3 mRNA in the ileum and colon have not, to our knowledge, been reported previously. It was striking that the highest level of IGFBP-3 expression in the ileum occurred in the midvillus level of the lamina propria, whereas in the colon IGFBP-3 was strongly expressed in the lamina propria adjacent to the midcrypt and surface epithelial cells. These regions correspond to sites of terminal differentiation of enterocytes (3), providing indirect evidence that, in vivo, locally expressed IGFBP-3 may play a role in enterocyte differentiation. It is also worth noting that, whereas Northern blot hybridization did not reveal a significant increase in IGFBP-3 in any bowel segment of SMP8-IGF-I-TG mice, in situ hybridization

---

**Fig. 7.** IGFBP-3 mRNA expression in SMP8-IGF-I-TG and WT mice by in situ hybridization. DF and BF micrographs show IGFBP-3 mRNA expression in the lamina propria of SMP8-IGF-I-TG and WT jejunum (Jej), ileum (Ile), and colon (Col). Bars = 100 μm.

---

**Fig. 8.** IGFBP-5 expression in the bowel of SMP8-IGF-I-TG and WT mice. A: DF and BF images demonstrate IGFBP-5 mRNA expression in the jejunum (Jej), ileum (Ile), and colon (Col) of SMP8-IGF-I-TG and WT mice. Bars = 100 μm. B: BF photomicrographs show higher-power magnification of ileum in 3 SMP8-IGF-I-TG and 1 WT mouse. Bars = 50 μm. Arrows in A and B indicate IGFBP-5 mRNA expression in the pericryptal area of the lamina propria in the ileum of SMP8-IGF-I-TG mice.
provided evidence for increased IGFBP-3 expression in the villus lamina propria of the ileum but not in other bowel segments. Because the ileum also showed increased expression of sucrase, a marker of enterocyte differentiation, this supports the possibility that IGFBP-3 may contribute to the increase in sucrase activity observed in the ileal mucosa of SMP8-IGF-I-TG mice. Although in situ hybridization is not strictly quantitative, it is important to note that it can be useful in situations such as the SMP8-IGF-I-TG mice, where increases in the relative proportion of the muscularis to the mucosa could attenuate any increase in expression of specific mRNAs in the mucosa when assessed by Northern blot on RNA from whole tissue.

Although these studies provide definitive evidence that mesenchymal cell-derived IGF-I can increase growth of ileal mucosal epithelium, these effects were somewhat less potent and more regionally restricted than those observed in other models in which circulating IGF-I is elevated by systemic administration (18, 30, 38) or due to transgene-mediated overexpression of growth hormone (39) or IGF-I (27). Circulating IGF-I was not altered in SMP8-IGF-I-TG mice compared with WT mice and in fact was similar to levels previously observed in WT mice in these other mouse models (27, 39). Together with findings that SMP8-IGF-I-TG mice had dramatic overgrowth of the muscularis layers in all bowel segments, our findings suggest that locally expressed, mesenchymal cell-derived IGF-I exerts less potent and less widespread effects on growth of the mucosal epithelium than circulating IGF-I. In addition, these data support a concept that locally expressed IGF-I derived from intestinal mesenchymal cells may predominantly or most potently regulate growth of the muscularis layers.

The potent mucosal effect of circulating IGF-I seen in other studies (18, 23, 27, 30, 38, 39) may relate to the fact that the mucosa receives a majority of the blood flow in the intestine (7, 19), facilitating potent endocrine actions of blood-borne IGF-I on the mucosa. Indeed, the muscularis and mucosal circulations are arranged in parallel, each branching from common small submucosal arteries and draining into common small veins in the submucosa (14). Thus IGF-I derived from the muscularis propria may not be able to access the mucosal epithelium. However, IGF-I derived from the muscularis mucosa, and particularly that from subepithelial myofibroblasts, would have ready access to vessels supplying the crypt and villus epithelium. Therefore, it is unlikely that the structural arrangement of the intestinal blood supply can entirely explain the modest overgrowth of the intestinal epithelium in SMP8-IGF-I-TG mice.

An alternate possibility for the modest and bowel segment-specific mucosal effect of IGF-I in SMP8-IGF-I-TG mice is that the strong elevation of IGF-I mRNA in the muscularis mucosa and subepithelial myofibroblasts of SMP8-IGF-I-TG mice does not lead to elevations in the encoded IGF-I protein. This could occur if the transgene-derived mRNA is not translated into protein or if the translated protein is degraded. We think these possibilities are unlikely because the potent effects of IGF-I on the muscularis in this model, both in the intestine and in other smooth muscle-rich tissues (40), indicate that the transgene product is translated, biologically active, and available, at least in the muscularis propria. The SMP8-IGF-I transgene encodes an identical precursor to the endogenous IGF-I mRNA (40). We do acknowledge that we have not verified that the encoded precursor or mature IGF-I is increased in the mucosa of SMP8-IGF-I-TG mice in parallel with increased IGF-I mRNA. This is because immunohistochemical quantification and localization of the IGF-I precursor or mature IGF-I in intestinal tissue is technically difficult, possibly because the peptide is rapidly cleared from tissues or is bound to IGFBPs and is not detectable by antibodies (reviewed in Ref. 21).

As well as providing novel evidence about mucosal growth, our studies extend the prior findings of Wang et al. (40) to demonstrate that mesenchymal cell-derived IGF-I can act as a potent autocrine regulator of mass of the muscularis propria throughout the intestine. Increased thickness and length of the muscularis propria and smooth muscle cell hyperplasia have been reported following small bowel resection (25), but adaptive responses in the muscularis layers have been much less studied than mucosal adaptation. Growth of the muscularis can be altered in disease. Decreases in thickness of the muscularis propria are associated with intestinal perforation in infants treated with dexamethasone (13). Interestingly, dexamethasone administration to newborn mice resulted in decreased proliferation of enteric smooth muscle cells, which correlated with decreased IGF-I immunoreactivity in the muscularis propria (12). Our characterization of trophic actions of mesenchymal cell-derived IGF-I on the muscle in the normal intestine provides an important basis for the use of the SMP8-IGF-I-TG mouse model to address the significance of autocrine actions of IGF-I on enteric muscle development and disease. It is clear that overexpression of IGF-I in SMP8-IGF-I-TG mice results in increased length of the intestine as well as increased thickness of the muscularis propria. This raises an intriguing question: what is the relative importance of the muscularis propria vs. the mucosa in determining length of the intestine? Increased length of the small intestine and colon occurs in SMP8-IGF-I-TG mice despite no increases in jejunal or colonic mucosal growth. This indicates that intestinal lengthening accompanies muscularis overgrowth even when there are no associated increases in growth of the mucosa.

It is possible that the intestinal lengthening observed in SMP8-IGF-I-TG mice is a result of chronic exposure to IGF-I and might not be seen in situations of acute increases in IGF-I expression in intestinal mesenchymal cells. In this regard, it would be interesting to develop a model with a cis-inducible element added to the α-SMA promoter to allow assessment of acute, inducible vs. chronic alterations in IGF-I expression in intestinal mesenchymal cells. Validation that the SMP8 promoter targets downstream genes to the
appropriate cell types is an important prerequisite to development of an inducible model. In conclusion, these studies provide new and conclusive evidence for paracrine effects of mesenchymal cell-derived IGF-I on the intestinal epithelium in vivo. This paracrine effect occurs preferentially in the ileum, whereas potent autocrine effects on muscularis growth occur throughout the intestine. In addition, we conclude that locally expressed, mesenchymal cell-derived IGF-I has distinct actions on intestinal IGFBP expression compared with circulating IGF-I.

We thank K. McNaughton for assistance with histology and E. Bruton for assistance with radioimmunoassays.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grant DK-40247 to P. K. Lund. K. L. Williams was supported by a predoctoral fellowship from the SPIRE program at the University of North Carolina, funded by the MORE division of the National Institutes of General Medical Sciences (GM-00678). These studies were facilitated by the molecular histopathology and animal cores of the University of North Carolina Center for Gastrointestinal Biology and Disease (NIDDK P30-DK-34987).

REFERENCES


