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The interplay of lipopolysaccharide-binding protein and cytokines in periodontal health and disease

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Abstract

Aim: Periodontal pathogenesis is characterized by Gram-negative bacteria activation of series of pro- and anti-inflammatory cytokines from host cells through the pathway of lipopolysaccharide (LPS), LPS-binding protein (LBP) and CD14. The present study investigated the expression profiles of interleukin (IL)-1β and IL-10 in periodontal health and disease, and examined the effects of *Escherichia coli* LPS and LBP interaction on the expression of IL-1β and IL-10 by human gingival fibroblasts (HGF).

Material and Methods: Gingival biopsies were collected from 44 subjects with chronic periodontitis and 15 periodontally healthy subjects. The expression of IL-1β and IL-10 was detected by immunohistochemistry. The mRNA expression of IL-1β and IL-10 in HGF was detected by RT-PCR with or without recombinant human LBP (rhLBP), while the peptides were analysed by an enzyme-linked immunosorbent assay.

Results: IL-1β was detected in both oral sulcular epithelia of healthy controls and periodontal pocket epithelia of patients. IL-10 was mainly expressed in the intercellular spaces of connective tissues. IL-1β displayed a reverse pattern of expression levels with reference to IL-10, and a negative correlation existed between LBP and the ratio of IL-1β/IL-10. rhLBP suppressed *E. coli* LPS-induced IL-1β expression by HGF.

Conclusion: An appropriate interplay of LBP and cytokines may have a beneficial effect on innate host defence, thereby contributing to periodontal homeostasis.

Key words: human gingival fibroblasts; interleukin-10; interleukin-1β; lipopolysaccharide; lipopolysaccharide-binding protein; periodontal pockets

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Periodontal disease is one of the most common infectious diseases in humans and it is characterized by inflammatory destruction of tooth-supporting hard and soft tissues, eventually resulting in loss of the teeth involved. The lipopolysaccharide (LPS)-mediated host responses appear to account for causing certain injury in periodontal disease. LPS acts as a potent stimulus to a variety of host cells, which subsequently results in the expression of pro-inflammatory cytokines and amplifies the related host immune response in periodontal diseases (Ren et al. 2005a). To prevent exaggerated responses to LPS, the host has evolved numerous LPS control mechanisms that exert their effects by intracellular processes, such as anti-inflammatory cytokines, or by extracellular processes, such as inhibitory LPS-binding proteins (LBP) (Thompson & Kitchens 2006).

LBP is a 60-kDa glycosylated protein synthesized by hepatocytes and it is released into the bloodstream upon acute-phase stimulation (Schumann et al. 1996). Its normal concentration in human serum ranges from 2 to 20 μg/ml and it can increase dramatically under the stimulation of inflammation. Extensive studies have found that LPS activation of the immuno-inflammatory response is modulated by LBP. It could

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activate various second-messenger systems through CD14–TLR4 pathways, resulting in the cytokine production from human gingival epithelial cells and human gingival fibroblasts (HGF) (Asai et al. 2001, Wang & Ohura 2002). These studies indicated that LBP may be involved in the initiation and development of periodontal disease. Further investigation is therefore needed to elaborate the potential relationship between LBP and LPS interactions and cytokines' production in periodontal pathogenesis.

Numerous studies have demonstrated that cytokine networks, composing of both pro- [e.g. IL-1β and tumour necrosis factor-α (TNF-α)] and anti-inflammatory cytokines (e.g. IL-10), play an important role in the initiation and progression of periodontal disease (Salvi & Lang 2005). IL-1 occurs in two forms as IL-1α and IL-1β, of which IL-1β has been particularly studied as a critical determinant of periodontal tissue destruction due to its pro-inflammatory and bone-resorptive properties (Taubmann & Kawai 2001). It was reported that IL-1β could stimulate fibroblasts to produce collagenase and lead to the destruction of connective tissues (Offenbacher 1996). Furthermore, significantly higher levels of IL-1β were found within the gingival crevicular fluid (GCF) of periodontitis patients, compared with those with healthy or gingivitis conditions (Feldner et al. 1994). This evidence indicates that IL-1β is one of the major stimulators of tissue breakdown in periodontal disease.

IL-10, an anti-inflammatory cytokine, plays a major role in suppressing immune and inflammatory responses (Al-Rasheed et al. 2003). It is mainly produced by human activated $CD8^+$ T cells, T-helper type 0 (Th0), Th1 and Th2-like $CD4^+$ T-cell, B-cells and LPS-activated monocytes. The expression of pro-inflammatory cytokines, including IL-1 and TNF-α, is inhibited by IL-10 (Wang et al. 1994). IL-10 in inflamed gingival tissues potentiated a local autoimmune response characterized by increasing the numbers of anti-collagen secreting cells (Gemmell et al. 1997). An in vivo study showed that IL-10 mRNA expression decreased at sites where *Actinobacillus actinomycetemcomitans* was detected (Hirose et al. 2001). Thus,the concept that IL-10 may be of importance in the control of periodontal disease progression has emerged from these studies (Gemmell et al. 1997).

In the past decades, the interactions of LBP and cytokines in infectious diseases were extensively documented. An in vitro study demonstrated that human intestinal epithelial cells could express LBP in response to IL-1β, IL-6 and TNF-α instead of LPS even in the absence of CD14 (Vreugdenhil et al. 1999). The level of LBP was controlled by transcriptional activation of the LBP gene, which was mediated by IL-1 and IL-6 released by macrophages (Kirschning et al. 1997). It was also reported that LBP-treated mouse macrophages could induce rapid expression of TNF and IL-1β by increasing cytokine mRNA stability (Mathison et al. 1992). In contrast, in the serum of patients with severe sepsis, high concentrations of LBP can markedly inhibit TNF-α production in response to LPS (Zweigner et al. 2001). To date, no study has been performed to reveal the interplay of LBP and cytokines in different stages of periodontal disease. In our previous studies, we demonstrated for the first time the local expression of LBP by human gingival epithelial cells (Ren et al. 2004) and that LBP could significantly down-regulate the production of IL-6 by HGF (Ren et al. 2005b). The present study further investigated the interrelationship between LBP and cytokine expression in periodontal health and disease as well as the potential modulatory effects of LBP on LPS-induced cytokine expression.

Material and Methods

Subjects

Forty-four Chinese adults with a mean age of 48.5 ± 8.9 years (22–65 years) were recruited into the study. Among them, 20 were smokers and 24 were non-smokers. Inclusion criteria were (1) presentation of untreated advanced chronic periodontitis, with probing depth (PD) $\geqslant 5.0$ mm, clinical attachment loss (AL) $\geqslant 3.0$ mm and radiographic evidence of alveolar bone loss on at least two teeth per quadrant, excluding the third molars; (2) healthy systemic condition; (3) no prior periodontal treatment; (4) no use of any immunosuppressive agents; (5) no antibiotics or anti-inflammatory drugs taken within the preceding 6 months. All the subjects were examined at a screening session to check their eligibility for the study. Then they received a baseline examination and a course of non-surgical periodontal therapy including oral hygiene instructions, scaling and root planing, and follow-up monitoring of treatment responses for at least 6 months with routine prophylaxis at an interval of 3 months. At the subsequent re-examination, all subjects exhibited unresolved periodontitis with remaining PD $\geqslant 6.0$ mm and bleeding on probing (BOP) in at least one quadrant of their dentitions and they required periodontal surgery.

Fifteen systemically and periodontally healthy subjects with a mean age of 23.4 ± 3.6 years who required tooth extraction for orthodontic treatment purposes were selected as control subjects. They were all non-smokers. Inclusion criteria were (1) systemically healthy condition; (2) no sites with PD > 4 mm or AL > 1 mm in the whole dentition; (3) no radiographic evidence of periodontal bone loss after a full-mouth radiographic examination; (4) a full-mouth score of BOP $< 15\%$ of sites; (5) no antibiotics or anti-inflammatory drugs taken within the preceding 6 months; and (6) no use of any immunosuppressive agents. The purposes and procedures of the study were explained and informed consents were obtained from all recruits, and the study protocol was approved by the Ethics Committee, Faculty of Dentistry, the University of Hong Kong.

Site selection and sampling

All patients were screened for their eligibility and selection of sampling sites, before collection of samples. They were subsequently re-examined, before periodontal surgery. Gingival biopsies were collected during periodontal surgery in unresolved periodontitis sites with PD $\geqslant 6$ mm and significant loss of alveolar bone following non-surgical treatment, consisting of (1) periodontal pocket tissues (PoTs), and (2) when collectable, clinically healthy tissues (HT-Ps) adjacent to the pocket sites with PD $\leqslant 3$ mm, absence of bleeding on probing and AL $\leqslant 1$ mm (Jin et al. 2004). Fifteen gingival biopsies were obtained from the 15 periodontally healthy subjects as controls (HT-Cs) during tooth extraction for orthodontic reasons.

Immunohistochemical staining

Immunoperoxidase staining procedures were performed as described below. Briefly, after deparaffin, slides were

treated with a high pressure for 3–4 min. and blocked in phosphate-buffered saline (PBS) containing 3% H_2O_2 for 10 min. to quench endogenous perioxidase activity at room temperature. After blocking non-specific binding in 10% normal horse serum, mouse anti-human IL-1β (Dako, Glostrup, Denmark), IL-10 (Dako) and LBP (Miometec Ltd., Greifswald, Germany) monoclonal antibodies were placed on tissue sections for 2 h at 37°C, respectively. Slides were then washed with PBS and incubated for 30 min. with biotinylated horse anti-mouse IgG antibody. The slides were washed with PBS again and incubated for 30 min. at 37°C with avidin-conjugated peroxidase. Finally, slides were incubated for 1 to 2 min. with the chromogen 3-3-diaminobenzidine (DAB) and counterstained with haematoxylin. After desiccation in graded ethanol (75%, 95% and 100%) and xylene, slides were permanently mounted. Negative control was performed by incubating with secondary antibody alone or Tris-buffered saline (TBS) instead of the primary antibody.

Preparation of HGF and cell culture

HGF were prepared as reported previously with modifications (Takada et al. 1991, Nemoto et al. 2000). They were prepared from the explants of normal gingival tissues in 15 periodontally healthy subjects (aged 18–25 years) with informed consent. The explants were minced finely into small pieces and distributed into a plastic 75-cm^2 cell culture flask with Dulbecco's modified Eagle's medium (DMEM/F-12) that contained 15% foetal bovine serum, 15 mM HEPES buffer, L-glutamine, pyridoxine hydrochloride, penicillin (100 U/ml), streptomycin (100 lg/ml) and insulin (4 lg/ml) (Invitrogen Corporation, Carlsbad, CA, USA). The culture flask was incubated at 37°C in 95% air and 5% CO_2. All tissue culture medium was removed and replaced with fresh medium every 7 days for 15 to 20 days. When the confluent HGF cell monolayers were formed, the cells were detached with 0.25% trypsin, washed with PBS and subcultured in 75-cm^2 plastic flasks with DMEM/F-12 supplemented with 10% foetal bovine serum. After two to three subcultures by trypsinization, homogeneous, slim, spindle-shaped cells growing in characteristic swirls were obtained. The cells were used as confluent monolayers between passages 4 and 7.

The harvested HGF ($1–5 \times 10^5$ cell/ml) were seeded into four-well plates or 75-cm^2 culture flasks and grown until confluent. The medium was replaced with fresh DMEM/F-12 supplemented with 1% foetal bovine serum, followed by incubation for 24 h with various concentrations of recombinant human LBP (rhLBP: 0.01–10 μg/ml, R&D Systems Inc., Minneapolis, MN, USA) and/or *Escherichia coli* LPS (0.01–1000 μg/ml, Sigma-Aldrich Co., St. Louis, MO, USA). At the end of incubation, the cell culture supernatants of nine subjects were collected and stored for assay of cytokines. The cells of six subjects in the 75-cm^2 flask were used for mRNA detection.

Sandwich enzyme-linked immunosorbent assay (ELISA)

The quantikine human IL-1β and IL-10 ELISA kits (R&D systems Inc.) were used to evaluate the levels of IL-1β and IL-10, respectively in the HGF culture supernatant, in accordance with the manufacturer's instructions. The sensitivity was determined as 1.0 pg/ml for IL-1β and 4.0 pg/ml for IL-10.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted according to the manufacturer's instruction for Trizol and quantified using a BioPhotometer (Eppendorf, Hamburg, Germany). Five micrograms of total RNA were used to synthesize first-strand cDNA using the ThermoSCRIPT™ reverse transcriptase system (Invitrogen Life Technologies) in a final volume of 20 µl. The synthesized cDNA was amplified using the PCR method. The mRNAs of IL-1β, IL-1Ra, and IL-10 in HGF were examined, respectively. β-actin was used as an internal standard. IL-1β primers were 5′ GGA TAT GGA GCA ACA AGT GG 3′ (sense), 5′ ATG TAC CAG TTG GGG AAC TG 3′ (antisense); IL-1ra primer sequences were 5′ CTC TTG CCA CTG CCT CT 3′(sense), 5′ GTA CTG CAG GCA GCT GT 3′ (antisense) (Roberts et al. 1997); IL-10 primers were 5′ ATG CCC CAA GCT GAG AAC CA 3′(sense), 5′ TCT CAA GGG GCT GGG TCA GC 3′ (antisense) (Aoyagi et al. 2000); and CD14 primers were 5′ CAA CTT CTC CGA ACC TCA GC 3′ (sense) and 5′ TAG GTC CTC GAG CGT CAG TT 3′ (antisense) (Roberts et al. 1997). The cycling conditions used were initial denaturation at 94°C for 5 min.' 30 $\sim$ 40 cycles of 94°C for 1 min., 58°C for 1 min. (IL-1β)/61°C for 1 min. (IL-10)/60°C for 1 min. (IL-1Ra)/and 72°C for 90s; and a final extension at 72°C for 10 min. Ten-microlitrr aliquots of the final PCR products were analysed by electrophoresis with 1.5% agarose gels and ethidium bromide. The bands were visualized under UV transillumination.

Image analysis

Haematoxylin and eosin-stained sections were used for morphologic analysis. The expression patterns and levels of IL-1β, IL-10 and LBP were evaluated by a single examiner (L. R.) using a computerized image analysis system (Leica Qwin, Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). The following two parameters were presented for assessment of the expression levels of IL-1β and IL-10, including proportion of positively stained area (area%) and the number of positive cells over unit area (cell/area) (Jin et al. 2004). For assessment of LBP expression levels, only area% was presented (Ren et al. 2004).

Statistical analysis

One-way analysis of variance (ANOVA) was performed to determine the significance of the differences among three or more groups for normally distributed variables. Spearman rank correlation analysis with two-tailed significance testing were performed to determine the correlation of expression levels of IL-10, IL-1β and LBP peptides, respectively. Regarding the statistics of cytokine analysis in cultured cells, all experiments were performed in triplicate to confirm the reproducibility of the results. IL-1β and IL-10 concentrations were presented as mean $\pm$ SD. The significance of the differences among the groups was analysed by ANOVA. Differences between data sets with a probability of <0.05 were regarded as statistically significant.

Results

The gingival biopsies consisted of 42 PoTs (not available in two subjects) with a mean PD of 7.5 $\pm$ 1.3 mm and a

mean bone loss of 62 ± 15%, and 33 HT-Ps (not available in 11 subjects) from 44 subjects with chronic periodontitis, as well as 15 HT-Cs with a mean PD of 1.7 ± 0.2 mm from 15 periodontally healthy subjects.

IL-1β, IL-10 and LBP protein expression patterns and levels

The IL-1β protein was detected in 67% (10/15) of HT-Cs, 76% (25/33) of HT-Ps and 83% (35/42) of PoTs. It was observed in the cytoplasm of granular and spinous layers in oral sulcular epithelia of healthy subjects and pocket epithelia of patients (Fig. 1a), as well as on macrophage-like cells and in the intercellular spaces of the underlying connective tissues (Fig. 1b). IL-10 protein was detected in 80% (12/15) of HT-Cs, 85% (28/33) of HT-Ps and 62% (26/42) of PoTs, mainly in the intercellular spaces of connective tissues beneath the gingival epithelia (Fig. 1c).

As shown in Table 1, IL-1β expression levels in PoTs were significantly higher than those in HT-Ps (cell/area, $p<0.05$). Within the patients, HT-Ps showed higher levels of IL-10 than those in PoTs (cell/area, $p<0.05$), while being lower compared with PoTs when expressed as area% ($p<0.01$). No significant difference existed between healthy controls and patients. No significant differences were found in the expression levels of IL-1β and IL-10 between smokers and non-smokers. The expression levels of LBP in HT-Cs were significantly higher than those in HT-Ps ($p<0.01$) and PoTs ($p<0.05$). A significant difference was also found between HT-Ps and PoTs ($p<0.05$).

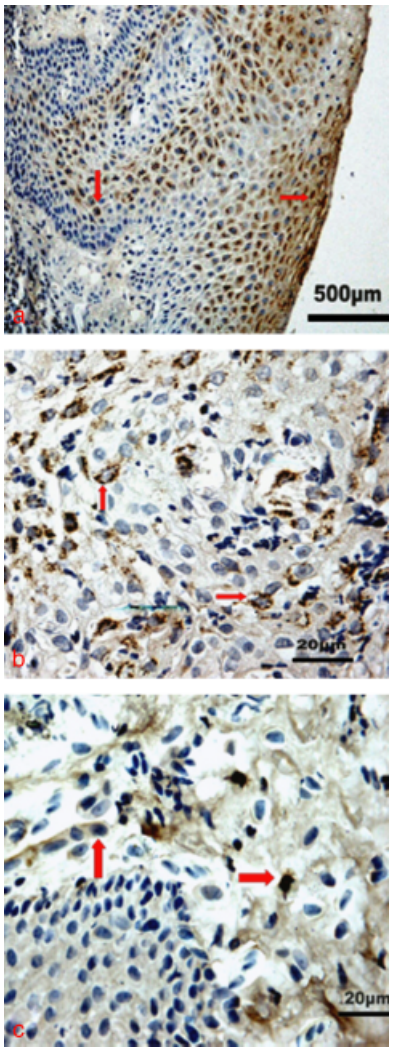

Fig. 1. Interleukin (IL)-1β and IL-10 protein expression in gingival tissues. (a) IL-1β expressed in the cytoplasm of granular and spinous layers in oral sulcular epithelia of healthy subjects and pocket epithelia of patients. (b) It was also found on macrophage-like cells and in the intercellular spaces of the underlying connective tissues. (c) IL-10 expressed in the intercellular spaces of connective tissues beneath the gingival epithelia.

Correlation of IL-1β, IL-10 and LBP protein expression

A significantly negative correlation existed between IL-1β and IL-10 levels in all tissue samples, HT-Ps and in both HT-Ps and PoTs, whereas no significant correlation was found in HT-Cs or PoTs (Table 2). Furthermore, a significantly negative correlation was found between LBP and the ratio of IL-1β/IL-10 expression levels in HT-Cs and HT-Ps, but none in PoTs (Table 3).

IL-1β and IL-10 expression by HGF in response to *E. coli* LPS with or without rhLBP

HGF could basally express IL-1β mRNA and rhLBP markedly down-regulated its expression. Similar results were found for CD14 mRNA expression. The IL-1β mRNA expression was significantly up-regulated by *E. coli* LPS. The up-regulated expression of IL-1β mRNA induced by *E. coli* LPS was significantly inhibited by rhLBP. IL-1ra and IL-10 mRNAs were not detected (Fig. 2).

Table 1. Expression levels (mean ± SD) of IL-1β, IL-10 and LBP proteins in various categories of gingival tissues

Proteins	PoT ($n=42$)		HT-P ($n=33$)		HT-C ($n=15$)	
	area%	cell/area	area%	cell/area	area%	cell/area
IL-1β	2.06 ± 0.17	1.03 ± 0.09*	1.96 ± 0.22	0.48 ± 0.15	1.93 ± 0.20	0.63 ± 0.15
IL-10	0.92 ± 0.04**	1.56 ± 0.33*	0.34 ± 0.03	2.76 ± 0.25	0.25 ± 0.13	1.89 ± 0.34
LBP	0.70 ± 0.09*#		0.38 ± 0.03*#		1.12 ± 0.42	

Significant difference from clinically healthy tissues (HT-P),
*$p<0.05$, **$p<0.01$.
Significant difference from periodontally healthy subjects as controls (HT-C),
#$p<0.05$, ##$p<0.01$.
n, number of samples (one sample per subject); IL, interleukin; PoTs, periodontal pocket tissues; LBP, lipopolysaccharide-binding protein; cell/area, the number of positive cells over unit area; HT-Ps, clinically healthy tissues; HT-Cs, periodontally healthy subjects as controls.

Table 2. Correlation (*r*) between IL-1β and IL-10 in various categories of gingival tissues

Samples	Area% *versus* area%	Area% *versus*. cell/area	Cell/area *versus* area%	Cell/area *versus* cell/area
All	−0.425#	−0.17	−0.2	−0.467#
PoTs	−0.215	−0.198	−0.144	−0.082
HT-Ps	−0.537#	−0.578#	−0.589#	−0.643#
HT-P & PoTs	−0.305	−0.278	−0.378*	−0.452*
HT-Cs	0.143	0.468	0.013	0.479

*$p<0.05$, #$p<0.01$.
Area%, proportion of positively stained area; cell/area, the number of positive cells over unit area; IL, interleukin; PoTs, periodontal pocket tissues; HT-Ps, clinically healthy tissues; HT-Cs, periodontally healthy subjects as controls.

Table 3. Correlation (r) between LBP and the ratio of IL-1β/IL-10 in various categories of gingival tissues

Samples	IL-1β/IL-10	
	area%	cell/area
PoTs	− 0.208	− 0.143
HT-Ps	− 0.478*	− 0.434*
HT-Cs	− 0.395*	− 0.452*

*$p < 0.05$.
Area%, proportion of positively stained area; cell/area, the number of positive cells over unit area IL, interleukin; PoTs, periodontal pocket tissues; LBP, lipopolysaccharide-binding protein; HT-Ps, clinically healthy tissues; HT-Cs, periodontally healthy subjects as controls.

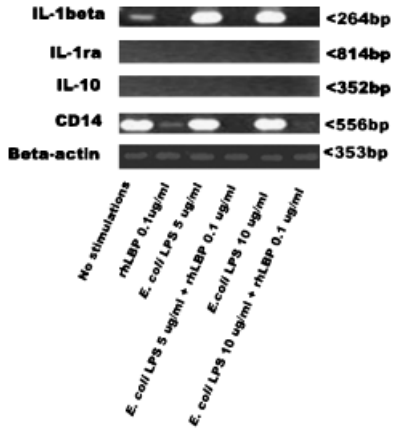

Fig. 2. Expression of interleukin (IL)-1β, IL-1ra, IL-10 and CD14 mRNAs by human gingival fibroblasts after a 24-h incubation with recombinant human lipopolysaccharide-binding protein (rhLBP) alone, *Escherichia coli* lipopolysaccharide (LPS) alone or combinations of *E. coli* LPS and rhLBP. The experiments were repeated three times.

The IL-1β protein was basally detected and it was significantly suppressed by rhLBP at various concentrations tested ($p < 0.01$). The IL-1β level increased slightly in the stimulation of *E. coli* LPS at the concentrations of 1–5 μg/ml, whereas its levels were markedly reduced in the presence of various rhLBP concentrations ($p < 0.01$). When *E. coli* LPS concentration increased over 10 μg/ml, the inhibitory effects of rhLBP on IL-1β levels shown above were diminished (Fig. 3). IL-10 protein was detected in the cell culture medium with or without *E. coli* LPS stimulation and its levels ranged from 6.78 to 25.75 pg/ml. No significant difference was found among various conditions with or without rhLBP.

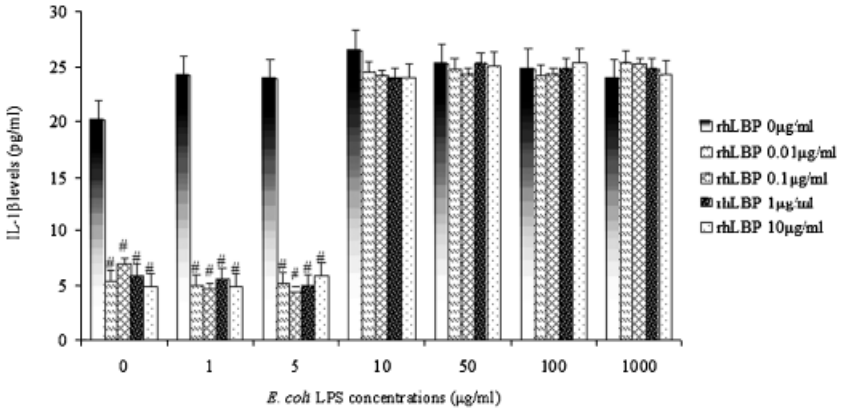

Fig. 3. Interleukin (IL)-1β protein levels in the supernatants of human gingival fibroblasts culture fluid after a 24-h incubation with various doses of recombinant human lipopolysaccharide-binding protein (rhLBP) alone, *Escherichia coli* lipopolysaccharide (LPS) alone or combinations of *E. coli* LPS and rhLBP.

Discussion

IL-1β could be expressed by many different cells, including macrophages, endothelial cells, epithelial cells and osteoblasts, in response to microorganisms, LPS or tissue injury (Dinarello 1987). The present findings were overall similar to those in a previous study (Hillmann et al. 1995). The observation of increased levels of IL-1β in PoTs as compared with those in clinically healthy tissues may indirectly support the view that the distribution of high numbers of IL-1β-positive cells in diseased sites is correlated with the extent or the degree of inflammation of connective tissues (Hou et al. 2003).

IL-10 is supposed to be important in control of periodontal disease progression (Gemmell et al. 1997). While controversy exists on its expression profile in periodontal health and disease, it has been shown that IL-10 levels in GCF were only detected in samples from periodontal patients (Gamonal et al. 2000), and T-cell lines derived from adult periodontitis subjects but not those from gingivitis subjects could produce IL-10 (Gemmell et al. 1995). In contrast, IL-10 expression in periodontitis lesions was lower than that in healthy gingiva (Yamazaki et al. 2001). In the current study, a higher expression level of IL-10 (cell/area) was noted in the clinically healthy gingival tissues than that in PoTs, while when IL-10 expression was presented as area%, its level in clinically healthy tissues was significantly lower than those in PoTs. These results suggest that the expression of IL-10 in periodontal tissues might be affected by various micro-environment factors. Recent data showed that IL-10 had pleiotropic effects in immunoinflammatory responses. In the early phases of inflammation, IL-10 expression was coordinated with other genes' expression that had potent pro-inflammatory and chemoattractant properties. Only at a late stage, did the function of IL-10 converge into a congruent attempt to limit the damage by dampening the inflammatory process (Mocellin et al. 2003). This may indirectly account for the diversity of the IL-10 expression pattern in periodontal health and disease.

Our previous studies showed the simultaneous expression pattern of LBP and mCD14 in human gingiva, in which mCD14 was mainly confined to the cells around the epithelium–connective tissue interface, whereas LBP peptides mainly expressed in the gingival epithelial cells (Ren et al. 2005a). It was worth noting that the expression pattern of IL-1β was similar to the LBP expression profile, while the localization of IL-10 was close to that of mCD14. These findings raise the question regarding the correlations that existed among them in periodontal tissues. In light of the extensive studies on cytokine networks involved in chronic periodontitis, the Th1 and Th2 paradigm was a framework for the investigation of cytokines in periodontal diseases (Gemmell et al. 1997). The characteristic cytokine products of Th1 and Th2 cells are mutually inhibitory for the differentiation and effector functions of the reciprocal phenotype (Gorska et al. 2003). It was demonstrated that IL-10 inhibited the production of IL-1 in monocytes and suppressed the up-regulation of IL-1 receptor genes (Dickensheets & Don-

nelly 1997). In polymorphonuclear leucocytes, the production of IL-1α, IL-1β, IL-8 and TNF-α was inhibited by IL-10 as well (Wang et al. 1994). In contrast, Deschner et al. (2000) provided evidence that IL-1β could suppress the secretion of IL-10 by periodontal ligament cells. In the current observations, it was noted that a significantly negative correlation existed between the levels of IL-1β and IL-10 proteins in clinically healthy tissues of the patients, which is in line with a recent finding that an inverse relationship existed between the levels of IL-1β and IL-10 in chronic periodontitis (Goutoudi et al. 2004), implying that a potential interlink might exist between them, and the dynamic interactions between pro- and anti-inflammatory cytokines may be crucial in periodontal pathogenesis.

On the other hand, it was reported that in the presence of LBP the expression of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, was all up-regulated in the stimulation of LPS (Schäfer et al. 1998). In contrast, an in vitro study found that IL-1β and cigarette smoke condensate induced the expression of LBP and CD14 by human airway epithelial cells (Regueiro et al. 2009). Although various studies revealed the interactions between LBP and cytokines, there is no information on the interplay of LBP and cytokines in association with periodontal health and disease. The current study demonstrated a significantly negative correlation between the expression level of LBP and the IL-1β/IL-10 ratio in healthy controls and clinically healthy tissues, implying that the interplay of LBP and cytokines might play a role in the local defence to bacterial endotoxin and maintenance of periodontal homeostasis. To some extent, it was suspected that LBP might function as an antimicrobial peptide-like protein such as human β-defensins (Dale et al. 2001, Lu et al. 2004, 2005). Further study is needed to confirm this hypothesis.

Currently, the roles of LBP in the activation of cells or neutralization of LPS remain unclear. We observed recently that rhLBP could significantly suppress IL-6 and CD14 expression by HGF (Ren et al. 2005b). The present study further examined the effects of LBP on IL-1β and IL-10 expression by HGF in response to *E. coli* LPS. Under a normal condition, activation of the immune system by LPS helps the host fight the invading pathogens at the right time and place. However, prolonged and uncontrolled activation causes high pro-inflammatory cytokine production in the blood and tissues (Rosenfeld & Shai 2006). LBP plays a dual role in both enabling and inhibiting cellular responses to LPS depending on the concentrations of LBP and LPS (Amura et al. 1998). A recent study found that at the concentrations of 0.1–1.0 μg/ml, rhLBP could enhance the LPS-induced TNF-α production by mouse microglial cell line in a concentration-dependent manner (Cooper et al. 2002). In contrast, the addition of up to 0.1 μg/ml rhLBP markedly inhibited TNF-α production on mouse macrophages (Amura et al. 1997). In the current study, the basal expression of IL-1β mRNAs in HGF could be promoted by *E. coli* LPS. rhLBP could markedly inhibit the basal expression of IL-1β mRNA and protein, and *E. coli* LPS up-regulated IL-1β mRNA and protein expression. With the increase of the LPS concentration, the inhibitory effects of rhLBP on IL-1β protein levels were diminished. It was interesting to note that both CD14 and IL-1β mRNAs could be inhibited by rhLBP in the presence of LPS, which implied that *E. coli* LPS-enhanced IL-1β production by HGF might be related to CD14. In general, the modulatory effects of LBP on the LPS-induced cellular responses might vary with different types of cells, the target mediators released by the cells concerned as well as the stimulators under investigation. In addition, no mRNAs of IL-10 were detected in the present study. The mean concentrations of IL-10 protein detected in HGF were at a very low level, despite the presence of various stimulations. In fact, in vivo studies showed that IL-10 was intensively expressed in the nuclei of inflammatory cells and fibroblasts of gingival connective tissues (Huang et al. 2003), but an in vitro study found contrasting results. It has been shown that almost no production of IL-10 by HGF was detected even under all kinds of stimulations (Nixon et al. 2000). Therefore, further studies would be required to explore the interactions of IL-10 and HGF and the relevant biological implications.

In summary, the current findings that IL-1β displayed a reverse pattern of expression levels with IL-10, and a negative correlation existed between LBP and the ratio of IL-1β/IL-10 implied that their interactions may play an important role in immuno-regulation of host responses to bacterial challenge. The in vitro results on the inhibitory effect of LBP on *E. coli* LPS-up-regulated IL-1β expression by HGF suggest that the appropriate expression of LBP may have beneficial effects on innate host defence, thereby contributing to periodontal homeostasis. Further studies are warranted to elaborate the molecular mechanisms of LBP regulation of cytokine expression and the relevant implications.

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Clinical Relevance

Scientific rationale for the study: Periodontal bacterial LPS can activate series of inflammatory cytokines and mediators from host cells through a pathway of LPS, LBP and CD14. The interactions of LBP and cytokines in periodontal diseases may play an important role in immuno-modulation of host responses to bacterial challenge.

Principal findings: A negative correlation existed between IL-1β, IL-10 and LBP in expression levels. rhLBP down-regulated *E. coli* LPS-induced IL-1β expression by HGF.

Practical implications: LBP might function as an antimicrobial peptide-like protein. Appropriate expression of LBP and its interplay with LPS may have a beneficial effect on innate host defence, thereby keeping the host protected from further insult of periodontal pathogens.