Induction of osteoclastogenesis by *Treponema denticola* lipooligosaccharide

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ABSTRACT

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Alveolar bone destruction is a characteristic feature of perio dontitis. *Treponema denticola* is known to be involved in period ontitis. To elucidate the role of *T. denticola* on alveolar bone destruction in periodontitis, the effects of lipooligosaccharide (LOS) from *T. denticola* on osteoclast formation and the mRNA expression of osteoclast differentiation factor (ODF) and osteo protegerin (OPG) were examined in a coculture system by using mouse calvaria and bone marrow cells. When the mouse calvaria and bone marrow cells were challenged with LOS (0.1-10 ㎍/mℓ) for 4 days, the number of tartrate-resistant acid phosphatase (TRAP) -positive multinucleated cells increased in a dose-dependent manner. The expression of ODF mRNA was increased, while OPG mRNA expression was decreased. Polymyxin B reverted the effect of LOS (10 ㎍/mℓ) on ODF and OPG mRNA expression to the control level. LOS (10 ㎍/mℓ) stimulated prostaglandin E_2 (PGE_2) production in the cocultures. Adding indomethacin, an inhibitor of prostagl andin synthesis, resulted in a reduction in the number of osteoclasts induced by LOS and abrogated the effect of *T. denticola* LOS on ODF and OPG mRNA expression. These results suggest that *T. denticola* LOS stimulates osteoclastogenesis and induces up-regulation of ODF and down-regulation of OPG through a

PGE_2-dependent mechanism.

Key word: *Treponema denticola*, Lipooligosaccharide, Osteoclastogenesis, Osteoclast differentiation factor, Osteoprotegerin

Induction of osteoclastogenesis by *Treponema denticola* lipooligosaccharide

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Ⅰ. INTRODUCTION

Periodontitis is an inflammatory disease and the loss of alveolar bone is a hallmark of this disease (Schwartz *et al.*, 1997). *Treponema denticola* is one of bacteria which are implicated in the etiology of periodontitis (Fenno *et al.*, 1998; Socransky *et al.*, 1998; Dewhirst *et al.*, 2000). This bacterium has multiple virulence factors that include adhesins, proteolytic and hydrolytic enzymes, cytopathic activity, and immunomodulation (Fenno *et al.*, 1998; Chan *et al.*, 2000). In the virulence factor associated with bone resorption, it was reported that the outer membrane of this bacterium increased Ca^{2+} release in an organ culture of fetal radii and ulnae (Gopalsami *et al.*, 1993). Although this suggested that the heat-stable material of *T. denticola* in the outer membrane might be involved in bone resorption, the components of this bacterium, which stimulate bone resorption, and the underlying mechanism has not been addressed. In previous study (Choi *et al.*,

1999), whole cell sonicates of *T. denticola* associated with aggressive periodontitis induced osteoclast formation. In this case, heat-stable components were involved. Taken together, these findings imply that heat-stable components from *T. denticola* may be involved in osteoclastogenesis. Lipopolysaccharide (LPS) is a heat stable component of gram negative bacteria. Yotis *et al.* (1995) reported that *T. denticola* possessed a lipopolysaccharide like molecule (8-14 kDa) that exhibited Limulus amoebocyte lysate clotting activity. Other research groups (Sela *et al.*, 1997; Rosen *et al.*, 1999) purified an approximately 14-21 kDa LOS from *T. denticola*. This LOS stimulated nitric oxide and TNF-α production in mouse macrophages and this induction was inhibited by polymyxin B (Rosen *et al.*, 1999). In addition, Schultz *et al.* (1998) reported that *T. denticola* Lipooligosaccharide (LOS) was quite different from the LPS of other gram negative bacteria. Therefore, it was interest to investigate the ability of *T. denticola* LOS to stimulate osteoclastogenesis.

Osteoclasts are multinucleated cells with bone resorbing activity, which play a crucial role in bone resorption. Osteoclast formation requires the presence of osteoblast/stromal cells (Takashi *et al.*, 1998). These cells express the osteoclast differentiation factor (ODF, also known as a receptor activator of the nuclear factor-κB ligand (RANKL)) that promotes osteoclastogenesis (Lacey *et al.*, 1998; Yasuda *et al.*, 1998). The osteoclast precursors that express RANK (the receptor for ODF) recognize ODF through a cell-to-cell interaction with osteoblasts, and differentiate into osteoclasts.

Osteoprotegerin (OPG), which is also secreted by osteoblast lineage cells, is a soluble decoy receptor that neutralizes the biological activity of ODF (Simonet *et al.*, 1997; Yasuda *et al.*, 1998; Udagawa *et al.*, 2000). Osteoclastogenesis is controlled by multiple factors such as 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$), parathyroid hormone (PTH), prostaglandin E_2 (PGE_2), and interleukin-1 (IL-1) (Suda *et al.*, 1997; Reddy *et al.*, 1998). The regulation of ODF/OPG expression by these agents (Hofbauer *et al.*, 1998; Vidal *et al.*, 1998; Nagai *et al.*, 1999; Brandstr¨m *et al.*, 2001) suggests that the effects of these factors on bone resorption may be mediated through control of ODF and/or OPG production, and that osteoclast formation is determined principally by the relative ODF to OPG ratio. Therefore, a shift to a higher ODF to OPG ratio may be a major cause of bone loss in many metabolic disorders, including osteoporosis and periodontitis (Hofbauer *et al.*, 2000). Recently, it was reported that the ODF-RANK interaction is not the sole pathway that leads osteoclast progenitors to differentiate into osteoclasts (Kobayashi *et al.*, 2000). Tumor necrosis factor-α (TNF-α), which is involved in bone resorption, can be substituted for the ODF to induce osteoclast differentiation.

In this study, in order to determine the mechanism of bone resorption induced by *T. denticola*, lipooligosaccharide (LOS) from *T. denticola* was isolated, and its effects on osteoclastogenesis and on expression of both ODF and OPG mRNA were investigated in a coculture system consisting of mouse calvaria and bone marrow cells. This is the first report showing that LOS from *T. denticola*

induced osteoclast formation and this process was dependent on the up-regulation of ODF expression and the down-regulation of OPG expression through PGE_2 synthesis.

Ⅱ. MATERIALS AND METHODS

1. Materials

The mice (ICR strain) were obtained from Bio Korea Co. (Seoul, Korea). The α-minimum essential medium (α-MEM), bovine serum albumin (BSA), and heat-inactivated fetal bovine serum (FBS) were purchased from GIBCO BRL (Grand Island, NY). Indomethacin, polymyxin B, LPS of *Escherichia coli*, and tartrate-resistant acid phosphatase (TRAP: a marker of osteoclast) staining kit were obtained from Sigma (St. Louis, MO).

2. Methods

A. Preparation of *T. denticola* sonicates

T. denticola (ATCC 33521) was cultured anaerobically in an OMIZ-PAT broth for 3-5 days, as described previously (Wyss *et al.*, 1996). The bacterial cells were harvested by centrifuging at 5,000 x g for 10 min at 4℃. The cells were then washed 3 times with phosphate buffered saline (PBS). The bacterial cells were then disrupted for 5 min using an ultrasonic processor (Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA) at an output power of 8 watts with 20 sec intervals. The cell debris was removed after centrifuging at 15,000 × g for 5 min at 4℃ and the supernatant was collected. The protein concentrations were determined using a Coomassie brilliant blue protein assay reagent (Pierce, Rockford,

IL).

B. Isolation of LOS

T. denticola LOS was isolated using the method reported by Walker *et al.* (1997). The bacteria were cultured and harvested as described above. The cell pellets were repeatedly frozen and thawed for 40 cycles and then centrifuged at 6,000 x g for 10 min to remove the cellular debris. The supernatant was centrifuged at 36,000 x g for 30 min at 4℃ and the resulting pellet containing the membrane fraction was washed twice in 60 mℓ of 0.05 M Tris-HCl (pH 7.2) and suspended in Tris-HCl. The detergent-soluble outer membrane fraction was obtained by extracting the membrane fraction with 1% Zwittergent 3.14 (Calbiochem. Co., La Jolla, CA). To obtain the LOS, the detergent soluble fraction was digested with proteinase K (50 ㎍/mℓ) at 37℃ overnight. Two volumes of 0.375 M $MgCl_2$ in 95% ethanol (-20℃) were added and kept at -20℃ for 40 min and then centrifuged at 15,000 x g for 20 min. The pellet was suspended in a solution containing 2% sodium dodecyl sulfate (SDS), 0.1 M EDTA, and 10 mM Tris-HCl (pH 8.0). Proteinase K digestion-ethanol precipitation was repeated twice. The LOS was purified by centrifugation at 48,000 x g for 2 h at 4℃ and suspended in a small volume of distilled water. After heating the LOS at 90℃ for 30 min, the LOS was quantified by lyophilization and the weight was measured. SDS-polyacrylamide gel electrophoresis (PAGE) of the LOS was conducted and the gels were silver-stained.

C. Preparation of primary calvaria and bone marrow cells

The osteoblastic cells were isolated from the calvariae of 1-2 day-old ICR mice as previously described (Choi *et al.*, 2001). The calvariae were digested in 10 mℓ of α-MEM containing 0.2% collagenase (Wako Pure Chemicals, Osaka, Japan) and 0.1% dispase (GIBCO BRL, Grand Island, NY) for 20 min at 37℃ with vigorous shaking, and then centrifuged at 1,500 × g for 5 min. The first supernatant was discarded and another 10 mℓ of the collagenase/dispase enzyme solution was added and incubated for 20 min. The digestion was repeated 4 times and the cells isolated by the last three digestions were combined as an osteoblastic cell population. They were cultured in α-MEM containing 10% FBS and antibiotics solution (100 U/mℓ of penicillin, 100 ㎍/mℓ of streptomycin, 25 ㎍/mℓ of amphotericin B) and used for the coculture system. The bone marrow cells were collected from 5-8 week-old mice. The ends of the tibiae and femurs were removed and the marrow cavity was flushed by slowly injecting media at one end using a 25 gauge needle. The marrow cells were washed and used for the coculture.

D. Osteoclast formation assay

The isolated calvaria cells were seeded at a concentration of 10^6 cells in a 10 cm culture dish and grown to confluence. The cells were then detached from the culture dishes by trypsin-EDTA (GIBCO BRL, Grand Island, NY). Subsequently, the cells (1×10^4 cells/well) were cocultured with the bone marrow cells (1×10^5

cells/well) in α-MEM containing 10% FBS in 48 well plates (Corning Inc., NY). The culture volume was made up to 400 ㎕ per well with α-MEM medium containing 10% FBS. Either the bacteria sonicates or LOS were added to the coculture with or without polymyxin B or indomethacin after exchanging the medium on day 3. The coculture was then maintained for an additional 4 days. Osteoclast differentiation was monitored using a TRAP staining kit according to the manufacturer's instruction. The TRAP-positive multinucleated cells showing more than 3 nuclei per well were counted as an osteoclast. ODF and OPG mRNA expression in the cocultures was determined after isolating the mRNA by TRIzol reagent (Life Technologies, Inc., Grand Island, NY).

E. RT-PCR

Expression of ODF and OPG mRNAs was determined by RT-PCR. The total RNA (1 ㎍) from the non-treated and treated cells was used as a template for cDNA synthesis in a 20 ㎕ of reaction volume using an RT kit (CLONTECH, Palo Alto, CA) according to the manufacturer's instructions. The RNA (1 ㎍) and oligo$(dT)_{18}$ primer (1 mM) were denatured at 70℃ for 5 min and incubated 1-2 min on ice. The denatured RNA and oligo$(dT)_{18}$ primers were added to the reaction mixture (1 U/㎕ Moloney murine leukemia virus (MMLV) reverse transcriptase; 1 reaction buffer; 500 ㎛ of each dATP, dCTP, dGTP and dTTP; 20 U of recombinant RNase inhibitor) and incubated at 42℃ for 60 min, followed by 94℃ for 5 min.

The cDNA (4 ㎕) was amplified by PCR in a 50 ㎕ reaction volume

containing the 1 x PCR reaction buffer, 200 μM of dNTPs, 200 pM of the forward and reverse primers, and 0.5 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) in a DNA thermal cycler (Biometra, Gottingen, Germany). The amplification reaction was performed for 35 cycles and primer sequences and annealing temperatures are presented in Table 1. The PCR products were separated by electrophoresis on a 1.5% agarose gel and visualized by ethidium bromide staining. The relative intensities of the gel bands are measured using an image-analyzing program (TINA 2.0e; Neuro-Image analysis Centre, Oxford, UK). In order to exclude contaminating DNA from the isolated RNA, the RNA was subjected to PCR without cDNA synthesis. In all preparations, no band was detected after PCR.

F. PGE_2 production assay

The osteoblastic cells (1 x 10^4 cells/well) were cocultured with the bone marrow cells (1 x 10^5 cells/well) in 400 ㎕ of α-MEM containing 10% FBS in 48 well plates (Corning Inc., NY). After cells were grown to confluence, the cocultures were treated with LOS (10 ㎍/㎖) in either the presence or absence of polymyxin B and incubated for an additional 24 h. PGE_2 production in the cocultures was determined by using a PGE_2 enzyme immunoassay kit according to the manufacturer's instructions (Amersham Pharmacia Biotech., Little Chalfont, Buckinghamshire, UK).

G. Statistical analyses.

The statistical significance of differences was determined by the Mann-Whitney U test. A p value <0.05 was considered significant.

Ⅲ. RESULTS

1. Effect of *T. denticola* LOS on osteoclast formation in coculture system

The purified lipopolysaccharide (LPS) preparation from *T. denticola* did not show a typical ladder-like band pattern in SDS-PAGE after staining with silver nitrate, as previously described (Sela *et al.*, 1997; Rosen *et al.*, 1999). It migrated to a position at 7 kDa of the protein marker and was not stained with Coomassie Brilliant blue (Fig 1). These data mean that the LPS preparation from *T. denticola* is LOS.

In the coculture treated with either the *T. denticola* sonicates (10 ㎍/㎖) or LOS (0.1-10 ㎍/㎖) for 4 days, a number of TRAP positive multinucleated cells were formed, while the non-treated cultures did not show TRAP positive multinucleated cells. LOS increased the number of osteoclasts in a dose-dependent manner (Fig 2).

2. Effect of *T. denticola* LOS on mRNA expression of ODF and OPG

ODF and OPG mRNA expression was investigated in the cocultures treated with LOS for 4 days by RT-PCR (Fig 3). The non-treated cells showed steady-state ODF and OPG mRNA expression levels, as shown in other reports (Horwood *et al.*, 1998; Nagai *et al.*, 1999). ODF mRNA expression was increased and OPG mRNA expression was decreased after stimulating with *T. denticola* LOS (0.1-10 ㎍/㎖).

Table 1. The sequence of primers for ODF, OPG, and β-actin.

Primer		Sequence	Annealing Temp. (℃)	Size (bp)
ODF	Forward	5'-ATCAGAAGACAGCACTCACT-3'	45.3	750
	Reverse	5'-ATCTAGGACATCCATGCTAATGTTC-3'		
OPG	Forward	5'-TGAGTGTGAGGAAGGGCGTTAC-3'	45.5	636
	Reverse	5'-TTCCTCGTTCTCTCAATCTC-3'		
β-actin	Forward	5'-GGACTCCTATGGTGGGTGACGAGG-3'	64.0	366
	Reverse	5'-GGGAGAGCATAGCCCTCGTAGAT-3'		

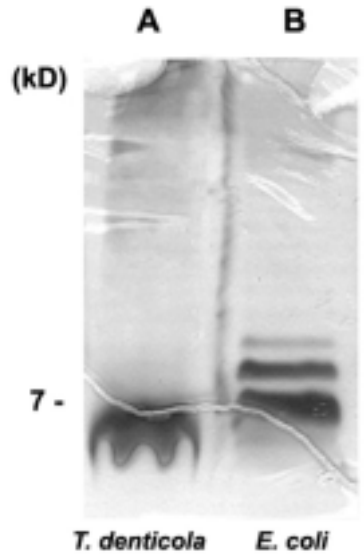

FIG 1. SDS-PAGE of purified LOS from *T. denticola*. Lanes: 1, LOS from *T. denticola*. 2, LPS from *Escherichia coli*. The 15% polyacrylamide gel was silver stained.

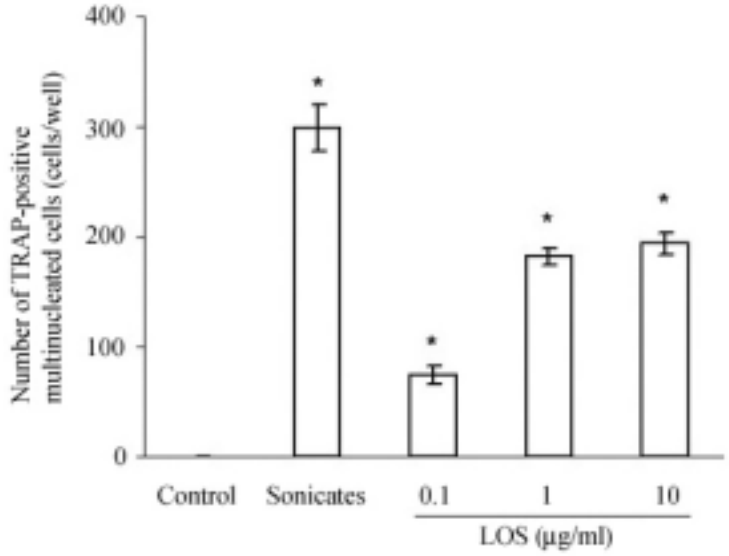

FIG 2. Formation of TRAP-positive multinucleated cells in the coculture system treated with whole-cell sonicates and LOS from *T. denticola*. Mouse bone marrow and calvaria cells were cocultured to confluence and treated with the *T. denticola* sonicates (10 ㎍/㎖) or LOS (0.1-10 ㎍/㎖) for an additional 4 days. The cells were then stained for TRAP. The TRAP-positive multinucleated cells containing more than three nuclei were counted as osteoclasts. The dada are the means _ standard errors for four cultures. * p value is <0.05 for a comparison with the results for the nontreated cultures.

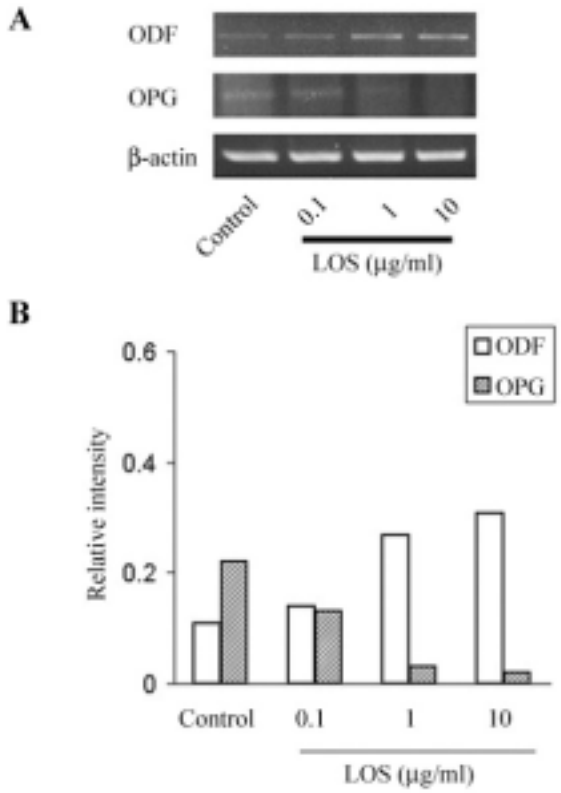

FIG 3. ODF and OPG mRNA expression in cocultures treated with *T. denticola* LOS. After the cocultres were treated with the LOS from *T. denticola* (0.1-10 ㎍/㎖), as described in the text, the ODF, OPG, and β-actin mRNA levels were examined by RT-PCR (A). Signals in the RT-PCR were quantified and normalized to β-actin mRNA expression by using an image analyzer (B). Representative results of three experiments that yield similar results are shown.

To confirm the effect of LOS on ODF and OPG mRNA expression, an inhibition assay was performed using polymyxin B, which is known to form a stable complex with the lipid A of LPS and neutralize LPS activity (Morrison *et al.*, 1976). Polymyxin B (50 ㎍/mℓ) abrogated the effect of LOS (10 ㎍/mℓ) on ODF and OPG mRNA expression (Fig 4).

3. Effect of indomethacin on osteoclast formation and expression of ODF and OPG mRNA regulated by *T. denticola* LOS

In order to determine whether PGE_2 was involved in osteoclastogenesis induced by *T. denticola* LOS, PGE_2 production in the coculture treated with LOS (10 ㎍/mℓ) in the presence or absence of polymyxin B was determined. A low level of PGE_2 was detected in the untreated cultures and the PGE_2 concentration was higher in the cultures treated with LOS. Polymyxin B (50 ㎍/mℓ) decreased the PGE_2 production stimulated by LOS (Fig 5). To confirm the involvement of PGE_2 in osteoclast formation and the expression of ODF and OPG mRNA, cocultures were treated with LOS in the presence or absence of indomethacin, which is an inhibitor of prostaglandin synthesis. In the coculture treated simultaneously with indomethacin (1 μM) and LOS (10 ㎍/mℓ) for 4 days, the number of TRAP-positive multinucleated cells was significantly decreased when compared to the cultures treated with LOS alone (Fig 6A). The ODF mRNA was down-regulated and the OPG mRNA was up-regulated by indomethacin (Fig 6B and C). Indomethacin alone did not affect osteoclast formation and the expression of ODF and OPG mRNA (data not shown).

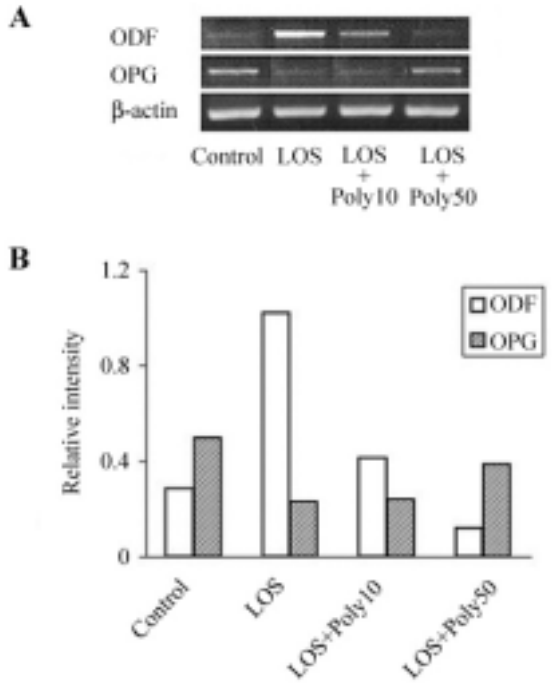

FIG 4. Effect of polymyxin B on the ODF and OPG mRNAs by *T. denticola* LOS. After the cocultures were treated with LOS (10 ㎍/㎖) in the presence or absence of polymyxin B (10, 50 ㎍/㎖) for 4 days, the ODF, OPG, and β-actin mRNA levels were examined by RT-PCR (A). The signals in the RT-PCR were quantified and normalized to β-actin mRNA expression by using an image analyzer (B). Representative results of three experiments that yield similar results are shown.

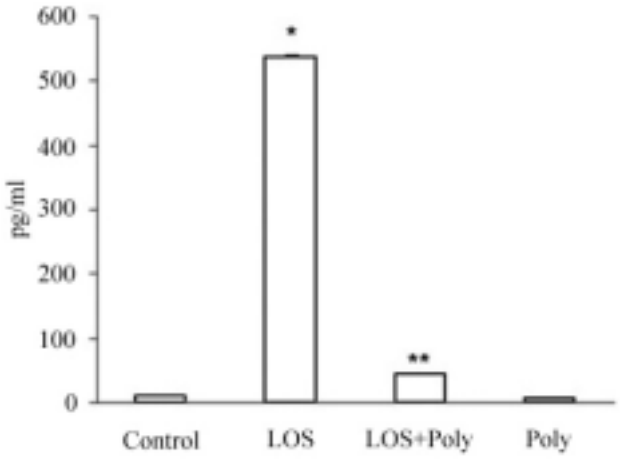

FIG 5. Effect of LOS on PGE_2 production in the coculture. The cocultures were treated with LOS (10 ㎍/㎖) with or without polymyxin B (50 ㎍/㎖) for 24 h. The PGE_2 concentration was determined by using a PGE_2 enzyme immunoassay kit. The data are the means _ standard errors for three cultures. * p value is <0.05 for a comparison with the results for the non-treated cultures. ** p value is <0.05 for a comparison with the results for the LOS-treated cultures.

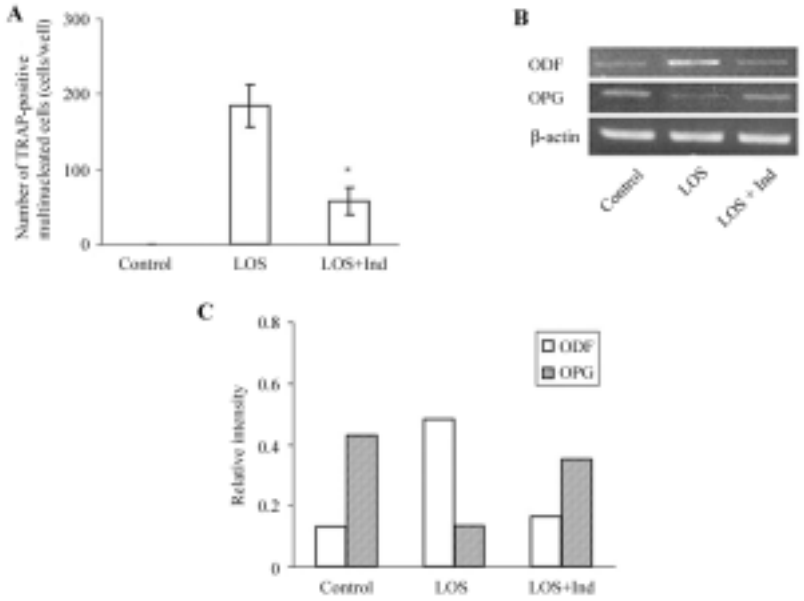

FIG 6. Effect of indomethacin on osteoclastogenesis and the expression of ODF and OPG mRNAs modulated by *T. denticola* LOS. The cocultures were simultaneously treated with LOS (10 ㎍/㎖), with or without indomethacin (1μM) for 4 days. The cells were then stained for TRAP to count the number of osteoclasts. The data are means _ standard errors for three cultures (A). The RNA was isolated from the cultured cells, and the ODF, OPG, and β-actin mRNA levels were analyzed by RT-PCR (B). The RT-PCR signals shown were quantified and normalized to β-actin mRNA expression by using an image analyzer. Representative results of three experiments that yielded similar results are shown (C). * p value is <0.05 for a comparison with the results for the LOS-treated cultures.

Ⅳ. DISCUSSION

To determine the role of *T. denticola* on bone resorption, the effects of *T. denticola* sonicates and LOS on osteoclast formation were examined. Osteoblasts/stromal cells play an essential role in osteoclastogenesis through ODF expression (Yasuda *et al.*, 1998). Therefore, to investigate the effect of *T. denticola* on osteoclastogenesis, a coculture system of mouse calvaria cells which contained primary osteoblasts and bone marrow cells which included osteoclast precursors was used. In the preliminary study, *T. denticola* sonicates stimulated osteoclastogenesis in coculture. This result means that some components of *T. denticola* may be involved in osteoclastogenesis. In the present study, *T. denticola* LOS was shown to stimulate osteoclast differentiation in a dose-dependent manner using a coculture of osteoblast and osteoclast precursors. This means that *T. denticola* LOS induces osteoclastogenesis. However, *T. denticola* sonicates induced more TRAP positive cells than LOS. In addition, when *T. denticola* sonicates were heat-treated, the osteoclast formation activity of sonicates was not completely inhibited (Choi *et al.*, 1999). Therefore, the involvement of some heat-labile components of *T. denticola* cannot be ruled out in osteoclastogenesis.

It was reported that the osteoblast/stromal cell lines that support osteoclastogenesis showed a much higher ODF mRNA level, whereas their OPG mRNA was drastically reduced in the presence of either PGE_2 or $1\alpha,25(OH)_2D_3$ (Nagai *et al.*, 1999). Another group also

reported that $1\alpha,25(OH)_2D_3$, PTH or IL-11 prompted an increase in the ODF to OPG ratio (Horwood *et al.*, 1998). These findings indicate that effects of these bone resorptive factors are mediated through the regulation of the production of ODF and its endogenous receptor antagonist, OPG and that the ODF:OPG ratio would appear to be an essential factor that determines the ability of osteoblastic cells to induce osteoclast formation. Since the discovery of ODF, it has been believed that the ODF is the sole factor responsible for inducing osteoclast differentiation. However, it was shown that TNF-α stimulated osteoclast formation via an ODF independent mechanism (Kobayashi *et al.*, 2000). In present study, the stimulatory effect of *T. denticola* LOS on osteoclast formation is mediated through ODF up-regulation and OPG down-regulation.

PGE_2 has been shown to play a role in osteoclastogenesis (Kaji *et al.*, 1996). Two studies on the involvement of PGE_2 in ODF/OPG mRNA expression in osteoblastic cells by LPS from *E. coli* showed different results. Kikuchi *et al.* (2001) reported that LPS increased the ODF mRNA level and an inhibitor of PGE_2 synthesis failed to block the effect of LPS after 2 h exposure, while OPG gene expression remained constant after LPS stimulation. This group suggested that LPS induced ODF mRNA in osteoblast directly, not via PGE_2 and did not affect OPG expression. In contrast, Sakuma *et al.* (2000) observed the effect of LPS for a longer period (24 h). They showed that LPS exposure time-dependently induced ODF mRNA expression. Induction for 4 h was not inhibited by indomethacin. However, induction for 24 h was partially inhibited by

indomethacin. Furthermore, LPS exposure increased OPG mRNA expression, but OPG levels that were induced by LPS exposure were unaffected by indomethacin. This report suggests that ODF is induced by LPS, in a PGE_2 dependent and in a PGE_2 independent manner, and OPG expression by LPS is increased independently of PGE_2. To determine if PGE_2 is involved in the effect of *T. denticola* LOS on ODF and OPG mRNA expression, we estimated the number of osteoclasts and the level of ODF and OPG mRNA expression in a coculture treated with LOS in the presence and absence of indomethacin for 4 days. *T. denticola* LOS stimulated PGE_2 production and osteoclast formation by LOS was reduced by indomethacin in coculture, which suggests the possibility that PGE_2 is involved in LOS-regulated ODF and OPG expression. Like LPS, *T. denticola* LOS increased ODF mRNA expression and this stimulating activity was inhibited by indomethacin. However, unlike regulation by LPS, OPG mRNA was down-regulated by *T. denticola* LOS and the OPG mRNA level recovered to the control level after treatment with indomethacin. Taken together, these findings indicate that PGE_2 is involved in the regulation of ODF/OPG gene expression by *T. denticola* LOS. The down-regulation of OPG gene expression by *T. denticola* LOS is somewhat different to the results of LPS reported previously (Sakuma *et al.*, 2000; Kikuchi *et al.*, 2001). The discrepancy may result from the different culture conditions used, (i.e., the cell type and the stimulation time) or from the structural differences between LPS and LOS.

It has been reported that PGE_2 and IL-1 are involved in osteoclast

formation by *Actinobacillus actinomycetemcomitans* LPS and that *Porphyromonas gingivalis* LPS promotes bone resorption, which is mediated by IL-1, IL-6, TNF-α, and PGE_2 (Miyata *et al.*, 1997; Ueda *et al.*, 1998; Zubery *et al.*, 1998; Chiang *et al.*, 1999). These studies suggest that cytokines, such as IL-1, TNF-α, and IL-6 are also involved in LPS-induced osteoclastogenesis. The involvement of pro-inflammatory cytokines in osteoclastogenesis by *T. denticola* LOS remains to be investigated.

In conclusion, here we provide evidence that the LOS from *T. denticola* stimulates osteoclastogenesis. ODF up-regulation and OPG down-regulation via PGE_2 are involved in osteoclastogenesis. *T. denticola* is known to be one of the major putative pathogens of periodontitis, a polymicrobial infection. Our results show that the pathogenesis induced by *T. denticola* may be one of the mechanisms of bone destruction in periodontitis.

V. CONCLUSION

Treponema denticola is known to be one of the major pathogens of periodontitis. To elucidate the role of *T. denticola* on alveolar bone destruction in periodontitis, the effect of lipooligosac charide(LOS) from *T. denticola* on osteoclast formation and the mRNA expression of ODF and OPG was examined in a coculture system.

1. LOS increased the number of osteoclasts in a dose-dependent manner.
2. LOS increased the expression of ODF mRNA but decreased OPG mRNA expression.
3. Polymyxin B abrogated the effect of LOS on ODF and OPG mRNA expression to the control level.
4. LOS stimulated PGE_2 production.
5. Indomethacin abrogated the effect of LOS on ODF and OPG mRNA expression.

LOS from *T. denticola* stimulates osteoclastogenesis and induces up-regulation of ODF and down-regulation of OPG through a PGE_2-dependent mechanism.

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국문요약

Treponema denticola lipooligosaccharide에 의한 파골세포 형성

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*Treponema denticola*는 치주염을 일으키는 주원인 균 중의 하나로, 치주염의 대표적 증상인 치조골 손상을 야기 시킨다. *T. denticola*에 의한 치조골 손상 기전을 이해하기 위하여 *T. denticola*에서 lipooligosaccharide (LOS)를 분리한 후 이를 이용하여 생쥐 두개골과 골수세포를 사용한 혼합배양에서 파골세포 형성효과, osteoclast differentiation factor (ODF)와 osteoprotegerin (OPG)의 mRNA 발현정도를 조사하였다. 파골세포의 형성은 파골세포의 분화 표식인자인 tartrate resistant acid phosphatase (TRAP)발현 다핵 세포의 수를 세어 평가하였다. 또한 ODF 및 OPG mRNA의 발현은 RT-PCR로, prostaglandin E_2 (PGE_2)의 농도는 면역효소 흡착법 (ELISA)으로 평가하였다. 생쥐 두개골과 골수세포를 LOS로 4일간 처치한 결과 농도에 비례하여 TRAP에 양성인 다핵 세포가 증가하였으며, OPG mRNA의 발현은 감소, ODF mRNA의 발현은 증가함을 확인하였다. Polymyxin B는 LOS에 의한 OPG와 ODF mRNA 발현 변화를 대조군 수준으로 복원시켰다. LOS는 혼합배양에서 PGE_2의 생성을 증가시켰다. PGE_2 생성 억제제인 indomethacin은 LOS에 의해 유도된 파골세포의 수를 감소시켰고, *T. denticola* LOS에 의한 ODF와 OPG mRNA의 발현 변화를 복원시켰다. 이상의 결과는 *T.*

denticola LOS에 의한 파골세포의 형성이 PGE_2에 의한 ODF의 증가 및 OPG의 감소에 의하여 매개됨을 시사한다.

핵심 단어: *Treponema denticola*, lipooligosaccharide, 파골세포 형성 기전, osteoclast differentiation factor, osteoprotegerin