The effect of bacterial γ-glutamyltranspeptidase on osteoclastogenesis

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감사의 글

연구 시작단계부터 본 논문의 완성까지 세심한 지도와 많은 배려를 해주시고 마지막까지 좋은 논문이 되도록 힘써주신 차정헌 교수님께 진심으로 감사를 드립니다. 논문 실험 시작부터 과정 내내 깊은 관심과 조언을 해주신 유윤정 교수님과 많은 충고와 조언으로 논문을 다듬어 주신 서정택 교수님께 감사 드립니다. 실험에 많은 도움을 주시고 항상 친절히 질문에 답해주신 이양신 선생님, 최호길 선생님께도 감사의 마음을 전합니다.

아울러 변함없는 사랑과 기도를 보내주신 양가 부모님, 든든한 후원자인 아내 유수정과 사랑스런 두 아들 영준, 호준에게 감사하며 하나님께 감사 드립니다.

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Abstract

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A novel bone-resorbing factor, γ-glutamyltranpeptidase (GGT), was recently cloned from mouse T-lymphoma cell cDNA library. Since the GGT is widely distributed in living organisms and *Bacillus subtilis* GGT exhibits high similarity in its primary structure and enzymatic characteristics with the mammalian GGTs, the *B. subtilis* GGT was examined for the osteoclast formation. The osteoclast formation was performed in a co-culture system of mouse calvaria-derived osteoblasts and bone marrow cells, and determined by tartrate resistant acid phosphatase staining. Conditioned medium from GGT-overproducing *B. subtilis* culture showed significantly higher activity of osteoclast formation when compared to those from wild-type and *ggt*-deleted *B. subtilis* culture. To understand the mechanism of how GGT induce osteoclastogenesis, *B. subtilis* GGT was cloned, expressed in *Escherichia coli* and purified using His-tag purification. The purified GGT stimulated mRNA expression of the receptor activator of nuclear factor-κB ligand, cyclooxygenase-2, heparin-binding epidermal growth factor-like growth factor, and amphiregulin in mouse calvaria-derived osteoblasts.

This report is the first demonstration that bacterial GGT is able to serve as the bone-resorbing factor and the osteoclastogenesis by the GGT is mediated by a RANKL-dependent pathway. Based on this study, it can be hypothesized that GGT in periodontopathic bacteria can be an important virulence factor for bone destruction in

periodontitis.

Keywords : γ-glutamyltranspeptidase, *Bacillus subtilis*, osteoclastogenesis, RANKL, COX-2, HB-EGF, amphiregulin

The effect of bacterial γ-glutamyltranspeptidase on osteoclastogenesis

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Ⅰ. Introduction

Osteoclasts are multinucleated cells with a bone resorbing activity and play a crucial role in bone resorption. They are derived from hemotopoietic cells through multiple steps including proliferation, expression of tartrate resistant acid phosphatase (TRAP), and fusion of cells (Suda et al., 1997; Hofbauer et al., 2000).

Osteoclast formation requires the presence of osteoblast or stromal cells (Takahashi et al., 1999). These cells express a receptor of the nuclear factor-κB(RANK) ligand (RANKL, also known as the osteoclast differentiation factor) that promotes osteoclastogenesis. The osteoclast precursors express RANK which interacts with RANKL, through cell-cell contact, the cells then differentiate into osteoclasts (Hsu et al., 1999). Thus, Osteoblast regulates osteoclast differentiation through RANKL-RANK interaction

(Lacey et al., 1998). Osteoprotegerin (OPG), which is also secreted by osteoblast lineage cells, is a soluble detoy receptor that neutralizes the biological activity of RANKL (Simonet et al., 1997; Yasuda et al., 1998; Udagawa et al., 2000). The regulation of RANKL by OPG suggests that bone resorption may be mediated through control of RANKL and /or OPG production and that ostesclast formation is determined principally by the ratio of RANKL to OPG (Hofbauer et al., 1998; Vidal et al., 1998; Nagai et al., 1999; Brändström et al., 2001).

RANKL expression in osteobalsts is upregulated by pro-resorptive hormones and cytokines, such as 1,25-dihydroxyvitamin D_3 (1 α,25$(OH)_2D_3$), parathyroid hormone, prostaglandin E_2 (PGE_2), and interlenkin-1 (Tsukii et al., 1998; Hofbauer et al., 1999; Walsh et al., 2003).

Recently, a novel bone-resorbing factor was isolated and cloned using an expression cloning technique from a BW5147 mouse T-lymphoma cell cDNA library. Sequencing analysis identified the factor as γ-glutamyltranpeptidase (GGT) (Niida et al., 2004). GGT, which is widely distributed in living organism (Tate et al., 1981), catalyzes the hydrolysis of γ-glutamyl compounds such as glutathione (Lieberman et al., 1995), as well as the hydrolysis of the amide bonds of glutamine to yield glutamic acid and ammonia, simillar to glutaminase. GGT can also catalyze the transfer of γ-glutamyl moieties to amino acids and peptides. *ywrD* might encode a second GGT and exhibit 31 and 27% identity with amino acid *B. subtilis* GGT. *ywrD* has no signal sequence.

The addition of purified GGT protein to mouse bone marrow culture effectively induced formation of osteoclasts. An antibody against GGT inhibited Osteoclast formation but not the enzymatic activity. They also demonstrated that an inactive form of GGT, the enzymatic

activity of which had been blocked by chemical modification with a specific inhibitor, acivicin, supported osteoclast formation. These results indicate that GGT acts on osteoclast formation independent of its own enzymatic activity. Furthermore, both native GGT and inactive GGT stimulated the expression of the RANKL mRNA and protein from bone marrow stromal cells. The osteoclastogenesis biological activity of GGT protein is in a manner independent of its enzymatic activity.

Since GGT is widely distributed in living organism, bacteria also have GGT. The GGT of gram-positive bacterea such as *Bacillus* species are extracellular enzymes (Ogawa et al., 1991; ogawa et al., 1997; Kunst et al., 1997), so they can be purified from the culture broth and the GGT of gram-negative bacteria such as *Escherichia coli* is present in the periplasmic space (Suzuki et al., 1986). It was reported that *Helicobacter pylori* GGT upregulates cyclooxygenase (COX)-2, heparin-binding epidermal growth factor-like growth factor (HB-EGF) and amphiregulin (AR) expression in human gastric cells (Busiello et al., 2004). The COX-2 is known as a factor of PGE_2 induction. The expression of RANKL is stimulated by systemic bone resorbing factors such as PGE_2. Above mechanisms suggest that the bacterial GGT may also stimulate RANKL expression and serve as a bone resorbing factor.

Thus, we examined whether bacterial GGT also has bone-resorbing activity and investigated the mechanism of how the GGT induces osteoclastogenesis.

Ⅱ. Materials and Method

1. Materials

The ICR mice 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). LPS of *Escherichia coli*, and tartrate-resistant acid phosphatase (TRAP: a marker of osteoclast) staining kit were obtained from Sigma (St. Louis, MO).

2. Preparation of primary calvaria and bone marrow cells.

The osteobblastic cells were isolated from the calvariae of 1- to 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, Grand Island, NY, USA) for 20 min at 37℃ with vigorous shaking and then centrifuged at 1,500 x g for 5 min. The first supernatant was discarded, another 10 ml of the collagenase-dispase enzyme solution was added, and the preparation was incubated for 20 min. The digestion procedure was repeated four 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. The bone marrow cells were collected from 5- to 8-week old mice. The ends of the tibiae and femurs were removed, and each marrow cavity was flushed by slowly injecting medium at one end with a 25-gauge needle. The marrow cells were washed and used for the co-culture.

3.Bacteria culture.

Wild-type (*B. subtilis* 168), *ggt*-deleted (MH2342), *ggt* and *ywrD*-

deleted (MH2353) and GGT-overproducing (MH2308) *B. subtilis* were obtained from Hideyuki Suzuki, Kyoto Univeristy and listed in Table 1. *B. subtilis* strains were inoculated on BHI agar plate and cultured at 37℃ for 18 hr. A single colony was inoculated and cultured at 37℃ for 96 hr in 10 ml Brain Heart Infusion (BHI) media. The bacteria were harvested by centrifugation and discarded. The supernatant was filtrated by 0.22 μm filter (Millipore, Bedford, Ireland). The filtrates were stored at -70℃ until use.

4. **Plasmid contruction.**

The pET21b plasmid was used for *B. subtilis* GGT protein expression in *E. coli*. The coding regions (residues 1 to 587) was amplified by PCR with *B. subtilis* chromosomal DNA template and primers (BsGGT-F and BsGGT-B), and cloned into pET21b using the *Eco*RI and *Xho*I sites, resulting in pET21b/BsGGT plasmid. The both strands of the insert fragment were sequenced by the Cosmogene Tec. (Cosmo, Korea) to confirm the fidelity of PCR.

5. Expression and purification of recombinant *B. subtilis* GGT protein

The recombinant *B. subtilis* GGT protein was expressed from pET21b/BsGGT in *E. coli* BL21(DE3). The recombinant *B. subtilis* GGT protein contained a T7 tag at its amino terminus and a His tag at its carboxyl terminus to facilitate the purification step. The overexpressed *B. subtilis* GGT protein formed inclusion body, which was isolated and dissolved in 8 M urea. The dissolved solution was purified by His tag affinity column as described in the manufacturer's instructions. The purified recombinant *B. subtilis* GGT protein was refolded by sequential dialytic removal of urea in 25 mM Tris buffer with 4, 2, 1, and 0 M urea steps. The amount of purified and refolded recombinant *B. subtilis* GGT protein was

determined by the Bradford protein assay (Bio-rad).

6. Osteoclast formation assay.

The isolated calvaria cells were seeded at a concentration of 10^6 cells per 10-cm culture dish and grown to confluence. The cells were then detached from the culture dishes with trypsin-EDTA (GIBCO BRL). Subsequently, the cells (1 x 10^4 cells/well) were cocultured with the bone marrow cells (1 x 10^5 cells/well) in α -MEM containing 10 % FBS in 48-welll plates (Corning Inc., Corning, N.Y.). The culture volume was adjusted to 400 μl per well with α-MEM containing 10% FBS. The conditioned media (50 or 100 μl) from wild-type, *ggt*-deleted, *ggt* and *ywrD*-deleted, and GGT-overporducing *B. subtilis* cultures or the purified GGT at the indicated concentration were added to each co-culture after the medium was exchanged on day 3. Also, BHI media (50 or 100 μl) or *E. coli* LPS (1 μg/ml) were added as negative and positive controls, respectively. The co-culture was then maintained for an additional 4 days. Osteoclast differentiation was monitored by using a TRAP staining kit according to the manufacturer's instructions. TRAP-positive multinucleated cells having more than three nuclei were defined as osteoclasts. The osteoclast cells per well were counted for osteoclast formation activity.

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

The mRNA Expression was determined by RT-PCR. Total RNA (1 μg) isolated from nontreated or treated mouse calvaria-derived osteoblast cells for 3 hr was used as a template for cDNA synthesis in a 20-μl reaction mixture performed with an RT kit (CLONTECH, Palo Alto, Calif.) according to the manufacturer's instructions. The RNA (1 μg) and oligo$(dT)_{18}$ primers (1 mM) were denatured at 70℃

for 5 min and incubated for 1 to 2 min on ice. The denatured RNA and oligo$(dT)_{18}$ primers were added to the reaction mixture (1 U of Moloney murine leukemia virus reverse transcriptase per μl, 1X reaction buffer, 500 μM 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 μg) was amplified by PCR in a 50-μl reaction mixture containing 1 X PCR reaction buffer, each deoxynucleoside triphosphate at a concentration of 200 μM, 200 pM forward primer, 200 pM reverse primer, and 0.5 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, United Kingdom) in a DNA thermal cycler (Biometra, Gottinge, Germany). The amplification reaction was performed for 35 cycles, and the primer sequences and annealing temperatures used are shown in Table 2. The PCR products were separated by electrophoresis on a 1.5% agarose gel, stained by ethidium bromide, and detected with Multilineage Light Cabinet (Alpha Innotech Corp. San Leandro, CA). The relative intensities of the gel bands were measured by using an image-analyzing program (frog2000 version3.1.2). 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.

Table 1. Bacterial strains and plasmids in this study

Strain or plasmid		Characteristic(s)
B. subtilis		
MH2275	*B. subtilis* 168	*trp*C2
MH2342	*B. subtilis* 168, but ggt-	*trpC2* △*ggt*::*cat*+
MH2308	plasmid with *B. subtilis ggt* gene (pMH2312) in sw153; GGT over-producer	
MH2350	*B. subtilis* 168, but *ywrD*-	*trpC2* △*ywrD*::*cat*+
MH2353	*B. subtilis* 168, but *ggt*- *ywr*D-	*trpC2* △*ggt*::*cat*+ △*ywrD*::*tet*+
Plasmid		
pMH2312		the *Hae*Ⅲ-*Bgl*Ⅱ fragment of pPL623 containing the *cam*+ gene was ligated with pMH2285 cleaved with *Hpa* Ⅰ

Table 2. Sequence of primers for *B.subtilis* GGT, RANKL, OPG, HB-EGF, AR, and β-actin

Molecule	Direction	Primer sequence	Annealing temp(℃)	Product size(bp)
GGT	Forward	5'-CCGGAATTCGATGAAAAGAACGTGGAAC-3'	55	1764
	Reverse	5'-CCGCTCGAGTTTACGTTTTAAATTAATGCCG-3'		
RANKL	Forward	5'-ATCAGAAGACACCACTCACT-3'	45.3	750
	Reverse	5'-ATCTAGGACATCCATGCTAATGTTC-3'		
OPG	Forward	5'-TGAGTGTGAGGAAGGGCGTT-3'	45.5	636
	Reverse	5'-TTCCTCGTTCTCTCAATCTC-3'		
HB-EGF	Forward	5'-CGGGAGGTGATCGTGCTCAGGG-3'	55	326
	Reverse	5'-CTGTAGTGTGGTCATATGTGTATAGGG-3'		
AR	Forward	5'-CAGGGGACTACGACTACTCAGA-3'	59	432
	Reverse	5'-GATAACGATGCCGATGCCAATA-3'		
COX-2	Forward	5'-GGGTTGCTGGGGGAAGAAATGTG-3'	60	479
	Reverse	5'-GGTGGCTGTTTTGGTAGGCTGTG-3'		
β-actin	Forward	5'-GGACTCCTATGGTGGGTGACGAGG-3'	58	366
	Reverse	5'-GGGAGAGCATAGCCCTCGTAGAT-3'		

Ⅲ. Results

1. Osteoclast-forming Activity of *B. subtilis* GGT in conditioned medium

To verify the osteoclast-froming activity of *B. subtilis* GGT, the conditioned media from wild-type, *ggt*-deleted, *ggt* and *ywrD*-deleted, GGT-overproducing *B. subtilis* were subjected to an in vitro osteoclast-forming assay using a co-culture system of mouse calvaria-derived osteoblasts and bone marrow cells. TRAP-positive multinucleated cells (MNCs) possessing more than three nuclei were defined as osteoclasts. The co-culture system was treated for 3 days with 50 or 100 μl of the conditioned media to induce TRAP-positive MNCs formation. The conditioned medium of GGT-overproducing *B. subtilis* stimutated the osteoclast-forming activity whereas other conditioned media didn't. The osteoclast-forming activity of the 50 μl conditioned medium (GGT-overproducing *B. subtilis*) is significantly high since it is comparable to that of *E. coli* LPS.(Fig. 1)

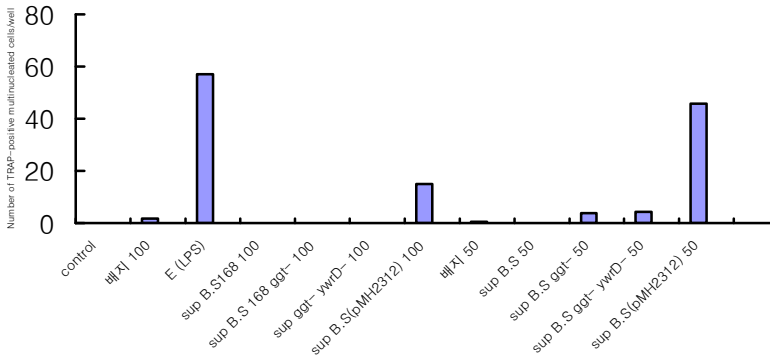

Figure 1. The effect of *B. subtilis* GGT in conditioned medium on osteoclast-forming activity.

Treatment with the 50 μl conditioned medium resulted in better osteoclast-forming activity than treatment with the 100 μl, suggesting that high dose of conditioned medium might be cytotoxic.

2. Purification of *B. subtilis* GGT

B. subtilis ggt gene was PCR-amplifed from *B. subtilis* chromosomal DNA and cloned in pET21-b expression vector, generating pET21b/BsGGT. The plasmid, pET21b/BsGGT containing *B. subtilis ggt* was screened with restriction enzymes, *Eco*RI and *Xho*I, and was sequenced to confirm the fidelity of PCR (data not shown). The plasmid was transformed, and expressed in BL21 strain. The over-expressed *B. subtilis* GGT was found to be in inclusion body (Fig. 2). The inclusion body was collected, denatured in 8 M urea, and purified using His-tag purification. The purified GGT was dialyzed against Tris buffer and the purified GGT was incubated at 37℃ for the autocatalytic process.

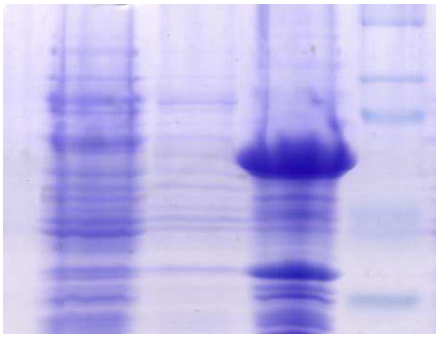

Figure 2. Overexpression of *B. subtilis* GGT in *E. coli*

The autocatalytic process was not observed until 2 days. A single major band of the GGT appeared after the purification (Fig. 3).

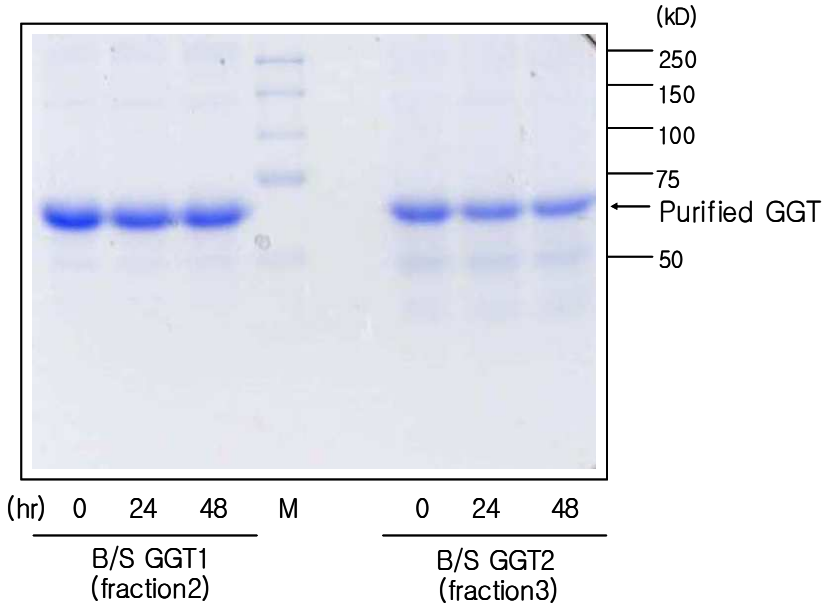

Figure 3. The purified *B. subtilis* GGT using His-Tag purificationwas incubated at 37℃ for the autocatalytic process.

3. Effect of Purified GGT on mRNA Expression of RANKL, OPG, HB-EGF, AR, and COX-2.

To observe the effect of the bacterial GGT on expression of RANKL, OPG, HB-EGF, AR and COX-2, the mRNA expression was measured using RT-PCR in mouse calvaria-derived osteoblasts treated for 3 hr with the purified GGT at the concentration of 0, 10, 100 and 1000 ng/ml (Figure 4 and Table 3). The RANKL mRNA expression was induced significantly by 2.1, 2.2, and 2.5 fold at the GGT concentration of 10, 100, 1000 ng/ml, respectively. In addition, since COX-2 is known as a factor to induce PGE_2, pro-resorptive factor, the COX-2 mRNA

expression was examined and resulted in induction by approximately 1.6 fold. Interestingly, the HB-EGF mRNA expression was induced by 1.7 fold at 10 and 100 ng/ml, and 2.0 fold at 1000 ng/ml as well as AR was also induced slightly by approximately 1.4 fold.

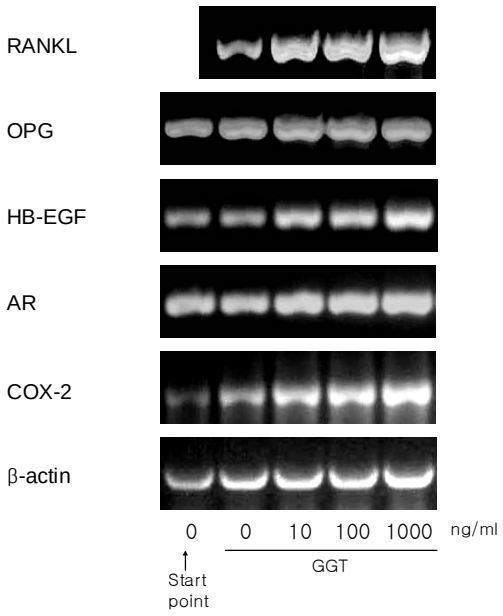

Figure 4. The effect of the purified GGT on m RNA expression of RANKL. OPG, HB-EGF, AR and COX-2 in osteoblasts. The calvaria-derived osteoblasts were incubated with the purified GGT at the concentration of 0, 10, 100, and 1000 ng/ml for 3 hr. Total RNA was extracted and subjected to RT-PCR analysis.

However, in the case of OPG, it was not induced at the concentration of 1000 ng/ml or slightly induced by 1.2 fold at 10 and 100 ng/ml.

Therefore, the mRNA expression of RANKL, HB-EGF, AR and COX-2 were induced by the GGT concentrations of 10, 100, and 1000 ng/ml while

that of OPG was induced very slightly, if any.

Table 3. The mRNA ratios of RANKL, OPG, HB-EGF, AR and COX-2 to β-actin in the calvaria-derived osteoblasts after 3 hr incubation with the purified GGT at the concentration of 10, 100, and 1000 ng/ml.

GGT concentration	RANKL/ β-actin	OPG/ β-actin	HB-EGF/ β-actin	AR/β-actin	Cox2/ β-actin
0 ng/ml	1	1	1	1	1
10 ng/ml	2.072	1.232	1.667	1.307	1.535
100 ng/ml	2.211	1.234	1.730	1.390	1.646
1000 ng/ml	2.472	1.057	1.975	1.405	1.576

Ⅳ. Discussion

The present study showed that *B. subtilis* GGT is able to serve as a bone-resorbing factor through a RANKL-dependent pathway as mammalian GGT does. It is the novel biological activity demonstrated with the prokaryotic GGT. Many previous studies of bacterial GGT have focused on the enzymatic functions. These studies have revealed that GGT catalyzes the first step in the degradation of glutathione and plays an important role in glutathione metabolism (Taniguchi et ai., 1998). In addition, the expression of mammalian GGT is elevated under certain conditions, such as carcinogenesis (Hanigan et al., 1994; Haniganet al., 1999; Taniguchi et al., 1985; Taniguchi et al., 1985) and it is used as a marker enzyme for many diseases. However, other biological activities of significance of GGT have not yet been demonstrated.

This study showed that the conditioned medium from GGT-overproducing *B. subtilis* induced osteoclast formation in the co-culture system. Fifty μl conditioned medium showed better activity for osteoclast formation than 100 μl broth did, indicating the bacterial broth may have some cytotoxic effect.

To verify the effect of GGT in osteoclast formation activity and to investigate the mechanism how GGT induces osteoclastogenesis, *B. subtilis* GGT was cloned, expressed in *E. coli*, and purified using His-tag purification. The purified GGT produced only one protein band, indicating no autocatalytic process occurred. It is possible that T7 tag at the amino terminus and His tag at the carboxyl terminus of GGT may interfere the autocatalytic process. Without the autocatalytic process, enzyme activity of GGT is not active. Because in mammalian GGT the osteocalst formation activity was independent

of its enzymatic activity (Niida et al., 2004), we continue to investigate further with the single polypeptide GGT.

The purified GGT significantly induced by up to 2.5 fold the mRNA expression of the receptor activator of nuclear factor-κB ligand (RANKL). This result suggested that the osteoclastogenesis by the bacterial GGT is mediated by a RANKL-dependent pathway and the bacterial GGT enzymatic activity is not required for the induction of RANKL expression like the mammalian GGT. Unlike RANKL, the expression of OPG, which blocks osteoclastogenesis induced by RANKL, was not affected by the GGT treatment in the osteobalsts, suggesting that the ratio of RANKL to OPG was increased to promote the direction of bone resorption.

It was previously reported that *Helicobacter pylori* GGT stimulated mRNA expression of COX-2, heparin-binding epidermal growth factor-like growth factor (HB-EGF) and amphiregulin (AR) in human gastric cells (AGS) (Busiello et al., 2004). Thus, it was examined whether *B. subtilis* GGT induce mRNA expression of COX-2, HB-EGF and AR in mouse calvaria-derived osteoblasts. The GGT stimulated mRNA expression of COX-2 by 1.6 fold, HB-EGF by 2.0 fold and AR by 1.4 fold in the osteoblasts. It has been reported that COX-2 is a transcription factor to induce PGE_2, pro-resorptive factor (Fujita et al., 2003). The RANKL-dependent pathway is essential to induce osteoclastogenesis by PGE_2 and other pro-resorptive factors such as 1,25(OH_3) vitamin D_3, TNF-α, IL-1β and IL-6 . Therefore, it can be postulated that PGE_2 is a main factor in the induction of RANKL expression in the osteoblasts by the GGT even though the induced PGE_2 secretion needs to be examined in the future. It is first report that GGT induced the expression of HB-EGF and AR in the osteoblasts. They could be new player as pro-resorptive factor in osteoclastogenesis. Therefore, it is interesting to investigate new

roles of those growth factors in osteoclastogenesis.

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국문 요약

파골세포 형성에 미치는 γ- glutamyltranspeptidase의 효과

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γ-glutamyltranspeptidase (GGT)는 새로운 골흡수 인자로서 최근에 쥐 T-lymphoma 세포의 cDNA library에서 클로닝되었다. GGT는 모든 생명체에 광범위하게 존재하며 특히 *Bacillus subtilis* GGT는 구조나 효소적 특성이 포유동물의 GGT와 매우 흡사해 *B. subtilis* GGT를 가지고 파골세포형성능을 실험하였다. 파골세포형성능은 두개골과 골수세포를 사용한 혼합배양을 이용하였고 tartrate-resistant acid phosphatase 염색으로 평가하였다. GGT 를 과발현하는 *B. subtilis* 배양액에서는 wild type이나 ggt-deleted *B. subtilis* 배양액보다 현저한 파골세포형성능이 나타났다. GGT가 어떤 기전에 의해 파골세포 분화에 관여하는지 알아보기 위해 *B. subtilis* GGT를 *Escherichia coli*에 클로닝하여 발현시키고 His-tag 정제방법으로 다량의 정제된 GGT를 취하였다. 정제된 GGT를 쥐 두개골의 조골세포에 처리한 결과 receptor activator of nuclear factor-κB ligand, cyclooxygenase-2, heparin-binding epidermal growth factor-like growth factor, amphiregulin의 mRNA 발현이 증가된 반면, OPG의 발현에는 거의 영향을 미치지 않았다. 이상의 결과는 세균의 GGT도 RANKL과 관련된 경로를 통해 골흡수와 파골세포 분화에 관여함을 나타낸다.

핵심단어 : γ-glutamyltranspeptidase, 파골세포형성능, *Bacillus subtilis*, RANKL, COX-2, HB-EGF, amphiregulin