Upregulated Leptin in Periodontitis Promotes Inflammatory Cytokine Expression in Periodontal Ligament Cells

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Background: Imbalance or disruption in the expression of inflammatory mediators contributes greatly to the breakdown of the periodontal supporting tissues. Leptin, through binding to its receptor (obesity-related leptin and leptin receptor [OBR]), has potent effects on immunity and inflammation. However, to date, researchers only indicated a role of leptin in periodontitis. No direct or valid evidence exists about how leptin and its receptor are regulated by local inflammation, what effects they have, and the underlying mechanisms.

Methods: Experimental periodontitis was induced by ligation of mandibular second molars in beagle dogs. The expression of leptin, OBR, and interleukin (IL)-1β was examined by immunohistochemistry. Meanwhile, recombinant human IL-1β was used to stimulate human periodontal ligament cells (hPDLCs) in vitro, and mRNA and protein levels of leptin were measured using real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. Then, mRNA and protein levels of IL-6 and IL-8 were measured using real-time PCR and ELISA, after stimulation with various concentrations of leptin, knocking down all or only the long form of OBR (OBRb) by small interfering RNA and incubation with multiple intracellular signaling pathway inhibitors, respectively.

Results: Leptin and OBR increased substantially in inflammatory periodontal tissues, which correlated well with the extent of inflammatory infiltration, and was a result of the upregulation in resident cells themselves. A high dose of leptin could induce the expression of mRNA and protein of IL-6 and IL-8 in hPDLCs through binding with OBRb and activating different intracellular signaling pathways.

Conclusion: Upregulated leptin and OBR in periodontitis stimulated proinflammatory cytokine expression in PDL cells to additionally promote local inflammation. *J Periodontol 2015;86:917-926.*

KEY WORDS

Interleukins; leptin; periodontitis; receptors, leptin.

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Leptin, since being discovered in 1994,[1] has caught great attention and provided a new link between nutrition, metabolism, and immune homeostasis.[2,3] Accumulating evidence also suggested a role of leptin in periodontitis. Compared with healthy controls, serum leptin levels increased substantially in patients with periodontitis and correlated positively with serum interleukin (IL)-1β, IL-6, and tumor necrosis factor-α, as well as clinical markers of periodontal breakdown, such as bleeding index (BI), probing depth (PD), and attachment loss.[4-7] After periodontal treatment, serum leptin levels decreased significantly.[8-10]

In recent years, leptin has been proven to be a potent modulator of immunity and inflammation.[3,11] It could, on the one hand, activate the innate and adaptive immune cells to promote a proinflammatory response but, on the other hand, induce resident cells, such as synovial fibroblasts, to secrete proinflammatory mediators and proteinases to accelerate local breakdowns.[12,13] Periodontal ligament cells (PDLCs), major resident cells of the periodontium, also have some characteristics of immune cells.[14] They participate actively in local immune defenses by secreting multiple proinflammatory cytokines, which provide signals to sustain local inflammation and tissue breakdown.[15] Thus, leptin might play roles in the pathogenesis of periodontitis partially through its effects on PDLCs.

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It was observed previously that obesity-related leptin and leptin receptor (OBR) were expressed abundantly by resident cells in healthy dental and periodontal tissues, and it was presumed that they played a protective role under healthy conditions, which might alter during periodontitis.[16] However, to the best of the authors' knowledge, to date no direct or valid evidence has come out to explain the following: 1) how exactly leptin and OBR are expressed in inflamed periodontal tissues; and 2) what effects they have on local inflammation. This study aims to systemically examine leptin and OBR expression in periodontal tissues during experimental periodontitis in beagle dogs in vivo and investigate the effects of leptin on proinflammatory cytokine expression of human PDLCs (hPDLCs) in vitro.

MATERIALS AND METHODS

Experimental Animals and Tissue Samples

Six healthy beagle dogs, aged 1 to 2 years and weighing 10 to 12.5 kg, were housed individually and fed once per day with soft food and water during the experiment. After supragingival scaling 1 week before the experiment, one side of the mandibular second molars was selected randomly, and cotton ligatures were placed around tooth cervixes to induce periodontitis. Throughout the experiment, the unligated teeth were maintained as healthy as possible by toothbrushing and applying 0.12% chlorhexidine twice a day, with the ligated teeth left untreated. At the end of 12 weeks, macroscopic and x-ray pictures were taken, and clinical markers, including Silness-Löe plaque index,[17] PD, and BI at the mesial and distal sites of each tooth, were recorded using a periodontal probe. After that, the animals were sacrificed by overdose of intravenous injection of sodium pentobarbital, followed by perfusion with 4% paraformaldehyde through the carotid arteries and veins. Dissected mandibular segments of posterior teeth with surrounding gingiva and alveolar bones, after fixation for an additional 1 month, were decalcified in EDTA, dehydrated, and embedded in paraffin. Then, mesio-distal serial sections were cut parallel to the long axis of the teeth with the microtome set at 5 μm. At least one section from each sample was examined with hematoxylin and eosin (H&E). The study protocol was evaluated and approved by the Experimental Animal Welfare Ethical Branch of Peking University Biomedical Ethics Committee (Protocol LA2010-032).

Immunohistologic Examinations of Tissue Samples

Leptin, OBR, and IL-1β expression in unligated and ligated periodontal tissues was examined as described previously.[16] Briefly, after deparaffinization with xylene and rehydration with descending concentrations of ethanol, endogenous peroxidase was blocked by treatment with 3% H_2O_2 for 10 minutes at room temperature. Antigen retrieval was achieved by 1 mg/mL trypsin digestion at 37°C for 10 minutes. After block with 10% normal goat serum at room temperature for 10 minutes, sections were incubated with rabbit antihuman leptin polyclonal antibody‖ (1:400), rabbit antihuman OBR polyclonal antibody¶ (1:100), or rabbit antihuman IL-1β polyclonal antibody# (1:400) in working solution at 4°C overnight. After washing in 0.01 *M* phosphate-buffered saline (pH 7.4), the location of leptin, OBR, and IL-1β was visualized using an immunohistochemistry** and a 3,3′-diaminobenzidine kit.†† Sections were finally counterstained with hematoxylin and mounted. The specificity of the immunoreaction was confirmed by incubation with normal rabbit immunoglobulin G.‡‡ Images were captured on a digital microscopic system.§§

Cell Culture and Stimulation

Primary cultured hPDLCs were isolated and cultured as described previously.[14] The study protocol was approved by the institutional review board of Peking University School and Hospital of Stomatology (Protocol PKUSSIRB-2011007). Cells from passages 4 to 6 were used in the following experiments. For mRNA examination, hPDLCs were seeded into 12-well plates at a density of 2×10^5/mL, whereas for protein detection, hPDLCs were seeded into 24-well plates at a density of 1×10^5/mL.

To determine the effects of proinflammatory cytokines on leptin production, hPDLCs were treated with 0 or 10 ng/mL recombinant human IL-1β‖‖ for 1, 2, 4, 6, 12, and 24 hours for mRNA and protein detection or were pretreated with cycloheximide¶¶ or brefeldin A (BFA)## for 30 minutes, followed by stimulation with 0 or 10 ng/mL IL-β for 2 hours for protein detection.

To determine the effects of leptin on IL-6 and IL-8 production, after serum starvation in Dulbecco's modified Eagle's medium*** containing 1% fetal bovine serum††† for 24 hours, hPDLCs were treated with 0, 1, 10, 100, or 1,000 ng/mL recombinant human leptin‡‡‡ for 3 and 6 hours for mRNA examination and 12 and 24 hours for protein examination.

‖ sc-842, Santa Cruz Biotechnology, Santa Cruz, CA.
¶ sc-8325, Santa Cruz Biotechnology.
# ab34837, Abcam, Cambridge, UK.
** PV-9001 kit, Zhongshan Golden Bridge Biotechnology, Beijing, China.
†† DAB kit, Zhongshan Golden Bridge Biotechnology.
‡‡ Santa Cruz Biotechnology.
§§ BX51/DP72, Olympus, Tokyo, Japan.
‖‖ PeproTech, Rocky Hill, NJ.
¶¶ Sigma, St. Louis, MO.
Gene Operation, Ann Arbor, MI.
*** Gibco, Thermo Fisher Scientific, Waltham, MA.
††† Hyclone, Logan, UT.
‡‡‡ R&D Systems, Minneapolis, MN.

RNA Interference of OBR

To determine the form of OBR involved in the effects of leptin on IL-6 and IL-8 production, specific small interfering RNA (siRNA) targeting mRNA of OBR§§§ and the long form of OBR (OBRb)‖‖‖‖ was used. hPDLCs were transfected with either OBR siRNA (5 nM) or OBRb siRNA (10 nM), respectively. A non-silencing control siRNA¶¶¶ was used as a negative control according to the instructions of the manufacturer. Forty-eight hours after transfection, cells were treated with 1,000 ng/mL leptin for 6 and 24 hours for mRNA and protein examination, respectively.

Intracellular Signaling Inhibition of OBRb

Intracellular signaling inhibitors for Janus-family tyrosine kinase 2 (JAK2) (AG490 [(*E*)-*N*-benzyl-2-cyano-3-(3,4-dihydroxyphenyl)prop-2-enamide]),### phosphatidylinositol 3-kinase (PI3K) (LY294002 [2-(4-morpholinyl)-8-phenyl-1(4*H*)-benzopyran-4-one]),**** extracellular signal-regulated kinase (ERK) (U0126 [1,4-diamino-2,3-dicyano-1,4-bis(*o*-aminophenylmercapto) butadiene]),†††† p38 mitogen-activated protein kinase (MAPK) (SB203580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1*H*-imidazole]),‡‡‡‡ and c-Jun N-terminal kinase [SP600125 (anthra[lsqb] 1,9-cd[rsqb]pyrazol-6(2*H*)-one)]§§§§ were used to determine the signaling pathway involved in the effect of leptin on IL-6 and IL-8 production in hPDLCs. After pretreatment with the inhibitors for 1 hour, hPDLCs were stimulated with 1,000 ng/mL leptin for 6 and 24 hours for mRNA and protein examination, respectively.

Measurement of mRNA and Protein Expression

The mRNA expression was determined by real-time polymerase chain reaction (PCR). Total RNA was extracted using an RNA extraction reagent.‖‖‖‖ Approximately 2 μg total RNA was converted to cDNA with a reverse transcription reagent kit.¶¶¶¶ Then real-time PCR was performed using a reagent#### in an thermocycler.***** GAPDH was used as an internal control. Data were presented and analyzed as relative levels calculated by the equation $2^{[minus]\Delta\Delta Ct}$.[18] The primers used are listed in supplementary Table 1 in online *Journal of Periodontology*.

Commercially available human enzyme-linked immunosorbent assay kits††††† were used for the measurement of leptin, IL-6, and IL-8 levels in the supernatants according to the instructions of the manufacturer.

Statistical Analyses

The normal distribution of all data were tested with Kolmogorov-Smirnov test. If normal distribution was confirmed, the statistical differences between different groups were analyzed by analysis of variance for comparison between three or more groups or Student *t* test for two groups. If normal distribution was denied, Mann-Whitney *U* test was used. All statistical analysis was performed using a software program.‡‡‡‡‡ Data were expressed as means ± SEMs. $P < 0.05$ was considered statistically significant.

RESULTS

Leptin, OBR, and IL-1β Expression in Healthy and Inflammatory Periodontal Tissues of Beagle Dogs

At week 12 of the experiment, clinical, radiographic, and H&E examinations all confirmed the establishment of periodontitis in beagle dogs (see supplementary Figs. 1 through 3 in online *Journal of Periodontology*).

In the unligated periodontal tissues of beagle dogs, leptin and OBR were expressed widely, with positive staining in gingival epithelium, PDL, and bone marrow, similar to results observed previously in healthy *Macaca fuscicularis* tissues.[16] Compared to the unligated tissues, the locations of positive leptin and OBR staining were exactly the same, but the intensity became significantly stronger and correlated positively with the extent of inflammatory infiltration.

Gingiva

In the ligated tissues, leptin and OBR staining decreased gradually from stratum basale to stratum spinosum, until completely negative in stratum corneum in oral epithelium. In sulcular epithelium, both basal cells and pickle cells showed leptin and OBR staining. Most of the junctional epithelium was destructed, whereas in the remainder, leptin and OBR staining reduced gradually as cells migrated to the tooth surface. Dense inflammatory infiltration was observed in the ligated gingival connective tissues (CTs). Leptin staining was positive in inflammatory cells (mostly round cells, such as lymphocytes, which has been proven in a previous publication[16]) and gingival fibroblasts near the capillaries, but it was negative in gingival fibroblasts away from the capillaries. However, OBR staining was almost negative (data not shown).

§§§ SI00035903, Qiagen, Hilden, Germany.
‖‖‖‖ SI05007611, Qiagen.
¶¶¶ Qiagen.
Santa Cruz Biotechnology.
**** Cell Signaling Technology, Danvers, MA.
†††† Santa Cruz Biotechnology.
‡‡‡‡ Santa Cruz Biotechnology.
§§§§ Santa Cruz Biotechnology.
‖‖‖‖ TRIzol reagent, Invitrogen, Thermo Fisher Scientific.
¶¶¶¶ ReverTra Ace qPCR RT Master Mix, Toyobo, Osaka, Japan.
FastStart Universal SYBR Green Master, Roche, Mannheim, Germany.
***** ABI 7500 Real-Time Thermocycler, Applied Biosystems, Thermo Fisher Scientific.
††††† Human ELISA kits for leptin, IL-6, and IL-8, Neobioscience Technology, Shenzhen, China.
‡‡‡‡‡ GraphPad Prism v.6.0, GraphPad Software, San Diego, CA.

PDL

Leptin and OBR were positive not only in cytoplasm of PDLCs but also in interstitial spaces of the ligament. In the ligated teeth, the cervical third of the PDL widened remarkably, with dilated capillaries and inflammatory infiltration. Leptin and OBR staining was strong in this part but gradually decreased from the central third to the apical third of the PDL (Fig. 1).

Alveolar Bone Marrow

Marrow spaces of crestal alveolar bone were infiltrated with a lot of inflammatory cells. Adipocytes and fibroblasts in the center and osteoblasts lining the marrow cavity showed strong positive staining of leptin and OBR. In contrast, in marrow spaces of the periapical region, in which inflammatory infiltration was not obvious, leptin and OBR staining in osteoblasts and adipocytes was weak (data not shown).

All capillaries were hyperproliferated, dilated, and engorged in gingival CT, PDL, and bone marrow. The lining of the endothelial cells showed strong staining of leptin and OBR, which became even stronger in inflammatory lesions. All negative controls were negative (data not shown).

In both unligated and ligated tissues, the features of IL-1β and leptin expression were similar to each other (see supplementary Fig. 4 in online *Journal of Periodontology*).

Effects of IL-1β on Leptin Expression in hPDLCs

IL-1β (10 ng/mL) increased leptin expression in hPDLCs time dependently. After stimulation, leptin mRNA rose to a peak at 6 hours (Fig. 2A), resulting in a significant rise of protein in supernatants at 12 hours (Fig. 2B).

Prompt increase of leptin concentration in supernatants at 2 hours (Fig. 2B) seemed discordant with mRNA transcription (Fig. 2A); thus, protein synthesis inhibitor (cycloheximide) and protein transport inhibitor (BFA) were used. The result was that BFA significantly decreased leptin levels, whereas cycloheximide had no influence (Fig. 2C).

Effects of Leptin on IL-6 and IL-8 Expression in hPDLCs

Concentrations of leptin used in the following experiments have been tested to have no influence on hPDLC growth during the test periods in vitro (data not shown).

Leptin increased IL-6 and IL-8 expression in hPDLCs dose dependently (Fig. 3). Low (1 and 10 ng/mL) and median (100 ng/mL) levels of leptin had no effects on IL-6 and IL-8 expression, but high (1,000 ng/mL) levels of leptin markedly increased both mRNA and protein expression in hPDLCs.

Effects of OBR RNA Interference on IL-6 and IL-8 Expression in hPDLCs

The efficiency of OBR and OBRb knockdown by RNA interference (RNAi) both reached ≈85% compared to negative controls (Figs. 4A and 4B). Knockdown of OBR and OBRb equally reduced the effects of 1,000 ng/mL leptin on IL-6 and IL-8 expression in hPDLCs to control levels in the experiment of direct leptin stimulation on hPDLCs (Figs. 4C through 4F).

Intracellular Signaling Pathway Activated by Leptin in hPDLCs

Relative to the control group, mRNA and protein expression of IL-6 was inhibited significantly by LY294002 and SB203580, partially inhibited by AG490, and promoted by U0126 and SP600125 (Figs. 5A and 5B).

Conversely, mRNA and protein expression of IL-8 was significantly inhibited by U0126 and SB203580, promoted by AG490 and LY294002, and not influenced by SP600125 (Figs. 5C and 5D).

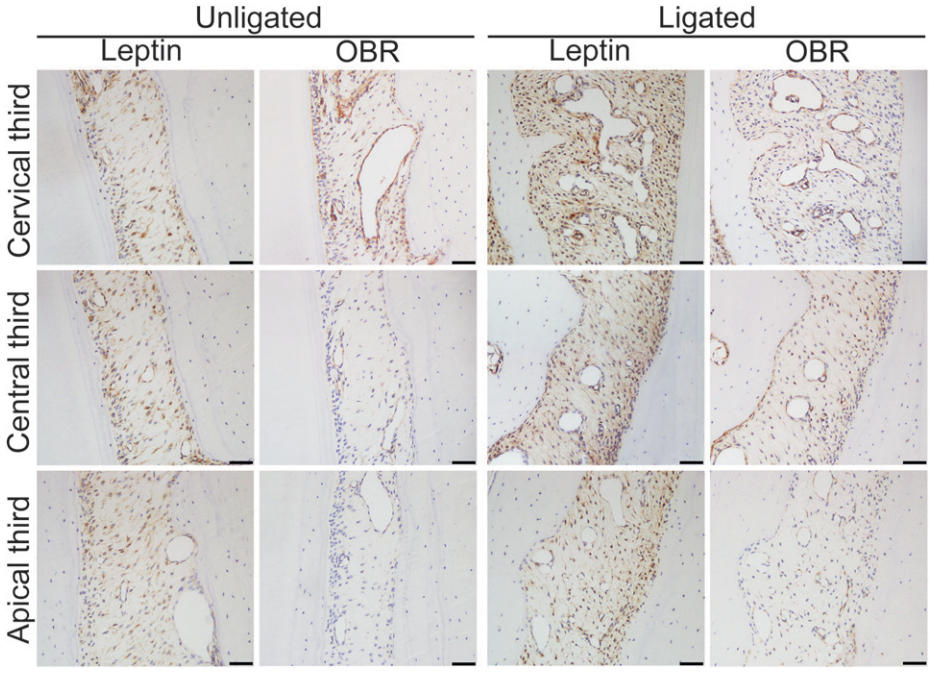

Figure 1.
Leptin and OBR staining in PDL at week 12 of experimental periodontitis in beagle dogs. Compared to the unligated tissues, leptin and OBR staining increased significantly in the cervical third, central third, and apical third of PDL in the ligated tissues. Magnification ×200. Scale bars = 50 μm.

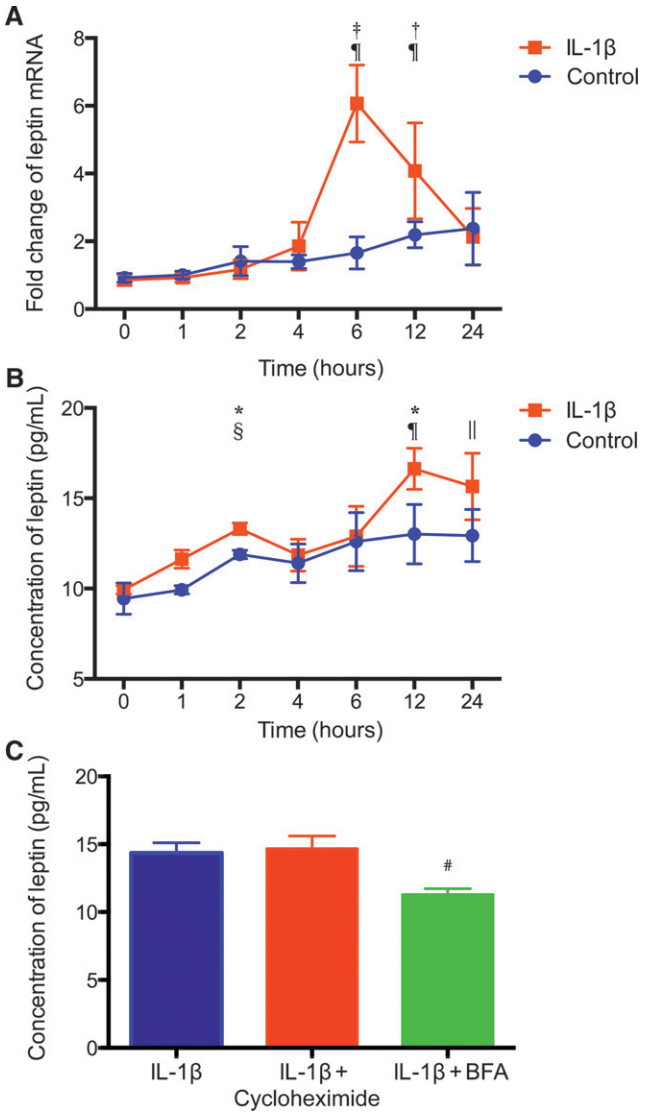

Figure 2.
A) *Leptin mRNA was upregulated time dependently in hPDLCs after IL-1β stimulation, with a peak at 6 hours.* ***B)*** *Leptin protein increased time dependently in hPDLCs after IL-1β stimulation, with two peaks at 2 and 12 hours. * P <0.05, † P <0.01, ‡ P <0.001, significant differences compared to controls at the same time; § P <0.05, || P <0.01, ¶ P <0.001, significant differences compared to 0 hours of the same group.* ***C)*** *BFA significantly inhibited leptin increase in supernatants of hPDLCs after IL-1β stimulation for 2 hours, but cycloheximide had no effects. # P <0.05, significant differences among groups.*

Thus, leptin-mediated IL-6 expression in hPDLCs was mainly through activating PI3K and p38 MAPK pathways and partially through the JAK2 pathway, whereas leptin-induced IL-8 expression was mainly mediated by ERK and p38 MAPK pathways.

DISCUSSION

To the best of the authors' knowledge, this is the first study to systematically observe leptin and OBR expression in ligature-induced periodontitis in vivo and to examine the effects of leptin on proinflammatory cytokine expression in hPDLCs and the underlying mechanisms.

In this study, leptin and OBR expression in the unligated periodontal tissues of beagle dogs is consistent with previous results showing that leptin and OBR could by expressed by gingival epithelial cells, gingival fibroblasts, and PDLCs of primates.[16] In addition, it was reported that leptin synthesis could be directly upregulated by inflammatory mediators.[19-21] In this study, leptin expression in vivo correlates positively with the extent of inflammatory infiltration. The relative intensity of leptin staining among tissues was similar to that of IL-1β. Both indicated that leptin expression in periodontal tissues could be regulated by periodontal inflammation. To mimic the inflammatory environment and to correspond to the IL-1β immunostaining in histologic sections of the beagle dogs, hPDLCs were stimulated in vitro, which further verified that the strengthened leptin staining in vivo was at least partially a result of the upregulation of resident cells in periodontal tissues instead of increased exudation secondary to increased serum leptin levels in periodontitis. In addition, the prompt increase of secreted leptin after 2 hours of stimulation of IL-1β in hPDLCs was the result of the release of preformed stores of leptin in hPDLCs, consistent with the results of adipocytes.[22-24] This suggested that hPDLCs could synthesize and store leptin in normal conditions, which could display dual effects, involving in the regular control of cellular functions and preparing to adapt to exogenous inflammatory stimuli rapidly.

It was noticeable that capillaries in gingival CT, PDL, and bone marrow of ligated tissues all proliferated, dilated, and engorged. This could provide great convenience for local inflammatory mediators to diffuse into serum, which not only caused the systemic inflammatory status in periodontitis but also reciprocally increased circulating leptin levels by inducing leptin release from adipose tissues, explaining the increase of serum leptin levels in patients with periodontitis compared with healthy controls.[4-6,25] Of greater importance, the increase of serum leptin levels has been reported to be an independent risk factor for cardiovascular diseases.[26] As periodontitis progresses, serum leptin levels could rise to twice that of healthy controls.[4] Thus, the excess leptin produced in periodontal tissues during periodontitis could be a link between periodontal and systemic conditions.

OBR exists in at least six alternatively spliced forms with cytoplasmic domains of different length: one long form (OBRb), four short forms (OBRa, OBRc, OBRd, and OBRf), and one soluble form (OBRe).[3] OBRb is the main functional form and is capable of activating intracellular signaling pathways. OBRs are the most widely expressed and are responsible for the

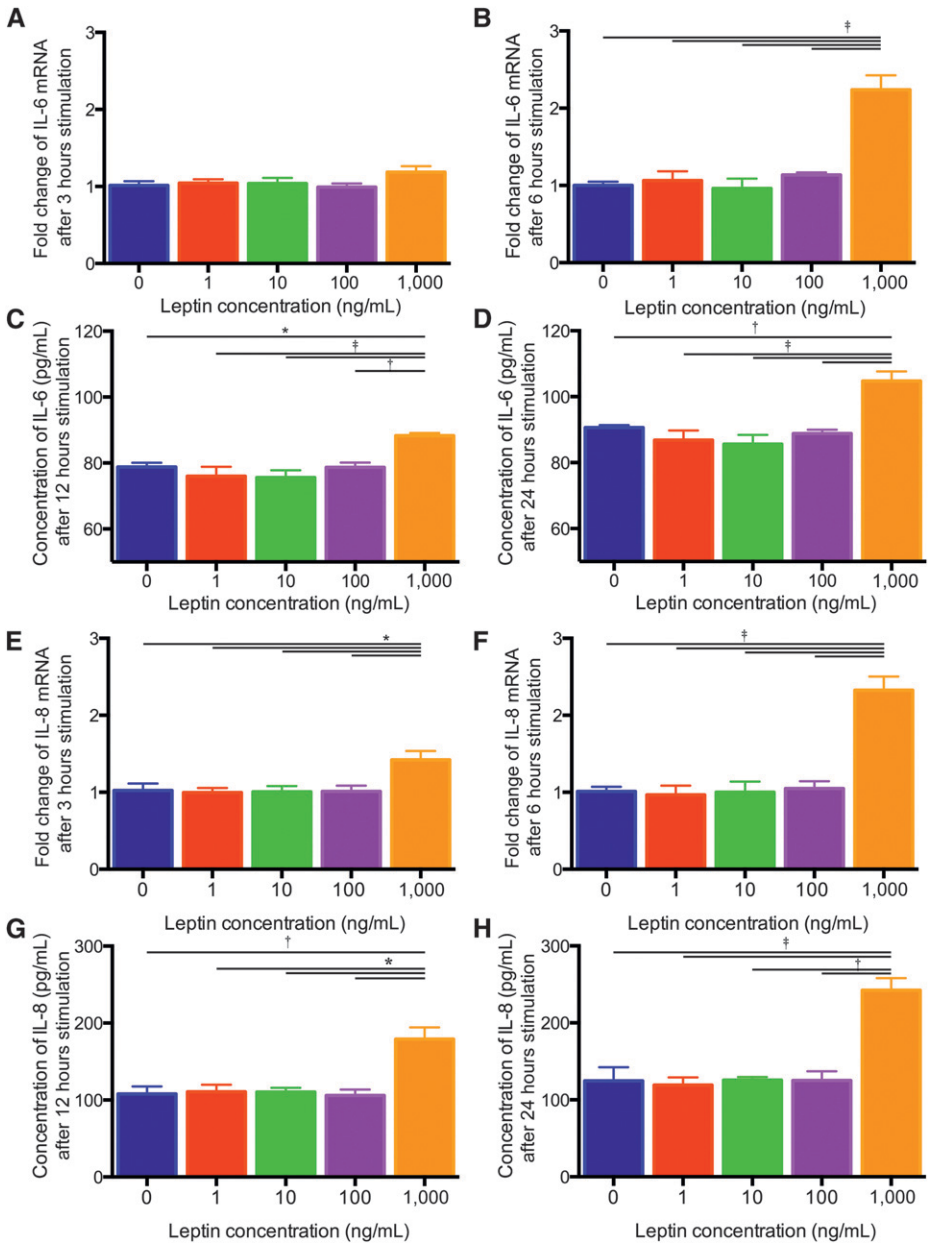

Figure 3.
Effects of leptin on IL-6 ***(A through D)*** *and IL-8* ***(E through H)*** *mRNA and protein expression in hPDLCs in vitro. Only 1,000 ng/mL leptin could significantly increase IL-6 and IL-8 expression in hPDLCs.* * P <0.05, † P <0.01, ‡ P <0.001.

transport and degradation of leptin. OBRe can only bind to leptin as a carrier protein. In the ligated tissues of this study, OBR expression, parallel to leptin expression, is observed to increase significantly in gingival epithelium, the central third of PDL, and bone marrow near the alveolar crest, suggesting an augmentation of transport and degradation of leptin in these tissues, as well as functional reinforcement of leptin through OBRb.

A hyper-responsive host immune system is the main cause of tissue breakdown in periodontitis. In studies of rheumatoid arthritis and osteoarthritis, leptin was able to induce IL-6 and IL-8 expression in synovial fibroblasts[12,13] and aggravate extracellular matrix degradation in synergy with IL-1β.[27] PDLCs are important targets for biologic mediators to regulate tissue breakdown and reconstruction. In recent years, they have been proven to exert immunomodulatory effects as well, which partially depends on secretion of various proinflammatory cytokines.[14,15,28,29] Among them, IL-6 is a potent proinflammatory cytokine linking the immune system and alveolar bone loss,[12,30] and IL-8 is a well-known chemoattractant and activator for neutrophils, lymphocytes, and macrophages.[13,31,32] Both are essential in the initiation and progression of periodontitis and are mostly upregulated in diseased PDLCs,[14,15] and so the effects of leptin on IL-6 and IL-8 production in hPDLCs were investigated. The low (1 and 10 ng/mL) and medium (100 ng/mL) concentrations of leptin used in this study cover serum leptin levels reported in clinical studies,[4,8,10,25] whereas the high concentration (1,000 ng/mL) of leptin reached the average level of leptin in gingival crevicular fluid of patients with periodontitis.[33] Remarkably, it turned out that only the high level of leptin could significantly upregulate IL-6 and IL-8 expression in hPDLCs. To make it more meaningful, the detected concentrations of IL-6 and IL-8 in supernatants of hPDLCs were 40 to 120 pg/mL and 100 to 250 pg/mL, respectively, much higher than those reported in gingival homogenates of patients with periodontitis, 34.01 ± 1.09 and 20.70 ± 0.31 pg/mL,[34] respectively. Therefore, it is reasonable to presume that, in periodontitis, upregulated leptin in the PDL could mediate an adequate amount of IL-6 and IL-8 production to contribute to sustained inflammatory breakdown, continuous migration, and hyper-responsiveness of immunoinflammatory cells.

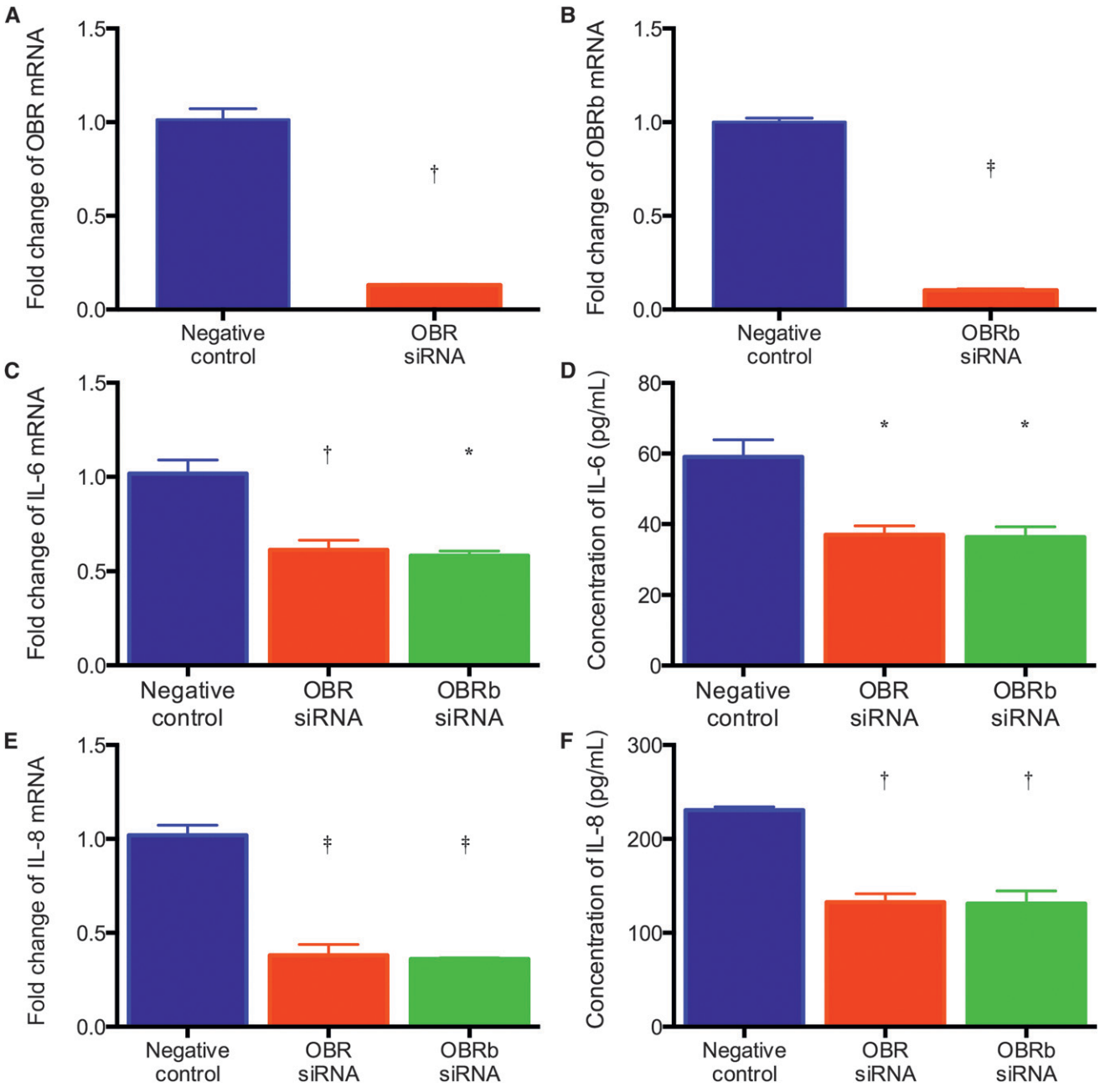

Figure 4.
*Efficacy of OBR and OBRb RNAi **(A** and **B)** and effects of OBR and OBRb RNAi on IL-6 **(C** and **D)** and IL-8 **(E** and **F)** expression in hPDLCs. * P <0.05, † P <0.01, ‡ P <0.001; significant differences compared to the negative control.*

The receptor form and intracellular signaling pathway involved in leptin-mediated IL-6 and IL-8 expression were further investigated. The RNAi experiment was performed, and it was found that, with similar knockdown efficiency, transfection of OBR siRNA and OBRb siRNA resulted in the same effects on IL-6 and IL-8 production. Thus, it could be verified that OBRb was essential for leptin-mediated IL-6 and IL-8 production. Then, inhibitors of the five most commonly activated intracellular signaling pathways after leptin binding to OBRb were used. Previous studies showed that ERK, p38 MAPK, and PI3K pathways were the usual pathways involved in inflammatory stimulation-mediating IL-6 and IL-8 production in hPDLCs.[35-37] Without exception, leptin-induced IL-6 and IL-8 also related to these three pathways. PI3K and p38 MAPK pathways took part in IL-6 production, whereas ERK and p38 MAPK

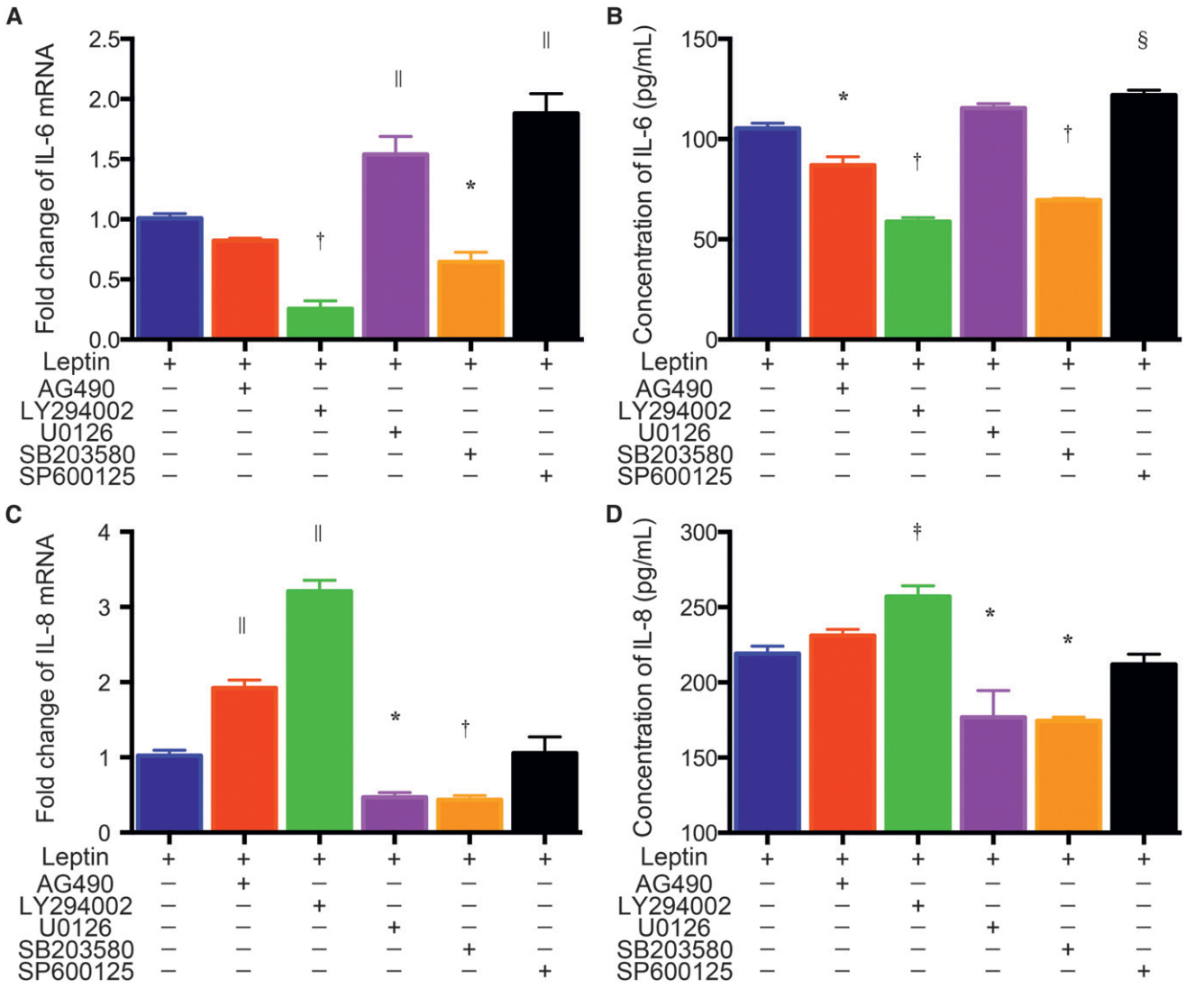

Figure 5.
Effects of intracellular signaling pathway inhibitors on IL-6 ***(A*** *and* ***B)*** *and IL-8* ***(C*** *and* ***D)*** *expression in hPDLCs.* * P <0.01, † P <0.001, *significant decreases compared to the control group;* ‡ P <0.05, § P <0.01, || P <0.001, *significant increases compared to the control group.*

pathways participated in IL-8 production. Notably, PI3K and ERK pathways counteracted in the expression of IL-6 and IL-8, indicating that leptin may selectively activate one of them during the progression of periodontitis to preferentially exert corresponding effects.

CONCLUSIONS

To the best of the authors' knowledge, for the first time, the expression of leptin and OBR has been proven to increase greatly in inflammatory periodontal tissues, which correlated well with the extent of the inflammatory infiltration, and was a result of the upregulation of resident cells themselves. Only a high dose of leptin, after binding to OBRb, could induce IL-6 production by mainly activating PI3K and p38 MAPK pathways and induce IL-8 production by ERK and p38 MAPK pathways in hPDLCs.

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