

Lactoferrin and oral diseases: current status and perspective in periodontitis

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Summary

Lactoferrin (Lf), an iron-binding glycoprotein able to chelate two ferric ions per molecule, is a component of human secretions synthesized by exocrine glands and neutrophils in infection/inflammation sites. Lactoferrin in saliva represents an important defence factor against bacterial injuries including those related to *Streptococcus mutans* and periodontopathic bacteria through its ability to decrease bacterial growth, biofilm development, iron overload, reactive oxygen formation and inflammatory processes.

A growing body of research suggests that inflammatory periodontal disease involves a failure of resolution pathways to restore tissue homeostasis. There is an important distinction between anti-inflammation and resolution; anti-inflammation is pharmacologic intervention in inflammatory pathways, whereas resolution involves biologic pathways restoring inflammatory homeostasis. An appropriate regulation of pro-inflammatory cytokine synthesis might be useful in reducing periodontal tissue destruction. Recently, the multifunctional IL-6 is emerging as an important factor able to modulate bone, iron and inflammatory homeostasis.

Here, we report an overview of Lf functions as well as for the first time Lf anti-inflammatory ability against pe-

riodontitis in in vitro model and observational clinical study. In in vitro model, represented by gingival fibroblasts infected with *Prevotella intermedia*, Lf exerted a potent anti-inflammatory activity. In the observational clinical trial performed through bovine Lf (bLf) topically administered to volunteers suffering from periodontitis, bLf decreased cytokines, including IL-6 in crevicular fluid, edema, bleeding, pocket depth, gingival and plaque index, thus improving clinical attachment levels.

Even if other clinical trials are required, these results provide strong evidence for a instead of an therapeutic potential of this multifunctional natural protein.

Key words: lactoferrin, oral cavity, periodontitis, inflammation.

Introduction

Lactoferrin (Lf), an 80-kDa iron-binding glycoprotein belonging to the transferrin family, is a component of human secretions including saliva, and it is synthesized by exocrine glands and neutrophils in infection and inflammation sites (1). Lf, containing 691 amino acid residues, is folded into two homologous lobes (N-lobe residues 1–333, and C-lobe residues 345–691) connected by a peptide (residues 334–344), which forms a 3-turn α -helix (Fig. 1). This glycoprotein, highly conserved among human, bovine, mouse, and porcine species, is able to reversibly chelate two ferric ions per molecule with high affinity ($K_d \sim 10$ –20 M) retaining ferric iron to pH values as low as 3.0, whereas transferrin retains ferric iron to pH of about 5.5 (1, 2, 3). The iron-binding affinity is high enough that, in the presence of Lf or transferrin, the concentration of free iron in body fluids cannot exceed 10^{-18} M, thus preventing the precipitation of this metal as insoluble hydroxides, inhibiting microbial growth and hindering the formation of reactive oxygen species and related inflammatory processes (1).

Lf concentration in human exocrine secretions is reported in Table 1. The reported concentrations increase in infection and/or inflammation sites due to the recruitment of neutrophils: 10^6 neutrophils secrete 15 μ g of Lf.

Antibacterial activity of lactoferrin related and unrelated to its iron withholding ability

The first function attributed to Lf was antibacterial activity depending on its ability to sequester iron necessary for bacterial growth and survival (5). This action of Lf was considered bacteriostatic, as reversible by the addition of ferric iron (6).

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Figure 1 - Structure of lactoferrin. From: Baker & Baker, 2004 (4).

However, bacterial pathogens are able to overcome iron limitation by means of two principal systems. The first is represented by the synthesis of small chelators, siderophores, which bind ferric ion with high affinity and transport it into bacteria through a specific receptor (7, 8). In addition to the synthesis of siderophores, some highly host-adapted bacterial species as *Porphyromonas gingivalis* acquire iron directly through surface receptors able to specifically bind Lf or other iron binding molecules as hemin and heme (9, 10, 11), and transport it across the outer membrane. The iron is then bound by a periplasmic iron-binding protein, FbpA, and transported into the bacteria via an inner membrane complex comprised of FbpB and FbpC (12).

An iron-independent bactericidal effect of Lf was also described (13). A direct interaction between Lf and lipopolysaccharide (LPS) of Gram-negative or lipoteichoic acid of Gram-positive bacteria is required for the lethal effect (14, 15, 16). Furthermore, it has been demonstrated that the binding of Lf to the lipid A of LPS (17, 18), induces a release of LPS. This bactericidal activity of Lf appears to be located in the N-terminal region as its derivative cationic peptide (residues 17-41), called lactoferricin (Lfcin), is several fold more active than the intact protein (19, 20, 21). More recently, the 1-11 residues in N-terminal region of Lf has shown to possess an antibacterial activity against *Streptococcus mutans* through its binding with bacterial DNA (22). Moreover, the Lf antibacterial activity related to its capability to release LPS can be annulled by high calcium concentration in the culture media (23). As Lf is also able to bind Ca(II) through the carboxylate groups of the sialic acid residues, present on two glycan chains, it cannot be ruled out that the release of LPS from Gram-negative bacteria can be also due to this additional binding property of Lf (23).

Lactoferrin in saliva

Saliva is composed of 98% water and its pH is around 6.64 (24, 25). Saliva composition consists of hormones, peptides, electrolytes, mucus, antibacterial compounds and various enzymes (26). Steroid hormones detectable in saliva comprise cortisol, androgens including testosterone and dehydroepiandrosterone, oestrogens and progesterone as well as aldosterone. Furthermore, saliva is rich in organic constituents as proteins, albumin, urea, uric acid, lactate, and creatinine (26). Inorganic compounds are also present in saliva; Na⁺, K⁺ and Ca²⁺ are the main cations and Cl⁻ and HCO₃⁻ the main anions (26). Furthermore, mar-

Table 1 - Lactoferrin concentration in human secretions.

Biological fluids	Concentration (mg/ml)
Colostrum	8
Milk	1.5-4
Tears	2
Saliva	0.008
Joint fluid	0.001
Vaginal secretion	0,008
Seminal fluid	0.112
Cerebrospinal fluid	Undetectable
Plasma	0.0004

kers of mucosal immunity as immunoglobulins, including IgA, IgM and IgG, α -amylase, lysozyme and Lf are detectable in saliva (27). Evidence is constantly increasing to support the use of saliva as a non-invasive tool for monitoring biomarkers in health and pathological human status (28).

However, it is important to follow some guidelines prior to saliva collection in order to minimize error variance and chances of methodological errors (29, 30). Food or drink intake must be avoided at least 2 h prior to sampling, due to variations in saliva secretion which in turn will negatively affect the results. Food or drink high in sugar content, caffeine or acidity can stimulate saliva flow, and acidity will lower mouth pH levels, both compromising antibody-antigen binding and enzyme activity thus leading to invalid immunoassay results. In addition, alcohol consumption 24 h prior to sampling should be avoided as it may cause increased saliva secretion (29, 30, 31).

Among salivary proteins, Lf is the most important factor of natural immunity. Its concentration corresponds to 1.23 mg/l in gingival crevicular fluids (GCF) and to 8.96 and 7.11 mg/l in unstimulated and stimulated saliva, respectively. The Lf concentration in oral cavity is related to different fluid samples to be assayed. In fact, unstimulated saliva mainly derives from 70% submandibular, 20% parotid, and 2% sublingual glands, while stimulated saliva mainly derives from 30% submandibular and 60% parotid glands (28). Moreover, the concentration of this glycoprotein in GCF is influenced by the amount of the excreted fluid which, in turn, depends from physiological or pathological status of the subject. The Lf concentration, measured /site, clearly shows that its levels increase from 36 ng/site in healthy humans to 63 and 90 ng/site in gingival and periodontal diseases, while its concentration calculated /ml is similar in health and pathological conditions (32).

Even if the detection of Lf as well as of other proteins in saliva, easily accessible source of potential local and systemic biomarkers of health and pathological status, the different amount of salivary flux in calculating the real concentration of the proteins must be considered.

This is a very critical point which can explain the conflicting data reported by different Authors on Lf oral concentration. Of note, several defence proteins (e.g. lysozyme, Lf and histatin-1) were found significantly more abundant in old (55–65 years old) than in young female (20–30 years old) subjects (33). These results, demonstrating that Lf synthesis in the saliva of females is age dependent, are in sharp contrast with the well ascertained data showing

that Lf synthesis is under the control of steroid hormones (34) and consequently, it decreases in menopausal and postmenopausal periods.

Summarizing, the changes of the Lf concentration depend not only from significant changes of saliva samples (unstimulated, stimulated) but they are also age-dependent. It was reported that Lf levels in GCF of patients with gingivitis, adult periodontitis (chronic periodontitis), and localized juvenile periodontitis (aggressive periodontitis) are similar, but higher than in normal subjects (32, 35, 36). Another study indicates that Lf level in GCF correlates with the clinical severity of periodontal diseases and the number of polymorphonuclear leucocytes (37). A decrease in Lf levels was observed in GCF and saliva after surgical periodontal treatment in chronic periodontitis (38). Similar Lf decrease in GCF and peripheral blood after oral hygiene procedures in experimental gingivitis using healthy volunteers was also detected (39). Lf is not synthesized in the healthy gingival tissues and elevated Lf levels in the GCF of inflamed tissues originate from invading inflammatory cells (40). Thus, Lf is released from neutrophils in GCF in response to the inflammatory condition of periodontitis as a potential host defence factor against periodontopathic bacteria and may be a good marker of periodontal diseases.

Influence of iron and lactoferrin on the lifestyle of oral microbiota

In human saliva, the iron content ranges from 0.1 to 1.0 μM depending on meals but it can increase for gingival bleeding due to infection and inflammatory processes. During the infection and inflammatory processes, the recruitment of neutrophils increases saliva Lf concentration from 20 to 60 $\mu\text{g/ml}$ (41).

Therefore, saliva represents an interesting model to investigate the influence of iron and Lf concentrations on bacterial infections. As matter of fact, the different ratio between iron and Lf plays an important role in the lifestyle of several bacteria (42, 43) by inducing aggregation and biofilm development (41). In particular, in a saliva pool well defined for iron and Lf content, apo-Lf (iron unsaturated form) was found to enhance *S. mutans* aggregates and biofilm formation, whereas iron-saturated Lf decreased aggregation and biofilm development (41). Similar behaviour has been recently described in the periodontopathogen *Aggregatibacter (Actinobacillus) actinomycetemcomitans*: iron limitation up-regulates its biofilm genes contributing to biofilm formation (44).

The reported data suggest that to assess the effect of Lf in oral cavity it is necessary to evaluate preliminarily the iron content of saliva. In fact, in periodontitis patients, the high iron concentration and the presence of hemin, which can form complexes with Lf, together with Lf degradation by bacterial and human enzymes (45, 46), could be responsible, in vivo, for the lack or reduced activity of Lf even if its concentration is increased following infection and inflammation.

Inhibition of bacterial adhesion on abiotic and cell surfaces by lactoferrin

Abiotic surfaces

Microbial adhesion and subsequent colonization, resulting in biofilm formation on abiotic surfaces such as dental surfaces and medical devices as dental prosthesis, represents

both a physiological process and a serious problem that can lead to oral illness. Efforts to control microbial adhesion by anti-adhesive new materials or compounds have had modest success once applied to the patient. Consequently, it could be very helpful to discover other compounds able to hinder microbial adhesion. The ability of Lf, in both apo- and iron-saturated form, to inhibit the adhesion of *S. mutans* to hydroxyapatite (HA), mimicking tooth surface (47), may represent an interesting function. The demonstration that Lf inhibits the adhesion of *S. mutans* to a salivary film and HA through residues 473–538 of its C-lobe (48), further helped to understand this activity, which is unrelated to its iron binding properties. Both apo- and iron-saturated bLf also inhibit adhesion of free and aggregated *S. mutans* cells to a dental polymer when Lfs were pre-coated to dental polymer or bound to both dental polymer and bacterial cells (41). Apo-Lf but not iron-saturated Lf also inhibits the attachment on HA of *Prevotella nigrescens* by binding to both HA and bacteria (49). Apo-Lf reduces the initial attachment of the commensal *Streptococcus gordonii* by iron sequestration, but not that of periodontopathogens *Fusobacterium nucleatum* and *P. gingivalis*. Interestingly, the initial attachment of mixed populations of *S. gordonii*/*F. nucleatum* and *S. gordonii*/*P. gingivalis* is significantly reduced in the presence respect to that observed in the absence of Lf (50). In other studies, Lf has been shown to inhibit the adhesion of *A. actinomycetemcomitans* and *P. intermedia* to reconstituted basement membrane, through ionic binding, and *P. intermedia* to bacterial adhesins by a specific binding of Lf (51).

The different nature of abiotic surfaces, the varying microbial adhesion mechanisms and the different in vitro experimental conditions could explain the different results obtained for inhibition of bacterial adhesion by apo- or iron-saturated Lf, which in some cases requires only ionic binding to biomaterials, and in others specific binding to bacterial structures, or both.

Cell surfaces

The ability of microbes to adhere, colonize and form biofilm on host cells is a crucial step for the development and persistence of infections.

A large number of Gram-positive and Gram-negative bacteria possess specific adhesins that mediate the adhesion process on epithelial host cells. Lf and Lfcin are all able to bind to bacterial Gram-negative and Gram-positive surfaces, as well as to host cells, by binding to glycosaminoglycans (GAGs) (52) and in particular to heparan sulphate (HS) (53) and the inhibition of bacterial adhesion seems generally to be mediated by Lf binding to both bacterial and host cell surfaces (1).

Evidences of anti-adhesive function of Lf against oral periodontopathogens was shown by Alugupally (1997) (51) who demonstrated that the Lf-dependent inhibition of the adhesion of *A. actinomycetemcomitans*, *P. intermedia* and *P. nigrescens* to fibroblasts can involve binding of lactoferrin to both the bacteria and host cells.

Recently, the Lf influence on *S. mutans* adhesion has been tested in vitro on epithelial cells. Lf at physiological concentration (20 $\mu\text{g/ml}$) is able to significantly decrease the adhesion efficiency of *S. mutans* to epithelial cells (Fig. 2).

Inhibition of microbial invasion of host cells by lactoferrin

Some mucosal pathogenic bacteria are capable not only

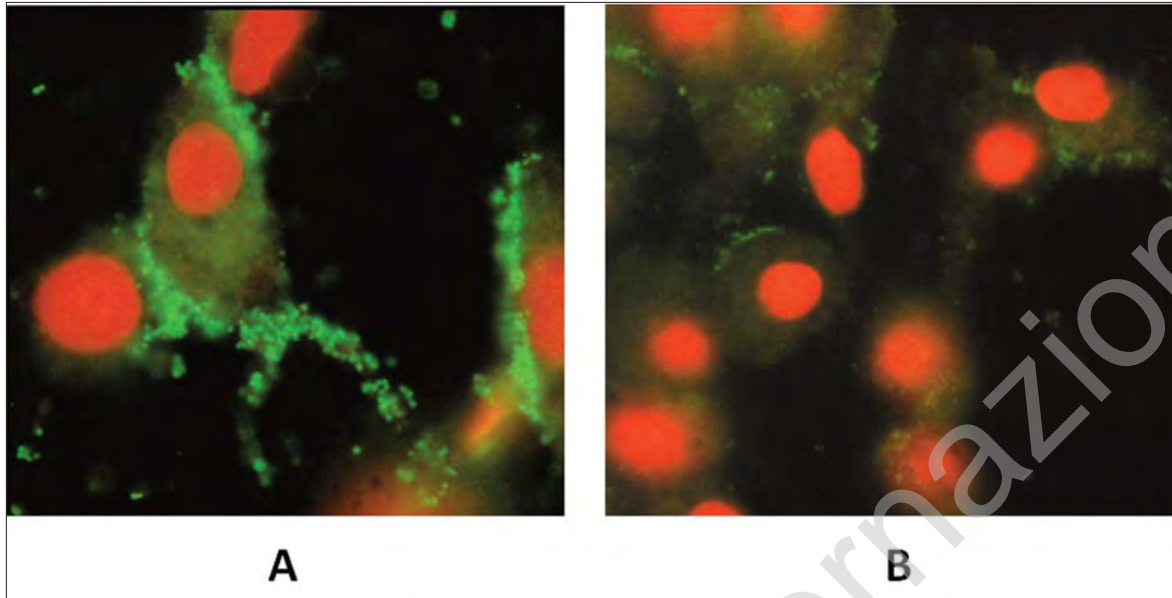


Figure 2 - Bovine lactoferrin in inhibiting adhesion efficiency of *Streptococcus mutans* on epithelial cells.
 Legend: Epifluorescence optical microscopy images of epithelial cells infected with *Streptococcus mutans*, stained with BacLight®LIVE/DEAD viability probe, in the absence (Panel A) or in the presence (Panel B) of bovine lactoferrin (20 µg/ml).

of adhering, but also of entering into non-professional phagocytes, such as epithelial cells. Inside host cells, bacteria are in a protective niche in which they can replicate and persist, thus avoiding host defences. Virulence determinants, such as surface proteins able to bind host cells, play a key role in the entry process inside the host cells. Lf has been shown to inhibit the entry of facultative intracellular bacteria, both Gram-negative and Gram-positive (1). Recently, the capability of *S. mutans* to enter inside gingival fibroblast cells has been reported (54). Preliminary experiments show the anti-invasive activity of Lf against *S. mutans* infecting gingival fibroblasts.

Anti-inflammatory activity of lactoferrin in oral diseases

Understanding the role of microbial communities in human health is emerging as a fundamental and fascinating microbiological challenge. In the battle against infectious diseases, finally there is the awareness that the discovery of novel connections between infection, inflammation and human diseases is a pivotal tool in the research of novel antibacterial drugs.

The increasingly frequent threats of bacterial resistance to antibiotics reinforce the necessity to understand the me-

chanisms that underlie pathogenicity and the interactions between pathogenic and non-pathogenic microbes co-existing on humans. These motivations are especially pertinent in the case of the human oral flora, which comprises at least 400 to 700 different bacterial species (55). Oral microorganisms constitute a complex and dynamical community, responsible for two important oral infectious diseases affecting virtually all humans: gingival and periodontal diseases. Differently from gingivitis, confined to gingival mucosa, periodontitis involves periodontium degeneration, alveolar bone resorption and gingival epithelium migration along tooth surface, and the resulting periodontal pockets.

It is well known that periodontal disease is associated to an inflammatory process that occurs in the tissues surrounding the teeth in response to the accumulation of subgingival bacterial plaque, mainly constituted by anaerobic Gram-negative facultative intracellular pathogens and their LPS, one of the factors responsible for the inflammatory reaction (56, 57, 58). Differently from commensal bacteria, intracellular bacteria induce the over-expression of several pro-inflammatory cytokines (59, 60, 61). In particular, increased levels of pro-inflammatory cytokines have

Table 2 - Synthesis of pro-inflammatory cytokines by gingival fibroblasts uninfected or infected with *Prevotella intermedia* in the absence or in the presence of bovine lactoferrin.

	Uninfected cells		Infected cells	
	None	bLf	None	bLf
IL-1β (ng/ml)	1.050±150	1.000 ± 97	4.800±421	900±180
IL-6 (ng/ml)	90±21	90±23	930±105	83±38
IL-8 (pg/ml)	2.100±241	2.150±210	9.450±750	5.800±550
TNF-α (pg/ml)	22±14	22±12	120±65	18±8

Table 3 - Clinical parameters and pro-inflammatory cytokine levels in gingival crevicular fluids before and after bovine lactoferrin topical administration.

	Baseline	After 4 weeks of topical administration of bovine lactoferrin
PPD	2.6±0.2	0.5±0.8
GI	0.8 ±0.1	0.5±0.1
PII	0.8 ±0.1	0.4±0.2
BOP (%)	32	0
CAL (mm)	1.50±0.7	0.50 ±0.2
IL-1β (ng/ml)	138±87	95 ±74
IL-6 (ng/ml)	1.35±0.8	0.64±0.32
IL-8 (pg/ml)	94±65	35±12
TNF-α (pg/ml)	31±21	3±2

Legend: The clinical parameters and pro-inflammatory cytokine concentrations are reported as mean values ± standard deviation deriving from a total of twenty-six teeth (2 teeth/subject). PPD: probing pocket depth; GI: gingival index; PII: plaque index; BOP: bleeding on probing; CAL: clinical attachment level.

been detected in the inflamed gingival tissue and GCF at infected and inflamed sites (62, 63, 64).

Although inflammation is an essential component of the host defence response to bacterial challenge, excessive cytokine production from host cells results in consequent periodontal tissue destruction. Therefore, chronic and progressive infection by anaerobic Gram-negative intracellular pathogens inducing the inflammatory process is responsible for alveolar bone destruction. In fact, the bone is a continuous remodelling between osteoblast (bone formation) and osteoclast (bone resorption), and its homeostasis is well controlled. Among soluble regulation factors influencing bone homeostasis, cytokines play an important role. It is well demonstrated that the pro-inflammatory cytokines having bone-resorption activity, act osteoblasts and stimulate osteoclastogenesis (i.e. bone resorption) through up-regulation of receptor activator of nuclear factor (NFκB) ligand (RANKL) and down-regulation of osteoprotegerin in osteoblasts (65, 66, 67).

Taken together these data reinforce the evidence that appropriate regulation of cytokine production by immunomodulators might be useful in reducing periodontal tissue destruction such as alveolar bone resorption. There is an important distinction between anti-inflammation and resolution; anti-inflammation is pharmacologic intervention in inflammatory pathways, whereas resolution involves biologic pathways restoring inflammatory homeostasis. Interestingly, the resolution of inflammation is an active, well-orchestrated return of tissue homeostasis. A growing body of research suggests that inflammatory periodontal disease involves a failure of resolution pathways to restore homeostasis.

At present, there is no ideal therapeutic approach to cure periodontitis and achieve an optimal periodontal tissue regeneration.

Recently, the milk derivative bovine Lf (bLf) is emerging as an important regulator of iron and inflammatory homeostasis exerting a potent effect in decreasing inflammatory host responses. The bLf ability in decreasing inflammatory processes is related to its ability to bound po-

tentially toxic-free iron (68) as well as to sequester LPS thus neutralizing its biological activity (69, 70). Moreover, bLf is also able to inhibit biofilm development (42, 43) thus decreasing inflammation due to the exopolysaccharides forming biofilm (71).

Recently, in several clinical trials on women suffering from anaemia, oral administration bLf has been found to be effective in decreasing serum levels of IL-6, a key factor of iron homeostasis, thus restoring the physiological distribution of iron between tissues and blood (72, 73, 74). As matter of fact, the iron deficiency in blood (iron deficiency anaemia) is related to iron overload in tissues as well as to high levels of serum IL-6 (73).

In addition to the local tooth and gum effects, periodontal disease has been shown to influence various systemic disorders and diseases as cardiovascular disease (75), and diabetes mellitus (76). Moreover, significant associations between periodontal disease and preterm low birth weight have been demonstrated (77). Recently, the prevention of preterm delivery, mainly associated to high levels of IL-6, has been obtained through bLf ability to decrease serum and cervicovaginal IL-6 (78).

As there is not ideal therapeutic approach to cure periodontitis, the anti-inflammatory efficacy of oral administration of bLf encourages new therapeutically approaches to cure periodontal diseases.

In vitro and in vivo influence of bovine lactoferrin on pro-inflammatory cytokine synthesis

As reported in the previous section, IL-6 is involved not only in bone resorption but also in iron homeostasis, where its over-expression induces iron overload in tissues and secretions and iron deficiency in blood (79). Consequently, we believe that the difficulty in resolving inflammatory periodontal disease also involves the failure of restoring iron homeostasis strictly related to inflammation through the over-expression of IL-6 (79). Therefore our opinion is that the resolution of inflammation in the context of periodontal disease should involve the decrease of iron overload in oral tissues and secretions.

In physiological conditions the availability of free iron in tissues and secretions do not exceed 10^{-18} M, while in pathological conditions the high concentration of free iron induces microbial multiplication, biofilm development, reactive oxygen species, cell damage and inflammation (1).

A strategy addressed to decrease iron overload could contribute to inhibit microbial growth and biofilm development as well as formation of reactive oxygen species, cell damage and related inflammation.

Here, we first report the anti-inflammatory activity of added bLf to infected gingival fibroblasts.

To mimic the in vivo environment, the synthesis of interleukin 1 (IL-1), IL-6, interleukin 8 (IL-8) and tumor necrosis factor-α (TNF-α) by human gingival fibroblasts infected or not with *P. intermedia*, an anaerobic Gram-negative intracellular pathogen associated to periodontitis, has been detected. The infection of monolayers was performed in the absence or presence of bLf at 20 µg/ml concentration corresponding to that detected in saliva of healthy subjects and found ineffective against *P. intermedia* growth. The absence of antibacterial activity of bLf in our in vitro model is pivotal to exclude the different cytokine synthesis related to the different number of viable bacteria. For this purpose semi-confluent monolayers were infected with *P. intermedia* at a multiplicity of infection 100 bacteria per cell

in the absence or in the presence of 20 µg/ml of bLf. After infection, the supernatants from uninfected and infected cells with or without bLf were collected and IL-1, IL-6, IL-8 and TNF-α concentrations were determined using standard enzyme-linked immunosorbent assays (ELISA) (Table 2).

The results clearly demonstrated that bLf added to uninfected monolayers did not modify cytokine synthesis, while it significantly decreased the synthesis of IL-1, IL-6, IL-8 and TNF-α by gingival fibroblasts infected with *P. intermedia*.

These *in vitro* results have encouraged us to carry out an observational preclinical study on volunteers suffering from mild chronic periodontitis. The calibration was performed before the study with 5 volunteer subjects among the researchers involved in the study of bLf functions. Then other volunteers suffering from mild chronic periodontitis among patients were enrolled at a private practice. Pregnant women as well as the subjects with other concomitant diseases were excluded from this study as well as smokers, subjects with ascertained allergic reactions to cow's milk, and subjects receiving antibiotic treatment within the previous 3 months.

As a result, 13 subjects (7 female and 6 men, age range 42–63 years) suffering from mild chronic periodontitis were enrolled in this observational preclinical trial after the understanding and written consent. The trial was conducted

according to the World Medical Association Declaration of Helsinki.

At each visit, GCFs to be analyzed were collected from two teeth affected by periodontitis/subject. All subjects were clinically evaluated in the following periodontal measurements: number of teeth present, plaque index (PII) (80), gingival index (GI) (81), probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP) (82). Two teeth affected by periodontitis were examined per subject. PII, GI, PD, CAL and BOP (%) were measured at four sites around each tooth.

All enrolled subjects were directed to topically place 100 mg of lyophilized bLf two times a day for 4 weeks after meal and correct oral hygiene as well as after extensive washings until the arrest of the putative gum bleeding. In particular, 100 mg of lyophilized bLf, contained in a pocket, were deposited in a little container in order to be adsorbed on soft bristles of the appropriate toothbrush and applied through a soft brush on the gums, particularly on the bleeding sites.

Periodontal clinical parameters consisting in PPD, GI, PII, BOP (%) and cytokine concentrations in GCFs were assayed in all subjects before (baseline) and after 1, 2 and 4 weeks of therapy. The parameters at the baseline and after 4 weeks of bLf topical treatment are reported as cumulated mean values because the total number of subjects in each group (seven women and six men) is very low (Ta-

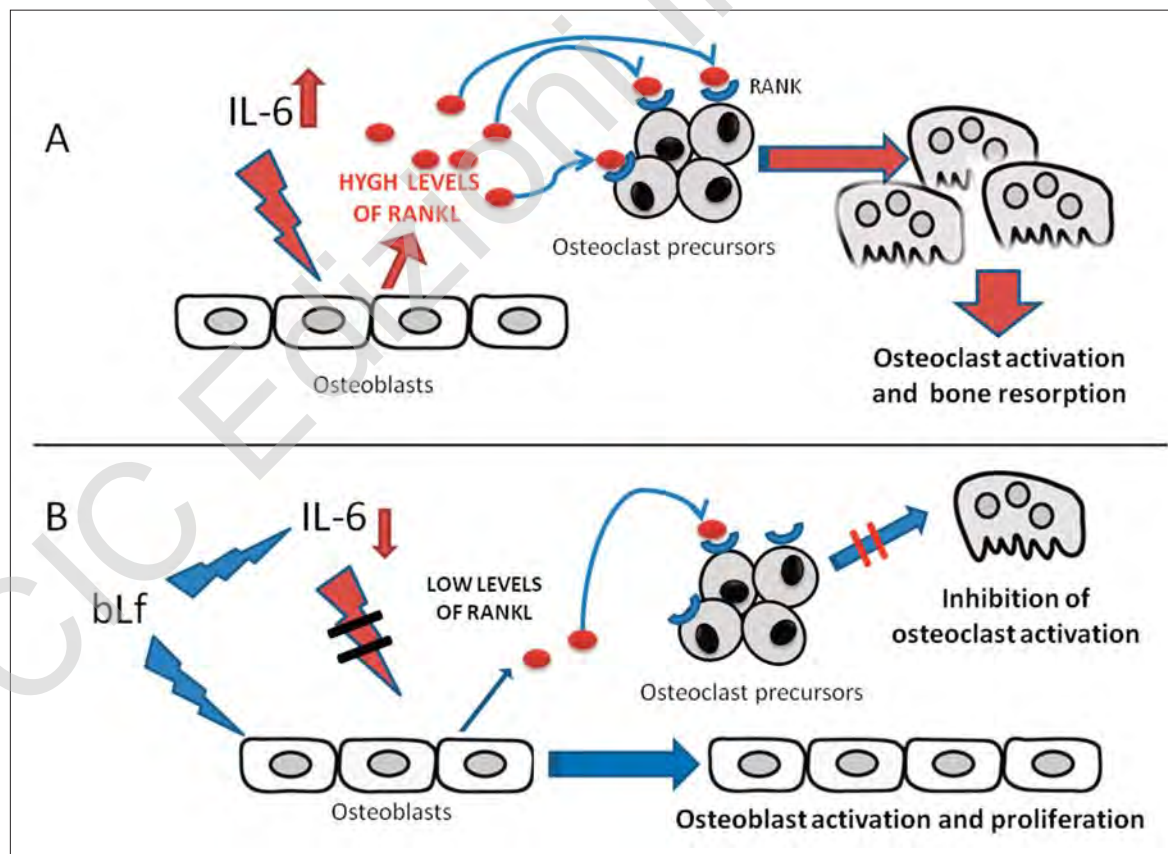


Figure 3 - Putative lactoferrin mechanism in inhibiting bone resorption. High levels of IL-6 induce bone resorption through the osteoclast activation induced by the osteoblast-mediated production of RANKL (Panel A). The topical administration of bLf by decreasing IL-6 levels and probably RANKL may induce both the inhibition of osteoclast activation and the activation and proliferation of osteoblast (Panel B). Legend: IL-6: interleukin-6; bLf: bovine lactoferrin; RANK: receptor activator of nuclear factor NFκB; RANKL: RANK ligand.

ble 3). Therefore, the data summarized in Table 3 correspond to the mean values deriving from a total of 13 subjects and 26 teeth.

From the data reported in Table 3, it can be observed that bLf topical administration decreased the concentrations of pro-inflammatory cytokines in GCF, thus decreasing oedema and inflammation. Moreover, after four weeks of topical bLf therapy, the absence of bleeding, the reduction of pocket depth, gingival and plaque index as well as an improvement of clinical attachment level were observed. Interestingly, no adverse events were reported in the subjects receiving topical administration of bLf.

In conclusion, Lf is a component of saliva and an important defence factor against bacterial injuries (1) including those related to *S. mutans* (54) and to other representative periodontopathic bacteria as *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia* (83). Periodontopathic bacteria reside as a biofilm in supragingival and subgingival plaque and Lf is able to reduce their biofilm development (84).

Even if in a small-scale clinical study, oral administration of bLf has been found to reduce *P. gingivalis* and *P. intermedia* in the subgingival plaque of chronic periodontitis patients thus suggesting that the inhibitory effects of Lf on biofilm development of periodontopathic bacteria can have a potential use in prevention, treatment of periodontal diseases (83).

However, it cannot be undervalued the importance of the inflammatory process associated with periodontal disease. Consequently, an appropriate regulation of pro-inflammatory cytokine synthesis might be useful in reducing periodontal tissue destruction. Recently, among pro-inflammatory cytokines, the multifunctional IL-6 is emerging as an important factor able to modulate bone, iron and inflammatory homeostasis (79, 67).

At present, there is no ideal therapeutic approach to cure periodontitis and achieve optimal periodontal tissue regeneration.

Here, firstly we report that the topical administration of bLf is able to reduced pro-inflammatory cytokines in GCF thus contemporary decreasing oedema and inflammation, bleeding, pocket depth, gingival and plaque index. This potent effect of bLf topically administered results in an important improvement of clinical attachment level, probably due to the ability of bLf to decrease the synthesis of IL-6 and RANKL thus resulting in the inhibition of bone resorption (Figure 3).

Even if other clinical trials are required, these results provide strong evidence for a role of bLf in curing periodontitis, thus extending the therapeutic potential of this multifunctional natural protein.

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