Induction of bone resorption-inducing factors in osteoblasts by *Actinobacillus actinomycetemcomitans*

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감사의 말씀

연구 시작 단계부터 본 논문의 완성까지 연구를 지도해 주시고 항상 조언을 아끼지 않으시고 마지막까지 저의 부족한 부분을 메워주려 애쓰신 유윤정 교수님께 감사 드립니다. 논문의 작성에서 논리적 사고와 과학적 서술의 중요성을 일깨워 주신 차정헌 교수님께 감사 드립니다. 많은 충고로 논문을 다듬어 주신 최봉규 교수님께 감사 드립니다. 실험에 많은 도움을 주시고 항상 친절히 질문에 답해주신 문선영 선생님께 감사의 마음을 전합니다.

언제나 저의 든든한 후원자인 사랑하는 아내와 아이들에게도 감사하며 조그만 성취를 허락해 주신 하나님께 감사 드립니다.

(이페이지는 원본과 교환)

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Abstract

Periodontitis is an inflammatory disorder and alveolar bone destruction is one of important characteristics. *Actinobacillus actinomycetemcomitans* is one of oral pathogens that causes periodontal disease. Osteoblasts play an important role in bone resorption. It has been reported that *A. actinomycetemcomitans* is able to adhere to and invade oral epithelial cells. However, it is unclear whether *A. actinomycetemcomitans* adheres to and invades osteoblasts. In this study, we examined the ability of *A. actinomycetemcomitans* for adherence and/or invasion to osteoblalsts and the effect of live or heat-killed *A. actinomycetemcomitans* on expression of bone resorption inducing factors such as receptor activator of NF-κB ligand (RANKL), macrophage inflammatory protein (MIP)-1α, tumor necrosis factor (TNF)-α, Interleukin (IL)-1β, and IL-6.

A. actinomycetemcomitans was able to adhere to and invade osteoblasts. The number of bacteria that adhered to osteoblasts is much higher than that of bacteria that invaded. In osteoblast Infected with live or heat-inactivated *A. actinomycetemcomitans*, mRNA level of RANKL, MIP-1α, TNF-α, IL-1β, and IL-6 was higher than that in non-treated osteoblasts. The osteoblasts infected with live *A. actinomycetemcomitans* expressed more mRNA of RANKL, MIP-1α, and IL-6 than those with the heat-inactivated *A. actinomycetemcomitans*. The induction level of TNF-α and IL-1β mRNA was similar in both cultures treated with live and heat-inactivated bacteria.

These findings suggest that *A. actinomycetemcomitans* can adhere to and invade osteoblasts and that the infection of *A. actinomycetemcomitans* in osteoblasts increases expression of bone resorption inducing factors such as RANKL, MIP-1α, and IL-6. These process may be involved in the increased osteoclastogenesis finally leads to bone resorption.

Key words : *Actinobacillus actinomycetemcomitans*, Osteoblast, RANKL, MIP-1α, IL-6, Invasion, Adhesion

Induction of bone resorption-inducing factors in osteoblasts by *Actinobacillus actinomycetemcomitans*

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Introduction

Bone resorption is induced by the osteoclast. The osteoclast is a multinucleated cell that removes bone matrix. It is differentiated from hematopoietic progenitor cells through multiple steps including migration, proliferation, expression of tartrate resistant acid phosphatase (TRAP), and fusion of cells. In this process, the osteoblast plays an important role via expression of osteoclastogenesis-inducing factors such as receptor activator of NF-κB ligand (RANKL, also known TRANCE, OPGL, and ODF), interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α (Boyle et al, 2003). RANKL is a recently discovered transmembrane molecule belonging to the TNF ligand superfamily expressed in osteoblasts, T cells, and synoviocytes. This molecule is essential for the process of osteoclast differentiation. Receptor activator of NF-κB (RANK), expressed in osteoclastic precursor cells, is a

receptor for RANKL and recognizes RANKL through cell-to-cell contacts. Thus, osteoblast regulates osteoclast differentiation through RANKL-RANK interaction (Hofbauer et al, 2000; Schoppet et al, 2002; Lacey et al, 1998).

Although RANKL is thought to be as the essential signal for full osteoclastic differentiation, there is RANKL-independent pathway. TNF-α directly regulates osteoclast differentiation by independent of RANKL-RANK interaction (Kobayashi et al, 2000). Macrophage inflammatory protein (MIP)-1 α, a member of the CC chemokines, is known to stimulate the formation of osteoclast-like multinucleated cells (Kukita et al, 1997). MIP-1α produced by human myeloma cells could act directly on osteoclast progenitor cells and induce osteoclastogenesis (Han et al, 2001).

Periodontitis is an inflammatory disease of periodontal tissue which surround the root of tooth. Alveolar bone resorption is one of representative clinical characteristics as cause of tooth loss. It has been well known that several periodontal pathogens can pass through epithelial barrier to invade deeper gingival tissue and destruct connective tissue. Several gram-negative bacteria-*Actinobacillus actinomycetecomitans, Porphyromonas gingivalis, Tannerella forsythensis, Eikenella corrodens, Prevotella intermedia, Fusobacterium nucleatum* and *Treponema denticola* - could invade deeper gingival tissue (Meyer et al, 1997). *A. actinomycetecomitans* is one of pathogens associated with chronic periodontitis and localized juvenile

periodontitis (LJP). LJP is a severe form of periodontitis and leads to rapid destruction of periodontal ligament and alveolar bone. Various structures *of A. actinomycetemcomitans* are associated with bone resorption. Saline extracted surface-associated material from *A. actinomycetemcomitans* stimulated bone resorption (Meghji et al, 1994). *A. actinomycetemcmitans* capsular-like polysaccharide stimulated osteoclast formation (Ueda et al, 1995; Nishihara et al, 1995). The bone resorbing mediator of *A. actinomycetemcomitans* was homologous to the molecular chaperone GroEL (Kirby et al, 1995). Lipopolysaccaride (LPS) from *A. actinomycetemcomitans* promoted osteoclastic differentiation (Ito et al, 1996). PGE_2 and IL-1α were involved in the differentiation and survival of osteoclasts induced by LPS from *A. actinomycetemcomitans* (Ueda et al, 1998).

A. actinomycetemcomitans was reported to invade endothelial cells (Schenkein et al, 2000). This property may provide this organism with access to the systemic circulation. *A. actinomycetemcomitans* invasion of epithelial cells is a multiple process which involves entry, escape from the vacuole, rapid multiplication, and intracellular and intercellular spread (Meyer et al, 1996). It was postulated that this process are involved in spreading of this bacterium to the gingival tissue and cause its destruction. The osteoblast is an important cell in osteoclast formation via expression of various osteoclast differentiation factors. However, the adhesion and invasion ability of *A.*

actinomycetemcomitans and the effects of the infection on osteoblasts remain unknown. We found here that *A. actinomycetemcomitans* can adhere to and invade osteoblasts and that its infection induces RANKL, MIP-1α, and IL-6. It suggests that it eventually may lead to up-regulate osteoclastogenesis.

Materials and Method

Materials

The mice (ICR strain) were obtained from Bio Korea Co. (Seoul, Korea). The α-minimum essential medium (α-MEM) and heat-inactivated bovine serum (FBS) were purchased from GIBCO BRL (Grand Island, NY). Gentamicin was obtained from Sigma (St. Louis, MO.)

Methods

Bacterial strains and growth conditions

A. actinomycetemcomitans Y4 (ATCC43718) was cultured in brain heart infusion media (BHI) at the condition of 37℃ and 10% CO_2 for 1 days. For preparation of heat-inactivated bacteria, bacterial cells were killed by incubating in 60℃ for 30min.

Preparation of primary osteoblasts.

The osteoblasts were isolated from the calvaria of 1-2-day old ICR mice. 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) for 20

min at 37℃ with a vigorous shaking, and, then, the digested solution was 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 procedure was repeated 4 times and the cells isolated by the last three digestions were combined as an osteoblasts. The isolated cells were cultured in α-MEM containing 10% FBS.

Adhesion and invasion assay

Osteoblasts (2×10^4) grown in 24-well culture plates were infected with $4x10^7$ *A. actinomycetemcomitans* (multiplicity of infection (MOI), 1:2000) for 3 h. The wells were then washed with phosphate-buffered saline (PBS) three times to remove nonadhering bacteria and the osteoblasts were lysed with PBS containing 0.5% Triton X-100 (100 μl/well) and then the lysate was diluted with PBS and plated on BHI agar. After indicated incubation for 1, 2 and 3 h, the colony forming unit (CFU) was determined as the invasive and adhesive cell number. For the invasion assay, the medium containing gentamicin (100 μg/ml) was added after bacterial contact and incubated for the indicated time to kill the bacteria that existed outside the cells. Bacterial suspension was discarded and washed with PBS twice. The cells were disrupted with PBS containing 0.5% Triton X-100. Dilution of the lysate was plated on BHI agar. After incubation, CFU was measured as the invasive cell

number.

Reverse-transcription polymerase chain-reaction (RT-PCR) method

Osteoblasts (2×10^5) grown in 6-well culture plates were infected with 4×10^8 live or heat-inactivated *A. actinomycetemcomitans* for 3 h. The mRNA expression level of RANKL, MIP-α, TNF-α, IL-1β, and IL-6 was determined by RT-PCR. RNA was extracted from infected cells by the Trizol-method. The cDNA was synthesized with cDNA synthesis kit (Bioneer, Daejeon, Korea) and followed by PCR amplication with specific primers for each cytokine. The total RNA (1μg) and oligo$(dT)_{18}$ primers were denatured at 70°C 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 unit/μl Moloney murine leukemia virus reverse transcriptase, 1× reaction buffer, 500 μM of each dATP, dCTP, dGTP, and dTTP, 20 units of recombinant RNase inhibitor), and incubated at 42°C for 60 min and then followed by 94°C for 5 min. The cDNA (4 μg) was amplified by PCR in a 50 μg reaction volume containing the 1× PCR reacton buffer, 200 μM dNTPs, 200 pM of the forward and reverse primers, and 0.5 units of Taq DNA polymerase (American Pharmacia Biotech., Little Chalfont, Buckinghamshire, UK) in a DNA thermal cycler (Biometra, Goettingen, Germany). The amplification reaction was performed for 35 cycles. The oligonucleotides used as primers for PCR and annealing temperatures are

shown in the Table 1. PCR products were electrophoresed on 1% agarose gel, stained with ethidium bromide, and detected with Multilineage Light Cabinet (Alpha Innotech Corp. San Leandro, CA). To determine involvement of material produced by *A. actinomycetemcomitans* in induction of RANKL, 4×10^8 *A. actinomycetemcomitans* were cultured in culture media of osteoblasts for 3 h and osteoblasts (2×10^5) were treated with this culture medium for 3 h.

Table 1. Sequence of primers for RANKL, MIP-1α, TNF-α, IL-1β, IL-6, and β-actin

Molecule	Direction	Primer sequence	Annealing temp (°C)	Product size (bp)
RANKL	Forward	5′-ATCAGAAGACAGCACTCACT-3′	45.3	750
	Reverse	5′-ATCTAGGACATCCATGCTAATGTTC-3′		
MIP-1α	Forward	5′-CAGCGAGTACCAGTCCCTTTT-3′	54.7	363
	Reverse	5′-CCTCGCTGCCTCCAAGA-3′		
TNF-α	Forward	5′-TTCTGTCTACTGAACTTCGGGGTGATCGGTCC-3′	60	468
	Reverse	5′-GTATGAGATAGCAAATCGGCTGACGGTGTGGG-3′		
IL-1β	Forward	5′-ATGGCAACTGTTCCTGAACTCAAGT-3′	50	563
	Reverse	5′-CAGGACAGGTATAGATTCTTTCCTTT-3′		
IL-6	Forward	5′-ATGAAGTTCCTCTCTGCAAGAGACT-3′	65	638
	Reverse	5′-CACTAGGTTTGCCGAGTAGATCTC-3′		
β-actin	Forward	5′-GGACTCCTATGGTGGGTGACGAGG-3′	58	366
	Reverse	5′-GGGAGAGCATAGCCCTCGTAGAT-3′		

Results

Adhesion and invasion of *A. actinomycetemcomitans* in osteoblasts.

At 1, 2, and 3 h of infection, the number of *A.actinomycetemcomitans* that adhere to osteoblasts was 1.6×10^5 CFU/well, 2.0×10^5 CFU/well, and 9.0×10^5 CFU/well, respectively. Aherence of *A.actinomycetemcomitans* increased with time through the 3 h assay. The number of adhesive bacteria increased slowly during first 2 h and increased rapidly after that time to reach at the maximum level of adhesion efficiency of 2.3% in 3 h (Table 2). In 1, 2, and 3 h of invasion assay, the number of bacteria within cells was 1 CFU/well, 13 CFU/well and 7 CFU/well, respectively. The number of internalized bacteria increased to reach at the maximum level after 2h and then decreased slowly in last 1h (Fig. 1). However, the number of internalized bacteria was much less than the adhesive ones.

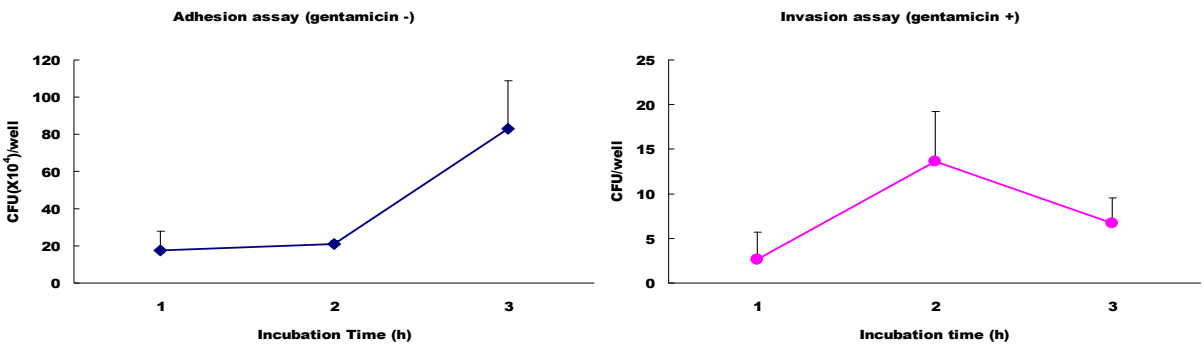

Fig. 1. Adhesion to and invasion of osteoblasts of *A. actinomycetemcomitans*. Time course of bacterial adhesion and invasion.

time / efficiency (%	Adhesion efficiency (%)	Invasion efficiency (%)
1 h	0.4%	0.000025%
2 h	0.5%	0.000033%
3 h	2.3%	0.000018%

Table 2. Adhesion and Invasion efficiency of *A. actinomycetemcomitans* to osteoblasts

Expression of RANKL, MIP-1α, and cytokines in osteoblasts after *A. actinomycetemcomitans* infection

To determine the effect of *A. actinomycetemcomitans* on osteoclastogenesis-inducing activity of osteoblasts, we measured the expression level of RANKL, MIP-1α, TNF-α, IL-1β, and IL-6 by RT-PCR in osteoblast after 3 h of *A. actinomycetemcomiitans* infection. In osteoblasts infected with *A. actinomycetemcomitans,* expression level of RANKL, MIP-1α, TNF-α, IL-1β, and IL-6 mRNA was increased (Fig. 2). To determine the involvement of secreted components from *A. actinomycetemcomitans* in RANKL induction, osteoblasts were treated with culture supernatant of *A. actinomycetemcomitans* and measured RANKL expression (Fig 3). Bacterial culture supernatant slightly increased expression of RANKL mRNA, but it is lower than that of bacteria infected cells.

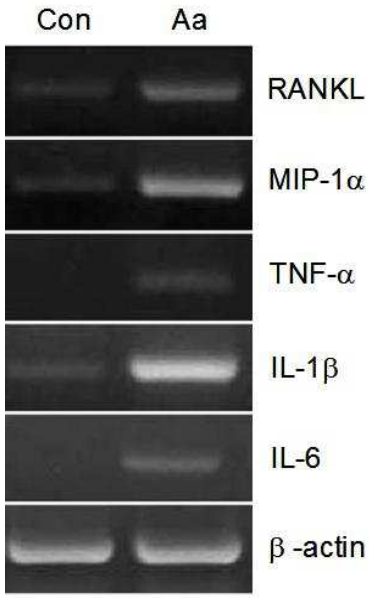

Fig. 2. Expression of RANKL, MIP-1α, TNF-α, IL-1β, and IL-6 mRNA in osteoblasts infected by *A. actinomycetemcomitans* .The Calvaria-derived osteoblats were infected with *A. actinomycetemcomitnas* for 3 h. Total RNA was extracted and subjected to RT-PCR analysis for RANKL, MIP-1α, TNF-α, IL-1β, IL-6, and β-actin .

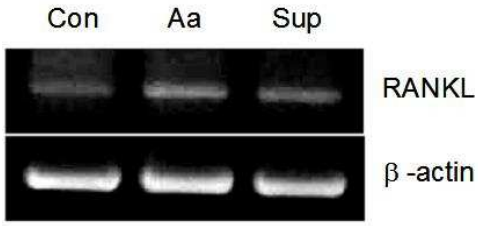

Fig 3. Expression of RANKL mRNA in osteoblasts infected by *A. actinomycetemcomitans* or treated with supernatant of *A. actinomycetemcomitans* culture media. Calvaria-derived osteoblasts were cultured in the absence of any stimulant. Osteoblasts were infected with *A. actinomycetemcomitans* for 3 h and the other osteoblasts were cultured in the

supernatant of *A. actinomycetemcomitans* culture media for the same time. The total RNA was extracted and subjected to RT-PCR analysis for RANKL and β-actin.

Effect of heat-inactivated *A. actinomycetemcomitans* on expression of RANKL, MIP-1α, and cytokines in osteoblasts

To compare the effect of heat-inactivated *A. actinomycetemcomitans* with that of live *A. actinomyctemcomitans*, RT-PCR analysis was done to detect mRNA of cytokines expressed by osteoblasts infected with live or heat-inactivated *A. actinomycetemcomitans* (Fig 4). In the RT-PCR analysis done in 3 h and 6 h, live *A. actinomycetemcomitans* and heat-inactivated *A. actinomycetemcomitans* increased expression of RANKL, MIP-1α, TNF-α, IL-1β, and IL-6. But osteoblasts infected with live *A.actinomycetemcomitans* expressed more mRNA of RANKL, MIP-1α, and IL-6 than osteoblasts infected with heat-inactivated *A.actinomycetemcomitans*. The expression level of TNF-α and IL-1β mRNA was similar in both cultures of 6 h.

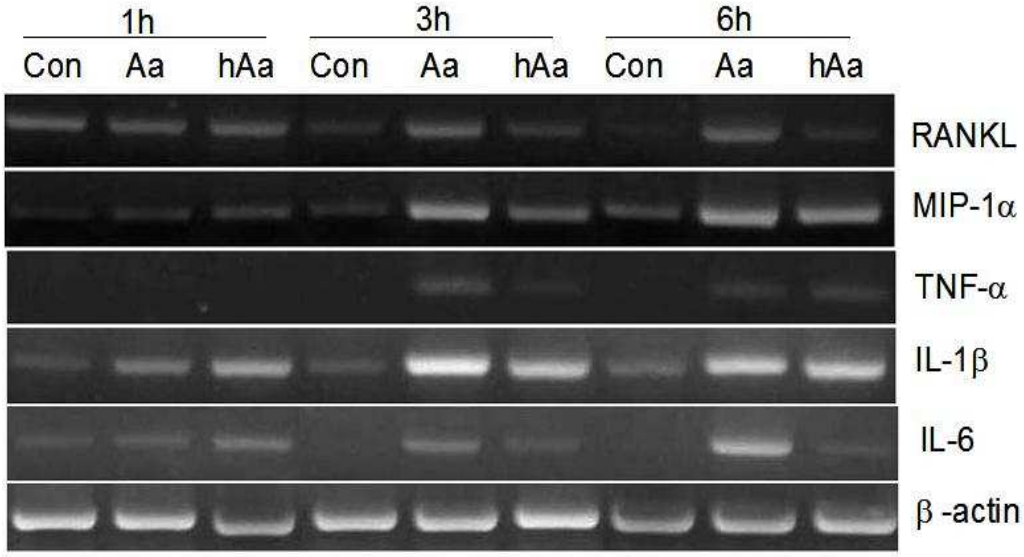

Fig. 4. Expression of RANKL, MIP-1α, TNF-α, IL-1β, and IL-6 mRNA in osteoblasts treated by heat-inactivated *A. actinomycetemcomitans.* Calvaria-derived osteoblasts were cultured in the presence of live or heat-inactivated *A. actinomycetemcomitnas* for indicated times. Total RNA was extracted and subjected to RT-PCR analysis for RANKL, MIP-1α, TNF-α, IL-1β, IL-6, and β-actin.

Discussion

The present study showed that *A. actinomycetemcomitans* can attach to and invade mouse osteoblasts and increase expression of bone resorption inducing factors such as RANKL, MIP-1α, IL-6, TNF-α, and IL-1β.

A. actinomycetemcomitans is known to invade various cells. Invasion of human gingival tissue in vivo by *A. actinomycetemcomitans* was clearly demonstrated. (Christersson et al, 1987) *A. actinomycetemcomitans* gains entry into epithelial cells and endothelial cells in vitro study (Meyer et al, 1996, 1997; Sreenivasan et al, 1993; Schenkein et al, 2000). However, it is unknown whether *A. actinomycetemcomitans* is able to adhere to and invade osteoblasts whether *A. actinomycetemcomitans* affects on function of bone cells. In this study, we firstly demonstrated that *A. actinomycetemcomitans* attached to and invaded mouse primary osteoblasts. Adhesion and invasion efficiency of *A. actinomycetemcomitans* to osteoblast was 2.3% and 1.8×10^{-5}% (13 CFU/well). Invasion efficiency of *A. actinomycetemcomitans* to epithelial and endothelial cells were 1% and 1.13%, respectively (Meyer et al, 1996; Harvey et al, 2000). Adhesion and invasion efficiency of *Porphyromonas gingivalis* to osteoblast was 3% and 0.8%, respectively (Okahashi et al, 2003). The invasion efficiency of *Streptococcus pyogenes* and *Staphylococcus aureus* was 6-7 CFU/well and 10-60 CFU/well, respectively (Okahashi et al, 2002;

Ellinton et al, 1999). In osteoblast, adhesion efficiency of *A. actinomycetemcomitans* was similar to that of *P. gingivalis* and invasion efficiency was similar to that of *S. aureus* and *S. pyogenes*. Invasion efficiency of *A. actinomycetemcomitans* to osteoblast was extremely lower than that to epithelial and endothelial cells. Previously, it is shown that invasiveness of *A. actinomycetemcomitans* to endothelial cells and epithelial cells plays role in spreading into deeper tissue of *A. actinomycetemcomitans* (Meyer et al, 1996). It is probably that internalization of *A. actinomycetemcomitans* to osteoblasts facilitate the progression of disease by protecting the organism from extracellular host defense and/or antibiotic therapy and by spreading to adjacent cells and tissues (Meyer et al, 1997).

Osteoblasts play a conductor in osteoclastogenesis by producing osteoclastogenesis inducing factors such as RANKL (Suda et al, 1999) and MIP-1α (Kukita et al, 1997). Therefore, it is important to determine the effect of *A. actinomycetemcomitans* infection on osteoclastogensis-inducing ability of osteoblasts. An increase in RANKL mRNA was detectable in 3 h infection and an increased expression of MIP-1α was also detected. It has not been clear whether MIP-1α act directly or indirectly on cells in the osteoclast lineage or not. More groups reported that MIP-1α alone increased osteoclast formation. Human MIP-1α increased osteoclast formation in human bone marrow culture (Han, 2001) and MIP-1α induced osteoclast differentiation in

rat bone marrow cultured on a calcified matrix (Kukita et al, 1997). But, in other study, MIP-1α alone did not stimulate osteoclastogenesis. MIP-1α only enhanced osteoclastogensis in the presence of RANKL or dihydroxyvitamin D_3 and It was suggested that MIP-1α is an up-regulator of osteoclastogenesis (Watanabe et al, 2004). Taken together, these suggest that infection of *A. actinomycetemcomitans* up-regulate osteoclastogensis-inducing activity of osteoblast via increase of RANKL and MIP-1α expression. In osteoclastogenesis by *A. actinomycetemcomitans*-infection, RANKL and MIP-1α may work together.

RANKL expression is controlled by various bone resorption-inducing factors. PGE_2, TNF-α, IL-1β, and IL-6 increase RANKL expression in osteoblastic cells (Fujita et al, 2003; Hofbauer et al, 2000; Palmqvist et al, 2002; Jimi 1999; Ishimi et al, 1990). In our study, RT-PCR revealed that *A. actinomycetemcomitans*-infection induced up-regulation of the mRNAs of TNF-α, IL-1β, and IL-6. These results suggest that increase of RANKL expression by infection of *A. actinomycetemcomitans* may be mediated by TNF-α, IL-1β, and IL-6.

We inactivated *A. actinomycetemcomitans* with heat-treatment, and compared its activity with that of live *A. actinomycetemcomitans*. Heat-treatment of *A. actinomycetemcomitans* reduced expression of RANKL, MIP-1α, and IL-6 induced by the live bacteria. But, the expression of IL-1β and

TNF-α was not affected by heat-treatment. In addition, the RANKL expression in culture treated with bacterial culture supernatant is lower than that of cultures treated with live bacteria. These suggest that adhesion and invasion of *A. actinomycetemcomitans* may be involved in expression of RANKL, MIP-1α, and IL-6. Although the expression level of RANKL, MIP-1α, and IL-6 was reduced by the heat inactivation, it was not completely inhibited. It indicates that other factors are also involved in the expression of RANKL, MIP-1α, and IL-6. It was reported that various components of *A. actinomycetemcomitans*, such as cell surface material, LPS, capsular polysaccharide, and heat-shock protein are involved in bone resorption (Ito et al. 1996; Kirby et al. 1995; Ueda et al.1995; Nishihara et al.1994,1995). Among these, LPS of *A. actinomycetemcomitans* increased expression of RANKL (Zou et al, 2002). The LPS and capsular polysaccharide are heat-stable component. Therefore, residual activity of heat-inactivated *A. actinomycetemcomitans* may be due to these components.

S. aureus, S. pyogenes, and P. gingivalis were also known to invade osteoblasts and affect on cell function. Osteoblasts infected with *S. aureus* induced high level of IL-6 and IL-12 (Bost et al. 1999). Infection by *S. pyogenes* induced RANKL expression in osteoblasts (Okahashi N, et al. 2003). *P. gingivalis* induced RANKL expression in osteoblasts through activator protein 1 signaling. (Okahashi et al, 2004). Regarding the invasion mechanism,

S. aureus internalization is related to actin microfilaments and microtubles of osteoblasts. In *S. pyogenes*, fibrinonectin-binding protein, such as F1 and Fba are considered to be an adhesin and invasin. The mechanism of invasion of *A. actinomycetemcomutans* into epithelial cell and endothelial cells is unclear. In endothelial cells, *A. actinomycetemcomitans* can invade endothelial cells using platelet-activating factor (PAF) receptors and this is depend on the presence of phosphorycholine on the bacterial cell surface (Schenkein et al, 2000). Transferrin receptor and integrin on epitheial cells is a possible invasion-specific receptor for *A. actinomycetemcomitans* (Meyer et al, 1997). Outer membrane protein 100 (Omp100) is localized on the surface of *A. actinomycetemcomitans* and, recently, it was demonstrated that Omp100 acts as adhesin, invasin, and inducer for IL-6 in epithelial cells (Asakawa et al, 2003). These suggest that invasion mechanism of each bacteria is different and depend on cell type and bacterial species. In the future, the mechanisn of *A. actinomycetemcomitans*-infection in osteoblast such as interaction of host cell receptor and bacterial cell component will be clarified.

A. actinomycetemcomitans can attach to and invade osteoblast, but the number of internalization of *A. actinomycetemcomitans* is lower than that of attached bacteria. In epithelial cell, the number of internalized *A. actinomycetemcomitans* is also lower than that of attached bacteria (Sreenivassan et al. 1993). In our assay system, we estimated the expression

of osteoclast-inducing factors in the presence of attached and internalized *A. actinomycetemcomitans*. Therefore, we will estimate relative importance of adhesion and invasion of *A. actinomycetemcomitans* in induction of bone-resorption inducing factors.

In summary, we found that *A. actinomycetemcomitans* can adhere to and invade osteoblasts and that the expression of RANKL, MIP-1α and IL-6 is increased by infection of *A. actinomycetemcomitans*. Regarding the number of adhesive and invading bacteria, Adhesion is probably more important than invasion in inducing bone resorbing factors. These results suggest that bone resorption is enhanced by *A. actinomycetemcomitans*-infection of osteoblasts and adhesion and invasion of *A. actinomycetemcomitans* into osteoblasts is an important step of pathogenesis in bone destruction induced by this bacteria.

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국문 요약

*Actinobacillus actinomycetemcomitans*에 의한 조골세포의 골흡수 유도인자의 생성증가

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치주염은 염증성 질환으로 치조골의 파괴가 중요한 임상적 특징이다. 조골세포는 골흡수를 유도하는 receptor activator of NF-κB ligand (RANKL), macrophage inflammatory protein (MIP)-1α, tumor necrosis factor (TNF)-α, Interleukin (IL)-1β 및 IL-6와 같은 cytokine을 생성하여 파골 세포 형성에 있어서 중요하다. 치주염의 원인균인 *Actinobacillus actinomycetemcomitans*의 상피세포 및 내피세포 부착/침투능에 대한 기전이 일부 밝혀져 있으나 본 세균의 조골세포 부착/침투능에 대해서는 알려져 있지 않다. 따라서 본 연구에서는 *A. actinomycetemcomitans*의 조골세포 부착/침투능 및 본 세균의 부착/침투에 의한 조골세포의 골흡수유도능 변화를 평가하였다.

*A. actinomycetemcomitans*는 조골세포에 부착하였으며 일부 세균은 세포 내로 침투하였다. 조골세포 표면에 부착한 세균의 수는 침투한 세균의 수 보다

많았다. *A. actinomycetemcomitans*에 의하여 감염된 조골세포에서 RANKL, MIP-1α, TNF-α, IL-1 β 및 IL-6 mRNA의 발현이 증가하였다. 열처리 세균으로 처리한 조골세포에서도 RANKL, MIP-1α, TNF-α, IL-1 β 및 IL-6의 발현이 증가하였으나 생균에 의하여 감염된 경우 열처리세균에 의한 경우 보다 RANKL, MIP-1α 및 IL-6 mRNA이 더 강하게 발현되었다.

이들 결과는 *A. actinomycetemcomitans*가 조골세포에 부착 및 침투를 할 수 있으며 *A. actinomycetemcomitans*에 의한 조골세포 감염이 조골세포의 RANKL, MIP-1α 및 IL-6의 생성을 증가시켜 치주염시 야기되는 골흡수에 관여할 수 있음을 시사한다.