

# Treponema denticola

. . . . .

## I.

. ,  
 ,  
 P. gingi -  
 valis A. actinomycetemcomitans

Spirochetes, Porphyromonas gingivalis  
Actinobacillus actinomycetemcomitans

가 . Treponema T. denticola  
lipopolysacchahride(LPS)  
가

50% 가 6), LPS

, 가가

1,2),

Treponema(T)

(genus) , T. denticola, T. pecti -  
novorum, T. socranskii T. vincentii

, T. denticola  
가 , 3),  
5), 5)

, 1 ,25 - dihydroxyvitamin  
D<sub>3</sub>(1 ,25(OH)<sub>2</sub>D<sub>3</sub>), IL - 1, IL - 6  
prostaglandin E (PGE<sub>2</sub>)  
7).

\* 96

(osteoclast differentiation factor;  
 ODF) <sup>8)</sup>,  
 ODF <sup>9,10,11)</sup>,  
 ODF  
 RANK(receptor activator of NF - B)  
 assay kit Bio - rad protein  
 TRAP - staining kit sigma(USA)  
 dispase Wako(Japan)  
 (1)  
 (2)  
 가 co - T. denticola  
 culture system (ATCC 33521) OMIZ - Pat  
<sup>12,13)</sup> . 50Mℓ  
 co - cul - OMIZ - Pat <sup>14)</sup>  
 ture system , T. denticola 4 , 5000xg 10  
 buffered saline(PBS) 50Mℓ phosphate  
 T. denticola 5000xg 10 4 ,  
 1Mℓ PBS  
 II.  
 Bio - rad protein assay kit  
 1.  
 5 8 ICR (2) ICR  
 , 1 2 1 2 ICR  
 ICR .  
 . 10 10Mℓ  
 0.2% DMEM 0.1% dispase가  
 37 10  
 alpha Minimum  
 Essential Medium( MEM), Dulbecco's  
 Modified Eagle Medium(DMEM), Antibiotic  
 and antimycotic sol., Sodium pyruvate, Non -  
 essential amino acid, Vitamin sol. GIBCO  
 BRL(USA) 10cm 2 x 10<sup>6</sup> 가  
 10% DMEM  
 1 ,25(OH)<sub>2</sub>D<sub>3</sub>, indomethacin, 2 10 %

10% DMSO가 DMEM 1 Mℓ  
 2 × 10<sup>6</sup> 가  
 liquid nitrogen tank

(3) ICR ICR  
 5 8 MEM  
 . 25 gauge (10  
 2000xg 5  
 10Mℓ (10  
 mM Tris · HCl, 0.83% ammonium chloride)  
 가 5 water bath 1 37  
 Well

(5) Tartrate resistant acid phosphatase  
 tartrate (tartrate  
 resistant acid phosphatase, TRAP)  
 가 (25  
 Mℓ citrate solution, 65Mℓ acetone, 8 Mℓ 37%  
 formaldehyde) 30  
 . TRAP kit(Sigma)  
 가 37  
 water bath 1 37  
 Well

(4) (10%  
 MEM) 200μℓ 가 III.  
 1 × 10<sup>4</sup> 1 × 10<sup>5</sup> 가  
 48well well 200 μℓ 1. T. denticola  
 3 . 3  
 1 , 25(OH)<sub>2</sub>D<sub>3</sub>, T. denticola  
 T. denticola 가  
 indomethacin 4 가 T.  
 denticola 25μg/Mℓ  
 80 30 4 TRAP  
 T.denticola  
 가 ,

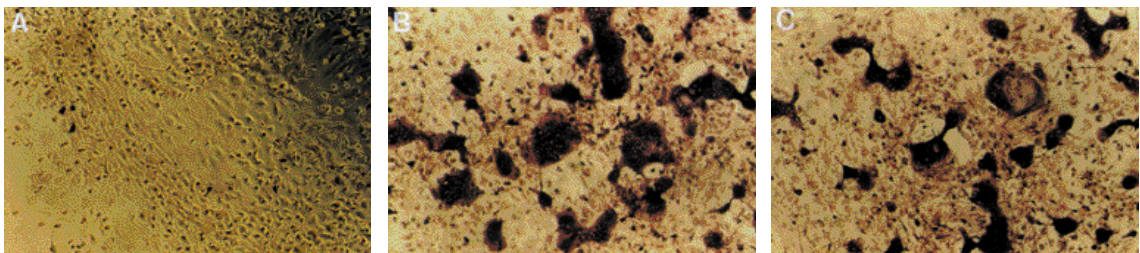


Figure 1. Osteoclast formation induced by extract of *T. denticola*. Mouse bone marrow cells and calvaria cells were co - cultured for 3 days. After changing the medium, cells were treated with extract of *T. denticola*(25μg/Mℓ, c) for an additional 4 days. 10<sup>-8</sup> M 1 ,25(OH)<sub>2</sub>D<sub>3</sub> treated(b) or non treated cells(a) were used as the positive control and negative control,

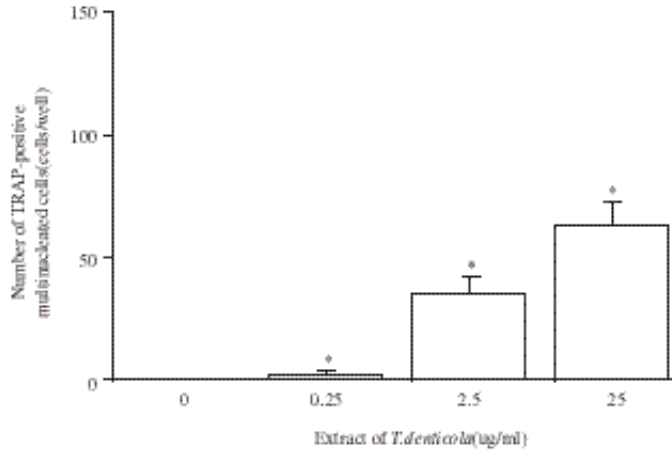


Figure 2. Concentration - dependent stimulation of osteoclast formation induced by extract of *T. denticola*. Mouse bone marrow cells and calvaria cells were co - cultured for 3 days. After changing the medium, cells were treated with various concentrations of extract of *T. den - ticola*. Then, cells were fixed and stained for TRAP. TRAP - positive multinucleated cells containing more than three nuclei were counted as osteoclast. The results were expressed

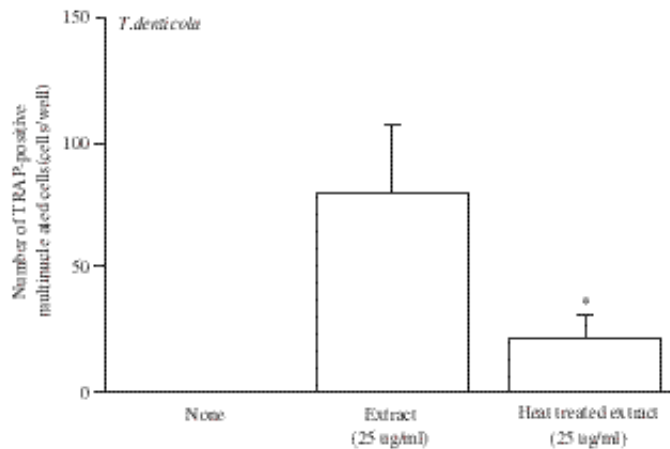


Figure 3. Effect of heat - treated extract of *T. denticola* on the osteoclast formation. Mouse bone marrow cells and calvaria cells were co - cultured for 3 days. After 3 days, cells were cul - tured in the presence of heat - treated extract of *T. denticola*(25ug/ml) for additional 4 days. Then, cells were fixed and stained for TRAP. TRAP - positive multinucleated cells con - taining more than three nuclei were counted as osteoclast. Results are expressed as the

$10^{-8}$  M  $1,25$  (OH) $_2$ D $_3$       $1,25$ (OH) $_2$ D $_3$       $25\mu\text{g}/\text{Ml}$  *T. denticola*  
 TRAP     TRAP     TRAP     가  
 (Figure 1).  
 $10^{-8}$  M     1

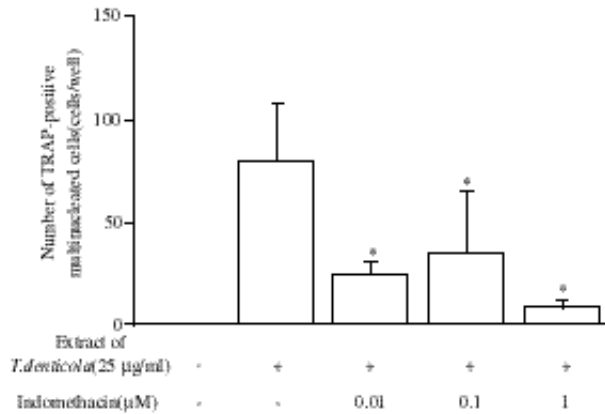


Figure 4. Effect of indomethacin on the osteoclast formation induced by extract of *T. denticola*. Mouse bone marrow cells and calvaria cells were co-cultured for 3 days. After 3 days, cells were cultured with extract of *T. denticola* (25 µg/ml) in the presence of various concentrations of indomethacin (0.01 to 1 µM) for additional 4 days. Then, cells were fixed and stained for TRAP. TRAP-positive multinucleated cells containing more than three nuclei were counted as osteoclast. Results are expressed as the mean ± SD of six cultures. \* :

2. *T. denticola* denticola  
 80 ± 28 ,  
 22 ± 9 T. denti -  
 0.25, 2.5, 25 µg/ cola  
 4 28%  
 MØ T. denticola 가 .  
 (Figure 3).

TRAP 4. *T. denticola*  
 indomethacin  
 MØ 2.5 µg/MØ 3 ± 1 36  
 ± 6 , 25 µg/MØ 64 ± 9 0.25 µg/  
 MØ TRAP 가  
 25 µg /MØ T. denticola  
 prostaglandin indomethacin  
 T. denticola  
 PGE<sub>2</sub> 가  
 3. T. denticola .  
 T. denticola 25 µg/  
 MØ 80 ± 28 ,  
 80 30 T. denticola indomethacin 0.01 µM  
 25 ± 5  
 MØ 4 31% indomethacin 0.1 µM  
 1 µM 36 ± 30  
 T. 9 ± 3 45% 11%

(Figure 4).

IV.

, macrophage - colony stimulating factor (M-CSF) osteoclast differentiation factor (ODF)

15,9).

가

가

15).

T. denticola

. vitamin D<sub>3</sub>

1, 25(OH)<sub>2</sub>D<sub>3</sub>

(co-culture system)

10<sup>-8</sup> M

16),

10<sup>-8</sup>M 1, 25(OH)<sub>2</sub>D<sub>3</sub>

, 25 µg/Mℓ T.

dneticola

T. denticola

1, 25(OH)<sub>2</sub>D<sub>3</sub>

(Figure 1),

0.25 25 µg/Mℓ

(Figure 2).

T. denticola가

가

Treponema

Porphyromonas gingi-

valis Actinobacillus actinomycetemcomitans

Treponema

Treponema

가

mycetemcomitans

. A. actino-

LPS<sup>17)</sup>

(Capsule)

18)

chaperone

heat shock protein

19,20),

Porphyromonas gingivalis

LPS<sup>21)</sup>

22)

(Fimbriae) 23)

Pasteurella multocida

pasteurella

multocida toxin (PMT)

가<sup>24)</sup>,

Salmonella typhimuri-

um

channel

porin

25)

Gopalsami<sup>6)</sup>

T. denticola

(outer sheath)

Ca<sup>2+</sup>

가

Ca<sup>2+</sup>

LPS 19), *P. gingivalis*  
 IL - 1 GM - CSF가<sup>23)</sup>, LPS  
 IL - 1 IL - 6가<sup>21)</sup>  
 IL - 1, PGE<sub>2</sub> TNF가  
<sup>22)</sup>, cytokine  
 75% (Figure 3).  
 Gopalsami<sup>6)</sup> PGE<sub>2</sub>  
 PGE<sub>2</sub> phsopholipid가  
 LPS phospholipase A<sub>2</sub> cyclooxygenase  
 indomethacin PGE<sub>2</sub>  
 cyclooxygenase PGE<sub>2</sub>  
 T. denticola  
 indomethacin  
 TRAP T. denticola  
 TRAP PGE<sub>2</sub>가  
 (Figure 4). PGE<sub>2</sub>  
 ODF 가<sup>8)</sup>  
 TRAP ODF  
 ODF  
 IL - 1 TRAP ODF가  
<sup>9,26)</sup>. 1 , 25(OH)<sub>2</sub>D<sub>3</sub>,  
 PGE<sub>2</sub>, IL - 11  
 ODF M - CSF T. denticola  
<sup>8,10)</sup>. T. denticola  
 가 가 PGE<sub>2</sub> cytokine  
 ODF M - 가  
 CSF cytokine PGE<sub>2</sub>  
 V.  
 A. actinomycetemcomitans  
 LPS IL - 1  
 PGE<sub>2</sub>가<sup>17,18)</sup>  
 chaperone  
 IL - 1 PGE<sub>2</sub>가

T. denticola가  
가 T. denticola  
가 .

(co - culture system)  
가 ,

tartrate resistant acid  
phosphatase(TRAP) TRAP

1. T. denticola 0.25  
25µg/Ml  
가 가 가 .

2.  
(25µg/Ml)

3. PGE<sub>2</sub> indomethacin  
(25µg/Ml)

T. denticola  
PGE<sub>2</sub>

가 .

## VI.

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- Abstract -

## Effect of Sonicated Extract of Treponema Denticola on Osteoclast Differentiation

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Alveolar bone destruction is a characteristic of periodontal disease. *Treponema denticola* are found in significantly increased numbers in the sites affected with periodontal disease. In order to clarify the role of *T. denticola* in destruction of alveolar bone in periodontal disease, this study was undertaken to determine the effect of sonicated extract of *T. denticola* on osteoclast differentiation in co-culture system of mouse bone marrow cells and calvaria cells. The ability of osteoclast formation was estimated by counting the number of tartrate resistant acid phosphatase (TRAP) positive cells. Sonicated extract of this bacteria stimulated osteoclast formation in a dose dependent manner ( $p < 0.05$ ). Indomethacin, an inhibitor of prostaglandin synthesis, decreased osteoclast formation induced by sonicated extract of this bacteria ( $p < 0.05$ ). Extract-induced osteoclast formation was decreased, when sonicated extract of bacteria was heated ( $p < 0.05$ ). These findings suggest that *T. denticola*

induces osteoclast differentiation, and protein component of this bacteria and  $PGE_2$  may play an important role in this process.

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Key words : *Treponema denticola*,  
Osteoclast differentiation, Prostaglandin  $E_2$