M1-like Macrophage Polarization Promotes Orthodontic Tooth Movement

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Abstract

Macrophages play a crucial role in inflammatory-mediated bone loss. Orthodontic tooth movement (OTM) is associated with inflammatory bone remodeling. However, whether and how macrophages contribute to mechanical force–induced OTM remains unknown. In this study, we hypothesized that polarization of M1-like macrophages may contribute to the OTM. Orthodontic nickel-titanium springs were applied to the upper first molars of rats or mice to induce OTM. The distance of OTM gradually increased after mechanical force was applied to the rats for 5 and 10 d. M1-like macrophage polarization and expression of M1 cytokine tumor necrosis factor (TNF)-α also increased after force application. More importantly, monocyte/macrophage depletion in mice by injection of clodronate liposomes decreased the distance of OTM and the number of tartrate-resistant acid phosphatase (TRAP)–positive osteoclasts and CD68^{+} macrophages, accompanied by reduced expressions of M1 markers TNF-α and inducible nitric oxide synthase (iNOS), whereas systemic transfusion of M1 macrophages in mice increased them. Further experiments showed that injection of recombinant TNF-α increased the distance of OTM and the number of TRAP-positive osteoclasts and CD68^{+} macrophages, as well as upregulated the expression of TNF-α and iNOS. Blockage of TNF-α by etanercept injection reduced the distance of OTM and the number of TRAP-positive osteoclasts and CD68^{+} macrophages, as well as decreased the levels of TNF-α and iNOS. These data suggest that M1-like macrophage polarization promotes alveolar bone resorption and consequent OTM after mechanical force application.

Keywords: orthodontics, biomechanical phenomena, bone resorption, inflammation, macrophage activation, osteoclasts

Introduction

Orthodontic tooth movement (OTM) induced by mechanical force is governed by local aseptic inflammation-associated alveolar bone remodeling. It includes osteoclastic bone resorption on the compression sides and osteoblastic bone formation on the tension sides (Garlet et al. 2007), along with increased levels of cytokines, chemokines, and leukocyte infiltration (Alhashimi et al. 2000; Krishnan and Davidovitch 2006; Meikle 2006; Garlet et al. 2007; Ren et al. 2007). Moreover, macrophage-like cells, which are found in the initial phase of OTM, may enhance bone and root resorption (Brudvik and Rygh 1993). However, the detailed role of macrophages in OTM remains unknown.

As progenitors of osteoclasts, macrophages directly mediate osteoclast differentiation and promote bone resorption (Teitelbaum 2000). Macrophages are also important in inflammation and host defense. Macrophages can be polarized into different phenotypes, including classically activated M1 macrophages and alternatively activated M2 macrophages (Mosser and Edwards 2008; Sica and Mantovani 2012). M1 macrophages mediate inflammation by producing tumor necrosis factor (TNF)-α, interleukins (IL)-1, IL-6, and inducible nitric oxide synthase (iNOS) (Dale et al. 2008); conversely, M2 macrophages function as anti-inflammatory agents by producing anti-inflammatory products (Gordon 2003). As important immune cells that mediate inflammation, macrophages play a crucial role in diseases characterized by inflammatory-mediated bone loss, such as rheumatoid arthritis and periodontal diseases (Arend and Dayer 1990; Shaddox et al. 2011). However, the detailed role of macrophages in OTM is largely unknown. Our previous study showed that enhanced M1/M2 macrophage ratio aggravates orthodontic root resorption (He et al. 2015); hence, the role of macrophage polarization in OTM should be elucidated. Considering that OTM is an

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inflammatory process, we hypothesized that M1-like macrophage polarization may promote OTM after force application.

Rising evidence suggests that fibroblasts are involved in inflammatory processes and interact with immune cells, such as macrophages (Smith et al. 1997; El Kasmi et al. 2014). Periodontal ligament cells (PDLCs) could interact with macrophages under normal or inflammatory conditions (Konermann et al. 2012). After orthodontic force application, PDLCs express elevated levels of inflammatory cytokines (Lee et al. 2012), including M1 stimulator interferon (IFN)-γ (He et al. 2015). However, the effect of PDLCs on macrophage polarization under mechanical force application remains unclear.

This study aims to examine 1) whether M1-like macrophage polarization affects OTM after mechanical force application and 2) what effect PDLCs have on macrophage polarization under mechanical force application.

Materials and Methods

Animals

Adult Sprague-Dawley rats (male, 180 to 200 g, 6 to 7 wk old) and C57BL/6 mice (male, 20 to 25 g, 8 wk old) were used in this study. Experimental protocols were approved by the Animal Use and Care Committee of Peking University (LA2013-92).

Application of Orthodontic Devices

Mechanical force was applied in rats as previously described with modification (Dunn et al. 2007). Briefly, a nickel-titanium coil spring (wire size, 0.2 mm; diameter, 1 mm; length, 4 mm; Smart Technology, Beijing, China) was connected between the maxillary first molar and incisors to provide a nearly constant force of approximately 60 g. Previous studies used 40 to 60 g of force to induce OTM (Dunn et al. 2007; MirHashemi et al. 2013). The contralateral side served as a control.

Mechanical force was applied in mice as previously described with modification (Taddei et al. 2012). Briefly, the spring (wire size, 0.2 mm; diameter, 1 mm; length, 1 mm) was bonded between the maxillary first molar and incisors to deliver a force of approximately 30 g for 7 d. The contralateral side served as a control.

Micro–computed tomography Scanning and Measurement of OTM Distance

The maxillae of rats and mice were scanned using a micro–computed tomography system (Inveon MMCT, Siemens, USA). OTM distance was measured as previously described (Cao et al. 2014). Briefly, the distance was measured between the midpoint of the distal marginal ridge of the first molar and the midpoint of the mesial marginal ridge of the second molar from the occlusal view of the 3-dimensional reconstructed image. Measurement was performed 3 times by a trained researcher blinded to the group design.

In Vivo Depletion of Monocytes/Macrophages

Mice were divided into 2 groups. One group was intravenously injected with clodronate liposomes (200 μL/mouse, 5 mg/mL; Clodrosome, Encapsula NanoSciences, USA) to deplete monocytes/macrophages (van Amerongen et al. 2007; Hunter et al. 2010); the other group was injected with control liposomes containing phosphate-buffered saline (PBS). All mice received injections every other day since 1 day prior to 7 d of force application. Each group comprised 5 to 6 mice. The effect of depletion was verified by the levels of $SSC^{low}CD11b^{high}$ monocytes in the peripheral blood using flow cytometry (Sunderkotter et al. 2004) and the number of $CD68^+$ macrophages in the maxillary alveolar bone.

Isolation of Bone Marrow–derived Macrophages and Adoptive Transfusion of M1 or M2 Macrophages

Bone marrow–derived macrophages were isolated and cultured as previously described (Zhang et al. 2013). Briefly, bone marrow cells of mice were flushed out from the bone marrow cavity of femurs and tibias. All nuclear cells were seeded at 1.5×10^7 onto 10-cm culture dishes (Corning). After initial incubation overnight, nonadherent cells were discarded. Adherent cells were continuously cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2mM glutamine, antibiotics (100 U/mL of penicillin and 100 mg/mL of streptomycin; Biofluids, USA), and murine GM-CSF (20 ng/mL; 315-03, Peprotech, USA) for 6 d. M1 or M2 polarization was induced as previously described with modification (Kou et al. 2015). The cells were stimulated with IFN-γ (20 ng/mL; 400-20, Peprotech, USA) and lipopolysaccharide (10 ng/mL; L2880, Sigma-Aldrich, USA) for 48 h to polarize into M1 macrophages or with IL-4 (10 ng/mL; 400-04, Peprotech, USA) for 48 h to polarize into M2 macrophages. M1 or M2 macrophages were characterized by real-time polymerase chain reaction.

Cells were collected and intravenously administered into mice (1.5×10^6/mouse) on the day of force application and received 7 d of force application. Each group comprised 5 to 6 mice. To track the transfusion cells in vivo, EGFP-labeled M1 macrophages were obtained from EGFP mice and administered into C57BL/6 mice (1×10^6/mouse).

Injection of Recombinant TNF-α and TNF-α Inhibitor Etanercept

Independent experiments were designed, and different periods were chosen according to the preliminary data for different experiment designs. The first batch of rats was randomly divided into 2 groups. The experiment group was intravenously injected with recombinant TNF-α (rTNF-α; 1 μg/rat; 400-14, Peprotech, USA), and the vehicle group was injected with PBS every day. Force was applied for 10 d.

Another batch of rats was randomly divided into 2 groups. The experiment group was intraperitoneally injected with TNF-α inhibitor etanercept (250 μg/rat; Wyeth, USA), and the vehicle group was injected with PBS every other day. Force was applied for 12 d. Each group comprised 5 to 6 rats.

Cell Culture and Treatments

The protocol was approved by the Ethical Guidelines of Peking University (PKUSSIRB-201311103) and performed with appropriate informed consents. Human PDLC isolation and static compressive force application on PDLCs were performed as previously described (Seo et al. 2004). The PDLCs were subjected to 1 g/cm^2 of continuous compressive force for 24 h. The supernatant was collected from primary cultured PDLCs with or without force loading for further experiments.

To detect whether PDLCs can influence macrophage polarization under mechanical force application, THP-1 human monocytic cells (1×10^6) were used to differentiate into macrophages in a 6-well plate with 2 mL RPMI 1640 medium containing 10% fetal bovine serum, 2mM glutamine, antibiotics, and 50 ng/mL PMA (P1585, Sigma, USA) for 24 h. The cells were then cultured with the supernatant of primary cultured PDLCs with or without force loading for 24 h.

Methods

The following are described in detail in the Appendix:

- Histology and tartrate-resistant acid phosphatase staining (TRAP)
- Immunohistochemistry and immunofluorescence staining
- Quantitative real-time polymerase chain reaction
- Western blot analysis

Statistical Analysis

Statistical analysis was performed with SPSS 13.0. All data were presented as mean ± SD and assessed by independent 2-tailed Student's *t* test or 1-way analysis of variance. Statistical significance was considered at $P < 0.05$.

Results

Orthodontic Force Application Promoted M1-like Macrophage Accumulation during OTM

Micro–computed tomography images showed that the distance of OTM in rats gradually increased to 324 and 409 μm after orthodontic force was applied for 5 and 10 d (Fig. 1A). After orthodontic force application, the infiltration of CD68$^+$ macrophages and expression of the proinflammatory cytokine TNF-α increased on the compression side of periodontal tissues (Fig. 1B, C). It should be mentioned that slight root resorption was also observed during OTM. M1 macrophages were accumulated in the periodontal tissues, near the alveolar bone and the root surface (Fig. 1B). Double immunostaining of CD68 and M1 marker iNOS or M2 marker CD163 showed that CD68$^+$iNOS$^+$ M1-like macrophages infiltrated on the compression side of periodontal tissues during OTM. After force application for 5 d, CD68$^+$iNOS$^+$ M1-like macrophages increased to 3.44% and maintained a relatively high level (3.30%) after 10 d compared with the control group (0.5%), whereas CD68$^+$CD163$^+$ M2-like macrophages were barely detected on the compressed side of periodontal tissues after force application (Fig. 1D, E).

Mechanical Force–induced OTM Was Repressed by Monocyte/Macrophage Depletion and Enhanced by M1 Macrophage Transfusion

After clodronate liposomes injection in mice, >70% of circulated monocytes was eliminated. CD68$^+$ macrophages in the maxillary alveolar bone also significantly decreased (Appendix Fig. 1), confirming the effectiveness of monocyte/macrophage depletion.

After monocyte/macrophage depletion, OTM distance significantly decreased to 41 μm, compared with 115 μm of the vehicle group ($P < 0.001$; Fig. 2A, B). With decreasing OTM distance, the number of tartrate-resistant acid phosphatase (TRAP)–positive osteoclasts and CD68$^+$ macrophages reduced on the compression side in the group treated with clodronate liposomes; the expression of M1 markers TNF-α and iNOS was downregulated as well (Fig. 2C, D).

We further determined the contribution of M1 polarization to OTM. M1 macrophages were confirmed by the high expressions of TNF-α and iNOS mRNA (Appendix Fig. 2A). EGFP-labeled cells were found in the compression side on day 7 during OTM, and the green fluorescent signals of EGFP were colocalized with part of CD68$^+$ macrophages (Appendix Fig. 2B). The increase in OTM distance induced by mechanical force in the vehicle group (113 μm) was enhanced by M1 macrophage transfusion (145 μm; $P < 0.01$; Fig. 3A, B). Correspondingly, the number of TRAP-positive osteoclasts and CD68$^+$ macrophages increased on the compression side in the M1 transfusion group; moreover, the expression of iNOS and TNF-α was also upregulated (Fig. 3C, D). These data indicate that M1 macrophages play an important role in alveolar bone resorption and consequent OTM. In addition, no significant change of the OTM distance was detected after M2 macrophage transfusion (Appendix Fig. 3), confirming the unique effect of M1 macrophages during OTM.

Mechanical Force–induced OTM Was Repressed by Blockage of TNF-α and Enhanced by Injection of rTNF-α

TNF-α could influence M1 macrophage activation and osteoclast differentiation (Azuma et al. 2000; Mosser and Edwards 2008). In this study, we enhanced or blocked TNF-α by systemically injected rTNF-α or TNF-α inhibitor to examine the effect of M1 macrophage activation on OTM.
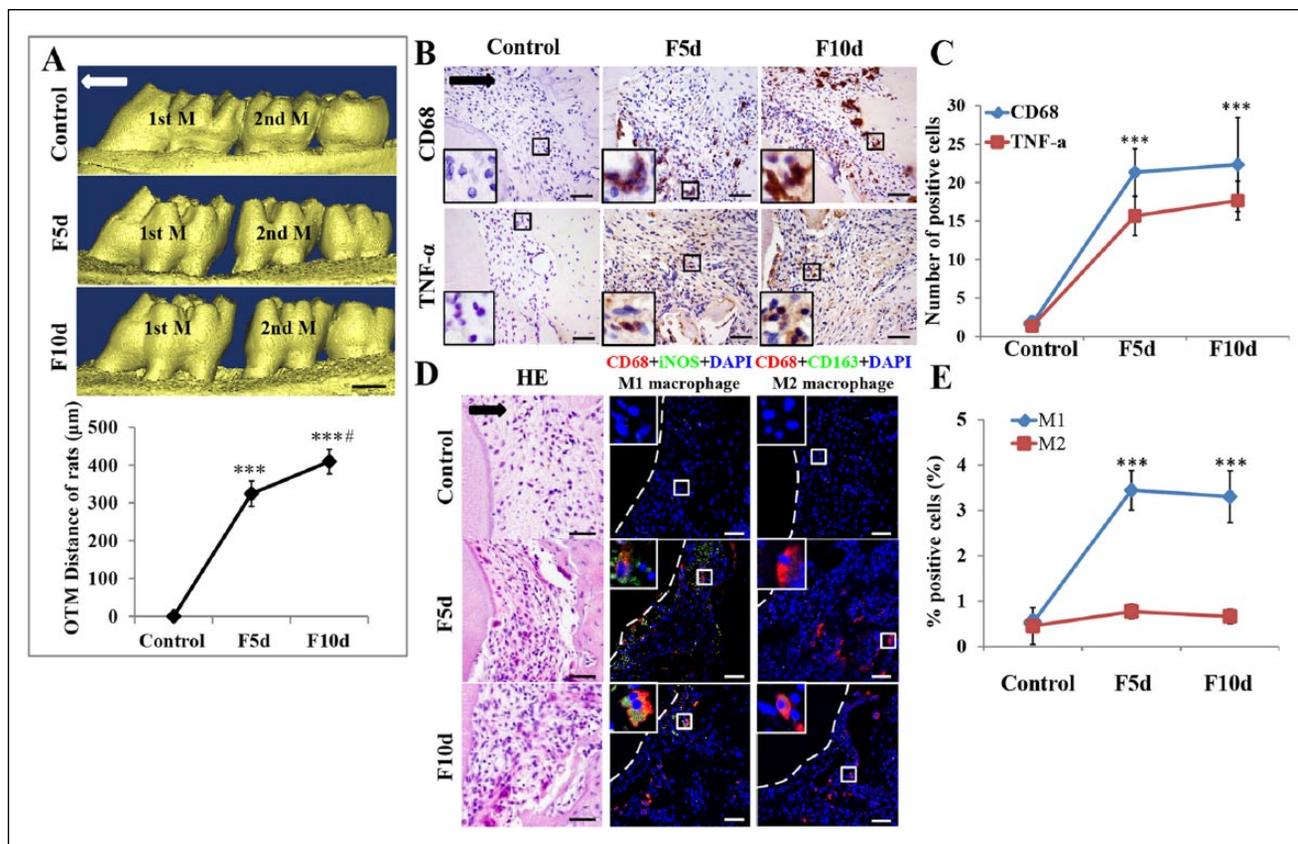
Figure 1. M1-like macrophages accumulated on the compression side of periodontal tissues after orthodontic force application. (**A**) Representative micro–computed tomography images and semiquantification analysis of the orthodontic tooth movement (OTM) distance in rats. OTM distance gradually increased after force was applied for 5 and 10 d (F5d and F10d). $n = 5$. $^{***}P < 0.001$ vs. control; $^{#}P < 0.05$ vs. F5d. 1st M, the first molar; 2nd M, the second molar. Arrow represents the direction of force application. Scale bar: 1,000 µm. (**B**) Representative immunohistochemical images of the compression side of distobuccal roots. The expression levels of CD68 and tumor necrosis factor α (TNF-α) were upregulated in F5d and F10d. Arrow represents the direction of force application. Large boxed areas show high-magnification views of the small boxed areas. Scale bar: 50 µm. (**C**) The number of CD68- and TNF-α-positive cells increased in F5d and F10d. $n = 5$; $^{***}P < 0.001$ vs. control. (**D**) Representative immunofluorescence images and hematoxylin and eosin staining on the compression side of distobuccal roots. The number of CD68-positive (red) and inducible nitric oxide synthase (iNOS)–positive (green) M1-like macrophages (merged yellow) increased in F5d and F10d, whereas the number of CD68- (red) and CD163-positive (green) M2-like macrophages (merged yellow) remained unchanged. Corresponding hematoxylin and eosin staining showed the histomorphology of the immunostained sections. Dashed lines mark the outline of the roots. Scale bar: 50 µm. Arrow represents the direction of force application. (**E**) Semiquantification of double-stained positive cells. M1 proportion increased after force application, whereas M2 proportion did not change. $n = 5$ or 6; M1-positive ratio: $^{***}P < 0.001$ vs. control.

After systemic injection of rTNF-α, the OTM distance increased to 522 µm, compared with 400 µm of the vehicle group ($P < 0.001$; Fig. 4A, B). By contrast, after TNF-α was blocked through systemic injection of TNF-α inhibitor etanercept, the OTM distance decreased to 291 µm, compared with 413 µm of the vehicle group ($P < 0.001$; Fig. 4E, F). In addition, systemic injection of rTNF-α increased the number of TRAP-positive osteoclasts and CD68$^+$ macrophages on the compression side and upregulated the expression of M1 markers iNOS and TNF-α (Fig. 4C, D). Conversely, blocking TNF-α by etanercept decreased the number of TRAP-positive osteoclasts and CD68$^+$ macrophages and downregulated the expression of iNOS and TNF-α (Fig. 4G, H). These data indicate that TNF-α-related M1 macrophage polarization influences bone resorption and consequent OTM.

Force Loading of PDLCs Promoted M1 Polarization of THP-1-derived Macrophages

Since we demonstrated that M1-like macrophages accumulated after force application, we further determined the effect of mechanical force loading of PDLCs on macrophage polarization. THP-1-derived macrophages were cultured with the supernatant from primary cultured PDLCs with or without compressive force loading. After incubation with the supernatants from force-treated PDLCs, the mRNA expression of M1 markers TNF-α, IL-1β, and IL-6 was upregulated in THP-1-derived macrophages; conversely, the expression of M2 markers DECTIN-1 and arginase-1 did not change, and IL-4R was downregulated (Fig. 5A). Western blot analysis showed that the protein expression of TNF-α in THP-1-derived macrophages
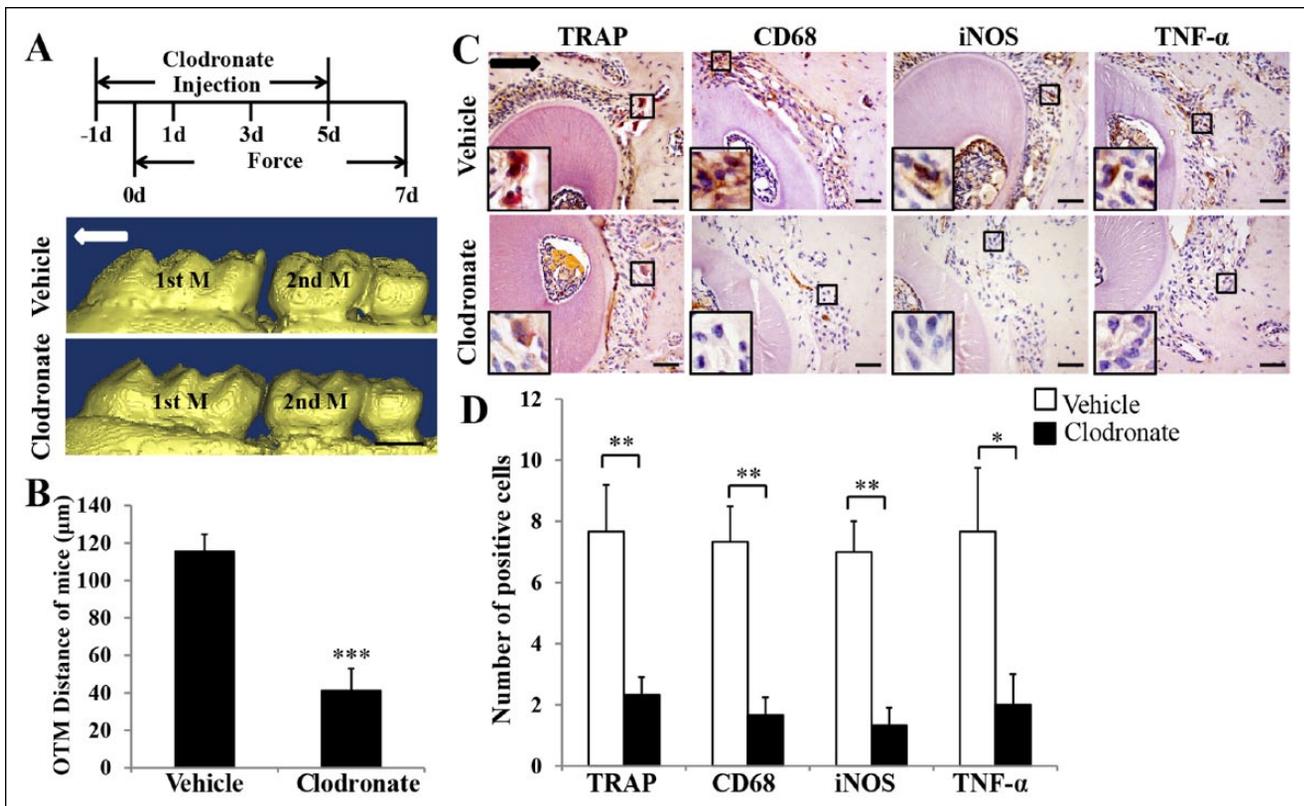
Figure 2. Monocyte/macrophage depletion by clodronate injection repressed orthodontic tooth movement (OTM). (**A**, **B**) Representative micro–computed tomography images and semiquantification analysis of OTM distance in mice. OTM distance significantly decreased in the clodronate-treated group after 7 d of force application. $n = 5$ or 6, [***]$P < 0.001$. 1st M, the first molar; 2nd M, the second molar. Scale bar: 500 µm. Arrow represents the direction of force application. (**C**) Representative tartrate-resistant acid phosphatase (TRAP) staining and immunohistochemical images of the compression side of the distal roots. Clodronate injection decreased the number of TRAP-positive osteoclasts and CD68^{+} macrophages, as well as the expression of inducible nitric oxide synthase (iNOS) and tumor necrosis factor α (TNF-α). Large boxed areas show high-magnification views of the small boxed areas. Scale bar: 50 µm. Arrow represents the direction of force application. (**D**) The number of TRAP-, CD68-, iNOS-, and TNF-α-positive cells decreased after clodronate injection. [*]$P < 0.05$, [**]$P < 0.01$.

was upregulated after incubation with the supernatant from force-treated PDLCs, whereas no changes were observed in the expression of arginase-1 (Fig. 5B). These data suggest that force-treated PDLCs may promote THP-1-derived macrophages to polarize into the M1 phenotype.

Discussion

In this study, we provided multiple lines of evidence that the accumulation of M1-like macrophages after force application contributed to OTM. First, force application promoted M1-like macrophage accumulation, leading to the elevated levels of M1 marker TNF-α during OTM. Second, OTM and M1 marker accumulation were enhanced by systemic transfusion of M1 macrophages and repressed by chemical depletion of monocytes/macrophages. Third, the blockage or enhancement of TNF-α led to the repression or increase of OTM and M1 markers. Finally, force loading of PDLCs upregulated the expression of M1 markers in macrophages, which may promote M1 polarization. These data suggest that M1-like macrophage polarization induced by mechanical force contributes to OTM.

In addition, TNF-α, which is a key signal of M1 macrophage activation, plays an important role during this process.

The accumulation of M1-like macrophages contributes to OTM under force application. M1 macrophages play essential functions in the onset and maintenance of inflammation and bone resorption based on their secretion of large amounts of iNOS and TNF-α (Dale et al. 2008) and their differentiation into osteoclasts (Udagawa et al. 1990). High levels of M1 cytokines IL-1β and TNF-α are present in chronic osteolytic diseases, including rheumatoid arthritis and periodontal diseases (Arend and Dayer 1990; Shaddox et al. 2011). TNF-α receptor–deficient mice demonstrate decreased OTM distance (Andrade et al. 2007). In this study, we found that CD68^{+} macrophages accumulated on the compression side of periodontal tissues, and we identified that the phenotype was mainly M1-like. Moreover, monocyte/macrophage depletion, which blocked macrophage accumulation and M1 marker expression, decreased OTM distance. To our knowledge, this study is the first to demonstrate that M1-like macrophage accumulation induced by force application in periodontal tissues contributes to alveolar bone resorption and consequent OTM. Previous
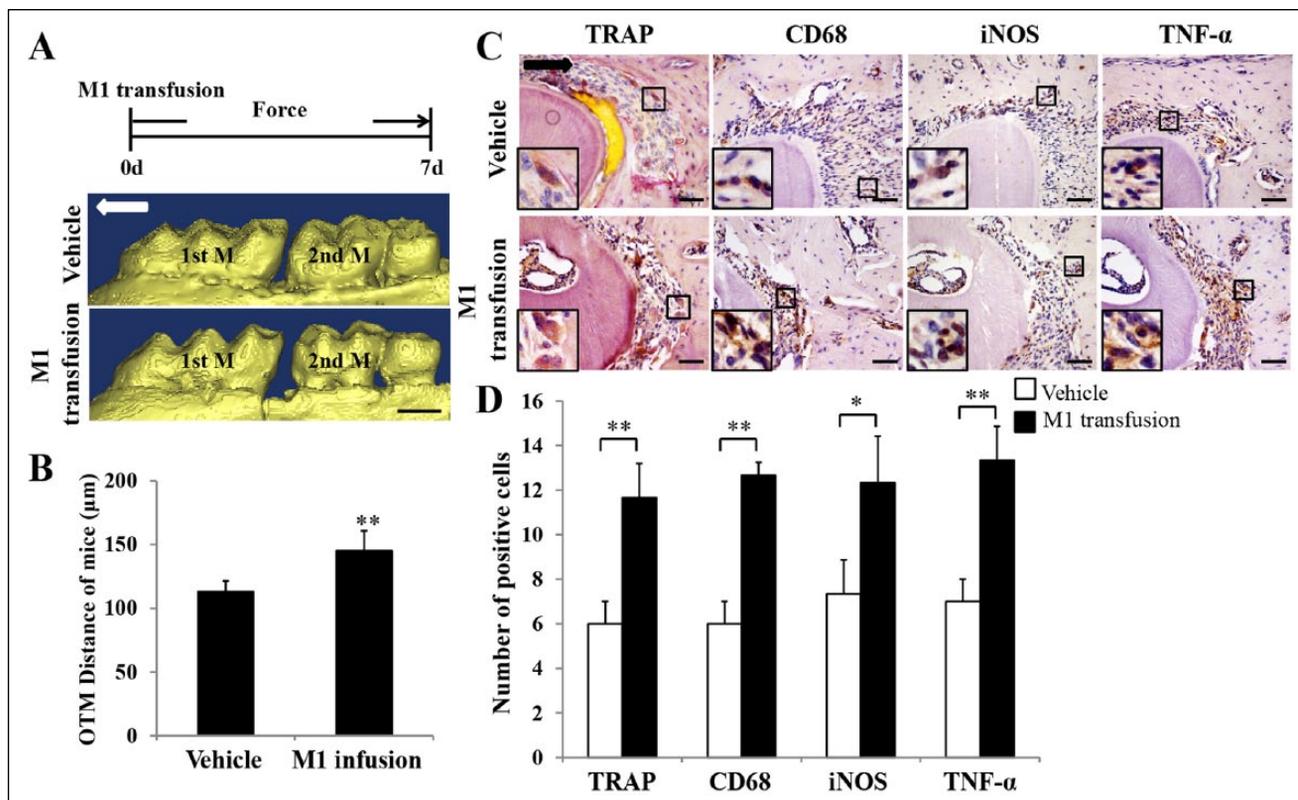
Figure 3. Adoptive transfusion of ex vivo polarized M1 macrophages enhanced orthodontic tooth movement (OTM). (**A**, **B**) Representative micro–computed tomography images and semiquantification analysis of OTM distance in mice. OTM distance significantly increased in the M1 infusion group after 7 d of force application. n = 5 or 6, $^{**}P < 0.01$. 1st M, the first molar; 2nd M, the second molar. Arrow represents the direction of force application. Scale bar: 500 μm. (**C**) Representative tartrate-resistant acid phosphatase (TRAP) staining and immunohistochemical images of the compression side of the distal roots. M1 infusion increased the number of TRAP-positive osteoclasts and CD68^{+} macrophages, as well as the expression of inducible nitric oxide synthase (iNOS) and tumor necrosis factor α (TNF-α). Large boxed areas show high-magnification views of the small boxed areas. Arrow represents the direction of force application. Scale bar: 50 μm. (**D**) The number of TRAP-, CD68-, iNOS-, and TNF-α-positive cells increased after M1 transfusion. $^{*}P < 0.05$, $^{**}P < 0.01$.

studies focused on how local elements affect osteoclastogenesis of osteoclast precursors (Teitelbaum 2000; Tyrovola et al. 2008). However, the source of accumulated macrophages, which are regarded as osteoclast precursors, is largely unknown. The effect of depletion suggested that circulated monocytes could be a source of M1 accumulation during OTM. This concept was consistent with previous studies, which reported that circulated monocytes are sources of infiltrating macrophages in pathologic processes (Ingersoll et al. 2011; Qian et al. 2011).

Systemic transfusion of M1 but not M2 macrophages promotes alveolar bone resorption and OTM. Previous studies have demonstrated that the adoptive transfer of M1 aggravated renal diseases compared with M0 (Ikezumi et al. 2003). Our results of M1 macrophage transfusion were in line with these previous findings. Moreover, no significant change of OTM distance was observed after M2 macrophage transfusion, confirming the unique effect of M1-like macrophages during OTM. Macrophages are plastic to change to different polarizations under different environmental conditions (Sindrilaru et al. 2011; Novak and Koh 2013). During OTM, force-induced

inflammatory microenvironment in periodontal tissues may promote or maintain M1 macrophage polarization, which may reduce the effect of M2 transfusion. How the transfused macrophages contribute to OTM is unclear. A previous study showed that macrophages transferred in vivo could traffic to the inflammatory sites (Wang et al. 2007). Our data showed that EGFP-labeled cells were found in the periodontal tissues on day 7 during OTM, indicating that portions of the transfused macrophages were recruited to the local periodontal tissues and may directly contribute to the OTM. However, only part of the CD68^{+} macrophages colocalized with the signals of EGFP, suggesting that transfused macrophages may also induce a systemic response and promote circulating monocytes/macrophages to accumulate in the local periodontal tissues and to increase bone resorption and OTM. Nevertheless, the relative contributions of the transfused and circulating monocytes/macrophages need to be further explored.

Systemic injection of rTNF-α enhanced alveolar bone resorption, consequent OTM, and the expression of M1 markers, whereas the injection of TNF-α inhibitor suppressed them. TNF-α could maintain the activation state of M1 macrophages
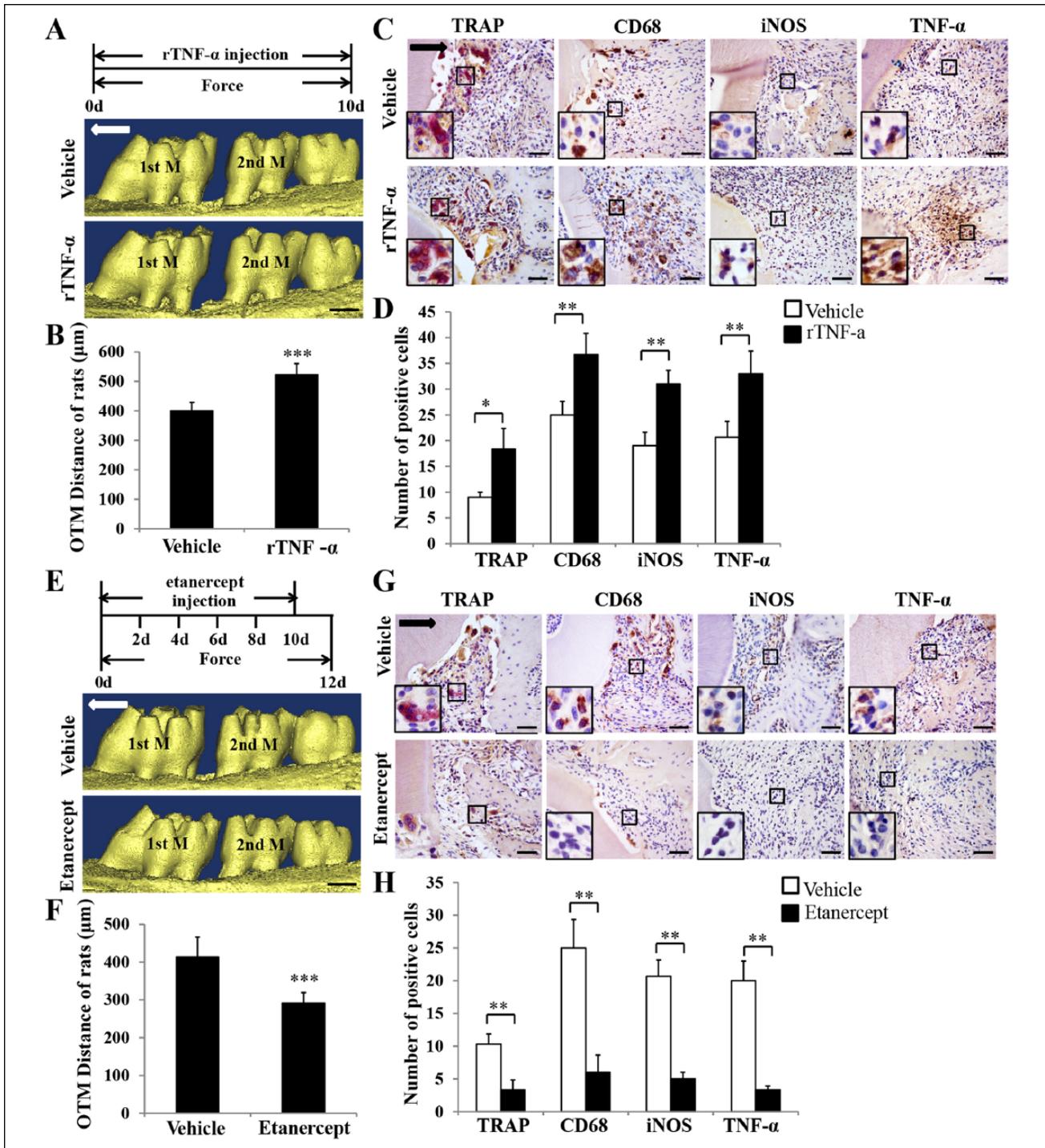
Figure 4. Blocking tumor necrosis factor α (TNF-α) by etanercept injection repressed orthodontic tooth movement (OTM), whereas injection of recombinant TNF-α (rTNF-α) enhanced OTM. (**A, B**) Representative micro–computed tomography images and semiquantification analysis of the OTM distance in rTNF-α-treated rats. OTM distance increased after 10 d of force application. Arrow represents the direction of force application. Scale bar: 1000 μm. $n = 5$ or 6, $^{***}P < 0.001$. (**C**) Representative tartrate-resistant acid phosphatase (TRAP) staining and immunohistochemical images of the compression side of distobuccal roots. Injection of rTNF-α increased the number of TRAP-positive osteoclasts and CD68^{+} macrophages, as well as the expression of inducible nitric oxide synthase (iNOS) and TNF-α. Large boxed areas show high-magnification views of the small boxed areas. Arrow represents the direction of force application. Scale bar: 50 μm. (**D**) The number of TRAP-, CD68-, iNOS-, and TNF-α-positive cells increased after rTNF-α injection. $^{*}P < 0.05$, $^{**}P < 0.01$. (**E, F**) Representative micro–computed tomography images and semiquantification analysis of the OTM distance in rats treated with TNF-α inhibitor etanercept. The OTM distance decreased after 12 d of force application. $n = 5$ or 6, $^{***}P < 0.001$. 1st M, the first molar; 2nd M, the second molar. Arrow represents the direction of force application. Scale bar: 1,000 μm. (**G**) Representative TRAP staining and immunohistochemical images of the compression side of distobuccal roots. Injection of the TNF-α inhibitor decreased the number of TRAP-positive osteoclasts and CD68^{+} macrophages, as well as the expression of iNOS and TNF-α. Arrow represents the direction of force application. Scale bar: 50 μm. (**H**) The number of TRAP-, CD68-, iNOS-, and TNF-α-positive cells decreased after TNF-α inhibitor injection. $^{**}P < 0.01$.

in an autocrine manner (Wang et al. 2006). The present study showed that TNF-α expression was upregulated during OTM. Moreover, enhancement of TNF-α increased the number of M1 macrophages and osteoclasts, accompanied by high expression of M1 markers; conversely, reverse effects were detected when TNF-α was suppressed. These data indicate that TNF-α may induce M1 macrophage activation and promote osteoclast differentiation under force application. Nevertheless, mouse and rat OTM models were chosen on the basis of their advantages in cell infusion and tooth movement distance, respectively. Our results suggest that TNF-α-related M1-like macrophages play an essential role in mechanical force–induced bone resorption and consequent OTM, which may provide insights into novel modalities to accelerate OTM.

Force-treated PDLCs in periodontal tissues may contribute to M1 macrophage polarization during OTM. Previous studies have shown that fibroblasts are important in the inflammatory process and can modulate macrophage polarization (Smith et al. 1997; El Kasmi et al. 2014). Pulmonary adventitial fibroblasts in pulmonary hypertension sustainedly produce proinflammatory cytokines and chemokines (Li et al. 2011). They could also activate a distinct proinflammatory macrophage phenotype (El Kasmi et al. 2014). Periodontal ligament fibroblasts also influence macrophages to express M2 markers under normal and inflammatory conditions (Konermann et al. 2012). Hence, determining the effect of PDLCs on macrophage polarization under mechanical force stimulation is indispensable. PDLCs are one of the most important cellular components in periodontal tissues. With mechanical force stimuli, PDLCs express multiple inflammatory elements (Lee et al. 2012). Previously we found that mechanical force upregulated IFN-γ expression in PDLCs in vivo and in vitro, which may contribute to M1 macrophage activation (He et al. 2015). In this study, we found that mechanical force promoted M1-like macrophage accumulation on the compression side of periodontal tissues. Additionally, the supernatant of force-loaded PDLCs promoted M1 polarization. These data suggest that force-loaded PDLCs may promote M1-like macrophage polarization during OTM. However, whether mechanical force directly influences macrophage polarization is unclear. Further studies are needed to explore the direct effect of mechanical force on macrophage polarization.

It should be mentioned that slight root resorption was also observed during OTM. The process of OTM may be accompanied by orthodontic root resorption. They shared similar histologic changes of hard tissue resorption by osteoclasts/
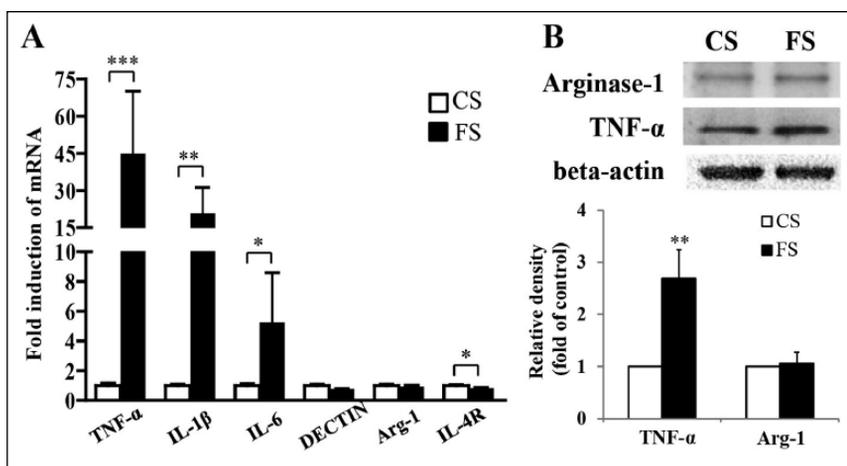

Figure 5. Supernatant from force-treated periodontal ligament cells (PDLCs) enhanced M1 markers of THP-1-induced macrophages. (**A**) Relative mRNA expression of M1/M2-related genes. The mRNA expression of M1 markers tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and IL-6 of THP-1-induced macrophages was upregulated after incubation with the supernatant of force-treated PDLCs for 24 h compared with those incubated with the supernatant of control PDLCs; the mRNA expression of M2 markers DECTIN-1 and arginase-1 did not change, and the expression of IL-4R was downregulated. $n = 3$, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. CS, control supernatant; FS, force-treated supernatant. (**B**) Western blot of TNF-α and arginase-1. TNF-α expression of THP-1-induced macrophages was upregulated after incubation with the supernatant of force-treated PDLCs for 24 h, whereas no changes were observed in arginase-1. Beta-actin served as an internal control for equal loading. Data represent 3 independent experiments.

odontoclasts and remodeling. However, clinically, significant tooth movement at the price of severe periodontal ligament damage and root resorption should be avoided. Further work should be done to illustrate the optimal force to gain substantial and stable tooth movement with less periodontal damage.

In conclusion, the accumulation of M1-like macrophages after mechanical force application promotes OTM. During this process, TNF-α, which is a key signal of M1 macrophage activation, plays an important role. These results elucidate the mechanisms of OTM and may provide some clues for accelerating OTM.

Author Contributions

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

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