Received: 1 May 2020 | Revised: 2 September 2020 | Accepted: 9 September 2020

DOI: 10.1096/fj.202001078R

RESEARCH ARTICLE

THE FASEB JOURNAL

T cells participate in bone remodeling during the rapid palatal expansion

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Funding information
National Science Foundation of China, Grant/Award Number: 81970909 and 51903003; National Natural Science Foundation of China, Grant/Award Number: U1704187; Natural Science Foundation of Beijing, Grant/Award Number: 7192290; Young Scientists Fund, Grant/Award Number: 20110203 and 20170109; Peking University Medicine Seed Fund for Interdisciplinary Research, Grant/Award Number: BMU2018MX007; Peking University Medicine Fund of Fostering Young Scholars' Scientific & Technological Innovation, Grant/Award Number: BMU2018PYB009; Fundamental Research Funds for the Central Universities

Abstract

Palatal expansion has been widely used for the treatment of transverse discrepancy or maxillae hypoplasia, but the biological mechanism of bone formation during this procedure is largely unknown. Osteoclasts, which could be regulated by T cells and other components of the immune system, play a crucial role in force-induced bone remodeling. However, whether T cells participate in the palatal expansion process remains to be determined. In this study, we conducted the tooth borne rapid palatal expansion model on the mouse, and detect whether the helper T cells (Th) and regulatory T cells (Treg) could affect osteoclasts and further bone formation. After bonding open spring palatal expanders for 3-day, 5-day, 7-day, and retention for 28-day, micro-computed tomography scanning, histologic, and immunofluorescence staining were conducted to evaluate how osteoclasts were regulated by T cells during the bone remodeling process. We revealed that the increased osteoclast number was downregulated at the end of the early stage of rapid palatal expansion. Type 1 helper T (Th1) cells and Type 17 helper T (Th17) cells increased initially and promoted osteoclastogenesis. Thereafter, the regulatory T (Treg) cells emerged and maintained a relatively high level at the late stage of the experiment to downregulate the osteoclast number by inhibiting Th1 and Th17 cells, which governed the new bone formation. In conclusion, orchestrated T cells are able to regulate osteoclasts at the early stage of rapid palatal expansion and further facilitate bone formation during retention. This study identifies that T cells participate in the palatal expansion procedure by

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; IF staining, immunofluorescence staining; IFN-γ, interferon-γ; IL-17, interleukin-17; micro-CT, micro-computed tomography; MSCs, mesenchymal stem cells; OTM, orthodontic tooth movement; PDL, periodontal ligament; RANKL, receptor activator of NF-kB ligand; Runx2, runt-related transcription factor 2; Th cells, helper T cells; Treg cells, regulatory T cells; Th1 cells, Type 1 helper T cells; Th17 cells, Type 17 helper T cells; TNF-α, tumor necrosis factor-α; TRAP staining, tartrate-resistant acid phosphatase staining.

Jing Li and Ting-Ting Yu contributed equally to this work.

regulating osteoclasts and implies the potential possibility for clinically modulating T cells to improve the palatal expansion efficacy.

KEYWORDS

bone formation, immunity, orthodontics, osteoclasts

1 | INTRODUCTION

Palatal expansion has been widely used for the transverse discrepancy or maxillae hypoplasia, which is the main approach to stimulate bone regeneration around the suture area during orthodontics treatment.[1-3] Palatal expansion is based on mechanical force-induced bone remodeling,[4,5] but its underlying biological mechanisms remains largely unknown. It has been reported that osteoclasts played a crucial role during orthodontic tooth movement (OTM) and palatal expansion process.[6,7] Osteoclasts can be regulated by various chemokines, cytokines, and inorganic molecules. Recent studies showed macrophage polarization could affect osteoclast activities in OTM,[8-13] and our previous study has proven that helper T (Th) cells can modulate osteoclasts and promote orthodontic tooth movement.[14,15] However, whether T cells are involved in palatal expansion-induced bone remodeling is still unknown.

T cells exert cellular immunity in the adaptive immune response, which plays important roles in local inflammatory reaction and host defense. Among the subsets of T cells, the reciprocal modulating effects between Th cells and regulatory T (Treg) cells are important in inflammatory reaction. Th cells can promote inflammation reaction by producing various cytokines, such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukins.[16] Besides, Th cells can promote osteoclastogenesis and lead to bone resorption in various diseases, such as arthritis and periodontitis.[17-19] Conversely, Treg cells are able to suppress inflammatory reaction, as they can selectively suppress the Th1 and Th17 cells as well as T cell related cytokines.[20,21] For bone remodeling, Treg cells can inhibit inflammatory-induced bone loss, and alleviate disease symptoms.[22-24]

In this study, we established a mouse palatal expansion model to explore how T cells regulate osteoclasts during the early stage of palatal expansion and found that osteoclastogenesis which was promoted by Th1 and Th17 cells was suppressed by Treg cells. This process ensured bone formation after retention.

2 | MATERIALS AND METHODS

2.1 | Animals

Six-week-old male C57/BL6 mice were used in this study, and they were bought from Vital River Laboratories (Beijing, China). We obtained approval from Institutional of Animal Care and Use Committee of Peking University before our study (LA2018251). We provided powder food and water for the mice, and housed them in the temperature of 22°C ± 1℃.

2.2 | Palatal expansion

An open spring made of a 0.012-inch nickel-titanium arch wire was used to provide orthopedic force for palatal expansion of mice (length 6 mm, wide 2 mm; Smart Technology, Beijing, China). The open spring was bonded between the upper incisors of mice by the flowable composite resin (3M ESPE, St. Paul, MN, USA) and could provide about 100-gram (g) expansion force. The control group was not applied force. 20 mice were randomly divided into the 3-day expansion group, the 5-day expansion group, the 7-day expansion group and the retention group (N = 5 per group). The retention group was applied 7-day expansion force, and after expansion, the open spring was removed immediately and the incisors of mice were bonded together for 28-day retention. After sacrificed the mice, we fixed the maxillae in 10% of phosphate-buffered formalin for 12 hours.

2.3 | Micro-computed tomography (Micro-CT) scanning

After fixed, we scanned the maxillae by Micro-CT (Inveon MMCT, Siemens, USA) for detecting the width change of the palatal suture and bone remodeling. The scanned image data were dealt, and then, the two/three-dimensional (2D/3D) models were reconstructed, and the changes of maxillary incisors, bone, and suture were measured. The middle part of the suture between incisors was measured for calculating the width changes by Image J (National Institutes of Health, Bethesda, MD, USA). The bone formation area and the angle between incisor and bone was measured by Image J (National Institutes of Health, Bethesda, MD, USA).

2.4 | Histology and tartrate-resistant acid phosphatase (TRAP) staining

We demineralize the samples for 7 days in 15% (w/v) of ethylenediaminetetraacetic acid (EDTA), and then, tissue

dehydration, fixed by paraffin. The samples were cut for histological sections with a 5-μm thickness in the direction of the axis of incisors. We applied hematoxylin eosin (HE) staining for the selected sections. The leukocyte acid phosphatase kit (387A, Sigma) was used for TRAP staining of these sections. The number and distribution of TRAP positive cells were observed and calculated by microscope (Eclipse 80i, Nikon, Tokyo, Japan) and the pictures were taken by a software (NIS-Elements D 4.00.03). The HE staining retention samples were observed by microscope (Eclipse 80i, Nikon, Tokyo, Japan) and the width of suture and bone formation area was measured by Image J (National Institutes of Health, Bethesda, MD, USA).

2.5 | Immunofluorescence (IF) staining

Selected sections were applied immunofluorescence staining. For antigen retrieval, they were treated by 0.125% of trypsin and Proteinase K (20 μg/mL) for 30 minutes. Second, 5% of bovine serum albumin was used to block these sections for 60 minutes. And then, they were incubated with antibodies of CD4 (1:100; 562891, BD bioscience, NJ, USA), IFN-γ (1:100; sc-57207, Santa Cruz, TX, USA), TNF-α (1:100; ab1793, Abcam, Cambridge, UK), IL-17 (1:100; ab79056, Abcam, Cambridge, UK), CD25 (1:100, NB600-564SS, Novus Biologicals, MN, USA), Foxp3 (1:100, MAB8214, Novus Biologicals, MN, USA), and Runx2 (1:100, sc-101145, Santa Cruz, TX, USA) at the temperature of 4°C overnight for 12 hours. At the second day, we washed the sections by phosphate-buffered saline (PBS) and then incubated them with secondary antibody for 1 hour (ZF-0311, ZF-0312, Zhongshan Golden Bridge Biotechnology, Beijing, China; A32733, A18750, Thermo Fisher Scientific, Waltham, MA, USA). The mounting medium with 4, 6-diamidino-2-phenylindole (DAPI; Zhongshan Golden Bridge Biotechnology, Beijing, China) was used to seal the sections. Then, we observed the sections by laser scanning confocal microscopy (LSM 510; Zeiss, Germany), and images were captured by software (LSM 5 Release 4.2 software).

2.6 | Statistical analysis

We performed the statistical analysis by SPSS 20.0 (SPSS Inc, Chicago, IL, USA). Compared the difference between the control group and the experimental group, when the statistics were normal distribution we used unpaired Student's *t* test; if not, we used Mann-Whitney Rank sum test. The one-way analysis of variance (one-way ANOVA) was used for comparing the difference among the groups. $P < .05$ was statistically significant.

3 | RESULTS

3.1 | The maxillary suture was expanded after force application

We established a tooth borne rapid palatal expansion model on the mouse by bonding an open spring between the upper incisors. After 7-day expansion, the palate was expanded along with the central incisors moved buccally (Figure 1A). We used micro-CT to evaluate the changes on the expanded suture site. The horizontal section of micro-CT showed the width of suture increased significantly from 0.06 to 0.24 mm after 7-day palatal expansion ($P < .001$, Figure 1B,C). The coronal reconstruction images also confirmed the suture width was largely increased along with the expansion time (Figure S1A). Interestingly, the shape of the bone formation area adjacent to the suture site on the coronal section was triangle-like. The average bone formation area was increased significantly after palatal expansion (from 0.12 mm^2 to 0.15 mm^2, $P < .001$, Figure 1D). Since this model established by the tooth borne palatal expansion, we wanted to check the relationship between incisors and maxillae. We found the lateral edge of the bone next to suture and mesial edge of homolateral incisor composed an acute angle, and average angle of bilateral sides was increased from 1.35° to 8.10° after 5-day expansion, and then, decreased from 8.10° to 6.23° at 7 days after expansion. (Figure S1B). These data indicated the palate suture could be opened and expanded efficiently on this tooth borne rapid palatal expansion model.

3.2 | The increased osteoclast number was downregulated at the end of the early stage of rapid palatal expansion

To further explore the histological changes during the early stage of palatal expansion, hematoxylin eosin (HE) staining was used to analyze (Figure 2A). After force application, the maxillary suture was expanded gradually, and the suture width was increased significantly between control and 7-day group (from 0.05 to 0.21 mm, $P < .001$, Figure 2B). Moreover, the morphological changes of maxillae were obvious along with palatal expansion. The periodontal ligament (PDL) was distracted and became longer, and the marrow cavity was enlarged and the edge of the bone became uneven during 7-day palatal expansion (Figure 2A). Similarly, the bone formation area next to the suture was triangle-like in HE staining, and the average bone formation area showed an increasing trend (from 0.05 to 0.10 mm^2, $P < .01$, Figure S2A,B). These HE staining results were consistent with the changes observed in micro-CT scanning.

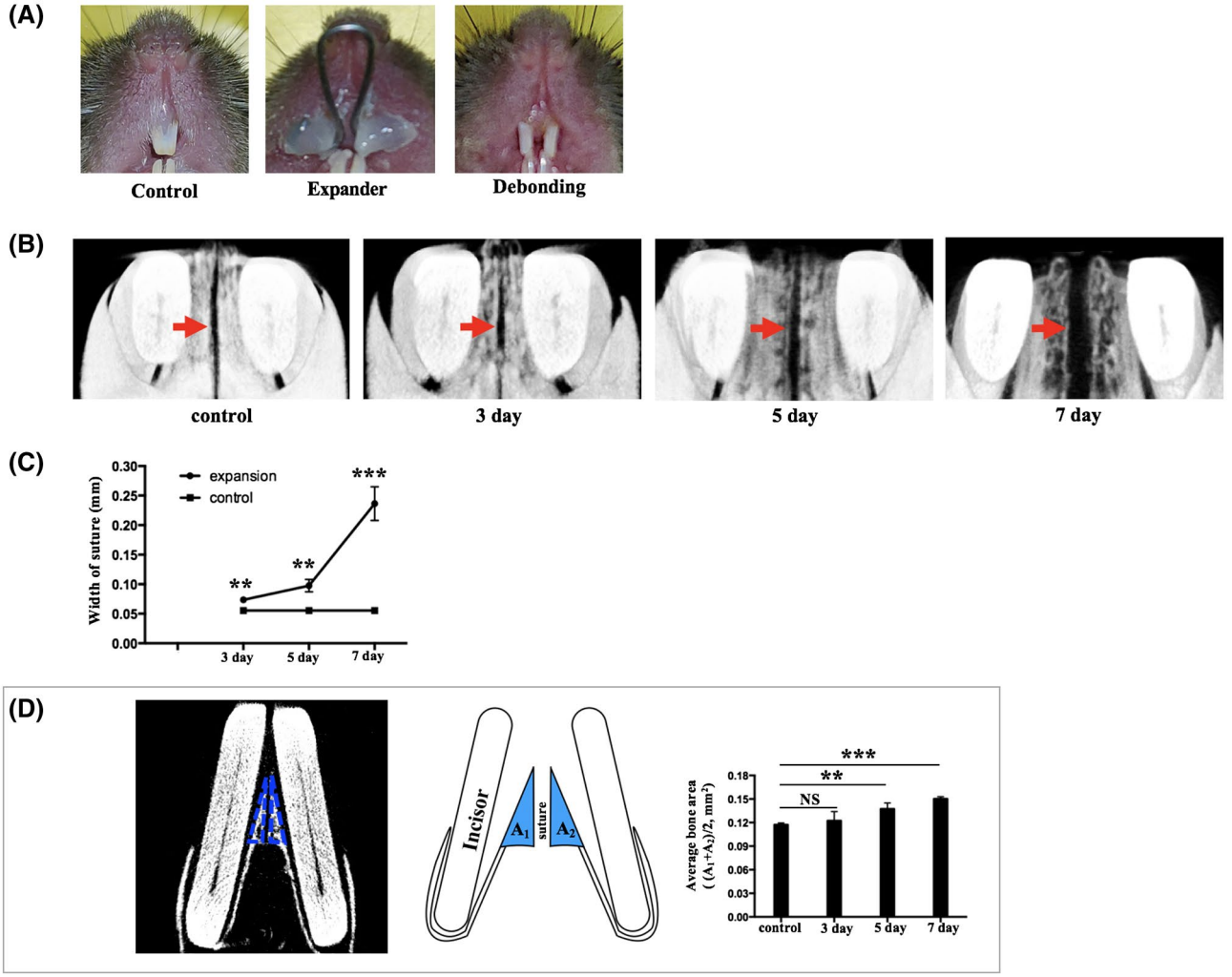

FIGURE 1 The maxillary sutures of mouse were expanded after force application. A, The early stage of rapid palatal expansion model was established, and the palate was expanded along with the central incisors moved buccally. B, The horizontal reconstruction images of micro-CT. The suture was expanded over time, and maxillae moved buccally. The red arrow indicated the maxillary suture. C, The width of suture increased after 7-day force application. D, The blue triangle showed bone formation area in coronal reconstruction picture. The bone formation area increased over time. A_1: left part of bone formation area; A_2: right part of bone formation area. Error bars, means ± SD; NS, not significant; N = 3-5 per group; $^{**}P < .01$; $^{***}P < .001$

Osteoclasts play important roles in the orthodontic bone reconstructions, and it has been proven that the osteoclasts were activated during palatal expansion and orthodontic tooth movement.[6,7] Therefore, to confirm the effect of mechanical force-induced osteoclastogenesis during palatal expansion, we conducted tartrate-resistant acid phosphatase (TRAP) staining to analyze the osteoclast activity. The TRAP positive cells appeared on day 3 after force application, then, the number peaked on day 5 and downregulated on day 7 (Figure 2C,D). The TRAP positive cells distributed along with the surface of the marrow cavity and the lateral edge of the bone, no similar feature was observed in control group. These results indicated that the mechanical expansion force could induce the bone remodeling by promoting osteoclastogenesis.

3.3 | Th1 and Th17 cells promote osteoclastogenesis during rapid palatal expansion

Osteoclastogenesis can be regulated by T cells and its secreted pro-inflammatory cytokines, such as TNF-α, IFN-γ, and IL-17.[25-27] It has been reported that mechanical force-induced osteoclastogenesis can be promoted by TNF-α during orthodontic bone remodeling.[9,14] Therefore, to confirm if T cells and T cell-associated cytokines are related to the osteoclasts variation during the early stage of palatal expansion, immunofluorescence staining (IF) was conducted.

The results showed that Th1 cells-derived IFN-γ was found in PDL and marrow cavity after force application. During the early stage of palatal expansion, the number of

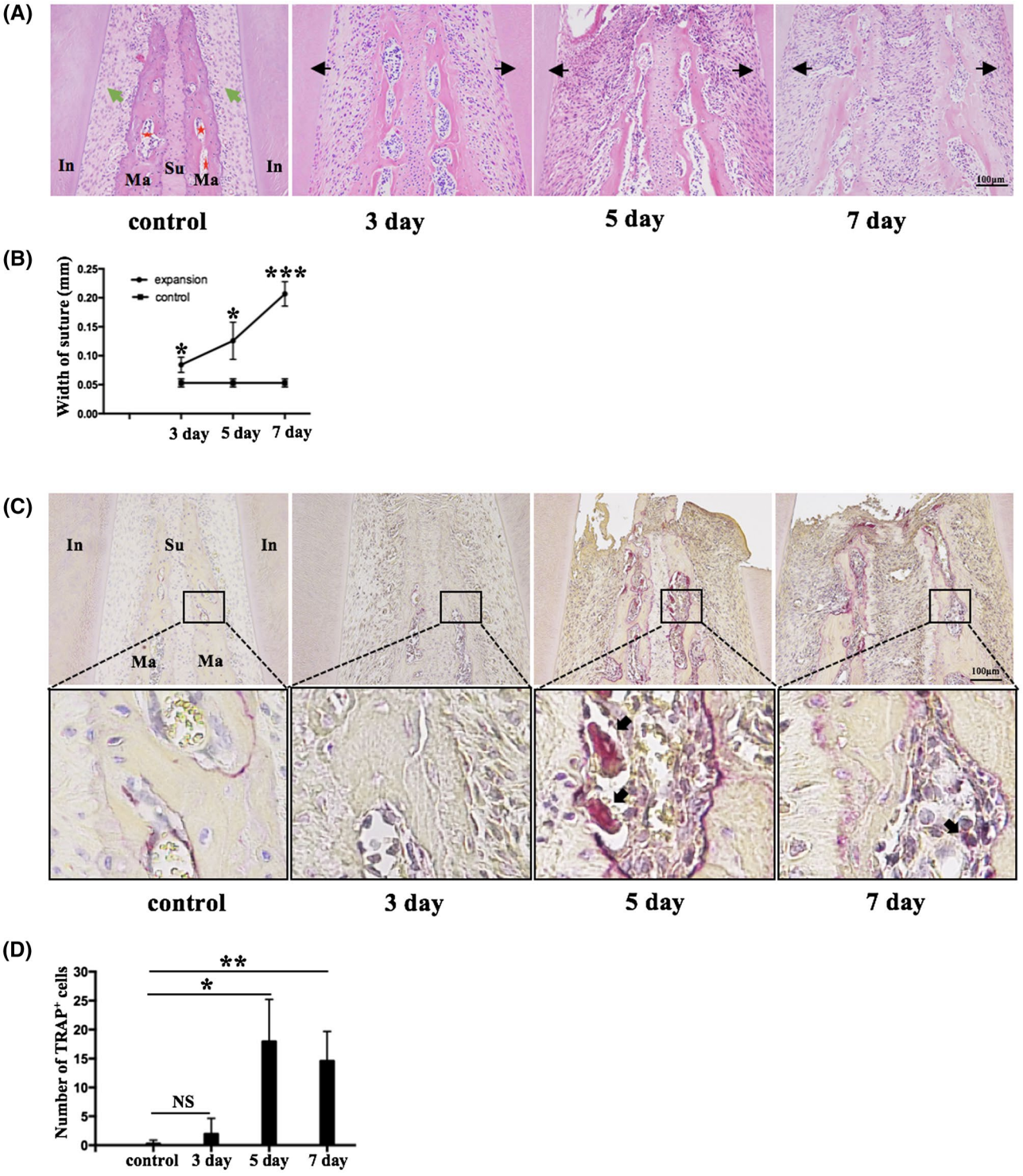

FIGURE 2 The number of osteoclast increased first and then decreased during the early stage of rapid palatal expansion. A, HE staining of the mouse maxillae and suture between incisors. Black arrow: the direction of expansion force; Blue arrow: the periodontal ligament; Red star: the marrow cavity. B, Statistical analysis of the width of suture measured from HE staining images. C, The tartrate-resistant acid phosphatase (TRAP) staining of the maxillae and suture. The $TRAP^+$ cells appeared after force application on day 3, and then, increased on day 5, and finally, decreased on day 7. The representative parts were marked by black rectangular frame and the high-magnification views of the small black rectangular frame area was showed. Black arrow: $TRAP^+$ cell. D, Semiquantification of $TRAP^+$ cells. In = Incisor, Ma = Maxillae, Su = Suture. Error bars, means ± SD; NS, not significant; N = 3 per group; $*P < .05$; $**P < .01$; $***P < .001$; Scale bars: 100 μm

IFN-γ$^+$CD4$^+$ T cells increased from day 3 to day 5 over time, and then, decreased on day 7 (Figure 3A,B), the variation of TNF-α$^+$CD4$^+$ T cells showed the same trend as well (Figure S3A,B). These results were consistent with the number of osteoclasts detected during the early stage of palatal expansion, which indicated that Th1 cells, as well as its secreted IFN-γ and TNF-α, are able to promote osteoclastogenesis induced by expansion force.

It was reported that the pro-inflammatory cytokine interleukin 17 (IL-17), derived from Th17, capable of promoting osteoclastogenesis.[19] Thus, to get further understanding of osteoclastogenesis during the expansion process, we detected

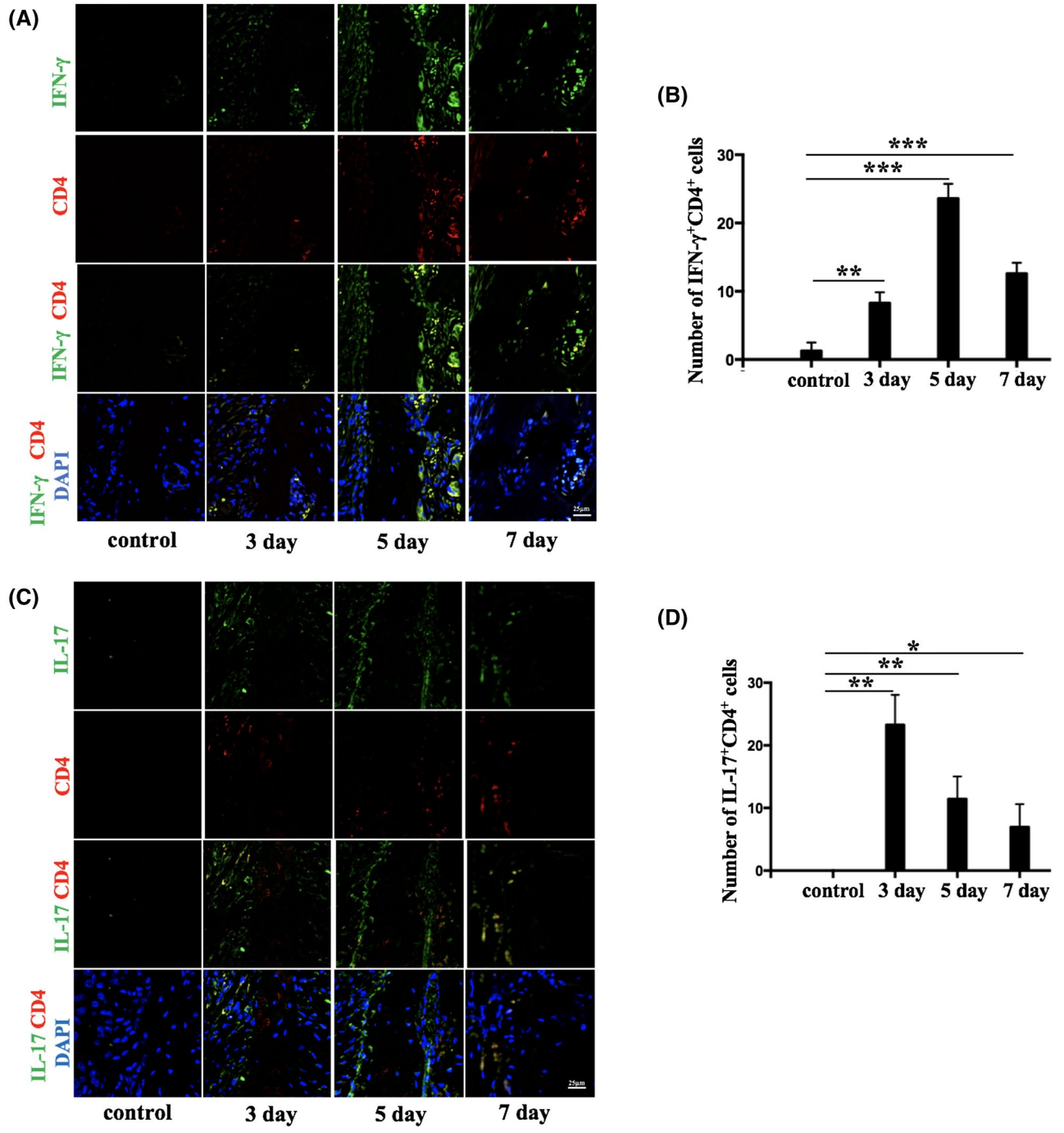

FIGURE 3 Changes of the T cells and the expression of T cell-associated cytokines after force application. A, Immunofluorescence staining of IFN-γ$^+$CD4$^+$ Th1 cells. Th1 cells appeared in the maxillary bone marrow and the periodontal ligament fibers after force application. The expression of IFN-γ (green) increased continuously after force application on day 3 and day 5, and decreased on day 7. B, Statistical analysis of Th1 cells. C, Immunofluorescence staining of IL-17$^+$CD4$^+$ Th17 cells. The day 3 group presented the highest number of IL-17$^+$CD4$^+$ Th17 cells, and these cells decreased continuously on day 5 and day 7. D, Statistical analysis of Th17 cells. Error bars, means ± SD; N = 3 per group. $^*P < .05$; $^{**}P < .01$; $^{***}P < .001$; Scale bars: 25 μm

the variation of IL-17 in our mouse palatal expansion model. The IF result showed that IL-17 was upregulated after 3 days force application (Figure 3C), and decreased at day 5 and day 7 over time. IL-17^{+}CD4^{+} Th17 cells showed the same variation trend as IL-17 during the early stage of rapid palatal expansion (Figure 3C,D).

These results demonstrated that Th1 and Th17 were involved in the early stage of rapid palatal expansion. The osteoclastogenesis was modulated by T cell-associated cytokines, including IFN-γ, TNF-α, and IL-17, which lead to the expansion force-induced bone resorption.

3.4 | Treg cells suppress osteoclastogenesis through inhibiting Th1 and Th17 cells at the end of early stage of rapid palatal expansion

Next, as it has been reported that CD4^{+}CD25^{+}Foxp3^{+} T cells (Treg cells) can specifically suppress pro-inflammatory Th1 and Th17 cells, and reduced the concentrations of T cell-associated cytokines as well.[20,21] To explore the underlying mechanism of how osteoclasts been modulated and the interplay between Th1/Th17/Treg cells after palatal expansion, CD4^{+}CD25^{+} Foxp3^{+} T cells were detected.

The IF staining showed that little Treg cell was found when there has no force applied in the control group. In the experimental group, after expansion for 3 days, Treg cells appeared and increased continuously on day 5. As time passed, the number of Treg cells maintained a high level until day 7, no significant difference was found between the group of day 5 and day 7 statistically ($P > .05$, Figure 4A,B).

These data suggested a correlation among the change of Th1, Th17, and Treg cells after palatal expansion. The increment of Treg cells inhibited the function of Th1 and Th17 cells, as well as the T cell-associated cytokines during the rapid palatal expansion process, especially at the end of the early stage of expansion (day 7), which lead to the decrease of osteoclastogenesis.

3.5 | Palatal expansion facilitates new bone formation in the suture area

Since our results showed that Treg cells were able to inhibit the elevation of osteoclasts number by suppressing the

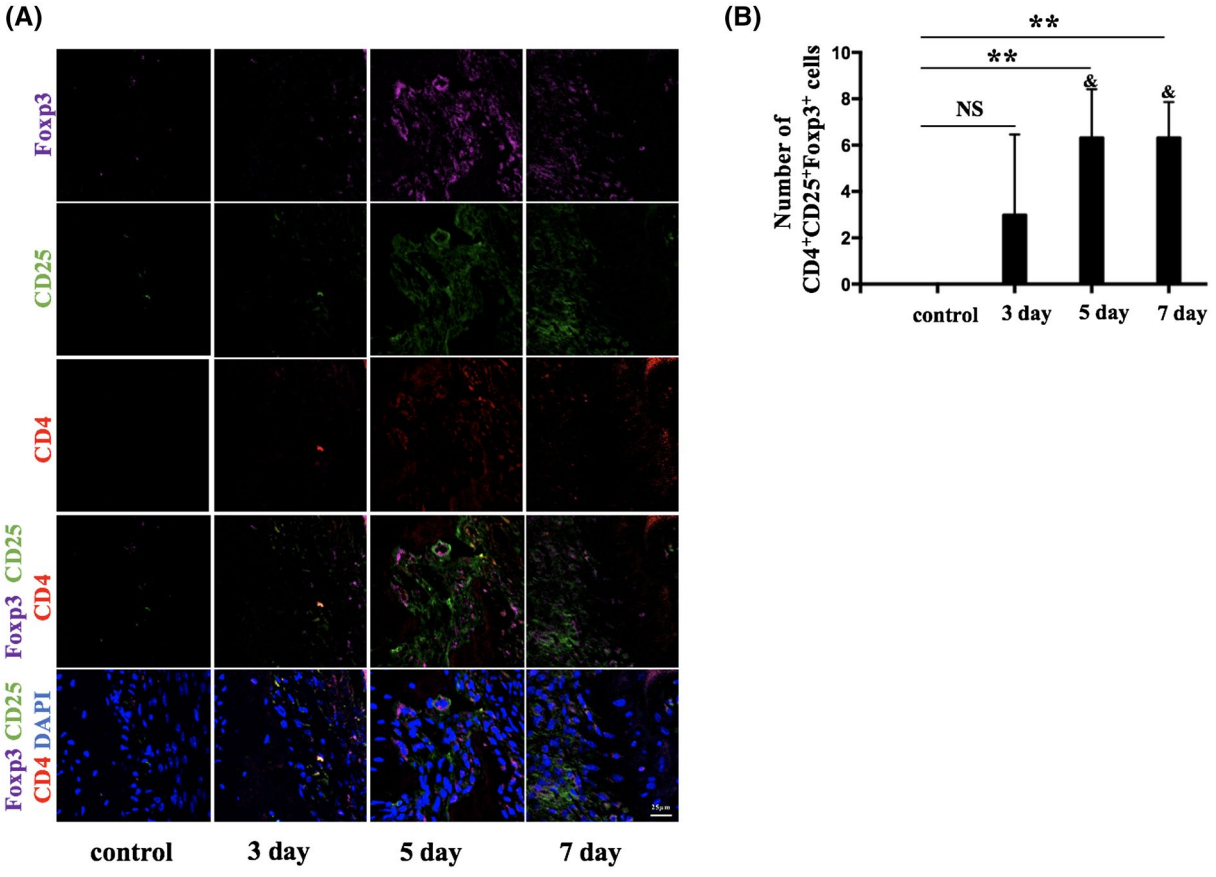

FIGURE 4 Changes of the regulatory T (Treg) cells during palatal expansion. A, Immunofluorescence staining of CD4^{+}CD25^{+}Foxp3^{+} Treg cells. Treg cells appeared on day 3, and increased on day 5 and slightly decreased on day 7. B, Semiquantification of CD4^{+}CD25^{+}Foxp3^{+} Treg cells. Error bars, means ± SD; NS, not significant; N = 3 per group. $^{**}P < .01$; $^{\&}P > .05$. Scale bars: 25 μm

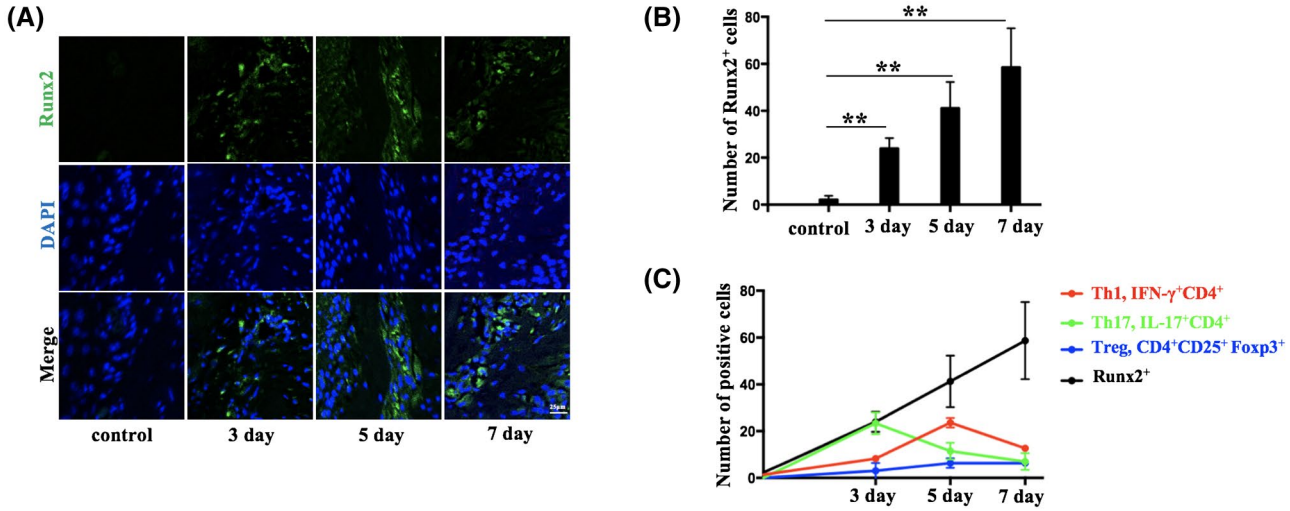

FIGURE 5 The new bone formed after retention. A, Representative immunofluorescence staining pictures of Runx2 (green). The Runx2 appeared after force application and distributed at PDL and marrow cavity. And the expression of Runx2 increased continuously during the early stage of rapid palatal expansion procedure. B, Statistical analysis of $Runx2^+$ cells. C, Statistical analysis of Th1, Th17, Treg, and $Runx2^+$ cells. Error bars, means ± SD; N = 3 per group; $^{**}P < .01$

function of Th1 and Th17 cells, we then evaluated the volume of new bone formation around the suture area during palatal expansion periods. To observe the new bone formation in our palatal expansion model, we detected the expression of Runx2 which is highly expressed in immature osteoblasts [28,29] first. The IF staining showed that the expression level of Runx2 elevated continuously after expansion (Figure 5A-C). These results indicated that with the increment of Runx2 positive cells, the osteogenesis around the suture area became promoted along with the expansion time.

To investigate if there has new bone formation after palatal expansion, we established the 28-day retention model on mouse by bonding upper incisors together with resin right after the 7-day expansion procedure. Observed by maxillae micro-CT scanning, the reconstruction images showed the volume of new bone formation increased significantly after 28-day retention when compared with 7-day expansion group (from 0.15 to 0.35 mm^2, $P < .01$, Figure S4A,B). Moreover, we used HE staining to analyze the histological changes of suture area after retention. Figure S4C showed that massive newly formed bone was stuck out to the suture in the retention group, which is consistent with the micro-CT scanning results. Moreover, the marrow cavity area decreased either. These data demonstrated that the osteogenesis around the suture area became continuously promoted with the time passed during expansion period. Retention helped to facilitate the osteogenesis process and result in massive bone formation.

4 | DISCUSSION

The variation and activation of osteoclasts contribute to the force-induced bone remodeling, during the process of orthodontics tooth movement and distraction osteogenesis.[6,7] It was reported that the number of osteoclasts increased significantly right after the force application and was mainly located at compression sides, their activation was able to induce bone resorption.[30] In our study, we established the teeth born rapid palatal expansion model on the mouse and made the procedure easily achievable. Along with the palatal expansion, the incisors showed buccally tipping movement, which stretched the local bone formation gradually. After expansion force application, osteoclasts were found on the outer margins and marrow cavity of new bone formation area, which indicated that these osteoclasts might participate in palatal expansion bone remodeling. As previous studies reported that the number of osteoclasts tended to increase initially, and then, decrease at day 7 during the OTM, which is consistent with our findings on palatal expansion animal model.[7,30]

The close relationship between the immune and bone systems has long been established since the 1970s.[31] It is now well studied that a number of immune cytokines are able to interact with bone cell activities and bone mass. Osteoclastogenesis can be modulated by RANKL signaling,[32] macrophage polarization,[8-10] and T cells activation.[16] It has been reported that the T cells are capable of modulating osteoclastogenesis[16] and participating in orthodontic bone remodeling.[14] Herein, we identified whether T cells, as well as their subsets, participate in modulating osteoclastogenesis induced by palatal expansion force, which have not been illustrated by previous studies. Our findings showed that local immune response was associated with expansion force-induced palatal expansion, helper T cells ($CD4^+$ T cells) was found accumulated in the maxillary bone marrow right after the force application, and their number changed along with the expansion periods. Moreover, $CD4^+$ T cells

were also found in the PDL tissue which is similar to that in OTM.[14]

During the activation and expansion of helper T cells ($CD4^+$ T cells), subsets of helper T (Th) cell can secrete specific types of cytokines and mediate distinct functions. Th1 cells were identified by secreting IFN-γ and TNF-α, which can directly or indirectly induce the differentiation of multinucleated osteoclasts from osteoclasts precursors. However, as a type of T cells-associated cytokines, IFN-γ has biphasic effects in the procedure of osteoclastogenesis. Some studies reported IFN-γ could inhibit osteoclastogenesis directly via suppressing RANKL signaling pathway,[26] but some other researchers also found IFN-γ played an important role on osteoclastogenesis under inflammation or T cell over-activated circumstances via RANKL-independent pathways.[33,34] As for TNF-α, which can be largely secreted by Th1 cells, it can directly or indirectly induce the differentiation of multinucleated osteoclasts from osteoclasts precursors.[25,27] The Th17 cytokine IL-17 is also considered as a strong stimulator of osteoclastogenesis via upregulating the RANK receptor on osteoclast precursors.[35] It has been reported that IL-17 is able to promote the expression of other osteoclastogenic cytokines including TNF-α.[36] In our study, we found that the variation of IFN-γ^+CD4^+ T cells, TNF-α^+CD4^+ T cells, and IL-17^+CD4^+ T cells were consistent with the trend of osteoclasts detected during the early stage of palatal expansion, which indicated that Th1 and Th17, as well as their secreted functional cytokines, including IFN-γ, TNF-α, and IL-17 are able to promote osteoclastogenesis induced by expansion force applied in the maxillary suture area. Interestingly, we also found that $CD4^+CD25^+Foxp3^+$ T cells (Treg cells) appeared after force application, and maintained a relatively high level at the end of the early stage (day 5-7) during the palatal expansion. Treg cells could selectively suppress the function of Th1 and Th17 cells through the reduction of TNF-α, IFN-γ, and IL-17 concentrations.[20,21] Based on our findings, we suggested that mechanical force induced an increasing trend of Th1, Th17, and their secreted cytokines initially, which caused the promotion of osteoclastogenesis. At the end of the early stage of palatal expansion, the function of Th1 and Th17 cells were inhibited by Treg, resulting in a decrease of osteoclastogenesis and promotion of new bone formation in the palatal suture area.

Palatal expansion is an effective method for treating palatal transverse discrepancy and induces bone formation in clinics. Continuous expansion force is effective for suture expansion and heavy force is benefit for bone formation in suture area.[37,38] After force application, the activation of osteoclasts and osteoblasts induce bone remodeling collectively. The mechanical force stimulates the activation of osteoblasts and contributes to bone formation; during this process, osteoclasts are also activated, and induce bone resorption. Different types of mechanical force is able to promote or inhibit osteoclast formation. Compressive mechanical force can accelerate osteoclastogenesis,[39] while cyclic stretch force and vibration force can inhibit osteoclastogenesis in vitro.[40,41] In vivo, orthodontic force mainly induces osteoclastogenesis at compression side of the bone.[30] Treg cells are able to inhibit osteoclastogenesis and reduce inflammatory bone loss in rodent models.[22,24] In our study, the increasing osteoclasts are inhibited by Treg cells which ensured the bone formation during and after the palatal expansion. Except for T cells, mesenchymal stem cells (MSCs) may also be functional involved during the bone formation process. Craniofacial sutures provide special microenvironment for MSCs, and they are important for bone homeostasis.[42,43] Moreover, mechanical force can stimulate the suture MSCs' osteogenic differentiation in rat.[44] Thus, the underlying mechanisms and the interaction between different types of cells involve in new bone formation around palatal suture area still need further exploration.

In this study, we clarified that $CD4^+$ T cells are able to regulate osteoclastogenesis during the early stage of rapid palatal expansion procedure. Treg cells facilitate the new bone formation in the palatal suture area through inhibiting the function of Th1 and Th17. Our study illustrated the interaction between immune cells and force-induced bone formation in palatal expansion model. These findings will promote our current understanding of rapid palatal expansion procedure. For further understanding the underlying mechanisms on palatal expansion, more mechanistic study should be done by using immunodeficiency mice model, palatal suture cell culture, and array analysis to detect the molecular regulation mechanism between T cells and osteoclasts.

ACKNOWLEDGMENTS

This work was supported by National Science Foundation of China 81970909 to DL, and 51903003 to TY; Joint Funds of the National Natural Science Foundation of China (Grant No. U1704187) to YQ; Natural Science Foundation of Beijing Municipality 7192290 to QL, Young Scientists Fund of PKUSS (No. 20110203, No. 20170109) to DL; the Peking University Medicine Seed Fund for Interdisciplinary Research (BMU2018MX007) to DL; and the Peking University Medicine Fund of Fostering Young Scholars' Scientific & Technological Innovation (BMU2018PYB009) to TY, which was supported by the "Fundamental Research Funds for the Central Universities."

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

D. Liu, Y. Zhou, J. Li, and T. Yu designed research; J. Li performed research; D. Liu, J. Li, T. Yu, H. Yan, Y. Qiao, and L. Wang analyzed data; D. Liu, Y. Zhou, J. Li, T. Yu, T. Zhang, and Q. Li interpreted data; J. Li, D. Liu, and T. Yu wrote manuscript. All authors critically revised the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

How to cite this article: Li J, Yu T-T, Yan H-C, et al. T cells participate in bone remodeling during the rapid palatal expansion. *The FASEB Journal*. 2020;34:15327–15337. https://doi.org/10.1096/fj.202001078R