Expression of IL-34 in Root Resorption by Excessive Orthodontic Force

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Abstract

Interleukin-34 (IL-34) was recently discovered and shown to mediate inflammation and osteoclastogenesis. An in vitro study reported that the expression of IL-34 is stimulated by tumor necrosis factor-α via nuclear factor-kB activation in osteoblasts. The present study focused on the expression of IL-34 in resorbed root cementum using an experimental rat model of tooth movement.

Experimental tooth movement was induced by a quad helix-type device. The upper first molar was palatally moved by the appliance with a force of 10 or 50 g for 21 days. We performed hematoxylin and eosin and immunohistochemistry staining for tartrateresistant acid phosphatase and IL-34 in the root resorption area.

In the heavy force (50g) group, few IL-34-positive odontoclasts were observed on day 7 in the root, but increased at days 14 and 21. Therefore, IL-34 may take part in the aggravation of root resorption.

Introduction

Orthodontically induced inflammatory root resorption (OIIRR) is one type of procedural accident that occurs during orthodontic treatment, and the apical root portion, especially in the incisor, is resorbed during orthodontic tooth movement (OTM). OIIRR progresses with a sterile inflammatory process and is induced by various factors (e.g., mechanical forces, morphology of tooth roots, treatment duration, and biologic messengers) (1,2).

Concerning the relationship between OIIRR and inflammatory cytokines, previous studies reported that interleukin (IL)-1 and IL-6 and tumor necrosis factor (TNF)-α are expressed in resorbed root in response to heavy forces (3–5). Furthermore, Nakano et al. (6) reported that the receptor activator of nuclear factor-kB (RANK) ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) are involved in OIIRR. These inflammatory cytokines participate in the progression of OIIRR.

IL-34, a new cytokine, was recently discovered by Lin et al. (7). IL-34 binds to the M-CSF receptor (also called c-fms) expressed on the cell surface of human monocytes and has the same function as M-CSF. Baud'huin et al. reported that RANKL-induced osteoclastogenesis was enhanced by IL-34 (8).

Therefore, we hypothesized that IL-34 induces cytokine production and may contribute to odontoclastogenesis and OIIRR. In the present study, the expression of IL-34 in resorbed root in response to a heavy force was examined in vivo.

Materials and Methods

Animals and application of the orthodontic devices

The protocol for animal experimentation was approved by the Ethics Committee for Animal Experiments at the Nihon University School of Dentistry at Matsudo (approval no. AP13MD003). A total of 18 male 8-week-old Wistar rats (body weight 350±10g; Sankyo Labo Service, Tokyo, Japan) were used for the experiments.

Animals were anesthetized with pentobarbital sodium (40 mg/kg body weight) for the application of orthodontic devices. Experimental tooth movement was performed using the method described by Hikida et al. (9). A quad helix-type device (diameter: 0.012 in, stainless steel wire; Tomy
International, Inc., Tokyo, Japan) was ligated to the maxillary first molar cleat by a 0.008-in stainless steel wire (Tomy International, Inc.). The upper first molar was palatally moved by the appliance with a force (10 or 50 g). The experimental period was 21 days. Rats were divided into three groups: control group (rats received no appliances); optimal force (OF) group (rats were subjected to a 10 g compression force); and heavy force (HF) group (rats were subjected to a 50 g compression force) (n=6 rats in each group).

**Fig. 1.** Experimental rat model of tooth movement.
Tooth movement was induced with a quad helix-type device (diameter: 0.012 in, stainless steel wire). The upper first molar was palatally moved by the appliance with a force (10 or 50 g). The experimental period was 21 days. Rats were divided into three groups: control group (rats received no appliances); optimal force (OF) group (rats were subjected to a 10 g compression force); and heavy force (HF) group (rats were subjected to a 50 g compression force) (n=6 rats in each group).

**Fig. 2.** The experimental schedule.
The rats were randomly assigned to three groups: control group (rats received no appliances); optimal force (OF) group (rats were subjected to a 10 g compression force); and heavy force (HF) group (rats were subjected to a 50 g compression force).

(Figs. 2 and 3).

**Tissue preparation**
Tissue preparation was performed as described by Hikida et al. (9). Each sample was sliced continuously into 4-μm sections in the frontal direction and prepared for hematoxylin and eosin (HE) and immunohistochemical staining. The periodontal tissue in the palatal part of the distal palatal root
at the left first upper molar was observed. Observations were conducted at the compression area (300-μm section of the root direction from the top of the alveolar bone surface on the palatal side) (Fig.3).

**Immunohistochemistry**

Immunohistochemistry staining was performed as described by Hayashi et al. (4). After washing the tissue sections in Tris-buffered saline, sections were incubated with polyclonal anti-rabbit tartrate-resistant acid phosphatase antibody (TRAP; working dilution, 1:100; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and polyclonal anti-goat IL-34 antibody (working dilution, 1:100; Biorbyt Ltd., Cambridge, UK) for 18 h at 4°C. The Histofine Simple Stain MAX-PO® kit (Nichirei, Co. Tokyo, Japan) was used to detect TRAP and IL-34 staining. The final color reactions were induced using the 3, 3’-diaminobenzidine tetrahydrochloride substrate reagent, and the sections were then counterstained with hematoxylin. As immunohistochemical controls, several sections were incubated with 0.01 M phosphate-buffered saline instead of primary antibody. Negative reactivity was observed in the control samples. TRAP-positive cells were counted as multinucleate odontoclasts on the surface of the cementum. The area resorbed in dentin, not localized in cementum, was defined as "root resorption" in this study.

**Quantitative evaluation**

Quantitative measurements were carried out as described by Chutimanutskul et al. (10). The slides were evaluated at a total magnification of 400 × with a fixed measurement frame (300 mm × 225 mm). IL-34-positive cells were counted as multinucleate odontoclasts on the surface of the cementum.

The values in each figure represent the mean ± standard deviation for each group. The Mann-Whitney U test was
used to compare the means of groups with \( p \) values of \(<0.01\) considered to indicate a significant difference.

**Results**

*Histological observation by HE staining*

Throughout the experimental period (days 7, 14, and 21), connective tissue fibers and fibroblasts were observed regularly in a horizontal direction from the root cementum toward the alveolar bone in the control group (0g). The alveolar bone and root surface were relatively smooth. A few resorbed lacunae were observed on the alveolar bone surface (Fig.4-a, b, c).

In the OF (10g) group, the arrangement of the fibers and fibroblasts was irregular and compressed on days 7 and 14. Resorbed lacunae with few multinucleated odontoclasts were observed on the surface of the palatal root (Fig.4-d, e). On day 21, root resorption lacunae with a few multinucleated odontoclasts were observed in the cementum (Fig.4-f).

In the HF (50g) group, some root resorbed lacunae with multinucleated odontoclasts were identified in the cementum on day 7 (Fig.4-g). Many resorbed lacunae were observed in the cementum on day 14 (Fig.4-h). On day 21, many root resorbed lacunae were detected (Fig.4-i). The area of root resorption gradually increased from day 7 through 21.

*Findings of TRAP*

In the control and OF groups, no resorbed lacunae with TRAP-positive odontoclasts were observed on the cementum during the experimental period (Fig.5-a, b, c, d, e, f). However, in the HF group, few root resorption lacunae with multinucleated TRAP-positive odontoclasts were observed in the cementum on day 7 (Fig.5-g). On days 14 and 21, many root resorbed lacunae with TRAP-positive multinucleated odontoclasts were detected (Fig.5-h, i).

*Protein expression of IL-34*

In the control group, IL-34-positive cells were rarely observed in the periodontal ligament (PDL) tissues during the experimental period (Fig.6-a, b, c). In the OF group, few IL-34-positive cells were observed in the cementum through 21 days (Fig.6-d, e, f). In the HF group, few IL-34-positive cells were detected in the cementum on day 7, but increased on days 14 and 21 (Fig.6-g, h, i).

In quantitative evaluations, the number of IL-34-positive odontoclasts was found to be significantly increased in the HF group on day 21 in comparison with the control and OF groups \((p<0.01)\) (Fig.7).

**Discussion**

In the present study, we investigated IL-34 expression in root resorbed lacunae using an experimental rat model of OTM. In the HF group, the heavy force increased the number of resorbed lacunae and TRAP-positive cells observed in comparison with the OF group on day 21 (Fig.5-i). Our results from HE and TRAP staining for the OF and HF groups largely support findings from previous studies (11-13). Furthermore, IL-34 expression was observed in...
odontoclasts in the HF group on days 7, 14, and 21 (Fig. 6-g, h, i). The number of IL-34-positive cells was significantly greater in the HF group than in the control and OP groups on day 21 (Fig. 7). Taken together, our results demonstrated that IL-34 was induced by heavy force during OTM.

RANKL (14, 15) and M-CSF play important roles in osteoclastogenesis and contribute to proliferation, survival, and differentiation of precursor cells (16). There is some evidence for the role of RANKL/RANK and the M-CSF/c-fms system in osteoclastogenesis during OTM. Yamaguchi (17) reported that RANKL is involved in the mechanism of OTM. Nishijima et al. (18) reported that the concentration of RANKL in gingival crevicular fluid increased during OTM.

Moreover, Kaku et al. (19) detected M-CSF gene expression in PDL cells during experimental tooth movement. Kitaura et al. (20) reported that M-CSF/c-fms system is involved in tooth movement by modulating osteoclastogenesis (21). Furthermore, Nakano et al. (6) demonstrated that RANKL/RANK and M-CSF/c-fms proteins were expressed in odontoclasts in response to heavy force. Therefore, RANKL/RANK and M-CSF/c-fms systems may modulate the progression of root resorption.

Concerning the relationship between IL-34 and osteoclastogenesis, Boström and Lundberg (22) reported that IL-34 stimulates RANKL-induced osteoclastogenesis of bone marrow macrophages. Recently, Baudhuin et al. (8) have shown IL-34 may be substituted for M-CSF to stimulate osteoclastogenesis in vitro. Taken together, these findings and the present results suggest that IL-34 may stimulate the production of RANKL and M-CSF and play a role in
odontoclasts differentiation in root resorbed cementum.

In the OF group, IL-34 expression was not detected, although IL-34 may contribute to bone resorption during OTM by optimum force. A previous study reported that M-CSF protein expression was also not detected during OTM (optimum force: 10g) (6). Furthermore, IL-34 expression is lower than that of M-CSF in gingival fibroblasts and PDL cells (22, 23). This may explain why IL-34 expression was not detected by immunohistochemistry in the present study.

Additionally, Chemel et al. (25) demonstrated that TNF-α and IL-1β increased the expression of IL-34 in rheumatoid arthritis (RA) synovial fibroblasts. Boström and Lundberg (22) reported that TNF-α and IL-1β enhanced the expression of IL-34 in human gingival fibroblasts. A previous study reported that TNF-α induced OIIRR in response to heavy force during tooth movement (5). Pereira et al. (26) reported that the IL-1β pathway is involved in susceptibility to OIIRR. Thus, IL-34, stimulated by TNF-α and IL-1β, mediates inflammation and odontoclastogenesis.

Conclusions
To our knowledge, the present study reports for the first time that IL-34 is expressed in resorbed lacunae of the cementum. On days 7, 14, and 21, IL-34 expression was induced in rat odontoclasts and PDL fibroblasts by heavy orthodontic force. Therefore, IL-34 may participate in the progression of root resorption. Considering the role of IL-34 in odontoclastogenesis and inflammation, this cytokine may play a role in the pathogenesis of OIIRR and therefore may be a new therapeutic target. Further investigations are needed to elucidate the role of IL-34 in the inflammatory process associated with OIIRR.

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References
1. Krishnan V, Davidovitch Z: Cellular, molecular, and tissue-level reactions to orthodontic force. Am J Orthod Dentofacial Orthop. 129: 469, e1–32, 2006.
2. Meikle MC: The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt.
1. Zhang D, Goetz W, Braumann B, Bourauel C, Jaeger A: Effect of soluble receptors to interleukin-1 and tumor necrosis factor alpha on experimentally induced root resorption in rats. J Periodontal Res, 38: 324–332, 2003.

2. Hayashi N, Yamaguchi M, Nakajima R, Utsunomiya T, Yamamoto H, Kasai K: T-helper 17 cells mediate the osteo/odontoclastogenesis induced by excessive orthodontic forces. Oral Dis, 18: 375–388, 2012.

3. Kojima T, Yamaguchi M, Yoshino T, Shimizu M, Yamada K, Goseki T, Kasai K: TNF-α and RANKL facilitates the development of orthodontically-induced root resorption. Open J Stomatol, 3: 52–58, 2013.

4. Nakano Y, Yamaguchi M, Fujita S, Asano M, Saito K, Kasai K: Expressions of RANKL/RANK and M-CSF/c-fms in root resorption by excessive orthodontic force. Eur J Orthod, 33: 335–343, 2011.

5. Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, Halenbeck R, Wu G, Zhou A, Behrens D, Hollenbaugh D, Linnemann T, Qin M, Wong J, Chu K, Doberstein SK, Williams LT: Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science, 320: 807–811, 2008.

6. Baudhuin M, Renaut R, Charrier C, Riet A, Moreau A, Brion R, Gouin F, Duplomb L, Heymann D: Interleukin-34 is expressed by giant cell tumours of bone and plays a key role in RANKL-induced osteoclastogenesis. J Pathol, 22: 77–86, 2010.

7. Hikida T, Yamaguchi M, Shimizu M, Kikuta J, Yoshino T, Kasai K: Comparison of orthodontic root resorption under heavy and jiggling forces during experimental tooth movement. Korean J Orthod, 46: 228–241, 2016.

8. Chutimanutskul W, Darendellier MA, Shen G, Petocz P, Swain MV: Changes in the physical properties of human premolar cementum after application of 4 weeks of controlled orthodontic forces, Eur J Orthod, 28: 313–318, 2006.

9. Chan E, Darendellier MA: Physical properties of root cementum: Part 5. Volumetric analysis of root resorption craters after application of light and heavy orthodontic forces. Am J Orthod Dentofacial Orthop, 127: 186–195, 2005.

10. Levander E, Malmgren O, Stenback K: Apical root resorption during orthodontic treatment of patients with multiple aplasia: a study of maxillary incisors. Eur J Orthodont, 20: 427–434, 1998.

11. Al-Qawasmi RA, Hartsfield JK Jr, Everett ET, Flury L, Liu L, Foroud TM, Macri JV, Roberts WE: Genetic predisposition to external apical root resorption in orthodontic patients linkage of chromosome-18 marker. J Dent Res, 82: 356–360, 2003.

12. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombo A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shallhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ: Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell, 93: 165–176, 1998.

13. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T: Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA, 95: 3597–3602, 1998.

14. Udagawa N, Takahashi N, Jimi E, Matsuzaki K, Tsurukai T, Itoh K, Nakagawa N, Yasuda H, Goto M, Tsuda E, Higashio K, Gillespie MT, Martin TJ, Suda T: Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. Bone, 25: 517–523, 1999.

15. Yamaguchi M: RANKL/RANKL/OPG during orthodontic tooth movement. Orthod Craniofacial Res, 12: 113–119, 2009.

16. Nishijima Y, Yamaguchi M, Kojima T, Aihara N, Nakajima R, Kasai K: Levels of RANKL and OPG in gingival crevicular fluid during orthodontic tooth movement and effect of compression force on releases from periodontal ligament cells in vitro. Orthod Craniofac Res, 9: 63–70, 2006.

17. Kaku M, Motokawa M, Tohma Y, Tsuka N, Koseki H, Sunagawa H, Arturo Marquez Hernandez R, Ohtani J, Fujita T, Kawata T, Tanne K: VEGF and M-CSF levels in periodontal tissue during tooth movement. Biomedical Research, 29: 181–187, 2008.

18. Kitaura H, Yoshimatsu M, Fujimura Y, Eguchi T, Kohara H, Yamaguchi A, Yoshida N: An anti-c-Fms antibody inhibits orthodontic tooth movement. J Dent Res, 87: 396–400, 2008.

19. Yamaguchi M, Fujita S, Yoshida T, Oikawa K, Utsunomiya T, Yamamoto H, Kasai K: Low-energy laser irradiation stimulates the tooth movement velocity via expression of M-CSF and c-fms. Orthodontic Waves, 66: 139–148, 2007.

20. Boström EA, Lundberg P: The newly discovered cytokine IL-34 is expressed in gingival fibroblasts, shows enhanced expression by pro-inflammatory cytokines, and stimulates osteoclast differentiation. PLoS One, 8: e81665, 2013.

21. Droin N, Solaré E: Editorial: cSF1R, CSF-1, and IL-34, a “menage a trois” conserved among vertebrates. J Leukoc Biol, 87: 745–747, 2010.

22. Kawabe M, Ohyama H, Kato-Kogoe N, Yamada N, Yamanegi K, Nishii H, Hirano H, Kishimoto H, Nakasho K: Expression of interleukin-34 and colony stimulating factor-1 in the stimulated periodontal ligament cells with tumor necrosis factor-α. Med Mol Morphol, 48: 169–176, 2015.

23. Chemel M, Le Goff B, Brion R, Cozic J, Berreur M, Amiaud J, Bougras G, Touchais S, Blanchard F, Heymann MF, Berthelot JM, Verrecchia F, Heymann D: Interleukin 34 expression is
associated with synovitis severity in rheumatoid arthritis patients. Ann Rheum Dis, 71: 150–154, 2012.

26. Pereira S, Nogueira L, Canova F, Lopez M, Silva HC: IRAK1 variant is protective for orthodontic-induced external apical root resorption. Oral Dis, 22: 658–664, 2016.