International Journal of

clinical pharmacology and therapeutics

Review

Bovine colostrum as a biologic in clinical medicine: A review – Part II: Clinical studies

Pharmacodynamics

Mucronulatol from propolis displays a strong apoptosis-inducing effect on human tumor cell lines

Clinical Trials

A Phase I study of CR002 against platelet-derived growth factor-D

Clinical and social correlates of the use of depot antipsychotic drugs in schizophrenia

Pharmacokinetics

Dose proportionality in the pharmacokinetics of the oral direct renin inhibitor aliskiren

Bioavailability Section

Vildagliptin/metformin fixed-dose combination tablets and a free combination of vildagliptin and metformin

Two cetirizine tablet formulations in healthy Indonesian volunteers

46/5

May 2008

Dustri

Dustri-Verlag
Dr. Karl Feistle
www.clinpharmacol.com

International Journal of Clinical Pharmacology and Therapeutics, Vol. 46 – No. 5/2008 (211-225)

ISSN 0946-1965

Bovine colostrum as a biologic in clinical medicine: A review – Part II:

Clinical studies

W.G. Struff[1] and G. Sprotte[2]

[1]Center for Transfusion Medicine Muenster, German Red Cross Blood Transfusion Service West gGmbH, Muenster, and [2]Clinic and Polyclinic for Anesthesiology, University of Wuerzburg, Pain Clinic for Outpatients, Wuerzburg, Germany

Key words
evidence-based medicine – immunotherapy – gastrointestinal infection – nutritional supplement – chronic idiopathic pain syndrome – polyvalent bovine colostrum concentrates (BCC) – clinical trials – diarrhea – cachexia – innate immunity

Abstract. The value of bovine colostrum as a biologic in medicine is documented in clinical trials and supported by relatively large databases containing case reports and anecdotal findings. The main actions include an antibacterial effect and modulation of the immune response. The ability of bovine colostrum concentrates (BCC are polyvalent bovine colostrum concentrates produced from the colostrums of several 100 cows) to neutralize lipopolysaccharides, i.e. endotoxins arising from Gram-negative bacterial pathogens and to inhibit enterogenic endotoxemia in animal models as shown in the last review to have its counterpart in patient therapy. Clinical trials with BCC provide evidence that oral application reduces the influx of LPS from the gut and this appears to be a major mechanism underlying its therapeutic effect in patients at risk for Gram-negative septic shock; data from two well-controlled clinical studies with a total of 100 surgical patients have shown that the inhibition of intestinal LPS absorption measured after the application of BCC not only reduced the LPS levels in the peripheral blood but also inflammatory parameters like IL-6 and CRP were found to be diminished. The usual daily dose of the commercially available BCC preparation, Lactobin® (LC1) is 10 – 20 g daily, but higher doses can be used in the majority of patients because of the low incidence of intolerance problems. In chronic diarrhea involving severe forms of secondary immunodeficiencies, patients receiving LC1 were disease-free for about 4 weeks but the response may be lower in patients with AIDS. BCC is effective in infants with hemorrhagic diarrhea caused by infections with enterohemorrhagic E. coli and reduces the likelihood of the disease progressing to a hemolytic uremic syndrome. The safety of newer BCC products obtained from BSE-free regions seems now beyond contention. In the case of LC1, which was used as a commercial dietary foodstuff in Germany until 1992 and tested in three Phase 1 and 5 clinical studies (two trials in patients with secondary immunodeficiencies, one in surgical patients with gastrointestinal disorders, one in patients undergoing open heart surgery and one in pediatric patients with EHEC infections), there were no cases of BSE-associated disease such as the new variant of Creutzfeldt-Jakob disease. Side effects of clinical relevance are limited to possible intolerance to lactose and sensitivity to milk proteins as these are also present in many commonly used foodstuffs. Important synergistic actions with conventional drug therapies have been observed with BCC including a reduction in LPS plasma levels in patients with Gram-negative bacterial infections treated with bactericidal antibiotics. In healthy persons there are only small concentrations of LPS detectable in peripheral blood (normal values: 3 – 10 pg/ ml plasma, i.e. approximately 0.1 EU/ml). In contrast, elevated systemic levels with concentrations > 300 pg/ml are common in patients with severe Gram-negative sepsis and septic shock. Raised LPS levels occur mainly in patients with Gram-negative bacterial infections who have been treated with bacteriocidal antibiotics. The LPS-lowering effects of BCC are probably due to the numerous active components present in BCC which have their origin in the innate humoral and adaptive immune system of their biologic source, the cow.

Received
September 10, 2007;
accepted
December 20, 2007

Correspondence to
Dr. G. Sprotte
Clinic and Polyclinic for Anesthesiology, University of Wuerzburg, Pain Clinic for Outpatients, Josef-Schneider-Straße 6, 97080 Wuerzburg, Germany
Sprotte_G@klinik.uni-wuerzburg.de

Introduction

Bovine colostrum has health-giving properties but widespread use in therapeutics in the past was limited because of technical factors affecting the manufacture such as the sensitivity of the lipid components in raw colostrum to oxidation and the need for cooling facilities during storage.

The standardization of the manufacture of BCC including collection procedures, pooling methods, production of homogenous

Abbreviations

AIDS	acquired immunodeficiency syndrome
AUC	area under curve
BCC	bovine colostrum concentrate
BSE	bovine spongiform encephalopathy
CD14	activation marker on monocytes, macrophages, neutrophil and dendritic cells
CNS	central nervous system
CRP	C-reactive protein
CVID	common variable immunodeficiency
E. coli	*Escherichia coli*
EHEC	enterohemorrhagic *Escherichia coli*
espP	*E. coli* secreted protease P
ETEC	enterotoxigenic *Escherichia coli*
ENC	endotoxin neutralization capacity
GCP	Good Clinical Practice
GI	gastrointestinal tract
GvHD	graft versus host disease
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
HUS	hemolytic uremic syndrome
H. pylori	*Helicobacter pylori*
IgG	immunoglobulin G
IL-4	interleukin-4
IL- 6	interleukin-6
IL-10	interleukin-10
IVIG	polyvalent intravenous immunoglobulin class G
LC1	Lactobin®
LC2N	Lactobin N®
LPS	lipopolysaccharide
MALT	mucosa-associated lymphatic tissue
MOV	multiorgan failure
NSAID	nonsteroidal anti-inflammatory drug
PAI	pathogenicity island
SCIG	subcutaneously administered immunoglobulins
SIRS	systemic inflammatory response syndrome
stcE	secreted protease of C1 esterase inhibitor from EHEC
STEC	Shiga toxin-producing *E. coli*
STx	Shiga toxin
TGF-β	transforming growth factor β
TLR4	Toll-like receptor-4
TNF-α	tumor necrosis factor α
WR	Walter Reed classification

batches and analytical characterization have been described in Part I of this review [Struff and Sprotte 2007]. Product homogeneity in particular is a strict prerequisite for clinical research including the use of BCC as a dietary supplement in the case of some groups of critically ill patients. Reports on hyperimmune BCC are not dealt with in detail here since they involve a different type of BCC (utilization of a limited number of colostra obtained from cows vaccinated with various antigens, e.g. *Shigella flexneri*, *E.coli* strains, various Protozoa, virus strains: see review by Ward et al. [2005].

Clinical research has involved both prospective and controlled clinical trials and three reviews have been published summarizing the most important of the earlier clinical trials with BCC [Davidson 1996, Kelly 2003, Korhonen et al. 1999]. These studies included the therapy of diarrhea not responding to commonly used medication, e.g. in patients with AIDS, those with severe primary and secondary immunodeficiencies and in the treatment of rotavirus infections in infants. A list of the most important clinical studies carried out using hyperimmune preparations and polyvalent BCC are shown in Table 1.

Part II of this review deals mainly with clinical studies carried out with polyvalent BCC, the pharmacodynamic potential of which has been tested in animal studies and appropriate in vitro experiments for review see [Struff and Sprotte 2007]. A selection of controlled clinical trials with polyvalent BCC are listed below and their designs and results discussed in the following paragraphs.

Clinical indications for BCC (current options using polyvalent preparations and conforming to evidence-based medicine standards)

Some of the clinical studies with the commercially available BC-concentrate, LC1 (Lactobin®, Biotest Pharma GmbH, Dreieich, Germany), have been double-blind and placebo-controlled and based on comprehensive pharmacodynamic investigations. Considerable information is available on the production and analysis of this product and it can be regarded as a prototype biologic for polyvalent BCC [Lissner et al. 1996, Stephan et al. 1990].

LC1 was developed for clinical use and was manufactured according to the GMP-guidelines and the studies have been carried out according to GCP. However, as precau-

Table 1. Clinical studies carried out using bovine colostrum concentrates (BCC) since 1970 (ethical indications only).

Indications investigated:	Number of patients enrolled	Investigators
1. Diarrhea:		
1.1. Diarrhea due to bacterial pathogens		
EHEC infections (in children)	30	[Huppertz et al. 1999]
Helicobacter pylori (infections in infants)	24	[Casswall et al. 1998]
Shigellosis (in infants)	70	[Ashraf et al. 2001]
Diarrhea due to infections with enterotoxic E. coli (ETEC) (in children)	60	[Mietens et al. 1979]
1.2. Diarrhea due to viral pathogens		
Therapy of acute rotaviral infections in children	75	[Hilpert et al. 1987]
Prophylaxis for rotaviral infections in hospitalized children	120	[Davidson et al. 1989]
Therapy of rotaviral infections in infants	75	[Mitra et al. 1995]
Therapy of rotaviral infections in infants	80	[Sarker et al. 1995]
Therapy of rotaviral infections in infants	135	[Ylitalo et al. 1998]
1.3. Diarrhea due to protozoal pathogens		
Chronic cryptosporidiosis in AIDS patients	24	[Greenberg and Cello 1996]
Cryptosporidiosis in HIV-infected patients	37 (Subgroup)	[Rump et al. 1992]
Cryptosporidiosis in patients with AIDS	21	[Tzipori et al. 1987]
Cryptosporidiosis in HIV-infected patients	5	[Nord et al. 1990]
Cryptosporidiosis in HIV-infected patients	25	[Plettenberg et al. 1993]
2. Neutralization of LPS (enterogenic translocation)		
Studies in patients undergoing abdominal surgery	40	[Bölke et al. 2002a]
Studies in patients undergoing cardiac surgery	60	[Bölke et al. 2002b]
3. Therapy of GI-infections with unknown pathogens		
Distal colitis	14	[Khan et al. 2002]
Sjögren's syndrome	20	[Pedersen et al. 2002]
Diarrhea in AIDS patients	25	[Plettenberg et al. 1993]

tion during the introduction of BSE safety measures, LC1 was replaced by LC2N (Lactobin N®, Dr. Wolz GmbH, Geisenheim, Germany), a colostrum preparation from New Zealand obtained from BSE-free cattle. On the other hand, a fingerprint analysis, based on the content of anti-LPS specificities per mg IgG indicated that the two preparations are equivalent [Waaga-Gasser, unpublished observations 2005].

A common finding in Phase II – III clinical studies and the Phase I studies in healthy volunteers with LC1 was the excellent tolerance and in view of the method of manufacture, this characteristic can presumably be extrapolated to LC2N without reservation [Struff and Sprotte 2007]. Adverse reactions are absent on repeated application of high doses and the only contraindications to the clinical application of BCC known so far are an intolerance to lactose and hypersensitivity to bovine milk proteins which disappear on withdrawal of BCC-treatment.

Use of BCC to treat chronic recurrent diarrhea in patients with AIDS and other forms of secondary immunodeficiencies

Prior to the introduction of the polypragmatic highly active antiretroviral therapy (HAART), diarrhea and weight loss occurred in more than 50% of patients with AIDS. In many patients, the course of this complication was serious and the prognosis poor. In about 30% of patients, evidence for enteric patho-

gens was lacking and only about half of those with identifiable etiologic pathogens causing diarrhea responded to antibiotics. Therapy of this disease using oral LC1 (10 g/day for 10 days) was investigated in a multicenter pilot study in 37 immunodeficiency patients with chronic diarrhea. Of these patients, 29 were HIV + WR 4-6 (Walter Reed classification), 2 had common variable immunodeficiency (CVID), 1 had an immunodeficiency of unknown etiology and 5 had graft versus host disease (GvHD) following allogeneic bone marrow transplantation. A good response, in some cases with long-lasting normalization of the diarrhea was obtained in 22 of the 33 treatment periods and only 4 patients with AIDS were non-responders. The mean daily stool frequency decreased from 7.4 before treatment with BCC to 2.2 at the end of the treatment. A recurrence of diarrhea was observed in 12 patients during the 4-week period post dose (41%) but 14 patients were free of diarrhea for periods longer than 4 weeks (48%). Intestinal cryptosporidiosis disappeared in 4 patients. The treatment was also successful in 4 of the 5 patients with GvHD and no serious side effects were recorded in any of the patients [Rump et al. 1992].

In a subsequent placebo-controlled Phase III clinical trial in 63 patients with HIV-infection, these findings could not be confirmed. The investigators attributed this to the introduction of the HAART therapy regimen given to the patients during the study since it was observed that this therapy caused marked changes in the spectrum of intestinal pathogens (unpublished report, Biotest Pharma GmbH, Dreieich, Germany).

BCC in the treatment of hemorrhagic diarrhea in infants

In a Phase II placebo-controlled, randomized clinical study, 30 children with acute hemorrhagic diarrhea due to infection with enterohemorrhagic *E. coli* (EHEC) were treated with LC1 [Huppertz et al. 1999]. During treatment the median value for stool frequency decreased from three to one per day, whereas during treatment with placebo there was no change ($p < 0.05$). In the patients treated with BCC, the duration required for a reduction in stool frequency of at least 50% was shorter ($p < 0.05$).

Treatment with BCC also increased the excretion of *E. coli* expressing intimin and EHEC-hemolysin although the differences did not reach statistical significance. The results of this study with colostrum are clinically important since therapy of EHEC diseases with antibiotics is controversial and an alternative and safe therapy other than BCC is not available. Indeed, a number of antibiotics actually increase the amount of Shiga toxin in vitro [Kimmit et al. 2000, Kohler et al. 2000, Yoh et al. 1999, Zhang et al. 2000] and Wong et al. [2000] showed in a prospective clinical study that antibiotic therapy in infants with EHEC infections increases the risk of developing a hemolytic uremic syndrome (HUS). Clinical studies carried out later confirmed that antibiotic therapy of EHEC infections, in particular in those patients already developing the syndrome, worsened the course of disease and the final outcome [Bell et al. 1997, Dundas et al. 2001, Tapper et al. 1995]. The results of the study of Huppertz et al. [1999] are supported by the findings of Lissner et al. [1996, 1998] where the serological analysis of LC1 reflecting a blockade of Shiga toxin (STx) production, points to the mode of action of BCC in the prophylaxis of HUS.

Severe diarrhea and HUS in children caused by EHEC have been reported from countries throughout the world and EHEC, like serotype O157, non-O157 are known to be important causative agents of these diseases. EHEC can persist in the gut of cattle, goats, sheep, horses and geese. These domestic animals constitute therefore direct or indirect infectious routes affecting man, mainly involving the ingestion of contaminated food such as raw milk and raw milk products, uncooked meat, non-pasteurized apple juice and mayonnaise. Infants living in rural districts are particular prone to infections from these sources [Huppertz and Karch 1998].

EHEC are associated with numerous virulence factors, the most important of which are the Shiga toxins, the Type III secretion system and the respective effector proteins encoded by the locus of enterocyte effacement and plasmid-encoded proteins such as EHEC-hemolysin, EspP and StcE (for review see [Bielaszewska and Karch 2005, Caprioli et al. 2005, Karch 2001, Nataro et al. 1998,

Orihuela et al. 2005]. In infants with severe hemorrhagic diarrhea due EHEC infections, the percentage who develop HUS is 5 – 10% of all cases [Huppertz and Karch 1998].

The clinical and economic impact of these infections and the need for hospital admission to treat dehydration and HUS is a growing burden on the health services [Tarr 1995].

A common problem in carrying out clinical trials in patients with HUS is dealing with epidemiological factors and their unpredictability and this problem arises because the reservoir for EHEC-type pathogens is difficult to identify [Huppertz et al. 1999]. The epidemiology problems in Germany involving infections with Shiga toxin-producing *E. coli* has been reviewed by Karch et al. [1997] and in a global context by Noris and Remuzzi [2005].

The strict avoidance of antibiotics in the therapy of HUS has been questioned recently but it is important to note that the therapeutic rationale for their use in HUS is based on only one meta-analysis of clinical studies, the protocols of which differ markedly in the quality standards applied [Phillips et al. 2005]. On the other hand, BCC preparations like LC1 and consequently LC2N are effective in HUS both as treatment and as prophylactic agents especially in children and are well tolerated.

Intra-enteral neutralization of lipopolysaccharides (endotoxins, LPS) with BCC in Gram-negative bacterial infections

The gut as an interface with the environment comes into direct contact with both foodstuffs and infectious organisms. In quantitative terms it is the largest organ of the immune system. The homeostatic mechanisms in the gastrointestinal wall involving perfusion with blood, local defense reactions (mucosa-associated lymphatic tissues, MALT) and thus the integrity of the gut wall are essential for the intake of nutrients and protection against invading pathogens and their toxins. The isolation of Gram-negative bacteria from the blood of patients with a sepsis syndrome or SIRS (systemic inflammatory response syndrome) frequently indicates a source of infection in the gastrointestinal or urinary tract. Prevention of the invasion of pathogens using bactericidal antibiotics is essential but can lead to the release of endotoxins CPS from the decaying pathogens followed by a massive secondary release of cytokines such as interleukins and tumor necrosis factor (TNF-α) from mononuclear cells with subsequent tissue damage. It is known that the release of these factors in traumatic conditions, e.g. following major surgery, can cause multiple organ failure [Livingston and Deitch 1995]. The extent of the damage to the GI-tract is dependent on the rate at which enterocytes are renewed and this depends critically on the rate of cell proliferation [Rittler et al. 2001].

In addition, any enduring deficit of dietary protein supply causes a suppression of cell-mediated immunity whereas early enteral nutrition of patients with intraabdominal injuries reduces the rate of complications like sepsis and pneumonia [Hasenberg et al. 2007, McClave et al. 1997, Moore et al. 1986].

On the basis of the results obtained in preclinical studies, two controlled clinical trials were carried out with LC1 to examine whether the components in the preparation are able to bind LPS and inhibit invasory processes in the gastrointestinal tract.

In the study of Bölke et al. [2002a] a total of 40 patients undergoing surgery of the gastrointestinal tract were randomized to receive either LC1 (56 g daily for 3 days preoperatively) or placebo and the course of the plasma endotoxin levels and the endotoxin neutralization capacity (ENC) were measured daily up to the 10th postoperative day. The results showed that the LPS-levels in the LC1-group, expressed as AUC, were significantly lower than those in the control group ($p < 0.05$). The difference between the two groups was apparent on the day of the operation and the day after. The fall in endotoxin neutralizing capacity (ENC) was significantly lower in the patients treated with the BCC and the return to initial values was faster than in the control group ($p < 0.006$). In the second study carried out by these authors, the effect of postoperative treatment with BCC on intestinal LPS translocation was examined in a randomized, placebo-controlled study in 60 patients undergoing open-heart surgery. The patients were treated perioperatively for 2 days with either 42 g LC1 or placebo. The

endotoxin concentration in the LC1-group was not significantly lower and the difference in the ENC compared to the control group did not reach statistical significance ($p = 0.06$). The difference in the concentration of interleukin-6 levels was also non-significant but the CRP-level was significantly lower. The investigators recommend that further studies should be carried out using a higher preoperative dose of BCC and should be carried out in a larger number of patients [Bölke et al. 2002b].

These two studies, performed according to GCP-guidelines in a total of 100 surgical patients, show that LC1 produces a marked and in part statistically significant decrease, measured postoperatively, in LPS in blood. This is further evidence that one of the events initiating the sepsis syndrome following hypovolemic insults in traumatized hypotensive patients is the entry of bacteria and lipopolysaccharides from the gut into the bloodstream [Bahrami et al. 1998, Claridge et al. 2000, Pfeiffer et al.1996].

Furthermore, anti-LPS therapy with monoclonal antibodies in patients with hemorrhage and trauma reduces septic complications [Demetriades et al. 1999]. It can be concluded from these findings, that if the GI-tract is a major source of LPS, as the evidence suggests, then elimination or reduction of intraluminal endotoxin, whilst maintaining the mucosal barrier function in intensive care patients, should prevent or reduce the complications of Gram-negative sepsis and the development of multiple organ failure (MOV) [Fitzal et al. 2001].

Of interest in this regard is a pilot study of Ulrich et al. [2001] in patients suffering from major burns where there is a high risk of Gram-negative sepsis with lethal outcome. These investigators determined the LPS level in plasma between the second and third day in such patients and observed a marked increase in the endotoxin concentration which reached a maximum after 47 hours. Of the 7 patients examined, 2 showed sharp increases in plasma LPS and these 2 patients subsequently developed sepsis and died.

As in the case of HUS patients, a further rationale for the application of BCC in trauma patients is that the use of bactericidal antibiotics can provoke or reinforce endotoxinemia [Holzheimer et al. 1996, Seifert et al. 2002].

LPS in mammals can be beneficial or detrimental in a concentration-dependent manner and these effects involve sensor molecules such as the Toll-like receptors [Beutler and Rietschel 2003]. The mechanisms by which BCCs neutralize LPS and prevent their uptake into the systemic circulation is not well understood but lactoferrin, probably the most important inhibitor in BCC of LPS, is able to bind the lipid A part of the LPS-molecule and this appears to act synergistically with the anti-LPS-specific antibodies in BCC in over-coming the effects of an infection [Appelmelk et al. 1994, Bellamy et al. 1992, Fomsgaard 1990].

BCC as an adjuvant in the therapy of patients with idiopathic pain syndrome

The studies carried out with LC1 in these patients to date have been mainly exploratory and often supported by unpublished observations referring back to earlier observations with patients receiving immunotherapy. Mondorf and Duswald [1979] had treated 54 patients with herpes zoster and 18 patients with herpes simplex with intravenous immunoglobulins class G (IVIG). During the infusion the patients experienced a relaxation in skin areas with severe lesions, and reported a complete relief of their concomitant neuralgia. In a further open design study carried out later, intravenous IgG (IVIG) was given to 6 patients receiving cytostatics to treat tumors and who concomitantly suffered from extended herpes simplex or zoster infections with severe attacks of neuralgia. All patients reported immediate pain relief during or shortly after the infusion of IVIG (5 – 10 g of IgG) [Mondorf et al. 1981].

New insights into the value of this approach have been obtained from investigations at the University Hospital, Wuerzburg, Germany. The research groups focussed on patients with intractable pain in whom pain relief with established therapeutic regimens had failed [Goebel et al. 2002a]. In a prospective multidose, open-label cohort study in 130 consecutive patients with chronic pain syndromes, including 48 cases with fibromyalgia and a total of 12 different types of pain syn-

drome, IVIG produced approximately 70% pain relief in 20% of patients and in about half of the patients the level of pain relief was above 25%. An interesting finding was that in patients responding to IVIG treatment, there was a return of almost incessant pain in a large proportion of those who had had an infection, e.g. common cold or influenza a short time before or simultaneously. In other patients with chronic pain syndromes, there were a higher proportion of patients with serum antibodies against intestinal pathogens when compared to healthy controls [Goebel et al. 2005]. These results prompted research groups in the Universities of Wuerzburg and Oxford to look for suitable diagnostic parameters in patients with defined pain syndromes prior to their enrollment in further studies with IVIG [Goebel et al. 2003].

In a clinical study in 40 patients with widespread pain, 26 of whom had fibromyalgia, there was a lack of anti-inflammatory and analgesic Th2 cytokine activity when compared to age- and sex-matched controls [Uçeyler et al. 2006]. In contrast to the control subjects, patients had a significantly lower relative gene expression and lower serum concentrations of the Th2 cytokines, IL-10 and IL-4.

These results prompted the investigators to perform an uncontrolled field trial at the Pain Center, University Hospital, Wuerzburg, Germany, in which BCC 20 g daily was administered to approximately 1,000 patients with different types of idiopathic syndromes. The aim of the investigation was to examine the hypothesis that some patients had a post-infectious autoimmune disease responsive to treatment with BCC. Significant pain relief occurred in 30 – 40% of patients depending on the duration and localization of the disease and there was a latency of 4 – 10 days after the treatment before the full effect was detectable [Sprotte, University Hospital, Wuerzburg: unpublished observations]. Patients with idiopathic facial pain syndromes, including trigeminus neuralgia, glossopharyngeal neuralgia, myoarthropathy of the jaw and atypical facial pain showed the highest response rate and the shortest latency period. Patients with chronic widespread pain, including fibromyalgia with and without irritable bowel syndrome also showed a marked improvement in symptoms. It was concluded that the patients in both groups had impairment in the mucosal barrier function in the gastrointestinal tract and this finding was supported by measurements of the resorption rates of various sugars [Goebel et al. 2002b]. In the case of patients with fibromyalgia, 31 of 40 who were examined by this method and who had not been treated with IVIG or BCC previously reported an adverse reaction after the application of the test sugars. This diagnostic tool is therefore not suitable for routine clinical use.

According to the experience obtained at the Wuerzburg Pain Center, patients with chronic pain showing a positive response to the treatment with BCC, can also be successfully treated with IVIG or SCIG and in many cases the results of these alternative treatments are superior to those using BCC. However, BCC has the advantage that patients responding can be treated in their homes (i.e. no infusion required).

Patients with chronic pain syndromes given BCC, in contrast to those without such symptoms, were often found to develop a previously unrecognized intolerance to the milk constituents in BCC limiting their use in these patients. Repeated intake of BCC has also caused exacerbation of atopic dermatitis with skin lesions. Although the tolerance and reponse to BCC is generally excellent, in children with chronic pain, atopy (allergic bronchial asthma, rhinitis and atopic dermatitis) is not an infrequent occurrence during treatment with BCC [Sprotte, University Hospital, Wuerzburg: unpublished observations].

Mechanisms of action of BCC

Colostrum, as starting material for BCC, in which the cellular components, most of the lipoproteins and some proteins such as lactalbumin have been removed, is a highly complex mixture of various effector molecules [Kelly 2003, van Hooijdonk et al. 2000]. This explains the wide range of pharmacodynamic effects described in in vitro systems, in animals and in clinical studies.

Synergisms of the colostral effector systems with the innate defense of a host, especially of patients infected with bacterial pathogens, colonizing the gastrointestinal mucosa appear to be suitable. For instance, the efficacy of antimicrobial peptides as

defensins produced permanently by the cells in high concentrations in the small intestine with a broad activity range against Gram-negative and Gram-positive bacteria might represent appropriate candidates in this regard [Wehkamp et al. 2007].

Discussion including a comment on other possible applications of BCC

One of the primary tasks of the immune system in mammals is the recognition of antigens and their eventual forced elimination. Bovine colostrums and BCC made from it carry out this task and also other important tasks such as the minimizing of inflammatory events which follow exposure to antigens. The ruminant is unique in that there is a selective passage of immunoglobulins class IgG into the mammary gland prior to parturition so that the colostrums contain large amounts of specific antibodies. Gorman and Halliwell [1989] and Kelly [2003], more recently described in more quantitative terms the constituents of the adaptive and the innate immune system also present in BCC acting together in a synergistic manner.

The efficacy of BCC therapy appears therefore to stem from those properties of native colostrum which are important for the health of newborn mammals and these include the transfer in the humoral components associated with innate and acquired maternal immunity and the concomitant provision of readily available nutritional substances such as essential amino acids.

The pharmacodynamic effects of BCC thus complement or replace those of standard therapies, e.g. with antibiotics, analgesics and anti-inflammatory drugs.

One of the sites of action of BCC is the mucosal surface of the gastrointestinal tract and active components presumably interact with the mucosa-associated lymphatic tissue (MALT). Although the proteins in BCC effector molecules, mainly IgG_1, are readily degradable in the small intestine, more than 20% of IgG_1 reaches the ileocecal valve in an active form [Roos et al. 1995]. As shown in Table 1, the most clinical studies with BCC up to now have involved the prophylaxis or the early treatment of infectious diseases originating in the GI-tract. In the case of some syndromes due to bacterial infection, adjuvant treatment using BCC in addition to an antibiotic is usually indicated and successful.

Unlike antibiotics, development of drug resistance with BCC is not thought to be a problem with BCC and there is no evidence that the constituents induce enzymes involved in the metabolism of xenobiotics.

In patients with Gram-negative sepsis the gut is usually the site for transfer of LPS via lymphatics into the systemic circulation. The mechanisms involved have been discussed in Part I and evidence has been provided for the intra-enteral neutralization of LPS by LC1 in patients.

The mechanisms of action of the active components in BCC is not known in detail and the earlier view that the γ-globulins (mainly IgG_1) are the most important active principle must be interpreted only as a working hypothesis. More recent investigations, however, such as those of Bölke et al. [2002a], have shown statistically significant decreases in LPS plasma levels in patients undergoing gastrointestinal surgery after treatment with LC1, and Seifert et al. [2002] using a rat model of Gram-negative septic shock, found a synergy between anti-LPS specificities (IgG_1) and lactoferrin which is also present in BCC and this phenomenon appears to have its clinical counterpart.

The LPS-release phenomenon, triggered on the other hand by bactericidal antibiotics, has been confirmed in clinical studies by several groups [Dofferhoff et al. 1991, Prins et al. 1995]. Certain types of β-lactam antibiotics seem to release more LPS than others and this property is strongly correlated with penicillin-binding protein-3 [Jackson and Knopp 1992].

These findings are relevant since there is much evidence to show that the gut is a locus minoris resistentiae for infections and a reservoir for noxious substances like LPS and Shiga-like toxins when the gut wall is damaged, e.g. during hypoperfusion states or during malnutrition.

A recently published review, however, casts doubt on the role of LPS as a major initiator of septic processes when the treatment of patients with anti TNF-α and anti-LPS interventions fail [Riedemann et al. 2003]. The neutralization in situ of LPS, a major toxin

from Gram-negative bacteria, by BCC is attributable to antitoxic effector molecules like lactoferrin and specific IgG antibodies. The combination of these pharmacological effects accounts for the efficacy of BCC in the prevention of Gram-negative septic shock in patients. A profound review article dealing with the pathophysiological role of CPS in clinical medicine was published by Alexander and Rietschel [2001].

Comparison of data on patients with sepsis of non-enteric origin with those having enterosepsis may not be very informative since the site of infection and stage of the disease have a marked influence on the course of the disease and the effects of therapy. Enteric sepsis comprises about 20% of all cases of sepsis [Martin 1991]. Evidence from experimental [Fitzal et al. 2001] and clinical studies [Demetriades et al. 1999] point to the gastrointestinal entry of bacteria and presence of LPS following trauma and hemorrhage with hypoperfusion of the gut wall as the initiating events in sepsis. Breakdown in the integrity of the gut barrier would aggravate organ injury in distal regions of the body because of mediators, in particular those carried in the mesenteric lymph [Magnotti et al. 1998]. On the other hand, the systemic administration of anti-endotoxin agents neutralizes the plasma endotoxin and can protect the vital organs against the injurious effects of these substances [Bahrami et al. 1997, Bahrami and Schlag 1995, Bauer and Welch 1996, Demetriades et al. 1999, Yao et al. 1995].

Sepsis is one of the most frequent causes of death after a major burn injury and is usually attributable to an infection involving Gram-negative organisms and the release of LPS [Ulrich et al. 2001]. When more than 40% of total body surface is affected, endotoxin plasma levels reach a maximum between the third and the fourth day [Ulrich et al. 2001, Winchurch et al. 1987, Yao et al. 1995]. The authors describe this as a consequence of increased colonization of the gut mucosa by Gram-negative organisms and concomitant influx of LPS from burnt tissue.

Nevertheless, LPS molecules resorbed continually from mucosal surfaces such as the GI-tract with a surface area of 300 m^2 have a physiological function within a limited range of concentrations within the mammalian defence networks.

Endotoxin is one of the most potent immunostimulants, which via the activation of Toll-like (TLRs) receptors acts as a permanent defense system against various pathogens. An infected host, on the other hand, would overreact in the presence of large quantities of LPS, in particular with a massive liberation of TNF-α and other inflammatory cytokines [Beutler and Rietschel 2003].

Irrespective of the pathological background, it is always correct in the case of sepsis treatment, along with folic acid application and antibiotic therapy, to inhibit endotoxins and the effects of these.

In a clinical trial in children with severe hemorrhagic diarrhea due to infection with enterohemorrhagic *E. coli* (EHEC), LC1 was able to eliminate these organisms and reduce the danger of developing a hemolytic uremic syndrome (HUS), a disease which in infants is often fatal or can cause severe damage [Huppertz et al. 1996]. This result provides evidence that the antibody specificities of the γ-globulins present in BCC are consistent with their ability to eliminate in the human GI-tract *Escherichia coli*-expressing *Shiga* toxins 1 and 2, intimin and EHEC-hemolysin [Lissner et al. 1996]. Because of the risks of antibiotic treatment in pediatric patients and its ineffectivity in preventing HUS, treatment with polyvalent BCC is the apparent first-choice therapy in patients with EHEC.

Safety aspects regarding the use of BCC in man

A risk analysis of the cases treated long-term with a polyvalent BCC primarily must involve the technical criteria of the production process, the results of pharmacodynamic investigations and the clinical studies and case reports, examples of which are described in this review.

i) The raw material e.g. for LC2N originates from animal herds kept under strict veterinary-controlled conditions. It is obtained from New Zealand colostrum and thus from colostrums originating from a country certified as BSE-free.
ii) The production of LC1 is carried out according to GMP. Homogeneity of production lots is attained by using a colostrum pool from several hundred colostrum col-

lections and strict compliance with the standards of production (GMP). Since Lactobin N (LCZN) is a complex mixture of proteins, this measure avoids the generation of new allergenic determinants in the production process. In this regard, particularly infants are at risk of developing cow's milk allergy after the cessation of breast-feeding when their intestinal immune system becomes exposed to these proteins in other foodstuffs [Heyman 1999].

iii) There is no addition of foreign materials during the production process of LC2N and LC1.

iv) Animal tests on LC1 have shown no evidence of toxicity (fingerprint analysis and microbiological test procedures based upon the IgG_1-concentration have demonstrated bioequivalence between LC1 and LC2N).

v) No adverse reactions were reported with LC1 in the three Phase 1 clinical trials in healthy subjects. In the clinical trials in patients, Lactobin produced no severe adverse reactions with the exception of patients with a prolonged history of chronic pain. Resorption tests with various sugars have shown that patients with chronic pain are able to absorb macromolecules from the gut [Goebel et al. 2002b]. The risk of sensitization reactions against non-degraded proteins means that BCC might therefore be contraindicated in patients with celiac disease and chronic enteritis such as Crohn's disease.

vi) Lactose intolerance: absorption of lactose requires lactase activity in the brush border of the sensitizing small intestine. Lactose intolerance can occur in infants and young children with acute diarrheal disease but the clinical significance of this is limited except in more severely affected children. Symptoms of lactose intolerance are relatively common in older children and adolescents but signs of intestinal injury resulting from it are uncommon. Lactose intolerance differs from milk protein sensitivity since a sensitivity to milk proteins involves the immune system and can cause lesions of the intestinal mucosa. During the first 3 months of life, 2 – 5% of infants exhibit symptoms of cow's milk protein intolerance which generally resolves itself before reaching the age of 1 year (for review see Hagemann [1999]).

Secondary lactase deficiency can occur later in life the etiology of which most often lies in an infection, e.g. due to rotavirus. Symptoms can also arise in celiac disease, Crohn's disease and immune-related diseases [Heyman 2006] or enteropathies as can occur in patients with chronic pain syndromes. The proportion of patients at the Wuerzburg Pain Center presenting with symptoms of intolerance to ingested lactose is approximately 10%. The lactose in BCC, like that in LC preparations, causes a reinforcement of pain sensations in addition to causing typical gastrointestinal disorders [Sprotte, unpublished observations].

Potential clinical indications for BCC

Some potential indications for the clinical application of BCC have been mentioned above. The multiple effector mechanisms contained in BCC can inhibit or destroy human pathogens and can neutralize virulence factors and liberated toxins. They are also involved in antiphlogistic effects. The question arises, whether other patient populations in ICU such as transplant recipients might also benefit from supplementary treatment with BCC. The selective decontamination of the gut in patients after multiple trauma, severe surgical stress and intense immunosuppression therapy with antibiotics has been the subject of controversial discussion since its introduction in 1984 [Stoutenbeck et al. 1984]. In spite of a reduction in mortality rates in clinical studies in ICU patients receiving combinations of non-absorbed antimicrobial and topical oropharyngeal antibiotics, concern exists regarding the development of bacterial resistance [de Jonge et. al. 2003, Vincent 2003]. Implementing the additional treatment with BCC into the current scheme of selective gut decontamination could represent an improvement but evidence in the form of data from appropriate clinical studies is needed. The rationale behind such therapy in ICU patients could be the eradication of aerobic, potentially pathogenic microorganisms from the stomach and gut and the preservation of useful indigenous anaerobic flora.

In the same context Bölke et al. [2002a] for example, advocate the perioperative treatment with BCC as a promising new strategy for patients undergoing liver transplantation, thereby protecting the transplanted organ for instance against a massive CPS translocation from the gut.

The inhibition of enteral LPS-transfer might also be beneficial in patients with malignant tumors since such patients may become anorexic due to increased activities of TNF-α induced by LPS. Increased TNF-α activities in tumor patients and the effects of this might have been attributed to induction of CD14 on monocytes in blood and within the tumor.

It can be speculated that reduction in the intestinal LPS-influx would decrease TNF-α activation and prevent anorexia in tumor patients. In support of this view is the observation that in patients with pancreatic cancer the expression of CD14 in both Kupffer cells and blood monocytes is increased. This change would render them more sensitive to bacterial LPS and increase the likelihood of a pro-inflammatory response with the consequent development of cachexia.

Bitzan et al. [1998] obtained evidence that the colonization of gastric epithelial cells by Helicobacter pylori can be blocked by polyvalent BCC such as LC1 and that this might be useful as an adjuvant in treating gastroduodenal disease including gastric malignancies when this organism is present. The causal relationship between the occurrence of *H. pylori* and a variety of gastric diseases has been reported in detail in a more recent review by Brenner and Rothenbacher [2005].

In a clinical study carried out in 24 infants with *H. pylori* infections, purified colostrum from cows vaccinated with the organism, however, failed to cure the infants of the disease [Casswall et al. 1998].

A further possible indication for the use of BCC is the treatment of injuries to the small intestine caused by nonsteroidal anti-inflammatory drugs (NSAID). The rationale for clinical investigations on this topic stems from animal experiments with BCC in rats was described in Part I of this review.

In two well-controlled clinical studies, Playford et al. [2001] used a non-invasive marker to determine the presence of gastrointestinal injury and increased gut permeability and to examine the effect of BCC (Viable Bioproducts, Turku, Finland) in patients taking NSAIDs. In one of these studies, a randomized crossover trial in healthy male subjects, changes in gut permeability, expressed as lactulose/rhamnose resorption ratios, were compared before and after a 5-day treatment with 50 mg indomethacin given orally 3 times daily whilst administering 125 ml colostrum or whey proteins (control) 3 times daily. The findings showed that indomethacin caused a 3-fold increase in gut permeability in the control arm but no significant increase during the coadministration of colostrum.

In the second study on the effect of colostral and control solutions (125 ml, for 7 days) on gut permeability in patients (n = 15) on long-term NSAID therapy, the initial permeability ratios were low and not influenced by coadministration of the test compounds. Further studies are therefore needed to confirm that bovine colostrum preparations prevent NSAID-induced gastrointestinal damage.

Cesarone et al. [2007] reported a clinical study in 144 subjects with ages in the range of 30 – 80 years, compared the efficacy of oral colostrum (900 mg/day for 2 months; Concon, Guna, Srl., Milano, Italy) with vaccination against influenza. The study population was divided into three groups: subjects receiving BCC who had been previously vaccinated against influenza virus, subjects without previous vaccination who also received BCC and Group 3 containing subjects who received no prophylactic treatment. The results showed that the average number of flu-episodes in subjects treated with colostrum was significantly lower than in subjects not receiving colostrum. The number of days with flu was 3 times higher in the non-colostrum-treated subjects. The incidence of events associated with flu was higher in the vaccinated than in the colostrum-treated individuals ($p < 0.05$). Preliminary data in healthy volunteers immunized with attenuated *Salmonella* typhi oral vaccine and who concomitantly consumed liquid prepacked bovine colostrum tended to have greater increases in specific IgA (ELISPOT assay and flow cytometry in vitro) in comparison to volunteers not treated with colostrum [He et al. 2001]. The increase in specific sIgA is regarded by the authors as the key mechanismof flu-prevention by BCC.

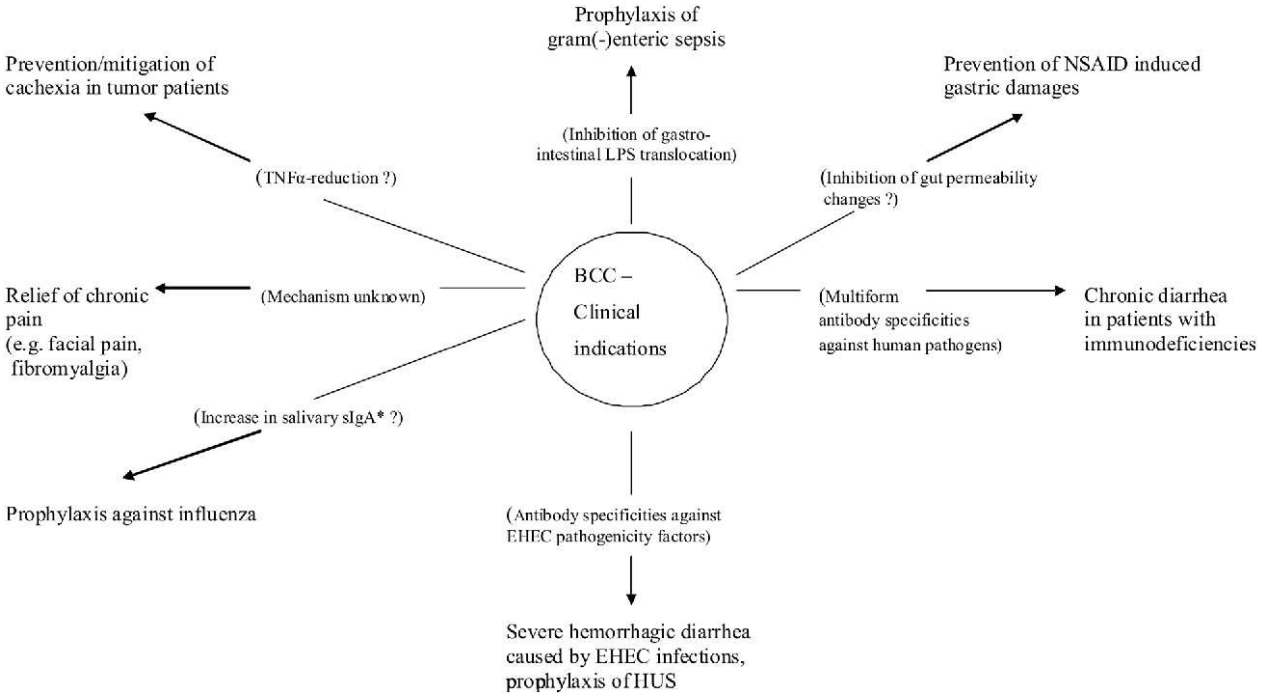

Figure 1. Diagram showing current clinical indications for the therapy with bovine colostrum concentrates (BCC). Information on possible pharmacodynamic mechanisms (in brackets) e.g. modulation of temporal response (increase of salivary sIgA) according to [He et al. 2001].

Safety and efficacy of BCC administration to humans – conclusions

In conclusion, the results of Phase I studies in human volunteers and Phase II/III studies in patients have demonstrated that the pharmacodynamic effects of BCC observed in preclinical experiments have their therapeutic counterpart in a number of clinically important diseases although there is a need for confirmatory investigations in most areas. On the other hand, the implications of the considerable volume of case study data, e.g. those held at the data bank of the Pain Clinic in Wuerzburg, should not be underestimated. The results of the studies are consistent with the view that BCC therapy is a "supportive" therapy, i.e. the pharmacodynamic mechanisms evolve from the multifold active components present and that these modulate the body defense processes (Figure 1 gives an outline of the present medical experience to treat patients with BCC).

The safety of BCC has been well-established in the clinical studies with LC1 and up to now no major or unpredictable risks have been reported. Intolerance to lactose, a component in BCC preparations, and milk proteins can occur but such reactions can generally be anticipated from the dietary habits of the patient.

Since it is clear that BCC preparations are safe and effective in a broad range of indications, this unique form of peroral passive immunization deserves attention.

Acknowledgment

The authors wish to thank Prof. Herbert Schmidt (University of Stuttgart-Hohenheim, Germany) and Dr. Nils Roos (Bundesforschungsanstalt für Ernährung und Lebensmittel, Kiel, Germany) for their competent advice.

References

Alexander C, Rietschel ET. Bacterial lipopolysaccharides and innate immunity. J Endotoxin Res. 2001; *7:* 167-202.

Appelmelk BJ, An YQ, Geerts M, Thijs BG, de Boer HA, MacLaren DM, de Graaf J, Nuijens JH. Lactoferrin is a lipid A-binding protein. Infect Immun. 1994; *62:* 2628-2632.

Ashraf H, Mahalanabis D, Mitra AK, Tzipori S, Fuchs GJ. Hyperimmune bovine colostrum in the treatment

of shigellosis in children: a double-blind, randomized, controlled trial. Acta Paediatr. 2001; *90:* 1373-1378.

Bahrami S, Schlag G. Significance of translocation/endotoxin in the development of systemic sepsis following trauma and/or hemorrhage. Prog Clin Biol Res. 1995; *392:* 197-208.

Bahrami S, Yao YM, Leichtfried G, Redl H, Schlag G, Di Padova FE. Monoclonal antibody to endotoxin attenuates hemorrhage-induced lung injury and mortality in rats. Crit Care Med. 1997; *25:* 1030-1036.

Bahrami S, Redl H, Schlag C. Der Darm als Ursache von Sepsis und Multiorganversagen. Wien Klin Wochenschr. 1998; *10 (Suppl 1):* 2-10.

Bauer MF, Welch RA. Characterization of an RTX toxin from enterohemorrhagic E. coli 0157:H7. Infect Immun. 1996; *64:* 167-175.

Bauer C, Riemer-Paxian I, Larsen R, Marzi I. Recombinant N-terminal fragment of bactericidal/permeability increasing protein (rBPI21) prevents shock-induced microcirculatory alterations in the liver. Crit Care Med. 1997; *25:* 1283-1288.

Bell BP, Griffin PM, Lozano P, Christie DL, Kobayashi JM, Tarr PI. Predictors of haemolytic uremic syndrome in children during a large outbreak of Escherichia coli 0157:H7 infections. Pediatrics. 1997; *100:* E12.

Bellamy W, Takase M, Wakabayashi H, Kawase K, Tomita M. Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin. J Appl Bacteriol. 1992; *73:* 472-479.

Berger D. Perioperative endotoxin leakage from the gastrointestinal tract can be prevented by enteral application of an immunoglobulin enriched milk preparation. In: Bennet D (ed) Glasgow, 24 – 28. Sept., Bologna: Monuzzi Editore. Int. Proceedings Division; 1996. p. 439-443.

Beutler B, Rietschel T. Innate immune sensing and its roots: the story of endotoxin. Nature Reviews Immunol. 2003; *3:* 169-176.

Bielaszewska M, Karch H. Consequence of enterohaemorrhagic Escherichia coli infection for the vascular endothelium. Thromb Haemost. 2005; *94:* 312-318.

Bitzan MM, Gold BD, Philpott DJ, Huesca M, Shermann PM, Karch H, Lissner R, Lingwood CA, Karmali MA. Inhibition of Helicobacter pylori and Helicobacter mustelae binding to lipid receptors by bovine colostrum. J Infect Dis. 1998; *177:* 955-961.

Bölke E, Jehle PM, Hausmann F, Daubler A, Wiedeck H, Steinbach G, Strock M, Orth K. Preoperative oral application of immunoglobulin-enriched colostrum milk and mediator response during abdominal surgery. Shock. 2002a; *17:* 9-12.

Bölke E, Orth K, Jehle PM, Schwarz A, Steinbach G, Schleich S, Ulmer C, Storck M, Hannekum A. Enteral application of an immunoglobulin-enriched colostrum milk preparation for reducing endotoxin translocation and acute phase response in patients undergoing coronary bypass surgery – a randomized placebo-controlled pilot trial. Wien Klin Wochenschr. 2002b; *114:* 923-928.

Brenner D, Rothenbacher D. Underestimation of the association of Helicobacter pylori infection and gastric cancer. Deutsches Ärzteblatt. 2005; *24:* 1381-1386.

Caprioli J, Peng L, Remuzzi G. The haemolytic uremic syndromes. Curr Opin Crit Carpe. 2005; *11:* 487-492.

Casswall TH, Sarker SA, Albert MJ, Fuchs GJ, Bergstrom M, Bjorck L, Hammarstrom L. Treatment of Helicobacter pylori infection in infants in rural Bangladesh with oral immunoglobulins from hyperimmune bovine colostrum. Aliment Pharmacol Ther. 1998; *12:* 563-568.

Cesarone MR et al. Prevention of influenza episodes with colostrum compared with vaccination in healthy and high risk cardiovascular subjects: the epidemiologic study in San Valentino. Clin Appl Thromb Hemostas. 2007; *13:* 130-136.

Claridge JA, Crabtree TD, Pettetier SJ, Butler K, Sawyer RG, Young JS. Persistent occult hypoperfusion is associated with a significant increase in infection rate and mortality in major trauma patients. J Trauma. 2000; *48:* 8-14; discussion 14-15.

Davidson GP, Whyte PB, Daniels E, Franklin K, Nunan H, McCloud PI, Moore AG, Moore DJ. Passive immunisation of children with bovine colostrum containing antibodies to human rotavirus. Lancet. 1989; *2:* 709-712. Comment in: Lancet. 1989; *2:* 1336.

Davidson GP. Passive protection against diarrheal disease. J Pediatr Gastroenterol Nutr. 1996; *25:* 207-212.

de Jonge E, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J, Kesecioglu J. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. Lancet. 2003; *362:* 1011-1016.

Demetriades D, Smith JS, Jacobson LE, Moncure M, Minei J, Nelson BJ, Scannon PJ. Bactericidal/permeability-increasing protein (rBPI21) in patients with hemorrhage due to trauma. Results of a multicenter phase II clinical trial. RBPI21 Acute Hemorrhagic Trauma Study Group. J Trauma. 1999; *46:* 667-676.

Dofferhoff AS, Nijland JH, de Vries-Hospers HG, Mulder PO, Weits J, Bom VJ. Effects of different types and combination of antimicrobial agents on endotoxin release from Gram-negative bacteria: an in vitro and in vivo study. Scand J Infect Dis. 1991; *23:* 745-747.

Dundas S, Todd WT, Stewart AI, Murdoch PS, Chaudhuri AK, Hutchinson SJ. The central Scotland E. coli O157H7 outbreak: risk factors for the haemolytic uremic syndrome and death among hospitalised patients. Clin Infect Dis. 2001; *33:* 923-931.

Fitzal F, Racz I, Hamar J, Redl H, Bahrami S. Immunglobuline enriched colostral milk reduces gut-derived endotoxemia in a rat hemorrhage model. Eur J Trauma. 2001; *27:* 257-263.

Fomsgaard A. Antibodies to lipopolysaccharides. Some diagnostic and protective aspects. APMIS. 1990; *98 (Suppl. 18):* 3-38.

Goebel A, Netal S, Schedel R, Sprotte G. Human immunoglobulin treatment of trigeminal neuralgia refractory to carbamazepine therapy. European Journal of Neuroscience. 2000; *12:* 360, abstract presentation at FENS-meeting in Brighton.

Goebel A, Netal S, Schedel R, Sprotte G. Human pooled immunoglobulin in the treatment of chronic pain syndromes. Pain Medicine. 2002a; *3:* 119-127.

Goebel A, Bühner S, Herzele K, Werich M, Schedel R, Roewer N, Lochs H, Sprotte G. Gastrointestinale Barrierefunktion bei Patienten mit Fibromyalgie. Der Schmerz. 2002b; *Suppl I:* 74.

Goebel A, Moore A, Weatherall R, Roewer N, Schedel R, Sprotte G. Intravenous immunoglobulin in the treatment of primary trigeminal neuralgia refractory to carbamazepine: a study protocol [ISRCTN 330 42138]. BMC Neurology. 2003; *3:* 1-7.

Goebel A, Vogel H, Caneris O, Bajwa Z, Clover L, Roewer N, Schedel R, Karch H, Sprotte G, Vincent A. Immune responses to Campylobacter and serum autoantibodies in patients with complex regional pain syndrome. J Neuroimmunol. 2005; *162:* 184-189.

Gorman NT, Halliwell REW. The immunoglobulins: Structure, Genetics and Function. In: Halliwell REW, Gorman NT (eds) Veterinary clinical immunology. Philadelphia-London: WB Saunder Comp; 1989. p. 19-59.

Greenberg PD, Cello JP. Treatment of severe diarrhea caused by Cryptosporidium parvum with oral bovine immunoglobulin concentrate in patients with AIDS. J Acquir Immune Defic Syndr Hum Retrovirol. 1996; *13:* 348-354.

Hasenberg T, Rittler P, Post S, Jauch KW, Senkal M, Spies C, Schwenk W, Shang E. Umfrage zur perioperativen Therapie bei elektiven Kolonresektionen in Deutschland 2006. Chirurg. 2007; *78:* 818-826.

He F, Tuomola E, Arvilommi H, Salminen S. Modulation of human humoral immune response through orally administered bovine colostrum.FEMS Immunol Med Microbiol. 2001; *31:* 93-96.

Heyman MB. Evaluation of the impact of food technology on the allergenicity of cow's milk proteins. Proc Nutr Soc. 1999; *58:* 587-592.

Heyman MB. Lactose intolerance in infants, children and adolescents. Pediatrics. 2006; *118:* 1279-1286.

Hilpert H, Brussow H, Mietens C, Sidoti J, Lerner L, Werchau H. Use of bovine milk concentrate containing antibody to rotavirus to treat rotavirus gastroenteritis in infants. J Infect Dis. 1987; *156:* 158-166.

Holzheimer RG et al. Different endotoxin release and IL-6 plasma levels after antibiotic administration in surgical intensive care patients. J Endotoxin Research. 1996; *3:* 1-17.

Huppertz HI, Busch D, Schmidt H, Aleksic S, Karch H. Diarrhea in young children associated with Escherichia coli non-O157 organisms that produce Shiga-like toxins. J Pediatr. 1996; *128:* 341-346.

Huppertz HI, Karch H. EHEC – enterohämorrhagische Escherichia coli: Ein neuer Erreger mit zunehmender Bedeutung für die Pädiatrie. Der Kinderarzt. 1998; *29:* 24-29.

Huppertz HI, Rutkowski S, Busch DH, Eisebit R, Lissner R, Karch H. Bovine colostrum ameliorates diarrhea in infection with diarrheagenic Escherichia coli, shiga-toxin-producing E. coli and E. coli expressing intimin and hemolysin. J Pediatr Gastroenterol Nutr. 1999; *29:* 452-456.

Jackson J, Knopp H. β-Lactam antibiotic-induced release of free endotoxin in vitro – comparison of penicillin binding protein (PBP), 2-specific imipenem an PBP-3 specific ceftoxidime. J Infect Dis. 1992; *165:* 1033-1041.

Kaper JB. Enterohemorrhagic Escherichia coli. Curr Opin Microbiol. 1998; *1:* 103-108.

Karch H. The role of virulence factors in enterohemorrhagic Escherichia coli/EHEC-associated haemolytic-uremic syndrome. Semin Thromb Hemost. 2001; *27:* 207-213.

Karch H, Huppertz HI, Bockemühl J, Schmidt H, Schwarzkopf A, Lissner R. Shiga toxin-producing Escherichia coli infections in Germany. J Food Protect. 1997; *30:* 1454-1457.

Kelly GS. Bovine colostrums: A review of clinical uses. Altern Med Rev. 2003; *8:* 378-394.

Khan Z, Macdonald C, Wicks AC, Holt MP, Floyd D, Ghosh S, Wright NA, Playford RJ. Use of the "nutriceutical", bovine colostrum, for the treatment of distal colitis: results from an initial study. Aliment Pharmacol Ther. 2002; *16:* 1917-1922.

Kimmitt PT, Harwood CR, Barer MR. Toxin gene expression by Shiga toxin-producing Escherichia coli: the role of antibiotics and the bacterial SOS response. Emerg Infect Dis. 2000; *6:* 458-465.

Kohler B, Karch H, Schmidt H. Antibacterials that are used as growth promoters in animal husbandry can affect the release of Shiga-toxin 2 converting bacteriophages and Shiga toxin 2 from Escherichia coli strains. Microbiology. 2000; *146:* 1085-1090.

Korhonen H, Marnila P, Gill HS. Bovine milk antibodies for health. Brit J Nutr. 1999; *84:* 1-24.

Lissner R, Schmidt H, Karch H. A standard immunoglobulin preparation produced from bovine colostra shows antibody reactivity and neutralization activity against Shiga-like toxins and EHEC-hemolysin of Escherichia coli O157:H7. J Infection. 1996; *24:* 378-383.

Lissner R, Thurmann PA, Merz G, Karch H. Antibody reactivity and fecal recovery of bovine immunoglobulins following oral administration of a colostrum concentrate from cows (Lactobin) to healthy volunteers. Int J Clin Pharmacol Ther. 1998; *5:* 239-245.

Livingston DH, Deitch EA. Multiple organ failure: a common problem in surgical intensive care unit patients. Ann Med. 1995; *27:* 13-20.

Magnotti LJ, Uppermann JS, Xu Da-Zhong, Qi Lu, Deitsch EA. Gut-derived mesenteric lymph but not portal blood increases endothelial cell permeability and promotes lung injury after hemorrhagic shock. Ann Surg. 1998; *228:* 518-527.

Martin MA. The respiratory distress syndrome in adults with Gram-negative sepsis. In: Vincent JL (ed) Update in intensive care and emergency medicine. Berlin-Heidelberg: Springer; 1991. p. 182-189.

Martin MA, Silverman HJ. Gram-negative sepsis and the adult respiratory distress syndrome. Clin Infect Dis. 1992; *14:* 1213-1228.

McClave SA, Green LM, Snider HL et al. Comparison of the safety of early enteral and parenteral nutrition in mild acute pancreatitis. J Parent Enter Nutr. 1997; *21:* 14-20.

Mietens C, Keinhorst H, Hilpert H, Gerber H, Amster H, Pahud JJ. Treatment of infantile E. coli gastroenteritis with specific bovine anti-E. coli milk immunoglobulins. Eur J Pediatr. 1979; *132:* 239-252.

Mitra AK, Mahalanabis D, Ashraf H, Unicomb L, Eeckels R, Tzipori S. Hyperimmune cow colostrum reduces diarrhea due to rotavirus: a double-blind, controlled clinical trial. Acta Paediatr. 1995; *84:* 996-1001.

Mondorf AW, Duswald KH. Intravenous immunoglobulin in high-risk patients. Efficacy and tolerance of long-term administration. In: Alving B, Finlayson JS (eds) Immunoglobulins: characteristics and uses of intravenous preparations. FDA (BOB) Workshop Bethesda, ML, USA, October 30 – 31; 1979. p. 37-48.

Mondorf AW et al. The immunoglobulin therapy of herpes infections in patients undergoing cytostatic treatment. Biotest Mitteilungen No 4 second edition. 1981; *1981:* 1-16 (ISSN 0343-3897).

Moore EE, Jones TN. Benefits of immediate jejunostomy after major abdominal trauma – a prospective randomized trial. Trauma. 1986; *26:* 874-881.

Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev. 1998; *11:* 142-201.

Nord J, Ma P, DiJohn D, Tzipori S, Tacket CO. Treatment with bovine hyperimmune colostrums of cryptosporidial diarrhea in AIDS patients. AIDS. 1990; *4:* 581-584.

Noris N, Remuzzi G. Hemolytic uremic syndrome. J Am Soc Nephrol. 2005; *16:* 1035-1056.

Orihuela CJ et al. Bacterial interactions with mucosal epithelial cells. In: Mestecky J et al. (eds) Mucosal Immunology 3rd ed. Amsterdam: J Elsevier Academic Press; 2005. p. 757-767.

Pedersen AM, Andersen TL, Reibel J, Holmstrup P, Nauntofte B. Oral findings in patients with primary Sjögren's syndrome and oral lichen planus – a preliminary study on the effects of bovine colostrum-containing oral hygiene products. Clin Oral Investig. 2002; *6:* 11-20.

Pfeiffer L, Ehrhardt N, Kretzschmar M, Urbaschek R, Schubert K, Schirrmeister W. Endotoxemia and multiple organ failure after polytrauma. Anaesthesiol Reanim. 1996; *21:* 91-96.

Phillips B, Tyerman K, Whiteley SM. Use of antibiotics in suspected haemolytic-uraemic syndrome. BMJ. 2005; *330:* 409-410.

Playford RJ, MacDonald CE, Calnan DP, Floyd DN, Podas T, Johnson W, Wicks AC, Bashir O, Marchbank T. Co-administration of the health-food supplement, bovine colostrum reduces the acute non-steroidal anti-inflammatory drug induced increase in intestinal permeability. Clinical Science. 2001; *100:* 627-633.

Plettenberg A, Stoehr A, Stellbrink HJ, Albrecht H, Meigel W. A preparation from bovine colostrum in the treatment of HIV-positive patients with chronic diarrhea. Clin Investig. 1993; *71:* 42-45.

Prins JM, van Agtmael MA, Kuijper EJ, van Deventer SJ, Speelman P. Antibiotic-induced endotoxin release in patiens with urosepsis – a double blind study comparing imipenem with ceftozidime. J Infect Dis. 1995; *172:* 886-891.

Riedemann NC, Guo RF, Ward PA. The enigma of sepsis. J Clin Invest. 2003; *112:* 460-467.

Rittler P, Demmelmair H, Koletzko B, Schildberg FW, Harthl WH. Effect of elective abdominal surgery on human colon protein synthesis in situ. Ann Surg. 2001; *233:* 39-44.

Roos N, Mahe S, Benamouzig R, Sick H, Rautureau J, Tome D. 15N-labeled immunoglobulins from bovine colostrum are partially resistant to digestion in human intestine. J Nutr. 1995; *125:* 1238-1244.

Rump JA, Arndt R, Arnold A, Bendick C, Dichtelmuller H, Franke M, Helm EB, Jager H, Kampmann B, Kolb P. Treatment of diarrhea in human immunodeficiency virusinfected patients with immunoglobulins from bovine colostrums. Clin Investig. 1992; *70:* 588-594.

Sarker SA, Majid N, Mahalanabis D. Alanine- and glucose-based hypo-osmolar oral rehydration solution in infants with persistent diarrhea: a controlled trial. Acta Paediatr. 1995; *84:* 775-780.

Seifert J, Molkewehrum M, Oesser S, Nebermann L, Schulze C. Endotoxin inactivation by enterally applied colostrum of different composition. Eur Surg Res. 2002; *34:* 68-72.

Stephan W, Dichtelmüller H, Lissner R. Antibodies from colostrum in oral immunotherapy. J Clin Chem Clin Biochem. 1990; *28:* 19-23.

Stoutenbeck CP, van Saene HK, Miranda DR, Zandstra DF. The effect of selective decontamination of the digestive tract on the colonization and infection rate in multiple trauma patients. Intensive Care Med. 1984; *10:* 185-192.

Struff WG, Sprotte G. Bovine colostrum as a biologic in clinical medicine: a review. Part I: Biotechnological standard, pharmacodynamic and pharmacokinetic characteristics and principles of treatment. Int J Clin Pharmacol Ther. 2007; *45:* 193-202.

Tapper D, Tarr P, Avner E, Brandt J, Waldhausen J. Lessons learned in the management of hemolytic uremic syndrome in children. J Pediatr Surg. 1995; *30:* 158-163.

Tarr PI. E. coli O157:H7: Clinical diagnostic and epidemiological aspects of human infection. Clin Infect Dis. 1995; *20:* 1-10.

Tzipori S, Roberton D, Cooper DA, White L. Chronic cryptosporidial diarrhea and hyperimmune cow colostrum. Lancet. 1987; *2:* 344-345.

Uçeyler N, Valenza R, Stock M, Schedel R, Sprotte G, Sommer C. Reduced levels of antiinflammatory cytokines in patients with chronic widespread pain. Arthritis Rheum. 2006; *54:* 2656-2664.

Ulrich D, Noah E-M, Pallua N. Plasma-endotoxin, procalcitonin, C-reactive protein and organ functions in patients with major burns. Handchir Mikrochir Plast Chir. 2001; *33:* 262-266.

van Hooijdonk AC, Kussendrager KD, Steijns JM. In vivo antimicrobial and antiviral activity of components in bovine milk and colostrum involved in non-specific defence. Br J Nutr. 2000; *84:* 127-134.

Vincent JL. Selective digestive decontamination for everyone, everywhere? Lancet. 2003; *362:* 1006-1007

Waaga-Gasser AM. IgG-Konzentrat aus bovinem Kolostrum zur Prophylaxe septischer Komplikationen nach Laparotomie von Patienten mit Peritonitis. Forschungsbericht 2005.

Ward L et al. Viral gastroenteritis vaccines. In: Mestecky J et al. (eds) Mucosal immunity. 3rd ed. Amsterdam: Elsevier Academic Press; 2005. p. 887-895.

Wehkamp J, Bals R, Kreft B, Schroeder JM, Stange EF. Innate immunity – the clinical significance of endogenous antimicrobial peptides in internal medicine and dermatology. Dtsch Arztebl. 2007; *104:* A 257-262.

Winchurch RA, Thupari JN, Munster AM. Endotoxemia in burn patients: level of circulating endotoxins are related to burn size. Surgery. 1987; *102:* 808-812.

Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI. The risk of the haemolytic-uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 infections. N Engl J Med. 2000; *342:* 1930-1936.

Yao YM, Sheng ZY, Tian HM, Yu Y, Wang YP, Yang HM, Guo ZR, Gao WY. The association of circulating endotoxaemia with the development of multiple organ failure in burned patients. Burns. 1995; *21:* 255-258.

Ylitalo S, Uhari M, Rasi S, Pudas J, Leppaluoto J. Rotaviral antibodies in the treatment of acute rotaviral gastroenteritis. Acta Paediatr. 1998; *87:* 264-267.

Yoh M, Frimpong EK, Voravuthikunchai SP, Honda T. Effect of subinhibitory concentrations of antimicrobial agents (quinolones and macrolide) on the production of verotoxin by enterohemorrhagic Escherichia coli 0157:H7. Can J Microbiol. 1999; *45:* 732-739.

Zhang X, McDaniel AD, Wolf LE, Keusch GT, Waldor MK, Acheson DW. Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. J Infect Dis. 2000; *181:* 664-670.