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Bovine milk antibodies for health

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The immunoglobulins of bovine colostrum provide the major antimicrobial protection against microbial infections and confer a passive immunity to the newborn calf until its own immune system matures. The concentration in colostrum of specific antibodies against pathogens can be raised by immunising cows with these pathogens or their antigens. Immune milk products are preparations made of such hyperimmune colostrum or antibodies enriched from it. These preparations can be used to give effective specific protection against different enteric diseases in calves and suckling pigs. Colostral immunoglobulin supplements designed for farm animals are commercially available in many countries. Also, some immune milk products containing specific antibodies against certain pathogens have been launched on the market. A number of clinical studies are currently in progress to evaluate the efficacy of immune milks in the prevention and treatment of various human infections, including those caused by antibiotic resistant bacteria. Bovine colostrum-based immune milk products have proven effective in prophylaxis against various infectious diseases in humans. Good results have been obtained with products targeted against rotavirus, *Shigella flexneri*, *Escherichia coli*, *Clostridium difficile*, *Streptococcus mutans*, *Cryptosporidium parvum* and *Helicobacter pylori*. Some successful attempts have been made to use immune milk in balancing gastrointestinal microbial flora. Immune milk products are promising examples of health-promoting functional foods, or nutraceuticals. This review summarises the recent progress in the development of these products and evaluates their potential as dietary supplements and in clinical nutrition.

Immune milk: Colostrum: Immunoglobulins: Passive immunisation

Introduction

Diarrhoeal diseases continue to represent a major threat to human health on a global scale. Factors such as malnutrition and HIV-immunocompromisation have exacerbated the incidence of acute and chronic acquired gastrointestinal infections, and the increase in global travel has ensured that new emerging strains of enteric pathogens can rapidly spread and become established on other continents. Current prophylactic or interventionist methods (vaccination or chemotherapy) are often ineffective at controlling disease and/or eliminating infection, and have created grave concerns over a rise in antibiotic-resistant strains through over-use. Even in cases where measures are effective, quite often the treatment regime is economically and logistically impossible to administer, particularly in developing countries. There is a real need for the development of new means to combat gastrointestinal (GI) tract infections, where the major criteria are effectiveness, affordability, ease of administration and safety. Borrowing molecules of immune defence from an immunised animal may provide an effective strategy in this combat.

It has long been recognised that maternal milk can offer passive protection to a newborn infant against enteric pathogens, primarily via the transfer of immunoglobulins and associated factors from mother to infant. The historical concept of 'immune milk', i.e. the transfer of passive immunity via lacteal antibodies, dates back to the 1950s (Campbell & Petersen, 1963; Lascelles, 1963). The underlying mechanisms of passive immunity, however, were only recognised in the early 1960s when the chemical structure of immunoglobuling (Igs) was elucidated. Particularly the identification of the mucosal or secretory immune system in the 1970s provided new insight into the role of secretory antibodies in the prevention or treatment of enteric infections in mammals (Lamm *et al.* 1978). The development of homologous (human-derived) antibodies into an effective treatment for enteric pathogens has subsequently received considerably less commercial attention than the utilisation of antibodies from the milk of heterologous species, particularly ruminants. Since the 1980s, an increasing number of studies have shown that immune milk preparations, based on bovine antibodies derived from the milk or colostrum of immunised cows,

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can be effective in the prevention or treatment of human and animal diseases caused by enteropathogenic microbes (for reviews see Reddy *et al.* 1988; Goldman, 1989; Boesman-Finkelstein & Finkelstein, 1991; Facon *et al.* 1993; Hammarström *et al.* 1994; Ruiz, 1994; Bogstedt *et al.* 1996; Davidson, 1996; Korhonen, 1998; Weiner *et al.* 1999). The efficacy of bovine immune milk products is mainly based on the antimicrobial activity of the specific antibodies and complement factors present in the preparation. This chapter reviews the current state of the art in the development of bovine immune milk preparations and their observed efficacy in clinical trials.

Development of antibody preparations

The progress made in understanding the underlying mechanisms of immunity has provoked renewed interest in the development of immune milk preparations for the prevention or treatment of microbial infections in humans and domestic animals. Also, the rapid development of modern fractionation technologies, based on membrane separation and chromatography, has enabled large-scale isolation of Igs from bovine colostrum and milk (Kothe *et al.* 1987; Abraham, 1988; Stott & Lucas, 1989; Korhonen *et al.* 1998). Basically, the approaches to the development of Ig-based preparations are either the concentration or isolation of Igs occurring naturally in colostrum or milk, or the hyperimmunisation of pregnant cows during the 'dry' period with antigens from pathogens in order to raise specific antibodies in the colostrum and milk.

Hyperimmunisation of cows with specific microbial antigens provides a method for inducing increased amounts of specific antibodies in the mammary secretions. The most common approach, described in many scientific articles and patent documents, is the repeated systemic inoculation of cows with an immunogen at the end of the lactation period and during the dry period (Korhonen *et al.* 1977; Saif *et al.* 1984; Linggood *et al.* 1990; Beck, 1990; Stolle, 1990). Also, immunisation via the mammary gland or by oral administration of the immunogen to pregnant or lactating cows has been tried but with only moderate success (Korhonen, 1973; Korhonen *et al.* 1977). High antibody titres in the blood and colostrum have been achieved using a combination of intramuscular and intramammary inoculations (Schaller *et al.* 1992). In most cases, the antibody response has depended on the nature of the adjuvant material used in the vaccine. In experimental studies, Freund's complete or incomplete adjuvant has been found to induce the strongest humoral (antibody-based) immune response (Schaller *et al.* 1992; Korhonen *et al.* 1994), but its commercial use is limited by concerns over possible side-effects. In most cases, this has led to the use of 'safer' aluminium hydroxide-based adjuvants for the immunisation of farm animals. In our experience, the health status of the cow is very important. There is still a need to explore further possibilities of optimising the immunisation protocol for the maximum yield and safe production of specific antibodies in the lacteal secretions, with a minimum of physiological stress to the immunised animals.

Efficacy of antibody preparations

The efficacy of orally administered bovine or human antibodies has been documented in numerous studies involving experimental animal models as well as clinical human trials (for reviews see Levine, 1991; Facon *et al.* 1993; Hammarström *et al.* 1994; Ruiz, 1994; Bogstedt *et al.* 1996; Davidson, 1996; Weiner *et al.* 1999). Some studies have provided evidence for the protective and therapeutic effects of Igs enriched from regular bovine cheese whey or colostrum from non-immunised cows against non-specific diarrhoeal diseases of newborn farm animals (Zaremba *et al.* 1993; Nousiainen *et al.* 1994) and humans (Fernandez *et al.* 1973; Lodinova-Zadnikova *et al.* 1987; Stephan *et al.* 1990). In animal studies, the efficacy of Ig supplements has proved variable (Mee & Mehra, 1995). In humans, promising results have been reported in the treatment of patients with antoimmune deficiency syndrome (AIDS) who received 10 g/d of normal bovine colostral Ig concentrate for 10 days (Stephan *et al.* 1990). Rump *et al.* (1992) used a similar preparation and dosage in human immunodeficiency virus-infected (HIV) patients suffering from chronic diarrhoea, and reported significant clinical benefits with no side-effects for the duration of the therapy.

Farm animal infections

There is ample evidence that Ig concentrates, purified Igs derived from colostrum or milk from hyperimmunised cows, can provide protection against rotavirus in calves (Saif & Smith, 1983; Castrucci *et al.* 1988; Tsunemitsu *et al.* 1989) and in agammaglobulinaemic piglets (Lecce *et al.* 1991). Schaller *et al.* (1992) demonstrated in a gnotobiotic piglet model that both viral shedding and diarrhoea were effectively reduced or eliminated in a dose-dependent manner, as a result of feeding Ig preparations containing antibodies specific for human rotavirus strains. Positive results have also been obtained in studies on the protection of newborn calves and piglets against enterotoxigenic *Escherichia coli* diarrhoea, using a colostral supplement from vaccinated cows or by vaccinating dams with purified antigens (Isaacson *et al.* 1980; Snodgrass *et al.* 1982; Moon & Bunn, 1993).

Human infections

Bacterial gastrointestinal infections. An increasing number of controlled clinical studies suggest that the oral administration of Ig preparations containing high titres of specific antibodies can provide effective protection and to some extent may also be of therapeutic value against gastrointestinal infections in humans. In most of these studies, the efficacy of the preparations has been tested against enteropathogenic *E. coli*, rotavirus and *Cryptosporidium* infections. Controlled clinical trials have also been carried out using hyperimmune bovine colostrum containing specific antibodies against *Shigella flexneri*, *Helicobacter pylori*, *Vibrio cholerae* and caries streptococci. Table 1 presents a summary of studies in which positive results against bacterial pathogenes have been achieved *in*

Table 1. Efficacy of bovine immune milk against bacterial infections *in vivo*

Subject	Target organism	Efficacy	Reference
	Campylobacter		
Chicken	*C. jejuni*	In prophylaxis >99 % decrease in number of *C. jejuni* in faeces. In therapeutic treatment 80–95 % lower level of *C. jejuni* in faeces.	Tsubokura *et al.* 1997
	Clostridium difficile		
Hamster	*C. difficile* enterotoxin	Protected against disease	Lyerly *et al.* 1991
Rat	*C. difficile* enterotoxin	Decreased enterotoxic symptoms	Kelly *et al.* 1996
	Escherichia coli		
Humans			
Infants	Enteropathogenic *E. coli*	Reduced *E. coli* in faeces	Mietens *et al.* 1979
Preterms and infants	Enteropathogenic *E. coli*	Reduced diarrhoea	Lodinova-Zadnikova *et al.* 1987
Healthy volunteers	Enterotoxigenic *E. coli* H10407	Prevented diarrhoea after experimental challenge	Tacket *et al.* 1988
117 children	Enteropathogenic *E. coli* and rotavirus	No reduction in incidence of diarrhoea or other parameters during 6 months' follow-up	Brunser *et al.* 1992
Preterms	Eight different enterobacteria	Positive effects on intestinal flora, reduction of enterobacteria, more effective than probiotics	Kushnareva *et al.* 1995
Healthy volunteers	Enterotoxigenic *E. coli*	Prevented diarrhoea after experimental challenge	Freedman *et al.* 1998
Animal models			
Mouse	Indigenous *E. coli*	Prevented indigenous infection after pharmacological impairment of intestinal microflora	Nomoto *et al.* 1992
Domestic animals			
Suckling pigs	Enterotoxigenic *E. coli*	Protected against fatal diarrhoea by *E. coli*	Isaacson *et al.* 1980
Neonatal calves	Enterotoxigenic *E. coli*	Prevented diarrhoeal disease after experimental challenge	Snodgrass *et al.* 1982
	Helicobacter		
Humans			
Adults	*H. pylori*	Attenuated gastritis symptoms	Tarpila *et al.* 1994
Children	*H. pylori*	Attenuated chronic inflammation of gastric antrum	Oona *et al.* 1997
Infants	*H. pylori*	No eradication or decrease in colonisation of gastric antrum	Casswall *et al.* 1998
Adults	*H. pylori*	No eradication or decrease in colonisation of gastric antrum	Opekun *et al.* 1999
Animal models			
Mouse	*H. felis*	Prevented infection after experimental challenge	Rehnberg-Laiho *et al.* 1995
Mouse	*H. felis*	Reduced colonisation of *H. felis* in gastric antrum	Marnila *et al.* 1996
	Klebsiella		
Mouse	*K. pneumoniae*	Prevented infection	Soboleva *et al.* 1991
	Proteus		
Mouse	*P. mirabilis*and *P. vulgaris*	Prevented infection	Soboleva *et al.* 1991
	Pseudomonas		
Mouse	*P. aeruginosa*	Prevented infection	Stephan *et al.* 1990; Soboleva *et al.* 1991
	Salmonella		
Mouse	*S. pullorum*	Delayed death	Campbell & Petersen, 1959
Mouse	*S. typhimurium*	Prevented infection	Stephan *et al.* 1990
Mouse	*S. typhimurium* and *S. enteritidis*	Prevented infection	Soboleva *et al.* 1991
	Shigella		
Human adults	*S. flexneri*	Prevented shigellosis	Tacket *et al.* 1992
	Streptococcus		
Human adults	*S. mutans*	Reduced *S. mutans* level in dental plaque	Filler *et al.* 1986, 1991
Human adults	*S. mutans*	Reduced proportion of *S. mutans* in dental plaque Elevated resting pH in plaque	Loimaranta *et al.* 1999b
Rat	*S. mutans*	Reduced caries development	Mikhalek *et al.* 1987
	Vibrio cholerae		
Rabbits	Cholera enterotoxin	Decreased mortality and intestinal fluid response	McClead & Gregory, 1984
Infant rabbits	Virulent cholera vibrios	Reduced diarrhoea	Boesman-Finkelstein *et al.* 1989

vivo, and examples of these studies are reviewed in more detail in the following examples.

Tacket *et al.* (1992) gave immune milk against *Sh. flexneri* 2a lipopolysaccharide to human volunteers two days before experimental challenge with a virulent strain of *Sh. flexneri*. The immune milk prevented the outbreak of the illness in all ten subjects whereas five out of eleven subjects fell ill in the control group which was treated similarly with an Ig preparation from non-immunised cows. Mietens *et al.* (1979) treated 60 infants having diarrhoea due to enteropathogenic *E. coli* for 10 days with 1 g/kg body weight of anti-enteropathogenic *E. coli* bovine Ig concentrate. The treatment was effective in eliminating the pathogen in forty-three of fifty-one children infected with strains present in the inoculum. A lyophilised Ig concentrate (prepared from colostrum of cows immunised with several enterotoxigenic *E. coli* serotypes, fimbria types, *E. coli* heat-labile enterotoxin, and cholera toxin) was shown to provide complete protection against enterotoxigenic *E. coli* infection in ten adult volunteers (Tacket *et al.* 1988). These results suggest that immune colostral or milk preparations could be useful in the prevention of traveller's diarrhoea. However, in a small-scale field trial carried out in Chile, Brunser *et al.* (1992) failed to demonstrate any protective benefit from supplementing an infant formula with milk-derived antibodies specific for major enteropathogenic *E. coli* serotypes (although this failure might be attributed to a low level of antibody in the formula). However, bovine Igs have proven effective in animal models in neutralising bacterial toxins in the gastrointestinal tract. McClead & Gregory (1984) reported that a specific bovine colostral Ig preparation against cholera enterotoxin was capable of decreasing mortality and intestinal fluid responses in rabbits exposed to cholera enterotoxin. The capability of bovine Igs to neutralise microbial toxins was also confirmed by Lyerly *et al.* (1991), who demonstrated that a colostral Ig preparation produced by hyperimmunising cows against *Clostridium difficile* toxoid protected hamsters against the manifestation of *C. difficile* disease. In another study, a basically similar preparation neutralised the cytotoxic effects of *C. difficile* toxins on rat ileum both *in vitro* and *in vivo* (Kelly *et al.* 1996). Thus, immune milk products may be clinically useful in the prevention and treatment of *C. difficile* diarrhoea and colitis.

Encouraging results, although not as good as those with cryptosporidiosis, have also been reported for the administration of an immune bovine colostral Ig concentrate, containing antibodies specific for *Helicobacter pylori*, to *H. pylori*-infected patients. This bacterium has been identified as the major aetiological agent of active chronic gastritis and peptic ulcer disease (Peek & Blaser, 1997). Both hyperimmune and nonimmune colostrum have been shown to kill *H. pylori* bacteria effectively *in vitro* (Korhonen *et al.* 1995), and the observed bactericidal activity is known to be associated with the antibody-complement system. A similar preparation containing specific antibodies for *H. felis* protected mice against *H. felis* infection (Rehnberg-Laiho *et al.* 1995). The protection was dependent on the presence of specific antibodies in milk, the control preparation having no protective effect. Similarly, Thomas *et al.* (1993) reported a strong negative correlation between the occurrence of *H. pylori* antibodies in the milk of Gambian mothers and the incidence of *H. pylori* infection in their small children. Preliminary clinical trials on chronic gastritis patients and children infected with *H. pylori* have shown that a daily treatment for 3–4 weeks with an immune anti-*H. pylori* bovine Ig concentrate, delivered in a daily dose of 20 g for adults and 12 g for children, can decrease the severity of the symptoms and the rate of *Helicobacter* colonisation in most subjects. However, total eradication of the *Helicobacter* infection was observed in only one out of nine adult patients and in none of the twenty treated children (Tarpila *et al.* 1994; Oona *et al.* 1997). The decrease observed in the severity of gastric inflammation suggests that the specific *H. pylori* antibodies may help to eliminate pro-inflammatory components secreted by *Helicobacter* and may also reduce (although not necessarily eliminate) bacterial colonisation in the gastric mucosa. These results are consistent with those of Casswall *et al.* (1998). *H. pylori* was eradicated from none of a group of small children in rural Bangladesh treated for 30 days with 1 g of a preparation containing *H. pylori*-specific antibodies. Correspondingly, Opekun *et al.* (1999) reported that immune bovine colostrum immunoglobulins were not effective in decreasing the number of *H. pylori* present in the gastric mucosa of infected volunteers. On the other hand, a reduction of colonisation in the gastric antrum but not the eradication of *H. felis* was observed in a mouse model when the mice were treated with an immune bovine Ig preparation (Marnila *et al.* 1996). Placebo-controlled clinical studies will be required to test the efficacy of immune colostral preparations as a potential adjunct to the chemotherapy currently practised in the treatment of *Helicobacter*-associated gastritis.

Oral infections. Oral pathogens, like fungal pathogens in immunocompromised patients or dental caries-promoting streptococci, should represent feasible targets for intervention with immune bovine Ig preparations. Recently, Tollemar *et al.* (1999) succeeded in reducing *Candida albicans* colonisation in the oral cavity of immunosuppressed bone marrow transplantation patients with bovine milk antibodies.

Dental caries is still one of the most common infectious diseases, especially in developing countries. Due to the potential side-effects of active immunisation against cariogenic mutans streptococci, passive immunity by an oral administration of antibodies is a more acceptable way of reducing colonisation and the virulence of these bacteria in human dentition. Studies in humans (Filler *et al.* 1986, 1991) and in rats (Mikhalek *et al.* 1987) have suggested that bovine milk-derived antibodies specific for *Streptococcus mutans* may confer effective protection against colonisation by mutans streptococci and the development of dental caries. Recent *in vitro* studies have shown that immune bovine colostrum, containing antibodies specific for *S. mutans* and *S. sobrinus*, was capable of inhibiting the bacterial enzymes producing sticky capsule glycopolysaccharides (Loimaranta *et al.* 1997), inhibiting in a dose-dependent manner the adherence of *S. mutans* cells to saliva-coated hydroxyapatite particles (which simulate the tooth surface); aggregating *S. mutans* cells (Loimaranta *et*

Table 2. Efficacy of bovine immune milk against viral infections *in vivo*

Subject	Virus	Efficacy	Reference
Humans			
Infants	Poliomyelitis vaccine of Sabin type 2	Prevented infection of gastrointestinal tract	Gonzaga *et al.* 1963
Infants	Rotavirus Wa 1	Prevented infection	Ebina *et al.* 1985
Children	Four human rotavirus serotypes	Prevented infection	Davidson *et al.* 1989
117 children	Enteropathogenic *E. coli* and rotavirus	No reduction in incidence of diarrhoea or other parameters during 6 months follow-up	Brunser *et al.* 1992
Infants	Human rotavirus	Reduced symptoms of infection but not incidence	Turner & Kelsey 1993
Small children	Four human rotavirus serotypes	Prevented infection	Davidson *et al.* 1994
Small children	Four human rotavirus serotypes	Shortened duration and decreased severity of diarrhoea	Mitra *et al.* 1995
Infants	Human rotavirus MO	Prevented diarrhoea	Ebina, 1996
Infants having acute rotaviral gastroenteritis	Four human rotavirus serotypes	Reduced duration of virus excretion in stools, no significant effect on diarrhoea	Hilpert *et al.* 1987
132 small children with rotaviral gastroenteritis	Rotavirus SA11	Not statistically significant, but trend-setting improvement in duration of diarrhoea, weight gain and stool frequency	Ylitalo *et al.* 1998
40 small children with rotaviral gastroenteritis	Four human rotavirus serotypes	Shortened duration of diarrhoea and reduced amount and frequency of stool	Sarker *et al.* 1998
Animal models			
Infant mice	Human rotavirus MO	Prevented infection after challenge	Ebina *et al.* 1992
Infant mice	Human rotavirus MO	Prevented infection after experimental challenge	Ebina, 1996
Domestic animals			
Calves	Bovine virus diarrhoea virus (BVDV)	Prevented infection of respiratory tract and protected against viraemia and leukopenia after experimental challenge.	Howard *et al.* 1989
Calves	Bovine rotavirus	Delayed onset of diarrhoea, reduced incidence, duration and severity of diarrhoea	Snodgrass *et al.* 1982
New-born calves	Bovine rotavirus 81/36F	Prevented diarrhoea after experimental infection	Castrucci *et al.* 1982
New-born calves	Bovine rotavirus	Prevented infection	Saif *et al.* 1987
Calves	Bovine rotavirus 81/36F	Prevented diarrhoea after experimental infection	Castrucci *et al.* 1988
New-born calves	Bovine rotavirus serotypes 1 and 2	Prevented infection	Tsunemitsu *et al.* 1989
Calves	Bovine rotavirus 81/36F	Prevented infection in most cases, decreased its severity	Castrucci *et al.* 1989
Neonatal calves	IgG from non-immunised cows, titres against RV proteins VP2, 4, 6 and 7	Protected against diarrhoea, no therapeutic effect on diarrhoea	Osame *et al.* 1991
Agammaglobulinaemic piglets	Simian rotavirus SA-11, not porcine rotavirus	Protected against North Carolina porcine rotavirus	Lecce *et al.* 1991
Gnotobiotic piglets	Human rotavirus	Prevented infection after challenge	Schaller *et al.* 1992
Calves	Bovine herpes virus 1	No prevention, attenuated clinical signs of infection after experimental challenge.	Bradshaw & Edwards, 1996
New-born calves	Recombinant SA-11 (P2G3) rotavirus like IND (P/5/G6) particles	Prevented bovine rotavirus shedding and diarrhoea	Fernandez *et al.* 1998
Foals	Mares immunised with SA11 (G3P2), H2 (G3P12) and Lincoln (G6P1)	Decreased morbidity, shortened duration of clinical signs of diarrhoea	Barrandeguy *et al.* 1998

al. 1998*b*), and augmenting the recognition, phagocytosis and killing of *S. mutans* by human polymorphonuclear (PMN) leucocytes (Loimaranta *et al.* 1999*a*). Further, the immune colostrum did not inhibit *in vitro* the natural antibacterial peroxidase-hypothiocyanate system of saliva but did, indeed, in certain circumstances act synergistically against *S. mutans* (Loimaranta *et al.* 1998*a*). In a short-term clinical trial, the immune colostrum also proved to be functional in *in vivo* conditions. Using immune colostrum as a mouth rinse for 3 days resulted in a higher resting pH in dental plaque and a lower proportion of caries streptococci in plaque microbial flora than in control groups (Loimaranta *et al.* 1999*b*). However, further clinical studies will be required to evaluate the *in vivo* efficacy of anticaries immune bovine Ig preparations.

Viral infections. Several clinical studies have shown that hyperimmune bovine colostrum, derived from cows immunised with different serotypes of human rotavirus, can

Table 3. Efficacy of bovine immune and non-immune milk against *Cryptosporidium* infections in humans

Subject	Route of administration of immune (1) or non-immune (2) milk	Efficacy	Reference
A three year old boy	Via nasogastric tube (1)	Vomiting and diarrhoea resolved in 5 days and oocysts absent from stools in 8 days	Tzipori *et al.* 1986
Three human patients	Oral (1)	Ceased diarrhoea in three of three patients	Tzipori *et al.* 1987
Five human AIDS patients	Continously via nasogastric tube (1)	Reduced diarrhoea in one of three cases and reduction of oocysts in stools in two of three patients	Nord *et al.* 1990
Human adult AIDS patients	Oral (10 g of Ig preparation daily) (2)	Relieved symptoms of intestinal inflammations. Cryptosporidia oocysts in stools absent	Stephan *et al.* 1990
One human AIDS patient	Direct duodenal infusion (1)	Ceased diarrhoea, stools formed, oocysts in stools absent	Ungar *et al.* 1990
29 HIV patients	Oral (10 g of Ig preparation daily) (2)	Normalized stool frequency in 21 of 29, cryptosporidiosis disappeared in five patients	Rump *et al.* 1992
Seven human AIDS patients	Oral (10 g of Ig preparation daily) (2)	Complete remission in three and partial in two of seven cryptosporidiosis patients	Plettenberg *et al.* 1993
4-year-old AIDS patient	Oral (10 g of Ig preparation daily) (2)	Permanent elimination of *Cryptosporidium*, improvement in diarrhoeal symptoms	Shield *et al.* 1993
Eight human AIDS patients	Oral (powder) (2)	Reduced diarrhoea, body weight stabilised, reduced oocysts in stools	Greenberg & Cello,1996
Healthy human adult volunteers	Oral (10 g of Ig preparation three times a day) (1)	Reduced diarrhoea and oocyst excretion after experimental challenge	Okhuysen *et al.* 1998

protect infants from acquiring rotavirus and also other viral infections during a reported outbreak (Table 2). Such concentrates also appear to be useful in the treatment of rotavirus-infected children. Ebina *et al.* (1985) and Ebina (1996) demonstrated that an antirotavirus antibody concentrate (Rota-colostrum®) could protect against infection, whereas infants fed commercial milk or purified antibodies (IgG, IgM, and IgA) were not protected against rotavirus. Davidson *et al.* (1989) fed bovine colostrum, containing high antibody titres against the four major human serotypes of rotavirus, to hospitalised children aged 3–15 months. Whereas none of the colostrum-fed children acquired symptomatic rotavirus infection during the treatment period, 14 % (9/65) of the control infants developed infection. A similar study was carried out in Hong Kong and India, confirming the above findings (Davidson *et al.* 1994). The authors concluded that the antibody titre is important for protection and that hyperimmune colostrum could protect against more than one rotavirus serotype. The importance of antibody levels was also reflected in another study (Turner & Kelsey, 1993) which showed that passive immunisation of healthy infants with hyperimmune colostrum was successful in reducing symptomatic rotavirus infection but had no effect on the actual incidence of the infection. Hilpert *et al.* (1987) tested the efficacy of a hyperimmune bovine colostral Ig concentrate in seventy-five hospitalised children with acute rotavirus gastroenteritis, when the children received Ig concentrate in a daily dose of 2 g/kg body weight for 5 days. A decrease in the duration of rotavirus excretion was noted, but there was no associated effect on the clinical symptoms. Brunser *et al.* (1992) used a milk formula with 1 % (w/w) of bovine milk immunoglobulin concentrate containing specific antibodies against simian rotavirus SA11 and enteropathogenic *E. coli* for 6 months. No protection against diarrhoea or beneficial effect during the disease was seen. In a double-blind controlled clinical study, carried out in Bangladesh (Mitra *et al.* 1995), a group of rotavirus-infected children aged 6–24 months was administered 100 ml of hyperimmune (HI) bovine colostrum per child three times daily for 3 days. As compared to the control group, who received non-immune colostrum, the children treated with HI colostrum showed a significant reduction in the duration and severity of diarrhoea. Ylitalo *et al.* (1998) used a similar mode of administration (100 ml of hyperimmune colostrum four times per day for 4 days for treating rotavirus-infected children) and observed a trend-setting but statistically non-significant improvement in all the evaluated variables (weight gain, duration of diarrhoea and number of stools). Sarker *et al.* (1998) treated children of age 4–24 months with 10 g of Ig concentrate in 20 ml of water four times per day and achieved a significant reduction in daily and total stool output as well as in the duration of diarrhoea. Altogether, it can be concluded that hyperimmune colostral preparations have potential not only in prophylaxis but also in the treatment of rotavirus infections, although adequate intake of specific Ig is crucial for achieving positive results.

Cryptosporidium infections. Very encouraging results have been obtained in clinical studies in which hyperimmune bovine colostrum, containing antibodies specific for the enteric protozoan parasite *Cryptosporidium parvum*, has been tested in immunocompromised patients (Table 3)

(Tzipori *et al.* 1986, 1987; Nord *et al.* 1990; Ungar *et al.* 1990; Williams, 1992; Okhuysen *et al.* 1998). In contrast, non-immune colostrum or non-specific bovine Ig concentrate has been shown to afford little protection against *Cryptosporidium* infection, again emphasising the importance of specific antibodies (Saxon & Weinstein, 1987; Stephan *et al.* 1990; Rump *et al.* 1992; Plettenberg *et al.* 1993; Shield *et al.* 1993; Greenberg & Cello, 1996). The preventive and therapeutic efficacy of specific antibodies is probably mainly due to their ability to neutralise the sporozoites released from the oocysts in the intestinal lumen before they penetrate the epithelial cells (Perryman *et al.* 1990). At present, no effective therapy exists for cryptosporidiosis, which is one of the leading contributors to mortality in AIDS patients.

Immune milk may also be effective against other parasites. A colostral Ig preparation against *Toxocara vitulorum* was found to protect mice against this helminth when larvae were fed to them (Rajapakse *et al.* 1994).

Taxonomy and immune milk

Bovine hyperimmune colostrum may at least in some cases exert its beneficial effects over the taxonomic borders. *Cryptosporidium parvum* immune colostrum proved effective in treating *C. serpensis*-infected snakes (Graczyk *et al.* 1998) and *Cryptosporidium* sp. infected geckos (Graczyk *et al.* 1999). Since birds are immunologically very close to reptiles and bovine immune milk can be effective in reptiles, it can be expected that bovine immunoglobulins are also functional in birds. Indeed, bovine Igs were as effective in the prophylaxis and therapy of *Campylobacter jejuni*-infected chickens as an IgY preparation isolated from hens' eggs (Tsubokura *et al.* 1997). With both preparations a substantial decrease in the number of *C. jejuni* bacteria in faeces was observed. It is not known whether bovine Igs can augment the effector functions of chicken or reptile leucocytes. However, bovine Igs augment the recognition, activation and phagocytosis of microbes by human leucocytes (Loimaranta *et al.* 1999*a*). These results open new views on the prophylaxis or treatment of gastrointestinal diseases in domestic or cultivated animals taxonomically distinct from mammals, e.g. fish, frogs and turtles.

Modulation of gastrointestinal microflora with immune milk preparations

The immune milk preparations used in most clinical studies have been made against a certain pathogen in order to prevent infection or to treat a disease. However, few studies have been carried out with the purpose of controlling or manipulating the gastrointestinal microbial flora in a more general manner, by using an immunoglobulin fraction of normal colostrum or immune milk preparations targeted against a wider variety of bacteria. Fernandez *et al.* (1973) reported positive results when treating children having prolonged infantile diarrhoea with lyophilised bovine colostrum from non-immunised cows.

Kushnareva *et al.* (1995) compared the effectiveness of a milk immunoglobulin preparation with bifidobacteria for the correction of intestinal microflora in human preterm infants with infectious inflammatory diseases. The Ig preparation was administered in a dose of 0.5 g/kg twice per day orally for 1–3 weeks. The treatment with the Ig preparation gave a more pronounced corrective effect on intestinal microflora than the use of bifidobacteria. Elimination of opportunistic lactose-negative enterobacteria, *Pseudomonas aeruginosa* and haemolytic forms of *E. coli* from the digestive tract as well as an increase of lactic acid bacteria were noted. This result suggests that immune milk preparations targeted against a wide variety of harmful pathogens may in future be of use in balancing the microflora of infants and small children suffering from gastrointestinal disturbances. A similar approach has been suggested already by Goldman (1989). Another situation where the prophylactic control of intestinal microflora might be necessary is one of patients under radiotherapy and chemotherapy (which often increases the probability of indigenous bacterial infections). Kobayashi *et al.* (1991*a*) used a model of endogenous infection based on X-ray irradiated mice. The death of mice after irradiation in this model was mainly caused by *E. coli* translocating from the intestine to various organs after a substantial decrease in the lymphocyte functions in gut-associated lymphoid tissues (GALT). GALT are composed of non-organised components such as intraepithelial and lamina propria lymphocytes and of various organised components like mesenteric lymph nodes and Peyer's patches. A bovine immune milk preparation (manufactured by Stolle Milk Biologicals International Inc.) containing specific antibodies against 26 different bacteria, given to mice orally before and after the irradiation, significantly increased the survival rate of the animals and decreased the numbers of Enterobacteriaceae detected from organs like the liver, lung and kidneys, as compared to controls given a milk preparation without specific antibodies (Kobayashi *et al.* 1991*b*; Ishida *et al.* 1992*a*). Also smaller numbers of enterobacteria were found in the intestines of the immune milk group than in the control group (Ishida *et al.* 1992*a*). In addition to the protective effects against severe infection with enteric *E. coli* and prolonging survival times after irradiation, several parameters reflecting the immune defence activity of the GALT were augmented (Ishida *et al.* 1992*a*). The mechanisms behind this effect are not known. However, one clue is that the immune milk group had a larger number of lactobacilli in the intestine than the control group. Lactobacilli are known to be potent immune stimulants (Ouwehand *et al.* 1997; Gill, 1998; Ouwehand & Salminen, 1998; Dugas *et al.* 1999). Also, other milk factors than immunoglobulins, e.g. lactoferrin, β-lactoglobulin and fatty acids, have been reported to have pathogen adhesion and translocation inhibiting effects (Ouwehand *et al.* 1997; Bitzan *et al.* 1998; Teraguchi & Kelsey, 1995).

The immune system also deteriorates with ageing. This is associated with autoimmune diseases, cancer and life-threatening infections. The prevention of a continuous stimulation of the immune system by extrinsic factors, such as the translocation of pathogenic microbes from the intestine, might protect aged individuals. Ishida *et al.* (1992*b*) tested the efficacy of the Stolle immune milk preparation described above in preventing an age-related

decrease in the immune competence of mice. Immune milk was administered from age 2 months for 6 or 16 months. The immune milk group had at the ages 8 and 18 months less serum antibodies against enteric bacteria, whereas several parameters reflecting GALT immune functions were at higher levels than in the control mice. The mechanisms involved are not known, but alterations in the intestinal flora, e.g. an increase in the amounts of lactobacilli, may result in augmenting the immune functions of GALT. Alternatively, specific immunoglobulins may directly augment the immune competence of GALT cells.

An immune milk preparation which is able to modulate GALT immune functions could be assumed to have other physiological effects as well. It was reported that the Stolle immune milk (see above) also had cholesterol lowering effects in human patients with primary hypercholesterolaemia and in humans with moderately raised plasma cholesterol. In a randomised, double-blind, placebo-controlled cross-over study, a daily dosage of 90 g of immune milk preparation for 8 weeks in patients with hypercholesterolaemia decreased the total serum cholesterol level by 8 % and LDL cholesterol by 4 % (Golay *et al.* 1990). In humans with moderately raised plasma cholesterol, a 10-week period with the same dosage resulted in a 5 % decrease of total and a 7 % decrease of LDL plasma cholesterol (Sharpe *et al.* 1994). A significant blood pressure lowering effect (5 mm Hg systolic and 4 mm Hg diastolic) was also seen in the latter trial. The mechanisms underlying these effects are not known. It is possible that the vaccination process of cows for the production of specific antibodies stimulates the production of biologically active compounds, including a low-molecular-weight hypotensive factor in the milk. On the other hand, skim milk containing specific antibodies against enterobacteria may have modulated the gastrointestinal microflora, which, in turn, is known to have far-reaching physiological effects including the lowering of cholesterol in rat models (Molin *et al.* 1992; Fukushima & Nakano, 1996).

Future prospects

Our current knowledge about the *in vivo* efficacy of immune bovine colostral or milk Ig concentrates suggests that these preparations could be effective in the prevention, and to a lesser extent also in the treatment, of specific microbial gastrointestinal diseases. Such preparations would be of particular importance for those microbial diseases which cannot be cured or are difficult to treat using current chemotherapy, such as rotaviruses, antibiotic-resistant enteropathogens and *Cryptosporidium*. Future aims to utilise bovine antibodies as intervention agents in the prevention or treatment of infection should determine, at an early stage of product development, the specific target for which the intervention product is intended. For example, antibodies which block the H antigen on fimbriae of enteropathogenic *E. coli* strains have proven useful as a prophylactic measure (Tacket *et al.* 1988). In many cases there is scope for improvement of the immunisation regimes, such that the ensuing bovine response produces high titres of strong binding-affinity antibodies, with polyclonal activity against a range of important pathogen determinants. In a related context, regulatory and ethical considerations for the immunisation regime should be taken into account, particularly with respect to the use of 'acceptable' immunopotentiating adjuvants and in relation to the frequency of immunisation doses.

The immunosupplementation of clinical diets and special infant formulas with specific antibodies appears, therefore, a challenging future approach. Also, the world-wide trend towards the development of health-promoting functional foods offers interesting opportunities for applications which contain specific antibody ingredients derived from hyper-immunised cows. However, the optimisation of the dietary regime still needs to be determined in many cases, from the viewpoint of dose, frequency, duration of use and (in the case of prophylactics) time of use prior to likely exposure to the pathogen. It is expected that such detailed information will only come from clinical trials.

The form in which bovine-derived antibodies are delivered to patients is also an area worth further consideration with respect to product development. Bovine IgG1 (the predominant colostral Ig) is relatively resistant to the conditions of the human gut and is thought to remain efficacious throughout the GI tract when delivered in colostrum. Contrary to this, it should be borne in mind that colostral proteins can act as immunogens in their own right, and in rare cases patients can present with atopic reactions to milk proteins, including sensitivity to bovine IgG (Bernhisel-Broadbent *et al.* 1991). Accordingly, intact colostrum-containing IgG may not be the appropriate treatment/prophylactic vehicle in these patients, and in cases where sensitivity to milk protein has been detected it may be necessary to develop a purified product based on pepsin-cleaved Ig, comprising two $F(ab)'_2$ fragments, which are less allergenic than the parent molecule (Lefranc-Millot *et al.* 1996).

There is increasing interest within the food and pharmaceutical industries to develop products which are targeted towards the manipulation of oral and intestinal microflora. Apart from specific antibodies, the possible benefits obtained from the application in the diet of specific antibodies together with probiotic bacteria should, therefore, be investigated. In the past, the commercial development of hyperimmune bovine colostral or milk-based preparations has been constrained by technological limitations. Recent developments in membrane separation techniques enable the concentration and isolation of antibodies from bovine colostrum and milk in an active form. There is, however, an obvious need to up-scale the technological processes so as to improve the economics related to the manufacture of hyperimmune colostral or milk preparations or ingredients. From a commercial viewpoint, the profitable exploitation of lacteal products from immunised cows may be limited to early colostrum, which contains the greatest concentration of Ig. A standardised approach to the production of commercial health-intervening bovine Ig products should be undertaken to ensure high product quality and consistency. It is therefore suggested that batch production be monitored by established *in vitro* testing of product efficacy (e.g. by *in*

vitro neutralisation tests against enteric viruses and bacteria).

Summary

In summary, there is a need to carry out well-controlled trials to establish the *in vivo* efficacy of the developed preparations before launching them on the market. On the other hand, since the safety of immune colostrum preparations has not been studied in the same manner as required for pharmaceuticals, further research is still needed to assess the potential allergenic, toxic and hormonal effects of immune milk preparations. The allergenic potential of bovine IgG may be a factor limiting its widespread use for disease prevention (Bernhisel-Broadbent *et al.* 1991). Is it also not known whether the long-term use of immune colostrum might, for example, influence the maturation of immune functions, or immunological tolerance in small children. So far, no significant health risks have been reported during or after oral ingestion of immune milk preparations, and this approach is generally regarded as a non-invasive, and therefore safe, intervention regime. It is anticipated that immune colostral or milk-based preparations, targeted at specific consumer groups, may in the future have remarkable potential to contribute to human health care, both as part of a health-promoting diet and as an alternative or a supplement to the medical treatment of specified human diseases.

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