

Lactoferrin: an iron-binding antimicrobial protein against *Escherichia coli* infection

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Abstract *Escherichia coli* (*E. coli*) are the most common aerobic gram-negative bacilli in a normal intestinal tract. They cause most of the intra-abdominal infections, wound infections associated with abdominal surgery, and septicemia. Most of these infections are of endogenous intestinal origin. Lactoferrin (LF) is an iron-binding glycoprotein found in milk and various external secretions. This protein has been found to have a number of biological functions, including antimicrobial, anti-cancer, antioxidant, and immunomodulatory effects. Partial degradation of LF

by pepsin can give rise to peptides termed lactoferricin (LFcin) with more potent antimicrobial activity. LF and LFcin have been shown to inhibit the growth of a number of pathogenic bacteria (including *E. coli* and antibiotic-resistant strains), fungi, and even viruses in both in vitro and in vivo studies. We previously demonstrated that both recombinant porcine LF (pLF) produced from yeast and a synthetic 20-residue porcine LFcin peptide exhibit antimicrobial activity in vitro. In one of our recent studies, we performed pathogen challenges, including pathogenic *E. coli*, *Staphylococcus aureus* and *Candida albicans*, of the digestive tract of a transgenic milk-fed animal model. The results showed that LF has broad spectrum antimicrobial activity in the digestive tract and protects the mucosa of the small intestine from injury. Our following study also revealed that pLF as a feedstuff additive enhances avian immunity, including antibody formation and cell-mediated immunity. All of these results suggest that LF could be a novel natural protein in the treatment and prevention of infections with *E. coli* or antibiotic-resistant bacteria strains.

Keywords Lactoferrin · *E. coli* infection · Host defense protein · Sepsis · Antimicrobial activity · Nosocomial infection

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Introduction

Escherichia coli (*E. coli*) strains are the most common aerobic gram-negative bacilli in the normal

intestinal tract. The bacteria are present whenever there is fecal contamination, and their presence is regarded as an indicator of fecal pollution. The species includes a variety of strains, which range in virulence from commensal to lethal. In human, *E. coli* can induce a variety of intestinal and extraintestinal infections. *E. coli* is the most common cause of acute uncomplicated urinary tract infection (UTI) in the community and one of the common causes of nosocomial urinary tract infection, intra-abdominal infections, wound infections associated with abdominal surgery, and septicemia (Murray et al. 2009).

In general, different strains are associated with different diseases. The diarrheagenic strains that cause gastrointestinal diseases fall into at least five groups: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC). The first three groups involve the small intestine and cause watery diarrhea in infants. The last two groups involve the large intestine and cause dysentery (Murray et al. 2009). Uropathogenic strains that have the ability to adhere to bladder mucosa often originate from the colon and must travel to the urethral opening to gain admission to the urinary tract. The strains that cause invasive diseases, such as meningitis and septicemia, almost always have a capsule composed of a polysaccharide for antiphagocytosis (Murray et al. 2009).

Normally, antibiotics are not used to treat diarrhea because the disease is often self-limiting. In suspected cases of EHEC, antibiotics are definitely not indicated because they may worsen disease (Salysers and Whitt 2002). The therapy for extraintestinal *E. coli* infections does consist of antibiotics. However, antibiotics have the disadvantage of adverse effects, safety problems during pregnancy and selective pressure that can induce the emergence of resistant strains (Camus et al. 2005; Vincent 2003). The rates of antibiotic resistance among *E. coli* strains have increased greatly over the past two decades, encouraging the search for novel antimicrobial strategies (Nickel 2007; Moreno et al. 2006). Lactoferrin, one of the natural cationic host defense proteins that have both antimicrobial and immunomodulatory activities, is a novel agent for treatment and prophylaxis of the extraintestinal infections and even for shortening the course of diarrheagenic *E. coli* infections.

Pathogenesis of *E. coli* infection

Pathogenic strains of *E. coli* differ from commensal organisms in that they possess certain virulence factors. Bacterial infection depends on the ability of the bacteria to adhere host cells, to escape from host immune response, to invade cells and tissues, or to produce toxins (Croten and Finlay 2010).

Adhesion is an essential and prerequisite step for the colonization of mucosal surfaces by microbial pathogens, and this colonization is required for the subsequent development of infection (Croten and Finlay 2010). Adherence of *E. coli* is mediated by plasmid-encoded adhesins. These proteins are expressed on the surface of the microorganisms, sometimes in the form of fimbriae or pili. The variable amino acids on the tip of the pili determine the binding specificity of bacteria. For example, the urogenic strains of *E. coli* express a specific P pilus that binds to cells lining the urinary tract and prevents the elimination of bacteria in voided urine. The ETEC strains adhere to the enterocytes via pili without invasion while EPEC strains adhere and efface the enterocytes with damages to the microvilli. Furthermore, the EIEC strains have the capacity to induce endocytosis and invasion into the epithelial cells (Murray et al. 2009).

Bacterial toxins are classified as endotoxins and exotoxins. Endotoxin is a lipopolysaccharide (LPS) that is a component of the outer cell wall of Gram-negative bacteria. The activity of endotoxins depends on the lipid A component of LPS, which is released at cell lysis. LPS binds to the cell surface receptor CD14 and is delivered to Toll-like receptor 4 (TLR-4), which transmits signals that lead to release of pro-inflammatory cytokines, systemic inflammatory responses and even septic shock (Kawai and Akira 2007). Exotoxins are secreted proteins that cause cellular injury and disease. Some diarrheagenic strains can adhere to enterocytes, secrete an enterotoxin, one kind of exotoxins, and cause gastroenteritis in children and travelers. The EHEC O157:H7 strains release Shigella-like toxins that cause damage to the colonic mucosa, and blood vessels in kidneys and elsewhere, leading to hemolysis and acute renal failure (Pennington 2010).

Lactoferrin

Lactoferrin (LF) is an iron-binding glycoprotein found in milk and various external secretions such

as saliva, tears, airway secretion, and the granules of neutrophils, implying an important role in innate immunity (Valenti and Antonini 2005). The protein, which is approximately 80 kDa, comprises two homologous lobes with one iron-binding site in each lobe. Lactoferrin has a number of biological functions, including antimicrobial, anti-tumor, antioxidant, and immunomodulatory effects. Partial degradation of LF by pepsin in the stomach, can give rise to peptides termed lactoferricin with more potent antimicrobial activity. LF and lactoferricin have been shown to inhibit the growth of a number of pathogenic bacteria (including antibiotic-resistant strains), fungi, and even viruses in both in vitro and in vivo studies (Tomita et al. 2002; Valenti and Antonini 2005). In mouse experiments, oral administration of bovine LF reduced the number of bacterial infections in the gastrointestinal tract (Tomita et al. 2002), while promoting the growth of bacteria with low iron requirements such as *Lactobacillus* and *Bifidobacteria*, which are generally believed to be beneficial to the host (Kim et al. 2002, 2004). Oral administration of LF also reduced experimentally induced UTIs and endotoxin-induced septic shock (Haversen et al. 2000; Lee et al. 1998). Another important function related to infection is the immunomodulatory effect of LF, which consists of both immunotropic and anti-inflammatory effects (Actor et al. 2009; Ando et al. 2010). This article will concentrate on the antimicrobial and immunomodulatory activities of LF first and will then discuss the effects LF on *E. coli* and the proposed clinical applications of LF.

Mechanisms of antimicrobial activity of lactoferrin

The antimicrobial activity of LF is well established. Originally, the bacteriostatic effect of intact LF was believed to be related to its ability to scavenge iron, the essential nutrient of microorganisms. Subsequent studies have shown that the highly cationic N-terminal regions of LF and its derived peptides, lactoferricin (LFcin17–30) and lactoferrampin (LFampin265–284), exert a potent bactericidal effect by interacting with the negatively charged elements in the bacterial membrane (Appelmelk et al. 1994; Chen et al. 2006; Zagulski et al. 1998; Flores-Villasenor et al. 2010; van der Kraan et al. 2004). These elements can be lipopolysaccharides (LPS) in Gram-negative bacteria,

and they are assumed to be lipoteichoic or teichoic acid in Gram-positive bacteria (Gonzalez-Chavez et al. 2009). The mechanism of the antifungal effect of LF is not clear at present. It is possible that its antifungal activity stems from its immunological effects (Kuipers et al. 1999; Legrand et al. 2004), or from its direct or indirect interaction with the cytoplasmic membrane (Viejo-Diaz et al. 2004). In our previous report, the destructive effects of LF and LFcin on the cellular membranes of *E. coli*, *S. aureus*, and *C. albicans* were directly observed under a scanning electron microscope (Chen et al. 2004a, b; Chen et al. 2006). LF has also been revealed to have antiviral activity. The mechanisms behind this function have not clearly defined, but it is likely that LF prevents the adhesion of the pathogens to host cells (Gonzalez-Chavez et al. 2009).

Lactoferrin also has a protease-like activity (Ling and Schryvers 2006). In studies involving *E. coli*, LF was found to degrade and impair the function of the surface expressed virulence factors of *E. coli*, thereby decreasing their ability to adhere to or invade host cells (Ochoa et al. 2008). Furthermore, LF has been found to prevent biofilm formation at sub-inhibitory concentrations by stimulating bacterial motility, which causes the bacteria to wander across the surface instead of forming cell clusters (Singh et al. 2002).

Mechanisms of immunomodulatory effect of lactoferrin

There are several studies demonstrating that LF has host-protecting properties, by its immunomodulatory action and its antimicrobial effect. Depending on the immune status of the host, LF can have an anti-inflammatory effect that downregulates the immune response and prevents septic shock and mortality (Kruzel et al. 2002; Lee et al. 1998; Zimecki et al. 2004). LF also acts as a promoter of the immune system. LF promotes the maturation of T-cell precursors into competent T helper cells and stimulates the differentiation of immature B cells into functional antigen presentation cells (Actor et al. 2009). Our recent studies on the effect of porcine LF (pLF) on avian immunity are in agreement with these findings. Recombinant pLF was used as a feedstuff additive in chickens vaccinated for the infectious bursal disease (IBD), a worldwide viral disease of chicken with high mortality. A high dose of pLF leads to significant

increases in serum IgA, IgG and IBD-specific antibodies. Porcine LF at either low or high doses enhances the expression of interferon- γ and IL-12, driving T lymphocytes toward Th1 rather than Th2 cells, and enhancing the immunity against microbial agents (Hung et al. 2010a, b).

The two contrasting effects of LF, immunotropic versus anti-inflammatory, have not been fully elucidated at present. Recent studies have implied that human LF may bind to LPS and CD-14 and then interfere with the formation of the CD14–LPS complex, resulting in the attenuation of the LPS/CD-14/TLR-4 signaling pathway that is essential for pathogenesis of sepsis. Human LF may stimulate the immune system by binding to CD-14 and then activating the TLR-4 mediated pathway while preventing overexpression of LPS-induced inflammation (Ando et al. 2010).

Effect of lactoferrin on intestinal *E. coli* infections

Diarrheal disease is still an important public health problem in developing countries. According to WHO estimates, diarrhea accounts for 18% of deaths in children before the age of 5 years, approximately 2 million deaths each year. *E. coli* is one of the major causes of infant diarrhea in developing countries and also causes traveler's diarrhea in adults (Bryce et al. 2005). A few outbreaks of EHEC disease, caused by *E. coli* O157:H7, have occurred in developed countries including the USA and Japan (Salyers and Whitt 2002; Shin et al. 1998). This strain can cause hemolytic uremic syndrome and even death (Cimolai et al. 1990). Breast feeding is the most effective intervention for protecting children younger than 5 years of age against diarrhea and all causes of mortality. Lactoferrin is one of the main anti-infective components in the milk (Ochoa et al. 2008).

In vitro studies

Many in vitro studies have revealed that LF inhibits adhesion of ETEC, EPEC, EHEC and EAEC to epithelial cells and intestinal mucosa (de Araujo and Giugliano 2001; de Oliveira et al. 2001; Giugliano et al. 1995; Ochoa et al. 2003). In addition to its bacteriostatic and bacteriocidal effects, LF has been found to exhibit a broad spectrum of anti-adhesion

activity. The EHEC O157:H7 strain is susceptible to the antimicrobial effects of bovine LF and its derived peptides (Shin et al. 1998) and its growth and attachment to intestinal epithelial cells are also inhibited by LF (Yekta et al. 2010). In the case of EPEC, both human and bovine LF block bacterial adhesion to cultured cells. At a concentration not affecting the growth of EPEC, LF blocks the attachment to and EPEC-induced actin polymerization in host cells. The mechanism of this inhibition comes from the ability of LF to cause degradation of the proteins of the type III secretory system that is necessary for bacterial contact and pore formation (Ochoa et al. 2003). Bovine LF also inhibits EAEC biofilm formation, an important sequel of bacterial adhesion (Ochoa et al. 2006). The combination treatment with antibiotics, such as cephalosporin and aminoglycoside, with LF or LFCin shows a synergistic effect against *E. coli* (Vorland et al. 1999; Sanchez and Watts 1999; Chen et al. 2004b).

We have previously demonstrated that both recombinant porcine LF (pLF) produced from yeast and a synthetic 20-residue porcine LFCin peptide exhibit antimicrobial activity on *E. coli* ATCC 25922, a surrogate of *E. coli* O157: H7, in vitro (Chen et al. 2004a; Chen et al. 2006). Under the scanning electron microscopy (Fig. 1), the *E. coli* cells showed either aggregative fragmentation (Fig. 1a) or display puncturing holes and membrane breakdown (Fig. 1c) after LF treatment for 2 or 4 h, respectively. The control groups of *E. coli* without LF treatment showed normal morphologies after 2 h (Fig. 1b) or 4 h (Fig. 1d) incubation in the same condition.

Animal studies

Orally administration of LF has been found to suppress enteric infection of *E. coli*. In one study of ETEC strain, the bacteria counts of ETEC in various sections of intestine is lower in the bLF treatment group than in the control group, implying the inhibitory effect of LF on the adherence of ETCE to the intestinal tract (Kawasaki et al. 2000). LF is able to reduce the endotoxemia and bacterial translocation to mesenteric lymph nodes and peritoneal cavity after enteral challenge of *E. coli* (Nebermann et al. 2001).

Our recent studies also revealed that probiotic *Lactobacillus casei* expressing human LF exhibit antibacterial activity on *E. coli* ATCC 25922 both in

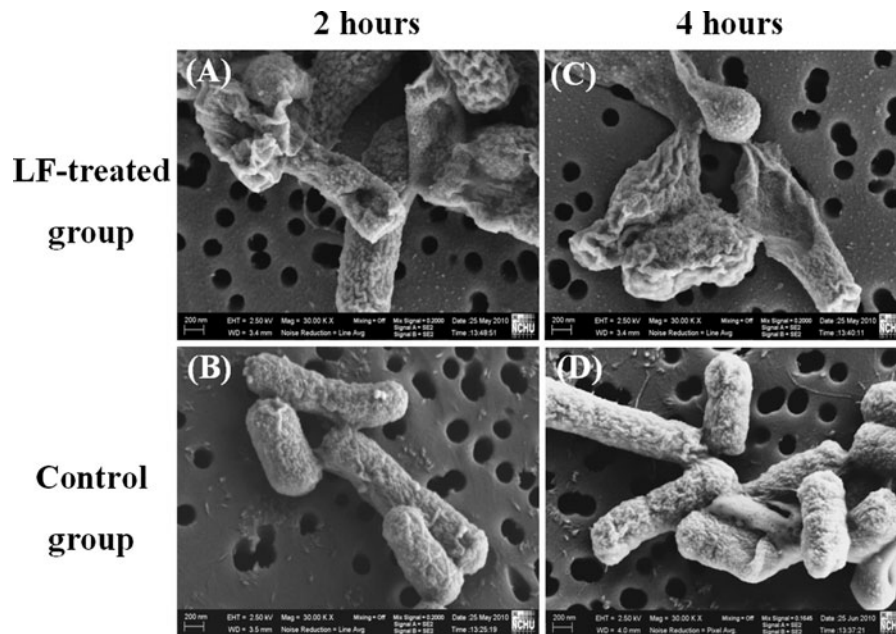


Fig. 1 In vitro antimicrobial activity assays of bovine LF by scanning electron microscopy (SEM). *E. coli* was cultured to logarithmic phase and resuspended 2×10^6 CFU/ml *E. coli* in 1% bacto peptone water (BPW) buffer for further 2 and 4 h incubation at 37°C without adding LF protein as a control group (a, c). In the test group, *E. coli* was cultured in the same

condition but adding with 1 mg/ml LF purified from cow milk for further 2 h (b) or 4 h (d). The samples were observed under 30,000 $\times$ magnifications with the JSM-6300 mode of scanning electron microscope. Data showed that *E. coli* after treated with lactoferrin for 4 h had much serious collapsed membrane phenomenon than treated with 2 h

vitro and in vivo (Chen et al. 2010). In another previous study, we demonstrated increased villous length in the small intestines of mouse pups fed pLF-enriched milk from transgenic females, compared with the length of villi in mice fed wild-type milk (Wu et al. 2007). LF has been demonstrated to enhance the growth of intestinal cells both in vitro and in vivo, which may be mediated by binding to specific LF receptors (Yen et al. 2009). The effect of LF on the intestinal cells may also be one of the mechanisms against enteric infections.

Effects of lactoferrin on extraintestinal *E. coli* infections and sepsis

Effect on UTI, meningitis and systemic infections

In vitro studies have demonstrated that LF or LFCin enhance the activity of antibiotics against *E. coli* strains isolated from cases of human UTI and bovine mastitis (Sanchez and Watts 1999; Longhi et al. 2009). Orally administration of LF or derived peptides is effective in

reducing *E. coli* infection at a remote site such as the urinary tract (Havarsen et al. 2000). Uropathogenic *E. coli* O6:K5 was inoculated into the mice urinary bladder to induce an experimental UTI. The number of bacteria in the urinary tract was significantly reduced by the LF treatment. Urinalysis revealed that hLF was excreted into the urine 2 h after feeding. Intact human LF of maternal origin has been detected in the urine of preterm infants fed human milk (Hutchens et al. 1991). These studies imply that LF may remain intact throughout the gastro intestinal (GI) tract and be absorbed into the blood when the integrity of the GI tract is compromised in some medical conditions (Legrand et al. 2004). Another report demonstrated that oral administration of recombinant hLF protects neonate rats against systemic infection by *E. coli* O18:K1:H7, a strain that can induce meningitis, from a challenge in the gastrointestinal tract. Neonate rats pretreated with hLF have less bacteremia and lower disease severity scores than those not pretreated. The in vitro studies in this report also suggested that hLF may act with other natural peptides, such as lysozyme, or may prime macrophages to kill *E. coli* in vivo (Edde et al. 2001).

Effects on septic shock

Gram-negative sepsis results from overwhelming systemic inflammation that is caused by excessive release of proinflammatory cytokines, which are induced in response to bacterial endotoxin (LPS). A number of immunotherapies, including anti-LPS antibodies and anti-tumor necrosis factor (TNF) antibodies, which block the action of endotoxin, have been proposed as potential therapies for sepsis but have failed in clinical studies (Baumgartner et al. 1985; Fisher et al. 1996; Ziegler et al. 1982). Lactoferrin has been found to have protective effects against septic shock induced by endotoxin or bacterial challenge by *E. coli* (Edde et al. 2001; Lee et al. 1998). Oral administration of LF significantly improves survival in neonatal piglets that are later challenged with intravenous *E. coli* endotoxin. The in vitro studies of the same report also demonstrated that LPS binding to monocytes/macrophages is inhibited by LF in a dose-dependent fashion (Lee et al. 1998). Overall, the proposed mechanisms against *E. coli* of LF are illustrated in Fig. 2.

Clinical studies of antimicrobial activity of lactoferrin

A few studies of bovine LF supplementation have been conducted to determine its effects on fecal flora, hepatitis C, *Helicobacter pylori* and anemia (Kaito et al. 2007; Nappi et al. 2009; Zullo et al. 2007). No evident adverse effect has been reported related to the clinical use of bovine LF. However, studies that address the effect on diarrhea and sepsis caused by *E. coli* are rare.

One multicenter, randomized, double-blind control trial enrolled 472 Italian very low birth weight neonates and revealed that bovine LF supplementation, alone or in combination with probiotic *Lactobacillus rhamnosus* GG reduces the incidence of later-onset sepsis. The antimicrobial effects of LF cover various pathogens, including Gram-negative bacteria, Gram-positive bacteria and invasive fungi (Manzoni et al. 2009). Another randomized, double-blind control study, which enrolled 140 Peruvian children with acute diarrhea and dehydration, revealed that addition of recombinant human LF and lysozyme to an oral rehydration solution reduces the duration and the

relapse of diarrhea. Bacteria, especially *E. coli*, are the predominant pathogens isolated in the stools of the children. This result supports the antimicrobial effects of combined treatment with LF and lysozyme but fails to discriminate the relative potency of LF and lysozyme (Zavaleta et al. 2007). A few clinical studies have demonstrated that LF reduces the severity of vomiting and diarrhea during rotavirus infection, reduces the incidence of lower respiratory tract illness, decreases *Giardia lamblia* colonization, and results in better growth of the affected children (King et al. 2007; Ochoa et al. 2008).

Clinical application

Prevention against *E. coli* infection and nosocomial infection

Based on the studies mentioned above, LF supplementation is a novel strategy for preventing *E. coli* infections, such as EHEC (O157:H7) infection, diarrheal diseases, and nosocomial infections due to *E. coli*. Preventing nosocomial infections and limiting the emergence of antibiotic-resistant strains of pathogens are two important issues in critical care medicine. It has been estimated that 50–60% of the nosocomial infection that occur each year in USA are caused by antibiotic-resistant strains (Jones 2001). Pathogen colonization of the nasopharynx and oropharynx predisposes patients to the development of nosocomial pneumonia, and bacterial overgrowth in the digestive tract may result in bacterial translocation and sepsis. Selective decontamination of the digestive tract (SDD), which aims to eradicate colonization of pathogens from digestive tract, is one of the strategies used to reduce ventilator associated pneumonia and sepsis in critically ill patients (Bonten and Grundmann 2003; de Jonge et al. 2003; Pittet et al. 2001).

In one of our recent studies, we performed pathogen challenges, including pathogenic *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 14053), through the gastrointestinal tract of a transgenic milk-fed mouse animal model. Compared with the control group, the group fed pLF-enriched milk had significant improvement in weight gain; less severity of illness; lower bacterial load in the intestinal tract, blood and liver; healthier microvilli

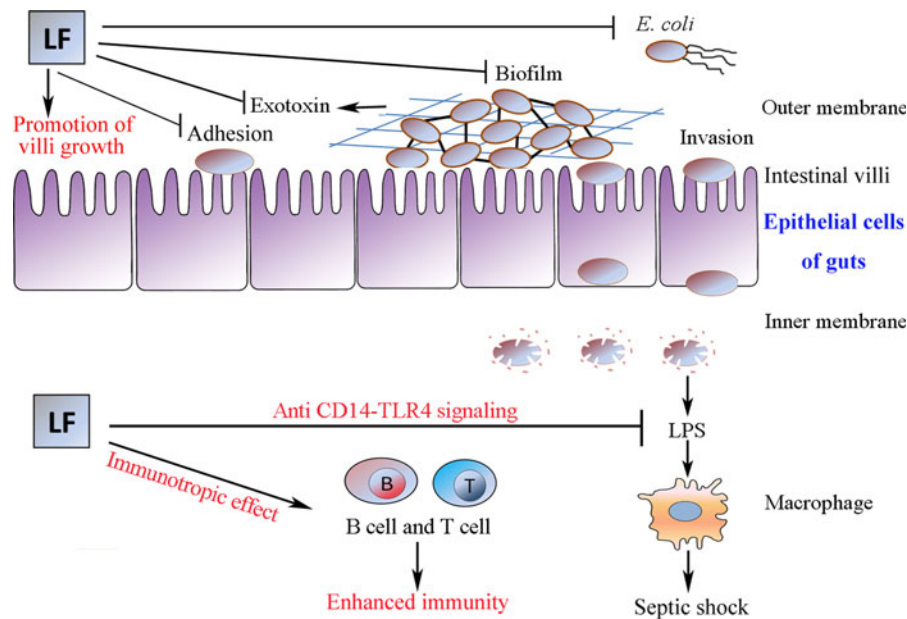


Fig. 2 Pathogenic mechanisms of gastro-intestinal *Escherichia coli* and the multifunctional properties of lactoferrin against *E. coli* infection. LF has the similar effects with bacteriocidal and bacteriostatic agent that can directly destroy free *E. coli*, perhaps even the *E. coli* with the antibiotic ability. Also, LF can avoid the formation of biofilm to reduce the effect of exotoxin after formation of biofilm. LF can inhibit the attachment and invasion of *E. coli* access to the gut cell through block the pathogenic ways of ETEC, EPEC, EIEC and

EHEC. LF has the ability to promote the growth of intestinal villi. After invasion into epithelial of gut cell, LPS was released from fragmented *E. coli*, at the time, LF can block the signaling transduction between LPS and CD14–TLR complex that existed in the macrophage to prevent or treat septic shock. LF can promote the maturation of B and T cells in functional and phenotypic to improve the well immune response. *LPS* lipopolysaccharides; *LF* lactoferrin

in the small intestine; and healthier alveoli in the lungs. Lactoferrin, which is effective against most pathogens while leaving the anaerobic flora undisturbed, could be an ideal regimen for SDD (Yen et al. 2009).

Combination therapy with antibiotics for *E. coli* infection and sepsis

Lactoferrin is considered to be a host defense protein involved in protection against a wide variety of microbial infections including many resistant strains, and in prevention against the systemic inflammatory response induced by endotoxins. LF and LFcin have been used in combination with antibiotics to treat *E. coli* including the resistant strains, *Helicobacter pylori* and hepatitis C infection (Sanchez and Watts 1999; Vorland et al. 1999; Chen et al. 2004b; Kaito et al. 2007; Zullo et al. 2007), and in these studies, adding LF/LFcin has produced positive results. This combination therapy is proposed to be an important

strategy for treating *E. coli* infections, especially those due to resistant strains and those with a fulminant course.

Discovery of many immunosuppressive agents has led to great progress in the treatment of cancers and autoimmune diseases. Oral administration of LF has been found to restore humoral immune response in immunocompromised mice after cyclophosphamide treatment (Artym et al. 2003). LF may be used as an adjuvant therapy aimed at reconstruction of B and T cells or in combination therapy with antibiotics when the immunocompromised patients have infectious diseases.

Limitation of the antimicrobial activity of lactoferrin

An in vitro study showed that recombinant human LF (rhLF) alone did not kill *E. coli*. Conversely, human milk lysozyme slightly reduced the number of *E. coli*, whereas a combination of rhLF and lysozyme had

evident bacteriocidal effect (Edde et al. 2001). A clinical trial in Peruvian children also added rhLF and lysozyme to an oral dehydration solution at the same time (Zavaleta et al. 2007). Our previous study also revealed that the natural immune defense mechanisms, perhaps secretory IgA and lysozyme, can overcome a pathogen challenge from the gastrointestinal tract. LF is one of the natural host defense protein that can act synergistically with other defense peptides or proteins to protect the host against pathogens (Yen et al. 2009). In addition, it is known that pathogens have developed countermeasures to resist the defense peptides/proteins, such as reducing the net negative charge of the bacterial membranes or the development of a capsule polysaccharide (CPS) to avoid the attack of the peptides/proteins (Campos et al. 2004; Llobet et al. 2008). The concept of combination therapy with LF and other natural defense peptides/proteins or therapeutic antibiotics is emerging as a new strategy to prevent or treat bacterial infection.

Conclusion

Lactoferrin is one of the natural host defense proteins that have multiple physiological functions. It has board-spectrum antimicrobial activity against Gram positive bacteria, Gram negative bacteria (including *E. coli*), and even antibiotic-resistant strains. The antimicrobial mechanisms include bacteriostatic, bactericidal, and anti-adhesion effects. It also plays an important role in host immunity with immunotropic and anti-inflammatory effects that protect the host against LPS-induced shock. Based on the growing body of evidence of LF's action against *E. coli*, LF is a novel natural host defense protein with potential utility in the prophylaxis and treatment of *E. coli* infection.

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