Td92, an outer membrane protein of *Treponema denticola*, induces osteoclatogenesis via PGE_2 mediated RANKL/OPG regulation

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Td92, an outer membrane protein of *Treponema denticola*, induces osteoclatogenesis via PGE_2 mediated RANKL/OPG regulation

Directed by Professor Yun-Jung Yoo

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사랑하는 베프 수영아, 결혼축하해!! 우린 결혼도 비슷하게 하는구나~
영화 죽은시인의사회의 한국판 주인공이라 할 수 있는 김성일 선생님. 학생들에 대한 식지 않는 가르침의 열정은 대한민국에서 가장 뜨거우실 것 같습니다. 학생으로서 느꼈던 그 감동은 10년이 지난 지금도 생생합니다. 선생님께 이 논문을 드릴 수 있어 기쁩니다.
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ABSTRACT

Td92, an outer membrane protein of *Treponema denticola*, induces osteoclatogenesis via PGE_2 mediated RANKL/OPG regulation

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(Directed by Professor Yun-Jung Yoo)

Periodontitis is a chronic inflammatory disease in periodontium which causes significant alveolar bone loss. Osteoclasts are bone-resorbing multinucleated cells. Osteoblasts regulate osteoclast differentiation by receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) expression.

Treponema denticola is one of the oral bacteria involved in periodontitis. Td92, Tp92 homolog of *T. denticola*, is a surface-exposed outer membrane protein that stimulates production of various proinflammatory mediators. However, the role of Td92 on alveolar bone resorption still remains unclear. To elucidate the role of Td92 on bone resorption, the effect of Td92 on osteoclast differentiation was evaluated in co-cultures of mouse calvariae-derived osteoblasts and bone marrow cells. The expression of RANKL, OPG, and PGE_2 in osteoblasts was estimated by ELISA. Td92 induced osteoclast formation in co-cultures. In osteoblasts, RANKL and PGE_2 expression was up-regulated while OPG expression was down-regulated by Td92. OPG inhibited Td92-induced osteoclast formation. NS398 or indomethacin, prostaglandin synthesis inhibitors, also inhibited Td92-induced osteoclast formation. The effect of Td92 on expressions of RANKL, OPG, and PGE_2 in osteoblasts was blocked by NS398 or indomethacin. These results suggest that Td92 promotes osteoclast formation through the regulation of RANKL and OPG productions via PGE_2 dependent mechanism.

Key words: *Treponema denticola*, Td92, osteoclast formation, periodontitis

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I. INTRODUCTION

Osteoclasts are tartrate-resistant acid phosphatase (TRAP) positive multinucleated cells with bone-resorbing activity. The osteoclast formation is induced by receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) expressed by osteoblast/stromal cells (1, 2). In bone, RANKL is an essential cytokine for osteoclastogenesis and it presents

in soluble- and membrane-bound form (3). RANKL binds to RANK, a receptor of RANKL, expressed on osteoclast precursors and by this interaction, osteoclast precursors differentiate into osteoclasts in the presence of macrophage-colony stimulating factor (M-CSF). Osteoprotegerin (OPG) produced by osteoblasts blocks osteoclastogenesis by interfering RANKL-RANK interaction (4). The levels of RANKL and OPG expression are regulated by several bone-resorbing factors such as 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2 D_3$), parathyroid hormone (PTH), prostaglandin E_2 (PGE_2) and lipopolysaccharide (LPS).

Periodontitis is a chronic inflammatory disease in the periodontal tissue with bacterial etiology. Alveolar bone is a periodontal tissue to support teeth. Also, alveolar bone loss observed in periodontitis is a non-reversible condition induced by stimulation of osteoclast formation. Therefore, understanding the mechanisms of bone resorption induced by periodontal pathogens can be an important knowledge for the prevention and treatment of periodontitis. It was reported that many of periodontal pathogens, such as

Actinobacillus actinomycetemcomitans, *Porphyromonas gingivalis*, and *Prevotella nigrescens* show the activity of bone resorption. LPS of *P. gingivalis* or *P. nigrescens* also have been implicated as an inducer of osteoclast formation in periodontitis (5, 6). *A. actinomycetemcomitans* has several osteoclastogenic factors such as LPS, capsular polysaccharides (CPs), and chaperone (cpn60) (7). Therefore, various components of oral bacteria including LPS, appear to be a potent activator of osteoclastogenesis in periodontitis (8-11).

An elevated level of *Treponema* species in subgingival plaque of periodontitis patients supports the hypothesis that *Treponema* species play an important role in periodontitis. There are 10 species of oral spirochetes which can be cultivated up to present time and *Treponema denticola* is one them. *T. denticola* is known to be involved in the early-onset periodontitis, chronic periodontitis, and necrotizing ulcerative gingivitis (12). *T. denticola* has various virulence factors such as proteolytic enzymes and cytolytic factors which involved in pathogenesis of periodontitis (13-17). Tp92 is a 92kDa *T.*

pallidum antigen that resides on the treponemal surface and it has been reported to have immunoprotective capabilities of inducing opsonization and phagocytosis, thus may be a useful vaccine candidate for syphilis (18). Tp92 homologs (88 to 92 kDa) were recently reported as highly conserved surface proteins of four representative oral spirochetes (*Treponema denticola, T. lecithinolyticum, T. maltophilum, and T. socranskii* subsp. s*ocranskii*) and they were shown to have amino-acid sequence identities of 37.9 to 49.3％ and similarities of 54.5 to 66.9％ to Tp92 (19). Tp92 homologs have been demonstrated to contribute to cell attachment, inflammation, and tissue destruction by inducing various proinflammatory factors. Td92 is a Tp92 homolog of *T. denticola* that is a highly conserved surface protein of *T. denticola* (19). Td92 is considered to be involved in cytopathogenic process of periodontal disease through binding to epithelial cells and inducing the expression of pro-inflammatory factors such as Interleukin (IL)-6, IL-8, COX-2, and PGE_2 in human monocytic cell line and periodontal ligament (PDL) cells (19). However, it is not yet to be identified for the role of Td92 in bone

resorption. Therefore, the effect of Td92 on osteoclast formation was evaluated in co-cultures of mouse calvaria-derived osteoblasts and bone marrow cells. Also, the involvement of RANKL, OPG, and PGE_2 in Td92-induced osteoclast formation was evaluated in osteoblast culture.

II. MATERIALS AND METHODS

1. Chemicals

LPS (*Escherichia coli* O26:B6), Indomethacin, CelLytic™ M, and Protease Inhibitor Cocktail were purchased from Sigma (St. Louis, MO, USA). NS398 was purchased from Calbiochem (San Diego, CA, USA). Human rM-CSF and Human rOPG were obtained from Peprotech (Rocky Hill, NJ, USA). Collagenase was purchased from Wako pure Chemicals (Osaka, Japan). Minimum Essential Medium alpha (α-MEM), Dispase, Fetal bovine serum (FBS), Dulbecco's phosphate-buffered Saline (PBS), 100X Antibiotic-Antimycotic (Ab), and 25% Trypsin-EDTA were purchased from Gibco BRL (Grand Lsland, NY, USA). The chemicals used for TRAP staining is as follows: Sodium Acetate Trihydrate, Fast Red Violet LB Salt, and Naphthol AS-MX phosphate were purchased from Sigma (St. Louis, MO, USA). Sodium (+)- Tartrate Dihydrate was purchased from Wako pure Chemicals (Osaka, Japan). Acetic Acid was purchased Junsei Chemicals (Tokyo, Japan).

Mice were obtained from Sankyo Laboratory Animal Center (Tokyo, Japan). Animal studies were performed after the experimental protocols approved by animal ethics committee of Yonsei University College of Dentistry.

2. Preparation of Td92

Recombinant Tp92 homolog of *T. denticola* (Td92) was thankfully given from B.K. Choi in Seoul National University. The Tp92 gene homolog of T. denticola was amplified from the genomic DNA by PCR. PCR was performed in a total volume of 50 μl containing 15 pmol of each primer, 1.25 U of Ex *Taq* polymerase (Perkin Elmer Cetus, Foster City, CA). The PCR products were cloned in *E. coli* by using the TA cloning vector pCR2.1-TOPO, and the inserts were isolated and cloned in *E. coli* M15 by using the expression vector pQE-30 as described previously (20). After the induction of *E. coli* with 1mM isopropyl-β-D-thiogalactopyranoside (IPTG), histidine-tagged recombinant proteins were purified by sonication, solubilizaiton with a detergent, and subsequent renaturation, followed by affinity chromatography using nickel-

nitrilotriacetic acid agarose (Qiagen, Valencia, CA) as described previously (20). Endotoxin present in *E. coli*, which may potentially contaminate the recombinant proteins, were removed using polymyxinB-agarose according to the instructions of the manufacturer (Sigma chemicals Co., St. Louis, MO, USA). The endotoxin decontamination of Td92 was verified using CHO/CD14/TLR4cells.

3. SDS-PAGE and immunoblot assay

The expression of the Td92 in *E. coli* was verified by immunoblotting using a monoclonal mouse antihistidine Ab (Qiagen, Alencia, CA, USA). *E. coli* M15 cells transformed with the recombinant plasmids were cultured in Luria-Bertani broth containing antibiotics and induced with IPTG. The *E. coli* lysates (20 μg of protein) were subjected to SDS-PAGE and subsequently transferred onto nitrocellulose membranes. The membranes were blocked with 2% bovine serum albumin (BSA) for 1h and allowed to react with antihistidine Ab for 1h. After being washed with PBS-0.2% Tween 20, the membranes were allowed

to react with alkaline phsphatase-labeled anti-mouse IgG for 1 h. After being washed with PBS-0.2% Tween 20, the membranes were developed with 5-bromo-4-chloro-3-indolylphosphate (165 μg/ml) and nitroblue tetrasolium (330 μg/ml). The expected molecular size of the prepared Td92 was approximately 92kDa (Fig. 1).

4. Preparation of primary osteoblasts

Mouse osteoblasts were isolated and cultured as described previous studies (21, 22). Twenty-five to thirty newborn ddY mice (1-day-old) were used for one preparation of osteoblasts. Mice were sacrificed in 70% alcohol and calvariae including frontal and parietal bones were detached anatomically. Calvariae were then collected in α-MEM and washed in 10 ml of α–MEM containing 0.2% collagenase and 0.1% dispase briefly to remove debris and blood cells. Calvariae were incubated in collagenase-dispase solution for 10 min at 37℃, in a 250 x *g* shaking water bath. The first supernatant was discarded and 10 ml of fresh solution was added and incubated at 37℃, in a 250 x *g* shaking water

bath for 20 min. The supernatant was collected and further incubation with fresh solution was repeated for four times. The last four supernatants were collected as a primary osteoblast population. Primary osteoblasts were cultured in α-MEM supplemented with 10% FBS and 1% Ab mixture for 3 days. Cells were detached by trypsin-EDTA, centrifuged, suspended in 90% FBS and 10% dimethyl sulfoxide, and stored at -80℃.

5. Osteoclast formation assay

Primary osteoblasts stored at -80 ℃ were cultured in α-MEM supplemented with 10% FBS and 1% Ab. Bone marrow cells were obtained from tibiae of 6-wk-old male ddY mice and cultured in the presence of M-CSF (50 ng/ml) for 16 hours prior to co-culture. Primary osteoblasts (8×10^3 cells) were co-cultured with bone marrow cells (8×10^4 cells) for 6 days in 200 μl α-MEM containing 10% FBS and 1% Ab in 96-well plates (NUNC, Denmark). Co-cultures were treated with 1-12.5 μg/ml Td92 or 0.001-1 μg/ml LPS in the absence or presence of OPG (100 ng/ml), NS398 (1 μM) or indomethacin (1

μM). The medium was refreshed on 3rd day. Osteoclast formation was evaluated by TRAP, a marker enzyme of osteoclasts, staining. TRAP staining was performed as described previously (23). Cells were fixed with 10% formaldehyde and with ethanol/acetone (1:1) solution. 100 μl of TRAP staining solution was added to each well and stained for 5 min. TRAP positive multinucleated cells containing more than 3 nuclei were counted as osteoclasts. The results obtained from a typical experiment of three independents are expressed as the mean ± SD of three cultures.

6. ELISA for cytokines and PGE_2

Primary osteoblasts ($2x10^4$ cells) were cultured in α-MEM containing 10% FBS and 1% Ab medium in 48-well culture plate until confluence. Cells were then further incubated in the presence or absence of Tp92 homolog (10 μg/ml) or LPS (0.01 μg/ml) for 3 days. Some cultures were treated in combination with NS398 (1 μM) or indomethacin (1 μM). The cell lysates were harvested to determine the concentration of RANKL and culture supernatants were used

to determine the concentration of OPG or PGE_2. To collect cell lysates, cultures wells were washed with DPBS and treated with CelLytic™ M for 15 min. The collected cells were centrifuged at 16,000 x *g* for 15 min to pellet cellular debris. Each sample was quantified using Bio-Rad Protein Assay kit prior to ELISA. The results obtained from a typical experiment of three independents are expressed as the mean ± SD of two cultures.

7. Statistical analysis

Statistical analysis was performed by a Student's *t*-test of Sigma Plot 8.0 to express the difference between the two groups. *P* value < 0.05 was considered to be statistically significant.

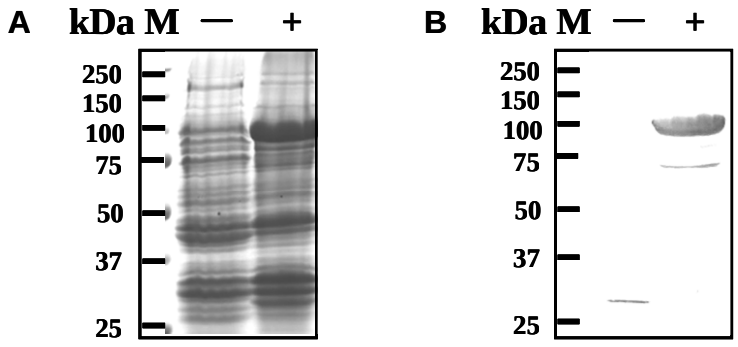

Figure 1. Expression of *td92* in *E. coli.* Expression of histidine-tagged recombinant proteins was analyzed by SDS-PAGE (10% polyacrylamide gel) (A) and immunoblot assay with anti-histidine antibody (B). M: Protein size marker; — : uninduced *E. coli* cell lysates; +: IPTG-induced *E. coli* cell lysates.

II. RESULTS

1. Effect of Td92 on osteoclast formation

To determine the effect of *T. denticola* Tp92 homolog (Td92) on osteoclast formation, co-cultures composed of osteoblasts and bone marrow cells were treated with Td92 (1 to 10 μg/ml), and TRAP-positive multinucleated cells were counted. Td92 stimulated the formation of TRAP-positive osteoclasts and the maximal number of osteoclasts was observed at 5-10 μg/ml (Fig. 2A and B). LPS, a positive control, showed the maximal effect of osteoclast formation at a concentration of 0.01-0.1 μg/ml (Fig. 2A and C). Endotoxin decontamination of Td92 was verified by heat treatment of Td92 at 99°C for 30 min. Heated Td92 (10 μg/ml) did not induce osteoclast formation while heated LPS still induced osteoclast formation in a similar manner with none-heated LPS (Fig. 2D), suggesting Td92 is a heat labile stimulator of osteoclast formation.

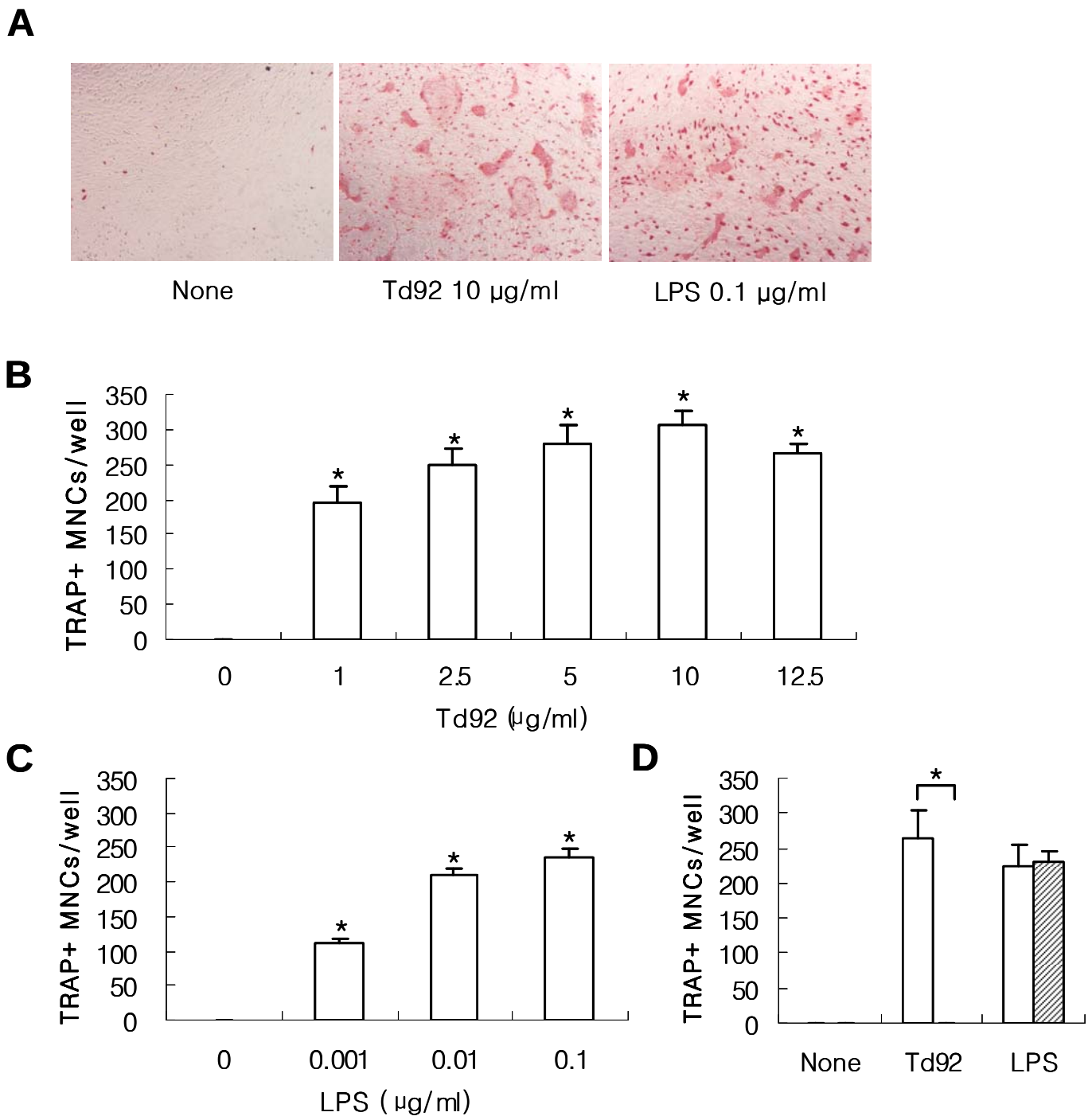

Figure 2. Effect of Td92 on osteoclast formation. Mouse calvariae-derived osteoblasts and bone marrow cells were co-cultured in the absence or presence of Td92 (1-12.5 μg/ml) or LPS (0.001-0.1 μg/ml) for 6 days and the cells were stained for TRAP (A). TRAP-positive multinucleated cells containing more than three nuclei were counted as osteoclasts (B, C). *, $P < 0.05$ *vs*. none-treated cells. Co-cultures were treated with Td92 (10 μg/ml, open bar), heated Td92 (10 μg/ml, oblique lined bar), LPS (0.1 μg/ml, open bar) or heated LPS (0.1 μg/ml, oblique lined bar) for 6 days (D). TRAP-positive multinucleated cells containing more than three nuclei were counted as osteoclasts. *, $P < 0.05$ *vs*. Td92 or LPS treated-cells.

2. Effect of Td92 on RANKL and OPG expression in osteoblasts

In order to assess the effect of Td92 on RANKL and OPG expression, Td92 (10 μg/ml) or heated Td92 was treated in osteoblasts. Also, the levels of RANKL in cell lysates and OPG in culture supernatants were assayed by ELISA. Td92 increased RANKL expression similar to LPS and its expression was 4.7-fold higher compared to none-treated group (Fig. 3A). Heated Td92 did not increase RANKL expression. In contrast to Td92, heat treatment of LPS did not block the LPS-induced RANKL expression. Td92 decreased OPG expression similar to LPS and its expression was 2.4-fold lower compared to none-treated group (Fig. 3B). Heated Td92 recovered the Td92-induced OPG decrease. In contrast to Td92, heat treatment of LPS did not recover the LPS-induced down-regulated OPG expression (Fig. 3C). To confirm the involvement of RANKL in Td92- induced osteoclast formation, co-cultures were treated with Td92 in the presence or absence of OPG. The osteoclast formation stimulated by Td92 was completely inhibited by the addition of OPG (Fig. 3D). These results suggest that the expression level of RANKL/

OPG in osteoblasts is crucially involved in Td92-stimulated osteoclast formation.

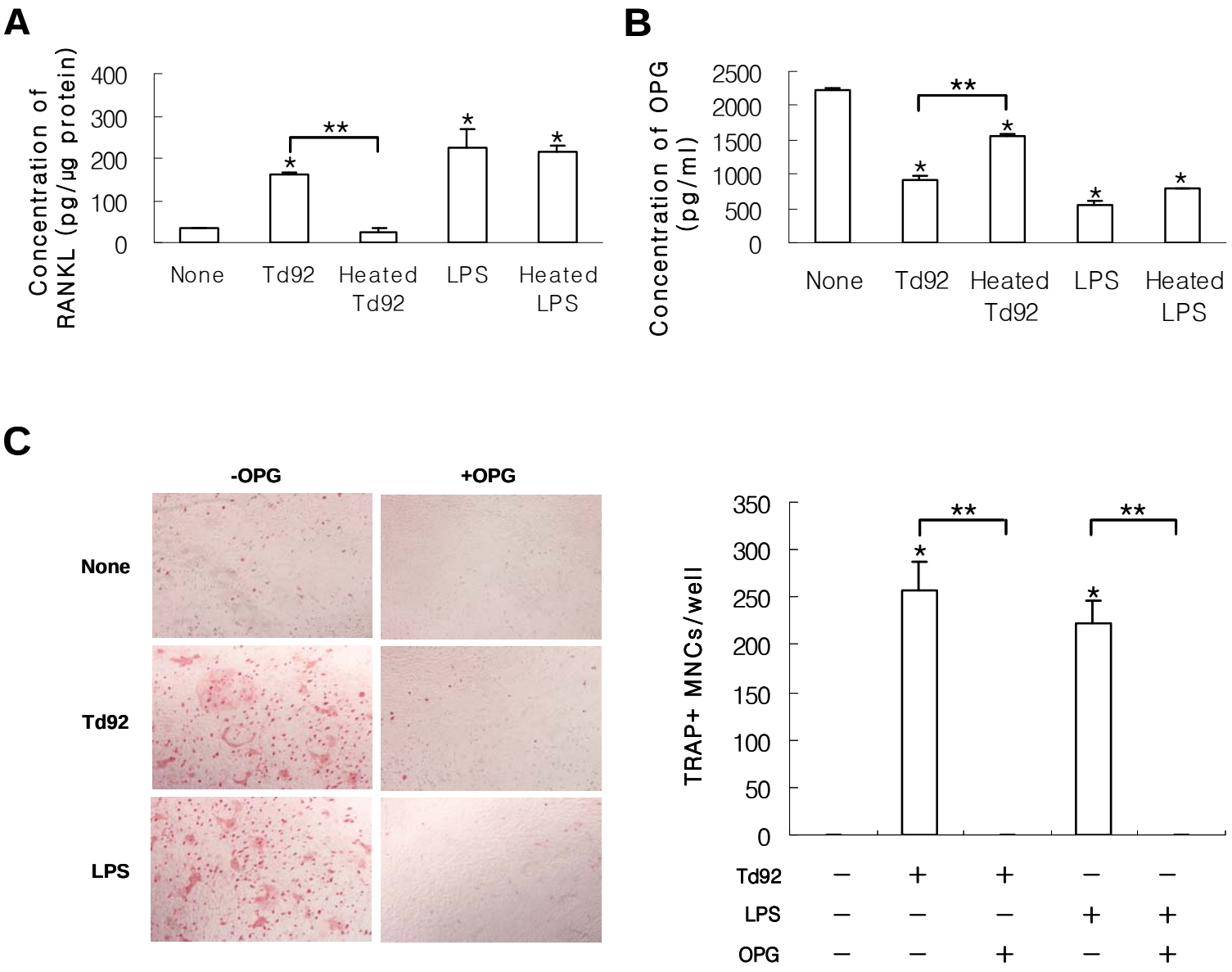

Figure 3. Effect of Td92 on RANKL and OPG expression in osteoblasts. Calvariae-derived osteoblasts were cultured with Td92 (10 μg/ml), heated Td92 (10 μg/ml), LPS (0.1 μg/ml) or heated LPS (0.1 μg/ml) for 3 days. The concentration of RANKL (A) and OPG (B) in cell lysates or culture supernatants, respectively, was determined by ELISA. *, $P < 0.05$ *vs.* none-treated cells, **, $P < 0.05$ *vs.* Td92 or LPS-treated cells. Osteoblasts and bone marrow cells were co-cultured with Td92 (10 μg/ml) or LPS (0.1 μg/ml) in the absence or presence of OPG (100 ng/ml) for 6 days (C). TRAP-positive multinucleated cells containing more than three nuclei were counted as osteoclasts. *, $P < 0.05$ *vs.* none-treated cells, **, $P < 0.05$ *vs.* Td92 or LPS-treated cells.

3. Effect of Td92 on PGE_2 expression in osteoblasts

In order to assess the involvement of PGE_2 in Td92- induced osteoclast formation, osteoblasts were stimulated with Td92 and the level of PGE_2 expression was measured by ELISA. Osteoblasts treated with Td92 or LPS showed increased level of PGE_2 expression (Fig. 4A). The increased PGE_2 expression, furthermore, was completely inhibited with the addition of NS398 or indomethacin, inhibitors of prostaglandin synthesis (Fig. 4A). The effect of NS398 or indomethacin on cell viability of osteoblasts was evaluated by MTT assay. NS398 or indomethacin did not affect cell viability (data not shown).

4. Effect of NS398 and indomethacin on RANKL/OPG expression altered by Td92

To investigate whether PGE_2 is involved in regulating RANKL and OPG expression in osteoblasts, osteoblasts were treated with Td92 in the absence or presence of NS398 or indomethacin. RANKL and OPG expression level was measured by ELISA. The RANKL expression induced by Td92 or LPS was

down-regulated by NS398 or indomethacin (Fig. 4B). Down-regulated OPG expression by Td92 or LPS was recovered by NS398 or indomethacin (Fig. 4C). These results indicate that RANKL and OPG expression altered by Tp92 in osteoblasts are closely related to the production of PGE_2.

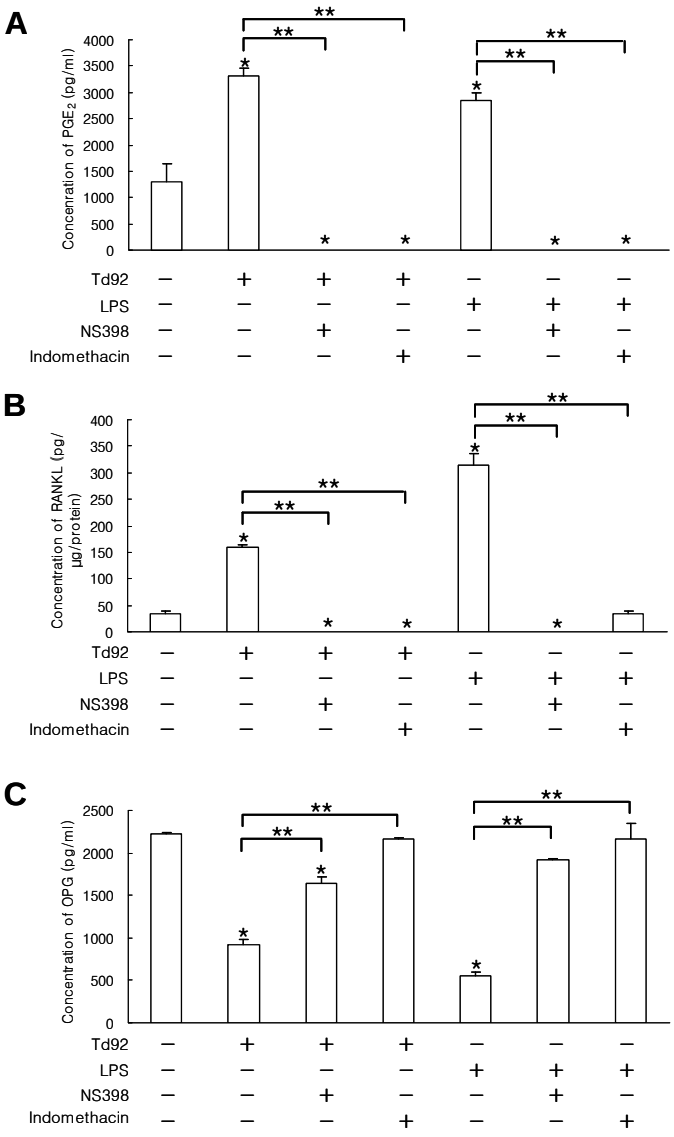

Figure 4. Effect of NS398 and indomethacin on Td92-regulated RANKL and OPG expression in osteoblasts. Osteoblasts were cultured with Td92 (10 μg/ml) or LPS (0.1 μg/ml) in the absence or presence of NS398 (1 μM) or indomethacin (1 μM). The cultures were incubated for 3 days, and the concentration of PGE_2 (A), RANKL (B), and OPG (C). was determined by ELISA. *, $P < 0.05$ *vs.* none-treated cells. **, $P < 0.05$ *vs.* Td92 or LPS-treated cells.

5. Effect of NS398 and indomethacin on osteoclast formation induced by Td92

To confirm the involvement of PGE_2 in Td92-induced osteoclast formation, Td92 was added to co-culture in the absence or presence of NS398 or indomethacin. Osteoclast formation induced by Td92 was inhibited by the addition of NS398 (1 μM) or indomethacin (1 μM) (Fig. 5). These results indicate that PGE_2 is critically involved in Td92-induced osteoclastogenesis.

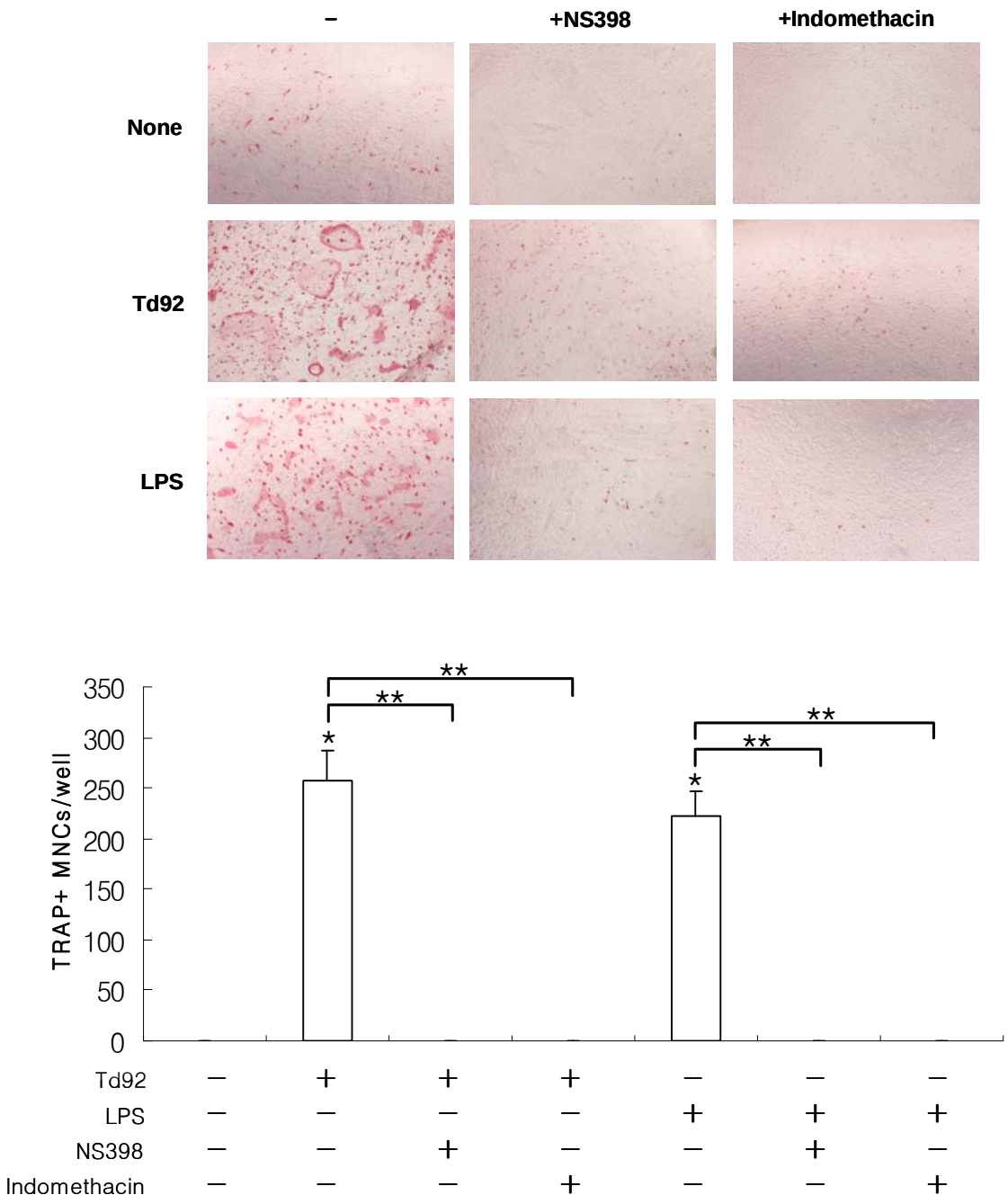

Figure 5. Effect of NS398 and indomethacin on Td92-induced osteoclast formation. Osteoblasts and bone marrow cells were co-cultured with Td92 (10 μg/ml) or LPS (0.1 μg/ml) in the absence or presence of NS398 (1 μM) or indomethacin (1 μM) for 6 days. TRAP-positive multinucleated cells containing more than three nuclei were counted as osteoclasts. *, $P < 0.05$ *vs*. none-treated cells. **, $P < 0.05$ *vs*. Td92 or LPS-treated cells.

III. DISCUSSION

Td92 has been proposed to contribute to the inflammation and osteoclastogenesis by inducing the production of tumor necrosis factor (TNF), IL-1β, IL-6, IL-8, and PGE_2 in THP-1 and PDL cells (19). Although it is possible that Td92 have osteoclastogenic ability by inducing osteoclastogenic cytokines, the exact role of Td92 on bone cells with the regulation of RANKL and OPG expressions remains unclear. For the first time, this study demonstrated that Td92 has stimulatory effect on osteoclastogenesis via RANKL/OPG/PGE_2 regulation.

Outer membrane proteins (OMPs) of *T. denticola* include major surface proteins (Msp), hemolysin/agglutinin, dentilisin, OppA, and HbpA/HbpB. Msp of *T. denticola* has pore-forming activity and adhesive activity (24, 25). Pore-forming activity of Msp was demonstrated via depolarization and increased conductance of the HeLa cell membranes (26). Msp retards Ca^{2+} release from endoplasmic reticulum stores and inhibits consequent Ca^{2+} influx by uncoupling store-operated channels (27). In the

animal model, Msp was elevated followed by greater Th-2-titled immune response and greator bone resorption was consequently observed in *T. denticola* infected model (28, 29). Dentilisin, chymotrypsin-like protease (CTLP), adheres to and lyses epithelial cells (30). Dentilisin also reduce proinflammatory cytokines such as IL-1β, IL-6, and TNF-α by degradation and penetrates epithelial cell layer through disrupting transepithelial resistance (TER) by likely degradading the tight junctional proteins such as ZO-1 (31, 32). *T. denticola* OppA, a solute binding protein involved in peptide uptake and environmental signaling in a wide range of bacteria, was demonstrated to bind to plasminogen and fibronectin (33). These previous studies suggest that these OMPs of *T. denticola* are valuable candidate for the virulence factor with binding activity, cytotoxic/antigenic activity, and cell stimulatory activity during host-*T. denticola* interaction in periodontitis. In the present study, Td92, one of the OMPs of *T. denticola*, was demonstrated as a stimulator of osteoclastogenesis. Outer membrane proteins (OMPs) detected in bacterial outer membrane is one of the first class molecules implicated in host-

bacterium interaction in the process of bacterial infection. Therefore, Td92, with other OMPs, could contribute to tissue destruction in periodontitis.

LPS, a representative potent inflammatory stimulator of gram-negative bacteria, binds to Toll-like receptor 4 (TLR4) and induces myeloid differentation protein 88 (MyD88), which then initiates the activation of downstream signaling pathways, leading to osteoclast formation through RANKL/OPG regulation in osteoblasts (8). Therefore, LPS is generally used as a positive control in the study of osteoclast formation of periodontitis. Prior to experiments, *E. coli* LPS from different manufactures and strains were tested and the maximal osteoclastogenic effect of LPS was seemed to shift in the range of 0.01 to 1 μg/ml depends on manufactures or the origins of *E. coli* (data not shown). In this study, 0.1 μg/ml of LPS from *E. coli* 026:B6 was used with maximal osteoclastogenic effect. When Td92 was compared with LPS, the osteoclastogenic activity of Td92 was similar to that of LPS.

Regarding periodontitis, RANKL and OPG are important cytokines which stimulates or inhibits bone destruction, respectively. Osteoblasts

infected with *P. gingivalis* exhibited elevated RANKL expression (34) and osteoblasts treated with LPS from *P. nigrescens* showed decreasing of OPG expression. The prevalence of *A. actinomycetemcomitans*, *P. gingivalis*, and *T. forsythensis* and level of RANKL in gingival crevicular fluid (GCF) of periodontitis patients was shown to be positively correlated. Other studies also demonstrated the increased RANKL expression level in GCF/gingival tissues of diseased sites is correlated to the incidence of periodontitis (35-38). RANKL has been proposed to be both Soluble- and membrane-bound form in association with osteoclastogenesis. Hofbauer *et al*. reported direct cell-to-cell contact with osteoblasts would allow continuous exposure of the membrane-bound RANKL to osteoclast precursors whereas the soluble form would explain the stimulatory effect of conditioned medium harvested from osteoblasts (4). Suda *et al*. reported direct cell-to-cell contact between osteoblasts and osteoclast progenitors is involved in osteoclastogenesis (39). Hikita *et al*. described the ectodomain shedding of membrane-bound RANKL (mRNA) *in vivo* by RANKL sheddases such as matrix metalloproteinase

(MMP14) (40). In order to determine which forms of RANKL expression is mainly involved in osteoclast formation by Td92, the level of RANKL in both supernatant and cell lysates harvested from osteoblast culture was examined. Cell lysates showed increased level of RANKL with Td92 treatment. However, culture supernatants did not show the detective level of RANKL in any of treated groups (data not shown). These results indicate that Td92 primarily induces membrane-bound RANKL in osteoblasts. This study also showed that Td92 markedly decreased the expression level of OPG. To confirm the involvement of RANKL/OPG expressions in Td92-induced osteoclastogenesis, OPG, a decoy receptor of RANKL, was added in Td92 treated co-cultures. Osteoclast formation induced by Td92 was significantly inhibited by OPG. This result reveals that Td92 is a potent virulence factor in bone-resorptive periodontitis by inducing osteoclast formation through RANKL up-regulation and OPG down-regulation.

Due to the Td92 is a recombinant protein, this study verified the endotoxin decontamination by utilizing NF-κB reporter cell line CHO/CD14/TLR4 cells

and heat-treatment. Td92 did not induce the NF-κB reporter in CHO cells to express membrane CD25 through TLR4-dependent NF-κB activation. However, LPS, a ligand of TLR4, induced NF-κB-regulated CD25 expression in flow cytometry (data not shown). When Td92 was heat-treated, osteoclast formation was completely inhibited and heat treatment of Td92 reversed the RANKL/OPG regulative activity of Td92. However, heat-treated LPS still possessed the same osteoclast formation activity and RANKL/OPG regulatory activity of none-heated LPS. Therefore, the effect of Td92 on osteoclast formation or RANKL/OPG regulation could be concluded as the effect of recombinant protein, not LPS.

Recent studies have shown the increased GCF-PGE_2 concentrations at the site of periodontitis, which notify PGE_2 is one of the major pathogenic molecules in periodontitis (41, 42). In bone, PGE_2 has been demonstrated to play a critical role as a mediator of RANKL-dependent osteoclastogenesis (43). Elevation of PGE_2 in LPS-induced osteoblasts suppressed OPG expression (39). Therefore, correlation between PGE_2 and RANKL/OPG is one of the

main focuses in the study of alveolar bone resorption of periodontitis. In the present study, Td92 significantly induced PGE_2 production in osteoblasts and this induction was completely inhibited by NS398/indomethacin, the inhibitors of PGE_2 synthesis. In addition, up-regulated RANKL expression by Td92 was completely inhibited by NS398/indomethacin. In contrast, down-regulated OPG expression by Td92 was recovered by NS398/indomethacin. NS398/indomethacin also further inhibited osteoclast formation induced by Td92. These results indicate that PGE_2 is critically involved in Td92-induced osteoclastogenesis by regulating RANKL/OPG expression in osteoblasts.

In osteoblasts, activated TLR4, a signal-transducing receptor for LPS, induces COX-2 which then stimulates PGE_2 (39). In this pathway, PGE_2 inhibits OPG expression and this finally induces osteoclastogenesis (44). Other studies also reported that LPS activates several downstream signaling pathways, which cause osteoclast differentiation through expression of RANKL in osteoblasts (45, 46). Although similarities between Td92 and LPS in inducing osteoclastogenesis were shown in this study, the precise

mechanisms of Td92 on alveolar bone resorption in periodontitis need further investigation within other osteoblast signaling pathways. Moreover, to better understand coincidence or differences among the other Tp92 homologs and the roles of *Treponema* species in alveolar bone resorption of periodontitis, Tp92 homologs of other *Treponema* species should be further investigated.

This study showed that Td92 induces osteoclastogenesis by up-regulating RANKL expression and down-regulating OPG expression and the regulation of RANKL/OPG is mediated by PGE_2 (Fig 6.). Therefore, this effect of Td92 may contribute to alveolar bone resorption of periodontitis.

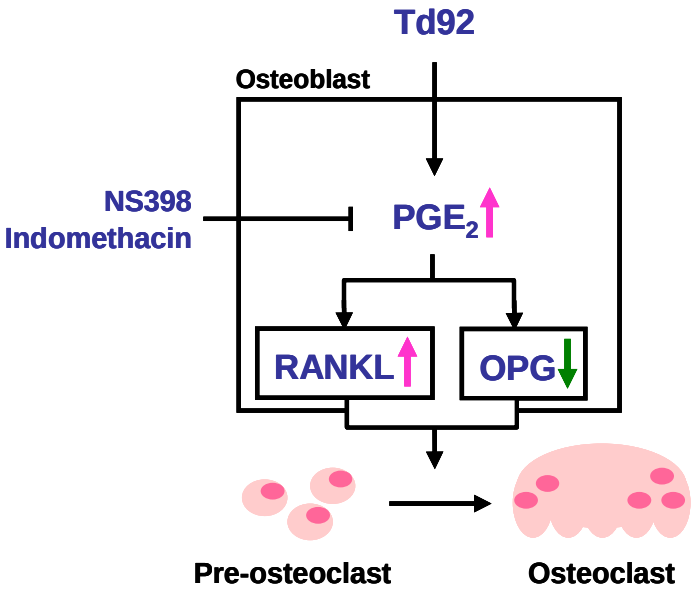

Figure 6. Td92 induces osteoclastogenesis by regulating RANKL/OPG/PGE_2.

(1) Takahashi N, Akatsu T, Udagawa N, et al. Osteoblastic cells are involved in osteoclast formation. *Endocrinology* 1988; **123**: 2600-2602.

(2) Udagawa N, Takahashi N, Akatsu T, et al. Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. *Proc Natl Acad Sci U S A* 1990; **87**: 7260-7264.

(3) Hofbauer LC, Kuhne CA, Viereck V. The OPG/RANKL/RANK system in metabolic bone diseases. *J Musculoskelet Neuronal Interact* 2004; **4**: 268-275.

(4) Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. *J Bone Miner Res* 2000; **15**: 2-12.

(5) Kiji M, Nagasawa T, Hormdee D, et al. Internal prostaglandin synthesis augments osteoprotegerin production in human gingival fibroblasts

stimulated by lipopolysaccharide. *Clin Exp Immunol* 2007; **149**: 327-334.

(6) Chung YH, Chang EJ, Kim SJ, et al. Lipopolysaccharide from Prevotella nigrescens stimulates osteoclastogenesis in cocultures of bone marrow mononuclear cells and primary osteoblasts. *J Periodontal Res* 2006; **41**: 288-296.

(7) Ohguchi Y, Ishihara Y, Ohguchi M, et al. Capsular polysaccharide from Actinobacillus actinomycetemcomitans inhibits IL-6 and IL-8 production in human gingival fibroblast. *J Periodontal Res* 2003; **38**: 191-197.

(8) Yang S, Takahashi N, Yamashita T, et al. Muramyl dipeptide enhances osteoclast formation induced by lipopolysaccharide, IL-1 alpha, and TNF-alpha through nucleotide-binding oligomerization domain 2-mediated signaling in osteoblasts. *J Immunol* 2005; **175**: 1956-1964.

(9) Wada N, Maeda H, Yoshimine Y, Akamine A. Lipopolysaccharide stimulates expression of osteoprotegerin and receptor activator of NF-

kappa B ligand in periodontal ligament fibroblasts through the induction of interleukin-1 beta and tumor necrosis factor-alpha. *Bone* 2004; **35**: 629-635.

(10) Daly CG, Seymour GJ, Kieser JB. Bacterial endotoxin: a role in chronic inflammatory periodontal disease? *J Oral Pathol* 1980; **9**: 1-15.

(11) Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol* 1995; **13**: 437-457.

(12) Sela MN. Role of Treponema denticola in periodontal diseases. *Crit Rev Oral Biol Med* 2001; **12**: 399-413.

(13) Uitto VJ, Grenier D, Chan EC, McBride BC. Isolation of a chymotrypsinlike enzyme from Treponema denticola. *Infect Immun* 1988; **56**: 2717-2722.

(14) Holt SC, Bramanti TE. Factors in virulence expression and their role in periodontal disease pathogenesis. *Crit Rev Oral Biol Med* 1991; **2**: 177-281.

(15) Syed SA, Makinen KK, Makinen PL, Chen CY, Muhammad Z. Proteolytic and oxidoreductase activity of Treponema denticola ATCC 35405 grown in an aerobic and anaerobic gaseous environment. *Res Microbiol* 1993; **144**: 317-326.

(16) Rosen G, Naor R, Kutner S, Sela MN. Characterization of fibrinolytic activities of Treponema denticola. *Infect Immun* 1994; **62**: 1749-1754.

(17) Rosen G, Naor R, Rahamim E, Yishai R, Sela MN. Proteases of Treponema denticola outer sheath and extracellular vesicles. *Infect Immun* 1995; **63**: 3973-3979.

(18) Cameron CE, Lukehart SA, Castro C, Molini B, Godornes C, Van Voorhis WC. Opsonic potential, protective capacity, and sequence conservation of the Treponema pallidum subspecies pallidum Tp92. *J Infect Dis* 2000; **181**: 1401-1413.

(19) Jun HK, Kang YM, Lee HR, Lee SH, Choi BK. Highly conserved surface proteins of oral spirochetes as adhesins and potent inducers of proinflammatory and osteoclastogenic factors. *Infect Immun* 2008; **76**:

2428-2438.

(20) Lee SH, Kim KK, Choi BK. Upregulation of intercellular adhesion molecule 1 and proinflammatory cytokines by the major surface proteins of Treponema maltophilum and Treponema lecithinolyticum, the phylogenetic group IV oral spirochetes associated with periodontitis and endodontic infections. *Infect Immun* 2005; **73**: 268-276.

(21) Choi HG, Kim JM, Kim BJ, Yoo YJ, Cha JH. Mouse strain-dependent osteoclastogenesis in response to lipopolysaccharide. *J Microbiol* 2007; **45**: 566-571.

(22) Choi BK, Moon SY, Cha JH, Kim KW, Yoo YJ. Prostaglandin E(2) is a main mediator in receptor activator of nuclear factor-kappaB ligand-dependent osteoclastogenesis induced by Porphyromonas gingivalis, Treponema denticola, and Treponema socranskii. *J Periodontol* 2005; **76**: 813-820.

(23) Suda T, Jimi E, Nakamura I, Takahashi N. Role of 1 alpha,25-

dihydroxyvitamin D3 in osteoclast differentiation and function. *Methods Enzymol* 1997; **282**: 223-235.

(24) Cullen PA, Haake DA, Adler B. Outer membrane proteins of pathogenic spirochetes. *FEMS Microbiol Rev* 2004; **28**: 291-318.

(25) Egli C, Leung WK, Muller KH, Hancock RE, McBride BC. Pore-forming properties of the major 53-kilodalton surface antigen from the outer sheath of Treponema denticola. *Infect Immun* 1993; **61**: 1694-1699.

(26) Mathers DA, Leung WK, Fenno JC, Hong Y, McBride BC. The major surface protein complex of Treponema denticola depolarizes and induces ion channels in HeLa cell membranes. *Infect Immun* 1996; **64**: 2904-2910.

(27) Wang Q, Ko KS, Kapus A, McCulloch CA, Ellen RP. A spirochete surface protein uncouples store-operated calcium channels in fibroblasts: a novel cytotoxic mechanism. *J Biol Chem* 2001; **276**: 23056-23064.

(28) Lee SF, Andrian E, Rowland E, Marquez IC. Immune response and

alveolar bone resorption in a mouse model of Treponema denticola infection. *Infect Immun* 2009; **77**: 694-698.

(29) Ishihara K, Okuda K. Molecular pathogenesis of the cell surface proteins and lipids from Treponema denticola. *FEMS Microbiol Lett* 1999; **181**: 199-204.

(30) Fenno JC, Hannam PM, Leung WK, Tamura M, Uitto VJ, McBride BC. Cytopathic effects of the major surface protein and the chymotrypsinlike protease of Treponema denticola. *Infect Immun* 1998; **66**: 1869-1877.

(31) Miyamoto M, Ishihara K, Okuda K. The Treponema denticola surface protease dentilisin degrades interleukin-1 beta (IL-1 beta), IL-6, and tumor necrosis factor alpha. *Infect Immun* 2006; **74**: 2462-2467.

(32) Chi B, Qi M, Kuramitsu HK. Role of dentilisin in Treponema denticola epithelial cell layer penetration. *Res Microbiol* 2003; **154**: 637-643.

(33) Fenno JC, Tamura M, Hannam PM, Wong GW, Chan RA, McBride BC. Identification of a Treponema denticola OppA homologue that binds

host proteins present in the subgingival environment. *Infect Immun* 2000; **68**: 1884-1892.

(34) Okahashi N, Inaba H, Nakagawa I, et al. Porphyromonas gingivalis induces receptor activator of NF-kappaB ligand expression in osteoblasts through the activator protein 1 pathway. *Infect Immun* 2004; **72**: 1706-1714.

(35) Liu D, Xu JK, Figliomeni L, et al. Expression of RANKL and OPG mRNA in periodontal disease: possible involvement in bone destruction. *Int J Mol Med* 2003; **11**: 17-21.

(36) Lu HK, Chen YL, Chang HC, Li CL, Kuo MY. Identification of the osteoprotegerin/receptor activator of nuclear factor-kappa B ligand system in gingival crevicular fluid and tissue of patients with chronic periodontitis. *J Periodontal Res* 2006; **41**: 354-360.

(37) Mogi M, Otogoto J, Ota N, Togari A. Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. *J Dent Res* 2004; **83**: 166-169.

(38) Vernal R, Chaparro A, Graumann R, Puente J, Valenzuela MA, Gamonal J. Levels of cytokine receptor activator of nuclear factor kappaB ligand in gingival crevicular fluid in untreated chronic periodontitis patients. *J Periodontol* 2004; **75**: 1586-1591.

(39) Suda K, Udagawa N, Sato N, et al. Suppression of osteoprotegerin expression by prostaglandin E2 is crucially involved in lipopolysaccharide-induced osteoclast formation. *J Immunol* 2004; **172**: 2504-2510.

(40) Hikita A, Yana I, Wakeyama H, et al. Negative regulation of osteoclastogenesis by ectodomain shedding of receptor activator of NF-kappaB ligand. *J Biol Chem* 2006; **281**: 36846-36855.

(41) Preshaw PM, Heasman PA. Prostaglandin E2 concentrations in gingival crevicular fluid: observations in untreated chronic periodontitis. *J Clin Periodontol* 2002; **29**: 15-20.

(42) Soder B, Jin LJ, Wickholm S. Granulocyte elastase, matrix metalloproteinase-8 and prostaglandin E2 in gingival crevicular fluid in

matched clinical sites in smokers and non-smokers with persistent periodontitis. *J Clin Periodontol* 2002; **29**: 384-391.

(43) Suzawa T, Miyaura C, Inada M, et al. The role of prostaglandin E receptor subtypes (EP1, EP2, EP3, and EP4) in bone resorption: an analysis using specific agonists for the respective EPs. *Endocrinology* 2000; **141**: 1554-1559.

(44) Ohshiba T, Miyaura C, Ito A. Role of prostaglandin E produced by osteoblasts in osteolysis due to bone metastasis. *Biochem Biophys Res Commun* 2003; **300**: 957-964.

(45) Zou W, Bar-Shavit Z. Dual modulation of osteoclast differentiation by lipopolysaccharide. *J Bone Miner Res* 2002; **17**: 1211-1218.

(46) Sato N, Takahashi N, Suda K, et al. MyD88 but not TRIF is essential for osteoclastogenesis induced by lipopolysaccharide, diacyl lipopeptide, and IL-1alpha. *J Exp Med* 2004; **200**: 601-611.

ABSTRACT (IN KOREAN)

*Treponema denticola*의 외막단백질인 Td92의 파골세포 형성 유도능

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김민영

치주염은 치주조직의 만성 염증성 질환으로 치조골의 흡수를 야기한다. 파골세포는 골 흡수 기능을 갖는 다핵의 세포이다. 조골세포는 파골세포 분화조절인자인 receptor activator or NF- κB (RANKL)와 osteoprotegerin (OPG) 를 발현하고 이를 통해 파골세포의 분화를 조절한다. *Treponema denticola* 는 치주염을 일으키는 원인 균들 중 하나이다. *T. dencitola*의 주외막단백질인 Tp92 homolog

(Td92) 는 다른 구강 나선균의 Tp92 homolog와 상동성을 보이며 염증인자와 세포결합에 관여하는 것으로 보고되어있으나 Td92의 치조골의 흡수에 대한 영향은 아직 알려져 있지 않다. 본 연구에서는 Td92의 골흡수 유도능을 평가하기 위해 생쥐의 두개골에서 분리한 조골세포와 경골에서 분리한 골수세포를 혼합배양하여 Td92의 파골세포의 형성능을 평가하였다. 또한 효소면역측정법을 이용하여 조골세포에서 Td92의 RANKL, OPG와 PGE_2의 생성 유도능을 분석하였다.

Td92는 혼합배양에서 파골세포의 형성을 농도의존적으로 유도하였다. Td92는 조골세포의 RANKL 및 PGE_2 발현을 증가시켰으며 OPG발현은 감소시켰다. OPG와 PGE_2 형성 억제인자인 NS398 및 indomethacin은 Td92에 의한 파골세포 형성을 억제하였다. 또한, NS398 및 indomethacin은 조골세포에서 Td92에 의한 RANKL 및

OPG발현의 변화를 억제하였다. 이들 결과는 *T. denticola*의 주외막 단백질인 Td92가 PGE_2를 매개로 조골세포의 RANKL 및 OPG의 발현을 조절하여 파골세포 형성을 유도함을 시사한다. 이를 통해 치주염의 주요 원인균인 *T. denticola* 의 주외막단백질이 치조골의 흡수를 일으키는 치주염의 병변에 밀접한 관련이 있음을 확인하였다.

핵심되는 말: *Treponema denticola*, 파골세포형성, Td92, 치주염