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The Role of Growth Factors in Intestinal Regeneration and Repair in Necrotizing Enterocolitis

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Abstract

Necrotizing enterocolitis (NEC) is a devastating intestinal disease resulting in major neonatal morbidity and mortality. The pathology is poorly understood, and means of preventing and treating NEC are limited. Several endogenous growth factors have been identified as having important roles in intestinal growth as well as aiding intestinal repair from injury or inflammation. In this review, we will discuss several growth factors as mediators of intestinal regeneration and repair as well as potential therapeutic agents for NEC.

Keywords

necrotizing enterocolitis; epidermal growth factor; insulin-like growth factor; hepatocyte growth factor; glucagon-like peptide-2; growth hormone

INTRODUCTION

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency encountered in the neonatal period.¹ The exact pathogenesis of NEC is unknown and likely multifactorial. Prematurity, aberrant bacterial colonization, hypoxia, and intestinal ischemia have all been implicated.² Localized intestinal mucosal injury is thought to result in an amplified cycle of bacterial invasion, immune activation, uncontrolled inflammation, and gut barrier failure, leading to necrosis, perforation, sepsis, and shock.²⁻⁴

In the dynamic milieu of the developing gut, growth factors play a critical role in intestinal development. Growth factors have also been established to be important mediators of gastrointestinal repair, with roles in cellular proliferation, differentiation, migration, and survival.⁵⁻⁸ It is logical to consider that absent or reduced levels of specific factors that are normally expressed during later periods of gestation may contribute to the development of NEC. As such, exogenous replacement of these key factor(s) may be of clinical value in the prevention and treatment of NEC.⁹ Epidermal growth factor (EGF), heparin-binding epidermal-like growth factor (HB-EGF), growth hormone (GH), insulin-like growth factor (IGF), glucagon-like peptide 2 (GLP-2), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), granulocyte colony stimulating factor (G-CSF), erythropoietin (Epo),

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and intestinal trefoil factor (ITF) have all been implicated in the pathogenesis and prevention of NEC, and will be the focus of the present review (the effects of these growth factors is summarized in Table 1).

EPIDERMAL GROWTH FACTOR

EGF and Normal Intestinal Development—EGF is a 53-amino acid peptide, established as a major trophic factor for the developing intestine.¹⁰⁻¹³ EGF is normally found in fluids that bathe the developing intestine, including amniotic fluid, fetal urine, breast milk, bile, and saliva.¹⁴ As gestation progresses, the concentration of EGF in amniotic fluid increases.^{13,15} In rabbits, exogenous in utero infusion of EGF stimulates intestinal growth and accelerates maturation of intestinal enzyme activity.¹⁶ Maternal colostrum and milk are the main sources of intestinal EGF in the postnatal period. EGF is resistant to proteolytic degradation across a range of gastric pH and as development progresses the vast majority of EGF is produced in the salivary glands, with minor contributions from the duodenal Bruner's glands, ulcer associated cell lineage, and the kidney.¹⁷⁻²⁰ Pharmacological stimulation of submandibular gland EGF secretion results in small intestine trophic effects.²¹

Under basal conditions, EGF signaling is crucial for intestinal epithelial cell proliferation and survival.²² The EGF receptor (EGFR) has tyrosine kinase activity and is localized primarily on the basolateral surface of the enterocytes, however, apical EGFR activity has been observed.²³⁻³⁰ Recent work from our laboratory has shown that the proliferative effects of EGF are preserved despite loss of the enterocyte EGFR, suggesting that EGFR activity in the underlying submucosa and muscularis layers of the bowel may also play a role.³¹ The importance of EGF in gut development is highlighted by the fact that global EGFR deletion is associated with embryonic lethality.³² Mice with global EGFR deletion that do survive to delivery die early in the neonatal period with a hemorrhagic enteritis that resembles NEC in humans.³²

EGF and Gastrointestinal Repair—The mucosal lining of the gastrointestinal tract consists of a single layer of columnar epithelial cells responsible for providing a barrier between the toxic luminal contents of the intestine and the subepithelial structures. Epithelial injury results in restitution, a process by which cells flatten and adopt a squamoid appearance to seal the wound, followed by epithelial proliferation and epithelial maturation and differentiation.^{33,34} EGF has established healing effects on the gastrointestinal mucosa following injury.^{17,35} Ulceration of the gastrointestinal epithelium induces expression of ulcer associated cell lineage, which has been shown to secrete EGF on the ulcer margin, contributing to ulcer repair.³⁶ The expression of EGFR protein is increased at the margin of mucosal ulcers, with a 75-fold increase in the number of cells expressing EGFR observed in a rat model of acetic acid-induced ulceration.³⁷

Reduced EGF levels have been associated with mucosal ulcer disease. Low salivary EGF levels have been recorded in patients with active duodenal ulcer disease.³⁸ Similarly, immunoreactive EGF levels in gastric juice and saliva are reduced in patients with gastric ulcers.^{39,40} Furthermore, pentagastrin stimulation of salivary and gastric EGF secretion is impaired in patients with active *Helicobacter pylori* and duodenal ulcer disease.⁴¹ For these patients, lack of EGF may set the stage for decreased mucosal resistance to stress and/or impaired ability for repair in the face of mucosal injury.

EGF supplementation has been demonstrated to improve mucosal repair and regeneration in several conditions. In a pig model, EGF has been shown to significantly reduce esophageal ulceration, stricture formation, and mucosal histological damage associated with sclerotherapy.⁴² In rats with gastric ulcers, orogastric EGF given in combination with

sucralfate improved ulcer healing.⁴³ A small study of humans treated with intravenous EGF demonstrated superior gastric ulcer healing with EGF as compared to antiulcer cetraxate hydrochloride treatment.⁴³ Oral EGF administration in patients with duodenal ulcer disease resulted in comparable success to treatment with cimetidine.⁴⁴ In a rat model of gluten-induced enteropathy, EGF supplementation has been shown to protect the intestine from the pathological changes associated with interferon-gamma and gliadin administration.⁴⁵ Similarly, acetic acid injury of the colon in rats was attenuated with administration of exogenous EGF.⁴⁶ EGF supplementation has been shown to prevent intestinal mucosal atrophy associated with elemental and parenteral nutrition.^{47,48,49} Human randomized controlled trials of EGF enemas for patients with left-sided ulcerative colitis demonstrated EGF to be superior to placebo in regards to disease activity, sigmoidoscopic findings, and histologic grading of injury.⁵⁰

Our laboratory has established a critical role for EGF in the process of intestinal adaptation following massive small bowel resection (SBR). Adaptation is a compensatory response, characterized by significant increases in villus height, crypt depth, and enterocyte proliferation, and resulting in increased absorptive mucosal surface area, allowing for adequate absorption of enteral nutrition despite significant loss of bowel length.⁵¹ Following SBR, salivary EGF levels are significantly increased with greater expression and activation of intestinal EGFR within the crypts and muscularis layer of the intestine.^{52,53} Systemic EGF stimulation enhances adaptation. EGF given exogenously after SBR or overexpressed in transgenic mice results in a magnified adaptation response.⁵⁴⁻⁵⁶ Perturbed EGFR activity inhibits the adaptation response. Removal of the major endogenous source of EGF via sialoadenectomy attenuates adaptation, an effect that is partially reversible with either systemic or oral administration of EGF.⁵⁷ Further, resection-induced adaptation after SBR is inhibited by systemic administration of EGFR inhibitors and in mutant mice (waved-2) that have generalized perturbed EGFR activity.^{58,59} These studies highlight the essential role of EGF in physiologic intestinal repair.

EGF and NEC—Several lines of evidence indicate an important role for EGF in the pathogenesis of NEC. Global EGFR deficiency in mice results in embryonic or early neonatal lethality with a hemorrhagic enteritis resembling human NEC.³² Waved-2 mice with deficient EGFR expression demonstrate increased susceptibility to agents associated with intestinal damage, such as dextran sodium sulfate.⁶⁰ In experimental NEC models, EGF has been shown to contribute to the pathogenesis of NEC. In a neonatal rat model of NEC, Dvorak and colleagues demonstrated significantly increased EGFR mRNA expression within the ileum of affected animals. Immunohistochemistry localized the EGFR expression to the epithelium, corresponding with the site of maximal injury. Enteral EGF supplementation prior to injury significantly decreased both the incidence and severity of NEC, as well as diminished EGFR expression.⁶¹

Increased intestinal permeability has been implicated in the pathogenesis of NEC.^{2-4,62,63} During the early postnatal period, greater intestinal permeability allows for immunoglobulin and growth factor absorption from the colostrum and milk.⁶⁴ However, insults to the immature intestinal barrier can result in mucosal damage, barrier failure, and initiation of inflammation.⁶⁵ Under normal conditions, the enterocyte is protected from the luminal contents by mucin-secreting goblet cells. A primary deficiency in the mucous layer may therefore result in intestinal injury. Studies of animal NEC models have demonstrated a decrease in the total number of goblet cells.⁶³ EGF supplementation is known to increase goblet cell density in short gut syndrome, while inhibition of the EGFR decreases goblet cell density.⁶⁶ In a neonatal rat model of NEC, treatment with EGF accelerated goblet cell maturation and mucin production and normalized the expression of tight junction proteins, restoring the intestinal barrier and resulting in prevention of NEC.⁶³ In addition, EGF plays

an important role in enterocyte migration and restitution following mucosal injury. EGF has been shown to enhance enterocyte migration and stimulate mucosal restitution in rat stomach and human colon.⁶⁷⁻⁶⁹

Another important component in the pathogenesis of NEC is a perturbation of the normal cellular barrier to bacteria and/or bacterial products.^{70,71} Prematurity is associated with limited gastrointestinal defenses, exposure to antibiotics, and feeding practices which contribute to the colonization of the gastrointestinal tract with select species of virulent bacteria. In addition, compared to mature animals, immature animals have been demonstrated to have greater adherence and translocation of pathogenic bacteria.⁷⁰ EGF may play a role in maintaining the intestinal barrier and preventing bacterial translocation. Supplementation of exogenous EGF to formula-fed newborn rabbits, burn injured mice, and rats receiving total parenteral nutrition has been shown to decrease bacterial translocation.^{48,72,73}

Dysregulated inflammation and immune system activation have been implicated in NEC development.^{4,74,75} Neonatal rat models of NEC have demonstrated elevated expression of pro-inflammatory IL-18 and IL-12, with increasing concentrations correlating with the degree of tissue damage.⁷⁶ Patients with NEC have elevated levels of pro-inflammatory mediators such as platelet activating factor, tumor necrosis factor-alpha, IL-8, and nitric oxide, as well as enhanced expression of anti-inflammatory cytokines, such as IL-10.⁷⁷⁻⁷⁹ Interestingly, IL-10 deficient mice are known to spontaneously develop enterocolitis.⁸⁰ Exogenous EGF administration in a neonatal rat NEC model attenuated the expression of IL-18 (a pro-inflammatory mediator) and increased production of IL-10 (an anti-inflammatory cytokine) in the ileum.⁸¹

Enterocyte apoptosis has been shown to precede gross morphological changes in neonatal rat models of NEC.⁸² Administration of pan-caspase inhibitors reduced the incidence of both apoptosis and NEC.⁸² Pro-apoptotic Bax mRNA levels have been shown to be markedly elevated following the induction of NEC in neonatal rat models, with antiapoptotic Bcl-2 mRNA levels markedly reduced. EGF supplementation in this model decreased pro-apoptotic Bax levels and increased antiapoptotic Bcl-2 mRNA levels.⁸³ In addition, increased autophagy has been demonstrated in the intestinal epithelium of NEC patients and in the ileum of NEC rats. In in-vivo and in-vitro models, EGF has been shown to inhibit intestinal autophagy.⁸⁴ These studies suggest that alterations in the balance of cell survival and death may provide a mechanism by which EGF maintains intestinal integrity and protects the epithelium against NEC.

In humans, an association between EGF production and NEC has been reported. Serum and salivary EGF levels are decreased in patients with NEC requiring surgical intervention as compared to age-matched controls without NEC.^{85,86} A study of urinary EGF revealed an increase from baseline in neonate urinary EGF levels, normalized to creatinine, at the time of NEC diagnosis.⁸⁷ No change from baseline was seen in neonates without NEC.⁸⁷ Urinary levels of EGF indicate that EGF production increases with gestational age.⁸⁸⁻⁹⁰

Given that the single most important risk factor for development of NEC is prematurity, a prospective cohort study demonstrated that salivary EGF levels are directly related to gestational age using both uni- and multivariate regression models.⁹ In this study, infants who developed NEC had lower initial EGF levels and demonstrated a greater increase in EGF between the first and second weeks of life than those infants who did not develop NEC.⁹

Human infants who receive breast milk are less likely to develop NEC than those infants feed formula.^{91,92} In a neonatal rat model of NEC, breast milk supplementation reduced the

severity of injury as compared to formula fed controls.⁹³ EGF is one of the major peptides present in biologically significant concentrations in human colostrum and milk, but is absent from all commercially available infant formulas.⁹⁴⁻⁹⁶ The concentration of EGF in maternal milk is inversely proportional to gestational age.⁹⁷ Notably, the greater the degree of prematurity, and therefore the greater risk of NEC, the higher the concentration of EGF present in breast milk. This illustrates a remarkable compensatory response to removal of the fetus from the EGF-rich amniotic fluid, and may explain, at least in part, that the protective effect of human milk against development of NEC.

In humans, the clinical use of EGF for treatment on NEC has been limited. A single case report of an 8 month old critically ill infant with unsalvageable intestinal necrosis treated with 4 day intravenous EGF infusion survived with full recovery of the intestine.⁹⁸ A small, randomized, prospective trial of intravenous recombinant EGF versus placebo control in premature infants with evidence of NEC demonstrated improved mucosal thickness and crypt surface area on rectal biopsy with recombinant EGF. A significant limitation of this study was the extrapolation of changes in the distal colon to that of the upper intestine. Changes in the distal colon reflect those of the upper intestine in animal models; however this has not been tested in humans. Importantly, no significant toxicities were reported with EGF administration.⁹⁹ The trophic effects of EGF are clear and further studies are warranted to test the possibility that EGF supplementation may prevent the development of NEC.

HEPARIN-BINDING EPIDERMAL-LIKE GROWTH FACTOR

HB-EGF is a 22-kDa glycoprotein that is a member of the EGF family and signals via the EGFR pathway to stimulate cellular growth and differentiation.¹⁰⁰ Unlike other members of the EGF family, HB-EGF is unique in its ability to bind strongly to heparin.¹⁰¹ Like EGF, HB-EGF is found in biologically significant quantities in amniotic fluid and human milk.¹⁰² HB-EGF expression has been demonstrated to be elevated in response to tissue damage, hypoxia, stress, and during wound healing and regeneration.¹⁰³⁻¹⁰⁵ In humans, intestinal tissue obtained at the time of operative intervention for NEC demonstrated higher HB-EGF mRNA expression at the healthy resection margin as compared to tissue adjacent to frankly necrotic tissue, raising the possibility that HB-EGF expression deficiency contributes to the pathogenesis of NEC or, alternatively, that HB-EGF overexpression at the healthy tissue margin promotes healing in response to tissue injury.¹⁰⁶

While at low endogenous basal levels in normal cells, HB-EGF may represent an immediate early response gene to hypoxia and stress.^{106,107} Intestinal ischemia results in tissue damage and reperfusion of ischemic intestinal tissues leads to a burst in free radical formation, causing further tissue injury. The Besner laboratory has established a protective role for HB-EGF in the intestinal ischemia/reperfusion response. HB-EGF expression increases after in vitro intestinal epithelial cells anoxia/reoxygenation injury and after intestinal ischemia/reperfusion injury.¹⁰⁸ In rats, HB-EGF supplementation enhanced and accelerated enterocyte restitution following intestinal ischemia/reperfusion injury. This early response to injury was inhibited with blocking of the EGFR.¹⁰⁹ HB-EGF has been demonstrated to preserve crypt cell proliferation and epithelial cell integrity, and decrease bacterial translocation following ischemia/reperfusion injury.¹¹⁰ Similarly, HB-EGF protects intestinal stem cells from hypoxic injury and promotes intestinal stem cell activation and survival.¹¹¹ Work from other laboratories suggests that HB-EGF is also implicated in regulating cell proliferation and apoptosis in response to ischemia/reperfusion injury.¹¹²

Ischemia is implicated as a contributing factor to the pathogenesis of NEC and the protective effects of HB-EGF have been seen following intestinal ischemia/reperfusion injury in animal models of NEC.¹⁰⁶ A neonatal rat model of NEC demonstrated a higher incidence of NEC in animals exposed to hypothermia, hypoxia, hypertonic feedings plus

lipopolysaccharide as compared to breast milk. Administration of HB-EGF in this model reduced the incidence and severity of NEC, and extended survival rate and survival time.¹⁰⁶ HB-EGF supplementation increased enterocyte restitution and proliferation, decreased intestinal permeability, and reduced rates of apoptosis.¹¹³⁻¹¹⁵ As well, HB-EGF resulted in preservation of microvascular blood flow.¹¹⁶ Overexpression of HB-EGF in mice results in decreased intestinal permeability and a decreased incidence of experimental NEC, effects that are inhibited with administration of a HB-EGF antagonist.¹¹⁷ Mice deficient in HB-EGF have significantly increased intestinal permeability and a higher incidence of experimental NEC, findings that are reversed with administration of exogenous HB-EGF.¹¹⁸ Recently, the Besner laboratory has demonstrated that HB-EGF acts synergistically with administered mesenchymal stem cells (MSC) in a newborn rat model of NEC. Rat pups who received both HB-EGF and MSC had decreased incidence of NEC, decreased intestinal permeability, and increased survival compared to rats who received HB-EGF or MSC alone.¹¹⁹

Both HB-EGF and EGF have been demonstrated to reduce the incidence of NEC in neonatal rat models, however, EGF has been shown to provide better protection at physiological doses, while HB-EGF protection requires pharmacological dosing.¹²⁰ HB-EGF production in a *Pichia pastoris* yeast system has been reported in preparation for human clinical trials.¹²¹

GLUCAGON-LIKE PEPTIDE 2

GLP-2 is a 33-amino acid peptide that regulates a wide range of actions on the intestine including growth, motility, nutrients, and blood flow. It is secreted from enteroendocrine L cells of the distal small intestine and colon in response to enteral nutrients, particularly fatty acids and glucose.^{122,123}

The full mechanism of GLP2 is not well understood. The GLP-2 receptor is a G-protein coupled receptor that is expressed in greatest concentration in the proximal bowel and decreases distally.¹²⁴ The receptor is localized to intestinal enteroendocrine cells, enteric neurons, and subepithelial myofibroblasts.¹²⁵⁻¹²⁹ However, the GLP-2 receptor is not found in crypt or villus enterocytes, suggesting that the GLP-2 requires downstream intermediaries to carry out its effects.¹³⁰ Earlier research has shown that the trophic effects of GLP-2 required the activation of enteric neurons.¹²⁷ In rat models of colitis, the anti-inflammatory effects of GLP-2 administration were abolished when a VIP antagonist was added. Additionally, immunohistochemical staining found GLP-2 activated VIP expressing neurons in the submucosa. This suggests that VIP is an important downstream mediator of GLP-2 initiated anti-inflammatory effects.¹³¹ However, exogenous administration to VIP knockout mice continued to demonstrate enhanced proliferation and bowel growth, indicating that VIP does not play a role in GLP-2 driven growth.¹³⁰

Another indirect mediator of the intestinal actions of GLP-2 is IGF-1, an enterotrophic factor that will be discussed later. IGF-1 was found to be required for GLP-2 induced small intestine growth as these effects were negated in IGF-1 knockout mice.¹³² Inducible deletion of the IGF-1 receptor has also been shown to abrogate any trophic effects of GLP-2.¹³³ Exogenous GLP-2 administration increases IGF1 mRNA levels in intestinal cells of rats and mice, and it has been found that GLP-2 may activate the subepithelial myofibroblasts to release IGF-1 via the PI3K/AKT pathway.¹³⁴⁻¹³⁶

While the exact role of GLP-2 in neonatal intestinal development remains unknown, it has been theorized that GLP-2 may have an important role in the ontogeny of the gut. The GLP-2 precursor, proglucagon, and prohormone convertase, the enzyme responsible for cleaving proglucagon into GLP-2, are both found in the intestine of rat fetuses.¹³⁷ GLP-2R

mRNA levels are significantly higher in the intestine of fetal/neonatal rats than in adult rats.^{138,139} Premature piglets are more responsive than neonatal piglets to exogenous GLP-2, demonstrating greater increases in the activity of BBM enzymes.¹⁴⁰ Similarly, more premature infants had greater levels of GLP-2 at baseline as well as a greater GLP-2 response after enteral feeding than infants born closer to term.¹⁴¹

The more immediate effects of GLP-2 involve slowing proximal motility as well as decreasing gastric emptying.¹⁴² However, over the long term, GLP-2 is an intestinotrophic factor and has been found to stimulate crypt cell proliferation, decrease epithelial apoptosis, enhance barrier function¹⁴³⁻¹⁴⁵ as well as increase the activity of brush border enzymes.^{139,140} Adult mice given subcutaneous injections of GLP-2 had increased small intestine weight and villus/crypt morphology.^{146,147} Piglets also had dose-dependent increases in intestinal weight, DNA/protein content, villus height, and decreased crypt/villus apoptosis with increased cell survival and proliferation.¹⁴⁸

Several studies have found that GLP-2 is an important regulator of nutrient absorption.^{139,149} Cheeseman and Tsang found that rats given intravenous GLP-2 were found to have increased glucose uptake as well as increased SGLT-1 protein.^{149,150} Similarly, other studies have found increased rates of fructose absorption with increased GLUT2 expression as well as increased SGLT1.¹⁵¹ GLP-2 has also been implicated in increasing mesenteric blood flow.¹⁵² TPN-fed piglets given intravascular infusions of GLP-2 demonstrated increased portal drained visceral blood flow, increased intestinal blood flow volume, as well as increased nitric oxide synthase activity.¹⁵³

While there are no specific publications regarding the use of GLP-2 in the context of NEC, there have been many studies with GLP-2 that have demonstrated its intestinal protective effects. GLP-2 has been beneficial in inflammatory bowel disease and is associated with increasing rates of remission and ability to taper steroids.¹⁵⁴ Mice with DSS-induced colitis given exogenous GLP2 also demonstrated preserved mucosal integrity, increased intestinal mass, and increased proliferation.¹⁵⁵ Increased proliferation and decreased apoptosis were seen in mice with indomethacin-induced enteritis.¹⁵⁶

GLP-2 is also believed to play an important role in resection-induced adaptation responses, as levels are increased immediately after resection.¹⁵⁷ Subcutaneous GLP-2 administration in rats after small bowel resection led to increases in mucosal mass.¹⁵⁸ Enhanced adaptation was also seen in GLP-2 treated rats after resection in the absence of enteral nutrition.¹⁵⁹ In infants with short bowel syndrome, GLP-2 levels correlated with length of remnant bowel as the shortened intestines had very low GLP-2 levels. Additionally, higher GLP-2 levels also correlated with the ability to better tolerate enteral feeding.¹⁶⁰ The ability of infants to wean from of TPN corresponded with higher GLP-2 levels.^{160,161} Patients with intestinal failure treated with GLP-2 have also demonstrated improved nutrient absorption and increased body weight.¹⁶²

GLP-2 has also been found to attenuate intestinal injury in necrotizing pancreatitis¹⁴⁵, burns¹⁶³, and ischemia-reperfusion injuries.^{129,164,165} Mice given GLP-2 demonstrated enhanced barrier function^{144,166} as well as decreased bacterial translocation.¹⁵⁶ Given its wide protective effects, GLP-2 may be a promising adjunct to the prevention or therapy of NEC.

GROWTH HORMONE

Growth Hormone (GH) is a 22kDA anabolic protein that is synthesized in the anterior pituitary and released into the systemic circulation with multiple tissue targets. GH has a significant role in postnatal growth as well as lipid and carbohydrate metabolism.^{167,168}

The GH receptor is a type 1 cytokine receptor that signals tyrosine kinase JAK2 pathways. GH receptors are expressed throughout the small and large intestine in the muscularis propria, submucosa, muscularis mucosa, lamina propria, and epithelial layers.¹⁶⁹ The widespread presence of GH receptors in the intestine suggests a direct effect of GH on growth.¹⁷⁰ In addition to direct stimulation on intestinal growth, the downstream effects of GH include the activation of STAT transcription factors leading to increased expression of IGF-1. Another intestinotrophic growth factor, IGF-1 is believed to be an important mediator for GH in the intestine.^{171,172}

The enterotrophic effects of GH have been demonstrated in transgenic mice with overexpression of GH.¹⁷³ Similarly, rats given GH with glutamine and/or high protein diet after small bowel resection demonstrated increased villus height/crypt depth, positive nitrogen balance, and bowel growth.^{174,175}

The potential benefits of GH in NEC have not been well established. One prospective study found that neonates who had surgery for NEC or gastroschisis and were randomized to receive GH had improved lipid utilization.¹⁷⁶ However, GH has been shown to enhance intestinal repair in various inflammatory states. When exposed to DSS-induced colitis, these mice also had increased survival, decreased inflammation, and increased crypt cell proliferation.¹⁷⁷ These same effects were seen in mice exposed to TNBS-induced colitis.¹⁷⁸ In the pediatric population, a randomized control trial studied the effects of patients with Crohn's disease who were treated with corticosteroids and GH or corticosteroids alone. While no differences in mucosal healing were seen by endoscopic evaluation, the GH group was found to have higher rates of clinical remission. The GH group also received the added benefit of increased linear growth. GH was well tolerated without any notable deleterious side effects.¹⁷⁹

Work in the laboratory has shown signs that GH enhances adaptation after small bowel resection, yielding mucosal hyperplasia, increased absorption, as well as increased length.^{180,181} GH has also yielded modest results in clinical trials with SBS patients. TPN dependent patients with SBS given recombinant GH were subsequently found to have increased absorption of nitrogen, carbohydrates, and fat.¹⁸² Other studies have found SBS patients to have increased absorption when GH was given with glutamine and a high protein, low-fat diet.^{183,184} However, a randomized open-label study in TPN dependent children with SBS found that GH did not improve weaning off TPN.¹⁸⁵

INSULIN-LIKE GROWTH FACTOR-1

IGF-1 is a small 70 amino acid polypeptide synthesized primarily in the liver but also in the gastrointestinal tract. IGF-1 is found in the fetal intestine and in human milk, suggesting a role in intestinal development.¹⁸⁶⁻¹⁸⁸ The IGF-1 Receptor (IGF-1R) is a transmembrane tyrosine kinase receptor with structural homology to insulin receptors. Activation of IGF-1R leads to autophosphorylation and activation of signaling cascades such as IRS-1/PI3K/AKT and GRB2/Ras/ERK pathways.¹⁸⁹ IGF-1 is regulated by GH, insulin, and caloric intake.¹⁹⁰

IGF-1 increases intestinal cell proliferation and increases intestinal growth.^{7,8,191-195} It is a key mediator of other trophic factors, namely GH and GLP-2.^{132-136,171,172,196} When oral IGF-1 was given to piglets, increased nutrient absorption, mucosal growth, intestinal weight, protein and DNA was observed.^{197,198}

IGF-1 can also demonstrate protective effects during inflammation and cytokine driven apoptosis.¹⁹⁹⁻²⁰¹ IGF-1 expression is increased in the intestine in animal models of IBD and in patient's with Crohn's.¹⁹⁵ In animal models of sepsis as well as in human patients,

decreased levels of IGF-1 correlate with increased bacterial translation. Further, exogenous IGF1 administration in mice was associated with decreased rates of translocation.²⁰²

Oxidative stress activates the cellular protective phosphatidylinositol 3-kinase (PI3-K) pathway. Additionally, activation of this pathway can be enhanced by exogenous IGF-1 administration during NEC and promotes cell survival.²⁰³⁻²⁰⁵ Premature neonates with persistently low levels of serum IGF-1 were found to be at increased risk of NEC.²⁰⁶

Baregamian et al. found that intestinal IGF-1R expression was elevated in vivo in a mouse pup model of NEC as well as in vitro rat and fetal human intestinal cell lines exposed to oxidative stress. Additionally, increased IGF-1R phosphorylation was observed in model intestinal epithelial cells while inhibition of IGF-1R lead to increased cellular apoptosis.²⁰⁷ Another study using a hypoxia/reoxygenation model for NEC in mice found that IGF-1 administration resulted in attenuated intestinal injury with decreased necrosis and decreased apoptotic indexes.²⁰⁸

GRANULOCYTE COLONY STIMULATING FACTOR

G-CSF is an 18.8-kDa glycoprotein involved in the regulation of neutrophil production. Neutrophils are key players in the human inflammatory and immune response, dysregulation of which has been implicated in the pathogenesis on NEC. G-CSF is found in amniotic fluid and human milk in physiologically significant concentrations.²⁰⁹⁻²¹¹ Consistent with the increased risk of NEC in premature infants, the concentration of G-CSF in cord blood of term infants is higher than that of preterm infants, and although concentrations in preterm infants increased under conditions of perinatal stress or infection, the increase in concentration was not to the degree observed in term infants.²¹² G-CSF receptors are expressed on both neutrophils and nonhematopoietic cells, including fetal and neonate enterocytes.²¹⁰⁻²¹³ Following mucosal injury a differential immune response is seen in adult and neonatal murine models of colitis, with neutrophil infiltration to the intestine seen in adult models of enterocolitis, and macrophage infiltration to the intestine in neonatal models.²¹⁴ Depletion of neutrophils has been shown to exacerbate NEC in animal models.²¹⁵

G-CSF has been established to be protective to the intestinal epithelium. In rats treated with the chemotherapeutic agent Paclitaxel addition of G-CSF resulted in decreased villus atrophy and enterocyte apoptosis, and decreased intestinal bacterial translocation.²¹⁶ In a rat NEC model, administration of G-CSF prevented mucosal injury.²¹⁷ A small pilot study of enteral G-CSF administered to preterm infants with stage I NEC reported shorter time to resolution of clinical and radiological signs of disease and prevention of disease progression following G-CSF treatment as compared to placebo controls.²¹⁸

ERYTHROPOIETIN

Epo is a 30.4-kDa glycoprotein, secreted by the fetal liver and adult kidney in response to anemia.²¹⁹ In addition to stimulating erythropoiesis, Epo has been implicated in immunologic modulation, anti-inflammatory effects, improved wound healing, and reduction in hypoxia induced apoptosis.²²⁰ Epo is present in amniotic fluid and breast milk.²²¹⁻²²⁶ Functional Epo receptors are present in fetal and postnatal small bowel.^{227,228} Fetal human enterocytes have been shown to decrease secretion of pro-inflammatory IL-8 in vitro in response to Epo, perhaps offering a protective effect in the setting of NEC.²²⁹ In a rat hypoxia NEC model, pretreatment with intraperitoneal Epo limited mucosal injury and resulted in decreased levels of the proinflammatory mediator nitric oxide.²³⁰ Oral Epo supplementation has also been shown in rat models to protect intestinal barrier function and reduce the incidence of NEC.²³¹ A retrospective cohort study of very low birth weight

infants given recombinant Epo for prevention or treatment of anemia demonstrated that the incidence of NEC was lower in those infants who received recombinant Epo.²³²

INTESTINAL TREFOIL FACTOR

The trefoil factor family (TFF) is a group of 7- to 14-kd polypeptides that are secreted by mucus producing epithelial cells, predominantly in the gastrointestinal tract. Three mammalian TFF members have been identified; TTF1 expressed by surface and pit mucus cells in the stomach, TTF2 expressed by mucus neck and glandular mucus cells of the stomach and Brunner's glands of the proximal duodenum, and TTF3 (also known as ITF) expressed by goblet cells of the intestine and colon.²³³ Trefoil peptides are established to be enterocyte protective and promote healing in response to gastrointestinal damage. Trefoil peptide expression is upregulated in ulcerative conditions such as peptic ulcer disease and inflammatory bowel disease.²³⁴⁻²³⁷ ITF promotes epithelial cell migration and inhibits apoptosis.^{238,239} TTF2 supplementation has been shown to protect the intestinal epithelium in rat models of colitis.²⁴⁰ ITF administration has been shown to prevent and heal injury from acute DSS-induced colitis and well as radiation and chemotherapy induced intestinal mucositis.^{241,242} ITF deficiency in mice is associated with extreme sensitivity to mucosal injury and failure to undergo mucosal repair.²⁴³ ITF has been demonstrated to be relatively deficient in premature rats, suggesting a possible role in the development on NEC in premature infants.²⁴⁴ Administration of ITF in a rat model of NEC reduced the severity of intestinal injury.²⁴⁵

KERATINOCYTE GROWTH FACTOR

KGF is a member of the fibroblast growth factor family and stimulates growth and differentiation of epithelial cells.²⁴⁶ Exogenous KGF supplementation in rats results in epithelial cell proliferation and induction of mucin-producing goblet cells.²⁴⁷ KGF and its receptor are present in the human fetal gastrointestinal tract and in vitro stimulation of human fetal enterocytes with KGF results in cellular proliferation.²⁴⁸ Expression of KGF is increased in surgical patients with inflammatory bowel disease and correlates with the degree of intestinal inflammation.²⁴⁹ In animal models of colitis, KGF administration reduces the extent of mucosal injury.²⁵⁰ KGF gene therapy in rats ameliorated acid-induced ulcerative colitis.²⁵¹ KGF supplementation decreases total parenteral nutrition related enterocyte apoptosis and results in increased expression of the antiapoptotic Bcl-2 protein.²⁵² In animal models of radiation and chemotherapy intestinal injury, KGF pretreatment increased enterocyte survival and mucosal thickness.^{253,254} Deficiency of KGF in mice is associated with severe colonic inflammation and delayed tissue repair in dextran sodium sulfate induced colitis.²⁵⁵ Recombinant KGF treatment has been studied in ulcerative colitis patients. In human phase II studies, recombinant KGF failed to induce remission in ulcerative colitis patients, however the maximal treatment dose may have been targeted too low for a beneficial effect to be seen. Importantly, no significant adverse effects of KGF treatment as compared to placebo were observed.²⁵⁶ While the intestinal protective effects of KGF are established, the role of KGF in NEC is currently unknown.

HEPATOCYTE GROWTH FACTOR

HGF is 94kDa glycoprotein that regulates cellular proliferation, survival, and angiogenesis in many different cells through MET receptor tyrosine kinase.^{257,258} It is present in breast milk as well as in fetal intestinal tissue.^{259,260} HGF is secreted by mesenchymal cells in an inactive form and is subsequently activated by HGF activator (HGFA), which is synthesized primarily in the liver and to a lesser extent in the intestine.^{261,262}

HGF plays an active role in the repair of injured intestinal tissue,²⁶²⁻²⁶⁶ however the mechanism is poorly understood. In response to tissue injury myofibroblasts, platelets, and

neutrophils were all found to release HGF which then interacts with receptors on the basolateral membrane of epithelial cells.²⁶⁷⁻²⁶⁹ HGF-deficient mice are embryonically lethal^{270,271}, while HGFA-deficient mice are born without any apparent abnormalities. However when exposed to DSS or acetic acid, HGFA-deficient mice experience increased mortality with impaired regeneration of injured colonic mucosa.²⁷²

After intestinal resection, HGF has been shown to increase epithelial proliferation as well as increase protein concentration and mucosal DNA content.^{273,274} In animal model of burns, HGF treatment leads to increased intestinal proliferation and preserved villus structures.²⁷⁵ Rats with DSS-induced colitis given intraperitoneal administration also had decreased weight loss, increased epithelial regeneration, and increased cellular proliferation.²⁷⁶ Rats with TNBS-induced colitis given intravenous HGF also demonstrated decreased ulcer coverage and increased epithelial proliferation.²⁷⁷

HGF inhibits intestinal epithelial apoptosis after ischemic reperfusion injury.²⁷⁸ Meldrum et al. used a NEC model with fetal small intestine epithelial cells that were exposed to hypoxic injury. These cells were then cultivated in mesenchymal stem cell-conditioned media and were found to have enhanced viability and proliferation with the supernatants from the cellular media to have increased levels of IL-6, VEGF, and HGF. This suggests that mesenchymal stem cells may release these factors in a paracrine manner to enhance cellular survival.²⁷⁹ However, transgenic mice who over-express HGF were found to have an increase in benign and malignant liver and mammary gland tumors.²⁸⁰⁻²⁸³ Thus, further research is needed to assess the safety of HGF as potential therapeutic agents in humans.

CONCLUSION

Growth factors play an important role in both the development of the gastrointestinal tract and the response to injury. These factors play important roles as mediators of cellular proliferation, migration, differentiation, and survival. In both the fetal and postnatal environment, the developing intestine is exposed to EGF, HB-EGF, GH, IGF, GLP-2, G-CSF, Epo, ITF, KGF, and HGF through luminal exposure to amniotic fluid and human milk. While the exact pathogenesis of NEC is unknown, the trophic and protective effects of growth factors in the intestine are clear. Recent evidence suggests that such factors may act synergistically to prevent intestinal injury.^{13,119,183,284-288} Further studies are warranted to test the possibility that supplementation of various factor(s) may prevent the development of this devastating disease.

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Table 1

Intestinal effects of growth factors as listed by references

	Intestintrophic	Increased Proliferation	Decreased Apoptosis	Increased Healing after injury/inflammation	Enhanced adaptation after resection or improved short gut syndrome	Restore barrier function/decrease bacterial translocation	Increased Blood flow
EGF	10-14, 21, 22, 49	22, 31	82-84	17, 25, 35-47, 49, 50, 60, 61, 66-68, 81, 98, 99	52-59, 66	48, 63, 72, 73	
HB-EGF		110, 112-115	112-114	103-115, 117, 119, 121		110, 113-114, 117-119	116
GLP-2	139, 143-148	143-145, 147, 148, 155, 157	148, 156	129, 154-156, 163-165	157-162	143-145, 156, 166	152, 153
GH	170, 171, 173	171, 177, 178		177-179	174-176, 180-184		
IGF-I	190-198		198-200, 203, 204, 207, 208	195, 199-201, 203-208	196	202	
G-CSF			216	216-218		216	
EPO				228-232		231	
ITF			238, 239, 243	234-237, 240-243, 245			
KGF		247, 248	252	249-251, 253-256			
HGF		265, 266, 273, 27-277	278	262-266, 270-278	273, 274		