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Review

# Milk immunoglobulins for health promotion

Raj Mehra<sup>a</sup>, Pertti Marnila<sup>b</sup>, Hannu Korhonen<sup>b,\*</sup>

<sup>a</sup>Moorepark Food Research Centre, Teagasc, Fermoy, Co. Cork, Ireland

<sup>b</sup>MTT Agrifood Research Finland, Biotechnology and Food Research, 31600 Jokioinen, Finland

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## Abstract

The biological function of bovine colostrum immunoglobulins is to provide the newborn calf with adequate passive immune protection against microbial infections. Immunoglobulin preparations designed for farm animals are commercially available, and some colostrum-based products are marketed also for humans as dietary supplements. The concentration of specific antibodies against a certain pathogenic microorganism can be raised in colostrum and milk by immunizing cows with this pathogen or its antigen. Advances in bioseparation and chromatographic techniques have made it possible to fractionate and enrich these antibodies and formulate so-called hyperimmune colostrum or milk preparations. Their efficacy in prevention and treatment of various microbial infections has been evaluated in numerous studies. Immune milk preparations have proven effective in prophylaxis against infections caused by a variety of gastrointestinal pathogens. Their therapeutic efficacy, however, seems more limited. A few commercial immune milk products are already on market and more applications can be expected in the coming years. This article reviews the recent progress made in isolation techniques of bovine immunoglobulins and the application of colostrum and immune milk preparations in fighting various microbial infectious diseases in humans.

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**Keywords:** Immunoglobulins; Isolation; Antibody; Immune milk; Applications

## Contents

1. Introduction . . . . .	1263
2. Immunoglobulins in milk and colostrum . . . . .	1263
3. Isolation and concentration of immunoglobulins . . . . .	1263
3.1. Current technologies for isolation and concentration of immunoglobulins . . . . .	1263
3.2. Effects of processing on immunoglobulin properties. . . . .	1264
3.3. Formulation of immunoglobulin products. . . . .	1265
4. Efficacy of immunoglobulin preparations. . . . .	1266
4.1. Milk, colostrum and immune products . . . . .	1266
4.2. Immunoglobulins and digestion . . . . .	1266
4.3. Recent studies on the efficacy of colostrum immunoglobulins . . . . .	1266
4.3.1. Caries streptococci . . . . .	1266
4.3.2. <i>Helicobacter pylori</i> . . . . .	1267
4.3.3. <i>Escherichia coli</i> . . . . .	1267
4.3.4. <i>Shigella</i> . . . . .	1267
4.3.5. <i>Clostridium difficile</i> . . . . .	1267
4.3.6. Other health benefits. . . . .	1268

\*Corresponding author. Tel.: +358 3 41883271; fax: +358 3 41883244.

E-mail address: [hannu.j.korhonen@mtt.fi](mailto:hannu.j.korhonen@mtt.fi) (H. Korhonen).

5. Conclusions .....	1268
References .....	1268

## 1. Introduction

Immunoglobulins (Igs), together with lactoferrin, lactoperoxidase and lysozyme form the very important antimicrobial system of bovine lacteal secretions. Igs are antibodies that are synthesized by mammals in response to antigenic or immunogenic stimuli such as bacteria and viruses, and thus provide protection against microbial infections. Bovine colostrum, which is a rich source of Igs, also confers passive immunity to the newborn during development of its own immune system (for a review see Lilius & Marnila, 2001). Igs with specific antibody activities can be increased in lacteal secretions through targeted hyperimmunization protocols, using vaccines containing inactivated pathogenic microbial material against which the Ig activities are desired. This increased pool of antibodies can be enriched further through concentration/fractionation techniques, leading to production of Ig products containing high antibody concentrations and titres. Such preparations may find beneficial applications in human and animal healthcare and well-being by preventing infections and controlling diseases (for reviews see Kelly, 2003; Korhonen, Marnila, & Gill, 2000; Lilius & Marnila, 2001). This review focuses on the current large-scale technologies employed to produce such specific Ig products, and the clinical studies undertaken to determine their efficacies against human and animal infections.

## 2. Immunoglobulins in milk and colostrum

The biological function of Igs in bovine milk and colostrum is to protect mammary gland against pathogens and to provide the calf with an immunological protection against surrounding pathogens. The IgG class antibodies comprise 80–90% of total Igs (about 50 gL<sup>-1</sup>) in the early bovine colostrum from the milking of the first day after parturition (Elfstrand, Lindmark-Månsson, Paulsson, Nyberg, & Åkesson, 2002) (Table 1). Thereafter, the Ig content decreases sharply during the next 2 days (Table 2).

As is the case with serum antibodies, the IgG antibodies (IgG is the dominant Ig class in colostrums), have a multitude of functions including opsonization, complement fixation, prevention of adhesion of pathogenic microbes to endothelial lining, inhibition of bacterial metabolism by blocking enzymes, agglutination of bacteria, and neutralization of toxins and viruses (Marnila & Korhonen, 2002). IgM antibodies, which are produced in smaller amounts than IgG, are considerably more efficient than IgG in most of the above-mentioned activities, especially in complement fixation, but it remains obscure how significant this mechanism is in milk or colostrum. In contrast, the

secretory IgA does not fix complement or opsonize bacteria, but agglutinates antigens, neutralizes viruses and bacterial toxins, and prevents the adhesion of enteropathogenic bacteria to mucosal epithelial cells (Marnila & Korhonen, 2002).

## 3. Isolation and concentration of immunoglobulins

### 3.1. Current technologies for isolation and concentration of immunoglobulins

Although the published scientific literature contains a substantial amount of information on methods and technologies used for isolation, extraction, concentration, and purification of Igs from milk/colostrum, not all the reported methods are suitable for large-scale production of Igs. Only those techniques that have the potential for scale up are described in this review. With the rapid development of new chromatographic and membrane-separation technologies in the last 20 years, it is now possible to isolate individual milk proteins at large-scale (Kelly & McDonagh, 2000; Korhonen, 2004; Maubois, 1998). A number of

Table 1  
Immunoglobulin concentration in the first milking colostrum and milk<sup>a</sup>

Immunoglobulin class	Molecular mass (kDa)	Concentration (g L <sup>-1</sup> )	
		Milk	Colostrum
IgG <sub>1</sub>	146–163	0.3–0.6	15–180
IgG <sub>2</sub>	146–154	0.06–0.12	1–3
IgG total		0.15–0.8	20–200
SIgA	385–430	0.05–0.1	1–6
IgM	900	0.04–0.1	3–9

<sup>a</sup>Data compiled from Marnila and Korhonen (2002) and Elfstrand et al. (2002).

Table 2  
The change in total immunoglobulin concentration of first nine milkings postpartum of five Ayrshire cows<sup>a</sup>

Milking postpartum	Concentration (g L <sup>-1</sup> )	
	Mean	Range
1.	52	35.0–70.0
2.	27	18.0–46.0
3.	9.0	5.2–15.2
4.	4.6	4.5–5.3
5.	3.4	2.5–3.9
6.	2.6	1.6–3.5
7.	2.3	1.5–2.9
8.	2.0	1.4–3.1
9.	1.6	1.0–2.6

<sup>a</sup>Data extracted from Korhonen (1977).

pilot- or industrial-scale methods for isolation and purification of Igs from colostrum or cheese whey, based on membrane-separation (Kothe, Dichtelmuller, Stephan, & Echentopf, 1987; Stott & Lucas, 1989) or membrane-separation and chromatographic techniques (Bottomley, 1993; Korhonen, Syväoja, Vasara, Kosunen, & Marnila, 1998) have been patented. The recovery rate of Igs using these methods varied from 25% to 70% of the level present in the starting material. Thomas, Cordle, Criswell, Westfall, and Barefoot (1992) developed a process for selective enrichment of Igs from whey by filtration through formed-in-place membranes on sintered stainless-steel tubes. The process employed both size exclusion and protein charge manipulation around the isoelectric point of proteins to selectively reject or pass components of the stream. IgG from cheese whey was enriched from 8% to 20% of total whey protein with 90% recovery of the IgG. More recently, Baruah and Belfort (2004) used the Predictive Aggregation Transport Model and microfiltration (0.1 µm pore size membrane) of transgenic goat milk with a helical hollow-fibre module operated at low uniform transmembrane pressure, low permeate flux rate, and permeate co-current flow to increase the yield of Ig in the permeate from less than 1% to over 95%. Similarly, microfiltration (0.1 µm membrane porosity) combined with ultrafiltration (UF; membrane cut-off 100 kDa) of bovine, equine and caprine colostrum resulted in at least 80% recovery of IgG in the microfiltrate, and IgG/TS purity of more than 90% with subsequent ultrafiltration (Piot, Fauquant, Madec, & Maubois, 2004). Mehra and Kelly (2004) also produced an Ig-rich fraction through microfiltration of cheese whey using tubular ceramic membranes (0.1 µm pore size). The Ig to protein ratio of this fraction was approximately 2.5–3.0 times that of the original cheese whey.

Membrane-separation of milk/colostrum whey Igs does not result in high purity or recovery of Ig fraction. Specific chromatographic techniques have been applied to further improve the yield and purity of the Ig preparations, but some of them may not be easily scaled-up. IgG was isolated from whey by immobilized metal-affinity chromatography (IMAC). UF concentrated whey was applied to IMAC column loaded with copper, and IgG fractions with purities of 52% for cheese whey (Fukumoto, Li-Chan, Kwan, & Nakai, 1994) and 77.2% and 53% for acid and cheddar cheese whey, respectively (Al-Mashikhi, Li-Chan, & Nakai, 1988) were isolated. An immunoaffinity chromatography method suitable for isolating pure IgG subclasses IgG<sub>1</sub> and IgG<sub>2</sub> from cheddar cheese whey or colostrum was reported by Akita and Li-Chan (1998). Immunoaffinity columns were made with specific egg yolk IgY raised against bovine IgG<sub>1</sub> and IgG<sub>2</sub>. Milk that was free of either IgG subclass was successfully produced by the selective removal of IgG<sub>1</sub> or IgG<sub>2</sub> subclasses by this technique. Expanded Bed Adsorption Chromatography (EBAC) was applied to isolate Igs from whey (Nielsen, Olander, & Lihme, 2002) using adsorbent with tailored ligand chemistry. EBAC provides significant advantages over conventional packed

bed column chromatography. The decisive characteristic of the adsorbent media to expand in the column when upward flow of liquid is applied allows free passage of particulate impurities in the feed stream through the column thus allowing crude, non-clarified feeds to be applied to the column. Ig purity of between 50% and 70% was achieved by this method. The first industrial implementation of EBAC in the dairy industry has been carried out for the separation of whey proteins by Dairy Farmers in Australia.

### 3.2. Effects of processing on immunoglobulin properties

Whey manufacturing processes such as acid and rennet treatments were shown to reduce antigen (*Escherichia coli* JCM13965) specific antibody activities (assayed using ELISA) of whole and skimmed colostrum IgG, IgA and IgM (Ando et al., 2005). It is probable that this loss of antibody activity during whey preparation was due to the involvement of Igs in casein precipitate. The extent of the decrease was lower during whey production from skimmed colostrum than from whole colostrum. Additionally, rennet whey retained more antibody activity during manufacture than acid whey. The IgG antibody activity was resistant to heat treatment at 72 °C for 15 s, and less stable at 80 °C for 15 s in whole and skimmed colostrum, and skimmed colostrum whey. On the other hand, the IgM activity in all colostrum-derived fractions was reduced by both heat treatments without any significant differences between the fractions. However, the SIgA antigen-specific antibody activity was more susceptible to whey preparation processes and heat treatments than IgG and IgM activities. The heat stability was markedly lower in whole colostrum than in other colostrum-derived fractions.

Using enzyme-linked immunosorbent assay (ELISA) and radial immunodiffusion (RID) techniques, IgG concentration and antigen-binding activities of IgG against lipopolysaccharide (FPS) fractions of 5 bacteria (*E. coli* O128B:12; *E. coli* O111:B4; *Salmonella flexneri* 1A; *S. enteritidis*; and *S. typhimurium*) were measured in commercially heat-treated fluid milks (Li-Chan, Kummer, Losso, Kits, & Makai, 1995). Established milk heat treatments, batch pasteurization (63 °C for 30 min) did not destroy the specific antigen-binding activities of IgG, while high-temperature short-time (HTST, 72 °C for 15 s) led to 25–40% loss of IgG concentration and a similar reduction in antigen-binding activities. On the other hand, ultra-high temperature (UHT) treatment (138 °C, 4 s) denatured all of the concentration and antigen-binding activities of IgG. In contrast, Mainer, Sánchez, Ena, and Calvo (1997) reported that bovine milk Igs could resist the HTST pasteurization treatment without affecting their structure. Only 1% of the IgG, 2% of the IgA, and 14% of the IgM concentrations were denatured when milk samples were heated in glass capillaries and a temperature-controlled water bath. Denaturation of IgG was determined using RID. In a similar further study, Mainer, Dominguez, Randrup, Sanchez, and Calvo (1999) also

showed that the HTST pasteurization had little effect on the activity of bovine colostrum IgG as the original rotavirus neutralizing activity was reduced by only 0.5%.

Pulsed electric fields (PEF) is an emerging non-thermal technology alternative to thermal processing. It has been reported to inactivate microorganisms through high-intensity electric fields, while contribution from thermal effect is minimal (Zhang, Molsalve-Gonzalez, Barbosa-Canovas, & Swanson, 1994). PEF treatment of purified bovine IgG solutions in buffers caused no detectable changes in the secondary structure or immunoactivity of IgG, as measured by circular dichroism (CD) (Li, Bomser & Zhang, 2005).

The effects of freeze-drying, microwave vacuum evaporation, and spray-drying of colostrum on the colostrum Igs and antigen-specific antibody-binding activities were reported by Chelack, Morley and Haines (1993). Total colostrum Igs was measured by RID, while antigen-specific antibody-binding activities against parainfluenza type 3 virus, bovine viral diarrhoea virus, bovine respiratory syncytial virus and infectious bovine rhinotracheitis virus were analysed by ELISA. The Ig content of colostrum was largely retained by all the dehydration methods investigated (93–99% retention). Results of the evaluation of control and dried colostrum for stability of antigen-specific antibody function showed that microwave vacuum evaporation caused the greatest loss of biological activities (~20%), followed by freeze-drying (~10%) and spray-drying (~5%). Spray drying was the most efficient, fast and cost-effective dehydration method in maintaining the bioactivity of Igs.

Bovine IgG isolated from cheese whey was membrane sterilized and aseptically added into cartons of UHT-treated milk (Fukumoto, Skura, & Nakai, 1994). The concentration of added bovine IgG was stable for more than 5 months during storage of the milk at different temperatures up to 35 °C. Freezing, storage as frozen, and storage of lyophilized colostrum at 4, 20 and 37 °C for at least 1 year did not have detrimental effect on Ig antibody activity against *Campylobacter jejuni* as determined by

ELISA (Husu et al., 1993). Bacterial fermentation of milk by yoghurt or probiotic bacterium (*Lactobacillus rhamnosus GG*) did not significantly reduce the biological activity of added colostrum antibodies to *Streptococcus mutans* (Wei, Marnila, & Korhonen, 2002). Antibody activity in fermented immune milk was maintained after storage for 50 days at 4 °C.

### 3.3. Formulation of immunoglobulin products

Current commercial Ig products are prepared from colostrum of non-vaccinated or immunized cows. Colostrum is collected, defatted, pasteurized under conditions that retain biological activity and dried. The products are in the form of spray dried and freeze-dried powders, and filtered colostrum whey liquids or concentrates. Dried products include whole colostrum powder, skim powders, skim colostrum protein concentrate and colostrum whey concentrates. It is not possible to list here every conceivable Ig ingredient/product that is commercially available in the global market. Some of the potential health promoting Ig ingredients are under proprietary development or clinical investigations. Table 3 lists some of the commercial Ig products that are described in the literature (Pakkanen & Aalto, 1997; Scammell, 2001). Gastrogard-R™ is a hyperimmune anti-rotavirus colostrum concentrate that was launched in Australia as a pharmaceutical product for prevention of rotavirus diarrhoea in infants and young children (Davidson, Daniels, Nunan, Moore, & Whyte, 1989; Davidson, Tam, & Kirubakaran, 1994). Intact™ is a colostrum concentrate from non-hyperimmunized cows that has been shown to be effective in running and rowing performance (Buckley, Abbott, Martin, Brinkworth, & Whyte, 1998; Buckley et al., 1999). Biotest Pharm GmbH (Germany) produces Lactimmunoglobulin Biotest, a colostrum product from non-immunized cows for human use. It has been tested for treatment of severe diarrhoea in AIDS patients (Stephan, Dichtelmuller, & Lissner, 1990). Viable Bioproducts Ltd. of Finland produces Bioenervi™, a sterile-filtered colostrum product that provides growth

Table 3  
Commercial colostrum and immune milk products

Product	Company	Claimed health benefits
Intact™ Gastrogard-R™	Numico RA (Australia) Northfield Laboratories, (Australia)	Immune enhancing, athletic performance Prevent diarrhoea caused by rotavirus in infants and children <4 years
PRO-IMMUNE 99 Proventra™ natural immune components	GalaGen Inc. (USA)	Prevent scours caused by <i>E. coli</i> in calves. Boosts immunity and enhances body's natural resistance
Lactimmunoglobulin Biotest	Biotest Pharm GmbH (Germany)	Product for humans treatment of diarrhoea in AIDS patients
ColostrumGold™ liquid Colostrumune™ powder First Defence <sup>R</sup>	Sterling Technology, Inc (USA) Immucell (USA)	Immune system booster Reduces mortality and morbidity from scours caused by <i>E. coli</i> K99 <sup>+</sup> and coronavirus in calves

factors and antimicrobial factors during strenuous physical activity performed by athletes (Mero, 1995). Ig preparations are further formulated into retail products such as powders in tins, sachets, capsules, chewable tablets and liquid clostrum whey. Consumer food products containing Ig supplements have also been formulated. Galagen Inc. manufactures a bovine colostrum-derived ingredient, Proventra, that contains a natural mixture of bioactive proteins including broad-spectrum Ig's, lactoferrin, lactoperoxidase, growth factors and cytokines ([www.savvynet.com/galagen](http://www.savvynet.com/galagen)). It has been demonstrated in clinical studies that Proventra weakens harmful microbial organisms, prevents the growth and attachment of these organisms to the gastrointestinal (GI) walls, and promotes general health. A number of Proventra-containing foods are on the market. Basic Plus<sup>TM</sup> is a milk-based refrigerated dietary

supplement containing active Kefir cultures and Proventra<sup>TM</sup> Natural Immune Components. Vistrum contains a mixture of beneficial probiotic cultures, Proventra<sup>TM</sup> Natural Immune Components and soluble fiber. PharmAssure<sup>TM</sup> Proventra<sup>TM</sup> is a dietary supplement that delivers Galagen's Proventra<sup>TM</sup> Natural Immune Components in tablet form. Other food products containing Proventra from Novartis Consumer Health and Wyeth-Ayerst are under development. The world's first liquid-drinkable colostrum product (ImmuNOVA) was launched in Finland by Novatreat, a Finnish company ([www.novatreat.com/englanti/immunova](http://www.novatreat.com/englanti/immunova)). ImmuNOVA is a dietary supplement that contains antibodies and growth factors of bovine colostrum in a biologically active state. It is intended mainly for hospital use as a nutritional preparation, but can also be used to boost immune system or treat

Table 4  
Recent human studies on the efficacy of bovine milk or colostrum Ig preparations

Microorganism used in immunization	Target disease	Treatment regimen	Efficacy	References
Virulence factors of <i>Str. mutans</i>	Dental caries	Mouth rinse twice daily for 14 days	Inhibited recolonization of <i>Str. mutans</i> after antibiotic treatment	Shimazaki et al. (2001)
<i>Str. mutans</i> and <i>Str. sobrinus</i>	Dental caries	Mouth rinse three times daily for 3 days	Higher resting pH and smaller proportion of <i>Str. mutans</i> in dental plaque	Loimaranta et al. (1999)
<i>Helicobacter pylori</i> urease	Gastritis	150 ml of yogurt with 1% avian IgY and probiotic bacteria 3 times per day for 4 weeks	Decreased values in urea breath test	Horie et al. (2004)
Five strains or one strain of <i>E. coli</i>	Diarrhoea	Once per day orally 0.5 g of IgG per kg of body weight, follow-up period for 6 months	Lower incidence of diarrhoea and shorter duration of diarrhoea episodes	Tawfeek et al. (2003)
<i>Shigella dysenteriae</i> antigen I	Shigellosis	100 mL orally three times per day for 3 days in combination with antibiotics	No significant difference in any clinical parameter	Ashraf et al. (2001)
<i>Clostridium difficile</i> toxin and <i>C. difficile</i> whole cells	<i>C. difficile</i> diarrhoea	Orally for 2 weeks as supportive treatment after antibiotic treatment	<i>C. difficile</i> toxins eradicated from 15 of 16 patients and no relapses in any patient during 11 month follow-up period	Van Dissel et al. (2005)
No immunization	Mild hyper-cholesterolemia	Orally 5 g of blood-derived IgG daily for 3 or 6 weeks	Both total cholesterol and LDL levels decreased from baselines	Earnest et al. (2005)
No immunization	Upper respiratory tract infections	60 g of colostrum protein daily for 8 weeks	Reduced significantly incidence of self-estimated symptoms of respiratory infections but no difference in duration	Brinkworth and Buckley (2003)
No immunization	Endotoxemia due to abdominal surgery	Colostrum preparation Lactobin <sup>®</sup> 52 g daily in 4 doses orally for 3 days before surgery	Lower levels of endotoxin and endotoxin neutralizing capacity in blood suggesting reduced endotoxemia due to surgery	Bölke, Jehle et al. (2002)
No immunization	Endotoxemia due to coronary surgery	Colostrum preparation Lactobin <sup>®</sup> 42 g daily doses orally for 2 days before surgery	Lower levels of CRP but no effect on perioperative endotoxemia	Bölke, Orth et al. (2002)

momentary bowel or intestine problems. New consumer products have been fortified with hyperimmune milk ingredients, containing specific antibodies that promote health and well-being, from Stolle Milk Biologics Inc. (SMBI) ([www.smbimilk.com/products](http://www.smbimilk.com/products)). These products include Stolle-Q<sup>TM</sup>, a powdered health drink being sold in Korea by Pulmuone; Stolle Dent<sup>TM</sup>, a new toothpaste sold by Lanfar International in Taiwan; Stolle Nutritional Tablets<sup>TM</sup> fortified with Stolle milk ingredients are distributed in Japan by Kanematsu Wellness Corporation, and NuVim<sup>R</sup>, a refrigerated patented dietary supplement beverage marketed in Asia, Australia and New Zealand. Lemaco (Egypt) has launched Extra Fit baby food with bovine colostrum and vegetables suitable for babies 6 months and over (Archibald, 2005). New Ig products continue to be introduced into the commercial market specifically for the healthcare sector. (Table 4)

#### 4. Efficacy of immunoglobulin preparations

##### 4.1. Milk, colostrum and immune products

Like Ig concentration, the antibody specificity also varies significantly in milk and colostrum of different cows depending on the spectre of antigens encountered during the animal's lifetime. All colostrum and milk contain detectable amounts of "natural" antibodies for most enterobacteria like *E. coli*, but the presence of antibodies to other antigens cannot be predicted (Korhonen et al., 2000; Kelly, 2003). The antibody titre against certain pathogen or its virulence factor can be raised up to several hundred times by immunizing the cow with microbial vaccines before parturition. Such an immune colostrum resulting from immunization regimes has fundamentally different properties and efficacies against pathogens than normal colostrum and these two concepts should, therefore, be differentiated from each other. This is emphasized by the fact that at present normal colostrum and preparations made from it are in many countries regarded as a food or dietary supplement, whereas the colostrum preparations from immunized cows are often regarded as pharmaceuticals or their regulatory status remains to be defined.

##### 4.2. Immunoglobulins and digestion

The low pH of gastric acid significantly reduces the activities of bovine milk Ig concentrates. Milk Ig's are degraded in the human GI tract into F(ab')<sub>2</sub>, Fab and Fc fragments by the intestinal proteolytic enzymes pepsin, trypsin, chymotrypsin, carboxypeptidase and elastase (for a review, see Reilly, Domingo, & Sandhu, 1997). However, the resulting F(ab')<sub>2</sub> and Fab fragments retain most of their natural activities in the oral cavity and oesophagus, and at least part of the neutralizing and adhesion inhibiting activities when in intestine. When 100 mL of whole milk supplemented with immune colostrum containing rotavirus

antibodies was administered to 105 children 3 times a day for 6 days, the rotavirus antibody activity was detected by ELISA in 521 (86%) of 602 faecal specimens obtained during the study (Pacyna et al., 2001). The secretory piece component of the IgA molecule makes SIgA more resistant to proteolytic digestive enzymes than other Ig classes (Reilly et al., 1997). The survival of IgG can be increased by encapsulation with gelatin (Kelly et al., 1997).

##### 4.3. Recent studies on the efficacy of colostrum immunoglobulins

Over the last 50 years, a large number of clinical studies have been carried out in humans and animals in order to establish the efficacy of immune milk preparations. These studies have been reviewed in many recent articles (Kelly, 2003; Korhonen et al., 2000; Lilius & Marnila, 2001). In this article, the most recent studies only will be reviewed.

###### 4.3.1. Caries streptococci

Bovine milk antibodies have been studied in local passive immunization to prevent dental caries (for a review see Koga, Oho, Shimazaki, and Nakano, 2002) since humans cannot be immunized actively against caries bacteria due to risk of side effects. The adherence of the principal caries pathogen *Streptococcus mutans* is a crucial factor in the colonization of the oral cavity. In recent studies bovine colostrum Igs have been shown specifically to block bacterial colonization factors when cows are immunized with proper bacterial structures. Mitoma et al. (2002) prepared a fusion of a saliva-binding alanine-rich region of a cell surface protein antigen (Pac) and a glucan-binding domain of the glucosyltransferase-I cell surface protein from *Str. mutans* and immunized cows with the fusion. The immune milk preparation made from colostrum of these cows proved effective in the prevention of dental caries development in an experimental rat model when given as concentrate once a day for 55 days. Shimazaki et al. (2001) examined the effect of immune milk containing antibodies against the above mentioned fusion protein, on adult subjects after an antibiotic (cetylpyridinium chloride) treatment. The immune milk inhibited significantly recolonization of *Str. mutans* in the saliva and plaque as compared to the control group. Also, the ratios of *Str. mutans* to total streptococci in saliva and plaque of the immune milk group were lower than in the control group.

These studies support the observations by Loimaranta et al. (1997) that the immune milk from cows immunized with a *Str. mutans* and *Str. sobrinus* whole cell vaccine inhibited in vitro glucosyltransferase and fructosyltransferase activities of *Str. mutans*, inhibited adherence of the bacteria to saliva-coated hydroxyapatite particles and promoted aggregation of *Str. mutans* (Loimaranta et al., 1998). When used as a mouth rinse for 3 days the immune preparation resulted in a higher resting pH in dental plaque of adults as compared with the control groups (Loimaranta et al., 1999). The relative number of mutans streptococci

decreased significantly after the immune product rinsing period when compared to the controls. Thus, the rinsing with bovine immune whey indicates favourable effects on human dental plaque by controlling *Str. mutans* in the human oral cavity.

Wei et al. (2002) supplemented pasteurized milk with the above immune preparation and fermented the milk with *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG). This probiotic bacteria was earlier shown to reduce caries risk in day care children when added in milk (Näse et al., 2001) and to decrease salivary *Str. mutans* counts in adult subjects when administered in cheese (Ahola et al., 2002). The immune preparation did not prevent the growth of LGG in milk or the fermentation. The specific antibodies had a synergistic *Str. mutans* adhesion inhibiting effect with LGG in vitro. In both LGG-fermented and UHT-treated milk, the activity of added anti-caries antibodies was maintained during the expected shelf-life of these products. From the anticariogenic point of view it may be beneficial to add bovine-specific antibodies against mutans streptococci to probiotic LGG-containing milk products.

#### 4.3.2. *Helicobacter pylori*

In a recent study colostrum specific Igs from cows immunized against whole cell *Helicobacter felis* protected mice from experimental *H. felis* infection (Marnila et al., 2003). A treatment with specific colostrum Igs has been reported to reduce the degree of gastric inflammation and *Helicobacter* colonization in mice (Casswall et al., 2002; Marnila et al., 2003) and the degree of inflammation in children (Oona et al., 1997).

Some studies suggest that ingesting live lactic acid bacteria suppress *H. pylori* infection (Sykora et al., 2005; Wang et al., 2004). Thus, a synergistic therapeutic effect could be achieved by combining Igs and probiotic bacteria. Horie et al. (2004) recently designed a yoghurt drink containing *Lb. acidophilus*, *bifidobacteria* spp. and added 1% of egg yolk IgY from hens immunized against *H. pylori* urease enzyme, a pathogenic factor of *H. pylori*, to yoghurt. Similarly, to above-described bovine colostrum IgG in sour milk, 85% of the egg yolk IgY antibodies remained stable and active in yoghurt for 3 weeks. In a clinical study administering 150 mL of IgY-yogurt for 3 times daily for 4 weeks to *H. pylori* positive subjects decreased significantly urea breath test values indicating decreased in vivo activity of urease enzyme as compared to the control group.

#### 4.3.3. *Escherichia coli*

Bovine colostrum Igs from cows immunized with *E. coli* UT5600/pSV6-strain ferric citrate receptor have recently been shown to be able to inhibit the iron uptake by the same strain and mastitic isolates of *E. coli* (Takemura, Hogan, & Smith, 2003). The effect of iron depletion and IgG was tested on growth of 15 *E. coli* strains. The results suggested that the colostrum IgG inhibits the iron uptake and growth in vitro, but has only little effect on the growth

in Fe-depleted media (Takemura, Hogan, & Smith, 2004). Immunizing cows against the *E. coli* ferric citrate receptor had only minimal effect on mastitis following intramammary challenge with *E. coli* 727-strain isolated originally from naturally occurring mastitis (Takemura, Hogan, Lin, & Smith, 2002).

An Ig enriched colostrum product IMMULAC™ made of colostrum from non-immunized cows reduced the mortality of mice due to oral challenge with enterohaemorrhagic *E. coli* O157:H7 (Funatogawa et al., 2002). The animals were given colostrum preparation for 3 weeks as a 5% solution starting from 1 h after the challenge with bacteria. The Ig preparation proved to be effective in inhibiting the colonization and growth of *E. coli* in the intestine of mice.

Tawfeek, Najim, and Al-Mashikhi (2003) supplemented a casein-based baby formula with a colostrum Ig concentrate from normal cows or cows immunized with polyvalent (five strains) or monovalent *E. coli* vaccines. The formulas were given to infants daily (0.5 g per kg body weight) for 7 days in a field trial in Iraq. The control groups received either a similar formula supplemented with a normal colostrum preparation or were breast-fed. Infants receiving the formula containing the immune preparation had a significantly lower incidence of diarrhoea than those given a formula containing the control preparation during the 6 months follow-up period. In the immune formula group the number of mean diarrhoea episodes was 1.9 and in the control groups 3.5. The severity of diarrhoea as judged by duration was 4.5 days and 6.5 days on average in the immune formula control group, respectively. The preparations were well tolerated.

#### 4.3.4. *Shigella*

There is evidence suggesting that shigellosis could be prevented by using colostrum Ig preparations from cows immunized against certain *Sh. flexneri* antigens (for reviews, see Kelly, 2003; Korhonen et al., 2000). However, when children suffering from already established *Sh. dysenteriae* infection were treated with 100 ml of hyper-immune colostrum preparation against *Sh. dysenteriae* antigen I in combination with an antibiotic (pivmecillinam) three times per day for 3 days, no difference was found in any clinical parameter when compared with patients treated with control preparation and antibiotic (Ashraf, Mahalanabis, Mitra, Tzipori, & Fuchs, 2001). This suggests that in shigellosis the specific colostrum antibodies can prevent the infection but are not effective in treatment of an already established one.

#### 4.3.5. *Clostridium difficile*

*Clostridium difficile* colonization results often from antibiotic treatments and cause severe infectious diarrhoea and colitis by releasing two kinds of exotoxins, A and B. In a hamster model an Ig preparation made from bovine immune colostrum from cows immunized with *C. difficile* toxins A and B has been shown to be effective in treatment



of experimental *C. difficile* diarrhoea (Kink & Williams, 1998). Recently, Van Dissel et al. (2005) used a preparation made of milk from cows immunized against *C. difficile* toxins and whole cell *C. difficile* as a supportive treatment for 2 weeks after a standard antibiotic treatment in an uncontrolled cohort study. Nine of 16 patients had a history of relapsing *C. difficile* diarrhoea. After the regimen in all but one patient *C. difficile* toxins had disappeared from faeces and during the follow-up period of 11 months none of the patients had another episode of *C. difficile* diarrhoea.

#### 4.3.6. Other health benefits

In earlier studies, the consumption of milk proteins has been thought to lower cholesterol in people with hypercholesterolaemia, a risk factor of cardiovascular disease. Recently Earnest, Jordan, Safir, Weaver, and Church (2005) reported that in a placebo-controlled trial a blood derived bovine Ig fraction reduced total cholesterol level from 6.33 mmol L<sup>-1</sup> to 5.98 or 5.97 mmol L<sup>-1</sup> in mildly hypercholesterolemic subjects when 5 g was administered daily for 3 or 6 weeks, respectively. The reduction was mainly due to decrease in LDL levels from baseline of 4.12 mmol L<sup>-1</sup> to 3.92 and 3.84 mmol L<sup>-1</sup>. The mechanism of the effect remains unknown, but the finding is encouraging.

During and after major surgical procedures endotoxemia and acute phase reactions may occur due to intestinal endotoxins reaching the bloodstream by translocation of bacterial products. Bölke, Jehle et al. (2002) and Bölke, Orth et al. (2002) treated 40 patients for 3 days before an abdominal surgery either with a colostrum preparation (52 g daily in four doses per day orally) Lactobin<sup>®</sup> or placebo and a single shot of antibiotics mezlocillin and metronidazole. The Lactobin<sup>®</sup> group showed lower levels of endotoxin and endotoxin neutralizing capacity in blood than the placebo group. The authors concluded that the colostrum preparation reduced endotoxemia suggesting stabilization of the gut barrier during surgery. In another study, 60 coronary bypass patients were given 42 g daily of either bovine colostrum preparation or placebo starting 2 days before surgery (Bölke, Jehle et al., 2002); Bölke, Orth et al., 2002). The colostrum preparation did reduce post-operative CRP-levels, but failed to reduce perioperative endotoxemia. It is not known whether these results can be attributed to colostrum Igs neutralizing endotoxins in intestine or to growth factors enforcing gut barrier.

The incidence of upper respiratory infections in adult males was reduced by an orally administered colostrum product Intact<sup>™</sup> (Brinkworth & Buckley, 2003). The authors suggest that the effect is more likely to be caused by indigenous growth factors present in colostrum than Igs.

## 5. Conclusions

Considerable progress has been made in isolation and enrichment techniques of immunoglobulins from bovine

colostrum and cheese whey. This has enabled the development of commercial Ig preparations targeted at health promotion in farm animals and humans. In order to improve the efficacy of such preparations customized immunization schemes of cows has been designed with the purpose of raising specific antibodies. The enhancement of specific antibody activities of milk Igs through targeted immunization and isolation/concentration technologies may in future have remarkable potential in producing Ig products for human health care, not only as part of a health-promoting diet, but also as an alternative or supplement to the medical cure of specified human diseases. In clinical trials, the Ig preparations have seldom caused any undesired symptoms and the health risks are mainly due to allergenic properties of whey proteins (Bernhisel-Broadbent, Yolken, & Sampson, 1991; Gingerich & McPhillips, 2005). However, the regulatory status of these products is still unclear in many countries or they are treated as pharmaceuticals (for a case study, see Hoerr & Bostwick, 2002).

Colostrum or milk Igs can prevent the attachment of pathogen to the epithelial lining which is a critical step in the establishment of infection. Thus, an orally administered bovine milk or colostrum Igs have proven effective in the prevention of orally mediated infections. On the other hand, in the treatment of already established infections promising therapeutic effects have been reported only in such diseases where the infection is maintained through a constant reattachment and reinfection, e.g. inside the oral cavity or the GI lumen, and where toxins or inflammatory compounds are involved, which can be neutralized by the specific Igs of colostrum preparations.

Incidences of diarrhoea and mortality rates are high among small children in developing countries. The prevention of diarrhoea should not only diminish mortality but also improve health, in general. Increasing antibiotic resistance among pathogens gives emphasis to the need to develop new means to prevent diseases by nutritional intervention. Modulation of the GI flora has turned out to be an integral part of health promotion also in developed countries. It is suggested that combining bovine milk or colostrum Igs with probiotic lactic acid bacteria could provide considerable prospects for health promotion in the future. Other bioactive components occurring naturally in colostrum, such as lactoferrin and growth factors may also have synergistic effects when addressing gastrointestinal disorders.

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