Abstract: Follicular dendritic cell-secreted protein (FDC-SP) is expressed in FDCs, human periodontal ligament (HPL) cells, and junctional epithelium. To evaluate the effects of interleukin-1 beta (IL-1β) on FDC-SP gene expression in immortalized HPL cells, FDC-SP mRNA and protein levels in HPL cells following stimulation by IL-1β were measured by real-time polymerase chain reaction and Western blotting. Luciferase (LUC), gel mobility shift, and chromatin immunoprecipitation (ChIP) analyses were performed to study the interaction between transcription factors and promoter regions in the human FDC-SP gene. IL-1β (1 ng/mL) induced the expression of FDC-SP mRNA and protein levels at 3 h, and reached maximum levels at 12 h. IL-1β increased LUC activities of constructs (−116FDCSP − −948FDCSP) including the FDC-SP gene promoter. Transcriptional inductions by IL-1β were partially inhibited by 3-base-pair (3-bp) mutations in the Yin Yang 1 (YY1), GATA, C/EBP2, or C/EBP3 in the −345FDCSP. IL-1β-induced −345FDCSP activities were inhibited by protein kinase A, tyrosine-kinase, mitogen-activated protein kinase (MEK)1/2, and PI3-kinase inhibitors. The results of gel shift and ChIP assays revealed that YY1, GATA, and C/EBP-β interacted with the YY1, GATA, C/EBP2, and C/EBP3 elements that were increased by IL-1β. These studies demonstrate that IL-1β increases FDC-SP gene transcription in HPL cells by targeting YY1, GATA, C/EBP2, and C/EBP3 in the human FDC-SP gene promoter.

Keywords: cytokine; follicular dendritic cell-secreted protein; IL-1β; inflammation; periodontal ligament; signaling pathway.

Introduction

Bacterial, host-related, and environmental influences are risk factors for periodontitis. These three factors may interact with each other and be involved, alone or in combination, in the progression of periodontal disease. Periodontitis is caused by specific periodontopathic bacteria, and it is evident that immune responses against bacterial products and the subsequent production of inflammatory cytokines are triggering events for periodontal tissue destruction (1, 2). Periodontitis is characterized by gum redness, swelling, bleeding, tooth mobility, and occlusal pain. The response to periodontal pathogens is determined by the nature and control of both innate and adaptive immune responses (2-4). Cytokines are produced by various cell types in response to bacterial
products, including macrophages, dendritic cells, T cells, epithelial cells, and fibroblasts. Cytokines play a central role in immune response. Production of inflammatory cytokines is regulated by mitogen-activated protein kinases, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and interferon regulatory factors. Interleukin-1beta (IL-1β) and IL-6 (both a pro-inflammatory cytokine) have a central role in the pathogenesis of periodontitis and rheumatoid arthritis (3-6). Periodontal disease is manifested by microbial, plaque-induced inflammation; therefore, therapeutic intervention is based on plaque removal. An appropriate immune response to bacteria may include lipopolysaccharides (LPS) and proteolytic enzymes, which reduce inflammation and boost immunity. The subsequent production of inflammatory cytokines could be essential in keeping the supporting structures of the periodontium healthy (4-8).

Human periodontal ligament (HPL) is made of periodontal cells that form connective tissue surrounding the tooth root and connecting two different mineralized tissues, the alveolar bone and root cementum (9). When HPL cells are destroyed by periodontal disease, life expectancy of the tooth will be shortened (10). One of the functions of HPL is to cushion the impact of occlusal force during chewing. HPL cells contain heterogeneous populations which possess multilineage potential and may play a pivotal role in the regeneration of periodontium. Recently, there have been several reports describing HPL fibroblasts that have a capacity to regenerate cementum and bone depending on the microenvironment (9-11). Such findings suggest that cells derived from HPL may play an important role in maintaining homeostasis of the periodontium, in addition to their role in regeneration of periodontal tissue (9-11).

Follicular dendritic cell-secreted protein (FDC-SP) is expressed in primary FDCs isolated from human tonsils. FDC-SP has a very restricted tissue distribution and is expressed by activated FDCs from tonsils and tumor necrosis factor-α activated FDC-like cell lines, but not by B cell lines (12). FDC-SP is also expressed in the parotid gland, prostate gland, trachea, HPL, and junctional epithelium (JE) (13-15). FDC-SP is a small secretory protein, structurally similar to statherin, which has a role in inhibiting calcium phosphate precipitation in saliva (13-16). FDC-SP in the JE was lost 3 h after treatment with LPS, but was detected again after a few days (15). Overexpression of FDC-SP could inhibit osteogenic differentiation and stabilize the fibroelastic characteristics of HPL cells, increasing the expression of osteoclastogenesis-related gene and receptor activator of nuclear factor kappa-B ligand (RANKL)/osteoprotegerin (OPG) ratio (17-20). FDC-SP is decreased in gingiva, including in JE of experimental periodontitis in rats, concurrently with the increase of IL-17 pro-inflammatory expression and the RANKL/OPG mRNA ratio (21). Porphyromonas gingivalis LPS upregulated FDC-SP expression in HPL cells (20). FDC-SP may play an important role for the host defense system within the gingival crevice (15). FDC-SP could be involved in the inflammation of periodontal tissue; whereas, the biological role of FDP-SP in periodontal disease is still unknown. The purpose of this study was to investigate the effects of IL-1β on FDC-SP gene expression in HPL cells.

Materials and Methods

Reagents
Alpha-minimum essential medium (α-MEM), ISOGEN II, recombinant human IL-1β, and herbimycin A (HA), a tyrosine kinase inhibitor, were obtained from Wako (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from JR Scientific (Woodland, CA, USA). Lipofectamine 2000, penicillin, streptomycin, and TrypLE Express enzyme were purchased from Invitrogen (Carlsbad, CA, USA). The PrimeScript RT reagent kit (Perfect Real Time) and SYBR Premix Ex Taq II (Tli RNase H Plus) were obtained from Takara (Tokyo, Japan). Anti-rabbit IgG (whole molecule)-horseradish peroxidase antibody goat serum, protein kinase C (PKC) inhibitor H7, protein kinase A (PKA) inhibitor KT5720, complete protease inhibitor cocktail, and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma-Aldrich Japan (Tokyo, Japan). Clarity plus Western ECL Blotting Substrate (Bio-Rad, Hercules, CA, USA). The pGL3-basic vector, pSV-β-galactosidase (β-Gal) control vector, and mitogen-activated protein kinase (MEK1/2) inhibitor U0126 were purchased from Promega (Madison, WI, USA). Phosphatidylinositol 3-kinase (PI3-K) inhibitor (LY294002) was obtained from Calbiochem (San Diego, CA, USA), and QuikChange Site-Directed Mutagenesis Kit was from Stratagene (La Jolla, CA, USA).

Cell culture
Immortalized HPL cells (22,23) were established by infection of human telomerase reverse transcriptase (hTERT) catalytic subunit (HPL-hTERT) and cultured at 37°C in 5% CO₂ and 95% air in α-MEM containing 10% FBS. The HPL cells were grown to confluence in 60 mm culture dishes in α-MEM containing 10% FBS, then cultured for 12 h in α-MEM without FBS, and stimulated with IL-1β (1 ng/mL). Total RNA was purified from
triplicate cultures at 3, 6, 12, and 24 h after stimulation and analyzed for genes that were stimulated by IL-1β.

**Real-time polymerase chain reaction (PCR)**

Total RNAs were isolated using Isogen II from HPL cells and used as a template for complementary DNA (cDNA) synthesis. cDNA was prepared using the PrimeScript RT reagent kit. Quantitative real-time PCR was performed using the following primer sets: FDC-SP forward, 5'-GCCAGTCACCTGCCCATTCT-3'; FDC-SP reverse, 5'-GAGGCAGATCCAGGTATTGGA-3'; GAPDH forward, 5'-GCCAGTCACCTGCCCATTCT-3'; and GAPDH reverse, 5'-ATGGTGGTGAGACGCCAGT-3' using the SYBR Premix Ex Taq II in a TP800 thermal cycler dice real-time system (Takara). The amplification reactions were performed in a total volume of 25 μL 2x SYBR Premix Ex Taq II (12.5 μL), 10 μM forward and reverse primers, 50 ng cDNA for FDC-SP, and 10 ng cDNA for GAPDH (glyceraldehyde-3-phosphate dehydrogenase [human]). To reduce variability between replicates, PCR premixes containing all reagents except cDNA were prepared and allocated into 0.2 mL PCR tubes. The thermal cycling conditions were 10 s at 95°C, 40 cycles of 5 s at 95°C, and 30 s at 60°C. Post-PCR melting curves confirmed the specificity of single-target amplification, and the expression of FDC-SP relative to GAPDH was determined in triplicate.

**Western blotting**

For the Western blotting analysis, lysates of HPL cells were separated in 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a Hybond 0.2 µm polyvinylidene difluoride (PVDF) membrane (GE Healthcare, Pittsburgh, PA, USA). The membrane was then incubated for 3 h with anti-FDC-SP polyclonal antibody (PAB20711; Abnoba, Taipei City, Taiwan) and anti-α tubulin monoclonal antibody (sc-5286; Santa Cruz Biotechnology Inc., Paso Robles, CA, USA). Rabbit anti-mouse IgG, H & L chain specific peroxidase conjugate was used for the secondary antibodies (Sigma-Aldrich, St, Louis, MO, USA). Immunoreactivities were detected using Clarity plus Western ECL Blotting Substrate.

**Luciferase assays**

Exponentially growing HPL cells were used for transient transfection assays. Various lengths of human FDC-SP gene promoter sequences (−116FDCSP, −116 - +60; −210FDCSP, −210 - +60; −345FDCSP, −345 - +60; −501FDCSP, −501 - +60; −717FDCSP, −717 - +60; and −948FDCSP, −948 - +60) were prepared by PCR amplification. Several lengths of human FDC-SP gene promoters were cloned into the multi-cloning SaeI site of the pGL3 basic luciferase (LUC) plasmid. Twenty-four hours after plating, cells at 60%-80% confluence were transfected using Lipofectamine 2000. The transfection mixture included 1 μg LUC plasmid and 2 μg β-Gal plasmid as a transfection control. Two days after transfection, the cells were cultured in α-MEM-deprived serum for 12 h, then stimulated with 1 ng/mL IL-1β for 12 h prior to harvest. The LUC activities were measured in accordance with the supplier’s protocol using a luminescence reader (AccuFlex Lumi 400; Aoka, Tokyo, Japan). β-Gal activity was separately determined to normalize the values. Mutation LUC constructs, mutation Ying Yang 1 (YY1) (−345mYY1, TTgAAgATAgTG), mutation GATA (−345mGATA, AAAAAgTTgTG), mutation CAAAT enhancer binding protein 2 (C/EBP2) (−345mCEBP2, AATAgATAgTG), mutation C/EBP3 (−345mCEBP3, GGTTgATgATgTG), and double mutation in C/EBP2 and C/EBP3 (−345mCEBP2 + mCEBP3) were made using the QuikChange Site-Directed Mutagenesis Kit within the context of the homologous −345 +60 FDC-SP promoter fragment. All LUC constructs were sequenced to verify the fidelity of the mutagenesis. Several types of protein kinase inhibitors were used for protein kinase inhibition. Two days after transfection, the cells were deprived of serum for 12 h, treated with PKC inhibitor H7 (5 μM), PKA inhibitor KT5720 (100 nM), MEK1/2 inhibitor U0126 (5 μM), and LY249002 (10 μM) for 30 min, or HA (1 μM) for 4 h, then incubated with IL-1β (1 ng/mL) for 12 h before harvesting.

**Gel shift analysis**

Confluent HPL cells incubated for 3, 6, and 12 h with IL-1β (1 ng/mL) in α-MEM without FBS were used to prepare the nuclear extracts. Double-stranded oligonucleotides encompassing the 5'-Cy5-labeled YY1, GATA, C/EBP2, and C/EBP3 sequences in the human FDC-SP promoter were prepared. Nuclear proteins (3 μg) were used for 20 min at room temperature with 2 pM Cy5-labeled YY1, GATA, C/EBP2, and C/EBP3 sequences in the human FDC-SP promoter were prepared. Nuclear proteins (3 μg) were incubated with 2 pM Cy5-labeled double-stranded oligonucleotide in a buffer containing 50 mM potassium chloride (KCl), 0.5 mM ethylenediaminetetraacetic acid (EDTA), 10 mM Tris-hydrochloride (HCl) (pH 7.9), 1 mM dithiothreitol, 0.04% octylphenoxypolyethoxy-ethanol (Nonidet P-40), 5% glycerol, and 1 μg of poly (deoxyinosinic-deoxycytidylic acid) sodium salt (poly dl-dc). After incubation, the DNA-protein complexes were separated by electrophoresis in 5% non-denaturing acrylamide gels run at 200 V at room temperature. After electrophoresis, the gels were scanned using a
Typhoon TRIO+ Variable Mode Imager (GE Healthcare). For competition experiments, 40-fold molar unlabeled oligonucleotides of YY1, GATA, C/EBP2, and C/EBP3 were used. The double-stranded oligonucleotide sequences were: YY1 For, 5'-CCAATTTCA-CATATGGAGATAGTTGA-3'; YY1 Rev, 5'-GATCTCAACTATCTCAATATGTGAAATTG-3'; GATA For, 5'-CCACAAAAGATTTTGGTATTTA-3'; GATA Rev, 5'-GATCTAAATACCAAAATCTTTTGTG-3'; C/EBP2 For, 5'-CGTAGACCAATGGAGCAACAAAGCCATGTCTACG-3'; C/EBP2 Rev, 5'-GATCTACATGTTGTTGTGTCATGTA-3'; C/EBP2 For, 5'-CGTAGACCAATGGAGCAACAAAGCCATGTCTACG-3'; C/EBP3 For, 5'-CAATATTACTATCTCAATATGTTGAAATTG-3'; and C/EBP3 Rev, 5'-GATCTAAATACCAAAATCTTTTGTG-3'.

**Chromatin immunoprecipitation (ChIP) assays**

To identify interactions between specific transcription factors and human FDC-SP gene promoters in vivo, ChIP assays were carried out using HPL cells. Confluent HPL cells in 100 mm dishes were stimulated by IL-1β (1 ng/mL) for 0, 3, 6, 12, and 24 h. The cells were fixed with 100 µL formaldehyde for 10 min to crosslink the protein-DNA complexes. The fixed cells were sonicated twice by a wash buffer (1 mM PMSF, and complete protease inhibitor cocktail in H2O), centrifuged for 5 min at 4°C. After the cells were resuspended in a 10-fold ChIP dilution buffer (0.01% SDS, 0.01 M EDTA, 0.05 M Tris-HCl, pH 8.1), lysate was sonicated to shear the protein-fragmentary DNA complexes. Sonicated cell supernatants were diluted in a 10-fold ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.9, 16.7 mM NaCl, 1 mM PMSF, and complete protease inhibitor cocktail in H2O). The diluted supernatants were assigned as a control which included non-specific protein-DNA complexes and precleared with 80 µL salmon sperm DNA/Protein A Agarose (50% slurry) for 30 min at 4°C with gentle agitation. For the immunoprecipitation of protein-DNA complexes, 2 µg of rabbit polyclonal anti-YY1 antibody (ab38422, Abcam, Cambridge, UK), GATA-6 (D61E4) XP Rabbit mAb (#5851, Cell Signaling Technology, Danvers, MA, USA), C/EBPβ (Δ 198) (SC-746, Santa Cruz Biotechnology, Inc.), and the appropriate unconjugated normal rabbit anti-IgG antibody (SC-2027, Santa Cruz, Biotechnology, Inc.) were used for 300 µL of precleared supernatant and incubated overnight at 4°C with constant rotation. Sixty µL of salmon sperm DNA/Protein A Agarose (50% slurry) was added for 1 h at 4°C with rotation to collect the antibody/histone complexes and pellet agarose by gentle centrifugation (1,000 rpm for 1 min). After removing the supernatant that contained unbound chromatin, the pellet was washed twice with 1 mL each of Low Salt buffer (0.1% SDS, 1% Triton-X100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl in double-distilled water [ddH2O]), High Salt buffer (0.1% SDS, 1% Triton-X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl in H2O), lithium chloride (LiCl) buffer (0.25 M LiCl, 0.1% NP-40, 1% deoxycholate, 0.5 mM EDTA, 0.01 M Tris-HCl, pH 8.1 in ddH2O), and 1 mL of Tris and EDTA (TE) buffer (10 mM Tris-HCl, pH 8.1, 1 mM EDTA in H2O). After the TE buffer was removed, Protein A Agarose/antibody/chromatin complexes were resuspended in a 250 µL elution buffer (1% SDS and 0.1 M NaHCO3 in H2O) and incubated at room temperature for 15 min with gentle rotation. After the spin down of the agarose beads, 20 µL 5 M NaCl was added to the supernatant for reverse cross-links, and 10 µL 0.5 M EDTA, 20 µL 1 M Tris-HCl, pH 6.5, and 1 µL 10 mg/mL proteinase K were added to the degradation of the antibodies and proteins. DNA was recovered by phenol/chloroform/isoamyl alcohol extraction and ethanol precipitation. The purified DNA was subjected to PCR amplification (1 cycle, 95°C for 3 min; amplification was performed for 35 cycles, denatured at 95°C, 15 s; 59 or 60°C, 15 s; 72°C, 1 min; and final extension was at 72°C, 1 min) mainly for the YY1, GATA, and C/EBP response elements within the human FDC-SP promoter using primer sets. In addition, KT5720 (100 nM), HA (1 µM), U0126 (5 µM), and LY249002 (10 µM) were used for protein kinases inhibition and stimulated with IL-1β (1 ng/mL) for 12 h to extract protein-DNA complexes. KAPA Taq EXtra HotStart was utilized for the PCR procedure, and the PCR products were separated on 2% agarose gels and visualized with ultraviolet light. Human FDC-SP promoter used the primers YY1 ChIP For, 5'-GTATTTTG-GTAGTTTTCATAGGGA-3'; YY1 ChIP Rev, 5'-GGCTTA-AAGGCTTCTCCCCCTTTCCCT-3'; GATA ChIP For, 5'-CATGTA-AAATGAGTTAATGTTTCCTTCTCCCCTCTTCCCTTTCCCT-3'; and GATA ChIP Rev, 5'-GATCTAAATACCAAAATCTTTTGTG-3'.

**Statistical analysis**

Triplicate samples were analyzed for each experiment, and experiments were replicated to ensure the consistency of the responses to drugs. Significant differences between the control and treatment groups were determined using one-way ANOVA.
Results

Effects of IL-1β on the expression of FDC-SP mRNA and protein levels in immortalized HPL cells

To study the effects of IL-1β on FDC-SP mRNA and protein levels in immortalized HPL cells, real-time PCR was performed using total RNA extracted from HPL cells. First, dose-response effects of IL-1β on the FDC-SP mRNA levels were studied by treating HPL cells with different concentrations of IL-1β for 12 h. IL-1β induced FDC-SP mRNA expressions at 1, 10, and 50 ng/mL (Fig. 1A). IL-1β (1 ng/mL) increased FDC-SP mRNA levels at 3 h and reached maximum at 12 h (Fig. 1B). IL-1β (1 ng/mL) induced FDC-SP protein levels at 3 h and reached maximum at 12 h and 24 h in HPL cells (Fig. 1C).

Luciferase assays for human FDC-SP gene promoter constructs

Subsequent studies were performed to investigate the transcriptional regulation of human FDC-SP by IL-1β in HPL cells. Transient transfection of human FDC-SP gene promoter constructs encompassing various-sized human FDC-SP gene promoters (−116FDCSP, −210FDCSP, −345FDCSP, −501FDCSP, −717FDCSP, −948FDCSP) was performed in HPL cells. The results of LUC assays showed that LUC activity of −116FDCSP was increased by IL-1β (1 ng/mL, 12 h). IL-1β-induced LUC activity of −210FDCSP was significantly higher than the activity of −116FDCSP, and IL-1β-induced LUC activity of −345FDCSP was significantly higher than the activity of −210FDCSP (Fig. 2). Promoter sequence of human FDC-SP gene from +23 to −355 contained Yin Yang 1 (YY1, nucleotides [ns] −69 to −53), inverted CCAAT box (nts −92 to −81), GATA (nts −122 to −106), CCAAT-enhancer-binding protein 1 (C/EBP1, nts −143 to −133), octamer-binding protein 1 (Oct-1, nts −159 to −146), C/EBP2 (nts −194 to −181), CAMP response element (CRE, nts −224 to −213), and C/EBP3 (nts −282 to −269) elements (Fig. 3). Next, we introduced 3-bp mutations within the −345FDCSP constructs in the YY1, GATA, C/EBP2, and C/EBP3 elements targeted by IL-1β. When mutation −345FDCSP constructs were used, LUC
activities induced by IL-1β were partially inhibited in the −345mYY1, −345mGATA, −345mC/EBP2, and −345mC/EBP3, and double mutation in −345mC/EBP2 and −345mC/EBP3 (−345 mC/EBP2 + mC/EBP3) (Fig. 4). These results suggested that YY1, GATA, C/EBP2, and C/EBP3 acted as functional response elements through which IL-1β regulated FDC-SP gene transcription. IL-1β-induced −345FDCSP activity was inhibited by PKA inhibitor KT5720, tyrosine kinase inhibitor HA, MEK1/2 inhibitor U0126, and PI3-K inhibitor LY249002. On the other hand, PKC inhibitor H7 could not inhibit the IL-1β-induced −345FDCSP activity (Fig. 5).

**Gel shift assay**

To identify the nuclear proteins that bind to YY1, GATA, C/EBP2, and CEBP3, double-stranded oligonucleotides were labeled with Cy5 and incubated with nuclear proteins (3 μg) extracted from HPL cells, which were stimulated with or without 1 ng/mL IL-1β for 0, 3, 6, and 12 h. With the nuclear extract from confluent control culture of HPL cells, shifts of YY1, GATA, C/EBP2, and C/EBP3-protein complexes were evident. After stimula-
tion by IL-1β (1 ng/mL), YY1, GATA, C/EBP2, and C/EBP3-protein complexes were increased at 3 h. YY1 and C/EBP3-protein complexes reached maximum at 6 h and 12 h. GATA-protein complex reached maximum at 12 h, and C/EBP2-protein complex did not increase after 12 h (Fig. 6). These DNA-protein complexes represent how specific interactions were confirmed by competition gel shifts using 40-fold molar excess of non-labeled YY1, GATA, C/EBP2, and CEBP3 to reduce DNA-protein complex formations (Fig. 7, lanes 3, 7, 11, and 15). GATA, YY1, and C/EBP2 could not compete with YY1, GATA, and C/EBP3-protein complex formations (Fig. 7, lanes 4, 8, and 16); however, C/EBP3 competed with the C/EBP2-protein complex formations (Fig. 7, lane 12).

Chromatin immunoprecipitation (ChIP) assays
ChIP assays were performed using HPL cells to study the in vivo interaction between specific transcription factors and YY1, GATA, C/EBP2, and C/EBP3 elements in the human FDC-SP gene promoter. YY1, GATA, and C/EBPβ transcription factors interacted with chromatin fragments containing YY1, GATA, C/EBP2, and C/EBP3. These interactions were increased by IL-1β (1 ng/mL) in a time dependent manner (Fig. 8). To investigate which signaling pathways can regulate YY1, GATA, and C/EBPβ bindings to YY1, GATA, C/EBP2, and C/EBP3 elements after stimulation by IL-1β, the PKA inhibitor KT5720, tyrosine kinase inhibitor HA, MEK1/2 inhibitor U0126, and PI3K inhibitor LY294002 were used with or without IL-1β treatment. YY1, GATA, and C/EBPβ binding to YY1, GATA, C/EBP2, and C/EBP3 were inhibited by KT5720, HA, U 0126, and LY294002 (Fig. 9).

Discussion
Among the most potent molecules of the innate immune system are the IL-1 family members. The IL-1 family is comprised of 11 members, possesses a conserved gene structure, and changes its physiological activities upon enzymatic degradation. IL-1β and IL-18 have pro-domains at their amino termini, which require cleavage by a protein assembly known as the inflammasome to generate the biologically active forms and to be secreted. Secreted IL-1β and IL-18 act on a variety of cells and mainly induce a Th1-type inflammatory response and neutrophil migration to infected loci (24,25).

In this study, IL-1β response regions that positively regulate human FDC-SP gene transcription in HPL cells are identified for the first time. The HPL is a very thin sheet-like structure that supports a tooth and connects it to alveolar bone. FDC-SP mRNA and protein levels are increased by IL-1β in HPL cells within 12 h (Fig. 1). IL-1β increases human FDC-SP gene transcription in HPL cells by targeting YY1, GATA, C/EBP2, and C/EBP3 elements in the human FDC-SP gene promoter. From the results of LUC assays, the region of IL-1β responding to the
proximal promoter (−116FDCSP) of the human FDC-SP gene (Fig. 2) is located, which contains YY1 and half of GATA response elements (Fig. 3). GATA and C/EBP2 elements are contained within −210FDCSP, and C/EBP3 is present in −345FDCSP (Fig. 2 and 3). LUC activities of −210FDCSP and −345FDCSP after stimulation by IL-1β are significantly higher than the IL-1β-induced −116FDCSP activity (Fig. 2). The results indicate that these four response elements (YY1, GATA, C/EBP2, and C/EBP3) are crucial for IL-1β-induced FDC-SP gene transcription. Transcriptional regulation by IL-1β is partially abrogated when 3-bp mutations within the −345FDCSP are constructed in YY1, GATA, C/EBP2, and C/EBP3 (Fig. 4). The involvement of YY1, GATA, C/EBP2, and C/EBP3 elements is further supported by gel shift assays in which nuclear proteins that form complexes with YY1, GATA, C/EBP2, and C/EBP3 are increased by IL-1β (Fig. 6). Competition gel shift assays show that YY1, GATA, C/EBP2, and C/EBP3-protein complex formations are specifically binding. Interestingly, a 40-fold molar excess of C/EBP3 competes with C/EBP2/protein complex formation; whereas, C/EBP2 cannot compete with the formation of C/EBP3-protein complex (Fig. 7), suggesting that the composition of C/EBP2- and C/EBP3-binding proteins are not the same.

YY1 can function as a transcriptional activator or repressor, and it can regulate normal biologic processes such as differentiation, replication, proliferation, and embryogenesis (26). YY1 is overexpressed in rheumatoid arthritis (RA) and is positively correlated with serum C-reactive protein and erythrocyte sedimentation rate in RA (27). GATA transcription factors (GATA-1 - 6) recognize the (A/T)GATA(A/G) motif, and GATA-4/5/6 are expressed in the cardiovascular system and in endoderm-derived tissues including the liver, lungs, pancreas, and gut (28). Targeted inhibition of GATA-6 attenuates airway inflammation and remodeling by regulating the protein caveolin-1 through toll-like receptor 2/myeloid differentiation primary response 88 (TLR2-MyD88) and nuclear factor (NF)-κB pathway (29). C/EBPs are a family of leucine zipper transcription factors involved in the regulation of various aspects of cellular differentiation, and they function in a variety of tissues (30). CEBPβ, a transcription factor required for the selective expression of an inflammatory gene, is a key activator of the IL-36α gene in murine macrophages (31). Tumor necrosis factor (TNF)-α promotes nuclear localization of C/EBPβ and C/EBP delta (δ) in response to inflammatory stress (32). Therefore, in this study, YY1, GATA, and C/EBPβ may have important roles in the regulation of human FDC-SP gene expression during the inflammation process.

The FDC-SP gene exists in chromosome 4q13 adjacent to proline-rich peptides from saliva and C-X-C chemokine receptors (12). FDC-SP is a secretory protein consisting of 85 amino acids that has a unique proline-rich region at C-terminal half. This proline-rich region shows a high degree of hydrophobicity in contrast to the highly conserved region in the N-terminal. The structure of FDC-SP is very similar to salivary protein statherin which is a small phosphoprotein consisting of 62 amino acids (13). FDC-SP is expressed in the tonsils, parotid gland, prostate gland, trachea, HPL, and JE (13-15). It can adsorb on the surface of bone and cementum adjacent to the HPL and onto tooth surface at the JE (14). FDC-SP in the JE is lost 3 h after treatment with LPS, but it is detected again after a few days (15). LPS from P. gingivalis increases the expression of FDC-SP in HPL cells (20). FDC-SP in JE is more strongly observed in P. gingivalis infected mice at early stage by immunostaining, but at the following stage, FDC-SP expression decreases due to severe inflammation (33). FDC-SP overexpression can inhibit osteogenic differentiation and stabilize the fibroblastic characteristics of HPL cells (17-19). These results suggest that FDC-SP may play an important role for the host defense system and for maintenance of the JE and HPL. Further study is necessary to elucidate the precise function of FDC-SP in the periodontium.

In conclusion, IL-1β increases FDC-SP gene transcription in HPL cells mediated through YY1, GATA, C/
EBP2, and C/EBP3 elements in the human FDC-SP gene promoter via PKA, tyrosine kinase, MEK1/2, and PI3-K pathways.

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Conflict of interest
The authors declare no conflict of interest.

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