Clinical Oral Investigations
https://doi.org/10.1007/s00784-019-02861-9

ORIGINAL ARTICLE

Expression of Toll-like receptors 2 and 4 and its association with matrix metalloproteinases in symptomatic and asymptomatic apical periodontitis

A. Fernández[1,2] · A. M. Cárdenas[1,3] · J. Astorga[1] · P. Veloso[1] · A. Alvarado[2] · P. Merino[1] · D. Pino[2] · D. Reyes-Court[4] · M. Hernández[1,5,6]

Received: 4 October 2018 / Accepted: 14 February 2019

Abstract

To determine Toll-like receptors (TLR)2 and TLR4 expression levels and associate them with matrix metalloproteinases (MMPs) in asymptomatic apical periodontitis (AAP), symptomatic apical periodontitis (SAP), and healthy controls. Apical tissue/lesion samples were obtained from chronic AAP ($n = 35$) and SAP ($n = 29$), and healthy periodontal ligament (HPL, $n = 10$) with indication of tooth extraction, respectively. mRNA expression levels of TLR2, TLR4, MMP-1, MMP-2, MMP-8, and MMP-13 were determined by real-time reverse-transcription polymerase chain reaction. The data were analyzed with Kruskal-Wallis and Dunn's pot hoc test ($p < 0.05$). The correlation coefficient was obtained using the Spearman correlation ($p < 0.05$). TLR2, MMP-1, MMP-2, and MMP-13 mRNA levels were the highest in SAP followed by AAP and controls ($p < 0.05$). TLR4 and MMP-8 were over expressed in AAP and SAP compared to HPL ($p < 0.05$). TLR2 positively correlated with TLR4, MMP-1, MMP-8, and MMP-13 in SAP ($p < 0.05$). TLR2 and TLR4 are overexpressed in apical lesions versus healthy periodontal ligament and correlate with collagenolytic MMPs. Particularly, TLR2 is overexpressed in SAP in association with MMP-1, MMP-8, and MMP-13. Our results suggest that the activation of TLR2 along with MMP overexpression might contribute to SAP clinical presentation and progression. TLRs, MMPs, and their interaction can explain the clinical presentations and evolution of apical periodontitis and might represent key targets for new diagnostic and treatment approaches.

Keywords Matrix metalloproteinases · Periapical periodontitis · Toll-like receptor 2 · Toll-like receptor 4

Introduction

Apical periodontitis (AP) is the result of the host's immune response to the persistent microbial infection of the dental pulp. Chronic forms of AP are characterized by the destruction of the peri-radicular tissues, particularly the alveolar bone, resulting in the formation of an osteolytic apical lesion. This response can be exacerbated and becomes symptomatic due to the imbalance between the endodontic infection and the host's immune response [1, 2], potentially representing an active form of the disease [3].

Toll-like receptors (TLRs) are central for pathogen recognition by innate immune cells during endodontic infection [4]. TLRs respond to pathogen-associated molecular patterns (PAMPs) and trigger intracellular signaling pathways that activate NF-κB and induce the expression of pro-inflammatory genes. TLR2 and TLR4 classically recognize Gram-positive's

✉ M. Hernández

[1] Laboratory of Periodontal Biology, Faculty of Dentistry, Universidad de Chile, Santiago, Chile

[2] Faculty of Dentistry, Universidad Andres Bello, Santiago, Chile

[3] Health sciences division, Faculty of Dentistry, Universidad Santo Tomás, Bucaramanga, Colombia

[4] Department of Surgery, Faculty of Dentistry, Universidad de Chile, Santiago, Chile

[5] Department of Oral Pathology and Medicine, Faculty of Dentistry, Universidad de Chile, Santiago, Chile

[6] Dentistry Unit, Faculty of Health Sciences, Universidad Autónoma de Chile, Santiago, Chile

Published online: 26 February 2019

lipoteichoic acids and Gram-negative's lipopolysaccharide (LPS), respectively [5]. Despite their key roles, few clinical studies have explored TLRs in apical lesions, limiting to their immunolocalization. TLR2 has been identified in mononuclear plasmocytes and macrophage-like cells in refractory AP [4]. TLR2 and TLR4 showed differential expression in periapical granulomas and radicular cysts with or without sinus tract, suggesting their participation in the reactivation of periapical inflammation [6]. Accordingly, experimental studies propose that TLR2 and TLR4 might play a relevant role in the progression of AP based on their ability to stimulate receptor activator of nuclear factor kappa-B ligand (RANKL) synthesis in osteoblasts [7], to promote survival of osteoclasts [8], and to induce pro-inflammatory cytokines [9] and matrix metalloproteinases (MMPs) [10].

MMPs are zinc-dependent enzymes that cleave components of the extracellular matrix. Bone resorption centrally involves the activity of collagenases (MMP-1, MMP-8, and MMP-13) and gelatinases (MMP-2 and MMP-9) that cooperatively and sequentially degrade native type I collagen and gelatin. In addition, they modulate bioactive non-matrix mediators, regulating the host's inflammatory response [11]. MMP-1, MMP-2, MMP-8, and MMP-9 immunolocalized in AL and their expression was reduced during the healing phase in experimental animal models, supporting their role in the initiation and progression of AP [12]. Many reports propose an important role for MMP-9 in human apical periodontitis, but much less is known regarding other relevant MMPs [13–16].

Previous studies support the interaction between TLRs and MMPs. The activation of TLR2 and TLR4 combined with the stimulation of mechanical force induced the secretion of MMPs by human periodontal fibroblasts via p38, JNK, and NF-kB, and TLR2 modulated MMP-9 expression in experimentally induced AP [10, 17, 18]. Up to now, there are no studies assessing whether TLR expression and MMPs can influence the clinical presentation of AP. We aimed to determine TLR2 and TLR4 expression levels and associate them with MMPs in asymptomatic apical periodontitis (AAP), symptomatic apical periodontitis (SAP), and healthy controls. The null hypothesis is that TLR2 and TLR4 expression is not associated with MMPs or clinical symptoms in endodontic apical lesions.

Materials and methods

Study design

Cross-sectional study. The study was approved by the Ethics Committee of the Faculty of Dentistry, Universidad de Chile (no. 2016/08) and the Ethics-Scientific Committee of the Central Metropolitan Health Service (no. 2017/70). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Patients and sample collection

Individuals consulting at the clinic of surgery, Faculty of Dentistry, Universidad Andrés Bello and the Public urgency hospital (HUAP), Santiago, Chile, were included if they had clinical and radiographic diagnosis of untreated AAP or SAP, and healthy periodontal ligament (HPL) from teeth having indication of extraction. The inclusion criteria were individuals ≥ 18 years, having teeth with clinical diagnosis of apical periodontitis in association with dental caries that did not respond to pulp sensitivity tests, and had a radiographic apical radiolucency ≥ 3 mm, according to previously defined criteria [3, 19]. Exclusion criteria were systemic diseases and/or antibiotic and/or anti-inflammatory consumption in the last 3 months prior to the study. SAP and AAP were diagnosed when clinical symptoms in response to percussion were present or absent, respectively, according to previously defined diagnostic terminology [20]. Apical lesions and healthy periodontal ligament were removed with sterile curettes and tweezers. Healthy periodontal ligament was extracted 2 mm below the amelocemental junction and 1 mm above the apex, washed with sterile 0.9% NaCl solution, and stored in 100 μL of RNAlater (Qiagen, Valencia, CA, USA) at − 80 °C until they were processed.

Isolation of RNA

Samples were homogenized and RNA extraction was performed using a commercial RNA commercial kit (Qiagen), following the protocol and recommendations of the manufacturer. RNA was quantified (260/280) using a spectrophotometer (Bio-Tek, Winooski, VT), and RNA integrity was evaluated in a microfluidic-based platform (Agilent 2100 Bioanalyzer; Agilent Technologies, Santa Clara, CA). Then, 1 μg of RNA from each sample was converted to single-stranded cDNA, using a reverse transcription kit (Thermo Fisher Scientific, Carlsbad, CA).

Expression of RNA

The mRNA expression levels were determined through real-time reverse-transcription polymerase chain reaction (SteponePlus®;Applied Biosystems, Singapore). 12.5 ng of cDNA were amplified using TLR 2, TLR4, MMP-1, MMP-2, MMP-8, and MMP-13 respective primer sets (Table 1) and KAPA SYBR ® Fast qPCR Kits (KAPA Biosystems, Woburn, MA, USA). The amplification process consisted of

Table 1 Primers used in AAP, SAP, and HPL samples

Target	Sequence forward	Sequence reverse
18S	CTCAACACGGGAAACCTCAC	CGTCCACCACTAAGAACG
TLR2	CTCTCGGTGTCGGAATGTC	AGGATCAGCAGGAACAGAGC
TLR4	CCCTCCCCTGTACCCTTC	TCCCTGCCTTGAATACCTTC
MMP-1	GCTAACCTTTGATGCTATAACTACGA	GGATTTGTGCGCATGTAGA
MMP-2	TGTGTTCTTTGCAGGGAATG	AGGCTGGTCAGTGGCTTG
MMP-8	AGCAGCCAAAAGAGAACCA	CCCATTTGGGTTTGGACTC
MMP-13	CCAGTCTCCGAGGAGAACA	AAAAACAGCTCCGCATCAAAC

the following steps: 95 °C for 3 min, 40 cycles of 95 °C for 30 s, 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s.

Analysis of results

Relative quantification of gene expression levels of TLR2 TLR4, MMP-1, MMP-2, MMP-8, and MMP-13 in AAP, SAP, and HPL controls was analyzed by $2^{-\Delta\Delta CT}$ method. The normalization of gene expression was made through the 18S rRNA, and HPL was used as a fold change reference.

The Shapiro-Wilk test was used to determine the distribution of the data. Inferential analyses were performed with chi-square test, Mann-Whitney or Kruskal-Wallis, and Dunn's pot hoc tests. The correlation coefficient between TLR and MMPs was obtained using the Spearman's correlation analysis. The level of significance was defined at $p < 0.05$. The statistical analysis was performed using STATA 12® (StataCorp LP, TX, USA). The figures were performed in GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA, USA). No formal sample-size calculation was performed due to the exploratory nature of this study.

Results

The study sample consisted of 74 individuals. Age, gender, tobacco smoking, and educational level for each diagnostic group are shown in Table 2.

TLR-mRNA expression levels in AAP, SAP, and control groups are presented in Fig. 1. The expression levels of TLR2 were the highest in SAP, followed by AAP and controls, with significant differences among the three groups ($p < 0.05$). Significantly higher expression levels of TLR4 mRNA were observed in SAP and AAP, compared to healthy controls ($p < 0.05$). No significant differences were found between SAP and AAP.

MMP mRNA expression levels in AAP, SAP, and control groups are presented in Fig. 2. MMP-1, MMP-2, and MMP-13 were the highest in SAP followed by AAP and controls, with statistically significant differences among the three groups ($p < 0.05$). MMP-8 was significantly higher in SAP and in AAP versus healthy controls ($p < 0.05$). No differences were found between SAP and AAP.

Table 3 shows the correlation matrix between TLR and MMP gene expression. In AAP, a positive correlation was found between TLR2 and TLR4. TLR2 positively correlated with MMP-13, whereas TLR4 positively correlated with MMP-8 and MMP-13 ($p < 0.05$). In SAP, positive correlations were found between TLR2 and TLR4. Additionally, positive correlations were identified between TLR2 and MMP-1, MMP-8, and MMP-13, whereas TLR4 positively correlated with MMP-8 and MMP-13 ($p < 0.05$).

Finally, we explored whether smoking influenced TLR and MMP. Table 4 shows their expression levels between non-smokers and smokers in AAP and SAP. Among them, MMP-2 was significantly lower in smokers compared to AAP ($p < 0.05$), and a similar tendency was found in SAP, but non-significant.

Table 2 Demographic parameters and smoking habit of study individuals. *AAP*, asymptomatic apical periodontitis; *SAP*, symptomatic apical periodontitis; *HPL*, healthy periodontal ligament

Parameters	HPL	AAP	SAP	*p*
Age (years, median (IQR))	22 (13)	47 (20)	34 (18)	0.0001*
Females (*n*, %)	7 (63.6%)	20 (54.1%)	15 (44.8%)	0.533
Smokers (*n*, %)	3 (27.3%)	14 (37.8%)	13 (44.8%)	0.585
Educational level (median)	Full high school	Full high school	Full high school	0.051

*$p < 0.05$

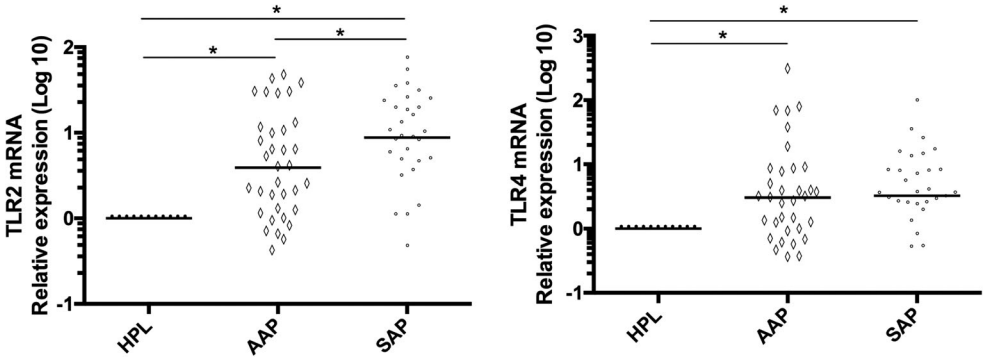

Fig 1 Relative gene expression of TLR2 and TLR4 in AAP, SAP, and HPL controls. Gene expression was normalized relative to 18S rRNA and expressed as the difference between $2^{-\Delta\Delta CT}$. Values on *y*-axis are shown in log10 scale. AAP, asymptomatic apical periodontitis; SAP, symptomatic apical periodontitis; HPL, healthy periodontal ligament. Bars represent the medians and asterisk $p < 0.05$

Discussion

The exacerbation of the clinical symptoms in SAP has been proposed to represent a progressive stage of apical lesions [3], which might be explained by immune activation in response to the bacterial challenge inside the root canal or apical lesion [21]. In the present study, we show that the symptomatic stage of AP-associated lesion may be conditioned by the participation of TLR in association with MMPs.

TLR2 and TLR4 have previously been identified in human apical tissues. Our results demonstrate that TLR2 and TLR4 were significantly over expressed in SAP and AAP versus HPL. However, only TLR2 expression levels were significantlty higher in SAP compared to AAP. In line with our results, a previous study reported higher localization of TLR2 in symptomatic than asymptomatic periapical granulomas or inflammatory periapical cyst in refractory lesions through immunohistochemical analysis [4]. A more recent study reported higher immunodetection of TLR2 and TLR4 in inflammatory cysts with sinus tracts compared to those with no sinus tracts [6], supporting a role for TLR in different clinical forms of acute exacerbation of AP. Our results also showed a positive correlation in the expression of TLR2 and TLR4 in both, AAP, and SAP. Experimentally induced AP in TLR2-knockout mice showed larger periapical lesions in association with a more pro-inflammatory phenotype induced by TLR4 over activation, suggesting a cooperative cross-talk between TRL2 and TLR4. In other study, TLR2 agonists upregulated osteoclastic genes, resulting in higher osteoclast formation and bone loss in wild type mice and osteoblast cell

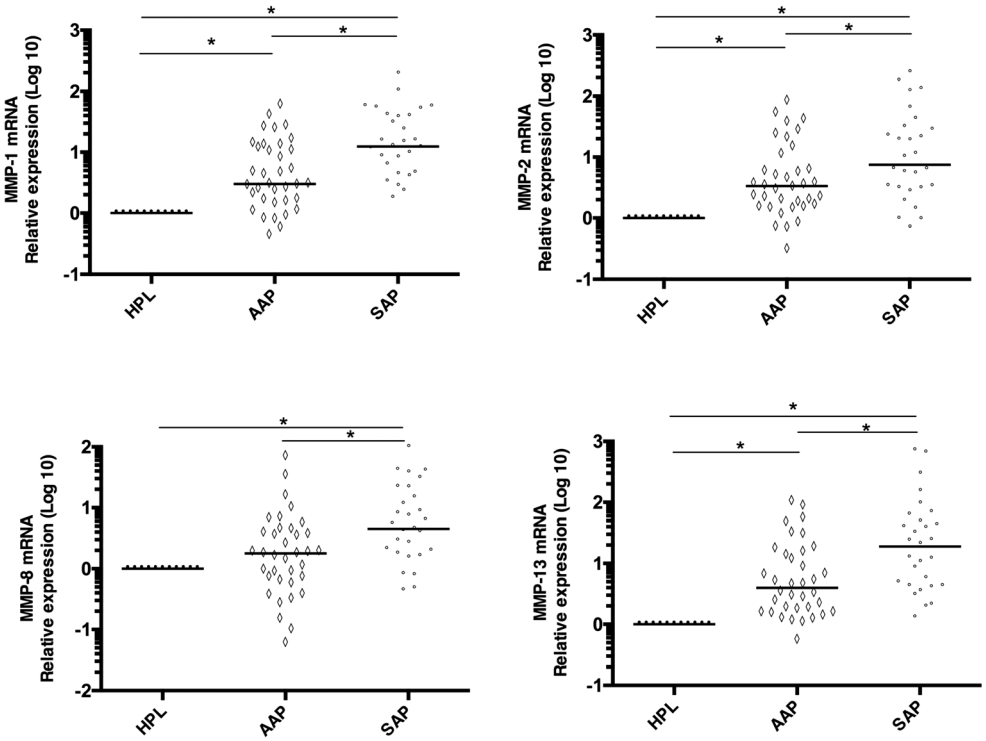

Fig 2 Relative gene expression of MMP-1, MMP-2, MMP-8, and MMP-13 in AAP, SAP, and HPL controls. Gene expression was normalized relative to 18S rRNA expressed as the difference between $^{2-\Delta\Delta CT}$. Values on *y*-axis are shown in log10 scale. AAP, asymptomatic apical periodontitis; SAP, symptomatic apical periodontitis; HPL, healthy periodontal ligament. Bars represent the medians and asterisk $p < 0.05$

Table 3 Correlation matrix between TLR and MMP gene expression in AAP and SAP

	AAP		SAP	
	TLR2	TLR4	TLR2	TLR4
TLR2	1	–	1	–
TLR4	0.592*	1	0.569*	1
MMP-1	0.341	0.346	0.472*	0.264
MMP-2	0.275	0.080	0.242	0.332
MMP-8	0.164	0.439*	0.632*	0.524*
MMP-13	0.504*	0.469*	0.389*	0.455*

AAP, asymptomatic apical periodontitis; *SAP*, symptomatic apical periodontitis

Spearman's correlation coefficient (*r*)

*$p < 0.05$

cultures, but not in TLR2-deficient mice [7], supporting that TLR2 also represents a key regulator of alveolar bone resorption [22, 23]. Overall, both TRL2 and TLR4 might interact in the settlement of the apical lesion during AAP, whereas the immunologically active stage in SAP could be triggered predominantly by TLR2 engagement.

A classic drawback from our study design is the young age of the control group, because orthodontics is a common indication particularly in young people. However, it has been previously validated in the literature based on the fact that healthy periodontal ligament represents the closest histophysiological counterpart for AP in humans [24, 25].

The interaction between periodontal inflammation and persistent bacterial infection upregulates MMPs, which contributes to the progressive breakdown of apical tissues. Collagenases (MMP-1, MMP-8, and MMP-13) process native collagen without unwinding the triple helical assembly of the molecule, generating ¾ ¼ fragments, whereas gelatinases (MMP-2 and MMP-9) further process denatured collagen in a cooperative manner [11, 26, 27]. In this study, we showed that MMP-1, MMP-2 and MMP-13 were significantly over expressed in SAP followed by AAP and controls. We also found significantly higher levels of MMP-8 mRNA in both AP forms compared to HPL. The results support that these MMPs participate in the pathogenesis of AP, but particularly MMP-1, MMP-2, and MMP-13 showed to be relevant in the active stage and/or clinical symptoms of apical chronic inflammatory lesions.

Clinical and experimental animal studies have also demonstrated high levels of MMP-2 in association with acute forms of AP. Previously, MMP-2 was detected in primary acute apical abscess and secondary apical abscess. Conversely, MMP2 forms were not detected in SAP with sinus tract [28]. Also, higher mRNA expression of MMP-2, MMP-7, and MMP-9 were reported in chronic apical abscesses compared to AAP [29], as well as higher MMP-2 and MMP-9 immunodetection in symptomatic apical lesions versus asymptomatic ones [16, 30]. All these antecedents in line with our results support a role for MMP-2 in the acute exacerbations and symptoms of chronic apical lesions. MMP-2 has been reported to have a central role in bone resorption in experimental models and has also been proposed to contribute to neuropathic pain symptoms during tissue injury [31, 32].

MMP-1 has been classically involved in physiologic matrix turnover [33], though a previous study reported higher MMP-1 mRNA in periapical lesions compared to healthy tissues [34]. Moreover, MMP-1 mRNA levels were over expressed in active lesions, defined as those having a higher RANKL/OPG ratio, along with the osteolytic cytokines, TNF-α, IFN-γ, IL-21, and IL-17A, in accordance with our results [34, 35].

Regarding MMP-8 and MMP-13, no previous reports are available in human symptomatic apical lesions. Still, our results are supported in part by previous reports from our research group. MMP-8 levels were elevated in gingival crevicular fluid (GCF) of teeth with AAP, SAP, and chronic marginal periodontitis compared to healthy controls [15, 36, 37]. MMP-13 on the other hand, was reported to increase in GCF from active periodontal lesions [38], whereas both MMP-8 and MMP-13, were involved in the development of apical lesions in experimentally induced AP [39]. They can also modulate bone resorption through osteoclast activation and differentiation, besides their classical role of periodontal tissue

Table 4 TLR and MMP gene expression between smokers and no smokers in AAP and SAP

	AAP		*p*	SAP		*p*
	NS (*n* = 23)	S (*n* = 13)		NS (*n* = 16)	S (*n* = 13)	
TLR2	3.25 (27.24)	3.91 (4.15)	0.83	7.80 (12.92)	11.96 (18.16)	0.38
TLR4	2.40 (7.58)	3.36 (3.00)	0.58	3.08 (7.425)	3.27 (4.30)	0.46
MMP-1	3.02 (12.06)	2.51 (4.39)	0.10	11.50 (33.08)	14.19 (26.66)	0.77
MMP-2	5.05 (18.68)	1.83 (1.44)	0.03*	18.22 (71.98)	5.50 (10.44)	0.35
MMP-8	1.61 (4.06)	1.93 (2.95)	0.41	7.67 (18.23)	3.21 (3.59)	0.29
MMP-13	3.44 (5.29)	5.29 (30.12)	0.66	25.41 (53.48)	7.73 (30.99)	0.26

AAP, asymptomatic apical periodontitis; *SAP*, symptomatic apical periodontitis; *NS*, no smokers; *S*, smokers. Results are expressed as medians and interquartile range (IQR). *$p < 0.05$

breakdown. In vivo and in vitro studies support that MMP-13 is required for the differentiation of pre-osteoclasts, osteoclasts activation, and osteolysis. Several mechanisms have been reported, which include the activation of osteoclast-secreted pro-MMP-9, cleaving of galectin-3, a known inhibitor of osteoclastogenesis, and by regulating the RANKL/OPG axis, among others [11].

In the current study, moderate positive correlations were found for TLR2, TLR4, and the studied MMPs in SAP and to a lesser extent in AAP, suggesting that TLR signaling, among others, induces MMP expression in association with the clinical presentation of AP. Particularly, our results demonstrated positive correlations between TLR2, TLR4, and collagenolytic MMPs (MMP-1, MMP-8, and MMP-13) in SAP. It is likely to hypothesize that PAMPs derived from exacerbated bacterial infection and/or DAMP released in SAP induce TLR overexpression and activation, which in turn induce collagenolytic MMP synthesis, aggravating periapical tissue breakdown. Specially, MMP-8 and MMP-13 have been recognized as key MMPs associated with periodontal tissue destruction [11, 33]. In support of our proposal, TLR2 and also TLR4 have been reported to activate the synthesis of MMP-1, MMP-8, MMP-9, and MMP-13 via p38, JNK, and NF-kB/AP-1 signaling in human fibroblasts and monocytic/macrophage cell lines [9, 18, 40, 41]; TLR2 activation and consequent recruitment of MyD88 was also reported to modulate the secretion of MMP-2 and MMP-9 in experimentally induced apical lesions [10]. MyD88 is an essential adaptor protein that has a key role in TLR signaling and triggers downstream cascades that activate NF-κB/AP-1, which regulates the expression of MMPs [42–45].

It is well-defined that smoking impacts on the progression and outcome of marginal periodontitis [46]; however, this is not yet clear in apical periodontitis [47, 48]. In this regard, a previous study reported that the frequency and severity of apical periodontitis were not affected by the smoking habits of individuals [47]. Though smoking habit was similarly distributed between groups in the present study, we explored whether smoking might influence TLR or MMP expression within AAP and SAP groups. Only MMP-2 was significantly lower in smokers compared to non-smokers AAP ($p < 0.05$), and a similar tendency was found in SAP, but non-significant. Supporting our results, a previous study reported lower of MMP-2 expression levels in smokers than non-smokers in (marginal) periodontitis patients [49]. Nonetheless further studies designed to assess the influence of tobacco on the levels of key inflammatory markers are needed.

Conclusions

TLR2 and TLR4 are overexpressed in apical lesions versus healthy periodontal ligament and correlate with collagenolytic MMPs. Particularly, TLR2 is overexpressed in SAP in association with MMP-1, MMP-8, and MMP-13. Our results suggest that the activation of TLR2 along with MMP overexpression might contribute to SAP clinical presentation and progression.

Acknowledgements We thank Peter Gebicke-Haerter and Andrés Tittarelli for their valuable support with bioanalyzer equipment. Alejandra Fernández thanks the fellowship CONICYT 21181377, from the Chilean Government.

Funding The work was supported by FONDECYT grant no. 1160741.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Menzies RA, Reiter AM, Lewis JR (2014) Assessment of apical periodontitis in dogs and humans: a review. J Vet Dent 31(1):8–21. https://doi.org/10.1177/089875641403100101
2. Croitoru IC, CraiToiu S, Petcu CM, Mihailescu OA, Pascu RM, Bobic AG, Agop Forna D, CraiToiu MM (2016) Clinical, imagistic and histopathological study of chronic apical periodontitis. Romanian J Morphol Embryol 57(2 Suppl):719–728
3. Salinas-Munoz M, Garrido-Flores M, Baeza M, Huaman-Chipana P, Garcia-Sesnich J, Bologna R, Vernal R, Hernandez M (2017) Bone resorptive activity in symptomatic and asymptomatic apical lesions of endodontic origin. Clin Oral Investig 21(8):2613–2618. https://doi.org/10.1007/s00784-017-2062-x
4. Desai SV, Love RM, Rich AM, Seymour GJ (2011) Toll-like receptor 2 expression in refractory periapical lesions. Int Endod J 44(10): 907–916. https://doi.org/10.1111/j.1365-2591.2011.01893.x
5. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, Takeda K, Akira S (1999) Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity 11(4):443–451
6. de Oliveira Rde C, Beghini M, Borges CR, Alves PM, de Araujo MS, Pereira SA, Rodrigues V Jr, Rodrigues DB (2014) Higher expression of galectin-3 and galectin-9 in periapical granulomas than in radicular cysts and an increased toll-like receptor-2 and toll-like receptor-4 expression are associated with reactivation of periapical inflammation. J Endod 40(2):199–203. https://doi.org/10.1016/j.joen.2013.10.031
7. Kassem A, Henning P, Lundberg P, Souza PP, Lindholm C, Lerner UH (2015) Porphyromonas gingivalis stimulates bone resorption by enhancing RANKL (receptor activator of NF-kappaB ligand)

through activation of toll-like receptor 2 in osteoblasts. J Biol Chem 290(33):20147–20158. https://doi.org/10.1074/jbc.M115.655787
8. Itoh K, Udagawa N, Kobayashi K, Suda K, Li X, Takami M, Okahashi N, Nishihara T, Takahashi N (2003) Lipopolysaccharide promotes the survival of osteoclasts via Toll-like receptor 4, but cytokine production of osteoclasts in response to lipopolysaccharide is different from that of macrophages. J Immunol 170(7):3688–3695
9. Tang L, Zhou XD, Wang Q, Zhang L, Wang Y, Li XY, Huang DM (2011) Expression of TRAF6 and pro-inflammatory cytokines through activation of TLR2, TLR4, NOD1, and NOD2 in human periodontal ligament fibroblasts. Arch Oral Biol 56(10):1064–1072. https://doi.org/10.1016/j.archoralbio.2011.02.020
10. Barreiros D, Nelson PF, Paula-Silva FWG, Oliveira KMH, Lucisano MP, Rossi A, Silva LAB, Kuchler EC, Silva RAB (2018) MMP2 and MMP9 are associated with apical periodontitis progression and might be modulated by TLR2 and MyD88. Braz Dent J 29(1):43–47. https://doi.org/10.1590/0103-6440201801731
11. Cavalla F, Hernández-Ríos P, Sorza T, Biguetti C, Hernández M (2017) Matrix metalloproteinases as regulators of periodontal inflammation. Int J Mol Sci 18(2):440
12. Paula-Silva FW, da Silva LA, Kapila YL (2010) Matrix metalloproteinase expression in teeth with apical periodontitis is differentially modulated by the modality of root canal treatment. J Endod 36(2):231–237. https://doi.org/10.1016/j.joen.2009.10.030
13. Lin SK, Chiang CP, Hong CY, Lin CP, Lan WH, Hsieh CC, Kuo MY (1997) Immunolocalization of interstitial collagenase (MMP-1) and tissue inhibitor of metalloproteinases-1 (TIMP-1) in radicular cysts. J Oral Pathol Med 26(10):458–463
14. Mundi Burgos V, Dezerega Piwonka A, Osorio Alfaro C, Dutzan Muñoz N, Franco Martínez ME, Ortega Pinto AV, Hernandez Rios M (2011) Inmunodetección de metaloproteinasas de matriz extracelular (MMPs) -2, -9, -13, -14 en lesiones apicales asociadas con periotontitis apical asintomática. Rev Clin Periodoncia Implantol Rehabil Oral 4(1):17–21
15. Baeza M, Garrido M, Hernandez-Rios P, Dezerega A, Garcia-Sesnich J, Strauss F, Aitken JP, Lesaffre E, Vanbelle S, Gamonal J, Brignardello-Petersen R, Tervahartiala T, Sorsa T, Hernandez M (2016) Diagnostic accuracy for apical and chronic periodontitis biomarkers in gingival crevicular fluid: an exploratory study. J Clin Periodontol 43(1):34–45. https://doi.org/10.1111/jcpe.12479
16. Pereira Faustino IS, Azevedo RS, Takahama A Jr (2016) Metalloproteinases 2 and 9 immunoexpression in periapical lesions from primary endodontic infection: possible relationship with the histopathological diagnosis and the presence of pain. J Endod 42(4):547–551. https://doi.org/10.1016/j.joen.2015.12.020
17. Agarwal S, Misra R, Aggarwal A (2010) Induction of metalloproteinases expression by TLR ligands in human fibroblast like synoviocytes from juvenile idiopathic arthritis patients. Indian J Med Res 131:771–779
18. Lisboa RA, Andrade MV, Cunha-Melo JR (2013) Toll-like receptor activation and mechanical force stimulation promote the secretion of matrix metalloproteinases 1, 3 and 10 of human periodontal fibroblasts via p38, JNK and NF-kB. Arch Oral Biol 58(6):731–739. https://doi.org/10.1016/j.archoralbio.2012.12.009
19. Gutmann JL, Baumgartner JC, Gluskin AH, Hartwell GR, Walton RE (2009) Identify and define all diagnostic terms for periapical/periradicular health and disease states. J Endod 35(12):1658–1674. https://doi.org/10.1016/j.joen.2009.09.028
20. Glickman GN (2009) AAE consensus conference on diagnostic terminology: background and perspectives. J Endod 35(12):1619–1620. https://doi.org/10.1016/j.joen.2009.09.029
21. Buonavoglia A, Latronico F, Pirani C, Greco MF, Corrente M, Prati C (2013) Symptomatic and asymptomatic apical periodontitis associated with red complex bacteria: clinical and microbiological evaluation. Odontology 101(1):84–88. https://doi.org/10.1007/s10266-011-0053-y
22. Hou L, Sasaki H, Stashenko P (2000) Toll-like receptor 4-deficient mice have reduced bone destruction following mixed anaerobic infection. Infect Immun 68(8):4681–4687
23. Rider D, Furusho H, Xu S, Trachtenberg AJ, Kuo WP, Hirai K, Susa M, Bahammam L, Stashenko P, Fujimura A, Sasaki H (2016) Elevated CD14 (cluster of differentiation 14) and toll-like receptor (TLR) 4 signaling deteriorate periapical inflammation in TLR2 deficient mice. Anat Rec (Hoboken) 299(9):1281–1292. https://doi.org/10.1002/ar.23383
24. Cavalla F, Reyes M, Vernal R, Alvarez C, Paredes R, Garcia-Sesnich J, Infante M, Farina V, Barron I, Hernandez M (2013) High levels of CXC ligand 12/stromal cell-derived factor 1 in apical lesions of endodontic origin associated with mast cell infiltration. J Endod 39(10):1234–1239. https://doi.org/10.1016/j.joen.2013.06.020
25. Garrido M, Dezerega A, Bordagaray MJ, Reyes M, Vernal R, Melgar-Rodriguez S, Ciuchi P, Paredes R, Garcia-Sesnich J, Ahumada-Montalva P, Hernandez M (2015) C-reactive protein expression is up-regulated in apical lesions of endodontic origin in association with interleukin-6. J Endod 41(4):464–469. https://doi.org/10.1016/j.joen.2014.12.021
26. Hernández P, Mäntylä P, Tervahartiala T, Sorsa T, Hernández M (2012) Análisis de MMPs en fluidos orales en el diagnóstico complementario de las enfermedades periodontales. Rev Clin Periodoncia Implantol Rehabil Oral 5(3):150–153
27. Sbardella D, Fasciglione GF, Gioia M, Ciaccio C, Tundo GR, Marini S, Coletta M (2012) Human matrix metalloproteinases: an ubiquitarian class of enzymes involved in several pathological processes. Mol Asp Med 33:119–208. https://doi.org/10.1016/j.mam.2011.10.015
28. Buzoglu HD, Unal H, Ulger C, Mert S, Kucukyildirim S, Er N (2009) The zymographic evaluation of gelatinase (MMP-2 and -9) levels in acute and chronic periapical abscesses. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 108(5):e121–e126. https://doi.org/10.1016/j.tripleo.2009.07.014
29. Letra A, Ghaneh G, Zhao M, Ray H Jr, Francisconi CF, Garlet GP, Silva RM (2013) MMP-7 and TIMP-1, new targets in predicting poor wound healing in apical periodontitis. J Endod 39(9):1141–1146. https://doi.org/10.1016/j.joen.2013.06.015
30. Ahmed GM, El-Baz AA, Hashem AA, Shalaan AK (2013) Expression levels of matrix metalloproteinase-9 and gram-negative bacteria in symptomatic and asymptomatic periapical lesions. J Endod 39(4):444–448. https://doi.org/10.1016/j.joen.2012.11.009
31. Kawasaki Y, Xu ZZ, Wang X, Park JY, Zhuang ZY, Tan PH, Gao YJ, Roy K, Corfas G, Lo EH, Ji RR (2008) Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. Nat Med 14(3):331–336. https://doi.org/10.1038/nm1723
32. Corotti MV, Zambuzzi WF, Paiva KB, Menezes R, Pinto LC, Lara VS, Granjeiro JM (2009) Immunolocalization of matrix metalloproteinases-2 and -9 during apical periodontitis development. Arch Oral Biol 54(8):764–771. https://doi.org/10.1016/j.archoralbio.2009.04.013
33. Hernandez M, Dutzan N, Garcia-Sesnich J, Abusleme L, Dezerega A, Silva N, Gonzalez FE, Vernal R, Sorsa T, Gamonal J (2011) Host-pathogen interactions in progressive chronic periodontitis. J Dent Res 90(10):1164–1170. https://doi.org/10.1177/0022034511401405
34. Hadziabdic N, Kurtovic-Kozaric A, Pojskic N, Sulejmanagic N, Todorovic L (2016) Gene-expression analysis of matrix metalloproteinases 1 and 2 and their tissue inhibitors in chronic periapical inflammatory lesions. J Oral Pathol Med 45(3):224–230. https://doi.org/10.1111/jop.12347
35. Trombone AP, Cavalla F, Silveira EM, Andreo CB, Francisconi CF, Fonseca AC, Letra A, Silva RM, Garlet GP (2016) MMP1-1607 polymorphism increases the risk for periapical lesion development

through the upregulation MMP-1 expression in association with pro-inflammatory milieu elements. J Appl Oral Sci 24(4):366–375. https://doi.org/10.1590/1678-775720160112

36. Shin SJ, Lee W, Lee JI, Baek SH, Kum KY, Shon WJ, Bae KS (2011) Matrix metalloproteinase-8 and substance P levels in gingival crevicular fluid during endodontic treatment of painful, nonvital teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 112(4): 548–554. https://doi.org/10.1016/j.tripleo.2011.04.026
37. Kuula H, Salo T, Pirila E, Tuomainen AM, Jauhiainen M, Uitto VJ, Tjaderhane L, Pussinen PJ, Sorsa T (2009) Local and systemic responses in matrix metalloproteinase 8-deficient mice during porphyromonas gingivalis-induced periodontitis. Infect Immun 77(2):850–859. https://doi.org/10.1128/IAI.00873-08
38. Hernandez M, Valenzuela MA, Lopez-Otin C, Alvarez J, Lopez JM, Vernal R, Gamonal J (2006) Matrix metalloproteinase-13 is highly expressed in destructive periodontal disease activity. J Periodontol 77(11):1863–1870. https://doi.org/10.1902/jop.2006.050461
39. Matsui H, Yamasaki M, Nakata K, Amano K, Nakamura H (2011) Expression of MMP-8 and MMP-13 in the development of periradicular lesions. Int Endod J 44(8):739–745. https://doi.org/10.1111/j.1365-2591.2011.01880.x
40. Hong CY, Lin SK, Kok SH, Cheng SJ, Lee MS, Wang TM, Chen CS, Lin LD, Wang JS (2004) The role of lipopolysaccharide in infectious bone resorption of periapical lesion. J Oral Pathol Med 33(3):162–169
41. Al-Rashed F, Kochumon S, Usmani S, Sindhu S, Ahmad R (2017) Pam3CSK4 induces MMP-9 expression in human monocytic THP-1 cells. Cell Physiol Biochem 41(5):1993–2003. https://doi.org/10.1159/000475298
42. Ahmad R, Shihab PK, Jasem S, Behbehani K (2014) FSL-1 induces MMP-9 production through TLR-2 and NF-kappaB /AP-1 signaling pathways in monocytic THP-1 cells. Cell Physiol Biochem 34(3):929–942. https://doi.org/10.1159/000366310
43. Lim R, Barker G, Lappas M (2014) The TLR2 ligand FSL-1 and the TLR5 ligand flagellin mediate pro-inflammatory and pro-labour response via MyD88/TRAF6/NF-kappaB-dependent signalling. Am J Reprod Immunol 71(5):401–417. https://doi.org/10.1111/aji.12229
44. Osorio C, Cavalla F, Paula-Lima A, Diaz-Araya G, Vernal R, Ahumada P, Gamonal J, Hernandez M (2015) H2 O2 activates matrix metalloproteinases through the nuclear factor kappa B pathway and Ca(2+) signals in human periodontal fibroblasts. J Periodontal Res 50(6):798–806. https://doi.org/10.1111/jre.12267
45. Sindhu S, Al-Roub A, Koshy M, Thomas R, Ahmad R (2016) Palmitate-induced MMP-9 expression in the human monocytic cells is mediated through the TLR4-MyD88 dependent mechanism. Cell Physiol Biochem 39(3):889–900. https://doi.org/10.1159/000447798
46. Nociti FH Jr, Casati MZ, Duarte PM (2015) Current perspective of the impact of smoking on the progression and treatment of periodontitis. Periodontol 67(1):187–210. https://doi.org/10.1111/prd.12063
47. Bergstrom J, Babcan J, Eliasson S (2004) Tobacco smoking and dental periapical condition. Eur J Oral Sci 112(2):115–120. https://doi.org/10.1111/j.1600-0722.2004.00112.x
48. Persic Bukmir R, Jurcevic Grgic M, Brumini G, Spalj S, Pezelj-Ribaric S, Brekalo Prso I (2016) Influence of tobacco smoking on dental periapical condition in a sample of Croatian adults. Wien Klin Wochenschr 128(7–8):260–265. https://doi.org/10.1007/s00508-015-0910-8
49. Senturk RA, Sezgin Y, Bulut S, Ozdemir BH (2018) The effects of smoking on the expression of gelatinases in chronic periodontitis: a cross-sectional study. Braz Oral Res 32:e114. https://doi.org/10.1590/1807-3107bor-2018.vol32.0114