Journal of Dental Research
2015, Vol. 94(10) 1463–1470

DOI: 10.1177/0022034515595003
jdr.sagepub.com

T Cells Are Required for Orthodontic Tooth Movement

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Abstract

The immune system plays a pivotal role during bone remodeling process. Orthodontic tooth movement (OTM) induces local inflammation in periodontium, but whether systemic immune response is involved in OTM remains unknown. In this study, we show that tooth movement distance was significantly reduced in T-cell–deficient immunocompromised mice compared with wild-type (WT) mice. Intravenous infusion of allogeneic T cells to the immunocompromised mice rescued the OTM distance. Correspondingly, increased numbers of tartrate-resistant acid phosphatase (TRAP)–positive osteoclasts were detected around the alveolar bone after OTM in WT mice but were barely detected in immunocompromised mice. Moreover, intravenous infusion of T cells rescued the number of TRAP-positive osteoclasts in the OTM area of the immunocompromised mice, thus suggesting T cells are required for OTM. We then reveal that OTM induced a significant elevation of type 1 T helper cell (Th1) cytokines tumor necrosis factor–α (TNF-α) and interferon-γ (IFN-γ) around periodontal tissue in WT but not in immunocompromised mice. Infusion of T cells could increase the levels of TNF-α and IFN-γ in periodontal tissues of immunocompromised mice. More interestingly, intraperitoneal injection of TNF-α inhibitor etanercept significantly reduced the distance of OTM in T-cell–infused immunocompromised mice. In summary, this study demonstrates a previously unrecognized mechanism that T cells are required for OTM depending on Th1-associated cytokines.

Keywords: orthodontics, bone remodeling, biomechanics, inflammation, immunity, cytokines

Introduction

In orthodontic tooth movement (OTM), alveolar bone remodeling is induced and regulated by constant exertion of mechanical force. During the OTM process, hyalinization is observed in the periodontal ligament (PDL) as a result of local aseptic inflammation (Reitan 1967). The components of the immune system greatly influence skeletal system homeostasis (Ogasawara et al. 2004; Yamaguchi et al. 2006). The shared molecules and pathways of the immune and skeletal system play a key role in bone remodeling (Krishnan and Davidovitch 2006). However, whether the systemic immune response is involved in OTM-induced alveolar bone remodeling remains unknown.

Immune system responses depend on the complex cooperation between innate and adaptive immunity. T cells are a vital part of adaptive immune response that initiate cellular immunity. The concept of osteoimmunology is an interdisciplinary research field that focuses on the molecular understanding of the interplay between the immune and skeletal systems (Horton et al. 1972; Takayanagi 2005, 2007). Furthermore, immune cells are formed in the bone marrow, where they interact with bone cells (Calvi et al. 2003; Mendez-Ferrer et al. 2010). T cells are believed to be a constant stimulator of bone destruction (Kong et al. 1999).

Tumor necrosis factor–α (TNF-α) and interferon-γ (IFN-γ) are crucial cytokines mainly produced by type 1 T helper cells (Th1). Expressions of interleukin (IL)–1β, IL-6, and TNF-α are elevated in gingival crevicular fluid during human OTM (Lowney et al. 1995; Uematsu et al. 1996). The function of IFN-γ in bone remodeling remains controversial (Gao et al. 2007; Kohara et al. 2011). Moreover, macrophages and dendritic cells, which are mediators of T-cell–associated immune response (Baba et al. 2011; Meeran 2013), are infiltrated in bone marrow and PDL. Therefore, we hypothesized that T cells could be involved in orthodontic force-induced alveolar bone remodeling.

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A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

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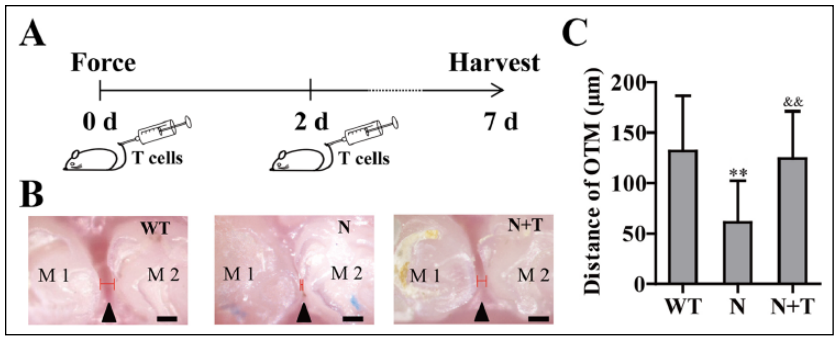

Figure 1. Reduced distance of tooth movement in immunocompromised mice and pan–T-cell infusion rescue tooth movement in immunocompromised mice. (**A**) Schedule of the experiment. Orthodontic force was applied to mice in 3 groups for 7 d. Infusion of T cells was performed at 0 d and 2 d; they were all harvested at 7 d. (**B**) Occlusal view of the first and second molars of the 3 groups after 7 d of force application in mice (n = 9–15). (**C**) Statistical results of tooth movement in 3 groups. WT, wild type; N, T-cell–deficient immunocompromised; N+T, immunocompromised mice with T-cell infusion. Scale bars indicate 200 μm. M1, first molar; M2, second molar. Red H-shaped lines indicate the measurement of orthodontic tooth movement (OTM) distance. Black triangles indicate OTM distance. $^{**}P < 0.01$ versus WT; $^{\&\&}P < 0.01$ versus N.

Researchers use T-cell infusion to evaluate the influence of T cells (Chang and Huang 2013; Wang et al. 2014). Attempts have been made to investigate the function of immunocomponents in OTM, but the role of T cells in OTM is unclear. In this study, we demonstrate a previously unrecognized mechanism by which T cells are required for OTM in a mouse model.

Materials and Methods

Animals

Eight-week-old male BALB/c mice (BALB/cAnNCrlVr) and BALB/c nude mice (CAnN.Cg-*Foxn1*^{nu}/Crl) provided by Vital River Laboratories (Beijing, China) were used. The Animal Use and Care Committee of Peking University approved the protocols of the experiments. Mice were housed under controlled temperature (22 ± 1 °C) in a 12-h/12-h light/dark cycle and were given free access to food and water.

Orthodontic force was applied to a mouse model by using a previously described method (Cao et al. 2014). A nickel–titanium coil spring (0.2 mm wire size, 1 mm in diameter and 1 mm in length; Smart Technology, Beijing, China) provided a force of 30 to 35 g (Taddei et al. 2012), as confirmed by a forcemeter (YS-31 2N; YDM Corporation, Tokyo, Japan) at activation. This spring was bonded using flowable restorative resin (3M ESPE, St. Paul, MN, USA) between the maxillary right first molar and maxillary incisors. The contralateral side was used as a control. The scheme for the animal experiment is shown in Figure 1A. All the mice were divided into 3 groups, including the wild-type (WT) group, T-cell–deficient immunocompromised (N) group, and immunocompromised mice with infusion of T cells (N+T) group. Orthodontic force was applied to the 3 groups for 7 d. Isolated T cells were infused to the N+T group twice at 0 d and 2 d. Each group had at least 9 mice. All the mice were sacrificed at 7 d, and the maxillae were fixed in 10% phosphate-buffered formalin. Another batch of WT mice was used to test the change of T cells and cytokines after OTM. Force side and control side of the upper jaw were collected separately at 2 d and 7 d. The former was used to collect the cell suspension of bone marrow followed by flow cytometry analysis; the latter were fixed and stained by immunohistochemistry. Peripheral blood was collected at 2 d, and the serum was obtained for enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA) of TNF-α, IFN-γ, IL-17, IL-10, and IL-4.

Measurement of Tooth Movement Distance

The maxillae were removed en bloc. Occlusal view of each maxilla was recorded using a stereo microscope (SWZ1000; Nikon, Tokyo, Japan). The OTM distance was measured between 2 easily located points (the midpoint of the distal-marginal ridge of the first molar and the midpoint of the mesial-marginal ridge of the second molar) using a modified form as previously described (Cao et al. 2014).

Histology and TRAP Staining

Detailed methods are described in the Appendix.

Immunohistochemistry

Detailed methods and all antibodies used in this study are described in the Appendix.

Flow Cytometry Analysis

Detailed methods and all antibodies used in this study are described in the Appendix.

Isolation and Infusion of Pan–T Cells

Pan–T cells were isolated from the spleen of the WT mouse using a mouse pan–T-cell isolation kit II (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. Isolated T cells were harvested and resuspended in 3% bovine serum albumin in phosphate-buffered saline (PBS). In brief, phycoerythrin (PE)–conjugated monoclonal antibodies against mouse CD3 and fluorescein isothiocyanate (FITC)–conjugated monoclonal antibodies against mouse CD4 were incubated with the cells at 4 °C for 30 min. Cells were washed and then analyzed by a flow cytometer. Approximately 2.0×10^6 of T cells were suspended in 100 μL PBS and injected intravenously (Liu et al. 2011). To confirm the efficiency of T-cell infusion, we labeled isolated T cells by incubating with carboxyfluorescein diacetate (CFSE) (Dojindo Laboratories, Rockville, MD, USA) for 30 min in 37 °C. Two hours after T-cell infusion, peripheral blood was collected from the tail vein.

Preparation of Cell Suspension in the Maxilla

The maxillae were removed en bloc and processed in PBS at 4 °C. Each of the maxilla was separated into force and control parts along the median palatine suture. The gingiva and molars were gently removed. Then, half of the maxilla was cut into pieces and gently ground. The suspension was filtered through a 100-μm cell strainer (BD Falcon, NY, USA), and we harvested the half-maxilla cell suspension.

Administration of CD25 Antibody, TNF-α Inhibitor, and IFN-γ Antibody

A batch of WT mice were randomly divided into 2 groups; mice were intravenously injected either with vehicle or with CD25 neutralizing antibody basiliximab (50 μg/mouse/time; Novartis, East Hanover, NJ, USA) at 0 and 2 d during OTM (Whited et al. 2015). Each group consisted of 6 mice. Another batch of mice were randomly divided into 2 groups; mice were intraperitoneally injected either with vehicle or with TNF-α inhibitor etanercept (100 μg/mouse/time; Wyeth, Dallas, TX, USA) at 0, 2, 4, and 6 d during OTM (He et al. 2015) in WT and N+T mice. Intraperitoneal injection either with vehicle or with IFN-γ neutralizing antibody (60 μg/mouse/time; Abcam, Cambridge, UK) in WT and N+T mice at 0, 2, 4 and 6 d during OTM was performed. Each group consisted of 5 to 9 mice.

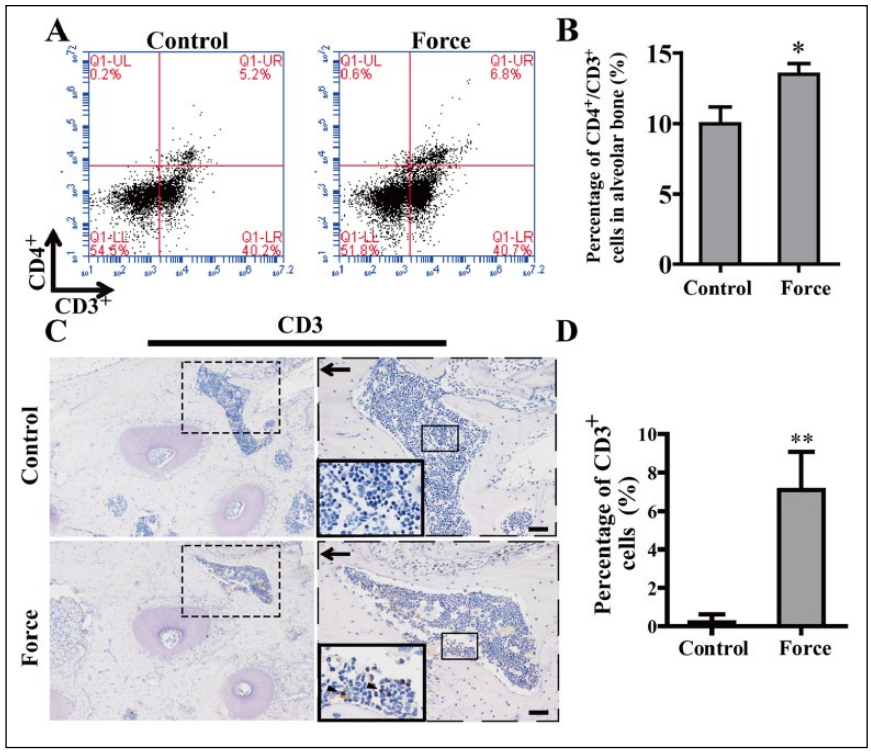

Figure 2. Increased ratio of $CD4^+/CD3^+$ cells in alveolar bone after force application. (**A**) Representative flow cytometry graphs show the $CD3^+$ and $CD4^+$ cells in the half of maxilla. Force: 2 d after force application; control: 2 d without force application. (**B**) Statistical analysis shows that the ratio of $CD4^+/CD3^+$ cells significantly increased in the force group compared with the control (n = 4). *P < 0.05. (**C**) Representative immunohistochemically stained images of alveolar bone marrow. $CD3^+$ cells appeared in the bone marrow around the active bone remodeling site in the force group, but few appeared in the control group. The rectangular shapes with dotted lines are the representative sites in the magnified pictures. Large solid-line-boxed areas show high-magnification views of the small-boxed areas. Arrows represent the direction of force application. Black triangles point to the $CD3^+$ cells. Scale bars indicate 50 μm. (**D**) Semi-quantification of percentage of $CD3^+$ cells in the alveolar bone marrow of the first molar (n = 3–5). Percentage of positive cells significantly increased in the force group compared with that of the control. **P < 0.01 versus control.

Statistical Analysis

The evaluation of each group was expressed as the mean ± SD. Comparison among the groups was statistically analyzed by 1-way analysis of variance (ANOVA), followed by the least significant difference (LSD) multiple-comparison test. Comparisons between groups were statistically analyzed by 2-sample t tests. $P < 0.05$ was considered statistically significant.

Results

Reduced Distance of Tooth Movement in Immunocompromised Mice

The results of flow cytometry analysis showed that 55.4% $CD3^+$ and 29.8% $CD4^+$ cells were present in the peripheral blood lymphocyte subpopulation in the WT mouse, which were higher compared with 2.5% and 0.3%, respectively, in immunocompromised mouse (Appendix Fig. 1). The OTM distance in the force-applied side of the WT mice resulted in 132 μm, whereas no movement was detected in the opposite side (data not shown). By contrast, OTM distance in the N group was significantly decreased (61 μm) compared with that of the WT group (Fig. 1B, C). In addition, we used CD25 neutralizing antibody basiliximab injection to repress the activation of T lymphocytes in WT mice. We found that the distance of OTM significantly decreased after injection of basiliximab compared with that of the vehicle group (Appendix Fig. 2), which further confirmed the effect of T cells on OTM.

Furthermore, we used micro–computed tomography (CT) analysis to define the bone mineral density (BMD). As shown in Appendix Figure 3A, BMD of femur from T-cell–deficient mice was lower than in WT mice ($P < 0.05$). Moreover, no significant difference in maxilla BMD was detected between the WT and N mice (Appendix Fig. 3B). Histology examination shows no significant difference between N and WT mice

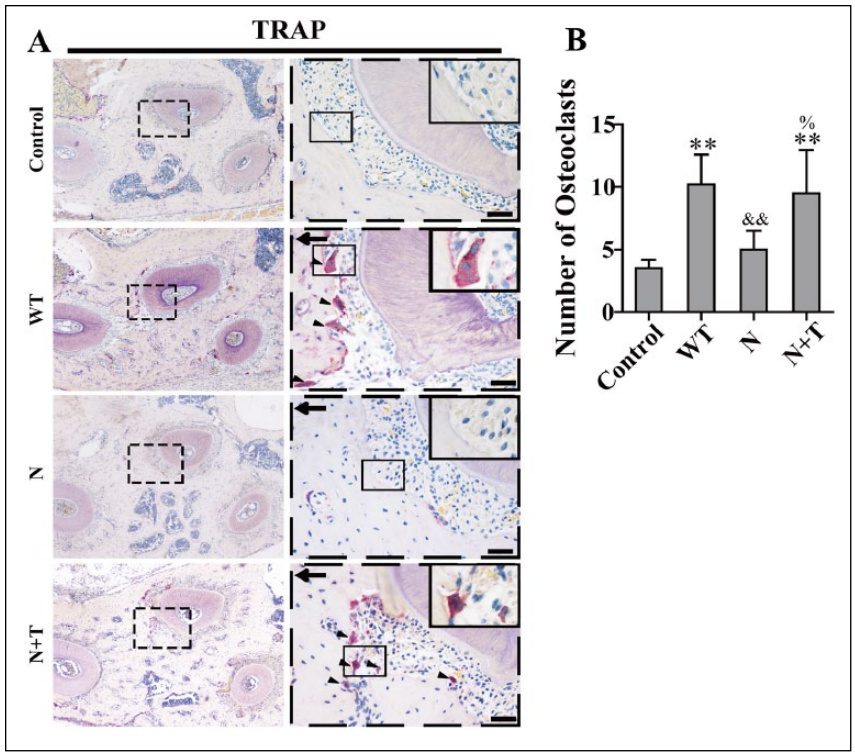

Figure 3. Changes of osteoclastogenesis after 7 d of force application. (**A**) Representative tartrate-resistant acid phosphatase (TRAP) staining images of palatal roots. TRAP-positive osteoclasts appeared near the compression side of the roots at the active bone resorption site in the WT and N+T groups compared with control, but few appeared in the N group. The rectangular shapes with dotted lines are the representative sites in the magnified pictures. Large solid-line-boxed areas show high-magnification views of the small-boxed areas. Arrows represent the direction of force application. Black triangles point to the TRAP-positive multinuclear osteoclasts. Scale bars indicate 40 μm. (**B**) Semi-quantification of TRAP-positive osteoclasts (n = 3–9). TRAP-positive cells were counted in high-power fields around each of the 3 roots of the first molar per sample. Number of positive cells significantly decreased in the N group compared with the WT group, whereas it significantly increased in the N+T group compared with the N group but no significant difference compared with the WT group. WT, wild type; N, T-cell–deficient immunocompromised; N+T, immunocompromised mice with T-cell infusion. $^{**}P < 0.01$ versus control, $^{\&\&}P < 0.01$ versus WT, and $^{\%}P < 0.05$ versus N.

in terms of bone structure and quantity (Appendix Fig. 3C, D). Thereby, these results suggest that the repressed OTM in N mice may not be due to the effect of T-cell deficiency on BMD.

Pan–T-Cell Infusion Rescues Decreased-Tooth Movement in Immunocompromised Mice

Immunocompromised mice presented reduced distance of tooth movement; thus, we used allogeneic T-cell infusion to explain its function. Flow cytometry analysis of isolated pan–T cells presented 95.9% $CD3^+$ cells and 71.2% $CD4^+$ cells (Appendix Fig. 4A). Peripheral blood smear showed that the green fluorescence signal of infused T cells was comparable with the CFSE-stained isolated T cells in vitro (Appendix Fig. 4B). After intravenous injection of T cells, the OTM distance was significantly rescued from 61 μm to 124 μm ($P < 0.01$) (Fig. 1B, C), which suggests that T cells may be required for the OTM process.

Increased Ratio of $CD4^+$/$CD3^+$ Cells in Alveolar Bone after Force Application

Given that the infusion of T cells rescued OTM in immunocompromised mice, we further confirmed whether the T cells in alveolar bone were changed in WT mouse. After OTM for 2 d, the ratio of $CD4^+/CD3^+$ cells in the maxilla bone marrow was significantly increased to 13.5% ($P < 0.05$) compared with 10.0% in the control group (Fig. 2A, B). In addition, percentage of $CD3^+$ cells was increased in the alveolar bone marrow in immunohistochemistry staining after 7 d of OTM compared with the control group (Fig. 2C, D), and TNF-α^+ and IFN-γ^+ cells in bone marrow were increased compared with the control group (Appendix Fig. 5A, B), which indicated the increased $CD3^+$ cells in bone marrow might be Th1 cells.

OTM-Induced Osteoclastogenesis Is Decreased in Immunocompromised Mice and Rescued by T-Cell Infusion

TRAP staining was conducted to confirm the effect of T cells on osteoclastogenesis during OTM. The number of TRAP-positive osteoclasts increased along the surface of the alveolar bone after orthodontic force application, compared with that of the control group, while no such feature was detected in the N group, thus suggesting the repressed activity of osteoclastogenesis in T-cell–deficient immunocompromised mice (Fig. 3A). More important, T-cell infusion rescued the repression of TRAP-positive osteoclasts in immunocompromised mice, which shows the active osteoclastogenesis in the N+T group (Fig. 3A). Generally, a good correlation existed between the distance of OTM and the number of TRAP-positive osteoclasts, as further measured semi-quantitatively (Fig. 3B). These findings indicate that T cells play an important role in the bone remodeling process induced by orthodontic force.

OTM-Induced Expression of Th1-Associated Cytokines Is Decreased in Immunocompromised Mice and Rescued by T-Cell Infusion

Regarding the effect of T-cell infusion on OTM and osteoclastogenesis, we further observed the changes of Th1-associated cytokines after OTM using immunohistochemistry staining.

After force application for 7 d, the periodontal tissue from the WT group presented increased expression of Th1-associated proinflammatory cytokine TNF-α on the compression side of periodontal tissues, especially in bone resorption lacunae, compared with that in the control group (Fig. 4A, B). In contrast, such features were barely detected in the immunocompromised mice, which were rescued by T-cell infusion in the N+T group (Fig. 4A, B). Another Th1-associated proinflammatory cytokine IFN-γ showed the same trend like TNF-α (Fig. 4C, D). The ELISA tests showed significant elevations of serum TNF-α after 2 d of orthodontic force application in groups WT, N, and N+T, compared with that of the control group (Appendix Fig. 5C). The elevation in the N group was to a lesser degree compared with the elevation in the WT group, but T-cell infusion to immunocompromised mice could potentiate the increase of serum TNF-α level (Appendix Fig. 5C). However, no significant change was found in serum IFN-γ, IL-4, IL-10, and IL-17 levels in these groups (Appendix Fig. 5C). These findings suggest that orthodontic force may also promote secretion of systemic Th1-associated cytokine TNF-α during OTM.

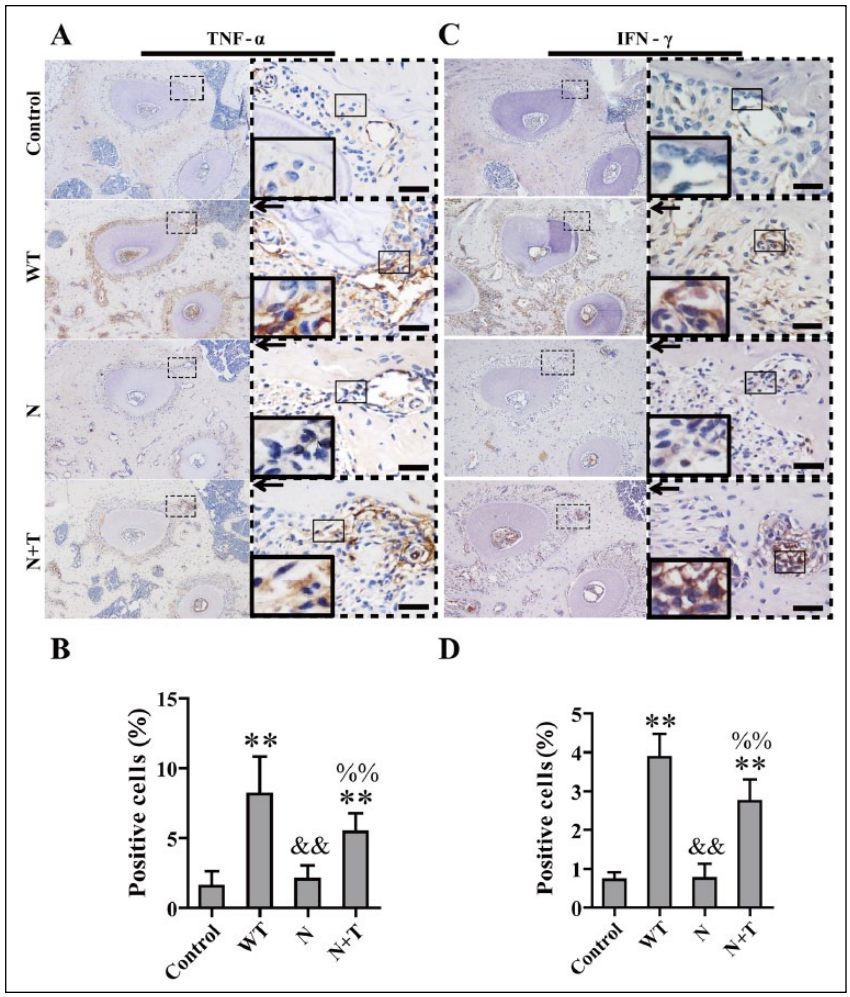

Figure 4. Different expression levels of T-cell–associated cytokines in the 3 groups. (**A**) Representative immunohistochemically stained images of the compression side of palatal roots. Tumor necrosis factor (TNF)–α staining was detected in the WT and N+T groups near the bone resorption site, which decreased in the N group. (**B**) Semi-quantification of positive cells. TNF-α expression significantly decreased in the N group but was rescued in the N+T group, compared with the WT group. (**C**) Representative immunohistochemically stained images of the tension side of mesial roots. Interferon-γ (IFN-γ) staining was observed in the WT and N+T groups along the alveolar bone near the periodontal ligament (PDL) but barely detected in the N group. The rectangular shapes with dotted lines are the representative sites in the magnified pictures. Large solid-line-boxed areas show high-magnification views of the small-boxed areas. Arrows represent the direction of force application. Scale bars: 30 μm. (**D**) IFN-γ expression decreased in the N group but increased in the N+T group. WT, wild type; N, T-cell–deficient immunocompromised; N+T, immunocompromised mice with T-cell infusion. $N = 5$; $^{**}P < 0.01$ versus control, $^{\&\&}P < 0.01$ versus WT, and $^{\%\%}P < 0.01$ versus N.

Blockage of TNF-α Represses the Enhancing Effect of T Cells on OTM

Local cytokines recovered and the OTM distance was rescued in N+T. To further explain the relationship between the expressions of local Th1-associated cytokines and the OTM distance in mice, we used etanercept to block TNF-α systemically both in WT and N+T mice and evaluated the change of OTM distance. At first, we found that blockage of TNF-α by etanercept injection in WT mice significantly repressed the force-induced expression of Th1-associated cytokine TNF-α in PDL and the number of TRAP-positive osteoclasts adjacent to alveolar bone (Appendix Fig. 6A–D). Correspondingly, the distance of OTM in WT mice was also reduced by blockage of TNF-α (Appendix Fig. 6E, F). Second, we found that the force-induced expression of Th1-associated cytokine TNF-α and the number of TRAP-positive osteoclasts in PDL were significantly reduced after etanercept administration in N+T mice compared with the vehicle group (Fig. 5A–D), as well as the OTM distance (Fig. 5E, F). Therefore, the enhancing effect of T cells on OTM in mice is repressed by blockage of TNF-α. However, no significant change of OTM distance was detected after blockage of IFN-γ in both the WT group and the N+T group (Appendix Fig. 7).

Discussion

In the present study, we provided evidence to show that T cells are required for mechanical force-induced OTM in mice. First, T-cell deficiency led to the decrease of force application–induced

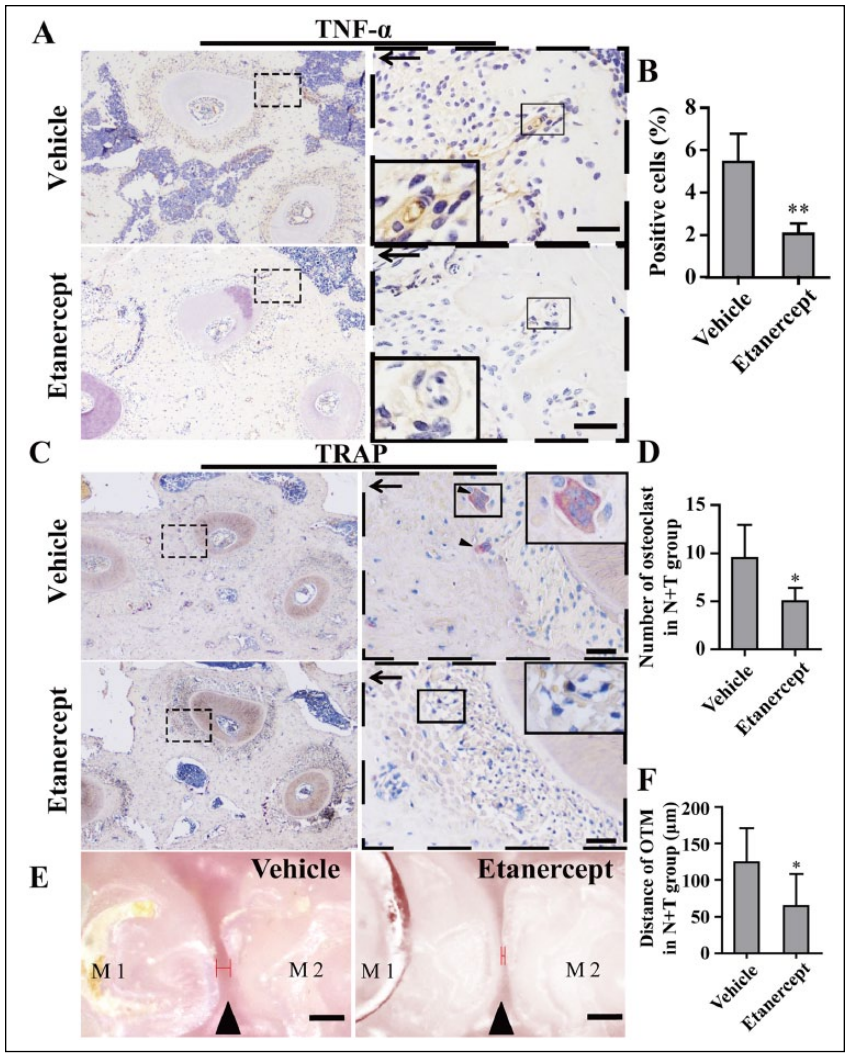

Figure 5. Injection of tumor necrosis factor (TNF)–α (etanercept) decreased the T-cell–associated cytokines and attenuated the orthodontic tooth movement (OTM) distance in the N+T group. (**A**) Representative immunohistochemically stained images of the compression side of palatal roots. Intraperitoneal injection of the TNF-α inhibitor etanercept decreased the expression of TNF-α. The rectangular shapes with dotted lines are the representative sites in the magnified pictures. Large solid-line-boxed areas show high-magnification views of the small-boxed areas. Arrows represent the direction of force application. Scale bars: 30 μm. (**B**) Semi-quantification of positive cells in the alveolar bone near the first molar (n = 3–5). Number of positive cells significantly decreased in etanercept compared with that in vehicle. $^{**}P < 0.01$ versus vehicle. (**C**) Representative tartrate-resistant acid phosphatase (TRAP) staining images of palatal roots. TRAP-positive osteoclasts appeared near the compression side of the roots at the active bone resorption site in the vehicle, but few appeared in etanercept. Arrows represent the direction of force application. The rectangular shapes with dotted lines are the representative sites in the magnified pictures. Large solid-line-boxed areas show high-magnification views of the small-boxed areas. Black triangles point to the TRAP-positive multinuclear osteoclasts. Scale bars: 40 μm. (**D**) Semi-quantification of TRAP-positive osteoclasts in the alveolar bone near the 3 roots of the first molar (n = 3–5). Number of positive cells significantly decreased in etanercept compared with that in vehicle. $^{*}P < 0.05$ versus vehicle. (**E**) Representative occlusal view of the first and second molars after 7 d of force application in the N+T group with intraperitoneal injection of vehicle and etanercept (n = 6–9). (**F**) Changes of OTM distance in the 2 groups. Intraperitoneal injection of the TNF-α inhibitor etanercept significantly decreased the distance of tooth movement compared with the vehicle group. Scale bars: 200 μm. N+T, immunocompromised mice with T-cell infusion; M1, first molar; M2, second molar. Black triangles indicate OTM distance. $^{*}P < 0.05$ versus vehicle.

osteoclastogenesis and OTM in mice, whereas T-cell infusion rescued them. Second, force-induced alveolar bone remodeling presented an increased ratio of $CD4^+/CD3^+$ cells and increased Th1 cells in the local bone marrow in WT mice. Third, force application–induced expression of Th1-associated cytokines during OTM was repressed in immunocompromised mice, while T-cell infusion rescued it. Fourth, blockage of TNF-α repressed the enhancing effect of T-cell infusion on OTM and osteoclastogenesis in immunocompromised mice. These results demonstrated that T cells are required for OTM, and the effects of T cells on OTM may depend on Th1-associated cytokines.

T cells are needed for the OTM process. OTM is currently known as a local inflammatory process of PDL and alveolar bone. In the present study, we used the N and N+T groups to confirm that systemic immune responses are involved in force application–induced OTM. Previous studies stated that OX6, CD40, and CD40 ligand, which are costimulatory molecules in adaptive immune response and further activate T cells, increased in quantity in PDL after OTM (Alhashimi et al. 2004; Baba et al. 2011). The increase in these molecules after orthodontic application might be one of the factors that contribute to the activation of T cells during OTM.

In the studies on the osteoimmune system, T cells are reportedly a constant stimulator of bone destruction (Takayanagi 2007; Zaidi 2007). However, their direct effect on osteoclastogenesis depends on the dynamic balance among the cytokines they produce (Takayanagi et al. 2000; Mirosavljevic et al. 2003). Local inflammation is usually activated by exogenous antigen and is amplified by T helper cells through the adaptive immune pathway in local immune organs, such as bone marrow (Rodeghero et al. 2013). Force application–induced alveolar bone remodeling in our mouse model showed an increased ratio of $CD4^+/CD3^+$ cells in the local bone marrow and elevated osteoclastogenesis. The results imply that T cells could mediate the osteoclastogenesis induced by force application and could further lead to OTM.

Researchers have previously attempted to investigate the function of immunocomponents in OTM, such as the influence of Th1-associated cytokines TNF-α (Andrade et al. 2007; Taddei et al. 2012) and IFN-γ (Alhashimi et al. 2000). OTM induces local expression of proinflammatory cytokines in gingival crevicular fluid (Basaran et al. 2006) and PDL (Ogasawara et al. 2004). In the present study, we confirmed that OTM was repressed because of T-cell deficiency. By contrast, T-cell

infusion systemically rescued the bone remodeling process, leading to OTM. Thus, T cells, a crucial regulator of the immune system, are required in OTM.

TNF-α and IFN-γ are cytokines from the Th1-associated immune response, particularly during the bone remodeling process (Hemeda et al. 2010).

TNF-α is a central regulator of inflammation (Elenkov and Chrousos 1999) and induces the maturation of osteoclasts (Cenci et al. 2000). In the present study, local expressions of TNF-α and osteoclastogenesis were induced in N+T mice after OTM. In force application–induced bone remodeling, the infused T cells may initiate the regulation of osteoclastogenesis by directly producing cytokines and associated immune responses. Etanercept, a soluble receptor construct, is capable of binding and neutralizing soluble and transmembrane TNF-α. Etanercept inhibits the expression of T-cell–associated production of TNF-α in the OTM process, thereby repressing the distance of OTM.

In the adaptive immune system, IFN-γ induces macrophage activation (Stout and Bottomly 1989). In the regulation of bone remodeling, most researchers speculate that IFN-γ inhibits osteoclastogenesis. Force-induced elevation of IFN-γ expression in PDL may serve as an equalizer of osteoclasts and osteoblasts because it inhibits osteoclastogenesis (Kohara et al. 2011). In our results, the expression of IFN-γ in PDL after OTM was elevated on the distal side of PDL in the N+T group. After systemically blocking IFN-γ, local osteohomeostasis and the bone remodeling process may be affected. Bone remodeling is a complex network of cells and cytokines, and our result did not show any change in OTM distance after IFN-γ blocking. IFN-γ might not be a direct inhibitor of bone remodeling but rather a stabilizer of force-induced orthodontic tooth movement.

Receptor activator of nuclear factor kappa-B ligand (RANKL) is a member of the tumor necrosis factor superfamily that is essential for osteoclast differentiation and bone resorption (Udagawa et al. 1999; Takayanagi et al. 2002). RANKL is expressed in osteoblasts and activated T cells during bone metabolism (Anderson et al. 1997; Colucci et al. 2004). Despite the secretion of Th1-associated cytokines, an increased number of T cells after force application may produce local functional RANKL, which directly contributes to osteoclast differentiation during OTM. This idea is consistent with our result that an orthodontic force-induced increase in the number of TRAP-positive osteoclasts was barely found in immunocompromised mice but was rescued by T-cell infusion. Moreover, our result showed that blocking TNF-α repressed the effect of T-cell infusion on osteoclastogenesis and OTM, thereby suggesting that Th1-associated cytokines play an important role in the T-cell–mediated OTM process.

To our knowledge, this study is the first to show that T cells are required for OTM, and the effects of T cells on OTM may depend on Th1-associated cytokines. The mechanism of T cells participating in OTM may present new insights into the regulation of force application–induced bone remodeling.

Author Contributions

Y. Yan, contributed to data acquisition and analysis, drafted the manuscript; F. Liu, contributed to data acquisition, drafted the manuscript; X. Kou, Y. Song, Y. Gan, contributed to conception, critically revised the manuscript; D. Liu, R. Yang, X. Wang, contributed to design, critically revised the manuscript; D. He, contributed to data analysis, drafted the manuscript; Y. Zhou, contributed to conception and design, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Acknowledgments

This study was supported by grants from International S&T cooperation program of China (Grant No. 2013DFB30360) and the National Natural Science Foundation of China (Grant No. 81470717 and No. 81300897). The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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