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Guest Editorial

Bovine colostrum – Therapeutic synergism involving immuno- modulation, nutritional supplementation and antibacterial action?

In this issue of the Journal, Struff and Sprutte review the pharmacology and clinical pharmacology of bovine colostrum preparations. Bovine colostrum has properties similar to human colostrum in stimulating the immune system and more than one of the many present constituents seems to be involved. Bovine colostrum preparations also have value as a nutritional supplement and the importance of this in regard to the pharmacological actions is becoming more clearly defined. The quality and safety of bovine colostrum are subjects of paramount importance. Heat treatment during manufacture can remove pathogens but can also impair efficacy. Thus, the material used in clinical investigations has to be carefully selected, processed and tested before use.

Bovine colostrum contains approximately 90 useful components, the most interesting of which are immune factors and growth factors. Colostrum is also a source of vitamins, minerals, amino acids, proteins, fats and carbohydrates for the newborn. The immune factors present include specific antibodies, immuno-

globulins, proline-rich polypeptides, lactoferrin, cytokines and trypsin inhibitors, lysozyme, leukocytes, lactoperoxidases and lactalbumins [Thapa 2005]. There are at least four groups of immune-enhancing factors present including growth factors, immunoglobulins, a putative permeability fraction and a fraction containing enzymes and peptides [van Hooijdonk et al. 2000].

Bovine colostrum has therapeutic effects in a variety of chronic infections of bacterial, viral, parasitic and fungal origin but it has also been used as a nutritional supplement to treat chronic fatigue syndrome, infectious diarrhea, sinusitis and fibromyalgia where it boosts the immune responses of the patient. Immunoglobulins in bovine colostrum preparations are thought to bestow protection against enteric infections [Sarkar et al. 1998] and eradicate certain microbial agents in the human gastrointestinal tract, e.g. Cryptosporidium, Shigella and toxin-producing E. coli. Bovine colostrum also contains antibodies against E. coli, Salmonella, Candida and H.-pylori infections [Stephan et al. 1990] where the preparations may prevent H. pylori from attaching to the lipid-binding sites in the gastric mucosa.

Clinical and experimental studies have thrown light on the pathogenic role of endotoxin in Gram-negative sepsis (for review see [Beutler and Rietschel 2003]) and the putative beneficial effects of bovine colostrum in this disease. Severe sepsis, and probably most prolonged critical illnesses, exhibit a paradoxical combination of both an increased activation and a marked depression of the immune system. Sepsis involves an imbalance between pro- and anti-inflammatory mediators produced by toxin-activated inflammatory cells where both proinflammatory mediators as well as immune paralysis are deleterious to the patient. Monoclonal antibodies and antagonists targeted against toxins and mediators have been used to prevent an overshoot in pro-

inflammatory effects but the results have been disappointing [Baumgartner 1991]. Intravenous immunoglobulins on the other hand can increase bactericidal activity in serum by virtue of the neutralizing (endotoxins and exotoxins) and opsonizing IgG and IgM antibodies present and the stimulation of phagocytosis. Mechanisms like these are thought to be responsible for the therapeutic properties of IgG-enriched colostrum preparations in sepsis since Fitzal and colleagues [2001] could show that when given orally, these preparations attenuate endotoxemia by reducing the intraluminal endotoxin content, reducing bacterial growth, preserving the intestinal wall and preventing the breakdown of the mucosal barrier. Breakdown of the mucosal barrier is a common finding in hemorrhage and leads to hemorrhage-induced endotoxemia [Fitzal et al. 2001]. Orally administered immunoglobulins may inhibit bacterial translocation through the bowel wall in intensive care patients with enteric (Gram-negative) septic shock and the mechanisms involved have been the subject of recent discussion: firstly, the primary site of action of the active immunoglobulins is the upper GI-tract where the translocation of pathogens and their toxins is "blocked". There are no other comparable therapeutic agents known which can both destroy microbial pathogens and neutralize concomitantly the toxic products of bacterial decay. Secondly, although the mechanism underlying these neutralizing processes is still unclear, the combination of distinct effector molecules and their well-known synergistic actions may have their origin in phylogenetic adaptation and the developments of complex defense mechanisms.

On the other hand, it is clear that bovine colostrum is not only a substance with immunological properties but it is also a natural product with characteristics of a balanced dietary supplement, i.e. it contains nutritional

components which contribute to the anabolic, energy, vitamin and trace element requirements of the body. Although the duration of treatment with bovine colostrum and intake (dose-response relationships) have not yet been optimized, bovine colostrum preparations could become useful therapeutic agents in severe human diseases involving impairment of immune defense systems.

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- Prof. Dr. A.M. Waaga-Gasser
Department of Surgery I,
Molecular Oncology and Immunology,
University of Würzburg,
Oberdürrbacher Straße 61
97080 Würzburg, Germany
Waaga-Gasser@chirurgie.uni-wuerzburg.de



Bovine colostrum as a biologic in clinical medicine: a review

Part I: Biotechnological standards, pharmacodynamic and pharmacokinetic characteristics and principles of treatment

W.G. Struff¹ and G. Sprotte²

¹Center for Transfusion Medicine, Münster, German Red Cross Blood Transfusion Service West gGmbH, Münster, and

²Clinic and Policlinic for Anesthesiology, University of Würzburg, Pain Clinic for Outpatients, Würzburg, Germany

Key words

bovine colostrum – pharmacodynamics – immunomodulation – lactoferrin – gastrointestinal infection – LPS – nutrition supplement – cytokines – supportive therapies

Abstract. Mammals supply their newborn before birth, at birth or shortly after birth with antibodies, immunocytes and humoral constituents. This “borrowed immunity” is a form of passive immunization to protect the newborn against environmental pathogens until it establishes its own pathogen recognition and disposal systems. In cows, goats, horses and some other animal species, most immunoglobulins are obtained from the colostrum, the first milk after birth, via the gut but in humans the majority of immunoglobulins, and those of the IgG-class in particular, are acquired from the mother by placental transport in the weeks prior to parturition. It has long been known that the consumption of bovine colostrum by humans has therapeutic effects e.g. in gastrointestinal infections, but only since the second half of the last century has it been possible to prepare stable, standardized preparations of colostrum. These biologics are administered to patients in combination with standard therapies as so-called balanced supportive diets. Investigations with standardized colostrum preparations in animal models of human disease and estimates of bovine IgG activity in the human GI-tract, described in this review, have provided preclinical data supporting the use of bovine colostrum in human diseases. On the other hand, the number of bovine colostrum products with a sufficiently large and reliable database is limited and the precise nature of the therapeutic targets is still being evaluated.

Introduction

Colostrum of bovine origin has been known in popular medicine for many years and has been used in the treatment of infectious diseases in humans and domestic ani-

mals including cattle and pig. After calving, cows generally produce more colostrum than needed to meet the requirements of the calf. In the past however, the medical and commercial

Abbreviations

BCC	bovine colostrum concentrate
BSE	bovine spongiform encephalopathy
E. coli	Escherichia coli
EBM	evidence-based medicine
EHEC	enterohemorrhagic Escherichia coli
ELISA	enzyme-linked immunosorbent assay
Fab	antigen-binding fragment
Fc	fragment crystalline
Gg3	gangliosyl ceramide 3
GIT	gastrointestinal tract
GMP	good manufacturing practice
H. pylori	Helicobacter pylori
IFN	interferon
IGF	insulin growth factor
IgG	immunoglobulin G
IL-1	interleukin-1
IL-2	interleukin-2
IVIG	intravenous immunoglobulin
LAB-CON	laboratory controls
LAL-test	limulus amoebocyte lysate test
LC1	Lactobin, Biotest GmbH, Dreieich, Germany
LC2N	Lactobin N, Dr. Wolz GmbH, Geisenheim, Germany
LPS	lipopolysaccharide (endotoxins)
¹⁵ N	Nitrogen 15 labeled
NSAIDs	nonsteroidal anti-inflammatory drugs
PE	phosphatidylethanolamine
SD	standard deviation
SLT	Shiga-like toxin
TGF	transforming growth factor
TNF- α	tumor necrosis factor- α
TNF- γ	tumor necrosis factor- γ
USP	U.S.-Pharmacopeia

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Correspondence to
W.G. Struff
Center for Transfusion
Medicine Münster,
German Red Cross
Blood Transfusion
Service West gGmbH,
Sperlichstraße 15,
48151 Münster,
Germany
w.struff@bsdwest.de

exploitation of the excess colostrum was hampered because of its low stability at room temperature and the need for efficient refrigeration facilities. Initial efforts by veterinarians were stimulated by advances in immunological research [Stokes and Bourne 1989] and attention was focussed on the specificity of the main antibodies of the IgG class present in bovine colostrum.

In clinical medicine, antibodies have been investigated for their ability to interact with specific targets (epitopes) in the tissues, to inactivate toxic substances including drugs and for the diagnosis and therapy of cancer. Another important application of antibodies under investigation, however, is their use in passive immunotherapy for the treatment of infectious diseases, particularly in patients with immune deficiencies. In most of these applications, the antibodies have been administered intravenously as immunoglobulin preparations (IVIG) of human origin produced from large plasma pools, usually comprising more than 5,000 donors. There is, however, increasing interest in the application of bovine colostrum concentrates containing 20 – 50% IgG in the treatment of infections and other diseases, primarily those affecting the gastrointestinal tract. As a result, some BC-concentrates have been used in trials as balanced dietary supplements, i.e. as supportive dietary treatment in patients with infectious disease, mainly of the gastrointestinal tract, receiving standard medication.

Ingested proteins are normally degraded by proteases to small peptides and amino acids in the stomach and intestines and subsequently absorbed. Proteolytic enzymes involved include pepsin, trypsin, chymotrypsin, carboxypeptidase and elastase which initially degrade antibodies of the IgG type into $F(ab')_2$, Fab and Fc fragments. The $F(ab')_2$ and Fab fragments, however, retain some of their antigen neutralizing activity locally in the gastrointestinal tract [Reilly et al. 1997].

This review describes the pharmacodynamic, pharmacokinetic and therapeutic effects of orally ingested colostrum concentrates of bovine origin. Part I deals with production standards and pharmacodynamic and pharmacokinetic data obtained in animal studies and clinical studies with commercially available products. The second part reviews clinical data obtained in controlled tri-

als and the relevance of these data for drug development and clinical use.

Bovine colostrum products

There are numerous products prepared from bovine colostrum (bovine colostrum concentrates; BCC) marketed in Europe. In the case of the majority, however, the clinical indications are unclear or are not supported by clinical data and the products themselves do not always meet quality standards appropriate for evidence based medicine (EBM). Furthermore, details on the manufacturing process, the origin and extraction of the raw material and whether the further processing steps conform to GMP-standard are not usually stated in the product descriptions. In the case of these preparations, the provision of quantitative analytical information, in particular regarding serology, is the exception rather than the rule and information on whether a colostrum concentrate or natural colostrum has been investigated is often missing in publications. There is also the problem that information on the homogeneity of production batches manufactured on a large scale is often missing and this is a possible explanation why pharmacodynamic information is only available for a few preparations. It is essential that reports on the properties of colostrum concentrates provide information on the active components present including the concentration of IgG or antibody titers. This information is required in order to meet the legal requirements applicable when evaluating the evidence for efficacy [Haber 2004].

The information given in this review on the development of two commercial products lactobin and lactobin N (LC1 and LC2N), and which belong to a later generation of BC preparations, illustrates some of these problems. LC1 was withdrawn from the market in the mid-1990s as a precautionary measure following the spread of BSE in several European countries. Its successor, LC2N, is manufactured from the colostrum of New Zealand cows, since cows in New Zealand are free of BSE and are maintained free by intensive cattle husbandry and strict veterinary control of beef and milk. According to the manufacturers, the measures taken (quality control of the

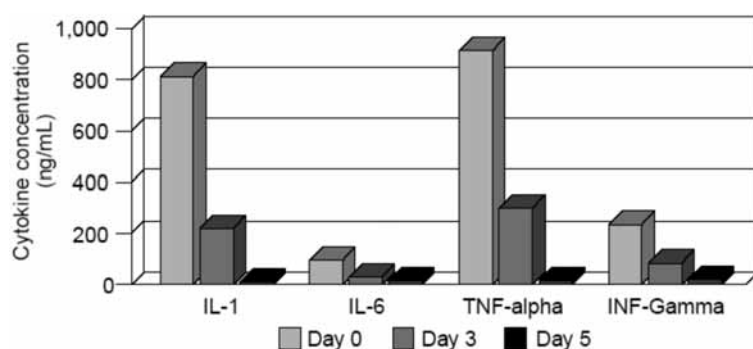


Figure 1. Decline in cytokine concentrations in bovine colostrum after parturition (concentrations in ng/ml) (from [Kelly 2003]).

end-product, veterinary supervision of cattle-breeding) are sufficient to remove any suspicion that transmissible pathogens are present in LC2N, the BCC product in the final stages of development and testing for clinical use. The measures include:

- Obtaining animal-derived raw material from countries in which the relevant infectious agents, e.g. transmissible spongiform encephalopathy (TSE), are reported to be absent.
- Screening animal-derived material for appropriate animal husbandry, feeding practices, health certification, tissue procurement and processing practices. In the case of LC2N, healthy cows are driven to grass throughout the year and would be expected to have a lower risk of infection by pathogens than animals kept in stables in close proximity to neighbouring cattle. Shortly before term the cows are segregated under veterinary supervision.
- Collecting colostrums by bulk tanker according to the product safety program of the New Zealand Ministry of Agriculture. In New Zealand, cattle breeding and rearing follows the principles of good husbandry, e.g. in the use of grass fertilizers and the avoidance of animal-derived feed-stuffs.
- Applying the most important pathogen elimination step which is pasteurization at 72 °C for 15 sec in EU-certified pasteurizers and preventing the introduction of foreign material into the final product in all steps of the production.

Quality requirements for BC concentrates used as supplementary-balanced dietary agents

In the manufacture of a production batch it is necessary to obtain a total of 100 single colostrum samples or more from animals in a specific region. This early milk from individual cows should be obtained within 48 hours after calving because on the 3rd day after calving there is a drastic fall in the content of IgG in the colostrum. The concentration of biological response modifiers (BMR) such as IL-1, IL-2, TNF- α and TNF- γ also falls drastically after the 2nd day [Kelly 2003]. Figure 1 shows the decline in cytokines in colostrum during the first few days after calving.

The problem of variation in commercially available BCC products due to differences in collection and production methods has been discussed by Kelly [2003].

The volume of colostrum milk produced by the cow shows marked interindividual variation (collections can be as great as 20 liters) and often only a small fraction is consumed by the calf. This contrasts with the situation in horses where the quantity of colostrum produced by the mare is adjusted biologically to meet the needs of the foal. In the case of LC2N production, colostrum is collected by designated farms over a defined period from cows segregated in individual herds. The fresh colostrum is filtered and chilled before further processing and a pasteurization step is included before spray-drying the final product.

Bioreactive components in bovine colostrum and antibacterial mechanisms

“Borrowed immunity” in the dog, sheep, goat, pig, horse and in cattle is transferred postpartum to the newborn in the colostrum and not via the placenta as is the case with IgG in the human. Thus, in the cow, the immune status of the calf will usually be determined by the antigens to which the mother has been exposed in the local environment and the calf will usually be sufficiently protected. It is thought that the eradication of living patho-

Table 1. Components and mechanisms of non-specific defense systems at the surface of the gastrointestinal mucosa.

Effector-system (localization)	Physiological function, efficacy
pH-value (stomach)	< pH 4 significant bactericidal (from < 3 overall), in addition the activation of proteases in the stomach and changes in antigenicity i.e. increase in the immunogenicity of antigens.
Bile salts (BS)	Inactivation of viruses with lipid-containing capsides. Free (non-conjugated) BS inactivate certain Enterobacteriaceae. Deconjugation of BS by some pathogens can take place (self-limiting effect). Forced elimination of foreign material in general (non-specific binding).
Epithelial layer	Expression of adhesion-receptors of enteropathogenic E. coli depends on age. High cellular turnover, thus limiting the duration of pathogen adherence: "Elevator effect" (an analogous principle is also seen in the mode of action of astringents: drugs which are able to remove pathogens adhering to the upper epithelial cell layer of the mucosa.)
Mucous	"Encapsulation" of bacteria and nematodes with accelerated excretion. Mucous has receptor-like molecular structures for pathogens. Serves as carrier substance for sIgA, lysozyme and lactoferrin.
Peristalsis	Most important elimination mechanism for bacteria in the small intestine.
Lysozyme	Synthesized by Paneth-cells; degrades peptidoglycan chains in the bacterial wall.
Lactoperoxidase system (saliva, stomach)	Killing of coliform organisms and Salmonella.
Bacterial flora of the intestinal	When present as a stable ecosystem, prevents colonization of the intestinal wall by pathogenic bacteria: competition for receptors or substrates, toxicity against invading organisms, beneficial effects on GI-peristalsis.
Liver	The phagocytosis capacity, in particular of the Kupffer cells, is a critical second-line of defense against microbial antigens and bacterial endotoxins (captured in the V. portae). Correspondingly high antibody titer against bacterial antigens in patients with liver damage [Bjornebe et al. 1972]. Elimination of LPS by Kupffer cells.

gens is optimized by this form of borrowed immunity since colostrum with its polyvalent character, contains components which support both specific (acquired) and inherited defense mechanisms [Stokes and Bourne 1989].

Specific humoral immunity, associated with the subclass IgG₁ antibodies facilitate the eradication of a pathogen by opsonization and is also able to neutralize pathogenicity factors such as lipopolysaccharides (LPS) and Shiga-like toxins (SLT) [Fitzal et al. 2001, Lissner et al. 1996]. The effector systems facilitating innate immunity in the calf are contained partly in the colostrum and partly at the site of action of IgG antibodies in the gastrointestinal tract where they are activated. In one of these processes, lactoferrin, a precursor molecule contained in colostrum, is transformed by pepsin in the stomach to lactoferricin B which has antibacterial properties [Bellamy et al. 1992] and lactoferrin molecules which are not metabolized bound ferric ions which are important growth factors for some bacterial pathogens.

Stokes and Bourne [1989] have described a synergism between bovine IgG-antibody

specificity and lysozyme present in colostrum. This is a phenomenon analogous to an attack on the structural wall (mural layer) of bacterial pathogens, Gram-positive bacteria in particular, adhering to the intestinal mucosa.

Table 1 lists the main mechanisms by which the GI-tract in mammals, where the primary target epitopes for colostrum are located, eliminates pathogens.

The composition of standard concentrates (BCC) of bovine origin used as a biologic and supplementary-balanced diet in patients

The gut is an interface with the environment and therefore with infectious organisms and food. The immune system in the mucosa of the gut is therefore continually processing signals from components of food and those associated with microbial agents. Dietary nutrients also have an effect on mucosal responses because during their metabolism they can provide an important stimulus to the in-

duction, differentiation and maintenance processes of the immune system in the mucosa [Cunningham-Rundles 2001].

Balanced dietary supplements are dietary formulas containing nutritional substances beneficial in diseases or disorders where there is a clear medical indication in line with the relevant regulatory guidelines. According to the German dietary regulations, the manufacture and marketing of such products must be based on "sensible medical and dietary principles". The claims made for the product (indications) must be proven by the manufacturer on the basis of known scientific data [Haber 2004].

The biologic BCC is a special form of foodstuff provided 1) it is devoid of substances introduced during manufacturing process and 2) is only offered commercially as a concentrate of the natural constituents present in the first-milk of cows. The purpose of the simultaneous purification and concentration process is to provide a stable final product containing a low level of oxidation-sensitive lipids. The concentrated proteins in the preparation, immunoglobulins in particular, are known to possess pharmacodynamic properties similar to those observed with other foodstuffs, e.g. the cholesterol-lowering action of pectin in apples.

Thus, BCC can be regarded as both a biologic and a foodstuff supplement which, in addition to purely nutritional constituents, has immunomodulatory properties. Adverse reactions with BCC only occur in subjects who are allergic to proteins present in cow's milk or who are intolerant to lactose.

BCC is obtained from a raw material that is subject to variation but this variation is compensated by pooling individual colostrum collections (> 200) and by collecting the material from animals of similar age which have been kept under comparable breeding conditions. These measures are an important part of the quality control to ensure that the product batches contain the same materials, i.e. have a high degree of homogeneity and are essential for establishing efficacy according to regulation criteria. Measurement of the global concentration of immunoglobulins (IgG, M and A), which on the basis of the precision and accuracy appeared to be a reliable detection method, was the only useful index of biological activity for BCC in the 1980s but this has been improved markedly by estimation of an-

tibody concentrations [Kelly 2003, Lissner et al. 1996, Rump et al. 1992, Stephan et al. 1990].

Whether immunoglobulins in BCC have a major modulatory effect on the immune response in addition to local intraenteral effects is not known, but a lack of dietary protein generally impairs the production of immunoglobulin A in the mucosa and the level of IgG [Bengmark 1999, Cunningham-Rundles 2001, Hulsewe et al. 1999]. BCC contains a large spectrum of neutralizing antibodies [Lissner et al. 1996, Rump et al. 1992, Stephan et al. 1990] but only publications involving LC1 and LC2N include information on the antibody spectrum of the test preparations used in controlled clinical trials. Batches of both preparations are manufactured using several hundred single collections of colostrum and it can be concluded that the homogeneity of both products is satisfactory. A fingerprint analysis has been carried out with LC1 and LC2N in order to determine if any differences are present in the nature of the two preparations. This analysis is useful because the content of antibodies against LPS (endotoxin) of two bacterial pathogens known to be important pathogenicity factors in Gram (-) septic shock provide an index of the pharmacodynamic potential. Furthermore, the results of the finger-print analysis are of particular value with regard to the intended indications of LC2N. According to the LAL-test, carried out according to the U.S.-Pharmacopeia (USP) [Guideline 1987], LC1 and LC2N had the same specific concentration (according to the IgG concentrations) of antibodies against LPS from *E. coli* and *S. typhimurium* (Waaga-Gasser 2005, personal communication). The specific lactoferrin concentration (also based on the IgG content) in LC2N, however, were much higher than those in LC1 [Knighton 2006]. It can be concluded, therefore, that both preparations have the potential to neutralize bacterial toxins.

Biological response modifiers are also present in the colostrum (Figure 1), but their concentration in BCC has not been reported so far. Such information, however is probably essential for the widespread use of BCC as balanced dietary supplement in severely ill patients because the efficacy of BCC in patients with polyarthritis and osteoarthritis is attributable to the presence of these substances [Nitsch and Nitsch 1996].

Table 2. Efficacy of orally administered milk and colostrum concentrates in animal models of infectious disease.

Animal model	Colostrum (type of product)	Results	Reference
Mice infected with <i>E. coli</i>	Whey protein from colostrum of vaccinated cows	Complete protection (dose-dependence)	Hilpert et al. 1977
Rabbit ileal loop model (enteropath. <i>E. coli</i>)	Bovine colostrum from vaccinated cows	Reduction of pathogens in the bowel	Zinkernagel et al. 1975
Rabbits infected with <i>Giardia muris</i>	Bovine milk containing antibodies against <i>G. muris</i>	Protection when treated early	Andrews and Hewlett 1981
Foals deprived of postnatal colostrum	Bovine colostrum administration	100% survival	Robinson et al. 1993
Calves infected with herpesvirus type 1	Hyperimmune colostrum	Efficacious (all animals survived)	Mechor et al. 1987
Calves infected with cryptosporidium parvum	Hyperimmune colostrum	Shorter period of diarrhea	Fayer et al. 1989
Rabbits infected with <i>V. cholerae</i>	Colostrum with specific antibodies	Protection	Boesmann-Finkelstein and Finkelstein 1989
Hamsters infected with <i>Clostridium difficile</i>	Colostrum from cows vaccinated with toxoid A, B	Protection after prophylactic treatment	Lyerly et al. 1991
Mice infected with <i>Pseudomonas aeruginosa</i> and <i>S. typhimurium</i>	Bovine colostrum – animals vaccinated with toxoid A, B	Protection after prophylactic treatment	Lyerly et al. 1991
Mice infected with <i>Pseudomonas aeruginosa</i> and <i>S. typhimurium</i>	BCC (Lactobin)	Protection	Stephan et al. 1990

In addition to the cytokines interleukin-1 β (IL-1 β), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ), bovine colostrum also contains numerous growth factors including insulin-like growth factor (IGF-I and II) and transforming growth factor (TGF- β). Lactoperoxidase activity and lactoferrin are also present [Kelly 2003].

The lipid fraction of LC1 has been shown to contain an additional anti-infectious effector system against *Helicobacter pylori*. LC1 is able to block the binding of *H. pylori* to phosphatidylethanolamine (PE) and gangliosyl ceramide (Gg3) in mucosa cells and, although LC1 lacks detectable antibodies (determined using immunoblotting) to *H. pylori*-surface proteins (adhesins), colostrum lipid residues contain PE and lyso-PE that bind to *H. pylori* in vitro. Colostrum lipids thus have the capacity to inhibit the interaction of *H. pylori*, and other pathogens expressing adhesin, with their target tissues [Bitzan et al. 1998].

Kelly [2003] has reported that the composition of pooled colostrum depends on the health status of the cows, the feeding practices used to maintain the animals, the timing of the colostrum collection periods and the

manufacturing process. It can be expected that a product manufactured from BC collected during the first 24 hours post-parturition has a higher concentration of immunoglobulins (Ig) and growth factors (Figure 1) than a product prepared from colostrum collected from the same cows over the first 4 days after parturition. According to Waaga-Gasser (2005, personal communication), fingerprint analyses as described above for comparing LC2N and LC1 could be used to determine the suitability of BCC for use in balanced substitution diets in patients with enteric inflammation, bacterial and viral diarrhea and early stage Gram (-) septic shock etc.

Pharmacodynamic data obtained in animal studies with BC concentrates

Preclinical trials carried out with colostrum and BCC are summarized in Table 2. They include studies in animal models of infectious disease and provide data on the humoral immunity associated with the three main classes of immunoglobulins (IgG, M

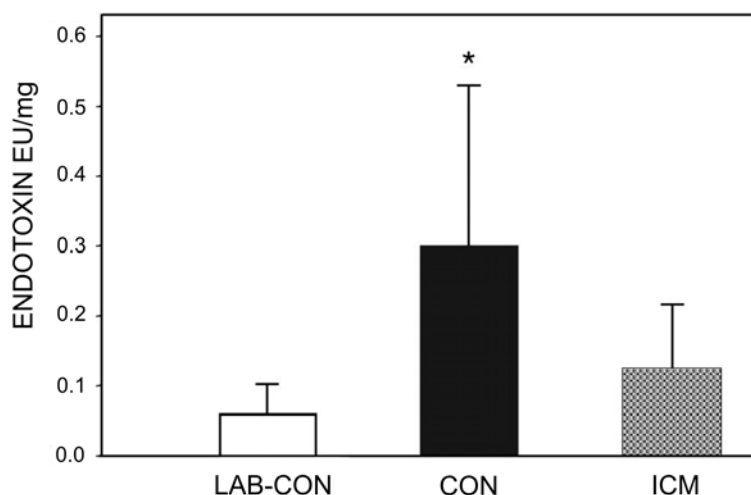


Figure 2. Plasma endotoxin concentrations in laboratory controls (LAB-CON) and 3 hours after the onset of hemorrhagic shock in rats pretreated either with 5 ml of 0.9% saline (CON) or 3 g/kg body-weight LC1 once daily for 5 days prior to hemorrhage. Data are presented as mean \pm SD (* indicates statistical significance compared to LAB-CON) [Fitzal et al. 2001].

and A), their effector systems, complement and phagocytic cells.

Playford and colleagues [2001] showed that bovine colostrum prevents the injurious effects of nonsteroidal anti-inflammatory drugs (NSAIDs) in the gut using a mouse (indomethacin-induced lesions) and a rat model (gastric lesions) and that the effects were dose-dependent and absent in control animals fed natural, unprocessed milk.

Experimental and clinical data support the view that the initiating events in sepsis following a hypovolemic insult (resulting from trauma and hypotension) include the entry of bacteria and endotoxin (LPS) into the bloodstream [Redl et al. 2000]. Using a rat hemorrhage model, Fitzal and colleagues [2001] found that treatment with LC1 significantly reduced the intraluminal LPS-content in the duodenum and that the concentration of endotoxin increased progressively along the gut from the duodenum to the jejunum, to the ileum and finally to the colon. LC1 pretreatment also reduced the level of endotoxin in plasma after hemorrhage (Figure 2). The 6-day survival rate after hemorrhage tended to be higher in the LC1 pretreated group compared to controls (66.7 vs. 40%) and these findings have since been confirmed Seifert et

al. [2002] where the endotoxin levels in plasma in an endotoxemia rat model fell by more than 50% after the enteral application of bovine colostrum.

Investigations on the stability of bovine IgG in the human GI-tract

It has long been known that bovine IgG₁ is resistant to the actions of stomach and intestinal proteases [Brock et al. 1978, Rham and Isliker 1977] and incubation experiments using colostrum, trypsin and pepsin and measurements of IgG₁ antibody specificity have confirmed these findings [McLead and Gregory 1984]. Hilpert and colleagues [1977] showed that immunoglobulins in milk are excreted intact or in the form of Ig-fragments in the stools. Similar findings have been reported for adults [Tacket et al. 1992].

The immunoglobulins in LC1 are relatively stable in artificial gastric juice pH 1.9 and after one hour the maximum fall in antibody titer against bacteria, viruses and toxins involved in diarrhea was only one titer step after which the titer remained constant for 8 hours. The antibody titer of LC1 against the viral pathogens and Shiga-like toxins examined was largely intact after incubation for 4–8 hours in artificial intestinal fluid (the antibodies against bacterial antigens under the experimental conditions used were less stable).

In a Phase I study in 7 healthy subjects, the kinetics in the gut (intra-enteral stability of IgG₁) was investigated using probes and ¹⁵N-labelled proteins in order to determine the breakdown of bovine IgG in various regions of human gastrointestinal tract (small intestine) [Roos et al. 1995]. The most important finding in this study was that only 20% of the orally administered ¹⁵N-protein was detectable down to the ileocecal valve, a value which was considerably higher than 4–7% found for normal dietary proteins (milk proteins: 7–10%) [Mahé et al. 1992]. The investigators concluded that in patients with gastrointestinal disorders the degradation of bovine immunoglobulins would be even less than that found in healthy subjects. The IgG recov-

ered in the ileum was present as F(ab')₂ fragments. Studies in infants showed that up to 50% of orally administered immunoglobulins can be recovered in the stools [Zinkernagel 1972].

In a further open Phase I clinical trial, 8 healthy subjects received a single 15 g dose from a batch of LC1 which had a raised antibody titer (1 : 100,000) against rotaviruses, an index of the amount of bovine IgG₁. This batch was prepared using colostrum from cows which had been vaccinated 6–8 weeks before calving with a bovine vaccine containing killed organisms of the rotavirus-genotype. The aim of the study were to examine if bovine IgG could be absorbed from the GIT and detected in plasma and urine. A further aim was to determine the elimination of animal immunoglobulins from the human intestine into the feces. Study subjects received a diet free of bovine proteins both before and during the study to avoid possible sources of analytical error. Bovine IgG was detectable in the stools of 3 subjects, 4.5, 7 and 11 hours after intake using immunoblot analysis and quantitation with ELISA. On the other hand, no bovine IgGs were detected in serum and urine of any of the 8 subjects and the investigators concluded that absorption of intact bovine IgG-molecules from the human intestine does not occur [Lissner et al. 1998, Stephan et al. 1990, (Merz 1994, personal communication)].

Discussion and conclusions

The phylogenesis of innate and adapted mammalian immunity over several million years has resulted in highly developed defense mechanisms. The method of acquisition of so-called perinatal “borrowed immunity” by the newborn, however, varies from species to species. In humans it is transferred mainly via the placental route to the foetus some weeks before term but in the cow this transfer occurs via the colostrum.

Current research on the pharmacodynamics of BCC is focussed on the antibacterial and antiviral properties, a primary target of these being the lipopolysaccharides of Gram-negative bacteria important as causative agents in septic shock of enteric origin. Two animal

models of this infection, Gram-negative sepsis in rats produced by hemorrhagic shock [Fitzal et al. 2001] and the action of bactericidal antibiotics administered to rats infected intraduodenally with 5×10^{10} E. coli [Seifert et al. 2002] have provided evidence that BCC administered in the diet can prevent the translocation of LPS across the intestinal wall.

Published data on BCC in this review have been used to identify clinical indications. However, although BCC is a biologic, it is more accurate to be classified as dietary supplement rather than a drug. Such products as represented by LC1 and LC2N are mixtures of various proteins (total protein content 83–86%), fat (<2%) and 7–8% of lactose.

BCC is free of the adverse reactions commonly seen with conventional drugs and this is because it is a substance composed only of naturally-occurring proteins and minor quantities of dietary lipids to which the human has been exposed to since the discovery of animal husbandary by primeval man.

It is known in veterinary medicine, however, that the intake of colostrum by animals can be a risk factor under certain conditions. Hemolytic disease of the newborn, associated with the absorption of colostrum antibodies for example, has been recorded in pigs, calves and horses but there are no reports of such events with BCC intake in man. These adverse reactions are thought to be due to the use of vaccines or the transfusion of erythrocytes sensitizing the dam.

The diversity of the antibody specificities in standardized BCC preparations [Stephan et al. 1990] has been discussed in this review with regard to possible additional treatment strategies: it is known that in patients with severe chronic diarrhea due to secondary immunodeficiencies such as AIDS and common variable immunodeficiency (CVID), a precise microbiological diagnosis is not always successful and can only be performed in specialized laboratories. Furthermore, treatment of these patients, and children in particular, with antibiotics and antidiarrheal drugs which often reduce gastrointestinal motility, carries risks because of the increased toxin release from pathogens such as enterohemorrhagic E. coli strains and the appearance of the hemolytic uremic syndrome (HUS). On the other hand, treatment of diarrhea symptoms with BCC, which contains an-

tibodies against LPS and Shiga-like toxins (SLT), as a supplement to standard therapy appears rational and may have advantages including a synergism between immunomodulatory, nutritional and antibacterial effects.

In three Phase I clinical trials with a total of 25 volunteers, the most important finding was 22% of ingested immunoglobulins (IgG₁) were still present in an active form after passing through the small intestine and these results were in accord with the results of stability tests in gastric juice and artificial intestinal fluid. It is of interest that no bovine IgG₁ absorbed from the GI-tract of the subjects was found in blood or urine. The results of these trials and analytical data obtained in animal models have led to Phase II and III clinical trials with BCC and these are reviewed in Part 2 of this review.

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