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ORIGINAL ARTICLE

Regulation of matrix metalloproteinase-1 by *Filifactor alocis* in human gingival and monocytic cells

Marjan Nokhbehsaim[1] · Andressa V. B. Nogueira[2] · Anna Damanaki[2] · Georgia Dalagiorgou[3] · Sigrun Eick[4] · Christos Adamopoulos[3] · Christina Piperi[3] · Efthimia K. Basdra[3] · Athanasios G. Papavassiliou[3] · James Deschner[2]

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Abstract

Objectives Periodontitis is a highly prevalent chronic inflammatory disease caused by periodontopathogens, such as *Filifactor alocis*. This study sought to examine the matrix metalloproteinase (MMP)-1 synthesis by monocytic and fibroblastic cells in response to *F. alocis* and to unravel the underlying cellular mechanisms.

Material and methods Gingival biopsies from periodontally healthy and periodontitis individuals were analyzed for the presence of *F. alocis* and MMP-1 by RT-PCR. Human gingival fibroblastic (HGF-1) and monocytic (THP-1) cells were stimulated with *F. alocis* in the presence and absence of a blocking toll-like receptor (TLR)2 antibody or specific inhibitors against MAPKs. MMP-1 expression and protein levels were studied by RT-PCR and ELISA, respectively.

Results *F. alocis* was highly prevalent in biopsies from periodontitis patients but barely present in the healthy gingiva. Significantly higher MMP-1 expression levels were found in the inflamed gingiva as compared with healthy biopsies. *F. alocis* caused a significant and dose-dependent MMP-1 upregulation in both cells. The stimulatory effect of *F. alocis* on MMP-1 was TLR2- and MAPK-dependent and more pronounced on THP-1 cells as compared with HGF-1 cells.

Conclusions Our results demonstrate that *F. alocis* and MMP-1 are more prevalent at periodontitis sites. Additionally, our study provides original evidence that *F. alocis* can stimulate MMP-1 production by fibroblastic and monocytic cells, suggesting that *F. alocis* may contribute to periodontal breakdown through MMP-1.

Clinical relevance *F. alocis* and MMP-1 are linked to each other and key players in periodontitis, which may have significant implications for future diagnostic and treatment strategies.

Keywords Periodontitis · *Filifactor alocis* · MMP-1 · Gingival fibroblast · Monocytes

Introduction

Periodontitis is a highly prevalent chronic inflammatory disease driven by a dysbiotic oral microbiota present in the dental biofilm [1]. It occurs as a result of complex interactions between the periodontopathic bacteria and the host response. The pathogenic bacteria along with their components and metabolic products activate the host immune system, resulting in an increased synthesis and release of proinflammatory mediators as well as tissue proteolytic enzymes such as matrix metalloproteinases (MMPs). The presence and persistence of this immunoinflammatory process in the periodontium leads to the destruction of the tooth-supporting tissues due to irreversible degradation of extracellular matrix and resorption of alveolar bone [1–3]. Periodontitis onset has been associated with the "classical" periodontopathogens such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum* [4]. *Filifactor alocis*, a gram-positive anaerobic bacterium, has been recognized as another significant periodontopathogen [5]. *F. alocis* has been considered one of the most prevalent pathogens in periodontitis and

✉ Andressa V. B. Nogueira
a.nogueira@uni-mainz.de

1 Section of Experimental Dento-Maxillo-Facial Medicine, Center of Dento-Maxillo-Facial Medicine, University of Bonn, Bonn 53111, Germany

2 Department of Periodontology and Operative Dentistry, University Medical Center of the Johannes Gutenberg University, Augustusplatz 2, 55131 Mainz, Germany

3 Department of Biological Chemistry, Medical School, National and Kapodistrian University of Athens, 157 72 Athens, Greece

4 Department of Periodontology, Laboratory of Oral Microbiology, University of Bern, Bern 3010, Switzerland

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mainly detected in the apical and middle thirds of the periodontal pocket [6]. In addition to the dental biofilm, saliva of periodontally diseased patients has also been demonstrated to contain *F. alocis* [7–9]. *F. alocis* possesses great resistance to oxidative stress, which facilitates survival and persistence of this pathogen in periodontal diseases [5]. *F. alocis* has been demonstrated to stimulate periodontal cells to release proinflammatory mediators [10, 11]. Moreover, *F. alocis* in combination with *P. gingivalis* has the ability to form a biofilm and to invade epithelial cells [12]. MMPs are a group of proteinases responsible for tissue remodeling and degradation of extracellular matrix. These proteases also play a key part in periodontal soft tissue degradation and hard tissue resorption [13]. MMP-1 is a collagenase and, in addition to its role in periodontal destruction, is also involved in many physiological processes such as embryonic development, reproduction, tissue remodeling, and pathophysiological conditions [14]. MMP-1 can degrade extracellular matrix such as types I and III collagen fibers, which are the most common components of the periodontal tissue matrix [14, 15]. Several studies have demonstrated increased MMP-1 levels in gingival crevicular fluid and gingival tissues of periodontitis patients as compared with periodontally healthy individuals [15–17]. In addition, MMP-1 levels are significantly reduced after periodontal treatment [16, 18, 19], proving the critical role of this protease in the etiopathogenesis of periodontal diseases. Although *F. alocis* seems to be strongly associated with periodontitis, it has yet to be elucidated whether this periodontopathogen can induce the production of MMPs by professional and accessory inflammatory cells in the periodontium. Therefore, the aim of this study was to examine the MMP-1 synthesis by monocytic and fibroblastic cells in response to *F. alocis* and to unravel the underlying cellular mechanisms. A better understanding of the pathogenic role and mechanisms of *F. alocis* could result in novel diagnostic and treatment strategies.

Materials and methods

Culture and treatment of cells

The human gingival fibroblast cell line (HGF-1) (ATCC® CRL-2014™) was purchased from LGC Standards (Wesel, Germany). The cryovial containing frozen cells was quickly thawed in a 37 °C water bath, and the cell suspension was carefully transferred to a centrifuge tube containing 9.0 ml of Dulbecco's minimal essential medium (DMEM, Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (FBS, Invitrogen), 100 units penicillin, and 100 μg/ml streptomycin (Invitrogen) and centrifuged at 125×*g* for 5 min at room temperature. After removal of the supernatant, the cell pellet was resuspended with growth medium and dispensed into a 75-cm^2 culture flask. For experiments, cells were seeded on 6-well cell culture plates (5×10^4 cells/well) and grown to 80% confluence.

The human acute monocytic leukemia cell line (THP-1) was purchased from CLS Cell Lines Service (Eppelheim, Germany). The cells from the cryovial were quickly thawed in a 37 °C water bath and transferred to a centrifuge tube containing 8 ml of culture medium and centrifuged at 300×*g* for 3 min. Afterwards, the supernatant was removed and the cell pellet was resuspended in RPMI 1640 medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% FBS, 100 units penicillin, and 100 μg/ml streptomycin. For experiments, 1×10^6 cells/ml were seeded to the culture plates.

Both types of cells were maintained in a humidified atmosphere of 5% CO_2 at 37 °C. The cell culture media was changed every other day, and the FBS concentration was reduced to 1% 1 day prior to the experiments. Cells were stimulated with the oral pathogenic bacterium *F. alocis* (ATCC 35896™), which was used as a lysate at different concentrations (OD_{660} 0.05, 0.1, and 0.2). Initially, the bacteria were precultivated on Schaedler agar plates (Oxoid, Basingstoke, UK) for 48 h in an anaerobic atmosphere and subsequently suspended in phosphate-buffered saline ($OD_{660nm} = 1$, equivalent to 1.2×10^9 bacterial cells/ml). Finally, bacteria were resuspended and lysed twice by ultrasonication (160 W for 15 min) resulting in complete bacterial killing. To unravel the mechanisms underlying the stimulatory effect of *F. alocis* on MMP-1 expression, cells were pretreated with a blocking toll-like receptor (TLR)2 antibody (anti-human TLR2 monoclonal antibody, eBioscience, San Diego, CA, USA) in a concentration of 10 μg/ml 45 min prior to stimulation with *F. alocis*. In further experiments, cells were preincubated with specific inhibitors of different pathways (MEK1/MEK2: U0126, 10 μM; p38: SB203580, 10 μM; JNK: SP600125, 10 μM; all purchased from Calbiochem, San Diego, CA, USA) 1 h prior to stimulation with *F. alocis* in order to unravel the intracellular mechanisms underlying the actions of *F. alocis* on MMP-1 regulation.

Human biopsies

The gingiva was obtained from 10 periodontally healthy donors and 10 periodontitis patients during wisdom tooth removal and dental extraction for periodontal reasons, respectively, in the Department of Oral Surgery at the University of Bonn [20]. Exclusion criteria were the presence of systemic diseases, use of medication as well as smoking. In order to distinguish between periodontal health and disease, gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL), and radiographic bone loss were assessed. When GI = 0 (no clinical inflammation), PPD ≤ 3 mm, no CAL, and no radiographic bone loss were found, the site

was categorized as periodontally healthy. Sites were classified as periodontitis, when GI > 1, PD ≥ 5 mm, CAL ≥ 3 mm, and radiographic bone loss were observed. Written informed consent and approval of the Ethics Committee of the University of Bonn were obtained (#043/11).

Hematoxylin and eosin staining

Gingival biopsies from both groups were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 2 days, followed by hydration and dehydration in an ascending ethanol series (AppliChem, Darmstadt, Germany), and subsequently embedded in paraffin (McCormick Scientific, Richmond, IL, USA). Next, tissue sections of 2.5-μm thickness were sliced, mounted on glass slides (Carl Roth), and dried at 37 °C overnight. Then, all sections were stained with hematoxylin and eosin (H&E) (Merck Eurolab, Darmstadt, Germany), dehydrated, and mounted with DePeX (SERVA Electrophoresis GmbH, Heidelberg, Germany). Finally, stained tissue sections were used to analyze the presence or absence of gingival inflammation by using an Axioskop 2 microscope (Carl Zeiss, Jena, Germany) with an AxioCam MRc camera and the AxioVision 4.7 software (Carl Zeiss).

Endpoint polymerase chain reaction

Total RNA was isolated from all gingival biopsies using the RNeasy Mini Kit (Qiagen, CA, USA) according to manufacturer's instructions. RNA concentration was determined by a NanoDrop ND-2000 (Thermo Fisher Scientific, Wilmington, DE, USA) spectrophotometer. Five hundred nanograms of total RNA was reversely transcribed, and cDNA was synthesized using the iScript™ Select cDNA Synthesis Kit (Bio-Rad Laboratories, Munich, Germany) at 42 °C for 90 min followed by 85 °C for 5 min. Two biopsies from each group were used for endpoint polymerase chain reaction (PCR) analysis, which was performed with a specific primer for *F. alocis* 16S rDNA (forward, 5′-CAGGTGGTTTAACAAGTTAG TGG-3′; reverse, 5′-CTAAGTTGTCCTTAGCTGTCTCG-3′, Microsynth AG, Balgach, Switzerland) [21] using the Maxima Hot Start Green PCR Master Mix K1061 (Thermo Scientific) following the manufacturer's instructions. Human actin (forward, 5′-GTGGGGCGCCCAGG CACCA-3′; reverse, 5′-CTCCTTAATGTCACGCACGA TTTC-3′) was used as a control for tissue. Further, as positive control, RNA extracted from *F. alocis* ATCC 35896 24-h culture was used. Reaction products were detected and verified for size on a 2% agarose gel with size markers (New England Biolabs, MA, USA) and stained with ethidium bromide. The intensity of density area was analyzed using the Quantity-One Program (Bio-Rad Laboratories, CA, USA).

Real-time RT-PCR

Quantitative RT-PCR was used in order to analyze MMP-1 mRNA expression and *F. alocis* in human gingival biopsies and MMP-1 mRNA expression in the HGF-1 and THP-1 cell lines in the presence and absence of *F. alocis*. Total RNA was extracted and reversely transcribed as described above. The gene expression of MMP-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as housekeeping gene as well as *F. alocis* was evaluated by quantitative RT-PCR using an iCycler iQ™ Real-Time PCR Detection System (Bio-Rad) and specific primers (QuantiTect Primer Assay, Qiagen). The 25-μl PCR reaction mixture containing 1 μl of cDNA was amplified as a template in 12.5 μl of SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad), 2.5 μl of primers, and RNase free water. The PCR mixture was heated initially at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 10 s and combined annealing/extension at 60 °C for 30 s. Gene expression data analysis was performed by using the comparative threshold cycle (CT) method.

ELISA

The levels of MMP-1 in HGF-1 or THP-1 cell supernatants were determined by using a commercially available enzyme-linked immunoassay (ELISA) detection kit (RayBiotech, Norcross, GA, USA) according to the manufacturer's instructions. The MMP-1 concentration was measured by spectrophotometry with a microplate reader (PowerWave X, BioTek Instruments, Winooski, VT, USA) at 450 nm with wavelength correction at 540 nm. Data were normalized by total protein concentration using Pierce BCA Protein Assay Kit (23227, Thermo Scientific, Pierce Biotechnology, Rockford, USA).

Statistical analysis

The statistical analysis was performed with the IBM SPSS Statistics 22 software. Mean values and standard errors of the mean (SEM) were calculated. Parametric (ANOVA followed by the post hoc Dunnett and Tukey's tests) and non-parametric (Wilcoxon and Mann-Whitney *U* tests) tests were used for statistical analyses. Differences between groups were considered significant at p value < 0.05.

Experiments were performed in triplicate and repeated at least twice.

Results

Presence of *F. alocis* and MMP-1 in human gingival biopsies

First, we sought to determine the presence of *F. alocis* and MMP-1 in gingival tissues from periodontally healthy subjects and periodontitis patients. As shown in Fig. 1a, b, gingival biopsies from periodontitis subjects demonstrated a pronounced immunoinflammatory infiltrate, as compared with the periodontally healthy gingiva. As analyzed by endpoint RT-PCR, *F. alocis* was present in gingiva samples from periodontitis patients. By contrast, no signals for this pathogen could be detected in the healthy gingiva (Fig. 1c). Further analysis by real-time RT-PCR confirmed the high prevalence of *F. alocis* in the inflamed gingiva (Fig. 1d). Although *F. alocis* was also detected in the periodontally healthy gingiva by real-time RT-PCR, the difference between the samples from both groups was significant ($p < 0.05$). Next, we studied the presence of MMP-1 in the gingival samples. As demonstrated in Fig. 1e, MMP-1 was found in the gingiva from both periodontally healthy and diseased subjects. However, the MMP-1 expression in the biopsies from periodontitis patients was significantly ($p < 0.05$) up-regulated by 15-fold (Fig. 1e).

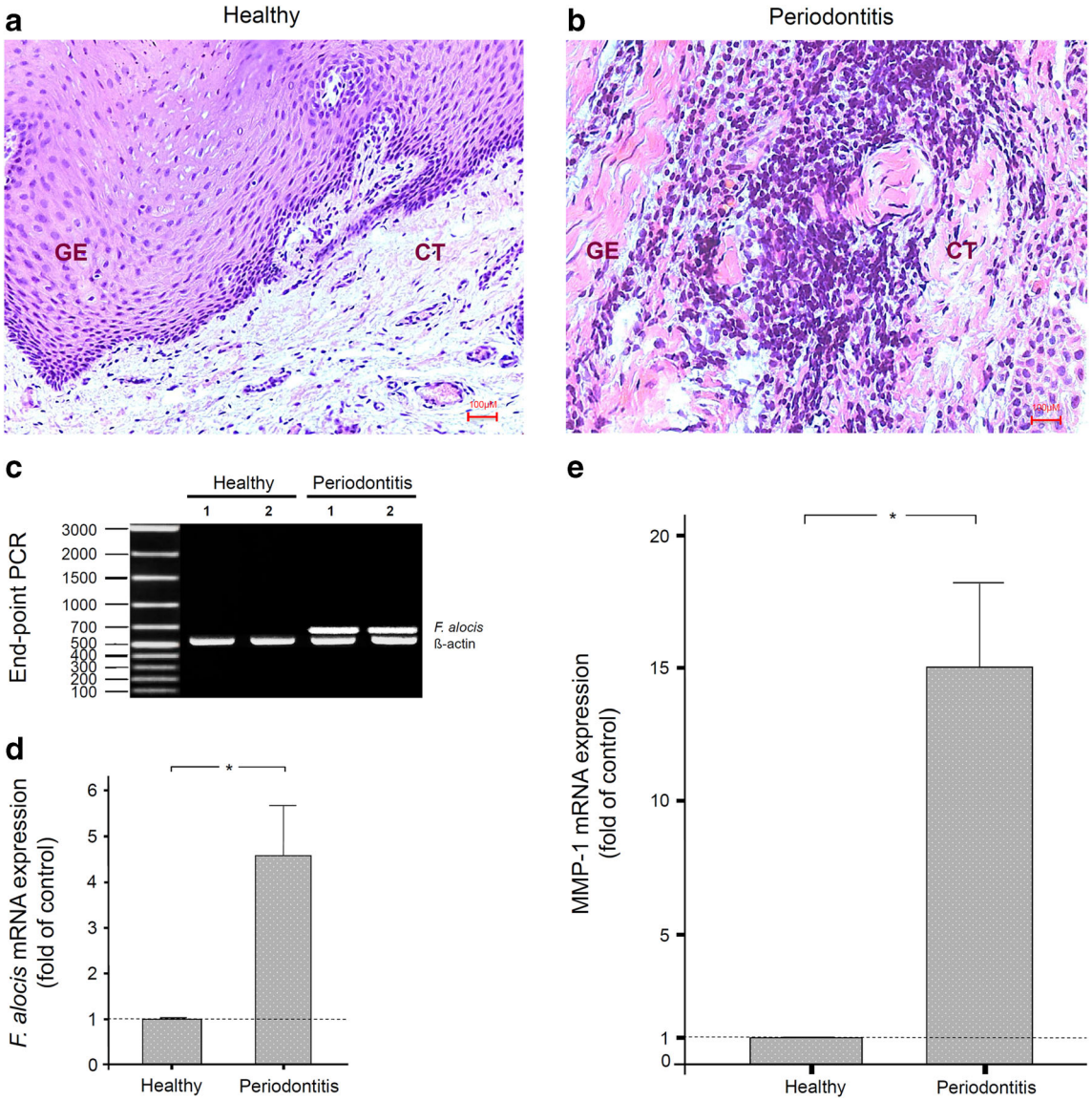

Fig. 1 Representative histological sections of gingival tissues stained with H&E from periodontally healthy subjects (**a**) and periodontitis patients (**b**). Presence or absence of *F. alocis* in gingival tissues of two representative periodontally healthy subjects and two representative periodontitis patients, as analyzed by endpoint RT-PCR (**c**). Levels of *F. alocis* (**d**) and MMP-1 (**e**) in the gingiva from periodontally healthy subjects ($n = 10$) and periodontitis patients ($n = 10$), as analyzed by real-time RT-PCR. Mean ± SEM. *Significant ($p < 0.05$) difference between groups

Regulation of MMP-1 by *F. alocis* in HGF-1 cells

Then, we studied the expression of MMP-1 in the presence and absence of the *F. alocis* in HGF-1 cells. *F. alocis* caused a significant ($p < 0.05$) increase in the MMP-1 expression at 1 and 2 days, as analyzed by real-time RT-PCR (Fig. 2a). Moreover, the stimulatory effect of *F. alocis* on the MMP-1 expression at 1 day was dose-dependent, with the highest MMP-1 expression level at an OD_{660} of 0.1 (Fig. 2b). Our findings at transcriptional level were paralleled at protein level, as demonstrated in Fig. 2c. The MMP1 protein levels were significantly ($p < 0.05$) higher in supernatants from *F. alocis*–stimulated HGF-1 cells as compared with those from untreated cells at 2 days, as examined by ELISA (Fig. 2c).

Regulation of MMP-1 by *F. alocis* in THP-1 cells

We also investigated the MMP-1 expression and protein levels in response to *F. alocis* in THP-1 cells. Like in HGF-1 cells, *F. alocis* caused a significant ($p < 0.05$) upregulation of MMP-1 in THP-1 cells at 1 and 2 days (Fig. 3a). However, the stimulatory effect was more pronounced in comparison with HGF-1 cells (Figs. 2a and 3a). Furthermore, the action of *F. alocis* on the MMP-1 expression was dose-dependent at 1 day, with the highest MMP-1 expression at the highest *F. alocis* concentration (Fig. 3b). Exposure of THP-1 cells to *F. alocis* was also associated with significantly increased MMP-1 protein levels in the supernatants at 2 days, as determined by ELISA (Fig. 3c).

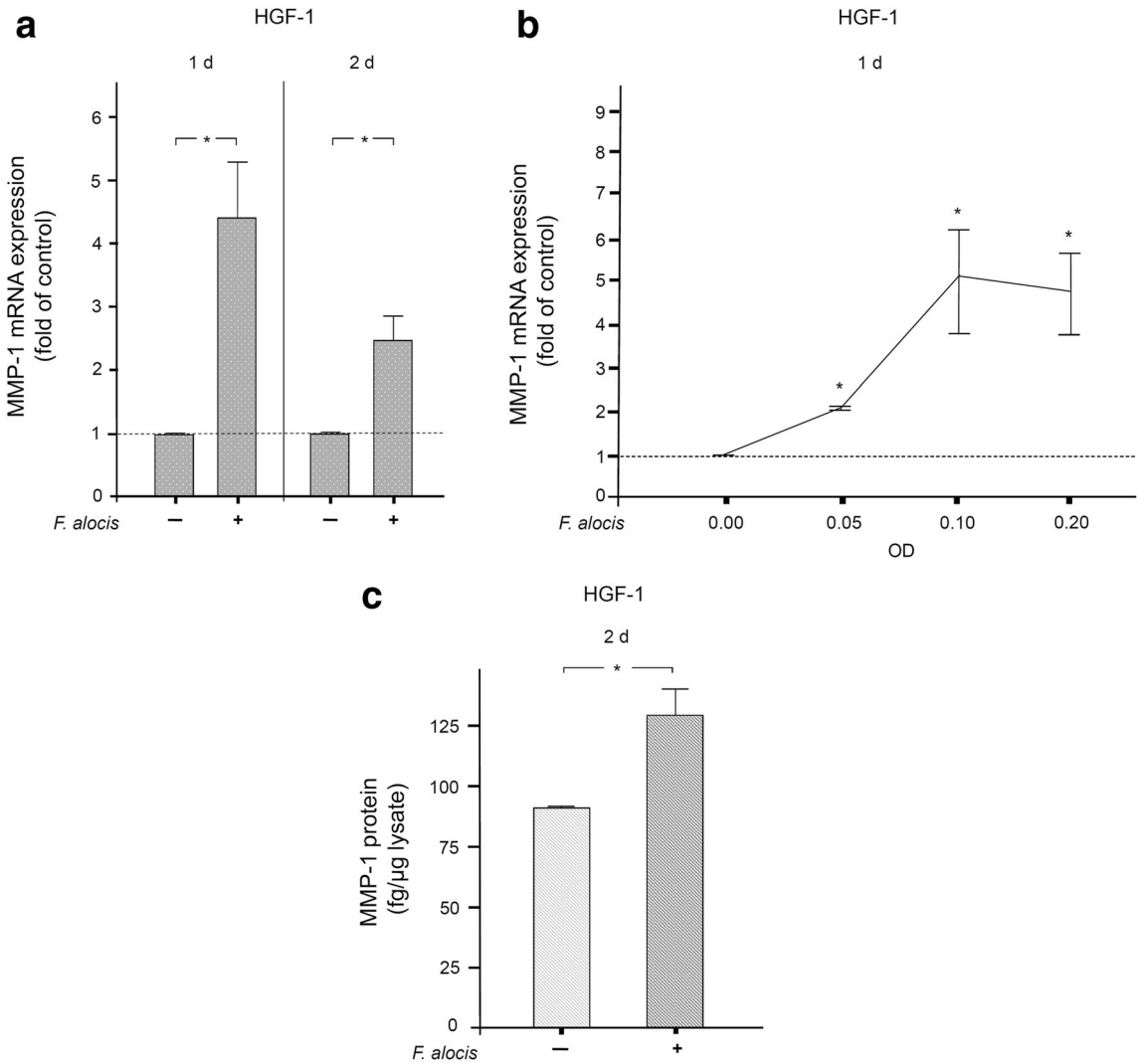

Fig. 2 MMP-1 gene expression in the presence and absence of *F. alocis* (OD_{660} 0.1) in HGF-1 cells at 1 day and 2 days, as analyzed by real-time RT-PCR (**a**). Mean ± SEM ($n = 12$). *Significant ($p < 0.05$) difference between groups. MMP-1 gene expression in response to various concentrations of *F. alocis* (OD_{660} 0.05, 0.1, and 0.2) in HGF-1 cells at 1 day, as analyzed by real-time RT-PCR (**b**). Unstimulated cells served as the control. Mean ± SEM ($n = 3$). *Significantly ($p < 0.05$) different from the control. MMP-1 protein level in supernatants of HGF-1 cells in the presence and absence of *F. alocis* (OD_{660} 0.1) at 2 days, as analyzed by ELISA (**c**). Mean ± SEM ($n = 18$). *Significant ($p < 0.05$) difference between groups

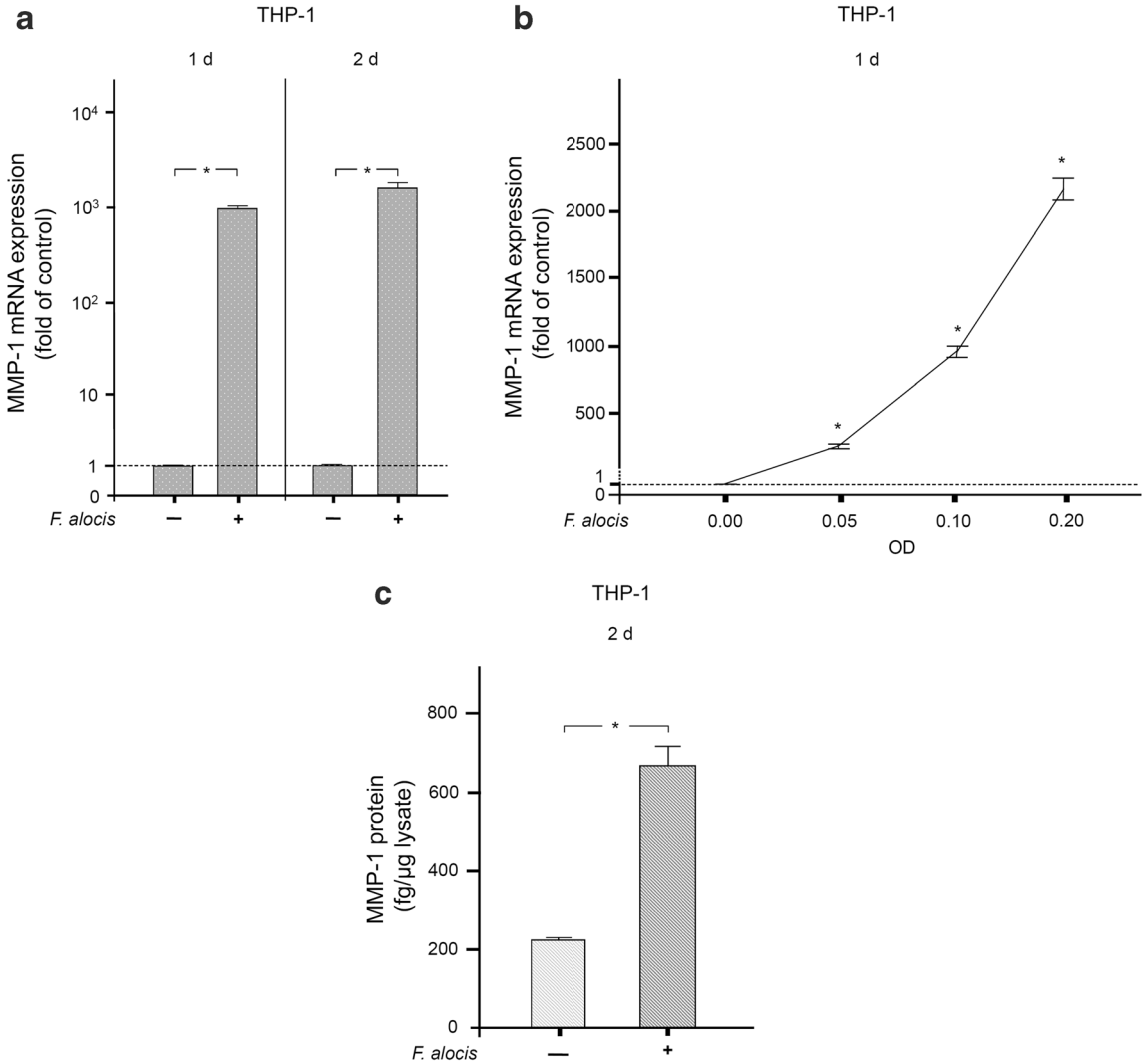

Fig. 3 MMP-1 gene expression in the presence and absence of *F. alocis* (OD_{660} 0.1) in THP-1 cells at 1 day and 2 days, as analyzed by real-time RT-PCR (**a**). Mean ± SEM ($n = 6$). *Significant ($p < 0.05$) difference between groups. MMP-1 gene expression in response to various concentrations of *F. alocis* (OD_{660} 0.05, 0.1, and 0.2) in THP-1 cells at 1 day, as analyzed by real-time RT-PCR (**b**). Unstimulated cells served as the control. Mean ± SEM ($n = 18$). *Significantly ($p < 0.05$) different from the control. MMP-1 protein level in supernatants of THP-1 cells in the presence and absence of *F. alocis* (OD_{660} 0.1) at 2 days, as analyzed by ELISA (**c**). Mean ± SEM ($n = 18$). *Significant ($p < 0.05$) difference between groups.

Involvement of TLR2 and intracellular pathways in the regulatory effects of *F. alocis*

First, we sought to clarify whether the effects of *F. alocis* on MMP-1 are mediated through the TLR2. As demonstrated in Fig. 4a, b, *F. alocis* caused a significant ($p < 0.05$) upregulation of MMP-1 in both HGF-1 and THP-1 cells. Interestingly, when both cell lines were preincubated with an anti-TLR2 blocking antibody, the stimulatory effect of *F. alocis* on MMP-1 was completely suppressed ($p < 0.05$) for HGF-1 cells and also strongly abolished ($p < 0.05$) for THP-1 cells at 1 day, as shown in Fig. 4a, b. Next, we studied the involvement of classical intracellular pathways associated with inflammation. Preincubation of HGF-1 and THP-1 cells with specific inhibitors for MEK1/MEK2 (U0126), p38 (SB203580), and JNK (SP600125) resulted in a significant ($p < 0.05$) downregulation of the *F. alocis*–induced MMP-1 upregulation (Fig. 4c, d). The most pronounced inhibitory effect on the *F. alocis*–stimulated MMP-1 expression was observed for the MEK1/MEK2 inhibitor in HGF-1 and THP-1 cells and also for the JNK inhibitor in THP-1 cells (Fig. 4c, d).

Discussion

The present study demonstrates that the periodontopathogen *F. alocis* and MMP-1 are more prevalent in the periodontally diseased gingiva. Moreover, our study shows for the first time that *F. alocis* is able to stimulate MMP-1 expression and protein synthesis in fibroblastic and monocytic cells, suggesting

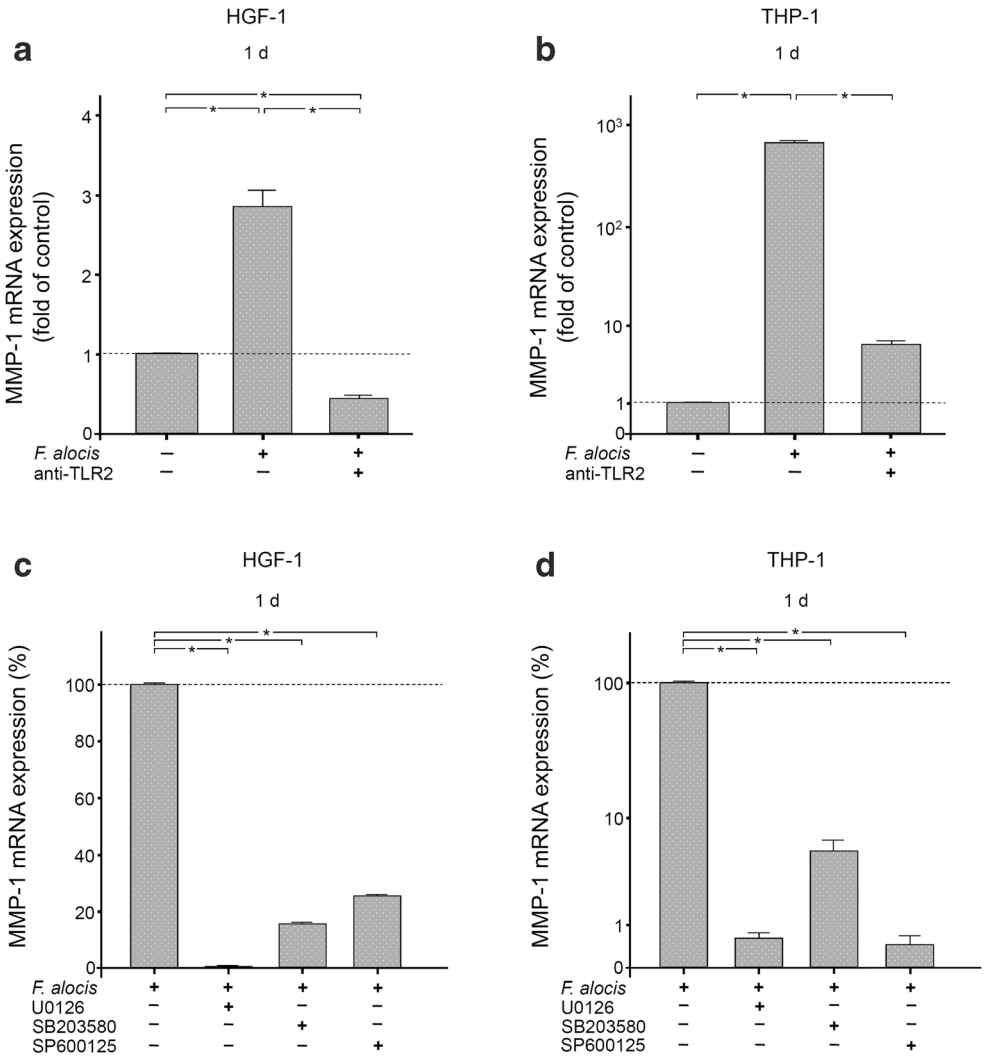

Fig. 4 MMP-1 gene expression in response to *F. alocis* (OD_{660} 0.1) in the presence and absence of an anti-TLR2 blocking antibody in HGF-1 (**a**) and THP-1 (**b**) cells at 1 day, as analyzed by real-time RT-PCR. Unstimulated HGF-1 and THP-1 cells served as the control. Mean ± SEM ($n = 3$). *Significant ($p < 0.05$) difference between groups. MMP-1 gene expression in response to *F. alocis* (OD_{660} 0.1) in the presence of specific inhibitors against MEK1/MEK2 (U0126; 10 μM), p38 (SB203580; 10 μM), and JNK (SP600125; 10 μM) in HGF-1 (**c**) and THP-1 (**d**) cells at 1 day, as analyzed by real-time RT-PCR. *F. alocis*-stimulated cells served as the control. Mean ± SEM ($n = 3$). *Significantly ($p < 0.05$) different from the control

that *F. alocis* may contribute to periodontal tissue destruction through the increase in this protease. Furthermore, the stimulatory effect of *F. alocis* on MMP-1 is much stronger on THP-1 cells as compared with HGF-1 cells.

The periodontopathogen *F. alocis* is a gram-positive, asaccharolytic, anaerobic bacterium with a high prevalence in periodontitis, which was confirmed in our study [5]. *F. alocis* has a number of virulence factors, enabling this bacterium in concert with other periodontopathogens to activate the host response [22]. As a result, a great number of proinflammatory mediators and proteases are released by professional and accessory immunoinflammatory cells. These molecules are mainly responsible for the soft tissue degradation and bone resorption in periodontitis. Key molecules in the periodontal breakdown are MMPs, such as MMP-1, which can degrade fibrillar collagens, mainly types I and III, which are the most prevalent extracellular matrix components in the gingiva and periodontal ligament [15, 23]. In addition, MMP-1 is capable of activating other proteases and cleaving chemokines such as monocyte chemoattractant proteins [24]. Increased MMP-1 levels have been measured in gingival crevicular fluid and the gingiva of periodontitis patients [15–17]. In addition, MMP-1 levels are decreased again after periodontal therapy [16, 18]. Our study could confirm the elevated MMP-1 expression in gingival tissues at sites of periodontitis, emphasizing the critical role of this protease in the etiopathogenesis of periodontitis. Coexistence of *F. alocis* and MMP-1 at sites of periodontitis prompted us to investigate whether *F. alocis* would be able to induce the production of MMP-1 in structural and infiltrating periodontal cells. Therefore, we analyzed the regulatory effect of *F. alocis* in fibroblastic (HGF-1) and monocytic (THP-1) cells, which play an important role in periodontal health and disease. Our experiments provide original evidence that *F. alocis* induces an increased MMP-1 expression and protein synthesis in both HGF-1 and THP-1 cells, suggesting that the upregulated MMP-1 expression in inflamed gingiva of periodontitis patients is, at least partly, caused by this pathogen. Interestingly, our results also revealed that the THP-1 cells, which mean the professional inflammatory cells, can mount a stronger response to *F. alocis*. Thus, this interesting observation may underline

the critical role of professional inflammatory cells in periodontal matrix destruction. Recently, it has been reported that *F. alocis* possesses a collagenolytic activity as a virulence factor [25]. Therefore, our data suggest that *F. alocis* can contribute to the proteolytic activity of sites of periodontitis directly by its own collagenolytic activity but also indirectly by stimulation of periodontal cells to produce MMP-1.

In the present study, the high levels of MMP-1 detected in *F. alocis*–stimulated HGF-1 and THP-1 cells were TLR2-dependent, demonstrating that at least this TLR is involved in the proteolytic actions of *F. alocis*. Unfortunately, the current knowledge on cell wall components of *F. alocis* is limited. Therefore, it can only be speculated that lipoteichoic acid and/or peptidoglycan, like in other gram-positive bacteria, may have interacted with TLR2 and thereby triggered the observed effects of *F. alocis* in our study. We then also focused on the downstream events occurring after receptor engagement. Interestingly, preincubation of HGF-1 and THP-1 cells with specific blockers against MEK1/2, JNK, or p38 resulted in inhibition of the *F. alocis*–upregulated MMP-1 expression, suggesting that the MAPK signaling is strongly involved in the stimulatory actions of *F. alocis* on MMP-1. However, other intracellular pathways might also have been used by *F. alocis* and should be considered in further studies. Recently, it has been shown that interaction of *F. alocis* with TLR2 causes granule exocytosis along with a transient ERK1/2 and sustained p38 MAPK activation in neutrophils, which further supports our observation that *F. alocis* exploits TLR2 and MAPKs for its cellular actions [26].

F. alocis was selected for our experiments due to its implication in periodontal diseases. So far, little information on its etiopathogenetic role and actions in periodontitis exist as compared with the "classical" periodontopathogens, such as *P. gingivalis*, *T. forsythia*, and *T. denticola*. In our study, endpoint RT-PCR was used to identify the presence of *F. alocis*. However, culture technique would have been ideal to detect *F. alocis* in gingival tissue samples. In our experiments, lysates of bacteria were used. As mentioned above, lipoteichoic acid and/or peptidoglycan could have been responsible for the observed effects of *F. alocis*. However, other virulence factors of *F. alocis* could also have been involved in the stimulatory actions of this pathogen on MMP-1. Moreover, periodontitis is a polymicrobial disease. Therefore, future studies should also examine the combined effects of *F. alocis* with other periodontopathogens on the production of MMP-1. In addition to the MMP-1 expression and protein synthesis, such studies could also focus on the activity of MMP-1 and its natural inhibitors. In our study, we analyzed the effects of *F. alocis* on fibroblasts and monocytes, because they are among the first cells encountered by the bacteria in periodontal diseases. However, periodontopathogens also get into contact with other structural and inflammatory cells of the gingiva, such as keratinocytes, macrophages, dendritic cells, and lymphocytes. In addition, periodontopathogens can also invade into deeper layers of the periodontal tissues. Thus, future studies should also focus on the effects of *F. alocis* on periodontal ligament cells, cementoblast cells, and alveolar bone cells.

Conclusions

Our results demonstrate that *F. alocis* and MMP-1 are more prevalent at sites of periodontitis. In addition, our study provides original evidence that *F. alocis* is able to induce MMP-1 expression and protein synthesis in fibroblastic and monocytic cells, which suggests that *F. alocis* may contribute to periodontal breakdown through activation of MMP-1 production. Furthermore, the stimulatory effect of *F. alocis* on MMP-1 is more pronounced on THP-1 cells in comparison with HGF-1 cells. Taken together, our study shows that *F. alocis* and MMP-1 are linked to each other and key players in periodontitis, which may have significant implications for future diagnostic and treatment strategies.

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Authors' contributions Defining the study aims: James Deschner; coordination of collaboration: James Deschner; planning the experiments: Marjan Nokhbehsaim, Anna Damanaki, Christina Piperi, Efthimia K. Basdra, Athanasios G. Papavassiliou, and James Deschner; growing the bacteria and preparing the bacterial lysate: Sigrun Eick; performing preexperiments: Andressa V. B. Nogueira and Anna Damanaki; performing the experiments: Marjan Nokhbehsaim, Anna Damanaki, Georgia Dalagiorgou, and Christos Adamopoulos; monitoring/supervising the experiments: Christina Piperi, Efthimia K. Basdra, Athanasios G. Papavassiliou, and James Deschner; analyzing and discussing the data: Marjan Nokhbehsaim, Andressa V. B. Nogueira, Anna Damanaki, Georgia Dalagiorgou, Christos Adamopoulos, and James Deschner; discussing the data: Sigrun Eick, Christina Piperi, Efthimia K. Basdra, and Athanasios G. Papavassiliou; creating the figures: Marjan Nokhbehsaim, Andressa V. B. Nogueira, and James Deschner; all authors contributed to the writing of the manuscript; all authors read and approved the final manuscript.

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Compliance with ethical standards

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

Research involving human participants All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the University of Bonn (043/11) 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.

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