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 immunoglobulin (Ig) 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,
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 I presents a summary of studies in which positive results against bacterial pathogens have been achieved in
Table 1. Efficacy of bovine immune milk against bacterial infections in vivo

<table>
<thead>
<tr>
<th>Subject</th>
<th>Target organism</th>
<th>Efficacy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>Campylobacter</td>
<td>In prophylaxis &gt;99 % decrease in number of C. jejuni in faeces. In therapeutic treatment 80–95 % lower level of C. jejuni in faeces.</td>
<td>Tsubokura et al. 1997</td>
</tr>
<tr>
<td></td>
<td>C. jejuni</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamster</td>
<td>Clostridium difficile</td>
<td>Protected against disease</td>
<td>Lyerly et al. 1991</td>
</tr>
<tr>
<td>Rat</td>
<td>C. difficile enterotoxin</td>
<td>Decreased enterotoxic symptoms</td>
<td>Kelly et al. 1996</td>
</tr>
<tr>
<td></td>
<td>C. difficile enterotoxin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humans</td>
<td>Escherichia coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants</td>
<td>Enteropathogenic E. coli</td>
<td>Reduced E. coli in faeces</td>
<td>Mietens et al. 1979</td>
</tr>
<tr>
<td>Preterms and infants</td>
<td>Enterotoxigenic E. coli H10407</td>
<td>Prevented diarrhoea after experimental challenge</td>
<td>Tacket et al. 1988</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>Enteropathogenic E. coli</td>
<td>Reduced diarrhoea</td>
<td>Lodiño-Zadnikova et al. 1987</td>
</tr>
<tr>
<td>117 children</td>
<td>Enteropathogenic E. coli</td>
<td>No reduction in incidence of diarrhoea or other parameters during 6 months’ follow-up</td>
<td>Brunser et al. 1992</td>
</tr>
<tr>
<td>Preterms</td>
<td>Eight different enterobacteria</td>
<td>Positive effects on intestinal flora, reduction of enterobacteria, more effective than probiotics</td>
<td>Kushnareva et al. 1995</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>Enterotoxigenic E. coli</td>
<td>Prevented diarrhoea after experimental challenge</td>
<td>Friedman et al. 1998</td>
</tr>
<tr>
<td>Animal models</td>
<td>Mouse Indigenous E. coli</td>
<td>Prevented indigenous infection after pharmacological impairment of intestinal microflora</td>
<td>Nomoto et al. 1992</td>
</tr>
<tr>
<td>Domestic animals</td>
<td>Mouse H. pylori</td>
<td>Attenuated gastritis symptoms</td>
<td>Tarpila et al. 1994</td>
</tr>
<tr>
<td>Suckling pigs</td>
<td>Mouse Enterotoxigenic E. coli</td>
<td>Prevented diarrhoeal disease after experimental challenge</td>
<td>Isaacson et al. 1980</td>
</tr>
<tr>
<td>Neonatal calves</td>
<td>Mouse Enterotoxigenic E. coli</td>
<td>Protected against fatal diarrhoea by E. coli</td>
<td>Snodgrass et al. 1982</td>
</tr>
<tr>
<td>Helicobacter</td>
<td>Helicobacter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humans</td>
<td>Adults H. pylori</td>
<td>Attenuated chronic inflammation of gastric antrum</td>
<td>Oona et al. 1997</td>
</tr>
<tr>
<td>Children</td>
<td>Infants H. pylori</td>
<td>No eradication or decrease in colonisation of gastric antrum</td>
<td>Caswall et al. 1998</td>
</tr>
<tr>
<td>Adults</td>
<td>H. pylori</td>
<td>No eradication or decrease in colonisation of gastric antrum</td>
<td>Opekun et al. 1999</td>
</tr>
<tr>
<td>Animal models</td>
<td>Mouse H. felis</td>
<td>Prevented infection after experimental challenge</td>
<td>Rehnberg-Laiho et al. 1995</td>
</tr>
<tr>
<td>Mouse</td>
<td>H. felis</td>
<td>Reduced colonisation of H. felis in gastric antrum</td>
<td>Mamila et al. 1996</td>
</tr>
<tr>
<td></td>
<td>Klebsiella K. pneumoniae</td>
<td>Prevented infection</td>
<td>Soboleva et al. 1991</td>
</tr>
<tr>
<td>Mouse</td>
<td>Proteus P. mirabilisand P. vulgaris</td>
<td>Prevented infection</td>
<td>Soboleva et al. 1991</td>
</tr>
<tr>
<td>Mouse</td>
<td>Pseudomonas P. aeruginosa</td>
<td>Prevented infection</td>
<td>Stephan et al. 1990; Soboleva et al. 1991</td>
</tr>
<tr>
<td>Mouse</td>
<td>Salmonella S. pullorum</td>
<td>Delayed death</td>
<td>Campbell &amp; Petersen, 1959</td>
</tr>
<tr>
<td>Mouse</td>
<td>S. typhimurium</td>
<td>Prevented infection</td>
<td>Stephan et al. 1990</td>
</tr>
<tr>
<td>Mouse</td>
<td>S. typhimurium and S. enteritidis</td>
<td>Prevented infection</td>
<td>Soboleva et al. 1991</td>
</tr>
<tr>
<td></td>
<td>Shigella S. flexneri</td>
<td>Prevented shigellosis</td>
<td>Tacket et al. 1992</td>
</tr>
<tr>
<td>Human adults</td>
<td>Streptococcus</td>
<td>Reduced S. mutans level in dental plaque</td>
<td>Filler et al. 1986, 1991</td>
</tr>
<tr>
<td>Human adults</td>
<td>S. mutans</td>
<td>Reduced proportion of S. mutans in dental plaque</td>
<td>Loimaranta et al. 1999b</td>
</tr>
<tr>
<td>Rat</td>
<td>S. mutans</td>
<td>Reduced caries development</td>
<td>Mikhalek et al. 1987</td>
</tr>
<tr>
<td>Rabbits</td>
<td>Vibrio cholerae</td>
<td>Decreased mortality and intestinal fluid response</td>
<td>McClead &amp; Gregory, 1984</td>
</tr>
<tr>
<td>Infant rabbits</td>
<td>Cholera enterotoxin</td>
<td>Reduced diarrhoea</td>
<td>Boesman-Finkelstein et al. 1989</td>
</tr>
</tbody>
</table>
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. Miëtens 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 incolum. 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 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 diarrhea 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 aetiologic 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 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, Tollemer 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 mutants 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 mutants 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 glycopoly saccharides (Loimarananta 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 (Loimarananta et
and killing of \textit{S. mutans} \textit{al.} 1998 (PMN) leucocytes \cite{Loimaranta1998}. Immune colostrum did not inhibit \textit{in vitro} \textit{S. mutans} against antibacterial peroxidase-hypothiocyanate system of saliva \cite{et al.1998}. Further, the immune colostrum also proved to be functional in \textit{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 \cite{Loimaranta1999}. However, further clinical studies will be required to evaluate the \textit{in vivo} efficacy of anticaries immune bovine Ig preparations.

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

\begin{table}[ht!]
\centering
\caption{Efficacy of bovine immune milk against viral infections \textit{in vivo}}
\begin{tabular}{|l|l|l|l|}
\hline
\textbf{Subject} & \textbf{Virus} & \textbf{Efficacy} & \textbf{Reference} \\
\hline
\textbf{Humans} & & & \\
Infants & Poliomyelitis vaccine of Sabin type 2 & Prevented infection \& gastrointestinal tract & Gonzaga \textit{et al.} 1963 \\
Infants & Rotavirus Wa 1 & Prevented infection & Ebina \textit{et al.} 1985 \\
Children & Four human rotavirus serotypes & Prevented infection & Davidson \textit{et al.} 1989 \\
117 children & Enteropathogenic \textit{E. coli} and rotavirus & No reduction in incidence of diarrhoea or other parameters during 6 months follow-up & Brunser \textit{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 \textit{et al.} 1994 \\
Small children & Four human rotavirus serotypes & Shortened duration and decreased severity of diarrhoea & Mitra \textit{et al.} 1995 \\
Infants & Human rotavirus MO & Prevented diarrhoea & Ebina, 1996 \\
Infants having acute rotoviral gastroenteritis & Four human rotavirus serotypes & Reduced duration of virus excretion in stools, no significant effect on diarrhoea & Hilpert \textit{et al.} 1987 \\
132 small children with rotoviral gastroenteritis & Rotavirus SA11 & Not statistically significant, but trend-setting improvement in duration of diarrhoea, weight gain and stool frequency & Ylitalo \textit{et al.} 1998 \\
40 small children with rotoviral gastroenteritis & Four human rotavirus serotypes & Shortened duration of diarrhoea and reduced amount and frequency of stool & Sarker \textit{et al.} 1998 \\
\hline
\textbf{Animal models} & & & \\
Infant mice & Human rotavirus MO & Prevented infection after challenge & Ebina \textit{et al.} 1992 \\
Infant mice & Human rotavirus MO & Prevented infection after experimental challenge & Ebina, 1996 \\
\hline
\textbf{Domestic animals} & & & \\
Calves & Bovine virus diarrhoea virus (BVDV) & Prevented infection of respiratory tract and protected against viraemia and leukopenia after experimental challenge. & Howard \textit{et al.} 1989 \\
Calves & Bovine rotavirus & Delayed onset of diarrhoea, reduced incidence, duration and severity of diarrhoea & Snodgrass \textit{et al.} 1982 \\
New-born calves & Bovine rotavirus 81/36F & Prevented diarrhoea after experimental infection & Castrucci \textit{et al.} 1982 \\
New-born calves & Bovine rotavirus & Prevented infection & Saif \textit{et al.} 1987 \\
Calves & Bovine rotavirus 81/36F & Prevented diarrhoea after experimental infection & Castrucci \textit{et al.} 1988 \\
New-born calves & Bovine rotavirus serotypes 1 and 2 & Prevented infection & Tsunemitsu \textit{et al.} 1989 \\
Calves & Bovine rotavirus 81/36F & Prevented infection in most cases, decreased its severity & Castrucci \textit{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 \textit{et al.} 1991 \\
Agammaglobulinaemic piglets & Simian rotavirus SA-11, not porcine rotavirus & Protected against North Carolina porcine rotavirus & Lecce \textit{et al.} 1991 \\
Gnotobiotic piglets & Human rotavirus & Prevented infection after challenge & Schaller \textit{et al.} 1992 \\
Calves & Bovine herpes virus 1 & No prevention, attenuated clinical signs of infection after experimental challenge. & Bradshaw \& Edwards, 1996 \\
Foals & Mares immunised with SA11 (G3P2), H2 (G3P12) and Lincoln (G6P1) & Decreased morbidity, shortened duration of clinical signs of diarrhoea & Barrandeguy \textit{et al.} 1998 \\
\hline
\end{tabular}
\end{table}
Table 3. Efficacy of bovine immune and non-immune milk against Cryptosporidium infections in humans

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

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 antitoxin 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 (Tsobukura 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. 1999a). 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 lact 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. (1991a) 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. 1991b; Ishida et al. 1992a). Also smaller numbers of enterobacteria were found in the intestines of the immune milk group than in the control group (Ishida et al. 1992a). 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. 1992a). 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. (1992b) 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 fimbrae of enteropathic 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 lactic 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). It is 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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