Matrix metalloproteinases and Th17 cytokines in the gingival crevicular fluid during orthodontic tooth movement



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DOI 10.23804/ejpd.2021.22.02.9

Abstract

Aim Matrix metalloproteinases (MMPs) contribute to remodeling in orthodontic tooth movement (OTM). Moreover, IL-17 can promote the production of MMPs. This study aimed to investigate the regulation of Th17 on MMPs expression during OTM.

Materials and methods Eighteen children undergoing orthodontic treatment were recruited. The gingival crevicular fluid (GCF) was collected at different 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. Th17 cell-related cytokines and MMPs expression were measured in GCF by Multiplex Luminex analyser. Human periodontal ligament (hPDL) tissues were stimulated by IL-17.

Results The levels of IL-17 and MMPs (MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13) of the study teeth at T2-T4 were significantly up-regulated compared with that of T0 and T1 and decreased to baseline level at T5. We found that the expression of IL-17 was correlated with MMPs. After rhIL-17 treatment, the expression of MMP-1, MMP-2, and MMP-9 were up-regulated significantly. The IL-17 expression was positively correlated with MMPs.

Conclusions IL-17 promotes the expression of MMP-1, MMP-2, and MMP-9 by hPDL cells, suggesting that IL-17 plays a crucial role in the remodeling during OTM.

KEYWORDS IL-17; Matrix metalloproteinases; Human periodontal ligament; Orthodontic tooth movement.

Introduction

Remodeling process of the extracellular matrix of the adjacent periodontium is involved in the orthodontic tooth movement (OTM) [Grant et al., 2013]. Matrix metalloproteinase (MMP) is one of inflammatory mediators and its balance with TIMPS is one of the main causes of remodeling during OTM.

Redlich reported that the expression of MMP-1 and its activity were increased in the compression gingival side using dog model [Redlich et al., 1996]. During active tooth movement, the expression of MMP-8 and MMP-13 in rat periodontal ligament (PDL) was also up-regulated [Takahashi et al., 2003]. In humans, the expression of MMP-1, MMP-8 MMP-2, MMP-9 and tissue inhibitors of metalloproteases (TIMP)-1 were up-regulated at sites of compression and tension during OTM [Cantarella et al., 2006; Ingman et al., 2005; Apajalahti et al., 2003; Bildt et al., 2009].

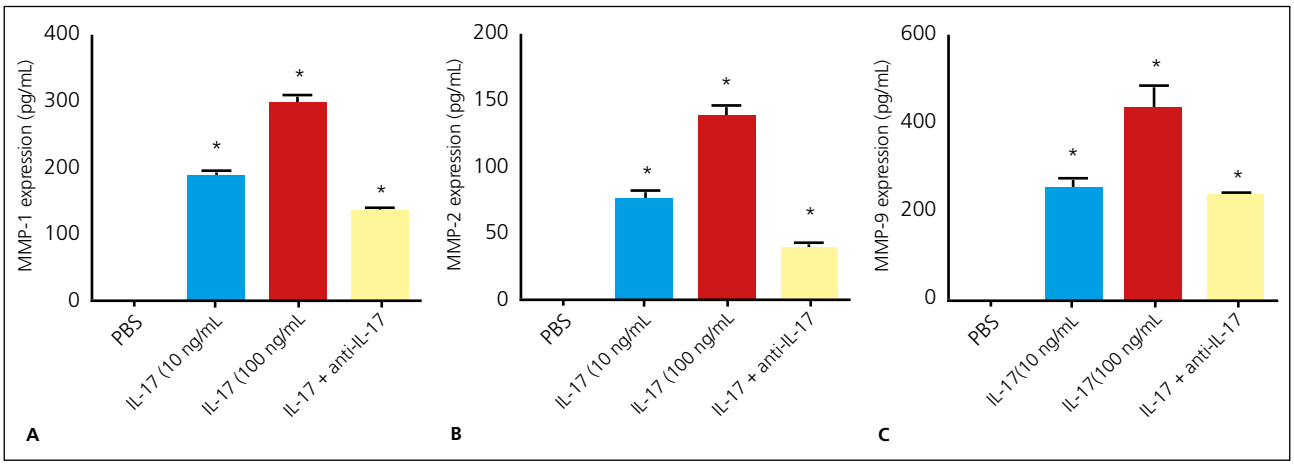
Interleukin-17a, commonly known as interleukin-17 (IL-17), is the first member of the Th17 cytokine family to be identified [Sfanos et al., 2008]. It is well known that IL-17 can promote the production of pro-inflammatory cytokines, chemokines and MMPs [Park et al., 2005]. It has been reported that IL-17 alleviate the invasion of cancer cells by promoting the expression of MMP-2 and MMP-9 [Wang et al., 2014; Li et al., 2011].

In this study, we used the Multiplex Luminex analyser, which enable multiple biomarkers to be measured on the same sample, to measure MMPs and IL-17. We also explored the effect of IL-17 on the expression MMPs by human PDL cells.

Materials and methods

Subjects

This study recruited 18 healthy subjects (12–18 years old, 8 males, 10 females) undergoing OTM. The subjects had full permanent dentition and their Little Irregularity index was between 3 and 5 mm in the anterior segment of the lower arch, which allowed for continuous archwire for alignment and leveling. The exclusion criteria were autoimmune diseases, deep overbite, caries or restorations in the anterior teeth and the use of any drugs (such as antibiotics, antihistamines, cortisone and hormones) that might interfere with OTM in the first six months of the study. This study was approved by the local ethics committee (No. 20180124) and informed consent was obtained by all the participants. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki. One week before the application of orthodontic treatment, the subjects received oral hygiene instruction and rinsed with 0.12% chlorhexidine gluconate twice a day for four consecutive weeks.



* compared with PBS group, P<0.05

FIG. 1 The expression of MMP-1, MMP-2, and MMP-9 by periodontal ligament (PDL) after stimulated by rhIL-17.

Orthodontic appliance placement

Orthodontic brackets (0.022×0.028-inch slot) were used for bonding from the first molar to the molar on both the lower and upper arches. Then, an archwire (0.014-inch Nickel-Titanium) was inserted into the brackets of the lower teeth and bonded with modular elastics. For the upper arch, only modular elastics were placed. The lower incisors were the selected study teeth and the upper incisors were selected as control teeth.

Gingival crevicular fluid (GCF) sampling

After removal of the gingival plaque, gingival crevice of subjects was carefully isolated using cotton rolls to avoid saliva contamination. The GCF samples were obtained by gently inserting 1–2 PerioPapers in the gingival crevice of test teeth or control teeth for 60s. The collection was repeated 3 times to obtain a proper amount of GCF. The collection time points were as follows: 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.

Human PDL cell culture

Human PDL tissues were obtained from the roots of premolars from six healthy young volunteers (three males, three females; 14–16 years of age) during orthodontic treatment. The PDL tissue specimens were cut into pieces and the cells were cultured in MEM medium (Wako, Osaka, Japan) supplemented with penicillin-G, gentamicin sulfate, amphotericin B and 10% fetal calf serum (Cell Culture Laboratories, Cleveland, OH, USA) at 37°C in a humidified incubator. The PDL cells (1×106) were stimulated by 10–100 ng/mL rhIL-17 (R&D Systems) and/or anti-IL-17 (100 ng/mL) for 72 hours to explore its effect on MMPs production.

Quantification of inflammatory mediators

According to the manufacturer's instructions, the concentrations of IL-17, MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 in GCF sample were determined using multiplex Luminex in duplicate.

Data analysis

All the data were showed as mean±SD. Levels of cytokines at different time points were analyzed by the Friedman test followed by Bonferroni-corrected Wilcoxon paired sign rank test as necessary. The Spearman correlation analysis was performed to analyse the correlation between different biomarker. Significance was set at P<0.05.

Results

The volume of GCF during treatment

Orthodontic treatment did not change the volume of GCF of the study and control teeth at both tension and pressure side of study teeth compared with baseline level (Table 1).

The expression of IL-17 and MMPs at different time points

The levels of IL-17, MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 at both tension and pressure side of study

Time points	Study teeth Tension side	Study teeth Pressure side	Control teeth Tension side	Control teeth Pressure side
T0	0.42 ± 0.11	0.53 ± 0.16	0.52 ± 0.22	0.62 ± 0.23
T1	0.57 ± 0.22	0.49 ± 0.21	0.47 ± 0.17	0.53 ± 0.18
T2	0.61 ± 0.23	0.56 ± 0.18	0.49 ± 0.15	0.59 ± 0.22
T3	0.54 ± 0.18	0.44 ± 0.21	0.58 ± 0.23	0.57 ± 0.19
T4	0.62 ± 0.25	0.52 ± 0.22	0.56 ± 0.19	0.61 ± 0.25
T5	0.48 ± 0.17	0.48 ± 0.21	0.61± 0.17	0.64 ± 0.27

TABLE 1 Observed oral hygiene status of 12- and 15-year-old adolescents measured using the simplified Debris Index (DI-S) according to socio-demographic and behavioural parameters.

All variables are expressed as mean ± standard deviation and the unit for volume was μL

Time points	T0	T1	T2	T3	T4	T5
IL-17 TS	29.1 ± 9.1	26.5 ± 7.3	41.7 ± 12.3	55.4 ± 16.3	62.3 ± 15.8	23.2 ± 8.9
IL-17 PS	21.1 ± 7.6	25.8 ± 9.1	34.6 ± 12.7	48.8 ± 16.1	59.3 ± 18.2	22.6 ± 7.3
MMP-1 TS	173.5±21.6	161.3±18.9	196.2±31.4	225.4±37.6	269.1±44.2	165.3±25.1
MMP-1 PS	181.6±32.7	159.9±21.8	207.3±34.5	231.6±30.5	258.3±35.4	169.8±20.4
MMP-2 TS	83.1 ± 21.1	79.4 ± 17.3	99.1 ± 18.3	134.5±29.8	159.6±31.3	79.2 ± 22.4
MMP-2 PS	77.3 ± 18.6	81.6 ± 23.8	95.3 ± 23.0	120.7±25.4	148.2±29.5	69.3 ± 26.8
MMP-3 TS	18.5 ± 4.1	17.4 ± 3.1	52.6 ± 11.8	77.5 ± 21.4	93.4 ± 26.4	19.9 ± 4.3
MMP-3 PS	19.9 ± 3.5	20.1 ± 3.7	48.9 ± 9.9	69.9 ± 18.2	89.5 ± 19.7	22.3 ± 4.7
MMP-8 TS	7.4 ± 2.9	5.5 ± 3.0	13.7 ± 4.1	21.3 ± 3.9	34.6 ± 4.8	6.9 ± 2.2
MMP-8 PS	8.2 ± 2.4	7.9 ± 2.8	12.6 ± 5.5	22.4 ± 4.3	39.6 ± 5.2	7.8 ± 2.7
MMP-9 TS	284.6±42.7	272.5±51.1	324.1±55.6	446.5±48.7	571.3±56.8	266.3±53.6
MMP-9 PS	273.1±51.2	268.5±49.8	317.8±61.2	431.9±52.8	542.3±54.7	251.6±43.6
MMP-13 TS	14.1 ± 2.3	16.3 ± 3.8	31.9 ± 6.1	71.8 ± 8.5	92.6 ± 11.3	12.1 ± 5.4
MMP-13 PS	15.6 ± 3.1	19.6 ± 4.9	27.6 ± 5.3	69.9 ± 7.4	82.9 ± 12.8	13.9 ± 3.6

TS for Tension side, PS for Pressure side.

TABLE 2 The expression of IL-17 and MMPs in gingival crevicular fluid of study tooth at different time points during orthodontic tooth movement.

teeth at T2-T4 were significantly up-regulated compared with that of T0 and T1 and decreased to baseline level at T5 (Table 2). For the control teeth, the cytokines at both tension and pressure side were not significantly different at different time points. We also found that the expression of IL-17 was positively correlated with MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 (Table 3).

Effects of rhIL-17 on the expression of MMPs by hPDL cells

After rhIL-17 treatment, the expression of MMP-1, MMP-2, and MMP-9 were up-regulated significantly (Fig. 1), whereas the expression of MMP-3, MMP-8, and MMP-13 were not affected (Data not shown). We also found that IL-17F and IL-23 had no effect on MMPs expression by hPDL cells (Data not shown).

Discussion

The matrix metalloproteinases are involved in different stages of collagen remodeling. The MMPs included several types: collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, and -11), membrane-type MMPs (MMP-14, -15, -16, and -17), and miscellaneous MMPs [Snoek-Van Beurdenand Von den Hoff, 2005].

The MMP expression had been reported in various studies. Apajalahti [2003] found up-regulated MMP-8 expression in the GCF after orthodontic treatment compared with baseline values and control teeth. In another study, Cantarella [2006]

Time points	T1	T2	T3	T4
MMP-1 TS	NS	0.67	0.58	0.49
MMP-1 PS	NS	0.59	0.46	0.51
MMP-2 TS	NS	0.44	0.52	0.57
MMP-2 PS	NS	0.53	0.49	0.48
MMP-3 TS	NS	0.51	0.61	0.63
MMP-3 PS	NS	0.48	0.55	0.52
MMP-8 TS	NS	0.61	0.64	0.48
MMP-8 PS	NS	0.49	0.51	0.53
MMP-9 TS	NS	0.66	0.63	0.42
MMP-9 PS	NS	0.57	0.52	0.55
MMP-13 TS	NS	0.39	0.47	0.46
MMP-13 PS	NS	0.51	0.42	0.47

TABLE 3
Comparison of the means of lip activity with and without LB, at the start and at the end of treatment.

TS for Tension side, PS for Pressure side

reported that the MMP-1 and MMP-2 levels increased at the both and tension pressure side after tooth movement. Chang [2008] found that MMP-3 distributed along the compressive site of the bony region within 3 days of orthodontic force compression in human bone samples. Leonardi [2007] indicated that MMP-13 might play an important role during tooth movement using Sprague–Dawley rats. The study of Grant [2013] found high levels of MMP-9 throughout OTM and related to speed of movement 4 hours after force application. Consistently, we found that MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 at both tension and pressure side of study teeth were significantly increased at T1-T4 and returned to baseline level at T5. However, the regulation of MMPs in OTM was not fully understood.

The role of IL-17 was reported in various chronic inflammatory and autoimmune diseases [Brennan and McInnes, 2008]. Previous study had showed that compressive force increased the production of IL-17 and its receptor by osteoblast-like cells, which affect osteoclastogenesis [Zhang et al., 2010]. Moreover, IL-17A has been proved to induce the invasion of cancer cells via up-regulating the expression of MMP-2 and MMP-9 [Wang et al., 2014; Li et al., 2011]. In this study, we also found that IL-17 expression was positively correlated with MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13. Therefore, we hypothesize that IL-17 could promote MMPs expression during OTM.

Our results also showed that rhIL-17 treatment up-regulated the expression of MMP-1, MMP-2, and MMP-9 by hPDL cells, while the MMP-3, MMP-8, and MMP-13 was not affected, suggesting that the regulation of MMP-3, MMP-8, and MMP-13 involved other pathway. Moreover, we also found that IL-17F and IL-23 had no effect on PDL cells.

Conclusion

In summary, we found that up-regulated IL-17 expression was positively correlated with MMPs. We also found the effect of Th17 on the expression MMP members by PDL cells, suggesting that IL-17 plays a crucial role in the remodeling during OTM.

Authors' contributions

T. Lin and B. Zhang created the concept and designed of the work and did a critical revision of the article. L. Yang and W. Zheng performed the experiment and analysed the data. All authors read and approved the final manuscript.

Funding

The authors financed the research with their own resources.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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