Original Article

Expression of LIF and LIFR in periodontal tissue during orthodontic tooth movement

Youde Liang[a,*]; Yi Zhou[b,*]; Tao Jiang[c]; Zhen Zhang[a]; Su Wang[d]; Yining Wang[e]

ABSTRACT

Objectives: To test the hypothesis that leukemia inhibitor factor (LIF) and LIF receptor (LIFR) are expressed in periodontal tissue and that their expression may be upregulated during orthodontic tooth movement.

Materials and Methods: Forces of 0.3 N were applied to move the upper left first molars mesially in 24 rats. These forces were kept constant for 3, 7, and 14 days and followed by animal sacrifice. The contralateral molars served as control. The rate of tooth movement was measured by Image J software. Paraffin-embedded sections of the upper jaws were prepared for histological and immunohistochemical analysis to test the LIF and LIFR expression.

Results: Loaded teeth showed a significantly higher rate of tooth movement. The periodontium of the moved teeth experienced tissue remodeling, while there was no obvious change in the contralateral controls. Furthermore, LIF and LIFR were expressed in the periodontal tissue, and there were statistically significant differences between the loaded and unloaded teeth at 3 and 14 days. LIF presented significantly higher expression on the tension side compared with the pressure side at 3 days.

Conclusion: Both LIF and LIFR exist in the periodontal tissue, and continuous orthodontic forces induce the upregulation of LIF/LIFR production, suggesting that LIF/LIFR may play important roles in periodontium remodeling. (*Angle Orthod.* 2011;81:600–608.)

KEY WORDS: Tooth movement; Orthodontic forces; LIF; LIFR; Periodontal tissue remodeling

INTRODUCTION

Orthodontic tooth movement (OTM) has been defined as the result of a biological reaction to the biomechanical stress generated by orthodontic forces, and this biologic response is a highly sophisticated process.[1] It is characterized mainly by remodeling changes of the periodontal ligament (PDL), alveolar bone, and sometimes the cementum.[2] Although the exact mechanism of periodontal tissue remodeling is not clearly understood, the upregulation or downregulation of cytokines, after mechanical stimulation, are essential for tooth movement.[3] Thus, it was considered of value to elucidate the complex cytokine cascade flow associated with inflammation-mediated tissue remodeling at the molecular level.

Leukemia inhibitory factor (LIF), one of the major members of the interleukin-6 (IL-6) cytokine family, has been involved in the process of bone remodeling. Since it was first reported to stimulate bone resorption by Abe et al.,[4] LIF has been confirmed by numerous experiments to be a powerful stimulator of bone resorption.[5–7] Furthermore, LIF regulates bone development and formation, showing a stimulatory or inhibitory effect in vitro.[8,9]

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As far as our knowledge goes, there has been no study testing the expression of LIF and LIF receptor (LIFR) in periodontal tissue. LIF has been detected in vitro in many cell types, including fibroblasts, bone marrow stromal cells, and osteoblasts. Based on the similarities between PDL cells and osteoblasts, it seems reasonable to speculate that LIF might express in normal periodontal tissue and function during remodeling. Several cytokines, including IL-1, interleukin-1 (IL-1) transforming growth factor β (TGF-β), and tumor necroses factor (TNF), stimulate LIF secretion from osteoblastic cells.[10,11] TNF-α, IL-1, and TGF-β levels were found to be elevated after OTM and thought to play important roles in both bone and soft tissue remodeling.[3] In addition, soft extracellular matrix (ECM) remodeling is tightly related to collagenases and tissue inhibitors of metalloproteinases (TIMPs). Researchers have demonstrated that LIF can regulate collagen synthesis[12] and stimulate the expression of collagenases and TIMPs in osteoblastic cells.[13]

Taken together, we hypothesized that LIF and LIFR express in periodontal tissue and that their expression may be upregulated during OTM. We used a rat model to detect the localization of LIF and LIFR in the periodontium and analyzed their expression levels during OTM.

MATERIALS AND METHODS

Animal Model of OTM

Twenty-four 7-week-old female Wistar rats (220 ± 20 g) received OTM for 3, 7, and 14 (n = 8) days. The left upper molar was moved mesially with an Ni-Ti closed coil spring (Grikin Advanced Materials Co Ltd, Beijing, China), which was fixed to the two upper incisors. Forces of 0.3 N were verified using a dynamometer during the fixation of the spring and followed up every day. To prevent slippage of the appliance, grooves were made along the lateral sides of the incisors. The right upper molar with only a ligature wire, but not a spring, was used as the control (Figure 1). Animals were acclimatized for 1 week prior to appliance placement and were fed powdered laboratory rat chow and tap water. The experiments followed the ethical permission guidelines for animal experiments and were approved by the Ethics Committee for Animal Research, Wuhan University, China.

Measurement of Tooth Movement

At the end of each experimental period, the animals were killed. The maxillary jaws were removed and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 24 hours. These samples were decalcified in 10% EDTA at room temperature for 4 weeks. After being dehydrated in ascending grades of alcohol, cleared in xylene, and paraffin embedded, three sets of 5-μm serial sections were cut parasagittally on a microtome (RM-2145, Leica, Solms, Germany) and mounted on glass slides.

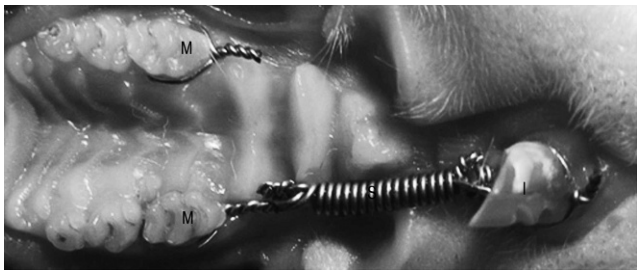

Figure 1. The orthodontic tooth movement model. A closed-coil spring (S) is attached to molar (M) and incisors (I), as described in the text.

Before decalcification, pictures of upper jaw samples were taken with a stereomicroscope (StemiSV 11, Zeiss, Jena, Germany) from the angle of the long axis of the molars. Image J was used to measure the distance between the cementum-enamel junctions (CEJs) from the first and second molar in three vertical sections per animal under a microscope equipped with an image system (Q-500 MCA, Leica). The rate of tooth movement was calculated by subtracting the distance between the CEJs on each day from that on the previous day and dividing this difference by the number of days between these two measurements. All measurements were repeated three times for each sample.

Immunohistochemistry and Histomorphometric Analysis

Some sections were stained with hematoxylin and eosin for light microscopy. Other sections were selected for immunohistochemical assay. The sections were dewaxed and rehydrated and endogenous peroxidase was inhibited by treatment with 3% H_2O_2 for 10 minutes at 25°C, incubated with goat serum for 20 minutes at 37°C, then incubated with polyclonal LIF (1:100, rabbit, Beijing Biosynthesis Biotechnology Co Ltd, Beijing, China) and LIFR antibody (1:50, rabbit, Santa Cruz Biotechnology, Santa Cruz, Calif) overnight at 4°C before being incubated at 37°C for 1 hour. Secondary antibody was added for 15 minutes and then incubated with the SP Kit (ZYMED Laboratories, San Francisco, Calif) for 15 minutes. Finally, the sections were developed with 3,3-diaminobenzidine (DAB; Zhongshan, Beijing, China) for 2 minutes. Counterstaining was performed by hematoxylin for 30 seconds. Breast cancer sections were used as positive controls. Negative controls were the sections in which primary antibodies were replaced with

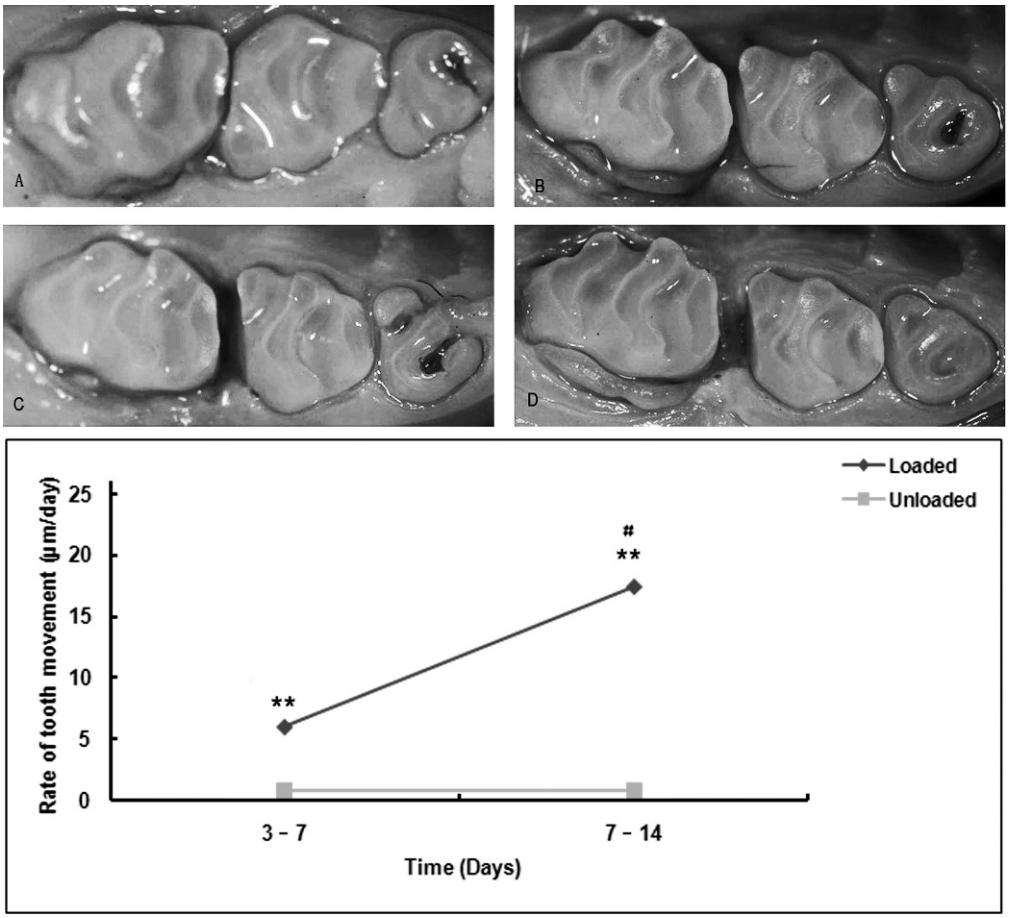

Figure 2. Tooth movement and rates of tooth movement. The unloaded (A) and loaded teeth after (B) 3, (C) 7, and (D) 14 days of orthodontic force application. # indicates comparisons across time within the group ($P < .05$); ** comparisons between groups at each time point ($P < .01$).

phosphate buffered saline (PBS). To identify osteoclastic cells, tartrate-resistant acid phosphatase (TRAP) activity was detected with a TRAP kit (Sigma, St Louis, Mo). Procedures were performed according to the instructions of the manufacturer.

The intensity of LIF and LIFR in PDL was measured using Image J. Three semiserial sections with 45-μm intervals were prepared from each animal, totaling 24 sections per group. The percentage of the LIF and LIFR immunopositive areas of the PDL was evaluated on the pressure and tension sides of the distobuccal root of the maxillary first molar via a computer-assisted image system (Q-500 MCA, Leica). Each image was captured in a personal computer, and brown staining of DAB was converted to a gray scale ranging from 0 (black) to 255 (close to white) units according to a previous study.[14] Signal intensity (gray) was determined by the optical density in the gray scale.

Statistical Analysis

A paired Student's *t*-test was used to evaluate differences between the unloaded and loaded teeth; comparisons among 3, 7, and 14 days were performed using one-way analysis of variance, and $P < .05$ was considered as the level of significance. The statistical analysis was performed with SPSS 13.0 for Windows.

RESULTS

Tooth Movement

In the loaded teeth, there was a continuous increase in space between the first and second molar from day 3 to 14, and the rate of tooth movement was significantly higher in the loaded teeth ($P < .05$; Figure 2), showing that the loaded molars were subject to slow movement in the mesial direction.

Histology

All of the specimens of the unloaded teeth exhibited a smooth cementum and a PDL without signs of alteration. In the loaded teeth, the PDL fibers were disoriented on both sides. The periodontal space on the distal side was wider than that of the contralateral control, while the space on the mesial side was

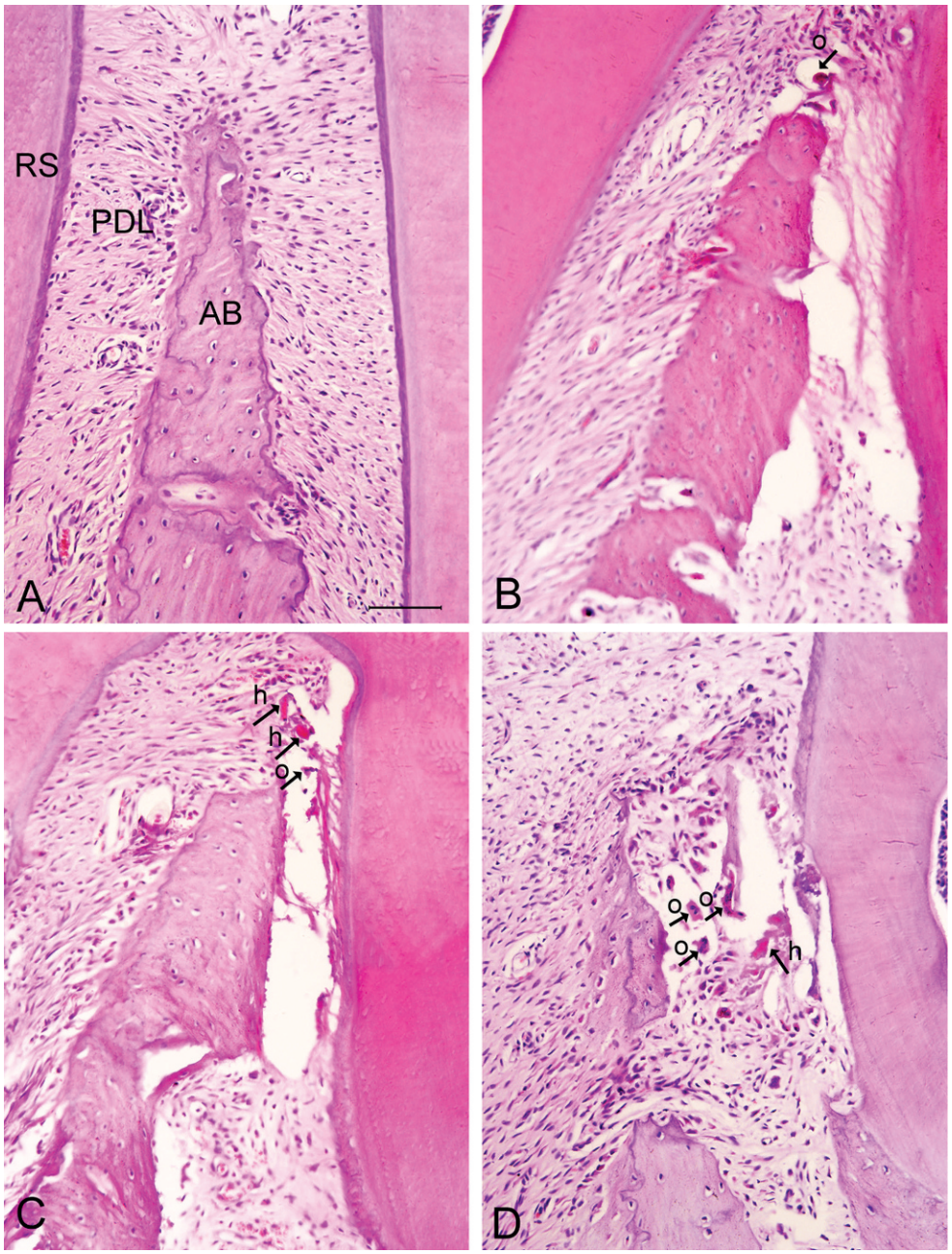

Figure 3. The histological findings in the unloaded (A) and loaded teeth after (B) 3, (C) 7, and (D) 14 days of orthodontic forces application. Bar = 50 μm. RS indicates root surface; PDL, periodontal ligament; AB, alveolar bone; o, osteoclasts; h, hyalinized tissue.

narrower. Hyalinization of the PDL was observed on the mesial side after 7 and 14 days. Furthermore, bone resorption was detected on the alveolar bone of the mesial side. After 14 days, the resorption of the cementum was noted on some parts of the root surface on the mesial side (Figure 3).

Immunolocalization

LIF immunoreactivity was observed in osteoblasts, cementoblasts, osteoclasts, fibroblasts, and endothelial cells in periodontal tissue from both loaded and unloaded teeth. Cell types expressing LIFR were similar to those expressing LIF, except that the expression of LIF was mainly in the cytoplasm (Figure 4). Furthermore, multinucleated osteoclast-like cells that were positively stained with TRAP were also positively stained with LIFR (Figure 5).

For LIF expression, statistically significant differences were found between the loaded and unloaded teeth on the distal side at 3 and 14 days ($P < .01$) but not at 7 days ($P > .05$). On the mesial side, LIF expression

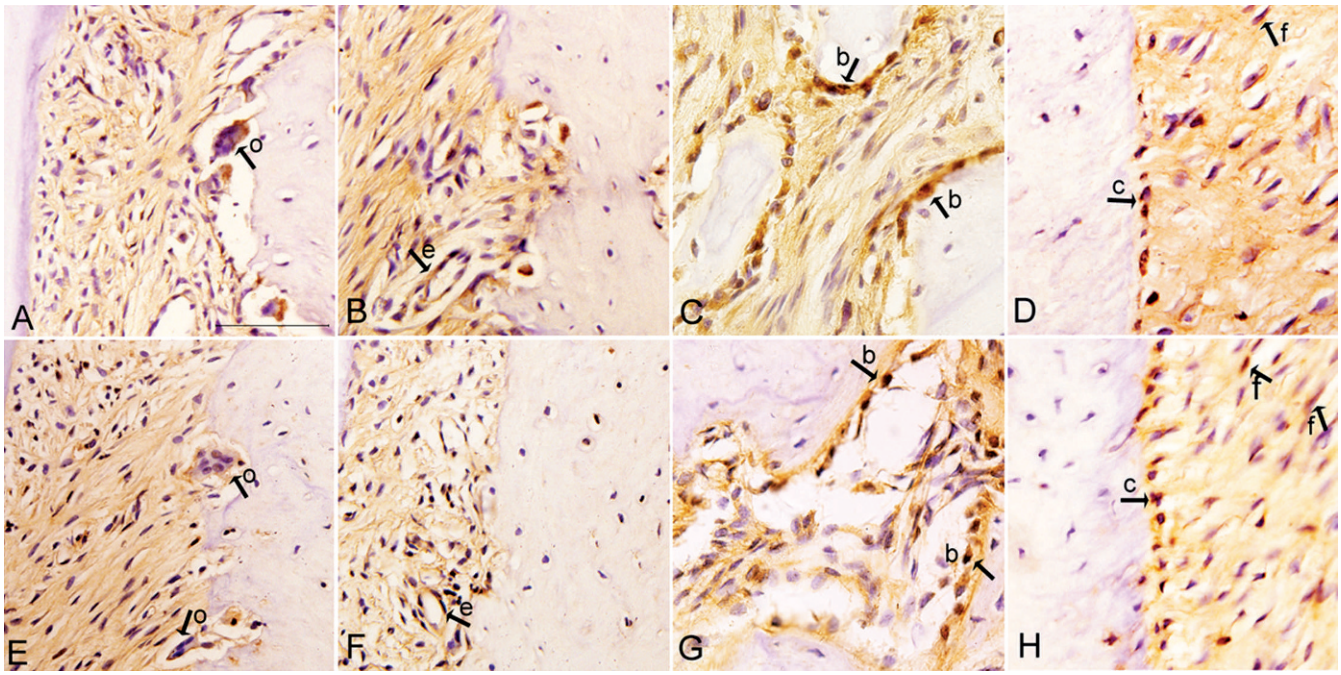

Figure 4. Leukemia inhibitor factor (A–D) and leukemia inhibitor factor receptor (E–H) were positive in osteoclasts (o), endothelial cells (e), osteoblasts (b), cementoblasts (c), and fibroblasts (f). Bar = 50 μm.

was also significantly higher in the loaded teeth on day 14 ($P < .05$) but not on day 3 or 7 (both $P > .05$). Furthermore, the expression of LIF was significantly stronger on the distal side than on the mesial side on day 3 ($P < .05$; Figures 6 and 7A).

For LIFR expression, statistically significant differences were found between the loaded and unloaded teeth on the distal side at 3 and 14 days ($P < .01$) but not at 7 days ($P > .05$). On the mesial side, LIFR expression was also significantly higher in the loaded teeth on day 14 ($P < .01$) but not on day 3 or 7 (both $P > .05$; Figures 7B and 8).

DISCUSSION

In this study, we first demonstrated that LIF and LIFR were located in the rat periodontal cells. Then, we modified the previously reported OTM system by setting ligature wires on both the loaded and unloaded molars, so that the influences of the ligature wires themselves on periodontal tissue were avoided. The increasing space between the first and second molars indicated that tooth movement took place. Furthermore, the histological assessment confirmed that the tooth movement induced periodontal remodeling.

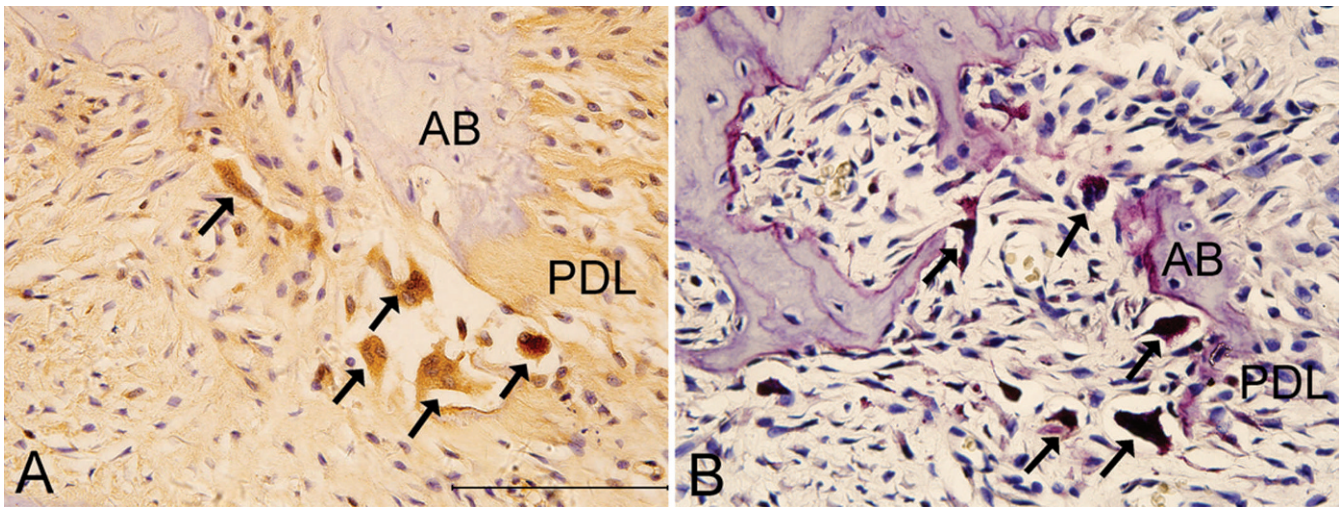

Figure 5. Leukemia inhibitor factor receptor was strongly positive in osteoclasts (A), which were TRAP positively stained (B). Bar = 50 μm.

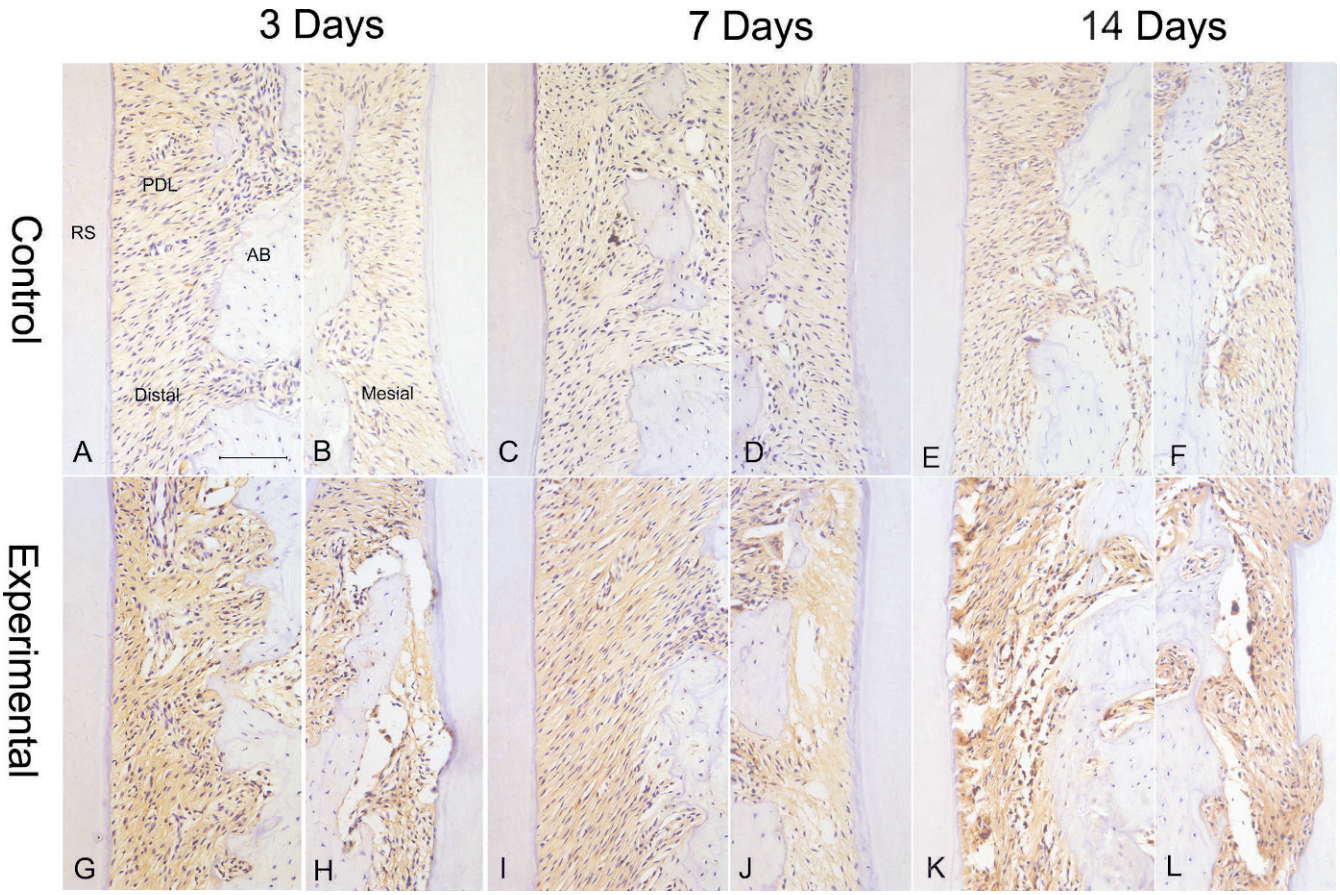

Figure 6. Leukemia inhibitor factor immunohistochemistry staining of the unloaded (A–F) and loaded (G–L) teeth after 3, 7, and 14 days of orthodontic forces application. Bar = 50 μm.

Based on this model, it was found that expression of both LIF and LIFR was higher of the loaded teeth than that of the unloaded teeth. This showed that the upregulation of LIF/LIFR may be associated with periodontal tissue remodeling. Moreover, the expression of LIF/LIFR was higher on the tension side than that on the pressure side.

Although the exact mechanism of periodontal tissue remodeling is not fully understood, there is evidence that cytokines evoke complicated cellular responses during OTM. Previous studies have implicated IL-1, TNF-α, and TGF-β level elevation after OTM, and they are thought to play key roles in both bone and soft tissue remodeling.[3] Although the production of LIF induced by IL-1 and TNF stimulation has not been reported in the PDL in vivo, LIF can be nevertheless stimulated by IL-1, TNF, and TGF-β in other cell types in vitro.[15–18] IL-1 induces the production of IL-6 and LIF through activating a cascade of intracellular signaling events, including the activation of the nuclear factor-κB (NF-κB) signaling pathways and mitogen-activated protein kinase (MAPK) family members, such as extracellular signal-regulated kinases and p38 in articular human chondrocytes.[19] However, IL-6 and LIF mRNA, stimulated by IL-1β and TNF-α, has been reported to involve the MAPK pathway but not the NF-κB signal-transducing pathway in gingival fibroblasts.[20] Furthermore, TGF-β not only regulates cellular functions through the Smad pathway but also induces other signaling pathways including the MAPK, the NF-κB, or phosphatidylinositol 3-kinases/RAC-alpha serine/threonene-protein (AKT) pathways.[21] Therefore, the upregulation of LIF may be related to or induced by the high level of IL-1, TNF, and TGF-β in the PDL. This suggests that LIF/LIFR expressions were induced by orthodontic forces, which seems to be consistent with the above reports.

There is no report on the roles of LIF in periodontal tissue remodeling. However, it has been confirmed that LIF is a powerful regulator of bone resorption[5–7] and formation.[8,9] PDL cells have been shown to possess osteogenic potential and to undergo osteoblastic differentiation in response to mechanical stimulus.[22] Osteoblasts, as important mechanosensing cells, secrete LIF and express LIF receptors.[23] Accordingly, the osteoblast-derived LIF stimulates

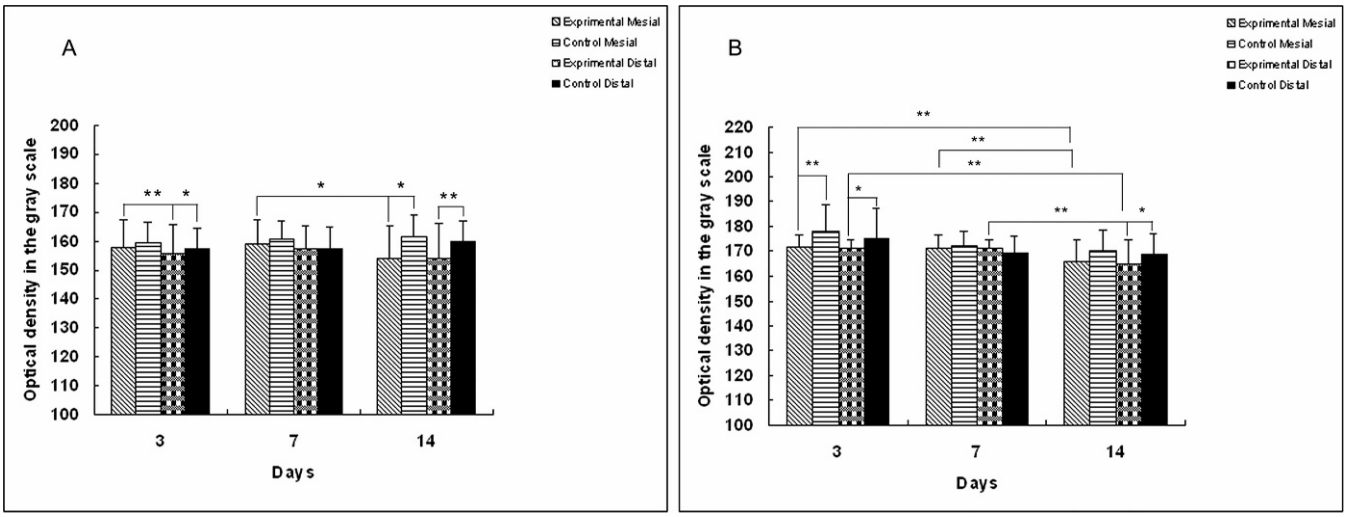

Figure 7. Immunostaining intensity for leukemia inhibitor factor (A) and leukemia inhibitor factor receptor (B) in periodontal tissue by the optical density in the gray scale (mean ± SD). * $P < .05$; ** $P < .01$.

mesencheymal progenitor differentiation toward the osteoblastic lineage[24] and promotes the proliferation of osteoblasts.[25] LIF was also reported to upregulate the expression of ECM-related genes, which were thought to be important for osteoblast differentiation.[26] Varghese et al.[13] reported that LIF stimulates the expression of MMP-13 and TIMP-1 in osteoblasts, which regulate the turnover of collagen and other

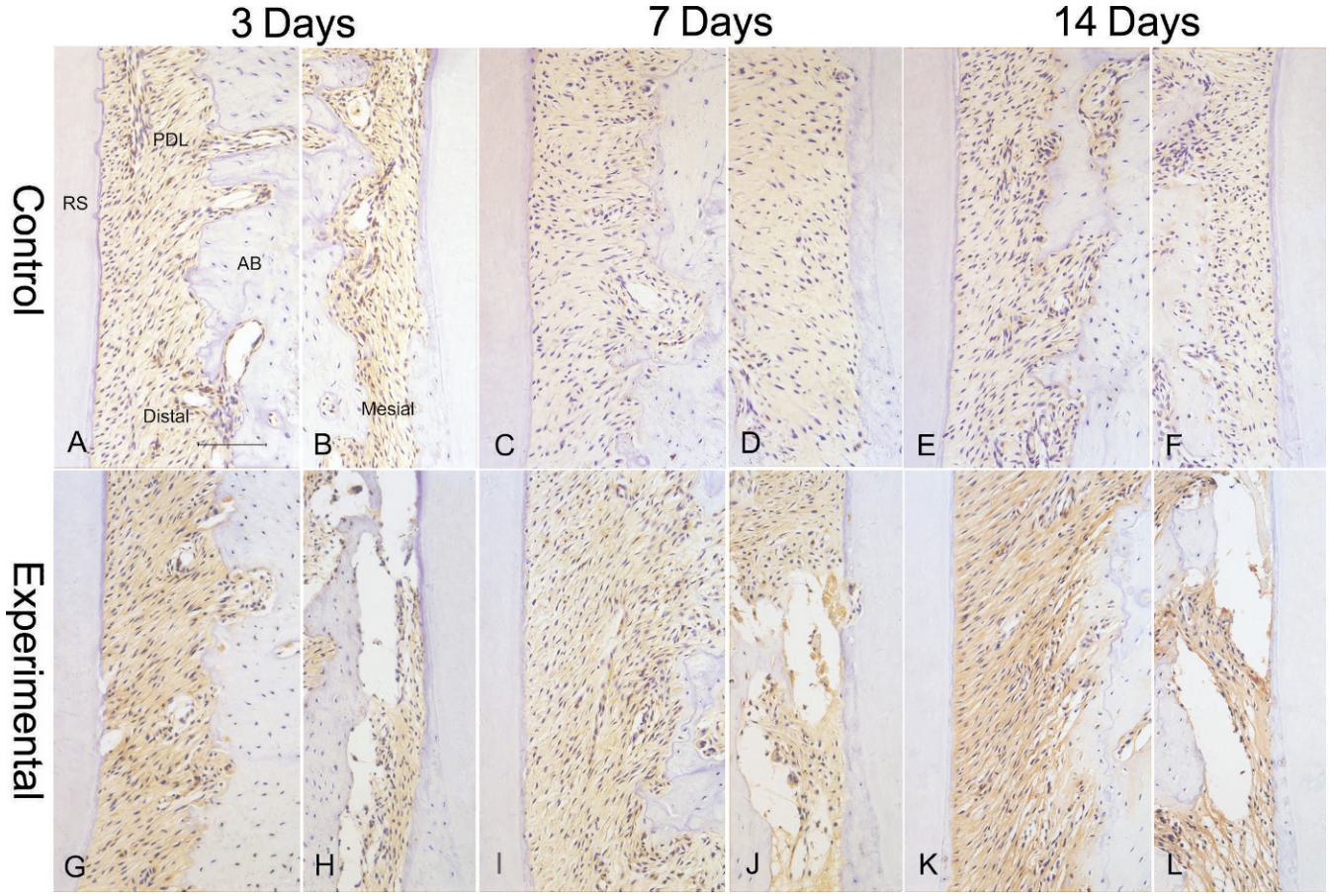

Figure 8. Leukemia inhibitor factor receptor immunohistochemistry staining of the unloaded (A–F) and loaded (G–L) teeth after 3, 7, and 14 days of orthodontic forces application. Bar = 50 μm.

components of bone matrix that may affect both bone formation and resorption. MMP-13 appears in both PDL and alveolar bone cells following the application of the orthodontic forces.[27] Because of the high similarities between PDL cells and osteoblasts, LIF may play roles in regulating PDL cell biological behavior and ECM remodeling during OTM.

We also found that the expressions of LIF/LIFR were higher on the tension side than those on the pressure side. The main characteristic of the tension side was alveolar bone formation, while it was bone resorption on the pressure side.[1,28] For bone remodeling, researches have shown that LIF increased osteoclastic differentiation via an indirect mechanism involving osteoblastic cells.[7,29] It seems reasonable that LIF/LIFR may directly participate in inducing bone formation on the tension side but indirectly activate osteoclasts to start the bone resorption on the compression side.

Another interesting finding is that LIFR was positively stained in osteoclast-like cells in the periodontium. Although LIFR was found to be expressed in osteoclast-like cells from a human giant cell tumor of the bone,[7] our finding is not consistent with the other report that it was negatively stained in osteoclasts.[11] The contradictory results might be explained by the different experiment models and the species used. Further investigation is needed to delineate the molecular mechanism of periodontium remodeling involving LIF-LIFR signaling.

CONCLUSION

- LIF and LIFR expressed in normal rat PDL and the expressions were markedly increased by the orthodontic forces. These findings suggested that LIF might play a pivotal role in periodontal remodeling during OTM.

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