

Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders¹⁻⁴

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ABSTRACT Colostrum is the specific first diet of mammalian neonates and is rich in immunoglobulins, antimicrobial peptides, and growth factors. In this article we review some of these constituents of human and bovine colostrum in comparison with those of mature milk. Recent studies suggest that colostrum fractions, or individual peptides present in colostrum, might be useful for the treatment of a wide variety of gastrointestinal conditions, including inflammatory bowel disease, nonsteroidal antiinflammatory drug-induced gut injury, and chemotherapy-induced mucositis. We therefore discuss the therapeutic possibilities of using whole colostrum, or individual peptides present in colostrum, for the treatment of various gastrointestinal diseases and the relative merits of the 2 approaches. *Am J Clin Nutr* 2000;72:5-14.

KEY WORDS Gastrointestinal tract, gastrointestinal disease, intestinal injury, repair, colostrum, milk, peptide growth factor, nutrition, nonsteroidal antiinflammatory drugs, review

INTRODUCTION

Colostrum is the first milk produced after birth and is particularly rich in immunoglobulins, antimicrobial peptides (eg, lactoferrin and lactoperoxidase), and other bioactive molecules, including growth factors. As is the milk that is subsequently produced, colostrum is important for the nutrition, growth, and development of newborn infants and contributes to the immunologic defense of neonates. The composition of mammary secretions changes continuously throughout the suckling period; however, for the purposes of this review we define colostrum as the milk produced in the first 48 h after birth.

Recent studies suggest that the peptide growth factors in colostrum might provide novel treatment options for a variety of gastrointestinal conditions. We initially provide a brief overview of the control of gut growth and the constituents of human and bovine colostrum. Next, we focus on the peptide growth factor constituents of colostrum and how their concentrations vary from those of the later occurring, mature milk. In the final section, we discuss the possibilities of using whole colostrum or individual peptides in the colostrum for the treatment of various gastrointestinal diseases and the relative merits of the 2 approaches. Because of the broad nature of these topics, the reader is referred to appropriate reviews of specified topics throughout the text.

OVERVIEW OF THE CONTROL OF GUT GROWTH AND REPAIR

The bowel shows a remarkable ability to respond to changes in dietary intake. Fasting results in marked atrophy of the intestine and this process can be rapidly reversed by refeeding. The molecular processes underlying these changes are poorly understood, although it has been proposed that humoral factors, local nutrition, and luminal growth factors are involved.

Hormonal factors

Cross-circulation experiments support the concept of circulating trophic factors influencing gut growth, although the identity of such factors remains unclear. Gastrin probably plays a role as a trophic factor for mucosal growth within the stomach and there is currently much interest in the role of glucagon-like peptide 2 (GLP-2) because systemic infusion of GLP-2 was shown to result in a general trophic response within the gut (1). In contrast, early enthusiasm for a major trophic role for the gut hormones peptide YY and cholecystokinin within the gastrointestinal tract has diminished because of the absent or weak response in gut growth when recombinant forms of the hormones are infused. A general review of the actions of gastrointestinal hormones and their actions is provided by Walsh (2).

Local nutrition

Circulating trophic factors are unlikely to explain regional variations in growth, as shown by studies using isolated loops of

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bowel or experiments involving ileojejunum transposition. Studies that showed direct effects on growth when nutrients are administered intraluminally to isolated loops (eg, 3) support the concept of the "luminal workload hypothesis." It is important to note, however, that not all studies showed a positive result, ie, hyperplasia of the loop (4).

Luminal growth factors

Peptide growth factors are constantly present in the gastrointestinal lumen, being secreted by glands, eg, epidermal growth factor (EGF) from the salivary glands, or ingested in foodstuffs such as milk and colostrum. The role of luminal growth factors in modulating intestinal growth in the normal adult gastrointestinal tract is, however, unclear because there is increasing evidence that the receptors for many of these peptides are restricted to the basolateral membranes of the mucosal cells, ie, are not present on the apical (luminal) membranes. The luminal ligands may therefore not be able to reach their receptor under normal circumstances in the adult nondamaged gut. This may not be the case, however, in the normal neonatal bowel or in the adult damaged gut because, in these conditions, the permeability of the bowel is increased. Furthermore, some studies have suggested that inflammation of the gastrointestinal tract, in conditions such as inflammatory bowel disease, might result in a shift in receptor distribution to include apical membranes (5). Some of these aspects are discussed in further detail later.

Role of peptides in the maintenance of mucosal mass and integrity

Tissue mass is dependent on the equilibrium established between cell production, migration, and loss (including apoptosis). Peptide growth factors in milk and colostrum can influence all of these aspects. For example, EGF stimulates cell proliferation and migration and also influences crypt fission, an identified mechanism by which new crypts are produced (6). Recent reports also suggested that peptides in colostrum and milk might influence the rate of programmed cell death (apoptosis) within the gut, acting via the *Fas/Fas* ligand (*FasL*) signaling system. *Fas* is a member of the tumor necrosis factor α -nerve growth factor receptor family and is expressed in various cells, including the gastrointestinal mucosa. Binding of *FasL* triggers apoptosis. The presence of soluble *Fas* in milk might therefore function as an alternative receptor site for any *FasL* produced within the mucosa by activated immune cells, thereby reducing the rate of mucosal apoptosis (7).

The gastrointestinal tract is constantly under attack from acid, proteolytic enzymes, and ingested noxious agents, such as aspirin or alcohol. The presence of multiple defense mechanisms—including the mucus-bicarbonate layer in the stomach, a rapid mucosal turnover, and a good blood supply—ensure that the mucosa remains intact most of the time. If a small area of injury is sustained, the healing process usually proceeds successfully via standard mechanisms. Surviving cells from the edge of the wound migrate over the denuded area to re-establish epithelial continuity. This process begins within a few minutes after injury and is termed *restitution*. This is followed by increased proliferation and remodeling, which begins \approx 24–48 h after the injury. Many factors, including peptide growth factors, stimulate these various processes and some of these are discussed below. Interested readers are referred to studies by Playford (8) and Murphy (9).

OVERVIEW OF TROPHIC FACTORS IN COLOSTRUM AND MILK

Colostrum and milk contain many factors that can influence cell growth, differentiation, and function. A full review of the influence of nutrients on gut growth and development is beyond the scope of this article but can be found in the review by Koletzko et al (10). Some of the major constituents of colostrum and milk that can interact with peptide growth factors are discussed briefly below.

Nonpeptide trophic factors

Several nonpeptide constituents of colostrum, when added to cells *in vitro* or when infused into animal models, have resulted in increased proliferation. These factors include glutamine, polyamines, and nucleotides. It is debatable whether these factors should be considered growth factors *per se* because the increased proliferation is not mediated by the classic receptor-ligand, secondary messenger system. Factors such as glutamine are therefore often referred to as preferred substrates. Nevertheless, these factors play an important role in maintaining gastrointestinal mucosal mass and modulating the immune system via multiple mechanisms, eg, altering intestinal flora and influencing the actions of growth factors. For example, the trophic response of EGF on the rat small intestinal cell line IE6 requires the presence of glutamine within the medium (11). These subject areas are reviewed further by Levy (12) and Carver and Barnes (13).

Hormones

It is well established that milk and colostrum contain many hormones, which, when infused systemically, influence a wide variety of end-organ systems. These systems include the hypothalamic-hypophyseal system (because milk contains prolactin, somatostatin, oxytocin, and luteinizing hormone-releasing hormone), thyroid gland (because milk contains thyroid-stimulating hormone, thyroxine, and calcitonin), sexual glands (because milk contains estrogen and progesterone), and adrenal and pancreatic glands. It is probable that at least some of these hormones (eg, luteinizing hormone-releasing hormone) influence plasma concentrations and the development of various end organs of suckling neonates (14) because of the passage of the hormones through the bowel wall into the systemic circulation. These hormones are likely to be less influential in adults because the lower permeability of the adult bowel is likely to restrict passage of most of these factors. However, it is important to appreciate that when these factors are administered to adult patients with a damaged bowel, eg, those with celiac or Crohn disease, the increased bowel permeability associated with these conditions might allow these hormones to reach their receptors and mediate pathophysiologic effects. Readers interested in the physiologic significance of hormones in milk in relation to neonatal development and the effect of hormones on milk production are referred to the work of Koldovsky (15, 16).

Cytokines

The protein molecules known as cytokines have a broad range of cellular function and are active in picomolar to nanomolar concentrations. In general, cytokines do not regulate normal cellular homeostasis but alter cellular metabolism during times of perturbation, eg, in response to inflammation (17).



Cytokines trigger acute cellular responses, such as chemotaxis, protein synthesis, and cellular differentiation. Colostrum and milk contain many cytokines, including interleukin (IL) 1 β , IL-6, IL-10, tumor necrosis factor α , and granulocyte, macrophage, and granulocyte-macrophage colony-stimulating factors. It is likely that in newborn animals and infants, these factors play an important role in modulating immunologic development, working in combination with the ingested maternal immunoglobulins and the nonspecific antibacterial components, such as lactoperoxidase, in colostrum.

Although cytokines and growth factors are often considered to be separate entities, it is important to appreciate that the distinction between them is sometimes blurred. For example, IL-8 has been shown to stimulate migration of the human colonic epithelial cell line LIM 1215 (18), an effect that is usually attributed to growth factors such as EGF and transforming growth factor (TGF) β . In addition, some studies have shown “cross-talk” between cytokines and growth factors. For example, Yasunaga et al (19) examined the molecular mechanisms underlying *Helicobacter pylori* (*H. pylori*)-induced gastric hyperproliferation in patients with large-fold gastritis. The presence of *H. pylori* caused the gastric mucosa to release the cytokine IL-1 β , which in turn resulted in the local production of hepatocyte growth factor. Further information regarding the functions of cytokines within the gastrointestinal tract can be found in a review by Przemioslo and Ciclitira (20), and a useful review of the cytokine constituents of human milk and their importance in the development of the neonatal immune system was published by Garofalo and Goldman (21).

Growth factors

Growth factors are so called because historically they have been identified by their ability to stimulate the growth of various cell lines in vitro but, in reality, the functions of these peptide-based molecules are considerably more diverse. Different names have been ascribed to molecular species as they have been identified. As characterization has become more sophisticated, however, it is apparent that some of these differently named species are structurally and functionally similar and may, in fact, be identical. Although there are many similarities among species, there are also marked species differences in the nature and concentration of growth factor constituents, eg, human colostrum has much higher concentrations of EGF than does the bovine equivalent, whereas the reverse is true for insulin-like growth factor (IGF) I and II. Further details of individual peptides that form the major peptide growth factor constituents of colostrum and milk are given in the next section.

MAJOR PEPTIDE GROWTH FACTOR CONSTITUENTS OF COLOSTRUM AND MILK

Epidermal growth factor receptor ligand family

This group of polypeptides, with the common property of binding to the EGF receptor (also known as the c-erb1 receptor), includes EGF itself, TGF- α , mammary-derived growth factor II (MDGF-II), and human milk growth factor III (HMGF-III), which might be the same molecule as EGF (*see below*). Other related polypeptides with these binding characteristics, but that are not present in significant concentrations in colostrum, are amphiregulin, betacellulin, and heparin-binding EGF (for a more comprehensive review of these peptides *see reference 22*).

Epidermal growth factor

EGF is a 53-amino acid peptide produced by the salivary glands and the Brunner's glands of the duodenum in adults. EGF is present in human colostrum (200 $\mu\text{g/L}$) and milk (30–50 $\mu\text{g/L}$) and in many other species but is not found in significant amounts in bovine secretions (23), although related molecules have been identified and characterized. In vitro experiments using gastric juice from preterm infants indicate that milk-borne EGF is not deactivated under typical gastric proteolytic conditions (24). In contrast, we showed that adult gastric juice digests EGF_{1–53} to an EGF_{1–49} form that has only 25% of the biological activity of the intact EGF molecule (25). Once EGF enters the small intestine, it is susceptible to proteolytic digestion under fasting conditions but is preserved in the presence of ingested food proteins (26).

There is controversy over the physiologic function of EGF in the gastrointestinal lumen under normal (nondamaged) conditions. Most studies examining the distribution of EGF receptor in the normal adult human gastrointestinal tract showed it to be present only on basolateral membranes and not on the apical (luminal) surfaces (27). The distribution of the EGF receptors might, however, vary between species, eg, autoradiographic studies identified apical receptors in the pig intestine (28). If EGF receptors are distributed only on the basolateral membranes of the normal adult human gut, then EGF in the intestinal lumen is unlikely to exert any biological activity, except at sites of injury. Evidence in favor of this role for EGF include the finding that rats that have had their salivary glands removed do not develop spontaneous ulcers or atrophy of the gut. However, compared with control animals, they do develop more extensive ulceration with diminished repair if artificial ulcers are induced (29). This has led to the suggestion that EGF acts as a “luminal surveillance peptide” in the adult gut, readily available to stimulate the repair process at sites of injury (8). It is important to note, however, that luminal EGF might gain access to basolateral receptors in the immature neonatal gut (30) because of its increased permeability. The EGF in colostrum and milk may therefore play a role in preventing bacterial translocation (31) and stimulating gut growth in suckling neonates.

Transforming growth factor α

TGF- α is a 50-amino acid molecule that is present in human colostrum and milk at much lower concentrations [2.2–7.2 $\mu\text{g/L}$ (32)] than is EGF. In contrast with EGF, TGF- α is produced within the mucosa throughout the gastrointestinal tract (33). Systemic administration of TGF- α stimulates gastrointestinal growth and repair, inhibits acid secretion, stimulates mucosal restitution after injury, and increases gastric mucin concentrations (22).

Within the small intestine and colon, TGF- α expression occurs mainly in the upper (nonproliferative) zones, which suggests that its physiologic role may be to influence differentiation and cell migration rather than cell proliferation. TGF- α may therefore play a complementary role to that of TGF- β (*see below*) in controlling the balance between proliferation and differentiation in the intestinal epithelium (34). Up-regulation of TGF- α expression has been shown to occur in the gastrointestinal mucosa at sites of injury as well as in the liver after partial hepatectomy, supporting a role for TGF- α in mucosal growth and repair (35). Further evidence for this role comes from research in mice that have had the TGF- α gene “knocked out” by homologous recombination. These rats have a relatively normal phenotype under control conditions but an increased sensitivity to colonic (36),



although not small intestinal (37), injury. These findings support the role of TGF- α in maintaining epithelial continuity but suggest that the relative importance of peptides such as this might vary from one region of the gut to another. Taken together, most studies suggest that the major physiologic role of TGF- α is to act as a mucosal-integrity peptide, maintaining normal epithelial function in the nondamaged mucosa (8).

Other peptides within this family are MDGF-II (38) and HMGF-III. HMGF-III has a molecular mass of ≈ 6 kDa and is the predominant growth factor in human milk, accounting for $\approx 75\%$ of total mitotic activity (39). There is uncertainty as to whether HMGF-III is a distinct molecule or is, in fact, the same as EGF.

Transforming growth factor β family

This family of molecules is structurally distinct from TGF- α and, in most systems, actually inhibits proliferation. There are ≥ 5 different isoforms of TGF- β and their major site of expression in the normal gastrointestinal tract is in the superficial zones, where they may inhibit proliferation once the cells have left the crypt region (34). TGF- β has many diverse functions; it is a potent chemoattractant for neutrophils and stimulates epithelial cell migration at wound sites (40). It is therefore likely to be a key player in stimulating restitution, the early phase of the repair process during which surviving cells from the edge of a wound migrate over the denuded area to reestablish epithelial continuity. TGF- β and TGF- β -like molecules are present in high concentrations in both bovine milk (1–2 mg/L) and colostrum (20–40 mg/L). These concentrations are sufficient to prevent indomethacin-induced gastric injury in rats (41), suggesting that the TGF- β in colostrum may be a key component in mediating its ability to maintain gastrointestinal integrity in suckling neonates. A TGF- β -like milk growth factor has been described as being associated with the casein fraction of cow milk; this has since been shown to be a mixture of TGF- β 1 and TGF- β 2, predominantly the β 2 form (85%) (42).

Insulin-like growth factors (somatomedins) and their binding proteins

IGF-I and IGF-II promote cell proliferation and differentiation (43). They are similar in structure to proinsulin and it is possible that they also exert insulin-like effects at high concentrations. The liver is a major site of IGF synthesis (44); IGF-I and IGF-II are both also expressed in particularly high amounts in the developing human fetal stomach and small intestine, with expression reaching a maximum soon after birth (45).

Bovine colostrum contains much higher concentrations of IGF-I than does human colostrum (500 compared with 18 $\mu\text{g/L}$) (46, 47), with lower concentrations in mature bovine milk (10 $\mu\text{g/L}$) (48). These growth factors are relatively stable to both heat and acidic conditions. They therefore survive the harsh conditions of both commercial milk processing and gastric acid to maintain their biological activity (49). IGF-I is known to promote protein accretion, ie, it is an anabolic agent (50) and is at least partly responsible for mediating the growth-promoting activity of growth hormone (GH). IGF-II is present in bovine milk and colostrum at much lower concentrations than is IGF-I, but like IGF-I, it has anabolic activity and has been shown to reduce the catabolic state in starved animals (51).

IGFs in bovine and human colostrum and milk are present in both free and bound forms. The amount of free IGF varies during the perinatal period, with most of the IGF-I in bovine colostrum

being present in the free form (ie, not associated with its binding protein), whereas the reverse is true in the antepartum period and in mature milk (52). Six IGF binding proteins (IGFBPs) have been identified and cloned. It was initially thought that the main function of IGFBPs was to act as carrier proteins, reducing the proteolytic digestion of IGF and limiting its biological activity because only the free forms of IGF are thought to have any major proliferative activity. Additional roles for IGFBPs have been suggested because it has been shown that different IGFBPs have distinct patterns of distribution in different tissues and their concentrations are altered in response to hormonal or nutrient status. Examples include the findings that administration of dexamethasone to rats increases hepatic production of IGFBP-1 (53) and that malnutrition of neonatal rats decreases serum IGF-I and IGF-II but increases serum IGFBP-2 (54). The detailed functions of IGFBPs are unclear, although it is probable that one of the roles of secreted or soluble IGFBP is to inhibit IGF-mediated proliferation or amino acid uptake by limiting the availability of free IGF to bind to its receptors. Conversely, cell surface- and cell matrix-associated IGFBPs may potentiate the actions of IGF by increasing local concentrations of IGF-I and IGF-II next to their receptors. A detailed review of IGFBPs was published by Rechler (55) and a general review of the role of IGFs and IGFBPs was published by Lund and Zimmermann (44). Changes in the secretion and mammary uptake of IGF-related peptides in the peripartum period of dairy cows have also been described (56).

Platelet-derived growth factor

Platelet-derived growth factor (PDGF) is an acid-stable molecule that was originally identified from platelets but is also synthesized and secreted by macrophages. It consists of 2 disulfide-linked polypeptides: chain A (14 kDa) and chain B (17 kDa). The dimer, therefore, exists in 3 isoforms (AA, AB, and BB) that bind to tyrosine kinase-type receptors. PDGF is a potent mitogen for fibroblasts and arterial smooth muscle cells and administration of exogenous PDGF has been shown to facilitate ulcer healing when administered orally to animals. Although PDGF is present in human and bovine milk and colostrum, most of the PDGF-like mitogenic activity in bovine milk is actually derived from bovine colostrum growth factor, which shares sequence homology with PDGF (57, 58). A general review of the effects of PDGF were published by Szabo and Sandor (59).

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a homodimeric 34–42-kD heparin-binding glycoprotein with potent angiogenic, mitogenic, and vascular permeability-enhancing factors that is related to PDGF (60). VEGF is present in human breast milk at a concentration of ≈ 75 $\mu\text{g/L}$ during the first week of lactation, and concentrations fall to ≈ 25 $\mu\text{g/L}$ during the second postnatal week (61). Specific receptors for VEGF have been identified on the apical membranes of the human colonic cell line Caco-2 (61) and also on the human cell line H-4. Although VEGF bound to these cell lines, it did not induce a proliferative response (61). The pathophysiologic role of VEGF is therefore unclear, although its angiogenic activity may play an important role in the healing of conditions such as peptic ulceration.

Lactoferrin

Lactoferrin is an iron binding glycoprotein (80 kDa) that is present in human colostrum at a concentration of ≈ 7 g/L, with

mature milk having a lower concentration (≈ 1 g/L). Bovine milk also contains lactoferrin, but the concentration is only $\approx 10\%$ of that of human milk (≈ 0.1 g/L) (62, 63). Lactoferrin exerts multiple effects, including facilitating iron absorption and acting as an antimicrobial agent (64, 65). In addition, lactoferrin has been shown to stimulate the growth of various cell lines in vitro, including fibroblasts and intestinal epithelial cells (66), suggesting that its presence in milk may be important in regulating gut growth in developing neonates.

Growth hormone and its releasing factor

Growth hormone (GH), along with its releasing factor (GHRF) and binding protein (67), is present in human and bovine colostrum and milk. Human GHRF concentrations have been reported to be ≈ 41 ng/L in colostrum, falling to ≈ 23 $\mu\text{g/L}$ in mature milk (68). Suckling neonates have high circulating concentrations of GH, probably because of a combination of GH and GHRF ingestion, which stimulates the neonate to release GH from the pituitary gland (69). Many of the growth-promoting effects of GH are mediated by release of IGF-I (70), although GH may also have direct mitogenic effects (71). There is increasing evidence that systemic GH plays important modulatory roles in gut growth and function. GH receptors have been reported to be present throughout the human gastrointestinal tract (72) and transgenic mice that overexpressed GH had higher total body weights and heavier small intestines than did control (nontransgenic) mice (71). The importance of GH in the lumen, however, is unclear. It is not known whether GH receptors are present on the apical membranes of enterocytes. Further studies examining the effect of GH in adults and neonates, when given via the lumen, are required to determine the pathophysiologic significance of GH in milk and colostrum.

Other less-well-defined peptides

Bovine and human milk contain several other peptides whose structure and function are less clearly defined, including

- 1) MDGF-I, a 62-kDa peptide that has been shown to stimulate the growth of mammary cells and enhance collagen production (73);
- 2) HMGF-I and -II, acidic polypeptides that are poorly characterized (74);
- 3) bovine colostrum growth factor, a 35-kDa molecule responsible for most of the mitogenic activity of bovine colostrum that appears to be biochemically similar to HMGF-II and possibly to PDGF (57, 58); and
- 4) other bovine MDGFs, such as b-MDGF-I, which has a molecular mass of ≈ 30 kDa and exhibits EGF-like activity, and b-MDGF-II, which is larger (50–150 kDa) (75).

Several other peptides reportedly exist; however, some of these were shown subsequently to be highly homologous with known existing molecules, whereas for others, the details of structure and function have not been elucidated. It is likely, however, that over the next few years, additional novel potent growth factors with clinical potential will be identified within colostrum and milk (76).

CLINICAL APPLICATIONS FOR THE GASTROENTEROLOGIST

Esophagitis and *H pylori*-related disease

Colostrum, milk, and recombinant peptides are unlikely to be of major clinical value for the treatment of reflux esophagitis or

H pylori-induced peptic ulceration. This is because acid-suppressant therapies, particularly proton-pump inhibitors, are highly efficacious and cheap (compared with recombinant peptides). Furthermore, standard *H pylori*-eradication regimens, usually consisting of a proton-pump inhibitor and 2 antibiotics for 7 d, have an eradication success rate of $>90\%$ and effectively provide a life-long cure for *H pylori*-induced peptic ulceration. There are, however, many serious gastrointestinal pathologies for which novel therapies might prove useful; these pathologies are discussed below.

Short-bowel syndrome

Some patients have an insufficient length of bowel to digest and absorb food adequately, usually as a result of massive intestinal resection for vascular insufficiency or after repeated operations for inflammatory bowel disease. Current therapeutic options are unpleasant and associated with a high risk of morbidity or mortality, eg, long-term parenteral (intravenous) feeding and small-bowel transplantation. Strategies to optimize the function of residual bowel and ultimately wean patients off total parenteral nutrition would therefore be of great benefit. There is evidence that growth factors could be instrumental in achieving this goal; eg, systemic administration of individual growth factors such as EGF have been shown to stimulate bowel growth in rats receiving total parenteral nutrition (77). In addition, oral administration of EGF helped restore glucose transport and phlorizin binding in rabbit intestines after jejunal resection (78), and colostrum supplementation of piglet feeding regimens resulted in a significant increase in intestinal proliferation (79). Colostrum supplementation may be of particular value in young children who have undergone intestinal resection because gut adaptation is more likely during early childhood than it is in adulthood.

Nonsteroidal antiinflammatory drug-induced gut injury

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely prescribed and are effective in the treatment of musculoskeletal injury and chronic arthritic conditions. Nevertheless, $\approx 2\%$ of subjects taking NSAIDs for 1 y suffer from gastrointestinal adverse effects, including bleeding, perforation, and stricture formation of the stomach and intestine (80). Acid suppressants and prostaglandin analogues have been shown to be effective in reducing gastric injury induced by NSAIDs but are less effective in preventing small intestinal injury. Novel therapeutic approaches to deal with these problems, such as the use of recombinant peptides, are therefore still required. A recent series of in vivo and in vitro studies support this idea; EGF (25) and TGF- α and TGF- β (81) have all been shown to reduce NSAID-induced gastric injury. The beneficial effects of recombinant growth factors on NSAID-induced small and large intestinal injury is, however, less well documented. It was shown recently that a defatted colostrum preparation, which is rich in the growth factors discussed earlier, reduced NSAID-induced gastric and intestinal injury in rats and mice (**Figure 1**) (81). This material was also shown to effectively reduce gastric erosions in human volunteers taking NSAIDs (J Hunter, personal communication, 1998). Further support for this approach comes from our recent finding that this defatted colostrum preparation reduced small intestinal permeability, which was used as a marker of intestinal damage in human volunteers taking clinically relevant doses of the drug indomethacin (82). Clinical trials involving patients taking NSAIDs long term are under way.



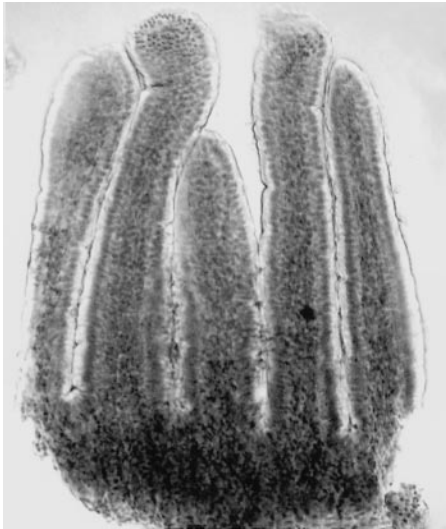


FIGURE 1. Effect of the administration of bovine colostrum, indomethacin, or both on nonsteroidal antiinflammatory drug-induced small intestinal injury in mice. Mice received placebo or colostrum supplementation in their drinking water for 14 d. Twenty-four hours before being killed, some animals also received 85 mg indomethacin/kg subcutaneously. The morphology of microdissected villi was determined throughout the small intestine (200 \times magnification). Top: Control mice did not receive indomethacin or colostrum and had long, slightly tapering, villi. Middle: Mice that received indomethacin alone had markedly shortened villi with bulbous expansion of the tips. Bottom: Mice that received indomethacin and colostrum showed much less marked changes to the villi. These results were published previously (81); however, the figure was not

Chemotherapy-induced mucositis

Current regimens for the treatment of cancers require patients to take much higher doses of chemotherapeutic agents than were used previously. As a result of these higher doses, toxic adverse effects on the bone marrow and gastrointestinal tract can be the factor limiting the dose or duration of treatment. Strategies to protect these tissues and encourage their recovery may facilitate the use of higher doses of chemotherapy, with greater potential for cure. For example, EGF enhances the repair of rat intestinal mucosa damaged by methotrexate (83), TGF- β ameliorates chemotherapy-induced mucositis (84), and administration of a cheese whey-derived preparation reduces methotrexate-induced gut injury in mice (85). Not all studies have shown favorable results, however, because EGF had only a minor beneficial effect in reducing mouth ulceration in a phase I clinical study of patients undergoing chemotherapy (86).

If peptides with growth stimulatory or inhibitory effects are to be used, the timing of administration is likely to be critical; growth-arresting factors might protect bone marrow or gut from the damaging effects of chemotherapy, which tend to affect areas with the highest cell turnover, if given before chemotherapy. In contrast, growth-stimulating factors might “rescue” recovery of injured areas if administered after chemotherapy. This latter approach is already being used clinically, eg, colony-stimulating growth factor is being used to stimulate bone marrow recovery after chemotherapy.

Inflammatory bowel disease

The etiology of ulcerative colitis and Crohn disease is unknown and, therefore, current treatment of these severe, incapacitating conditions has to be on an empiric basis. Studies examining the effect of administration of EGF, PDGF, TGF- β or IGF-I in animal models of colitis have had encouraging results (87), and a cheese whey growth factor extract containing several of these growth factors had positive results in a similar model (88). Other peptides, not present in milk or colostrum in significant concentrations, under study as potential therapeutic agents for these conditions include keratinocyte growth factor (89) and trefoil peptides (90). These studies are in the very early (animal model) stages and the agents are unlikely to be in standard clinical use for many years.

Milk-derived products are already in clinical use for the treatment of inflammatory bowel disease; casein-based enteral feeds are used for the treatment of Crohn disease and their efficacy might be due, in part, to the presence of MDGFs in the preparation, which are preserved during the processing of the milk protein (*see above*). In addition, clinical trials of the use of colostrum enemas for the treatment of ulcerative colitis and resistant proctitis are under way and the results are awaited with interest.

Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a severe life-threatening illness of young children that causes severe ulceration of the small and large bowel. Its etiology is unclear, although there are many possible risk factors, including prematurity, enteric infections, intestinal ischemia, and abnormal immune responses. Although many proinflammatory molecules are likely to be involved in the etiology of NEC, there is currently interest in the role of the phospholipid-mediator platelet activating factor (PAF), which is produced by intestinal flora and inflammatory

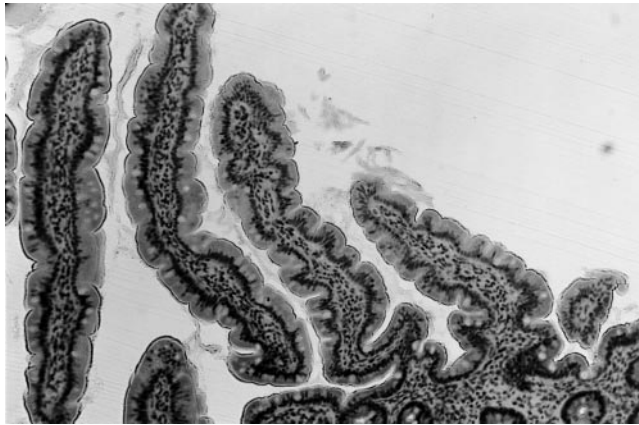
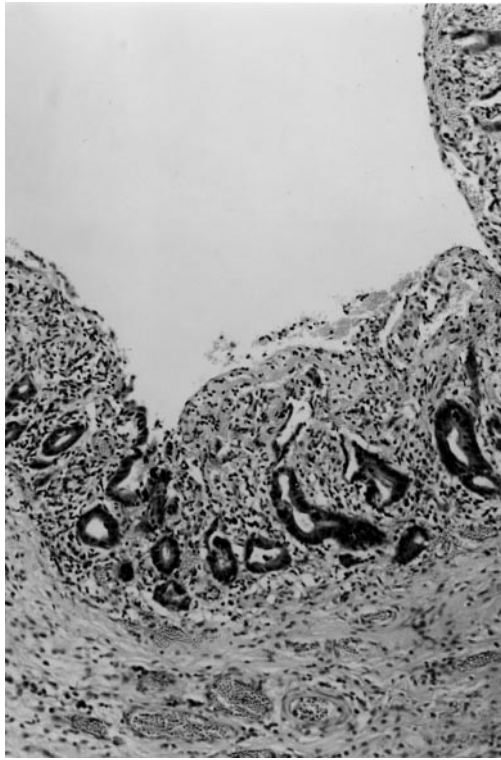


FIGURE 2. Hematoxylin and eosin stain of small intestinal biopsies of a child with necrotizing enterocolitis (200 \times magnification) before (top) and 7 d after (bottom) infusion of epidermal growth factor. Before therapy the mucosa is virtually completely ulcerated and after therapy the mucosa is almost completely regenerated. Details of this case report were published previously (94); however, the figure was not.

cells during the development of NEC. The finding that human colostrum contains the enzyme PAF acetylhydrolase (91), which degrades PAF, might therefore be relevant in explaining why human milk feeds protect against the development of NEC. These areas are discussed further by others (91–93). Although the molecular mechanisms underlying the development of NEC are unclear, there is no doubt that once it is established, it is associated with a very high mortality rate. Current treatment consists of general supportive measures consisting of fluid-replacement and antibiotic therapy, although intestinal resection is often

required. There is therefore a need for novel therapeutic approaches, eg, the use of peptides to stimulate the repair process. Support for this idea comes from a recent case study in which a continuous infusion of EGF resulted in a remarkable restorative effect on gut histology in a child with NEC (**Figure 2**) (94). Larger clinical trials are ongoing.

Infective diarrhea

Most cases of infective diarrhea resolve spontaneously and only occasionally require a short course of antibiotics. For immunocompromised subjects, such as those with HIV infection, prophylaxis against the unusual organisms that they are susceptible to, eg, *Cryptosporidium*, may be beneficial. Hyperimmune milk or colostrum preparations have been shown to be of benefit in the prevention and treatment of infection and to increase weight gain in both clinical and veterinary practice, eg, vaccination of cows with specific viruses or bacteria to produce hyperimmune milk has been shown to be beneficial in the prevention and treatment of enteropathic infections due to *Escherichia coli* (95) and rotavirus (96). The use of whole hyperimmune colostrum rather than specific antibodies purified from milk (97) or other sources has the added value of potentially stimulating the repair process (due to the presence of growth factors) as well as facilitating the eradication of the infection by mechanisms involving nonspecific antibacterial factors in colostrum and milk.

SHOULD WE USE SINGLE OR MULTIPLE PEPTIDES AND HOW SHOULD THEY BE ADMINISTERED?

Advances in molecular biology techniques now allow the large-scale production of individual recombinant peptides. Some of these have already found a place in clinical practice, eg, erythropoietin for the treatment of renal failure–induced anemia and interferon for the treatment of viral hepatitis. The use of growth factors for the prevention and treatment of gastrointestinal disease is, however, at a much earlier stage of development (98).

Although the potent growth factor activity of many of these peptides appears advantageous for stimulating the repair process, there is concern over their potential risks. Systemically administered growth factors could induce proliferation in other regions of the body that harbor premalignant cells. In contrast, lumenally administered growth factors, given orally or via enema, could be delivered at much higher local concentrations. A further advantage of luminal administration is that a proliferative response could be specifically targeted to affect only injured areas. This could be achieved by administering a growth factor, such as EGF, whose receptors are normally restricted to basolateral membranes because it is only at sites of injury that these receptors would be exposed. If the luminal administration of growth factors is to be effective, they must be protected from proteolytic digestion in the stomach and intestine (26). Possible strategies would be to deliver the growth factors in site-specific delivery formulations, to coadminister acid suppressants to reduce proteolytic digestion within the stomach (25), or to coadminister proteins that would act as competitive substrates for the proteolytic enzymes—milk proteins such as casein are particularly beneficial in this regard (26).

Until recently, most research has focused on the use of a single peptide for the treatment of a particular condition. There is now increasing evidence, however, that administration of a combination of many peptides, whether purified or recombinantly

produced, can result in additive or synergistic activity. For example, the coadministration of GH and IGF-I stimulate anabolism (99) and the coadministration of bovine lactoferrin and EGF stimulate the growth of the rat intestinal epithelial cell line IEC-18 (66). Orally administered colostrum-derived preparations therefore appear to be an attractive therapeutic option because they contain many different growth factors in a formulation that provides inherent protection against proteolytic digestion.


Other approaches currently under scrutiny include 1) altering the volume and nature of the components of mature milk [eg, GH (100), prolactin, and colony-stimulating factor 1 (101)] before administering the milk to animals and 2) using genetic modification technology to improve milk's healing and protective properties. With the use of recombinant technology, the production of the required peptides, including human homologues, can be specifically targeted to the breast tissue of the animal by using specific promoters such as the β -lactoglobulin gene (102). This approach, therefore, provides the potential to specifically modify bovine or ovine milk to increase its content of beneficial peptides, including human homologues. These products could then be used in a way similar to that of colostrum for the prevention and treatment of gut injury. Interested readers are referred to the excellent review by Dalrymple and Garner (103).

Several bovine colostrum preparations are already available in health-food shops and, as for any other milk product for human consumption, their manufacture is regulated by food hygiene standards. All of these colostrum preparations are pasteurized, microfiltered, or otherwise treated to prevent the risk of contamination with enteropathogens and the concentration of endotoxins in these preparations is similar to that of standard commercial milk. If colostrum or modified milk products are to be used in clinical practice, several issues regarding their safety will, however, need to be addressed. It is unlikely that human colostrum or milk will find a major role in clinical practice because of its limited supply and because of concerns regarding the transmission of infectious agents such as HIV or cytomegalovirus. It is therefore likely that further research into the commercial aspects of using purified peptides to treat gastrointestinal diseases will focus on milk and colostrum derived from ruminants. Regulatory authorities require bovine herds to be certified free from bovine spongiform encephalopathy and require sheep, which are being used in several studies to produce recombinant peptides in milk (102, 103), to be free from the ovine equivalent of bovine spongiform encephalopathy, scrapie.

An additional area of research concerns the use of recombinant hormones, such as bovine somatotropin, to increase milk yields. Although approval for the use of bovine somatotropin was granted by the US Food and Drug Administration in 1993, the European Union banned its use until at least the end of 1999 and there is continuing controversy regarding the safety of its use. For further discussion of the use of bovine somatotropin, readers are referred to the article by Morris (104). Commercially available bovine colostrum preparations are essentially cell free because they are microfiltered during the production process; therefore, theoretic concerns about graft versus host disease are probably unwarranted. However, graft versus host disease is a concern if fresh, nonfiltered products are used. Our own (unpublished) studies of several of the commercially available colostrum products showed that their bioactivity, determined by cell proliferation assays, is maintained for many months when the products are frozen or stored at 4°C. In addition, we found that dried formulations have

biological activity similar to that of liquid forms when prepared in equivalent concentrations of protein.

Current farming methods allow the production of large amounts of bovine colostrum for clinical use. It is important that batch variations during production be kept to a minimum to ensure consistency of the product produced and that processing methods be developed to prevent deactivation. Such preparations have the advantage of being perceived as "natural" products, which might result in greater patient acceptance and compliance. Further therapeutic advantages might also be gained by developing formulations specifically tailored for individual conditions, eg, the use of a hyperimmune milk or colostrum formulation for the treatment of immunocompromised patients who have gut disease, thereby reducing the incidence of gut infection while stimulating gut repair.

In summary, research examining the potential benefits of using recombinant peptides or colostrum-derived preparations for a wide range of gastroenterologic conditions is underway. Early results are encouraging and we envisage the standard use of these products in the clinical management of gastrointestinal diseases within the next decade. 

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