The Expression Profile of Lipopolysaccharide-Binding Protein, Membrane-Bound CD14, and Toll-Like Receptors 2 and 4 in Chronic Periodontitis

Lei Ren,* W. Keung Leung,* Richard P. Darveau,† and Lijian Jin*

Background: This study aimed to investigate the interrelationship of in vivo expression of lipopolysaccharide-binding protein (LBP) and membrane-bound CD14 (mCD14) in human gingival tissues as well as the coexpression of Toll-like receptors (TLR) 2 and 4 in association with periodontal conditions.

Methods: Gingival biopsies were collected from 43 subjects with chronic periodontitis, including periodontal pocket tissues (PoTs) and clinically healthy gingival tissues (HT-Ps), and from 15 periodontally healthy subjects as controls (HT-Cs). The expression of LBP, CD14, TLR 2, and TLR 4 was detected by immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR).

Results: LBP and mCD14 peptides were simultaneously detected in 91% of PoTs, 85% of HT-Ps, and 100% of HT-Cs. LBP and mCD14 mRNAs were simultaneously detected in 55% of PoTs, 55% of HT-Ps, and 75% of HT-Cs. The expression of LBP was confined to the gingival epithelium, whereas mCD14 was observed around the epithelium-connective tissue interface. A positive correlation existed between LBP and mCD14 peptides in both detection expression ($r_s = 0.608; P < 0.001$) and expression levels ($r = 0.304; P < 0.05$) of these two molecules. In PoTs, TLR 2 was detected in both pocket epithelia and macrophage-like cells in connective tissues, whereas TLR 4 was predominantly detected in connective tissues. In HT-Ps and HT-Cs, a weak expression of TLR 2 was found in gingival epithelia, and no TLR 4 expression was detected. In PoTs, mCD14 was codetected on CD68-labeled macrophages in the underlying connective tissues of pocket epithelium as well as on CD1a-labeled dendritic cells in the pocket epithelium and connective tissues interface. No similar expression profile was detected in HT-Ps and HT-Cs.

Conclusions: This study suggests that the in vivo expression of LBP and mCD14 may be interrelated. Altered cellular expression profiles of mCD14 and TLR 2 and 4 in periodontal pocket tissues imply that these pattern recognition receptors may play a role in periodontal pathogenesis. J Periodontol 2005;76:1950-1959.

KEY WORDS
Antigens, CD14; gingiva; immunohistochemistry; lipopolysaccharide receptors; reverse transcriptase polymerase chain reaction; Toll-like receptors.

Human periodontitis is an infectious disease characterized by the inflammatory destruction of tooth-supporting structures. The tissue destruction is initiated by the interaction of periodontopathogens and host cells. The lipopolysaccharide (LPS)-mediated host responses appear to cause certain injury in periodontal disease. The inflammatory response to bacterial LPS is a part of the host innate immune response which provides the first line of defense against invading pathogens. LPS acts as a potent stimulus to a variety of host cells via LPS, LPS-binding protein (LBP), and CD14 pathway, which subsequently results in the expression of proinflammatory cytokines and amplifies the related host immune response in periodontal diseases. As the essential host pattern recognition receptors, LBP and CD14, as well as other related molecules such as Toll-like receptors (TLRs) 2 and 4, may therefore play important roles in periodontal pathogenesis.

CD14 is mainly expressed on monocytes and macrophages and acts as a key receptor in inducing an inflammatory response triggered by bacterial
LPS. It is attached to the cell surface by a glycosylphosphatidylinositol (GPI) anchor. CD14 has two forms, i.e., soluble CD14 (sCD14) and membrane-bound CD14 (mCD14), which function through different mechanisms. It has been claimed that the site of amino acids 57 and 64 is the LPS-binding domain of human sCD14; although based on the experiments with this domain-deletion mutant, the mCD14 is able to bind LPS and *Escherichia coli* as well. These studies suggest that the same domains of sCD14 and mCD14 function differently. mCD14 molecules were originally shown to be expressed by cells of the myeloid lineage, such as monocytes, macrophages, and PMNs. Recently, B cells, liver parenchymal cells, gingival fibroblasts, and microglial cells have also been found to express this protein.

LBP is a glycosylated protein synthesized by hepatocytes, and it is released into the bloodstream upon acute-phase stimulation. Its normal concentration in human serum ranges from 2 to 20 µg/ml, and it can rise dramatically under the stimulation of inflammation. The level of LBP is controlled by transcriptional activation of the LBP gene, which is mediated by interleukin (IL)-1 and IL-6 released by macrophages. Although it is well appreciated that LBP is mainly produced in the liver, recent studies have shown the evidence that LBP mRNA can be expressed by human respiratory type II epithelial cells, human intestinal epithelial cells, and gingival epithelial cells, suggesting that LBP may be produced by non-hepatocytes as well in humans, and its role need to be further defined.

As two major endotoxin binding proteins of host, the detailed mechanisms about how LBP and CD14 interact with LPS are well studied. LBP has two-sided effects in challenge with LPS. As a lipid transfer protein, high concentrations of LBP can neutralize LPS by transferring it to high-density lipoproteins and subsequently result in detoxification of LPS. In contrast, low concentrations of LBP, CD14, and TLRs increase the sensitivity of the host to LPS-induced, uncontrolled acute inflammation that could result in the production of proinflammatory cytokines and animal death. These findings suggest that cellular recognition of microbial LPS could be viewed as a process that involves at least LBP, CD14, and TLRs. In recent years, TLRs were found to be the essential components in innate and acquired immunity. TLR 2 and 4 are the most well-defined members in the TLR superfamily, TLR 4 works downstream of CD14, and it is responsible for delivering an LPS signal, whereas TLR 2 is involved in *Porphyromonas gingivalis* LPS-induced cellular activation. Presently, although increasing in vitro evidence shows the interactions of LBP, CD14, and TLRs in response to the challenge of LPS, no in vivo study has been performed to reveal the interrelationship of the cellular expression of these pattern recognition receptors in association with periodontal conditions and to elaborate the clinical implications.

In recent studies, we showed for the first time, to our knowledge, the local expression of LBP in human gingival tissues, and both LBP and mCD14 peptide expression levels in periodontally healthy subjects were significantly higher than in patients with chronic periodontitis. It is hypothesized that 1) potential links might exist between LBP and mCD14 expression as well as their related expression of TLR 2 and 4 in various periodontal conditions; and 2) the appropriate interactions of LPS, LBP, and mCD14 with TLR 2 and 4 might contribute to periodontal homeostasis. Therefore, the present study further investigated the simultaneous expression profiles of LBP, mCD14, and TLR 2 and 4 in periodontal pocket tissues and healthy gingival tissues in an attempt to explore the potential role of these pattern recognition receptors in the pathogenesis of periodontal diseases.

**MATERIALS AND METHODS**

**Subjects**

Forty-three Chinese adults (22 males and 21 females) with a mean age of 47.9 ± 3.7 years (22 to 65 years) were recruited for the study from January 2002 to February 2004. Inclusion criteria were: 1) presentation of untreated advanced chronic periodontitis, with a probing depth (PD) ≥5.0 mm, clinical attachment loss (AL) ≥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; and 5) no antibiotics or anti-inflammatory drugs taken within the preceding 6 months. All subjects were examined at a screening session for checking their suitability for the study. 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 reexamination session for evaluation of treatment outcomes, all of the subjects exhibited unresolved periodontitis with remaining PD ≥6.0 mm and bleeding on probing (BOP) in at least one quadrant of their dentitions, and they required periodontal surgery as an essential part of the definitive treatment plan.

Fifteen systemically and periodontally healthy subjects (seven males and eight females) with a mean age of 23.4 ± 3.6 years who required tooth extraction for orthodontic treatment purposes were selected as control subjects. Inclusion criteria were: 1) a systemically healthy condition; 2) no sites with PD >4 mm or AL...
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>1 mm in the whole dentition; 3) no radiographic evidence of periodontal bone loss after full-mouth radiographs; 4) a full-mouth score of BOP <15% of sites; 5) no use of any immunosuppressive agents; and 6) no use of antibiotics, anti-inflammatory drugs, or immunosuppressive agents within the preceding 6 months. 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.

Collection of Samples
All patients were screened for their suitability and selection of sampling sites prior to collection of samples. They were subsequently reexamined again prior to periodontal surgery. Gingival biopsies were collected through internal beveled incisions during periodontal surgery. When possible, two biopsies were obtained from each patient: 1) periodontal pocket tissues (PoTs) from unresolved periodontitis sites with PD ≥6 mm and significant loss of alveolar bone following non-surgical treatment; and 2) clinically healthy tissues (HT-Ps) adjacent to the pocket sites with PD ≤3 mm, absence of BOP, and AL ≤1 mm. Fifteen gingival biopsies were obtained from 15 periodontally healthy subjects as controls (HT-Cs) during tooth extraction for orthodontic reasons. These sampled sites met the following criteria: 1) PD not exceeding 3 mm; 2) absence of BOP; 3) AL not exceeding 1 mm; and 4) no radiographic evidence of alveolar bone loss.

Immunohistochemistry
Serial paraffin sections of biopsies were cut 4 µm thick, mounted onto slides, and used for immunohistochemistry procedures. CD68 and CD1a were used as the markers of macrophages and immature dendritic cells, respectively. Immunoperoxidase staining procedures were performed as described below. Briefly, after deparaffinization, slides were treated with high pressure for 3 to 4 minutes and immersed in phosphate buffered saline (PBS) containing 3% H2O2 for 10 minutes to quench endogenous peroxidase activity. Non-specific immunoglobulin binding was blocked with 10% normal rabbit serum for 30 minutes. After incubation with mouse anti-human mCD14 monoclonal antibody, a rabbit anti-mouse IgG secondary antibody†† at a dilution of 1:100 was applied to the first staining. After blocking non-specific binding in 10% normal rabbit serum, sections stained with anti-human CD14 antibody were incubated with mouse anti-human LBP, CD68, and CD1a monoclonal antibodies, respectively. Slides were washed with PBS and incubated for 30 minutes with biotinylated horse anti-mouse IgG antibody. The slides were washed with PBS again and incubated for 30 minutes at 37°C with avidin-conjugated peroxidase. Incubation with the DAB in TBS-0.003% H2O2 resulted in brown precipitates. Finally, sections were dehydrated and permanently mounted. Negative control experiments were performed by incubation of the sections with secondary antibody alone or TBS instead of the primary antibodies.

Image Analysis
The expression levels of LBP and mCD14 peptides were evaluated and quantitatively analyzed by a single examiner (LR) using a computerized image analysis

† Biometec, Greifswald, Germany.
§ NeoMarkers, Fremont, CA.
¶ Dako, Glostrup, Denmark.
‖ NeoMarkers.
# Imgenex, San Diego, CA.
* Imgenex.
†† Dako.
‡‡ Dako.
§§ Roche, Mannheim, Germany.
system with a digital camera and software. The proportion of positively stained area over the total area of the specimen was calculated and presented as area % \((\times 10^2)\) for LBP\(^{10}\) and area % for mCD14,\(^{15}\) respectively.

**Reverse Transcription-Polymerase Chain Reaction (RT-PCR)**

**Isolation and extraction of total RNA.** Total RNA in gingival tissues was isolated according to the manufacturer's instructions.\(^{##}\) In brief, the fresh gingival tissue was homogenized with 350 µl lysis buffer\(^{***}\) for 20 to 40 seconds, until a complete lysate was obtained. The lysate was centrifuged for 3 minutes at 10,000 g, and the supernatant was transferred to a fresh tube. The supernatant was mixed with 350 µl 70% ethanol, and 700 µl sample was transferred into a spin column\(^{†††}\) and centrifuged for 15 seconds at 8,000 g. The column was washed with wash buffer\(^{†††}\) twice, and 30 to 50 µl RNase-free water were pipetted onto the membrane,\(^{§§§}\) and centrifuged for 1 minute at 8,000 g to elute RNA.

**cDNA synthesis.** Total RNA was reverse-transcribed.\(^{¶¶¶}\) Briefly, the isolated RNA (1 µg) was preincubated with 0.4 µg 12–18 oligo (dT) and 10 mM dNTP at 65°C for 5 minutes. The mixture was incubated with 25 mM MgCl\(_2\), 0.1M DTT, RNase inhibitor, and reverse transcriptase\(^{¶¶¶}\) at 50°C for 50 minutes. The reversed transcription procedure stopped at 85°C for 15 minutes.

**PCR.** The synthesized cDNA was amplified by PCR method. A total volume of 50 µl containing 0.4 U Taq polymerase and specific primers derived from the human LBP sequence/or human CD14 sequence, 1.5 mM MgCl\(_2\), 100 µM dNTP, and reaction buffer were used. The primers used to amplify the human LBP gene were 5’ AAG GCC TGA GTC TCA GCA TCT 3’ (sense) and 5’ CAG GCT GCC GTG TGT GAA GAC 3’ (antisense)\(^{17}\); and human CD14 primers were 5’ CAA CTT CTC CGA ACC TCA GC 3’ (sense) and 5’ TAG GTC CTC GAG CGT CAG TT 3’ (antisense)\(^{18}\).

**β-actin gene** was amplified by using primer 5’ GCT CGT CGA CAA CGG CTC 3’ (sense) and 5’ CAA ACA TGA TCT GGG TCA TCT C TCT C 3’ (antisense)\(^{19}\) as an internal standard. The cycling conditions used were initial denaturation at 94°C for 5 minutes; 40 cycles of 94°C for 1 minute, 55°C (LBP) for 1 minute/57°C (CD14) for 1 minute, and 72°C for 90s; and a final extension at 72°C for 10 minutes. Ten-microliter aliquots of final PCR products were analyzed by electrophoresis with 1.5% agarose gels and ethidium bromide. The bands were visualized under ultraviolet (UV) transillumination.

**Statistical Analysis**

The chi-square test was performed to determine the significance of the differences in codetection frequency of LBP and mCD14 between healthy controls and patients under various conditions. The differences between data sets with a probability of <0.05 were regarded as statistically significant. Spearman rank and linear correlation analyses with two-tailed significance testing were performed, respectively, to determine the correlation of detection frequency of LBP with mCD14 and expression levels of LBP and mCD14 peptides.

**RESULTS**

**Samples**

Gingival biopsies consisted of 42 PoTs (mean PD of 7.5 ± 1.3 mm) and 33 HT-Ps from 43 subjects with chronic periodontitis. Of these subjects, 23 patients including five current smokers, four former smokers, and 14 non-smokers contributed 22 PoTs and 13 HT-Ps for immunohistochemical analysis; whereas 20 patients, including six current smokers, four former smokers, and 10 non-smokers contributed 20 PoTs and 20 HT-Ps for detection of LBP and mCD14 mRNAs. Fifteen HT-Cs were obtained from 15 non-smoking, healthy subjects as controls. Among them, seven samples were used for immunohistochemical analysis and eight samples for RT-PCR analysis.

**LBP and CD14 Expression**

No significant difference was found in expression patterns and levels of both LBP and mCD14 among the patients who were smokers, ex-smokers, or non-smokers. No significant correlation was found between the expression patterns and levels of LBP and mCD14 and the age of subjects, either within the patient group or within the control group. Therefore, individual results in each group were pooled, respectively, for data analysis and presentation. The coexpression of LBP and mCD14 was detected by immunohistochemical double staining in gingival tissues (Fig. 1), which was consistent with our recent findings.\(^{10,15}\) mCD14 was mainly confined to the cells around epithelium-connective tissue interface in all samples, whereas LBP mainly expressed in the outer layer of gingival epithelium, which was especially confined to the cytoplasm of granular and keratinized layers. In the underlying connecting tissues of the epithelium, LBP was mainly detected on the surface of vascular endothelial cells and/or inside the lumen of blood vessels, which was reported elsewhere.\(^{10}\) LBP and mCD14 peptides were codetected in 89% (31/
of the samples from patients (PoTs and HT-Ps), 91% (20/22) in PoTs, and 85% (11/13) in HT-Ps, as well as in 100% (seven/seven) of HT-Cs (Table 1). LBP and mCD14 peptide expression levels were significantly higher in healthy controls than those in diseased tissues, which were reported elsewhere.10,15 Overall, a positive correlation existed between LBP and mCD14 peptides in detection expression ($r_s = 0.608$, corrected for ties; $P < 0.001$) and expression levels ($r = 0.304$; $P < 0.05$) of these two molecules. A significant correlation was also found between the detection expression of LBP and mCD14 peptides in patients ($r_s = 0.796$ in PoT; $P < 0.001$; $r_s = 0.804$ in HT-P; $P < 0.01$) and healthy control subjects ($r_s = 1.0$; $P < 0.02$).

The expression of LBP and mCD14 mRNAs is presented in Figure 2. CD14 mRNA was detected in all categories of samples, whereas LBP message was detected in over 50% of various categories of gingival tissues. LBP and mCD14 mRNAs were codetected in 55% of PoTs (11/20), 55% of HT-Ps (11/20), and 75% of HT-Cs (six/eight), and no significant difference was found in detection frequency among them.

**TLR 2 and TLR 4 Expression**

The expression of TLR 2 and 4 peptides was detected as shown in Figure 3. The negative control is shown in Figure 3A. In PoTs, TLR 2 expressed in both pocket epithelia and underlying connective tissues (Fig. 3B), in which TLR 2 mainly expressed on the membrane of pocket epithelial cells in granular and keratinized layers (Fig. 3C) and the macrophages-like cells in connective tissues (Fig. 3D). In HT-Ps, TLR 2 expressed in the same area of gingival epithelial cells, whereas the expression density was weaker than that of PoTs (Figs. 3E and 3F). Furthermore, TLR 2 staining was hardly found in connective tissues of HT-Ps. The expression pattern of TLR 2 in HT-Cs was similar to that of HT-Ps. TLR 4 was predominantly detected on the membrane of cells in connective tissues of PoTs (Fig. 3G), and no expression was found in pocket epithelium. In HT-Ps and HT-Cs, no TLR 4 expression was detected (Fig. 3H).**

**DISCUSSION**

It is well recognized that LPS constitutes one of the major virulent factors on the surface of pathogenic Gram-negative bacteria and interacts with host cells via pattern recognition receptors, such as LBP, CD14, and TLRs. Detailed mechanisms that show how these molecules in an oral environment interact with LPS remain to be elucidated. In general, LPS binds LBP and CD14 in various forms, which results in either activation of the host cells or neutralization of LPS.20
mCD14 binds LBP-opsonized Gram-negative bacteria and augments phagocytosis, by which LPS first binds LBP and then mCD14 on the surface of macrophages. LBP then mediates LPS transport into the macrophage liposomes in a concentration-dependent manner. Regarding the cellular activation by LPS, mCD14 facilitates LPS interactions with one or more pattern recognition elements, e.g., TLR 2 and 4, which transduce signals into the cell with or without LBP. Therefore, LBP plays a central role in mediating cellular responses to LPS.

Extensive evidence shows that LPS is an important etiologic factor in the pathogenesis of periodontitis. It has been reported that the local concentrations of LBP and CD14 in gingival tissues and gingival crevice may be the arbiters for the immunobiological effects of LPS in the periodontal diseases. Further studies on LPS interactions with LBP and CD14 may enhance the understanding of periodontal pathogenesis. Although various in vitro studies revealed the mechanisms of LBP and mCD14 interactions in response to LPS, no in vivo report is available on the interrelationship of LBP and mCD14 in association with periodontal health and disease. The present study 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 cytoplasm of keratinized and granular cells. These results revealed that LBP and mCD14 expressed on separate cells and at different compartments of gingival tissues. Moreover, most tissue samples expressed both LBP and mCD14 peptides as well as the corresponding mRNAs, whereas no significant difference was found in the detection frequency of peptides and mRNAs among various clinical categories of gingival tissues. The expression patterns of LBP and mCD14 peptides were similar in

Table 1.

Coexpression of LBP and CD14 Peptides in Gingival Tissues From Subjects With Chronic Periodontitis and Healthy Controls

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+ = detectable; − = not detectable.

Figure 2.

mRNA expression of LBP and CD14 in various categories of gingival tissues. β-actin was used as the internal control. Lanes 1 and 4: periodontal pocket tissues from subjects with chronic periodontitis; lanes 3 and 6: clinically healthy tissues from subjects with chronic periodontitis; and lanes 2, 5, and 7: periodontally healthy tissues from healthy control subjects.
periodontally healthy subjects and patients with chronic periodontitis. No significant correlation was found between the expression patterns or detection frequency of these two proteins and smoking status as well as the age of subjects. It is interesting to note that a significantly positive correlation existed between LBP and mCD14 peptides in both detection expression and expression levels in gingival tissues, suggesting that a potential interlink might exist between them despite of their expression at different compartments of gingival tissues. This observation is consistent with a recent study that showed a positive correlation between the levels of mCD14 expression on macrophages and their phagocytic activity toward LBP-opsonized *E. coli*.21 Furthermore, in the present study, the detection frequency of LBP mRNA (55%) was lower than that of peptides (over 85%); it may be that LBP peptides observed in gingival tissues consisted of those that expressed in both epithelium and blood vessels, although...
the endothelial cells could not produce LBP and the LBP peptides observed in blood vessels may derive from serum. It was therefore postulated that the LBP mRNA detected in the gingival tissues was mainly derived from gingival epithelial cells.

The present study further investigated the expression of TLR 2 and 4 in gingival tissues. In periodontal pocket tissues, TLR 2 was detected in both pocket epithelia and the macrophage-like cells in underlying connective tissues, whereas TLR 4 was predominantly detected in connective tissues. In healthy gingival tissues, only weak expression of TLR 2 could be detected in gingival epithelia, and no TLR 4 expression was detected. Overall, these observations are consistent with recent findings that TLR 2 and 4 expression is related to the severity of periodontal inflammation. Considering the expression location of these target molecules in the present study, TLR 2 and 4 appear to express at the respective compartments of LBP and mCD14 peptides. A recent study has demonstrated that LBP can transfer a variety of bacterial ligands including the compounds derived from both Gram-positive and Gram-negative bacteria to CD14 present in different complexes of TLRs. It is known that P. gingivalis LPS induces cellular activation via a TLR 4–MD-2–MyD88-dependent pathway, whereas some unknown bacterial components in P. gingivalis LPS and its lipid A may induce cellular activation via TLR 2. In the present study, we found that the expression of TLR 2 and 4 was more markedly detected in pocket tissues as compared to healthy tissues. In contrast, LBP and mCD14 were less expressed in pocket tissues than in healthy tissues. Taken together, our findings imply that the appropriate interactions of bacterial LPS, LBP, and mCD14 with TLRs might contribute to periodontal homeostasis.

Our previous study showed that mCD14 expression was confined to the cells around the epithelium-connective tissue interface, and its expression levels in periodontal pocket tissues were significantly lower than in healthy control tissues. These results raise the question concerning what cell types might be involved in the expression of mCD14 and what would be the relevant physiopathological effects. It has recently been reported that matured dendritic cells (DC) could be derived from human CD14-positive mononuclear cells, and its implication remains unclear in vivo. Therefore, in the present study, macrophages and dendritic cells were further investigated to evaluate their association with the mCD14 expression profile in periodontally healthy subjects and patients with chronic periodontitis. CD68 was the marker for mature macrophage, whereas CD1a was the marker for the DC, especially the immature Langerhans cells. It was found that in periodontal pocket tissues, mCD14 was codetected on CD68-labeled macrophages in the underlying connective tissues of pocket epithelium as well as on CD1a-labeled dendritic cells in the pocket epithelium and connective tissues interface. No similar expression profile was detected in healthy tissues. Currently, the roles of macrophages in periodontal diseases remain controversial. Two different phenotypes of monocytes (CD14+/CD16− and CD14+/CD16+) were recently identified. The CD14+/CD16− monocyte was regarded as the proinflammatory phenotype, and it increased significantly in patients with sepsis. Furthermore, functionally different macrophages were also identified according to the difference in surface proteins, which exhibited a different localization pattern depending on the stage of inflammation.
These results suggested that different phenotypes or functions of macrophages, present within normal and inflamed gingiva, might play an important role in antigen presentation and regulation of local immune response. On the other hand, DCs have been demonstrated to function as a link between innate and acquired immunity.36 According to our observations, a conversion potential might exist among the mCD14-positive cells in the presence of differentiation and/or other stimulatory signals, such as LPS and cytokines.38 Therefore, various phenotypes and functions of macrophages and the DCs might take part in the bacteria-host interactions through different regulation mechanisms, which may contribute to periodontal health and disease.

The present study suggests that the in vivo expression of LBP and mCD14 may be interrelated. The expression patterns of mCD14 and TLR 2 and 4 appear to be associated with different periodontal conditions. Altered cellular expression profiles of these pattern recognition receptors in periodontal pocket tissues imply that these molecules may play a role in periodontal pathogenesis. Further in vivo and in vitro investigations are warranted to elucidate the molecular mechanisms of LBP-mCD14-TLR interactions for clarification of their roles in periodontal pathogenesis.

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