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Periodontitis, periodontopathic bacteria and lactoferrin

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Abstract Lactoferrin (LF) is a component of saliva and is suspected to be a defense factor against oral pathogens including *Streptococcus mutans* and *Candida albicans*. Periodontitis is a very common oral disease caused by periodontopathic bacteria. Antimicrobial activities and other biological effects of LF against representative periodontopathic bacteria, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*, have been widely studied. Association of polymorphisms in LF with incidence of aggressive periodontitis and the role of LF in the gingival crevicular fluid as a marker of periodontitis severity have also been reported. Periodontopathic bacteria reside as a biofilm in supragingival and subgingival plaque. Our recent study indicated that LF exhibits antibacterial activity against planktonic forms of *P. gingivalis* and *P. intermedia* at higher concentrations, and furthermore, LF effectively inhibits biofilm formation and reduces the established biofilm of these bacteria at physiological concentrations. A small-scale clinical study indicated that oral administration of bovine LF reduces *P. gingivalis* and *P. intermedia* in the subgingival plaque of chronic periodontitis patients. LF seems to be a biofilm inhibitor of periodontopathic bacteria in vitro and in vivo.

Keywords Lactoferrin · Periodontitis · Periodontopathic bacteria · Plaque · Biofilm

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Introduction

Intake of milk and dairy products has been thought to prevent periodontal disease (Al-Zahrani 2006). Among dairy products, lactic acid foods such as yogurt and lactic acid drinks were the most effective at preventing disease in an epidemiological study (Shimazaki et al. 2008). Related to this, administration of probiotic *Lactobacillus salivarius* was proven to improve periodontal clinical parameters in smokers (Shimauchi et al. 2008) and reduce a periodontopathic bacterium, *Tannerella forsythia*, in subgingival plaque (Mayanagi et al 2009).

Lactoferrin (LF), an 80-kDa iron-binding glycoprotein of the transferrin family, is a component of

exocrine secretions such as milk and saliva, and is present in neutrophil granules (Levay and Viljoen 1995). LF is thought to play a role in innate defense and exhibits a diverse range of biological activities, including antimicrobial activities, antiviral activities, antioxidant activities, immunomodulation, modulation of cell growth, and binding of several bioactive compounds, such as lipopolysaccharide (LPS) (Baveye et al. 1999, Chierici 2001). LF is one of the antimicrobial factors in saliva (Tenovuo 2002). Previously, we studied its effects on the oral pathogen *Candida albicans* (Wakabayashi et al. 1998, Takakura et al. 2003). Moreover, LF has been studied for its antimicrobial activity against periodontopathic bacteria and its relationship with periodontal diseases (Kalmar and Arnold 1988, Aguilera et al. 1998). In inflammatory conditions, LF is also secreted from neutrophils to gingival crevicular fluids (GCF) (Eberhard et al. 2006). LF may be beneficial for the prevention and treatment of periodontal diseases as a food component derived from milk.

Periodontal diseases are generally classified into aggressive periodontitis and chronic periodontitis. In this article, we review previous studies regarding the effects and interactions of LF on the representative periodontopathic bacteria, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*, and the relationship between LF and these diseases (Table 1). We also report our recent studies examining the in vitro anti-biofilm activity of LF against *P. gingivalis* and *P. intermedia*, and a clinical study of LF in patients with chronic periodontitis, especially focusing on periodontopathic bacteria in the subgingival plaque.

Aggressive periodontitis and *A. actinomycetemcomitans*

The most important causative organism in aggressive periodontitis is *A. actinomycetemcomitans*. The first report delineating the effect of LF on periodontopathic bacteria showed killing of *A. actinomycetemcomitans* by human LF (hLF) (Kalmar and Arnold 1988). This killing activity required an unsaturated (iron- and anion-free) LF. Bovine LF (bLF) and hLF inhibit the adhesion of *A. actinomycetemcomitans* and *P. intermedia* to fibroblasts and epithelial cells (Alugupalli and Kalfas 1995). Another report indicated that iron-free apo-LF killed smooth lab strains, but not rough clinical isolates, of *A. actinomycetemcomitans*. On the other hand, increasing the iron saturation of LF resulted in increased inhibition of *A. actinomycetemcomitans* binding to buccal epithelial cells (Fine and Furgang 2002). In relation to this report, they indicated that the level of bound iron and iron-binding capacity in LF is reduced in localized aggressive periodontitis (Fine et al. 2002). This group reported that an hLF variant comprising Lys29/not Arg in the N-terminal region exhibited increased

Table 1 Summary of studies on LF and periodontitis

LF species	LF activities or findings	References
Human	Higher level of LF in GCF of periodontitis patients	Friedman et al. (1983)
Human	Killing of *A. actinomycetemcomitans*	Kalmar and Arnold (1988)
Bovine	Inhibition of *P. intermedia* binding to subepithelial matrix proteins	Alugupalli et al. (1994)
Human and bovine	Inhibition of *A. actinomycetemcomitans* and *P. intermedia* binding to human cells	Alugupalli and Kalfas (1995)
Human	Growth-inhibition of *P. gingivalis*	Aguilera et al. (1998)
Human	Hemoglobin receptor-dependent growth-inhibition of *P. gingivalis*	Shi et al. (2000)
Human	Association of LF SNPs with aggressive periodontitis	Velliyagounder et al. (2003), Jordan et al. (2005)
Human	Occurrence of inflammatory LF peptides in saliva of chronic periodontitis patients	Komine et al. (2007)
Bovine	Reduction of *P. gingivalis* and *P. intermedia* in subgingival plaque of chronic periodontitis patients	Kondo et al. (2008)
Human and bovine	Growth- and biofilm-inhibition of *P. gingivalis* and *P. intermedia*	Wakabayashi et al. (2009)

antibacterial activity against *Streptococcus mutans* and *Streptococcus mitis*, but not *A. actinomycetemcomitans*, and increased transcriptional activation for tracheal antimicrobial peptide mRNA (Velliyagounder et al. 2003). This hLF variant was frequent in patients with localized juvenile periodontitis in the USA (Velliyagounder et al. 2003). The association between this hLF gene's polymorphisms and aggressive periodontitis was also found in Taiwanese patients (Wu et al. 2009). Another hLF variant comprising Ala11/not Thr in the N-terminal region was associated with aggressive periodontitis in African-American patients, but not Caucasian patients (Jordan et al. 2005). The iron-binding capacity and biological activities of the lactoferricin (LFcin) region at the N-terminal of hLF may alter the composition of *A. actinomycetemcomitans* in the microbial flora of the oral cavity and influence the incidence of aggressive periodontitis.

Chronic periodontitis, *P. gingivalis*, and *P. intermedia*

Porphyromonas gingivalis and *Prevotella intermedia* are gram-negative black-pigmenting anaerobes, which are etiologic agents of chronic adult periodontitis (Loesche 1999). hLF was shown to exhibit growth inhibitory activity against *P. gingivalis*, but not *P. intermedia* (Aguilera et al. 1998, Shi et al. 2000) and LFcin B indicated a weak antibacterial activity against *P. gingivalis* (Shi et al. 2000). hLF binds to *P. gingivalis*, *P. intermedia*, and *Prevotella melaninogenica* (Kalfas et al. 1991). hLF binds to the fimbrillin of *P. gingivalis* fimbriae through hLF oligosaccharide (Sojar et al. 1998) and to the hemoglobin receptor protein of this bacterium (Shi et al. 2000). bLF inhibits binding of *P. intermedia* to plasma and subepithelial matrix proteins, fibronectin, collagen type I and type IV, laminin, and fibrinogen, and dissociates the bacterial complexes with these proteins (Alugupalli et al. 1994). hLF and bLF also inhibits adhesion of *P. intermedia* to epithelial cells (Alugupalli and Kalfas 1995). On the other hand, hLF is degraded strongly by *P. gingivalis* and weakly by *P. intermedia* (Alugupalli and Kalfas 1996; de Lillo et al. 1996). A recent study showed that hLF peptides with no anti-*Escherichia coli* activity and reduced iron-chelating capacity are present in the parotid saliva of chronic periodontitis patients (Komine et al. 2007). Although they suggested that the peptides exhibit inflammatory activity and are produced by proteolysis with proteinase 3 co-localizing in the parotid saliva, the protease seems to be of host, but not bacterial. LF is a host defense factor against periodontopathic bacteria, but the protein may be degraded in chronic periodontitis.

LF as a marker of periodontal diseases

Initially, it was reported that hLF levels in GCF of patients with gingivitis, adult periodontitis (chronic periodontitis), and localized juvenile periodontitis (aggressive periodontitis) were similar, but higher than in normal subjects (Friedman et al. 1983). Another study indicated that the hLF level in GCF correlates with the clinical severity of periodontal diseases and the number of polymorphonuclear leucocytes (Adonogianaki et al. 1993). A decrease in hLF levels was observed in GCF and saliva after surgical periodontal treatment in chronic periodontitis (Jentsch et al. 2004), and in GCF and peripheral blood after oral hygiene procedures in experimental gingivitis using healthy volunteers (Ozdemir et al. 2009). hLF is not synthesized in the healthy gingival tissues and elevated hLF levels in the GCF of inflamed tissues originate from invading inflammatory cells (Eberhard et al. 2006). Thus, hLF is released from neutrophils to GCF in response to the inflammatory condition of periodontitis as a potential host defense factor against periodontopathic bacteria and may be a good marker of periodontal diseases.

Inhibition of *P. gingivalis* and *P. intermedia* biofilms by LF

P. gingivalis and *P. intermedia* reside as a biofilm in the subgingival plaque (Davey and Costerton 2006, Marsh 1992). Recently, we have investigated the effect of LF on the planktonic growth and biofilms of *P. gingivalis* and *P. intermedia* (Wakabayashi et al. 2009). It was indicated that hLF, iron-free apo-bLF, and iron-saturated holo-bLF can suppress planktonic growth of *P. gingivalis* and *P. intermedia* over 5 h of incubation. Furthermore, it was found that various iron-bound (apo, native, and holo) forms of bLF and

hLF inhibit biofilm formation of these bacteria even at lower concentrations (≥8 μg/ml). LF alone or in combination with antibiotics used for periodontal disease chemotherapy including ciprofloxacin, clarithromycin, and minocycline also reduced the pre-formed biofilms of these bacteria. These findings suggest a role for LF in the inhibition of periodontopathic bacteria in the oral cavity and its potential usefulness for prevention, treatment, and adjunct therapy of periodontal diseases.

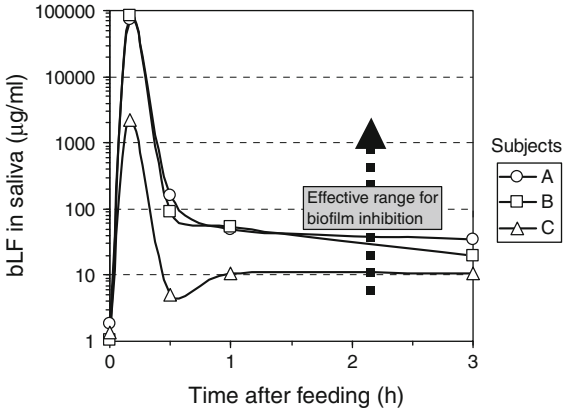

Fig. 1 Kinetics of bLF administered to the oral cavity. A tablet containing 0.3 g of bLF was given and melted in the oral cavity of three volunteers. Unstimulated saliva was collected from immediately before administration to 3 h after administration. The saliva was centrifuged at 3,000 rpm for 3 min and bLF concentration in the supernatant was measured by an automated latex assay. The effective range for biofilm inhibition of *P. gingivalis* and *P. intermedia* (8–2,000 μg/ml) was also indicated in the figure (Wakabayashi et al. 2009)

Clinical study in chronic periodontitis

Before we performed a clinical study in chronic periodontitis patients, kinetics of administered LF in the oral cavity were examined. Troche-type tablets containing 0.3 g of bLF were produced. One tablet was given to three volunteers and melted in the oral cavity. Unstimulated saliva was collected before and after administration, and the bLF concentration in the saliva was measured. As shown in Fig. 1, bLF levels in saliva rose up to 2,000–80,000 μg/ml immediately after feeding. Although the bLF levels fell rapidly, they remained at 10–50 μg/ml up to 3 h after feeding. These bLF levels are within the effective range for biofilm inhibition of periodontopathic bacteria (Wakabayashi et al. 2009). Next, we did a small-scale clinical trial of bLF in patients with mild chronic periodontitis (Kondo et al. 2008). Eight patients were

Table 2 Change in bacterial copy number in subgingival plaque after administration of a placebo or bLF tablet

Bacteria	Change in copy number from the baseline (Log_{10}/paperpoint)		
	1 week	1 month	3 months
Total bacteria			
Placebo group	0.26 ± 0.16	0.49 ± 0.20	0.17 ± 0.21
LF group	0.34 ± 0.20	−0.21 ± 0.14	−0.05 ± 0.20
P value	0.9746	0.0075	0.7024
P. gingivalis			
Placebo group	0.35 ± 0.29	0.21 ± 0.21	1.04 ± 0.39
LF group	−0.12 ± 0.39	−0.57 ± 0.40	−0.20 ± 0.32
P value	0.4754	0.0289	0.0289
P. intermedia			
Placebo group	0.42 ± 0.32	0.43 ± 0.44	0.38 ± 0.42
LF group	−0.65 ± 0.42	−0.62 ± 0.38	−0.46 ± 0.44
P value	0.0233	0.1008	0.1638

A small-scale clinical trial of bLF was conducted in 18 patients with mild chronic periodontitis in a randomized double-blind manner (Kondo et al. 2008). Eight patients were administered tablet containing 0.3 g of bLF and 10 patients were administered a placebo tablet. The patients were given two tablets three times a day for 3 months. Two teeth from each patient were subjected to bacteriological analysis ($n = 20$ for the placebo group and $n = 16$ for the bLF group). Copy number of total bacteria, *P. gingivalis*, and *P. intermedia* in the subgingival plaque was measured by quantitative PCR, and change in copy number from the baseline was assessed. Statistical analysis was done between the two groups with the Mann-Whitney U test

administered tablet containing 0.3 g of bLF and 10 patients were administered a placebo tablet. The patients were given two tablets three times a day for 3 months in a double-blind manner. In the bLF group, a significant reduction was shown in changes in the number of total bacteria (after 1 month), *P. gingivalis* (after 1 and 3 months), and *P. intermedia* (after 1 week) in the subgingival plaque, as compared to the placebo group (Table 2). In addition, a trend towards a reduction in GCF levels of hLF was observed in the bLF group. bLF was also detected in GCF, as well as in saliva, of bLF-administered patients. The bLF may have penetrated into GCF and inhibited or/and reduced the biofilm of the periodontopathic bacteria in the subgingival plaque. This finding suggests that bLF is a good candidate food-component to promote periodontal health.

Conclusions

There appears no doubt from the literature that LF is an important host defense factor against periodontopathic bacteria and may influence the course of periodontal diseases. Like other bacteria, the biofilm form of periodontopathic bacteria exhibits lower susceptibility or resistance to antibiotics (Larsen 2002, Takahashi et al. 2006). Therefore, the inhibitory effects of LF on biofilm development and the established biofilm of periodontopathic bacteria have potential uses in prevention, treatment, and combination therapy of periodontal diseases. In addition, the antibacterial, anti-adhesive, anti-inflammatory, and immunomodulatory activities of LF may have beneficial effects on prevention of periodontal diseases.

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