In vivo antimicrobial and antiviral activity of components in bovine milk and colostrum involved in non-specific defence

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The *in vivo* evidence of the antimicrobial and antiviral activity of bovine milk and colostrum derived components are reviewed with special emphasis on lactoferrin and lactoperoxidase. Their mode of action and the rationale for their application in efficacy trials with rodents, farm animals, fish and humans, to give protection against infectious agents, are described. A distinction is made between efficacy obtained by oral and non-oral administration of these non-specific defence factors which can be commercially applied in large quantities due to major achievements in dairy technology. From the *in vivo* studies one can infer that lactoferrin and lactoperoxidase are very promising, naturally occurring antimicrobials for use in fish farming, husbandry, oral hygiene and functional foods. Other promising milk-derived compounds include lipids, from which anti-infective degradation products are generated during digestion, and antimicrobial peptides hidden in the casein molecules.

Bovine: Milk: Colostrum: Antimicrobial: Antiviral: Lactoferrin: Lactoperoxidase: Lipids

Introduction

Milk and colostrum contain several antimicrobial factors which exert both specific and non-specific bacteriostatic and bactericidal activity. These factors are transferred from the mother to the neonate and contribute to the protection against infectious diseases. For many species the milk derived antimicrobial system is crucial for survival of the newborn.

During the first few days postpartum, the specific activity of the different immunoglobulins is the dominant factor for immunity. The specificity is a reflection of the bacterial and viral pressure of the environment and therefore protects the neonate against the prevailing contaminating organisms. Through targeted immunisation of cows, bovine milk antibodies can be raised with a substantially higher activity against predetermined bacteria and viruses and several companies are now isolating these specific antibodies for both pharmaceutical, food and feed applications.

Non-specific antimicrobial and antiviral factors are also important for the host defence system and probably act synergistically with the specific antibodies. In bovine milk and colostrum, lactoferrin and lactoperoxidase are the most dominant and best studied non-specific antimicrobial components and many *in vitro* experiments have proven their activity against all kinds of micro-organisms. Lysozyme is a potent antimicrobial enzyme but, in contrast to human milk, the concentration in bovine milk and colostrum is probably too low to significantly contribute to the overall bacteriostatic and bactericidal activity.

In this review, we will focus on the *in vivo* evidence of the antimicrobial and antiviral activity of bovine milk and colostrum-derived components. Several of these components are now commercialised or under clinical testing for both therapeutic and preventive use against infectious diseases in animals and humans. More recent research shows that several peptides present in milk, or generated in the digestive tract through enzymatic degradation of the major milk proteins, may as well play an antimicrobial role *in vivo*. Furthermore, several components or metabolites of the fat phase in bovine milk have been identified that contribute to the host defence against infection by enhancing the gastrointestinal killing of pathogens.

Lactoferrin

Mode of action

The mechanism by which lactoferrin exerts its antimicrobial and antiviral activity *in vivo* is complex and in many cases still poorly understood. Two basic biochemical properties of lactoferrin contribute to its involvement in the host defence: the extremely powerful iron-binding capability and the strong interaction with other molecules and surfaces. The antimicrobial effects can be direct through bacteriostatic and bactericidal activity or indirect through activation of a complex series of reactions leading

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to a protective immune response after infection (Sánchez *et al.* 1992; Levay & Viljoen, 1995; Lönnerdal & Iyer, 1995). The direct bacteriostatic effect of lactoferrin is well established by *in vitro* experiments and many studies have proven that iron deprivation was the underlying antimicrobial mechanism because the properties disappeared when the lactoferrin molecule was saturated with iron (Naidu & Arnold, 1997). In addition to this bacteriostatic effect, lactoferrin also exhibits an iron-independent bactericidal activity (Arnold *et al.* 1980; Naidu & Arnold, 1997). The bactericidal activity is related to the direct binding of lactoferrin to the microbial membrane, which alters the membrane permeability through dispersion of lipopolysaccharides and leads to death of the organism. Both intact lactoferrin and partially hydrolysed lactoferrin may kill the microbes via this binding mechanism. In the case of hydrolysed lactoferrin, the activity is attributed to a peptide (lactoferricin) derived from the N-terminal region (Tomita, 1994). This observation may be relevant for oral administration of lactoferrin because the proteolytic enzymes in the gastrointestinal tract may (partially) degrade lactoferrin, although it is well known that the proteolytic degradation is limited in infants (Lönnerdal, 1996).

Lactoferrin plays a role in the cellular defence system which comprises a close interaction between neutrophils, lymphocytes, macrophages and their secretory products upon microbial invasion. Lactoferrin may influence this defence system in several ways. The regulation of macrophage activity and proliferation of lymphocytes are reported functions of lactoferrin. The mechanism underlying these functions and their *in vivo* significance needs to be elucidated.

The most important pool of systemic lactoferrin is found in the polymorphonuclear neutrophils (Sánchez *et al.* 1992; Levay & Viljoen, 1995). It is well established that this pool of lactoferrin is crucial for the protection of the host against infection and inflammation. Upon contamination with micro-organisms the neutrophils will capture the invader (phagocytosis) and at the same time specific granules discharge lactoferrin into the blood. Due to the high affinity of lactoferrin for iron, a hypoferraemic state is generated, which prevents the pathogen from acquiring sufficient iron for growth (Sánchez *et al.* 1992). This is the immediate acute effect. The protective role of lactoferrin during inflammation is less clear but is probably related to the indirect influence on the production of cytokines, in particular the tumour necrosis factor alpha (Zagulski *et al.* 1989; Machnicki *et al.* 1993).

Endogenous lactoferrin

Plasma lactoferrin is released from neutrophils during infection, inflammation, tumour development and iron overload, demonstrating its multi-functional biological role (Bullen, 1987; Levay & Viljoen, 1995). The direct evidence that lactoferrin plays a crucial role in the body's defence against micro-organisms is seen in patients with a lactoferrin deficiency. Several studies revealed that patients with a high susceptibility for infections lacked the protective effect of lactoferrin from the neutrophils (Breton-Gorius *et al.* 1980; Boxer *et al.* 1982). Spitznagel *et al.* (1972) demonstrated that polymorphonuclear neutrophils with no lactoferrin lost their bactericidal activity. In general, it can be stated that the susceptibility to infections is increased when the lactoferrin production is reduced, for instance as a result of malnutrition, pre- or postoperative starvation, hepatic failure or after iron saturation following parenteral administration (Gaunt & Seal, 1984). Diabetic patients also suffer from recurrent infections and it was suggested that the inhibition of endogenous lactoferrin and lysozyme by glucose-modified proteins is responsible for this loss of the antibacterial protection (Li *et al*. 1995). This once more demonstrates the important role of lactoferrin against infectious diseases.

Rudney *et al.* (1995) studied the *in vitro* and *in vivo* binding of saliva proteins to oral streptococci and demonstrated that lactoferrin was one of the proteins that interacted with different strains. It was suggested that this might have a positive influence on the microbial ecology of tooth surfaces. Another manifestation of the antimicrobial effect of endogenous lactoferrin is the defence of the mammary gland against infections. During lactation lactoferrin probably does not play an important protective role against invading micro-organisms in the cow's udder because of the low concentration and the high citrate level (Reiter, 1985). However, in the non-lactating udder, the concentration of lactoferrin is considerably higher and the condition more favourable for antimicrobial activity. Indeed Reiter & Bramley (1975) showed that infusion of the non-lactating udder with *Escherichia.coli* did not lead to mastitis, whereas with the same treatment, the lactating udder became infected. If the dry udder was infused with both *E. coli* and iron, the multiplication of the pathogen took place, indicating the protective role of lactoferrin via an iron-chelating mechanism.

Non-oral administration of lactoferrin

A few studies have been carried out to investigate the antibacterial and antiviral effect of intravenously or intraperitoneally administered lactoferrin. Zagulski *et al.* (1985) showed that bovine lactoferrin given intravenously to rabbits considerably prolonged survival time after a systemic experimental infection with a lethal dose of *E. coli*. A subsequent study with mice confirmed the observation that a single intravenously administered dose of lactoferrin given 24 hours before an *E. coli* challenge protected the animals, resulting in a lower mortality rate compared to a control group without lactoferrin (Zagulski *et al.* 1989). No difference in protection was found between bovine and human lactoferrin. A single dose of iron given just before or after the bacterial challenge suppressed the protective effect of lactoferrin but only during the first week. Subsequent doses of iron enhanced the killing of bacteria. In later studies Zagulski *et al.* (1998) showed that the clearance of *E. coli* was strongly accelerated in blood, liver, lungs, spleen and kidney after intravenous application of bovine lactoferrin.

Apparently, lactoferrin not only acts via an iron sequestering mechanism but also stimulates other delayed non-specific responses to improve protection against

infections. These responses are associated with the indirect effect of lactoferrin on the production of plasma cytokines, compounds produced by immune cells during infection and inflammation to coordinate the defence against pathogens. In a study with mice, it was observed that the production of tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) were regulated by lactoferrin. This resulted in a suppression of inflammation decreased mortality (Machnicki *et al.* 1993). Lee *et al.* (1998) used a germ-free, colostrum-deprived, immunologically 'virgin' piglet model to evaluate the protective effect of bovine lactoferrin against the endotoxin lipopolysaccaride (LPS). Compared to bovine serum albumin (BSA) as the control, the feeding of lactoferrin prior to LPS challenge resulted in substantial reduction in mortality after 48 hours: 73·7 % versus only 16·7 % in the group with lactoferrin.

The antiviral activity of lactoferrin was demonstrated *in vivo* against the Friend virus complex which is associated with the formation of leukaemia cells (Lu *et al.* 1987). Mice were injected interperitoneally with iron-saturated human lactoferrin and challenged with a lethal dosage of the virus. The survival of the lactoferrin-treated animals was significantly increased. This effect could not be explained by the direct binding of lactoferrin to the virus and it was speculated that the action on certain cells inhibitory to the virus were responsible for the protection.

Shimizu *et al.* (1996) injected mice intraperitoneally with lactoferrin before and after an infection with murine cytomegalovirus. The administration prior to injection protected the mice from death whereas no protection was found when lactoferrin was given after or together with the virus. The antiviral activity of lactoferrin was also found to be indirect and caused by the augmentation of T-cell dependent natural killer cell activity.

Oral administration of lactoferrin to animals

A number of animal studies with oral administration of lactoferrin have been carried out to investigate the *in vivo* bactericidal efficacy. Bullen *et al.* (1972) studied the effect of lactoferrin on the bacterial flora of suckling guinea piglets receiving mothers milk, which is as rich in lactoferrin as human milk. Challenging the piglets with *E. coli* resulted in lactobacilli-dominant flora for the animals receiving mothers' milk, whereas the animals on an artificial diet displayed a coliform dominant flora. When suckling piglets also received iron, the protective effect of the mothers' milk disappeared and a coliform-rich flora developed, supporting the view that protection was due to lactoferrin in milk.

The bacteriostatic effect of orally administered lactoferrin was also observed in mice challenged with strains of *Clostridium* (Teraguchi *et al.* 1995*a*). The addition of lactoferrin and partially hydrolysed lactoferrin resulted in a clear suppression of the *in vivo* growth of the *Clostridium* strains as evidenced from decreased numbers in the faeces. In a similar experiment, it was also shown that lactoferrin significantly reduces bacterial translocation, probably because of prevention of bacterial overgrowth in the gut (Teraguchi *et al.* 1995*b*).

Another interesting finding is that lactoferrin can protect fish against bacterial infection. Oral administration of bovine lactoferrin to rainbow trout increased survival rates after an intraperitoneal challenge with *Vibrio anguillarum* (Sakai *et al.* 1993). An enhanced resistance against streptococci was also observed. No *in vitro* bactericidal effect against these organisms was observed. In a subsequent study it was found that the phagocytic activity of kidney cells was increased in fish receiving lactoferrin (Sakai *et al.* 1995).

In a study initiated by Kussendrager (unpublished) a similar effect of lactoferrin on Atlantic salmon was found. In this experiment, a small number of fish were injected with *Aeromonas salmonicida* and transferred to a tank to simulate naturally transmitted infection. Mortality of the control group reached a level of 14 % whereas in the lactoferrin fed group, the mortality remained at a level of 2 %. In the carp *Cyprinus carpio* L., bovine lactoferrin in the fish feed for 14 days reduced the levels of typical stress markers (e.g. catecholamines) after a period of hypoxia (Kakuta, 1998).

Feeding mice bovine lactoferrin for 4 weeks resulted in an increase of the total amounts of the immunoglobulins IgA and IgG in the intestinal fluid, versus a non-lactoferrin control group. This increase was reflected in the higher secretion of IgA and IgG by the Peyer's patches in the mucosa of the intestine. The secretion of IgA and IgG by immune cells in the spleen was also increased in the mice fed lactoferrin in the diet. The authors concluded that lactoferrin acts as an immune stimulating factor on the mucosal and the systemic immune system and that its binding to the mucosal cells is required for activation (Debbabi *et al.* 1998).

Oral administration of lactoferrin to humans

The most natural means of administering lactoferrin is by breast-feeding. Reiter (1985) estimated that breast-fed human infants ingest about 3 g lactoferrin per day during the first week of life. A calf drinking 2 litres of colostrum ingests about 2 g of lactoferrin per day. Lactoferrin is not easily digested by the enzymes in the intestinal tract and could be recovered from the faeces of breast-fed infants with an intact iron-binding capacity, indicating that antibacterial activity can take place during the gastro-intestinal passage via the iron-chelating mechanism. However, even if lactoferrin is partially digested, it still has antibacterial activity via the direct interaction with micro-organisms as pointed out earlier. It is well recognised that human milk has a protective effect against infection in infants, especially against enteric infections (Roberts *et al.* 1992). The gut flora of breast-fed infants, in contrast to that of formula-fed infants, is much richer in bifidobacteria and lactobacilli. Such a flora is normally associated with an increased resistance against colonisation of pathogens. Lactoferrin in conjunction with other factors in milk likely contributes to this favourable microbial ecosystem in the gut.

Roberts *et al.* (1992) investigated the infant faecal flora after feeding formula with and without addition of lactoferrin. The observation was that half the babies displayed a bifidus flora in the case of lactoferrin

supplementation but this flora was not as rich in bifidus as that of breast-fed infants. In the latter case, the bifidus flora was established earlier and remained stable during the breast-fed period whereas, in the case of lactoferrin-containing formula, the bifidus flora developed later at the age of three months. The question remains whether the occurrence of a bifidus flora is the correct marker for the antibacterial activity of lactoferrin. Stronger evidence of the protective effect of lactoferrin against enteric infections in humans was found by Trümpler *et al.* (1989) with neutropenic patients receiving chemotherapy for acute myelogenous leukaemia. Patients receiving human lactoferrin coated with an acid stable substance had a significantly lower incidence of bacteraemia due to reduced multiplication and/or systemic spread of enterobacteriaceae. This reduction was explained by the iron-binding effect of lactoferrin.

Lactoperoxidase

Mode of action

The detailed chemistry of the antibacterial activity of lactoperoxidase (LPO) in combination with its two co-factors, H_2O_2 and SCN^-, has been fully elucidated and explained elsewhere in this volume (see article by Kussendrager & Hooydonk)

The biological function of the lactoperoxidase system (LP-s) is predominantly that of defence against microbial infections and many *in vitro* studies showed the bacteriostatic and bactericidal effect against a broad spectrum of micro-organisms (Pruitt & Reiter, 1985; Wolfson & Sumner, 1993). Several applications now exist where the LP-s is commercially used as a natural preservative (De Wit & Van Hooydonk, 1996).

Other reported biological functions of LP-s are antiviral activity (Courtois *et al.*1990), tumoricidal activity (Stanislawski *et al.* 1989) and protection against H_2O_2-mediated peroxidation (Reiter & Perraudin, 1991). The significance of these functions has still to be proven *in vivo*.

Various members of the peroxidase family play a role in the defence of mammals including eosinophilic peroxidase in intestinal tissues (Rytomaa & Teir, 1961), myeloperoxidase in leucocytes (Klebanoff, 1970), thyroid peroxidase in cell membranes (Ohtaki *et al.* 1980) and lactoperoxidase in saliva, milk and tears (Reiter & Oram, 1967). In this paragraph, we only discuss the *in vivo* antimicrobial activity of milk and saliva lactoperoxidase.

Compared to bovine milk, the concentration of LPO in human milk is very low. In contrast, in saliva of infants, the level is relatively high and significant amounts are ingested during suckling and fasting. We may expect that this saliva-derived LPO and SCN^- contributes to the peroxidase activity in the human intestine because the enzyme is very resistant against proteolysis (Reiter & Perraudin, 1991). Calves receive most of the LPO via milk and colostrum.

SCN^- occurs ubiquitously in tissue and secretions of mammals, making H_2O_2 normally the limiting factor for the activity of LP-s (Reiter & Perraudin, 1991). The SCN^- level in bovine milk reflects the level in serum and is increased during infection of the udder, due to leakage from the blood. Clinical trials proved that addition of SCN^- to milk for preservation purposes is not harmful for man (Dahlberg *et al.* 1984).

Whereas the systemic supply of H_2O_2 is generated by the polymorphonuclear neutrophils during phagocytosis, in the mouth and intestine it is the flora that may excrete H_2O_2 to trigger the LP-s activity (Reiter & Perraudin, 1991). In this way, LPO probably continuously contributes (together with other antimicrobial factors such as lactoferrin and lysozyme) to the maintenance of a healthy, non-cariogenic and non-infectious flora. LP-s is now added to toothpaste, mouthwashes, artificial saliva, chewing gum and calf starters to augment the *in vivo* protection against infections. To provide sufficient and a continuous source of H_2O_2, peroxidogenic enzymes such as glucose oxidase may be added to the system.

LP-s administration to saliva

A number of clinical trials have been undertaken to investigate the activation of lactoperoxidase in saliva with H_2O_2-generating enzymes.

In a paper by Hoogendoorn (1985), a positive effect of toothpaste supplemented with amyloglucosidase and glucose oxidase was reported from several clinical studies. The activation of lactoperoxidase by these enzymes prevented a fall in the tooth pH of the surface, reduced plaque accumulation and suppressed carious lesion and gingivitis. Also in patients suffering from recurrent oral ulcerations, the same system reduced the occurrence and provided a long-lasting protection.

Supplementation of both LPO and peroxidogenic enzymes to toothpaste also proved to be beneficial for oral health care. In a trial with 25 healthy humans increased levels of $HOSCN/OSCN^-$ were found in the saliva of the subjects receiving a toothpaste containing LP-s (Leriander-Lumikari *et al.* 1993). This is clearly an indication of an elevated antimicrobial system in the mouth due to the addition of LP-s. Van Steenberghe *et al.* (1994) demonstrated the protective effect of LP-s containing toothpaste in patients suffering from radiation-induced xerostomia. Patients treated with the test toothpaste showed less plaque formation and a lower incidence of gingival inflammation.

LP-s administration to calves and piglets

In contrast to infants, the saliva of calves contains little LPO and they receive most of the protective enzyme via colostrum and milk. SCN^- is secreted in the calf's stomach and it was found that lactobacilli isolated from the abomasal fluid generated H_2O_2. Thus, in calves the conditions seem favourable for the formation of LP-s activity *in vivo*. Despite the continuous ingestion of the antibacterial components from colostrum or milk, the frequency of diarrhoea in calves during the first week is high and antibiotics are often required for recovery.

Reiter and Oram (1967) performed *in vivo* experiments with calves using a non-pathogenic *E. coli* (no adhesion to the intestinal wall of the calf). The strain was orally administered followed by feeding raw milk containing glucose oxidase/glucose and extra SCN^-. Compared to the

control containing a reducing compound to inactivate indigenous LPO, the recoverable organisms were reduced by as much as 4 log cycles. An interesting observation was that *Lactobacillus lactis* could replace the enzyme as a source of H_2O_2. This suggests that the activity of endogenous LPO may be enhanced and contribute to beneficial antibacterial effects upon eating of fermented milk products. This possible secondary effect of probiotics has, to our knowledge, never been explored.

In a larger field trial in Sweden (Reiter *et al.* 1981), 5-d-old calves were removed from a herd to calf-fattening units, which frequently led to a high incidence of diarrhoea. It was observed that the LP-s-fed calves remained more healthy and the weight gain was significantly higher. Similar trials over a period of 5 years in the UK confirmed the health-promoting effect of LP-s during calf rearing under practical conditions (Reiter & Perraudin, 1991).

With the current pressure on the use of antibiotics, isolated bovine LPO appears to be an attractive, natural antibacterial compound for supplementation of calf starters and several successful trials have been conducted with milk replacers containing LP-s (Waterhouse & Mullan, 1980*a*, *b*). Reiter (1985) also reported a trial with piglets and showed a significant protective effect of bovine LP-s against an *E. coli* challenge. The piglets that received LP-s containing diluted colostrum remained unaffected, whereas the controls developed severe diarrhoea.

LP-s plus lactoferrin administration to calves

Recently, the Dutch TNO research institute performed a feeding trial with thirty 7-d-old calves (Van Leeuwen *et al.* 1998). The calves were split into two groups of 15 animals and test results recorded for 13 days. The control group received a commercial cow milk replacer and the test group the same diet, supplemented with a combination of LP-s and lactoferrin (LF) (obtained from DMV International). The final concentration of the active components in the milk, on dry basis, was: lactoperoxidase (LP), 200 ppm; KSCN, 120 ppm; 2 Na_2CO_3.3 H_2O_2; 225 ppm and LF 1000 ppm. The main conclusions of the trial were:

- The incidence and severity of diarrhoea was less in the animals receiving LP-s + LF.
- The blood immunoglobulin level in the test group increased whereas the level decreased in the control group.
- Morphological and histological measurements showed more normal, finger shaped and higher villi at the distal jejunum of the calves in the supplemented group.
- *E.coli* counts were lower in the jejunum, colon and faeces for calves receiving LP-s + LF.
- The weight gain was slightly higher in the test group but the difference was not significant.

Still *et al.* (1989) reported a similar trial in which calves aged 1–3 days were infected with *E.coli* and subsequently fed with a combination of LP-s and LF. All infected calves had diarrhoea within 1–2 days but the treated animals recovered much faster, without suffering from hypothermia or clinical depression as was observed in the untreated animals.

Indeed, it may well be that the combined antibacterial and antiviral activity of LP-s and LF is more powerful than each of the components alone but this needs further verification in studies where the combination is compared with the separate components.

LP-s administration to fish

Together with the Danish Institute for Fishery Technology and Agriculture Kussendrager (unpublished) undertook a trial which involved feeding bovine LP-s to rainbow trout fry.

The test group received a standard diet containing LP-s which was sprayed on the pellets together with the oil phase. The LP-s (derived from DMV International) consisted of LPO, glucose oxidase, KSCN and KI. Potassium iodide was added to obtain a more powerful antibacterial system with a broader spectrum. The mortality during the weaning period is normally high, mainly due to infections by *Flexibacter psychrophilus* and *Octomis salmonis*. Growth and mortality was recorded over a period of 10 weeks and it was observed that the growth rate was not significantly different between the control and the LP-s group. However, the difference in mortality was highly significant, resulting in a relative reduction of the accumulated mortality by 30 % for the test group.

In a second trial Kussendrager (unpublished) investigated the efficacy of LP-s against *Lepeophtheirus salmonis*, a type of sea lice, which is a major threat in salmon farming in Norway, Scotland and other salmon-producing countries. Atlantic salmon were brought in a tank with ozonated seawater and challenged with *L. salmonis*. After the lice developed into pre-adult stages, the fish were split into two groups and the test group was transferred to a tank containing LP-s and the control group to a tank only containing seawater. After 48 hours treatment, the test group showed a 58 % reduction in lice whereas the lice levels in the untreated group remained the same. This pilot experiment indicated that a lactoperoxidase system may be a good alternative for the excessive high levels of hydrogen peroxide (over 1000 ppm) normally used to prevent sea lice-induced mortality in practice.

Lipids

The antimicrobial and antiviral activities of lipids are currently receiving increasing interest, especially in the context of the preparation of infant formula and medical food.

Milk contains a complex mixture of lipids ranging from simple triglycerides comprising 98 % of the fat phase in milk to minor lipids such as phospholipids, which are mainly concentrated in the fat globule membrane (Renner *et al.* 1989). Phosphatidylethanolamine, phosphatidylcholine and sphingomyelin are the predominant fractions of phospholipids. The biological function of these components is still unclear but *in vitro* results indicate that metabolic breakdown products of triglycerides and phospholipids may possess antimicrobial and antiviral activity.

Isaacs *et al.* (1995) conducted an *in vitro* study in which fatty acids and monoglycerides were added to

milk and infant formula. They found that fatty acids and monoglycerides with chain lengths varying from 8 to 12 carbons were more antiviral and antibacterial than long-chain monoglycerides.

Edwards *et al.* (1994) compared the faecal concentration of short-chain fatty acids in breast-fed infants and formula-fed infants and observed a significant difference in the pattern of these faecal fatty acids. Breast-fed babies showed a predominantly acetic–lactic acid profile, whereas the formula-fed babies had higher concentrations of propionic and butyric acids. The authors speculated that short-chain fatty acids play a role in maintaining a stable favourable flora by inhibitory actions preventing colonisation of enterogenic pathogens. However, the difference between the flora of breast- and formula-fed infants could not be explained by the profile of these fatty acids.

The protective effect of phospholipids against gastric ulceration was investigated by Kivinen *et al.* (1992) in a human study. Milk phospholipids were given to human volunteers and the protective effects against an aspirin challenge was investigated. Gastric mucosal damage was almost completely absent in the presence of phospholipids, suggesting that these components derived from milk may contribute in the prevention of gastritis, which is associated with the colonisation by *Helicobacter pylori*.

In a recent study by Sprong *et al.* (1998), the effect of phospholipids on gastrointestinal survival and translocation of *Listeria* was investigated in rats. The test group received sweet butter milk known to be rich in phospholipids and the control group skim milk. The conclusion was that the phospholipids in butter milk improved the host defence against *Listeria* by enhancing the gastrointestinal killing of the pathogen. This observation is important and may throw a new light on the biological role of phospholipids in milk.

Conclusion and outlook

As shown above lactoferrin and lactoperoxidase are well-characterised protective factors from milk and colostrum. Although their *in vivo* mode of action is not fully understood, various supplement studies reveal their inhibitory actions against pathogens such as bacteria and viruses, and because of this, both compounds have found their way on to the market.

Interest in understanding the composition of cow's milk and colostrum is initiated by the desire of infant formula manufacturers to adjust cow's milk-based infant formulas so as to mimic as closely as possible, human breast milk. In a recent review Rudloff & Kunz (1997) compared the protein and non-protein components in human milk, bovine milk and infant formulas. In human milk glycoproteins, glycolipids and lactose-derived oligosaccharides are now considered to be soluble receptors for pathogenic micro-organisms, viruses or endotoxins, and hence may exert anti-infective properties. There are indications that some of these components may also be present in cow's milk. Schanbacher *et al.* (1997) have recently indicated bioactive peptides released from α_s- or β-caseins that may contribute to the antimicrobial defence in the mammary gland, and possibly the suckling neonate. *In vitro* studies have shown that a 39 amino acids long C-terminal fragment of bovine α_{s2}-casein inhibits the growth of *E.coli* and *Staphylococcus* strains (Zucht *et al.* 1995).

Future research will identify and characterise new components from either mature milk or colostrum that offer protection against infections and modulate the control of inflammation, and will reveal whether the primary function is targeted at protection of the milk gland or the neonate. Dairy technology has expanded enormously over the last years and it is envisaged that the technology for manufacturing minor bioactive components in milk or colostrum will be realised in the near future. Thus it will become possible to isolate these compounds for inclusion in health-promoting functional foods, nutraceutical products, veterinary and health care products.

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