IGF-I augments resection-induced mucosal hyperplasia by altering enterocyte kinetics

Elizabeth M. Dahly,1 Ziwen Guo,2 and Denise M. Ney1

1Department of Nutritional Sciences, University of Wisconsin-Madison, Madison 53706; and 2Department of Pathology and Laboratory Medicine, University of Wisconsin Hospitals and Clinics, Madison, Wisconsin 53792

Submitted 10 January 2003; accepted in final form 19 May 2003

The role that alterations in enterocyte kinetics play in regulating intestinal adaptive growth after resection is incompletely understood (13). For instance, a few reports showed increases in both enterocyte proliferation and apoptosis in association with intestinal adaptive growth after bowel resection (8, 9). Thus it has been suggested that proliferation and apoptosis are coordinately regulated (8). However, other observations of disparate rates of proliferation and apoptosis dispute the notion that proliferation and apoptosis are regulated by the same stimuli (2, 33, 35). Thus further knowledge of both enterocyte proliferation and apoptosis is a vital step in elucidating the factors responsible for the adaptive response to resection and the ability of IGF-I to induce resection-induced adaptation.

In this study, we determine structural adaptation and concomitant changes in enterocyte proliferation, apoptosis, and migration of the jejunum in growing, parenterally fed rats after mid small bowel resection or small bowel transection, and treatment with IGF-I or vehicle. Our objective was to determine if exogenous IGF-I augments the adaptive response to mid small bowel resection.

METHODS

Animals and experimental design. The animal facilities and protocols were approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee. Male, Sprague-Dawley rats (Harlan, Madison, WI) initially weighing 200–225 g were housed in individual stainless steel, wire bottom cages with free access to water in a room maintained at 22°C on a 12:12-h light-dark cycle.

The experimental design included four parenterally fed treatment groups run concurrently in a 2 × 2 factorial treatment design as follows: gut resection (R; n = 13), resection + IGF-I (R + I; n = 13), gut transection (T; n = 13), and transection + IGF-I (T + I; n = 12). The experiment began with 14 animals in each group immediately after surgery; one animal died during surgery. During the administration of exclusive total parenteral nutrition (TPN), one to two animals were removed from each group due to loss of catheter patency. Thus 7 days after surgery, 85–93% of the animals inhibit (e.g., IGFBP-3) intestinal adaptation after bowel resection (7, 22, 45).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Animals were injected at the same time of day (0900) and chemically killed (2) concurrent with continuous infusion of TPN for 6 days (rhIGF-I supplied through the courtesy of Ross Labs, Columbus, OH) (2). On the day of surgery (day 0), rats were anesthetized, given ampicillin (200 mg/kg sc) to prevent infection (2, 4), and prepped for surgery (2). Resected animals underwent a 70% mid jejunoileal resection as previously described in detail (2). Briefly, resected animals had bowel from 15 cm distal to the ligament of Treitz until 15 cm proximal to the cecum removed and continuity restored with an end-to-end jejunoileal anastomosis. Transected animals received a transection cut in the ileum (2) and suturing to reestablish continuity. The TPN catheter was placed in the superior vena cava via the external jugular vein (16) after closing the midline abdominal incision.

Immediately after surgery (day 0), infusion of a nutritionally complete, semipurified powdered diet ad libitum (3). For 24–48 h after surgery, animals received oxymorphone HCl for pain management and ampicillin to prevent infection (200 mg/kg iv every 12 h) (2, 4). Body weights were recorded daily. Changes in body weight due to resection or IGF-I treatments were calculated as the difference between body weights on the day animals were killed (day 7) and the preoperative body weights on the day of surgery (day 0). Resected rats lost ~5 g of intestinal tissue due to surgery. Urine was collected daily into vials containing a final concentration 0.01% boric acid and then stored at 4°C before determination of nitrogen concentration as previously outlined (43). After 7 days of TPN, rats were anesthetized, killed by exsanguination, and the jejunum was obtained, processed, and analyzed for histology and mucosal mass, protein, and DNA as previously described (2).

IGF-I administration and assessment. IGF-I-treated animals received 3.2 mg recombinant human IGF-I (rhIGF-I)-kg body wt⁻¹-day⁻¹ concurrent with continuous infusion of TPN for 6 days (rhIGF-I supplied through the courtesy of Genentech, South San Francisco, CA). Confirmation of IGF-I administration was assessed by analyzing total serum IGF-I concentrations by radioimmunoassay (19). Serum levels of IGFBP-3 were estimated by modified Western ligand blotting (7, 14).

Jejunal kinetics: enterocyte proliferation and migration. Bromo-deoxyuridine (BrdU) incorporation into proliferating (S phase) cells in jejunal tissue sections was used to determine enterocyte proliferation and migration rates (12). Animals received an intravenous bolus of BrdU (Sigma Chemical, St. Louis, MO) at a dose of 0.2 mg/g body wt. Animals were injected at the same time of day (0900) and killed 1 or 25 h later (n = 4–7 animals per time point per group). The 24-h difference in time points and the same injection time (0900) were selected to account for circadian patterns (26). The paraffin-embedded tissue was cut into 5-μm sections, processed for immunoperoxidase staining of BrdU-labeled cells (5, 15), and counterstained with Mayer’s hematoxylin (Sigma Chemical).

One investigator, blinded to the treatment groups, assessed the presence of seven well-oriented crypt-villus axes per animal under a light microscope. For each animal, the total number of cells and the number and position of BrdU-labeled cells were recorded. The scoring was initiated with the cell at the base of the crypt that was designated cell position 1 and continued to the crypt-villus junction in the 1-h animals and until the last labeled cell in the villus of the 25-h animals. From these data, three parameters were assessed. First, enterocyte proliferation was assessed by counting the number of labeled cells in a column and the total number of cells per column in the crypts of animals injected 1 h before death. The proliferation index was determined by expressing the number of labeled crypt cells to total crypt cells as a percentage. Second, patterns of labeling were identified in animals injected 1 h before death from labeling index distribution curves based on group means that plotted cell position vs. percent labeling at each position similar to Steeb et al. (31). Third, migration rate was assessed by comparing both median-labeled cell (MLC) or foremost-labeled cell (FLC) positions on each crypt-villus axis at 1 or 25 h. FLC, defined as the 50th percentile value at which labeling occurred, can be thought of as the “centrally” labeled cell and was used as a descriptive means of location in the asymmetric distributions. FLC, defined as the highest labeled cell from the base of the crypt, can be thought of as the “leader” cell. Migration rates were calculated as the difference between means of MLC or FLC positions in animals injected at 1 or 25 h and were considered significant if there was a lack of significant interaction in the two-way ANOVA. The sample size (n = 6 animals per group) for each treatment group was determined by the protected least-significant differences technique in SAS was used to determine significant differences between group means at P ≤ 0.05. Outcomes were predicted in an additive manner if there was a lack of significant interaction in the two-way ANOVA. One-way ANOVA followed by the protected least-significant differences technique in SAS was used to determine significant differences between group means at P ≤ 0.05.

Changes in body weight were assessed by repeated-measures ANOVA, incorporating the treatment factors, resection and IGF-I, time effects, and interactions of these factors. Wilks’ Lambda was used to determine significance at P ≤ 0.05. Migration rates were assessed by contrast analysis that compared means of the four TPN groups while accounting for group variation. A three-way ANOVA with main effects for time, resection, and IGF-I was used to isolate the effects of resection and IGF-I on migration rate. Resection and IGF-I were considered to have an effect on migration rate if the interactions of resection × time and IGF-I × time, respectively, were significant (P ≤ 0.05). Data are presented as means ± SE. Statistics were performed on transformed data for parameters of apoptosis because residual plots indicated that there was unequal variance between groups. The sample...
size is 12–13 rats per group for jejunal structure and composition data and 4–7 rats per group for enterocyte proliferation, apoptosis, and migration data, unless otherwise stated.

RESULTS

Body weight, nitrogen balance, serum IGF-I, and serum IGFBPs. Daily body weights are shown in Fig. 1. There were no significant differences in body weights among the groups before surgery (day −4 to day −1), on the day of surgery (day 0), or on the day after surgery (day 1). After 24 h of IGF-I infusion, IGF-I-treated animals began to grow significantly more than animals not receiving IGF-I (day 2), and this pattern continued through day 7 (main effects, 2-way ANOVA, IGF-I, \( P < 0.0001 \)). All animals gained weight, suggesting the systemic nutrition was sufficient to promote whole body anabolism. The amount of TPN solution infused was not significantly different between groups.

Body weight gains in resection and transection animals not given IGF-I were not significantly different after 7 days of TPN (R, 10 ± 2; T, 12 ± 2 g body wt gain/7 days). IGF-I treatment for 6 days nearly tripled body weight gain in both resected and transected rats compared with non-IGF-I-treated animals (main effects, 2-way ANOVA, IGF-I, \( P < 0.0001 \); R + I, 29 ± 2; T + I, 33 ± 2 g body wt gain/7 days). Repeated-measures analysis of body weight confirmed that IGF-I administration significantly increased body weight (main effects, IGF-I \( \times \) time, \( P < 0.0001 \)) and indicated that resection had no overall effect on body weight.

Total nitrogen retention data over 7 days of TPN are summarized in Fig. 1, inset. Nitrogen intake was not significantly different among groups, but nitrogen excretion was significantly greater in animals not given IGF-I (data not shown; main effects, 2-way ANOVA, IGF-I, \( P < 0.0018 \)). Thus treatment with IGF-I resulted in significantly greater nitrogen retention in both resected and transected rats. There were no significant differences in nitrogen retention between resected and transected animals maintained with TPN without IGF-I. Cumulative nitrogen retention was positively correlated with changes in body weight (\( r = 0.76, P < 0.0003, n = 4–5 \) animals per group), suggesting that increases in body weight reflected, in part, increases in lean body mass.

Coinfusion of IGF-I with the TPN solution was confirmed by serum IGF-I concentrations. Total serum IGF-I concentrations were over twofold greater in animals treated with IGF-I than in animals maintained with TPN alone (R, 362 ± 11; T, 337 ± 23; R + I, 882 ± 44; T + I, 857 ± 43 μg/l). Both IGF-I and resection increased serum IGFBP-3 at 38–43 kDa, IGFBP-1, -2, -4, and -5 at 30–34 kDa, and total IGFBPs (Fig. 2; main effects, resection or IGF-I, 2-way ANOVA, \( P < 0.0142 \)) (7). Neither resection nor IGF-I treatments significantly affected IGFBP-6 at 24 kDa.

Jejunal composition and histology. Resection and IGF-I had additive effects to significantly increase jejunal mucosal dry mass and concentrations of protein and DNA as evidenced by significant main effects for resection and IGF-I without significant interaction (Fig. 3; main effects, resection or IGF-I, 2-way ANOVA, \( P < 0.0001 \)). Parallel significant increases in the concentrations of mucosal protein and DNA with resection or IGF-I administration indicate that intestinal resection or IGF-I treatment induced mucosal hyperplasia. Resection or IGF-I treatment significantly increased villus height; however, IGF-I treatment did not increase villus height further in resection animals compared with animals receiving resection alone (Fig. 4; main effects, resection \( \times \) IGF-I, 2-way ANOVA, \( P = 0.0005 \)). IGF-I may have heightened villus height in an additive manner as it did mucosal mass and cellularity.

Fig. 1. Daily body weights of 4 groups of rats maintained exclusively with total parenteral nutrition (TPN) for 7 days after ileal transection (T), transection + recombinant human insulin-like growth factor-I (rhIGF-I; T + I), 70% midjejunoeileal resection (R), or resection + rhIGF-I (R + I). Body weights were not significantly different among groups during the presurgery phase (day −4 to day −1), on the day of surgery (day 0), or on the day after surgery (day 1). Values are means ± SE; \( n = 12–13 \) animals/group; \( \ast P < 0.0001 \) vs. R and T for days 2–7. Inset: nitrogen (N) retention over 7 days. Values are means ± SE; \( n = 4–5 \) animals/group. Means with different superscripts are significantly different.

AJP-Regul Integr Comp Physiol • VOL 285 • OCTOBER 2003 • www.ajpregu.org
had the adaptive response been assessed after more cycles of enterocyte turnover took place.

**Enterocyte proliferation in the jejunum.** Resection significantly increased both the number of cells in a crypt column and the number of BrdU-labeled cells in the crypt column (Table 1; main effects, 2-way ANOVA, resection, $P < 0.0192$). Animals receiving resection alone also had significantly more mitotic cells per column than transected animals not given IGF-I (Table 1). The proliferation index, or the percentage of cells in the crypt incorporating BrdU, as well as the mitotic index, was not significantly different between groups. Constant labeling indexes are often seen after resection (40). Based on a greater absolute number of crypt cells that incorporated BrdU and a greater number of mitotic cells per crypt column, this suggests resection increased proliferation by contributing to a greater number of cell births. Likewise, IGF-I increased proliferation by significantly increasing the number of cells in a crypt column, the number of BrdU-labeled cells in the crypt column, and the number of mitotic cells per crypt column (Table 1; main effects, 2-way ANOVA, IGF-I, $P < 0.0117$).

To further assess the mitogenic properties of resection and/or IGF-I on the jejunal epithelium, we constructed BrdU-labeling distribution profiles (31) for each group based on data from animals injected with BrdU 1 h before death (Fig. 5). By dividing the crypts into thirds based on cell position (cp) (lower, cp 1–10; mid, cp 11–20; upper, cp 21–30), we determined three trends from these curves. First, resection animals receiving no growth factor had the greatest average labeling indexes in the lower-third of the crypt (45%) compared with the other TPN groups (27–32%). Second, IGF-I treatment increased the average labeling indexes in midcrypt positions compared with groups receiving no growth factor (~50 vs. 35%). Third, IGF-I administration increased the average labeling indexes in the upper-third of the crypt compared with groups receiving no growth factor (15 vs. 2%).

In summary, resection or IGF-I treatment significantly increased jejunal proliferation by increasing crypt cell populations and establishing a greater number of cell births. Increased proliferation with resection...
was due to increasing the proportion of proliferative cells in lower positions of the proliferative zone whereas IGF-I treatment increased proliferation at higher positions of the proliferative zone as shown by the increased average labeling indexes in the mid- and upper crypt. Thus IGF-I increased the overall area of the proliferative compartment in the crypt.

Enterocyte apoptosis in the jejunum. In the crypt, resection significantly decreased the mean apoptotic index over 50% in animals not given IGF-I based on 1-way ANOVA due to significant interaction in the main effects (Table 2). Like resection, IGF-I treatment also decreased the mean apoptotic index in the crypt compared with transected animals given TPN alone (Table 2). However, IGF-I treatment did not further decrease the crypt apoptotic index in resected animals. Unlike the effects of resection or IGF-I to decrease apoptosis in the crypt, neither resection nor IGF-I treatment significantly affected the mean apoptotic index in the villus.

Enterocyte migration rate in the jejunum. Migration rate data are shown in Table 3. There were no significant differences in MLC, i.e., the central cell, or FLC, i.e., the leader cell, positions between resected and transected animals injected 25 h before death. However, the FLC position was significantly higher (15 cell positions) in resected animals injected 25 h before death compared with transected animals maintained with TPN alone. Thus resection significantly increased the migration rate of the FLC. Resection also tended to increase the migration rate of the MLC (P = 0.0634).

IGF-I treatment, unlike resection, resulted in a significantly greater MLC and FLC positions in animals injected 1 h before death, consistent with an increase in the proliferative zone of the crypt. In animals injected 25 h before death, the FLC was also greater in IGF-I-treated animals compared with transected animals given TPN alone; however, IGF-I did not increase migration rate further in resected animals.

Table 1. Enterocyte proliferation in the jejunal crypt

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cells per Crypt Column, N</th>
<th>BrdU-Labeled Cells per Crypt Column, n</th>
<th>Proliferation Index, %</th>
<th>Mitotic Cells per Column, n</th>
<th>Mitotic Index, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>30 ± 1a</td>
<td>7 ± 1b</td>
<td>23 ± 1</td>
<td>0.65 ± 0.05a</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>R</td>
<td>33 ± 1b</td>
<td>9 ± 1a</td>
<td>27 ± 1</td>
<td>0.88 ± 0.09a</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>T + I</td>
<td>32 ± 1a</td>
<td>9 ± 1a</td>
<td>27 ± 1</td>
<td>0.96 ± 0.06a</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>R + I</td>
<td>35 ± 1a</td>
<td>10 ± 1a</td>
<td>28 ± 2</td>
<td>0.93 ± 0.06a</td>
<td>2.8 ± 0.2</td>
</tr>
</tbody>
</table>

Two-way ANOVA

<table>
<thead>
<tr>
<th>Main effect</th>
<th>Resection</th>
<th>IGF-I</th>
<th>Resection × IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0079</td>
<td>0.0117</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0.0192</td>
<td>0.0114</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5–7 animals/group. Means in the same column with different superscripts are significantly different based on 1-way ANOVA and protected least significant differences (PLSD). Mitotic index expressed as the percentage of mitotic cells to total cells in the crypt. BrdU, bromo-2′-deoxyuridine; IGF-I, insulin-like growth factor-I; R, resected; R + I, R + IGF-I; T, transected; T + I, T + IGF-I; NS, nonsignificant.
DISCUSSION

Multiple factors, including luminal nutrients, pancreaticobiliary secretions and humoral factors, have been implicated in controlling the endogenous intestinal adaptive response after bowel resection. Furthermore, the ability of exogenous anabolic agents, such as IGF-I, to enhance intestinal adaptation after resection establishes the importance of growth factors in the adaptive process (5, 17, 38). In this report, we extend knowledge regarding the independent effects of resection and/or IGF-I as it allows examination of how eliminating the growth-promoting signals stimulated by enteral nutrients, while maintaining adequate nutritional status of the animals, influences intestinal composition and structure, as well as enterocyte kinetics. Thus our design permits us to isolate the effects on intestinal growth due solely to resection and/or IGF-I. Here, we show that the ability of IGF-I to augment resection-induced increases in jejunal mucosal mass and concentrations of protein and DNA is consistent with additive increases in enterocyte proliferation due to resection and IGF-I. In contrast, IGF-I does not modify enterocyte apoptosis or enterocyte migration beyond the responses induced by resection alone.

The effect of IGF-I to additively increase jejunal mucosal growth and cellularity in parenterally fed, resected rats is similar to IGF-I’s ability to induce small intestinal adaptation after mid small bowel resection in orally (17, 38) or gastrostomy (44, 45)-fed animals. Yet, our parenterally fed mid small bowel resected animals had a net body weight gain that was nearly tripled with IGF-I treatment, whereas animals fed orally or intragastrically in the presence (45) or in the absence (17, 44, 45) of IGF-I had a net body weight loss over 7 days after mid small bowel resection. The more anabolic condition of our TPN rats was reflected not only in an overall positive nitrogen balance but also in similar concentrations of total circulating IGF-I in resection compared with transection animals. This is in contrast to the weight loss and ~16% decrease in plasma IGF-I after resection and gastrostomy feeding (45).

Resection and IGF-I significantly increased serum IGFBPs in an additive manner. This suggests that a potential decrease in the pool of circulating free IGF-I that is available to IGF-I receptors does not hinder the additive response in resection-induced intestinal growth in IGF-I-treated animals. Moreover, the resection-induced adaptation was not associated with alterations in abundance of jejunal IGF-I mRNA based on preliminary studies with this model (1).

The ability of both resection and IGF-I to induce mucosal hyperplasia was associated with increased enterocyte proliferation, based on a greater absolute number of BrdU-labeled cells, a greater number of mitotic cells, and an increased total cell number in the crypt. The resection-induced increase is consistent

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Apoptotic cells per column, n</th>
<th>Apoptotic index, %</th>
<th>Apoptotic cells per column, n</th>
<th>Apoptotic index, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>0.22 ± 0.06b</td>
<td>0.85 ± 0.24a</td>
<td>0.29 ± 0.09</td>
<td>0.40 ± 0.14</td>
</tr>
<tr>
<td>R</td>
<td>0.08 ± 0.02b</td>
<td>0.28 ± 0.08b</td>
<td>0.15 ± 0.07</td>
<td>0.20 ± 0.10</td>
</tr>
<tr>
<td>T + I</td>
<td>0.09 ± 0.03b</td>
<td>0.24 ± 0.09b</td>
<td>0.24 ± 0.07</td>
<td>0.28 ± 0.08</td>
</tr>
<tr>
<td>R + I</td>
<td>0.12 ± 0.04ab</td>
<td>0.36 ± 0.12b</td>
<td>0.26 ± 0.08</td>
<td>0.31 ± 0.10</td>
</tr>
</tbody>
</table>

Two-way ANOVA

Main effect
- Resection: NS
- IGF-I: NS
- Resection × IGF-I: 0.0286 NS 0.0212 NS

Values are means ± SE; n = 5–6 animals/group. Means in the same column with different superscripts are significantly different based on 1-way ANOVA and PLSD. Apoptotic index is the percentage of total cells in the crypt or villus that are apoptotic.
with increases in epithelial proliferation that have been described after mid small bowel resection and oral feeding (8, 9, 30, 36). This heightened proliferative activity may have been accomplished by a decrease in the cell cycle time in enterocytes in lower cell positions (31). The ability of IGF-I to augment intestinal adaptation is consistent with the ability of acute exogenous IGF-I to stimulate proliferation in a distal small bowel resection and cecectomy model (5) and in intact rats fed orally (25, 31, 32) or parenterally (3) and with the resection and cecectomy model (5) and in intact rats fed IGF-I to stimulate proliferation in a distal small bowel (31). The ability of IGF-I to augment intestinal adaptation is consistent with the ability of chronic endogenous IGF-I excess to increase mitogenesis threefold in IGF-I transgenic mice (39). Nevertheless, others have suggested that the taller villi and deeper crypts that characterize the adaptive response to resection are maintained by concomitant increases in enterocyte proliferation and apoptosis. In particular, resection significantly increased intestinal growth in conjunction with increases in both proliferation and apoptosis in orally fed rabbits (36) and mice (8, 9). The discrepancies that exist between incidences of apoptosis after mid small bowel resection and TPN (2), oral feeding (2, 8, 9, 36), and/or administration of growth factors must be interpreted cautiously as differences in methods of detecting apoptosis exist (2).

Resection but not IGF-I significantly increased enterocyte migration rate. The resection-induced increase in migration was similar to that reported in orally fed rats (40). The IGF-I-induced significant increase in proliferation but no significant increase in migration after resection is consistent with the ~40% increase in proliferation but no increase in migration in formula-fed piglets treated with IGF-I compared with vehicle for 14 days (10). Yet, IGF-I administration stimulated increases in migration rate in gastrectomy-fed infant rats (23) and in a distal small bowel resection and cecectomy model (5) and tended to increase migration in our previous report of parenterally fed rats with intact gut (21). We cannot reconcile our contrasting observations of IGF-I-induced effects on

### Table 3. Enterocyte migration rate in the jejunum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median Labeled Cell Position at 1 h</th>
<th>Migration rate</th>
<th>Foremost Labeled Cell Position at 1 h</th>
<th>Migration rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>10 ± 1b</td>
<td>17 ± 2b</td>
<td>17 ± 1b</td>
<td>27 ± 2a</td>
</tr>
<tr>
<td>R</td>
<td>10 ± 1b</td>
<td>24 ± 2b</td>
<td>17 ± 1b</td>
<td>59 ± 5a</td>
</tr>
<tr>
<td>T + I</td>
<td>14 ± 1*</td>
<td>20 ± 2ab</td>
<td>22 ± 1*</td>
<td>54 ± 1a</td>
</tr>
<tr>
<td>R + I</td>
<td>14 ± 1a</td>
<td>20 ± 2ab</td>
<td>21 ± 1a</td>
<td>59 ± 2a</td>
</tr>
</tbody>
</table>

Two-way ANOVA

<table>
<thead>
<tr>
<th>Main effect</th>
<th>NS</th>
<th>NS</th>
<th>NS*</th>
<th>0.0005</th>
<th>0.0001</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resection</td>
<td>0.0005</td>
<td>NS</td>
<td>NS</td>
<td>0.0046</td>
<td>0.0009</td>
<td>NS</td>
</tr>
<tr>
<td>IGF-I</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Resection × IGF-I</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4–7 animals/group per time point. Means in the same column with different superscripts are significantly different based on 1-way ANOVA and PLSD. Migration rate is cell positions moved over 24 h. Migration rate is calculated using the difference in foremost labeled cell or median labeled cell positions between 25 and 1 h. *P = 0.0634.

We speculate that the inability of IGF-I to decrease apoptosis further after resection is due to the fact that incidences of apoptosis in the presence of resection and/or IGF-I in parenterally fed rats are already at low levels we previously observed in orally fed animals (3). This may also be due to the ability of resection or IGF-I to impact on the same cellular signaling pathways leading to enterocyte apoptosis. Specifically, alterations in the proapoptotic Bax proteins and the antiapoptotic Bcl-2 subfamily of proteins are associated with changes in enterocyte apoptosis after small bowel resection (34, 35) or administration of IGF-I (39). Furthermore, apoptosis may be intimately linked with villus height, and the equally tall villi in parenterally fed resected and/or IGF-I-treated animals are all associated with similar low levels of crypt cell apoptosis. Nevertheless, others have suggested that the taller villi and deeper crypts that characterize the adaptive response to resection are maintained by concomitant increases in enterocyte proliferation and apoptosis. In particular, resection significantly increased intestinal growth in conjunction with increases in both proliferation and apoptosis in orally fed rabbits (36) and mice (8, 9). The discrepancies that exist between incidences of apoptosis after mid small bowel resection and TPN (2), oral feeding (2, 8, 9, 36), and/or administration of growth factors must be interpreted cautiously as differences in methods of detecting apoptosis exist (2).
migration rate with a species-specific phenomenon, so it is perhaps a function of the distinct animal models or the statistical methods used to determine migration.

Nevertheless, we observe the calculated migration rate was ~60–85% greater for all treatment groups based on FLC rather than MLC positions. This suggests the migration velocity increases as cells move up the crypt-villus axis (26). The factors driving the greater migration with resection are unknown. Yet, it seems clear that proliferation within the intestinal crypts and enterocyte migration along the crypt-villus axis are independently regulated as enhanced proliferation does not necessarily drive migration (11, 24).

In conclusion, we have shown that IGF-I augments increases in mucosal mass and concentrations of protein and DNA stimulated by the endogenous response to resection. The mucosal hyperplasia induced by resection alone was associated with increased enterocyte proliferation and migration and decreased apoptosis. IGF-I additively increased the resection-induced growth and hyperplasia in association with increased enterocyte proliferation and expansion of the proliferative compartment in the crypt. IGF-I did not confer any significant additional effect beyond resection alone with respect to enterocyte apoptosis or migration. The enhancement of the endogenous hyperplastic response to resection by IGF-I treatment substantiates the role of IGF-I as an intestinal mitogen that promotes tissue regeneration. The lack of a further decrease in apoptosis in resected rats given IGF-I may suggest that IGF-I therapy would pose a modest risk for cancer when used after intestinal resection. Introduction of oral feeding after acute treatment with IGF-I after resection may help to sustain the intestinotropic effects of IGF-I (6).

We thank M. Gillingham, M. Grahn, D. Huss, and K. Kritsch for assistance with animal care, W. K. Pui for the BrdU cell kinetic measurements, and Dr. M. Clayton for expert statistical advice.

Jejunal composition, apoptosis, and some proliferation data from the T and R groups were previously published (2) but are included in this report to establish a baseline for the additive effects of IGF-I resection within the experimental design utilized.

DISCLOSURES

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-42835, a United States Department of Agriculture National Needs Graduate Fellowship, and funds from the College of Agriculture and Life Sciences, University of Wisconsin-Madison.

REFERENCES


