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ORIGINAL ARTICLE

The Changes of Th17 Cytokines Expression and Its Correlation with Receptor Activator of Nuclear Factor Kappa B Ligand During Orthodontic Tooth Movement

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ABSTRACT

Background: IL-17 is reported to be associated with the pathophysiology of Orthodontic Tooth Movement (OTM) by affecting osteoclastogenesis. **Objective:** To explore the changes of Th17 cytokines (IL-17, IL-23 and IL-27) expression and its correlation with receptor activator of nuclear factor kappa B ligand during orthodontic tooth movement. **Methods:** Thirty patients who needed extraction of the first premolar during orthodontic treatment were included. The gingival crevicular fluid was sampled at the day of application (T0), one hour (T1), 24 hours (T2), one week (T3), 4 weeks (T4) and 12 weeks (T5) after the application of orthodontic force. The expression of Th17 cytokines and receptor activator of nuclear factor kappa B ligand (RANKL) as well as their correlations were measured by using enzyme-linked immunosorbent assay. **Results:** The levels of IL-17A, IL-17F, IL-23 and IL-27 at both tension and pressure side of study teeth at T2-T4 were significantly higher comparing with that of T0 and T1. Moreover, the expression of IL-27 at both tension and pressure sides of study teeth at T2-T4 were significantly lower comparing with that of T0 and T1. At T5, IL-17A, IL-17F, IL-23 and IL-27 returned to baseline level. For the control group, the cytokines were not significantly different at various time points. The expression of IL-17A, IL-17F and IL-23 were positively correlated with RANKL expression at T2-T4, whereas the IL-27 was negatively correlated with RANKL expression at T2-T4. **Conclusion:** This study proved preliminary evidence that Th17 inflammation may be involved in the regulation of OTM.

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Keywords: Gingival Crevicular Fluid, Orthodontic Tooth Movement, Th17 Cytokines

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INTRODUCTION

Orthodontic Tooth Movement (OTM) is featured by the rebuilding of Periodontal Ligament (PDL) and alveolar bone (1). Cytokines and hormones are produced during OTM and are found in Gingival Crevicular Fluid (GCF) (2). The determination of GCF's components is a method for determining markers during OTM (3). Many studies have shown that orthodontic appliances produced mechanical loading induced the expression signaling molecules in GCF (4-6). It has been reported IL-17 which is mainly produced by Th17 cells, induces osteoclastogenesis directly from monocytes (7). Besides, IL-17 promotes the production of receptor activator of nuclear factor kappa B ligand (RANKL) production from osteoblasts and involved in bone absorption in periodontitis (8-10). Besides, the previous study found that compressive force promotes the up-regulation of the IL-17 and corresponding receptors by MC3T3-E1 cells, followed by the induction of osteoclastogenesis (8). In current study, the levels of Th17 cytokines and RANKL and analyzed their relationship in GCF levels during OTM were detected.

MATERIALS AND METHODS

Patients. Thirty patients (12-20 years old, 7 males) who required extraction of the first premolar during orthodontic treatment were included. Seven days before the application of orthodontic force, the patients were asked to rinse with 0.12% chlorhexidine gluconate. All subjects underwent extraction of maxillary first premolars and distalization of maxillary canine teeth through fixed orthodontic treatment; thus, the canine tooth was studied. The contralateral canine tooth that did not undergo orthodontic treatment was selected as control. The orthodontic force was applied according to method described by Iwasaki using a force of 150 g (11). Patients with autoimmune diseases, type 1 or type 2 diabetes, osteoporosis, systemic antibiotics or anti-inflammatory or hormone drugs in the first six months of the study were excluded. The study was approved by Guangzhou medical university ethical committee (No. 20180902) and informed consent was signed by all the participants or the guardians of participants.

GCF Sampling. After removing the plaque from the gingiva, these areas were isolated using cotton rolls to reduce saliva contamination. Absorbent strips of paper (Periopaper, Interstate Drug Exchange, Amityville, NY, USA) were inserted into the gingival crevice and were kept for 30 s for GCF collection. GCF samples were collected from the tension and pressure sides of the tooth at 7 time points: the day of application (T0), one hour (T1), 24 hours (T2), one week (T3), 4 weeks (T4) and 12 weeks (T5) after the application of orthodontic force. T0 and T1 represented baseline level and short variation of cytokine levels after application of orthodontic force. T2 was chosen since the cytokine reached peak levels one day after orthodontic force. T3 was the turnover time for enzymes. T4 was the remodeling stage of the socket. T5 represent the outcome of application of orthodontic force. The GCF volume was confirmed by pre-calibrated Periotron 8000. The paper strips were stored at -80 ºC for further analysis.

Enzyme-linked Immunosorbent Assay (ELISA). According to the manufacturer's instructions, the levels of IL-17A, IL-17F, IL-23, IL-27, RANKL in GCF sample were measured using ELISA kit (Minneapolis R&D System) in duplicate. The detection limits of the assays were as follows: IL-17A, 15.6 pg/mL, IL-17F, 15.6 pg/mL, IL-23, 39 pg/mL, IL-27, 156 pg/mL, RANKL, 78.1 pg/mL.

Statistical Analysis. All the data were presented as mean ± SD. Expression of cytokines at different time points were analyzed by the Friedman test and Bonferroni-corrected Wilcoxon paired sign rank test a necessary. Spearman Rank Sum analysis was performed for correlation analysis and $p<0.05$ was considered as significant difference.

RESULTS

Changes Over Time in the Volume of GCF.

The average volume of GCF at the tension and pressure side of the study and control teeth was not significantly different comparing with baseline level (Table 1).

Table 1. The volume of gingival crevicular fluid at different time points during orthodontic tooth movement.

Time points	Study teeth Tension side	Study teeth Pressure side	Control teeth Mesiobuccal side	Control teeth Distobuccal side
T0	0.53 ± 0.21	0.62 ± 0.18	0.48 ± 0.14	0.57 ± 0.17
T1	0.46 ± 0.13	0.57 ± 0.26	0.55 ± 0.19	0.54 ± 0.22
T2	0.58 ± 0.23	0.41 ± 0.13	0.42 ± 0.22	0.48 ± 0.16
T3	0.41 ± 0.11	0.59 ± 0.23	0.57 ± 0.18	0.55 ± 0.22
T4	0.61 ± 0.27	0.51 ± 0.15	0.48 ± 0.17	0.43 ± 0.13
T5	0.55 ± 0.19	0.57 ± 0.23	0.58 ± 0.25	0.50 ± 0.16

All variables are expressed as mean ± standard deviation and the unit for volume was μL. For all the volume of T1-T5, there is no significant difference compared with baseline level.

Th17 Cytokines at Different Time Points.

As described in Tables 2, the expression of IL-17A and RANKL at both tension and pressure sides of study teeth at T2-T4 were significantly higher comparing with that of T0 and T1. Moreover, the expression of IL-27 at both tension and pressure sides of study teeth at T1-T4 were significantly lower comparing with that of T0 and T1. At T5, the levels of IL-17A, IL-17F, IL-23 and RANKL returned to baseline level. For the control teeth, the cytokines at both tension and pressure side were not significantly different at different time points (Table 3).

Table 2. The levels of Th17 cytokines and RANKL in gingival crevicular fluid of study tooth at various time points during orthodontic tooth movement.

Time points	T0	T1	T2	T3	T4	T5
IL-17A TS	25.2 ± 7.2	27.1 ± 6.9	38.5 ± 11.2*	47.2 ± 15.1*	57.2 ± 17.8*	26.8 ± 8.3
IL-17A PS	23.6 ± 6.5	24.3 ± 6.8	32.1 ± 13.7*	40.1 ± 10.9*	45.6 ± 13.7*	24.2 ± 5.8
IL-17F TS	51.3 ± 13.5	58.2 ± 17.8	73.4 ± 20.8*	96.5 ± 22.6*	99.3 ± 21.4*	55.7 ± 15.7
IL-17F PS	47.9 ± 15.6	55.6 ± 18.5	77.4 ± 19.6*	89.3 ± 24.7*	95.7 ± 25.6*	44.8 ± 12.9
IL-23 TS	62.4 ± 24.6	69.3 ± 22.7	95.6 ± 23.9*	123.4±33.7*	138.5±39.9*	66.9 ± 26.6
IL-23 PS	64.9 ± 21.3	68.2 ± 23.8	88.7 ± 25.1*	112.7±31.6*	127.6±28.4*	71.2 ± 20.8
IL-27 TS	348.2±58.7	335.6±61.8*	229.1±47.8*	197.3±35.4*	176.4±33.9*	324.2±55.3
IL-27 PS	319.1±49.9	301.8±47.2*	211.5±51.4*	182.6±39.6*	165.1±41.2*	315.8±46.2
RANKL TS	1.2 ± 0.4	1.3 ± 0.3	1.9 ± 0.5*	2.4 ± 0.3*	2.5 ± 0.3*	1.2 ± 0.3
RANKL PS	1.1 ± 0.3	1.2 ± 0.4	2.1 ± 0.4*	2.5 ± 0.5*	2.9 ± 0.5*	1.3 ± 0.2

TS for Tension side, PS for Pressure side. All variables are expressed as mean ± SD and the unit for cytokine expression was pg/mL. *Compared with T0 or T1 level, $p<0.05$.

The expression of IL-17A, IL-17F and IL-23 were positively correlated with RANKL expression at T2-T4, whereas the IL-27 was negatively correlated with RANKL expression at T2-T4 (Table 4).

Table 3. The expression of Th17 cytokines and RANKL in gingival crevicular fluid of control tooth at different time points during orthodontic tooth movement.

Time points	T0	T1	T2	T3	T4	T5
IL-17A MS	26.3 ± 8.1	25.0 ± 5.8	24.1 ± 7.3	25.7 ± 7.2	28.2 ± 6.8	25.5 ± 6.6
IL-17A DS	24.7 ± 6.1	25.8 ± 7.2	22.9 ± 6.7	28.1 ± 8.3	24.5 ± 7.4	25.3 ± 5.8
IL-17F MS	50.7 ± 12.9	55.4 ± 18.1	57.2 ± 16.6	48.4 ± 19.6	51.3 ± 21.7	56.8 ± 13.2
IL-17F DS	48.1 ± 17.7	52.4 ± 15.2	51.3 ± 18.3	58.9 ± 12.6	53.7 ± 17.3	42.9 ± 13.1
IL-23 MS	58.3 ± 21.5	64.2 ± 23.6	55.7 ± 18.3	61.3 ± 17.8	62.6 ± 21.0	65.3 ± 21.4
IL-23 DS	61.7 ± 17.9	67.6 ± 20.7	58.7 ± 15.6	63.9 ± 21.2	62.4 ± 22.8	70.8 ± 24.4
IL-27 MS	326.4±47.6	346.7±40.8	359.3±58.7	318.2±41.3	336.7±50.7	335.4 ±51.2
IL-27 DS	323.4±38.7	315.9±48.3	330.1±46.3	307.5±36.1	305.5±41.1	326.7 ± 41.8
RANKL MS	1.1 ± 0.3	1.2 ± 0.4	1.2 ± 0.3	1.2 ± 0.3	1.3 ± 0.2	1.2 ± 0.3
RANKL DS	1.2 ± 0.3	1.3 ± 0.3	1.2 ± 0.4	1.1 ± 0.1	1.3 ± 0.2	1.1 ± 0.3

MS for Mesiobuccal side, DS for Distobuccal side. All variables are expressed as mean ± standard deviation and the unit for cytokine expression was pg/mL. All the cytokines at both tension and pressure side were not significantly different at different time points ($p>0.05$).

Table 4. The correlation of Th17 cytokines and RANKL in gingival crevicular fluid of study tooth at different time points during orthodontic tooth movement.

Time points	T2			T3			T4		
	r	R^2	*P*	*r*	R^2	*P*	*r*	R^2	*P*
IL-17A TS	0.61	0.37	0.02	0.63	0.39	0.02	0.67	0.45	0.03
IL-17A PS	0.57	0.32	0.03	0.67	0.45	0.04	0.64	0.41	0.02
IL-17F TS	0.47	0.22	0.02	0.58	0.37	0.02	0.52	0.27	0.04
IL-17F PS	0.52	0.27	0.04	0.49	0.24	0.02	0.57	0.32	0.04
IL-23 TS	0.55	0.3	0.02	0.52	0.27	0.03	0.51	0.26	0.02
IL-23 PS	0.49	0.24	0.03	0.62	0.38	0.01	0.63	0.40	0.02
IL-27 TS	-0.57	0.32	0.01	-0.55	0.30	0.03	-0.62	0.38	0.03
IL-27 PS	-0.61	0.37	0.01	-0.49	0.24	0.02	-0.58	0.34	0.01

TS for Tension side, PS for Pressure side.

DISCUSSION

Stimulation and inflammation during OTM accelerate blood flow and change the composition of crevice fluid. Therefore, the expression of some cytokines related to osteoclast activity in GCF, such as RANKL and osteoprotegerin, had been used as biomarkers for OTM (12,13). IL-17 has been reported to participate in different chronic diseases (14). Zhang's study showed that the expression of IL-17 and IL-17 receptor by osteoblast-like cells were induced after compressive force, which were further regulated osteoclastogenesis (15). Moreover, IL-17 can affect the differentiation and role of osteoclasts by prostaglandin E2 (16). In addition, Hayashi reported that IL-17 during OTM promoted the formation of odontoclasts through the induction of IL-6 by periodontal ligament tissues (17). Yamagu *et al.* found that T-helper 17 cells up-regulated the expression of IL-17, RANKL and RANK after excessive orthodontic stress, which resulted in root resorption (18). All the mentioned studies were performed in cell or animal models. In this study, the Th17 related cytokines in GCF of patients during OTM were explored. It is found that the expression of IL-17A, IL-17F and IL-23 at both tension and pressure sides of teeth were enhanced after orthodontic treatment. However, Th17 related cytokines decreased to baseline level 12 weeks after treatment. On the other hand, IL-27, an anti-inflammatory cytokine, was significantly inhibited during OTM. Our studies suggested Th17 inflammation was deeply involved in the pathogenesis of OTM, which is consistent with previous studies. RANK/RANKL/osteoprotegerin (OPG) system has a very important role in osteoclast differentiation. RANKL released by osteoblasts and bone marrow stromal cells, is necessary for the induction of osteoclastogenesis. Previous studies have shown that RANK／RANKL/OPG system plays an important role

in osteoclast activation during orthodontic tooth movement (19). Previous researches found that IL-17 regulated the RANKL signaling pathway by inducing the up-regulation of RANK receptor expressed by osteoclast precursor cells. Moreover, studies have confirmed that IL-17 promotes osteoclast differentiation through RANKL from fibroblast-like synoviocytes (20). In the present study, it was also found that elevated Th17 cytokines were correlated with RANKL expression, suggesting the important roles of Th17 cytokines in RANK⁄RANKL/OPG system (Figure 1).

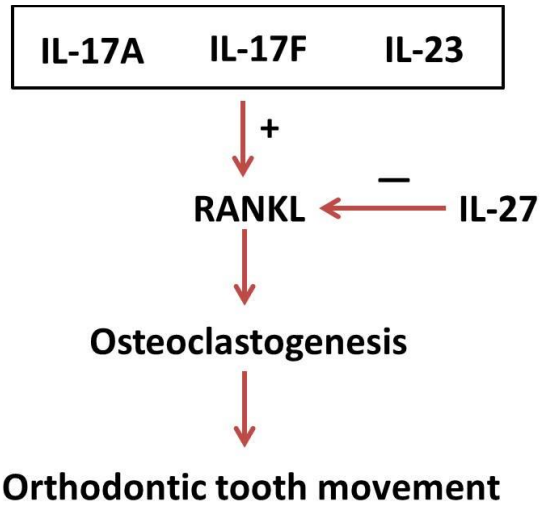

Figure 1. Schematic of the correlation of Th17 cytokines and RANKL during orthodontic tooth movement.

There were some limitations in this study. First, the study sample is not large. Second, the direct effect of Th17 cytokines on RANK⁄RANKL/OPG system was not explored. In conclusion, this study proved preliminary evidence that Th17 inflammation had important role in regulation of OTM. However, the detailed mechanism needed further exploration.

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