Role and Function of Growth Factors in Infant Nutrition

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Mammalian milk contains numerous secreted factors with wide-ranging biologic and physicochemical activities. Cow’s milk in the diet has been viewed primarily as a source of nourishment through supply of amino acids; however, in addition to their nutrient value, many milk proteins show biologic activity involved in the maintenance, repair, and proliferation of cells. Such actions are ascribed to a category of milk constituents termed growth factors. These are usually low-molecular-weight proteins that initiate a biologic response in target cells through binding to specific cell-surface receptors. Once bound to the cell surface, the growth factor–receptor complex is internalized, triggering an increase in cell size and number. In the process, various cellular pathways are stimulated, including nutrient uptake and synthesis of protein, DNA, and RNA. Concentrations of known growth factors and other bioactive factors in bovine milk are shown in Table 1. Although there are differences in the concentrations of specific growth factors in human and bovine milk (1), both are rich sources (Table 1).

It is currently believed that maternally derived growth factors may play a significant role in maturation and function of the immature gastrointestinal tract and the immune system. Indeed, some growth factors in milk from early lactation occur at higher concentrations than those in maternal blood, supporting the case for their function in neonatal development. Knowledge of these milk growth factors and their actions has expanded as we explore the biologic value of milk. At least some—such as epidermal growth factor (EGF)—can survive digestion in the neonatal gut, and there is evidence for their intact intestinal absorption (2). Moreover, various studies have shown biologic responses to enterally administered growth factors in neonatal animals (2). Nevertheless, it has proved difficult to identify a clear physiologic requirement for specific milk-derived growth factors in infant development. This may reflect in part the fact that growth factors do not act in isolation, but rather it is the mixture of growth factors in milk that provides the optimal biologic response.
TABLE 1. Major cell mitogens in mature bovine milk: approximate concentrations and responsive cell lines

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentrations</th>
<th>Responsive cell/tissue types</th>
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<tbody>
<tr>
<td><strong>Growth factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin-like growth factor 1 (µg/l)</td>
<td>5.0</td>
<td>Mammary, intestinal, fetal</td>
</tr>
<tr>
<td>Insulin-like growth factor 2 (µg/l)</td>
<td>1.0</td>
<td>Chondrocytes, adipocytes, mesodermal</td>
</tr>
<tr>
<td>Transforming growth factor (µg/l)</td>
<td>3.7–4.3</td>
<td>Fibroblasts, endothelial, lymphocytes</td>
</tr>
<tr>
<td>Fibroblast growth factor (ng/l whey)</td>
<td>5.8 (acidic)</td>
<td>Fibroblasts, endothelial, astrocytes</td>
</tr>
<tr>
<td>Epidermal growth factor (µg/l)</td>
<td>19.8 (basic)</td>
<td>Neuroblasts, keratinocytes, myoblasts</td>
</tr>
<tr>
<td></td>
<td>&lt;2.0</td>
<td>Intestinal, fibroblasts, endothelial</td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (µg/l)</td>
<td>80–100</td>
<td>Mammary, epithelial, fetal, granulosa</td>
</tr>
<tr>
<td>Relaxin (µg/l)</td>
<td>2–4</td>
<td>Mammary tissues, fibroblasts</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>6–8</td>
<td>Lymphocytes, mammary</td>
</tr>
<tr>
<td><strong>Major proteins (g/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein α, β, κ</td>
<td>26</td>
<td>Hybridomas, colonic, lymphocytes</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>1.2–2.0</td>
<td>Lymphocytes, hybridoma</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>1.2</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>0.3</td>
<td>Lymphocytes, hybridoma</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>0.05</td>
<td>Epithelial, myoblasts, fibroblasts</td>
</tr>
<tr>
<td>Proteose-peptone PP3</td>
<td>0.3</td>
<td>Hybridoma</td>
</tr>
</tbody>
</table>

The most compelling evidence for a physiologic function of a milk-derived growth factor has been obtained for transforming growth factor β (TGF-β). This growth factor has pleiotropic actions, including a key role in regulation of immune function. Human colostrum contains high levels of TGF-β, with slightly lower levels in mature milk. Our own studies in rats have shown a similar pattern of milk TGF-β, and we found that the neonatal gut expresses it only at low levels (3). These data suggest that maternal milk provides the infant with high levels of TGF-β at a stage of development when the neonatal intestine has minimal endogenous production. The physiologic role of milk TGF-β is supported by data in gene knockout mice. Null mice born to heterozygous mothers survive during lactation, but then die of a massive inflammatory response after weaning (4). Taken together, the evidence is strong that milk TGF-β survives digestion by the neonatal gut, crosses the infant intestinal barrier, and is present in maternal milk at concentrations sufficient to exert immune protective effects in peripheral tissue.

If milk growth factors do indeed play a significant role in growth, maturation, and repair of infant tissues, there could be important implications for the formula-fed infant, as commercial infant formulas appear to be devoid of growth factor activity (2,5). This presumably reflects destruction during processing, because bovine milk contains overall growth-factor activity similar to that of human milk (6–9). This lack of bioactive factors may play a role in the reported differences in response to infection and the development of allergy and atopic disease between breast-fed and formula-fed infants (10).
THE BOVINE MILK GROWTH FACTORS AND ENRICHMENT STRATEGIES

From the preceding brief review of growth factors in milk, it can be predicted that there are commercial prospects for the dairy and biopharmaceutical industries to exploit the growth-factor mixture that occurs naturally in bovine milk and milk products. Indeed, Donnet-Hughes et al. (10) recently reported the efficacy of a TGF-β-enriched diet in inducing remission in adolescents with Crohn’s disease.

Recognition of this potential opportunity has led us to undertake the systematic isolation of bovine milk growth factors and to develop commercially scalable enrichment strategies. Whereas the whey fraction of bovine milk contains a rich composite of cell growth factors, unconcentrated milk or whey alone cannot sustain long-term culture of mammalian cells (11). To improve cell growth performance, we have developed scalable purification strategies to enrich bioactive proteins from unprocessed bovine milk or whey. This process uses ion-exchange chromatography to separate growth factors with basic isoelectric charge from major anionic milk proteins. The strategy was first applied to colostrum, yielding a potent fraction that stimulated growth of L6 rat myoblasts, BALB/c 3T3 mouse fibroblasts, and baby hamster kidney cells (12). Further fractionation of the bovine colostrum extract by gel filtration identified a number of stimulatory fractions for each cell type, suggesting significant mitogen enrichment by the chromatographic process. Subsequently, cation exchange was applied to concentrate cell growth-promoting factors in cheese whey, a readily available source of bovine milk proteins (13).

Purification of cheddar whey by cation exchange yielded a fraction containing <1% of the whey proteins but with growth-promoting activity in mouse BALB/c 3T3 fibroblasts, human skin fibroblasts, and rat L6 myoblasts greater than the response achieved by using 5% bovine serum (Table 2). This study also showed that removal of immunoglobulin and heat treatment of the extract failed to inactivate its cell growth–promoting ability. A more thorough examination of whey growth factor extract has been made recently (14). Measurement of activity in epithelial and fibroblastic cells revealed that the material is an important source of mitogens for mesodermally derived cell cultures (Table 2).

In contrast to its growth-promoting capacity in mesodermal cell lines, whey growth-factor extract has been found to be inhibitory toward proliferation of...
TABLE 3. Known growth factors and concentration determined in cation exchange–derived whey protein extract

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>Concentration (ng/mg extract)</th>
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<tr>
<td>IGF-1</td>
<td>22.7</td>
</tr>
<tr>
<td>IGF-2</td>
<td>23.9</td>
</tr>
<tr>
<td>FGF-1 (acidic)</td>
<td>0.19</td>
</tr>
<tr>
<td>FGF-2 (basic)</td>
<td>0.66</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>4.0</td>
</tr>
<tr>
<td>TGF-β</td>
<td></td>
</tr>
</tbody>
</table>

FGF, fibroblast growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor.

epithelial cell lines—including rat, canine, and mink lung epithelial cells—reflecting levels of a cell inhibitor, possibly TGF-β, in the material (15). To identify cell mitogens responsible for biologic activity of the cation-exchange extract, the concentration of several known growth factors has been measured in the material (Table 3). The extract contains a variety of growth factors including high concentrations of insulin-like growth factor (IGF) I and II, fibroblast and platelet-derived growth factors (FGF, PDGF), as well as TGF-β (Table 3).

Evidence for unidentified growth factors in the whey protein extract has been presented recently (16). In a cell-culture study of individual and proportionally mixed

FIG. 1. Known growth factors in bovine milk do not account for total biologic activity of a bovine whey extract (WGFE). Fibroblast growth in the presence of recombinant and whey growth factors at the following concentrations: insulin-like growth factor (IGF)-1, 20 mg/ml; IGF-2, 20 ng/ml; platelet-derived growth factor AB, 10 ng/ml; transforming growth factor β2, 1 ng/ml; fibroblast growth factor 2, 1 ng/ml; and WGFE, 1 mg/ml. *p < 0.05 versus all other treatments.
growth factors identified in the bovine milk extract (PDGF, IGF-I and -II, bFGF, and TGF-β), biologic effects of this cocktail accounted for only 51% of growth-promoting activity in fibroblast cells compared with that of whey growth-factor extract produced by cation-exchange chromatography (Fig. 1). This finding implied that the bulk of the fibroblast-stimulating activity present in bovine milk remains uncharacterized. One of these unknown factors was recently purified to homogeneity by Dunbar et al. (17) and was identified as bovine β-cellulin, a member of the EGF family. This is the first demonstration of β-cellulin in milk.

CLINICAL AND NUTRITIONAL APPLICATIONS FOR BOVINE MILK GROWTH-FACTOR EXTRACT

The bovine milk growth-factor extract represents a rich source of growth factors, which we are now evaluating for clinical use in the prevention and treatment of cell and tissue damage. These studies have included enteral and topical application of whey growth-factor extract in several animal models of gastrointestinal and oral mucosal injury.

Methotrexate-induced Small Intestinal Damage

There are now no truly effective treatments for chemotherapy-induced intestinal mucositis, a debilitating side effect of cancer treatment with a shared pathophysiology common to many chemotherapeutic regimens. The degree of intestinal toxicity—often characterized by malaise, nausea, vomiting, anorexia, and diarrhea—can limit the duration and dose intensity of chemotherapy. In some cases, particularly with malnourished cancer patients, this may necessitate dose reduction, or even termination of chemotherapy. Agents capable of reducing or preventing chemotherapy-induced mucositis would provide a useful therapeutic adjunct, especially if they could be given orally before chemotherapy. Efficacy of whey growth-factor extract against intestinal mucositis has been demonstrated in a recent study in rats (18). Male Sprague–Dawley rats were subcutaneously injected with the chemotherapeutic drug, methotrexate (2.5 mg/kg), daily on days 1, 2, and 3, to induce severe damage in the small bowel and bacterial translocation across the gut. Whey extract (15–514 mg/day) was given orally for 5–12 days, beginning on day 1, with controls fed an isonitrogenous diet. Histologic indices of villus and crypt integrity, and quantitation of bacterial translocation into mesenteric lymph nodes, were used to assess the potential efficacy of the extract.

Administration of the whey extract for 5 days increased the villus surface length index in the jejunum and ileum by 52 and 56%, respectively \((p < 0.001)\), compared with controls not receiving the whey extract. The crypt area index was increased by 64% \((p < 0.001)\) in the jejunum, but not significantly increased in the duodenum or ileum compared with the control group (Fig. 2A). Similarly, bacterial translocation (incidence and number of colonies) was significantly reduced by feeding the whey extract (Fig. 2B).

In summary, whey growth-factor extract, given orally, reduced methotrexate-
induced damage in the small bowel, suggesting clinical potential for this extract in the treatment of intestinal mucositis.

Chemotherapy-induced Oral Mucositis

Ulceration, inflammation, and secondary infection of the oral mucosa are further dose-limiting side effects of chemotherapy, referred to as oral mucositis. Biologically active factors have been considered elsewhere for their efficacy in preventing or treating oral mucositis. For example, granulocyte colony-stimulating factor (G-CSF) (19), keratinocyte growth factor (KGF) (20), interleukin 2 (IL-2) (21), and TGF-β3 (22,23) reduce the severity of oral mucositis when applied in experimental animal models. Clinical application of these agents may be limited by the prohibitive costs and technical problems associated with large-scale manufacture by recombinant technologies. Whey growth-factor extract contains a variety of antibacterial factors, cytokines, and growth factors that might provide effective treatment for oral mucositis.

To test this theory, the extract was applied topically to the oral mucosa of Syrian hamsters to measure its preventive activity against chemotherapy-induced oral mucositis. The extract was given in the cheek pouch of hamsters as a solution and in a hydrogel-based vehicle. Cheek pouch treatments were applied 3 times over the 28-hour period before injection of the chemotherapy agent 5-fluorouracil (5-FU), and were repeated twice daily until the second injection of 5-FU was given. Assessment of the oral cavity included tracing the ulcer margin for determining ulcer area by image analysis. Animals also were weighed daily as a measure of well-being.
Application of extract before and during treatment with the chemotherapeutic agent reduced the area of ulcers on days 9 to 13 ($p < 0.05$ to $p < 0.001$), whereas reduction in body weight with the vehicle-treated group was significantly greater than that for hamsters treated with whey growth-factor extract ($p < 0.05$ to $p < 0.001$ between days 8 and 15). Data derived from curve fitting the mucositis indices indicated that ulcer-area values for the whey-treated animals were smaller than those for vehicle-treated hamsters on day 9 ($p < 0.007$). Ulcers also were reduced to 10% of their maximal size earlier with the whey extract than in vehicle-treated hamsters (12.5 ± 0.3 vs. 13.7 ± 0.3 days, respectively; $p < 0.008$). The maximal rate of ulcer-area reduction occurred earlier in the whey extract–treated group (day 9.5 ± 0.5) than in the vehicle-treated group (day 12.3 ± 0.4; $p < 0.0006$). Furthermore, the area under the ulcer-area curve was significantly smaller ($p < 0.004$) in the whey group (140.0 mm$^2$ ± 31.2) than that in the vehicle group (257.8 mm$^2$ ± 23.5).

Several constituents of whey growth-factor extract are likely to confer protection to the oral mucosa, including antiapoptotic, cell-inhibitory, and antimicrobial factors. This finding suggests that the whey growth-factor extract may provide a valuable source of topically delivered proteins for clinical application in preventing severe oral mucositis caused by chemotherapy.

**Immune Modulation of the Neonatal Intestine**

Oral tolerance to foreign enteral antigens is not fully developed in early neonatal life, and epidemiologic evidence supports a role for maternal milk in modulating this response. This activity may be mediated in part through milk growth factors and cytokines, particularly TGF-β. If so, the lack of growth factors in commercial infant formulas may play a part in the increased susceptibility of formula-fed infants to infection, food allergy, and autoimmune disease (10). As a first step in evaluating this hypothesis, we recently showed that enteral administration of a whey growth-factor extract to naturally suckling rat pups can downregulate immune activation to a specifically administered food antigen, ovalbumin. This was assessed by lymphocyte proliferation and by nonspecific downregulation of major histocompatibility complex (MHC) I as well as transferrin receptor in the intestine (unpublished data). We are now extending those studies to determine the effect of dietary supplementation with whey growth-factor extracts in rat pups reared on an artificial, growth-factor–deficient formula as a means of evaluating the potential benefit to formula-fed infants.

**CONCLUSIONS**

The future investigation of growth factors in milk offers exciting challenges over the next decade. It is likely to be directed at three areas: (a) characterization of currently defined milk mitogens; (b) gaining a better understanding of the physiologic significance of ingested growth factors; and (c) discovering other therapeutic applications for purified milk fractions. We have developed a novel method of extracting biologically active milk peptides, which can modulate growth and proliferation of
cultured cells. The same extract offers benefits to the gastrointestinal tract in vivo when given around the time of ablative cytotoxic drug regimens. The beneficial activity probably works through combined effects of different growth factors and components offering anti-infective, antiproliferative, and antiapoptotic activity. The results presented are important in the light of current clinical demands for ways to treat mucositis. Administration of a natural milk extract could be used either to reduce symptoms of mucositis in standard cytotoxic therapy regimens, or alternatively to increase the dosage of chemotherapeutic agents, thereby improving patient prognosis. The product might also be used to treat other diseases, or for immaturity of the gastrointestinal tract.

REFERENCES


**DISCUSSION**

**Dr. Endres:** Several companies are marketing colostrum that is claimed to have high concentrations of growth factor. If you analyze these products, very often the concentrations are not particularly high. What do you think about such preparations?

**Dr. Read:** Growth factor in colostrum it is definitely higher than in mature milk. In general it ranges from severalfold up to 10 times as high, so in that respect, it is certainly an enriched preparation. However, you destroy growth factors during the preparation of infant formula, and I am not sure whether the processes that these manufacturers apply would allow these factors to survive. That would need investigating. You certainly would not be supplementing up to normal physiologic levels in milk. The potential effects of colostrum supplementation are an interesting area of study, and they may well be related to the actions of the immunoglobulins present rather than to those of the growth factors.

**Dr. Räihä:** We heard from Dr. De Curtis about using probiotics to prevent necrotizing enterocolitis (NEC). From your last slide, it seems that it might be possible to do some clinical studies using purified or concentrated growth factors in cases of NEC. Do you know if any studies of this kind have been done?

**Dr. Read:** Clinical studies with growth factors in infants have been very limited, but this is certainly an interest of ours, and premature infants would be the logical place to start. There are many problems with gut function in premature infants, which these agents have the potential to improve, including barrier function and so on. We are starting to investigate milk growth-factor extracts clinically in adults, but I agree that applications in premature infants also have potential.

**Dr. Haschke:** Are there any data available on gut closure when acidified growth factor extract is given, or is any research in this area planned?

**Dr. Read:** We are certainly interested in looking at the phenomenon of gut closure and indeed at barrier function as well. The steps we are taking now are to repeat some of our neonatal studies in formula-fed rat pups, which can be then tested on the background of a very low growth factor supplemented with whey extract. Gut closure is one of the areas we are looking at. From our initial studies, normal neonatal rat pups show very low permeability to macromolecules—for example, if you give them an ovalbumin gavage and look at the levels that appear in plasma. However, formula-fed pups have significantly higher permeability, so this is a very interesting system for looking at whey extracts. We do not have enough information yet to give you any answers.

**Dr. Haschke:** Do you have any other speculations about how the whey-derived growth factors help to induce oral tolerance rather than gut closure?

**Dr. Read:** I could not say that we understand the mechanism. It may be related to antigen presentation in the gut, or to TGF-β content, but I do not think we really understand this well enough yet for me to comment further.

**Dr. Lyonnetti:** I was impressed to see the amount of TGF-β in human milk. TGF-β has a
variety of functions—it induces extracellular matrix deposition, and it also is a powerful immunosuppressant, inhibiting γ interferon production, which switches off the Th1 immune response. Can you discuss these functions? Is there any effect, for example, on oral tolerance?

**Dr. Read:** TGF-β is a very important component of whey extract and milk for the reasons I discussed earlier. Its immune-suppressant activity may well be an important part of the mechanism of the immune-modulating effects of this material.

**Dr. Hernell:** It is obvious that milk contains various growth factors and other biologically active substances, but there also is some evidence that biologic activity may be produced during the digestion of milk proteins. Have you any idea what happens when you digest bovine milk? Will you create new biologically active growth factors, and what happens to your extract when you expose it to digestion?

**Dr. Read:** This is a key question. We do not have a good answer yet, because it is very hard to study the survival of growth factors in a mixture of undetermined biologic activity. We would like to do that, and I think there are ways in which we can mimic the digestive processes *in vitro* and then apply this to some of the cell lines. We have done some similar studies with specific growth factors, and there is certainly evidence that other proteins in milk help to protect the growth factors. IGF, for example, is protected by the presence of casein in particular, so it is the survival in the environment that is relevant. Some of the growth factors, or other bioactive molecules that you alluded to, have precursor forms, and TGF-β is one of them. It may well be that the precursor form helps the molecule to survive on its way to its optimal location, and perhaps TGF-β receptor 3 helps to guide it along as well. The IGFs also have binding proteins that would dissociate during the digestive process, so the dynamics of their activity in the gut are very hard to predict. We do not yet have enough understanding of what occurs in such a complex environment to be able to say exactly what happens.

**Dr. Boza:** In your neonatal rat pup model, do you also have controls that are fed with rat milk?

**Dr. Read:** The controls in that experiment were naturally suckled. The treated pups were gavaged with a very small amount of additional protein per day, and the controls were gavaged with saline. In our studies on formula-fed rat pups, we have not got the ultimate control, which would involve feeding rat milk through a stomach tube, simply because it is virtually impossible to collect the amount of rat milk that we would need for that experiment. We have collected rat milk for analysis, and we would very much like to do that experiment, but I think the rat is probably not the best species! You would need to do those studies in piglets.

**Dr. Boza:** I have another question on your current clinical trials in which you are adding this growth-factor mixture. Are you adding it fresh, or is it incorporated into a formula of some kind?

**Dr. Read:** In the adult patients with chronic leg ulcers, the growth factors are being applied with a dressing onto the chronic wound. In the mucositis trials, we have developed a mouth patch that slowly dissolves over a number of hours, and the patients put that on the inside of their mouths for a day before they receive chemotherapy. The aim is preventive.

**Dr. Karogiozolu:** Have you performed any studies with your mixture on enterostomized rats, and will one of your prospective studies be on infants with short bowel syndrome?

**Dr. Read:** We have not given this particular extract to animals with short bowel. We have been doing quite a bit of work with short bowel, and the extract may have some benefits. I do not think these would be growth benefits because the extract does not appear to stimulate a lot of normal bowel growth. IGF-1, for example, probably has clinical potential in the treatment of short bowel, but it would be best given systemically because it does not have the same effect when given enterally. I think the benefits are likely to be related to the fact that with short
bowel comes a range of other problems such as leaky gut and so on, and the extract could well be a good nutritional supplement for those other side effects of short-bowel syndrome.

Dr. Tato: Many proteases for these growth factors also are present in milk. What is the effect of that?

Dr. Read: The growth factors seem quite stable in milk, so the proteases that are present in milk do not cause rapid degradation. It is the gut proteases that seem to affect them most. These growth factors also are relatively stable in plasma: they are cleared rapidly from plasma, but they do not tend to be degraded in plasma, probably because a lot of them have a complex structure of cysteine bridges that makes them resistant to proteolytic digestion.