The pathogenesis of resection-associated intestinal adaptation

Brad W. Warner
Washington University School of Medicine in St. Louis

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After massive small-bowel resection, the remnant bowel compensates by a process termed adaptation. Adaptation is characterized by villus elongation and crypt deepening, which increases the capacity for absorption and digestion per unit length. The mechanisms/mediators of this important response are multiple. The purpose of this review is to highlight the major basic contributions in elucidating a more comprehensive understanding of this process. (Cell Mol Gastroenterol Hepatol 2016;2:429–438; http://dx.doi.org/10.1016/j.jcmgh.2016.05.001)

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Intestinal adaptation is an important response to massive small-bowel resection (SBR) and represents a mitogenic signal to the intestine culminating in a compensatory expansion in mucosal digestive and absorptive surface area per unit length. Clinically, adaptation is heralded by the gradual tolerance of enteral nutrition that could not be tolerated at earlier time points. A complete adaptation response allows for tolerance of all nutrition to be absorbed from the gut, without the need for supplemental parenteral feeding. The expression of several immediate–early genes within the remnant bowel has been recorded to be increased during hours of intestinal resection.1,2 Similarly, in a murine model of SBR, alterations in wet weight as well as DNA and protein content in the remnant bowel are increased as soon as 24 hours, but before the initiation of enteral feeding.3

Adaptation is characterized structurally by taller villi and deeper crypts, as well as enhanced rates of enterocyte proliferation and apoptosis. Although these features are a renowned characteristic of adaptation in animal models of massive SBR, similar structural alterations have not been described consistently in human beings. In one study, the intestine was evaluated in a uniform population of infants with neonatal necrotizing enterocolitis who required bowel resection.4 Comparing villus height and crypt depth at the normal margin of tissue at the time of resection with the time of ostomy takedown showed significant increases in both parameters. In another report, a 70%–75% increase in villus height was documented in the small intestines of 13 patients at 2 years after jejun-ileal bypass.5 In addition, significant increases in crypt depth and cell number/crypt in the colons of 12 patients with jejunocolonic anastomosis compared with healthy controls was identified at a mean of 9.8 years after resection.6 Unfortunately, the histologic status of the small intestine was not evaluated in that study. In contrast, other studies have failed to show changes in rates of enterocyte proliferation, crypt depth, or villus height in the small intestine of patients with short-gut syndrome compared with controls.7–9 All of the earlier-mentioned human studies comprised small numbers of patients, variable lengths of resected intestine, assorted amounts of enteral feeding, and analysis at single time points after SBR. Despite these limitations, animal models for studying resection-induced adaptation continue to provide important mechanistic insights.

Mechanisms of Adaptation

The mechanisms and mediators of intestinal adaptation are multifactorial and include intraluminal nutrients, gastrointestinal secretions, as well as hormones10,11 (Figure 1). In general, most research has focused on various growth factors and how they affect rates of enterocyte proliferation as the primary driver of resection-induced mucosal growth. It should be considered, however, that enhanced rates of enterocyte proliferation actually may occur secondary to growth of subepithelial structures.

Intraluminal Nutrients

Enteral nutrients appear to stimulate intestinal adaptation via several mechanisms including direct contact with epithelial cells as well as stimulated secretion of trophic gastrointestinal hormones and pancreaticobiliary secretions.12 The contributions of luminal nutrients to the adaptive response of the intestine is underscored by the

Abbreviations used in this paper: EGF, epidermal growth factor; GH, growth hormone; GLP-2, glucagon-like peptide-2; IGF-1, insulin-like growth factor-1; LA, lactate-accumulator; PN, parenteral nutrition; Rb, retinoblastoma protein; SBBO, small-bowel bacterial overgrowth; SBR, small-bowel resection.
observations that gut mucosal atrophy is associated with starvation and is reversed by refeeding. Furthermore, surgical transposition of a segment of the ileum into the more proximal intestinal stream results in structural and functional “jejunalization” of the transposed ileum.\textsuperscript{13,14} Not only is the presence of luminal nutrition important for adaptation, but so is the nutritional composition. Luminal administration of non-nutrient substrates has little effect on adaptation. More complex nutrients requiring more metabolic energy to absorb and digest have been suggested to induce the greatest adaptation response, presumably by virtue of an increased functional workload of the enterocyte. Enteral fats appear to be the most trophic of the macronutrients in inducing adaptation.\textsuperscript{15} More specifically, longer-chain and more polyunsaturated fats as present in fish oil may provide an even greater adaptive stimulus.\textsuperscript{16–18}

**Gastrointestinal Secretions**

Multiple experimental observations have contributed to the notion that endogenous gastrointestinal secretions are important for adaptation. Experimental models in which the ampulla of Vater is transposed surgically to areas more distal in the gastrointestinal tract induces villus hyperplasia beyond the transposed segment.\textsuperscript{19,20} Bile alone has been shown to stimulate intestinal RNA and DNA content when delivered directly to the mid–small bowel, but the effect seems to be more profound when combined with pancreatic secretions.\textsuperscript{20} In other studies, pancreatic secretions seem to be more trophic to the intestinal mucosa when compared with bile.\textsuperscript{20} Further evidence that pancreaticobiliary secretions are important for postresection adaptation is the observation that somatostatin, an agent that dramatically diminishes the output of endogenous gastrointestinal
secretions, also is associated with an inhibited adaptation response.21

**Humoral Factors**

A surgical model of vascular parabiosis in which 2 rats share a common circulation has provided one of the most compelling studies endorsing the contributions of hormones to resection-induced adaptation.22 In that report, intestinal resection in one animal was associated with adaptive changes in the intestine of the other unoperated animal. Multiple endogenous humoral factors that have been suggested to play a role in intestinal adaptation including growth hormone, insulin-like growth factor, glucagon-like peptide-2, epidermal growth factor (EGF), leptin, thyroxine, and corticosteroids, to name but a few.23 Many of these factors either have been found to be increased in the serum of patients who have undergone SBR, or exogenous administration of these agents after SBR has resulted in enhanced parameters of adaptation.

**Growth Hormone**

Growth hormone (GH) is a 191-amino acid, single-chain protein produced in the anterior pituitary gland. This growth factor is known to be a major regulator of postnatal growth in mammals as well as play an important role in the regulation of lipid and carbohydrate metabolism.24,25 Because GH has been shown to induce growth and proliferation in many different tissues and cell lines, its role in the setting of short-gut syndrome has been studied extensively. The receptor for GH has been found throughout the intestine: in cells of the muscularis propria, submucosa, muscularis mucosa, lamina propria, and intestinal epithelium.26 Because of the widespread distribution for this receptor in the gut, GH has been proposed to stimulate intestinal growth directly. In addition to stimulating growth of the intestinal layers directly, GH is a major stimulus for the production of insulin-like growth factor-1 (IGF-1), another intestinotrophic hormone, whose role in intestinal adaptation will be discussed in later.

In animal studies, exogenous GH has resulted in significantly increased small-bowel length, mucosal height, jejunal villus height, and/or glutamine and leucine transport in animals that had undergone intestinal resection.27–29 In contrast, other reports have failed to show an effect of GH on postresection mucosal growth.30,31 GH appears to be more effective in combination with glutamine, an enterocyte-preferred fuel. Several, but not all, animal studies evaluating GH plus glutamine have shown improvements in structural measures of intestinal adaptation.2–35

In a clinical trial, Byrne et al36 showed clinical benefit by administering GH and glutamine in 10 patients with short-bowel syndrome who had been on long-term parenteral nutrition. This study inspired multiple subsequent clinical trials with mixed results. In a 2010 Cochrane Review, Wales et al37 analyzed 5 clinical trials of GH with or without glutamine and suggested a positive effect of GH on weight gain and energy absorption. In the majority of trials, the effects were short-lived and returned to baseline shortly after cessation of therapy. The evidence to recommend this therapy therefore was inconclusive and the clinical utility of this treatment was questioned. Somatropin is a recombinant form of human GH and recently has been shown to enhance fat-free mass through the stimulation of protein synthesis and to decrease proteolysis in response to feeding.38 In that study, improvements in de novo synthesis and intestinal absorption increased glutamine availability over the physiologic range, suggesting that beneficial effects of GH may not require supplemental glutamine.

**IGF-1**

IGF-1 is a hormone produced chiefly in the liver and to a lesser degree in the gastrointestinal tract and has commanded much attention as an enterotrophic hormone. Similar to GH, IGF-1 has been shown to enhance the rates of enterocyte proliferation after SBR.39 These observations, along with the localization of IGF-1 production, its receptor, and regulatory binding proteins in the intestine, make IGF-1 an attractive target for modulating adaptation responses.40,41

It has been considered that IGF-1 is the mediator of the effects attributed to GH.32,42 Both functional and structural parameters of adaptation have been shown to be amplified by IGF-1. Vanderhoof et al43 found an increase in the activity of the ileal digestive enzymes sucrase, maltase, and leucine aminopeptidase when IGF-1 was given after SBR. In rats with short-bowel syndrome, IGF-1 treatment allowed the rats to be weaned from parenteral nutrition. In that study, IGF-1-treated short-bowel rats also were found to have greater body weights and increased lean body mass.

In addition to the effects of IGF-1 on enterocytes, our laboratory has shown a possible effect of IGF-1 on the smooth muscle of the intestine. We performed SBR procedures on transgenic mice who overexpressed IGF-1 specifically in smooth muscle cells.44 We found that these mice increased the length of their remnant intestine far more (approximately 2-fold) than nontransgenic control mice that also underwent SBR. Of note, these transgenic mice did not show the normal adaptive response of increasing villus height and crypt depth in the early phases of adaptation. The intestinal lengthening response preceded villus growth, which was noted at later postoperative time points. These experiments suggest that the IGF-1–stimulated muscular lengthening might be an important trigger for enhanced villus and crypt growth. As such, it is plausible to consider that enterocyte proliferation occurs secondary to growth of the underlying mesenchyme, as opposed to being the primary stimulus for villus lengthening.

In contrast with the positive findings described earlier, adaptation responses appear to be preserved after SBR in both IGF-1-null mice as well as in a strain of mice in whom IGF-1–receptor expression was disrupted specifically in enterocytes.45 These findings have several implications. First, they suggest that enterocytes are not a major cell
compartment for IGF-1-receptor signaling during adaptation. Thus, the beneficial effects of exogenous IGF-1 may involve IGF-1 receptors in other cells within the bowel wall. This notion would be supported by the magnified intestinal lengthening shown after SBR in mesenchymal IGF-1 transgenic mice as described earlier. In addition, these findings would offer the possibility that other ligands (such as insulin or IGF-2) for the IGF receptor may be able to compensate for the lack of IGF-1 expression. Despite the significant preclinical work that has been performed, no human clinical trials with IGF-1 have been reported.

Glucagon-Like Peptide-2

Glucagon-like peptide-2 (GLP-2) is an enterotrophic hormone and a member of the pituitary adenylate cyclase activating peptide glucagon superfamily. GLP-2 is synthesized in enteroendocrine L cells of the distal ileum and proximal colon. Within this 33-amino acid protein, the second amino acid in the sequence is alanine, which makes the hormone sensitive to degradation by the exopeptidase dipeptidyl peptidase-4. Substitution of glycine for alanine at position 2 makes a synthetic analog of GLP-2 (teduglutide) that is resistant to enzymatic degradation and significantly extends its half-life.

GLP 2 exerts its effects through the GLP-2 receptor, which has been identified on intestinal enteroendocrine cells, enteric neurons, and subepithelial myofibroblasts. Secretion of GLP-2 by intestinal L cells is driven by both direct stimulation of nutrients in the distal bowel and vagally mediated pathways, which are activated by the presence of nutrients in the proximal bowel. Ingestion of nutrients, particularly long-chain fatty acids, plays a major role in GLP-2 secretion. In patients with short-bowel syndrome, the presence of a colon in continuity with the small intestine is important for nutrient-stimulated increases in GLP-2. This finding may help explain why the presence of the colon reduces the likelihood that a patient with short-bowel syndrome will require parenteral nutrition.

The intestinal effects of GLP-2 have been studied both in animal models and in human clinical trials. When given to rodents, GLP-2 stimulates intestinal mucosal growth. In addition to elongated intestinal villi and crypts, GLP-2 administration augments rates of crypt cell proliferation and attenuates rates of apoptosis. Other effects mediated by GLP-2 include reduced gastric motility, inhibited gastric acid secretion, and increased mesenteric blood flow.

Epidermal Growth Factor

Human EGF is a 53-amino acid protein found in platelets, macrophages, urine, saliva, breast milk, and plasma. EGF is a member of a family of ligands sharing a common EGF receptor, which also includes transforming growth factor, heparin-binding EGF-like growth factor, amphiregulin, epiregulin, epigen, betacellulin, and neuuregulins 1–4. This growth factor has been shown to induce growth in the epithelia of multiple tissues to include skin, lung, tracheal, corneal, and gastrointestinal tract.

An important role for EGF as a mediator of adaptation initially was suggested by a study in which EGF was administered to rats after SBR and showed significant increases in weight gain as well as other parameters of adaptation. Through several subsequent experimental paradigms, enhanced resection-induced adaptation responses have been verified after stimulation of the EGF receptor either by exogenous EGF, in EGF transgenic mice, or administration of another EGF-receptor ligand (transforming growth factor-α). Alternatively, inhibiting EGF-receptor signaling by removing the submandibular glands, a major source of endogenous EGF in the mouse, or administration of a pharmacologic EGF-receptor inhibitor all resulted in attenuated adaptation responses.

Because the intestinal mucosa is a very dynamic organ containing some of the most rapidly proliferating cells in the body, the relationship between rates of cell production and cell death must be precise. Any imbalance may result in either intestinal mucosal atrophy or neoplasia. In studies focused on mechanisms for EGF-receptor regulation of proliferation showed that expression of the cell-cycle inhibitor p21 was increased and paradoxically required for EGF-directed proliferation of enterocytes in vitro. In this study, a critical region of the p21 promoter was found to be activated by EGF-receptor stimulation. This promoter activity required activated extracellular signal-regulated kinase 1/2 and contained a putative binding site for the transcription factor Sp1. The requirement for this cell-cycle regulatory protein was verified in earlier experiments in which p21-null mice showed no induction of enterocyte proliferation after the stimulus of SBR. In seeking potential mechanisms for how p21 regulates adaptation, we initially expanded upon the observation that p21 affects stem cell populations within bone marrow. We therefore sought to determine the effect of p21 deficiency on intestinal stem cells. In these studies, we were unable to show differences in the expression of several stem cell markers or numbers of crypt-base columnar cells in p21-null vs control mice. However, we did identify increased expression of another cell-cycle inhibitor retinoblastoma protein (Rb) within the crypt cells of the p21-deficient mice. The significance of Rb expression was established.

Teduglutide is now approved for clinical use in parenteral nutrition–dependent adults with short-bowel syndrome.
by genetically inactivating a single Rb allele in the p21-null animals, which restored enterocyte proliferation and adaptation responses. In another study, rates of enterocyte proliferation and villus growth were magnified when Rb expression was completely disrupted within the intestinal epithelium of unoperated mice.83

Independent of p21, EGF-receptor stimulation has been shown to inactivate Rb directly by phosphorylation in cultured enterocytes.85 One simple explanation for how Rb deficiency results in enterocyte proliferation is the fact that the activity of this cell-cycle inhibitor is attenuated. Alternatively, enhanced IGF-2 expression has been shown to be associated with Rb deficiency in enterocytes.87 In this study, genetic disruption of IGF-2 expression in intestinal Rb-deficient mice prevented the mucosal hyperplasia associated with Rb deficiency. Because acute disruption of intestinal Rb expression after intestinal resection results in amplified adaptation responses,86 future experiments focused on illuminating this previously unrecognized role for Rb as a critical player in the molecular mechanism of resection-induced enterocyte proliferation and adaptation appear justified.

Similar to proliferation, rates of enterocyte apoptosis also are increased after SBR.87–90 Because rates of enterocyte production must be matched perfectly by rates of enterocyte loss, these findings made biological sense. It appears that the proapoptotic Bcl-2 family member Bax is a major mediator of resection-induced enterocyte apoptosis. Bax expression is increased in the intestine after SBR,88,91 and coincides with reduced expression of the antiapoptotic Bcl-2 family member Bcl-w.91 Indeed, the proliferative crypt compartment is the site for the greatest changes in Bax and Bcl-w expression.92 When intestinal resections were performed in Bax-null mice, the expected increase in enterocyte apoptosis did not occur, despite normal induction of enterocyte proliferation.93 In the setting of intestinal resection, apoptotic and adaptive responses are preserved in both tumor necrosis factor-receptor 1-null and Fas-null mice.94 These results suggest that the mechanism for increased enterocyte apoptosis after massive SBR does not appear to involve the extrinsic, death receptor–mediated pathway. Furthermore, the apoptosis response to SBR is not a simple passive response to increased rates of enterocyte proliferation.

The ultimate utility of focusing on both the rates of proliferation and apoptosis is that future growth factor and/or pharmacologic therapy targeted to stimulate proliferation while at the same time inhibit apoptosis may result in an even greater expanded mucosal surface area than either intervention alone. The benefits of this dual therapeutic approach was suggested by co-administration of EGF (to inhibit apoptosis and stimulate proliferation) and a pharmacologic apoptosis (pan-caspase) inhibitor after SBR, resulting in greater mucosal growth.95 Explicit understanding of these mechanisms will be necessary to optimize this novel therapy. A summary of key signaling events that have been established to be involved with EGF-directed enhanced enterocyte proliferation and attenuated rates of apoptosis is presented in Figure 2.

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**Figure 2.** Key signaling events that have been established to play a role in mechanisms for how EGF amplifies resection-induced intestinal adaptation. ERK, extracellular signal-regulated kinase.

**Angiogenesis**

We previously identified a significant induction of capillary growth within adapting intestinal villi.96 It presently is unclear whether this angiogenic response occurs as a result of stimulated enterocyte production or whether this angiogenic response is a primary signal to induce enterocyte proliferation. Photoacoustic microscopy applied to live mice immediately after intestinal resection showed that both blood flow and arterial oxygen saturation were reduced and oxygen extraction was increased within the remnant intestine.97 This immediate response was associated with increased expression of hypoxia-inducing factor-1α.98 It therefore is possible that the immediate response to SBR results in a hypoxic milieu that may initiate a series of hypoxia-regulated genes capable of signaling for enterocyte proliferation. This notion is supported by a study that reported rescued adaptation responses in the intestine of vascular endothelial growth factor–deficient mice after SBR.99

One proangiogenic chemokine is CXCL-5, which has been shown to be increased in the adapting intestine after SBR.100 The expression of endothelial CXCL-5 appears to be modulated by EGF.100 Indeed, genetic disruption of CXCL-5 expression prevents adaptive angiogenesis after SBR.101 In this study, it was surprising that villus growth occurred despite the lack of an angiogenic response. In a subsequent
series of experiments, CXCL-5 null mice were found to have impaired intestinal lipid absorption after SBR. It therefore is plausible to conclude that the angiogenic response to intestinal resection is more important for functional, rather than structural, adaptation.

Gut Microbiome

Small-bowel bacterial overgrowth (SBBO) and catheter-related bloodstream infections are 2 of the most common complications in patients with intestinal failure and directly impact morbidity and mortality. SBO generally results from the development of dilated loops of intestine with impaired peristalsis. This anatomic alteration sets the stage for stasis, disruption of the enteric flora, secretory diarrhea, malabsorption, gut mucosal inflammation, D-lactic acid production, and bacterial translocation into either the portal circulation or mesenteric lymph nodes. Despite these well-known events, data supporting the occurrence of bacterial translocation and microbiologic features of SBBO in human beings are both limited and indirect. Prior studies have used cultures of duodenal aspirate, absorption of various sugar markers as a surrogate for intestinal permeability, or hydrogen and/or $^{13}$C-D-xylose breath testing. Reliance on culturable organisms alone is restricted by the fact that less than 50% of bacterial species in the gut cannot be cultured.

Recent advances in high-throughput sequencing of the 16S ribosomal RNA gene of luminal gut bacteria have established a significant association between the intestinal microbiome and various intestinal epithelial and metabolic responses to a wide spectrum of diseases and conditions. By using 16S sequencing, massive SBR has been shown in several animal models (mouse, piglet) to be associated with significant alterations in the gut microbiome.

There have been limited pediatric clinical studies involving the gut microbiome in the context of short-bowel syndrome. In one report, the gut microbiota in 11 children with short-bowel syndrome was studied and a reduced bacterial diversity was found to be associated with an increased relative abundance of Proteobacteria. A confounding variable of this study was that the majority of patients on parenteral nutrition (PN) were receiving antibiotics at the time of stool sampling. Another 6 patients already had been weaned from PN. In another study of 23 children with intestinal failure, there was also reduced bacterial diversity associated with an increased relative abundance of Proteobacteria in patients who required PN, although there was an overabundance of Lactobacilli in patients who already had been weaned from PN. In the PN patients, Proteobacteria was associated with a greater degree of liver injury. These data offer the possibility that the gut microbiome may be a major contributor in the pathogenesis of cholestasis and hepatic injury in patients with intestinal failure.

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studied 16 patients with short-gut syndrome and showed a marked dysbiosis in fecal microbiota, with a predominance of the Lactobacillus/Leuconostoc group, whereas Clostridium and Bacteroides were under-represented. The presence of fecal lactate (56% of patients) was used to define a lactate-accumulator (LA) group, whereas an absence of fecal lactate (44% of patients) defined a non-lactate-accumulator group. The LA group had lower serum HC03- levels and were at risk of D-encephalopathic reactions. Furthermore, all patients in the non-lactate-accumulator group and those accumulating preferentially L isoform in the LA group had never developed D-acidosis. The D/L fecal lactate ratio therefore may be a relevant index to predict the risk for D-lactate encephalopathy. There was a recent case report of a child with D-lactic acidosis and short-bowel syndrome who was managed successfully by fecal transplantation.

Through metagenomic and biochemical analysis, the intestinal microbiota of genetically obese mice have been shown to have an increased capacity for energy harvest from the diet. In that study, transfer of stool into the gastrointestinal tract of germ-free mice resulted in a significant increase in body fat. Stool from obese mice show a proportional increase in Firmicutes phyla (which includes the genus Lactobacillus) in their intestinal lumen. It therefore is plausible to investigate whether the altered intestinal microbiota in patients with intestinal failure adapts to provide greater energy harvest for the host.

The paucity of published data regarding direct interrogation of the microbiota in the setting of intestinal failure represents a significant gap in our understanding of this important morbidity. These data will direct a more informed scientific rationale for current therapeutic interventions such as antibiotic administration, prebiotics, probiotics, surgical reduction in small-bowel caliber, or even future interventions such as microbiota manipulation via fecal transplantation.

Conclusions

Adaptation is critical for survival after massive intestinal loss. In children with intestinal failure, roughly half will have a complete adaptation response and be weaned completely from parenteral nutrition. Within the other half of patients, an equal proportion will either die or require a small-bowel transplant. Current 5-year graft survival rates after intestinal transplantation are roughly 50%, with significant patient morbidity associated with significant immunosuppression. Basic research designed to elucidate specific mechanisms for resection-induced adaptation responses therefore are critical for the future design of more targeted, innovative therapies to enhance this important response.

References


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